

THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE

Report of the Governing Body,
1935.

CHELSEA BRIDGE ROAD,
LONDON, S.W. 1.

May 21st, 1935.

The Lister Institute of Preventive Medicine,

CHELSEA BRIDGE ROAD, LONDON, S.W. 1.
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* *Recognised Teacher of the University of London.*

ANNUAL GENERAL MEETING
OF
The Lister Institute of Preventive Medicine,
May 21st, 1935.

REPORT OF THE GOVERNING BODY.

The Governing Body has the honour to present the Institute's 41st Annual Report.

GOVERNING BODY.

The personnel of the Governing Body has undergone one change during the year. Owing to his public commitments Sir William Goschen, one of the representatives of Lord Moyne, was compelled to resign his seat upon the Board and has been succeeded by Lord Horder.

At the meeting held last year, the Council re-elected Professor W. Bulloch, Professor A. E. Boycott and Professor A. Harden as its representatives on the Governing Body until December 31st, 1935.

COUNCIL.

At the Annual General Meeting last year the three members of Council retiring were Lord Mildmay of Flete, representing the Royal Agricultural Society, Professor A. Harden and Professor J. C. G. Ledingham, representatives of the Members. Each of these representatives was re-appointed a member of the Council. A vacancy created in the representation of the Members of the Institute by the death of Sir Walter Fletcher, was filled by the appointment of Professor R. Robison.

The three members of Council, who in accordance with the Articles of Association retire this year by rotation, but who are eligible for re-election, are Professor R. T. Hewlett, Dr. L. C. Parkes and Dr. Harriette Chick, three representatives of the Members of the Institute.

MEMBERS.

The Governing Body regrets to announce the death during the year of Sir James Kingston Fowler, who served as a member of the Governing Body from 1918 till 1931, of Professor Georges Dreyer who had represented the University of Oxford upon the Council since 1920, and of Sir John Rose Bradford who was an original member of the Council of the Institute, served as a member of the Governing Body from its inception in 1899 to 1918, and acted as Chairman from 1912 to 1914.

STAFF.

The Director, at the invitation of the Johns Hopkins University, delivered the Herter Lectures on "Studies on Virus Problems" at Baltimore in December last.

Dr. A. Felix by arrangement with the Colonial Office, spent two months in Palestine investigating the curative properties of a new Anti-typhoid serum, in collaboration with physicians at Jaffa, Telaviv and Jerusalem.

Dr. C. Russell Amies completed his appointment as an Institute Research Fellow in Bacteriology on December 31st. He is continuing to work at the Institute under the auspices of the British Empire Cancer Campaign.

Dr. L. A. Elson, Jenner Memorial Research Student and Mr. V. N. Patwardhan, Grocers' Company Research Student, completed the tenure of their studentships on May 31st and September 30th respectively.

During the year Mr. S. S. Randall has continued to hold an Institute Research Studentship in Biochemistry and Mr. R. O. Jones the Morna Macleod Research Studentship, which is endowed by Mr. W. A. Macleod. Mr. Eric Downing, B.Sc. was appointed Grocers' Company Research Student on April 1st and is attached to the Department of Biochemistry.

RESEARCH WORK.

Before surveying the scientific work carried out during the year in the various departments, the Governing Body desires again to record its appreciation of the continued co-operation it has enjoyed with the Medical Research Council. Salaries of the staff of the National Collection of Type Cultures, of Miss Hume and Dr. Zilva and his assistants and part salaries of Professor Korenchevsky and Miss Hume's assistant have been furnished by the Council, the Institute providing, as hitherto, accommodation for each of these workers and the necessary materials for their researches.

The Governing Body also desires to record its gratitude to the Rockefeller Foundation, which through its European Assistant Director of Medical Sciences, Dr. Daniel P. O'Brien, has made a grant of £3,400 to the Institute for the purchase of a velocity and an equilibrium centrifuge with their accessories to be manufactured in Professor Svedberg's workshop in Upsala. Dr. A. S. MacFarlane (Beit Memorial Research Fellow), now working in the Institute, has had considerable experience of their working while carrying out research on the serum proteins under Professor Svedberg's direction. The provision of accommodation for the centrifuges and their maintenance after installation will be a charge upon the funds of the Institute.

It is anticipated that this new and valuable equipment will, when it becomes available, find useful application in exact investigations on the proteins of serum and of many problems in biochemistry, serology and virus research.

The hospitality of the Institute's laboratories has this year been extended to three foreign guests displaced from scientific posts in Germany, viz., Professor Ph. Ellinger, Miss E. Klieneberger and Dr. M. Gutstein, late Physician and honorary research worker at the Virchow Hospital, Berlin.

DEPARTMENT OF BACTERIOLOGY, SEROLOGY AND EXPERIMENTAL PATHOLOGY.

Studies on Viruses.

Vaccinia. Cultivation of the virus on artificial media. Dr. G. H. Eagles has conducted two further series of successful passages of vaccinia virus in a cell-free medium. In one series elementary bodies, obtained from Berkefeld "V" filtrates of dermal virus centrifuged at high speed, were used as seed. In another a Berkefeld "V" filtrate of neuro-testicular virus was employed. Investigation has been made into possible sources of error in the technique employed and in the interpretation of the results obtained. No reason has been found for the fact that in certain instances an initial seeding may be carried through serial subculture while others, under apparently identical conditions, fail. It is assumed, therefore, that vaccinia virus may with difficulty, and under conditions which cannot at present be controlled at will, be cultivated in the absence of viable cells. Until further evidence on the nature of the virus and its essential growth requirements are available, the problem of simplifying its cultivation must remain unsettled.

Elementary body suspensions as a possible substitute for ordinary vaccine lymph. Elementary bodies from dermal lymph were obtained by filtration through Berkefeld "V" candles and high speed centrifugation of the filtrates. The deposits were re-constituted in (1) normal saline, (2) saline plus a small percentage of plain agar and (3) plain broth. After storage in the cold for varying periods tests were made to determine their survival. When suspended in plain broth elementary bodies were found to be highly active after a period of 400 days in the cold. Survival was well maintained in saline to which agar had been added while in saline alone, or in buffered phosphate, a rapid decline in infective titre was the rule. Animal experiments showed these suspensions to be in every way comparable to ordinary lymph. They possess the advantage of bacteriological sterility and represent the virus in the purest form available.

Human vaccinations have not yet been made with these suspensions but their possibilities in this respect will be investigated when an opportunity occurs.

Elementary bodies as antigen in complement-fixation with antivaccinal serum. Dr. M. H. Finlayson, formerly Finkelstein (Research Fellow in Bacteriology), before his departure to South Africa, completed his investigation into the power of suspensions of elementary bodies from vaccinia to bind complement in the presence of antivaccinal serum. Complement is fixed in the presence of vaccinia hyperimmune rabbit serum. This activity of the suspensions is reduced but not abolished by filtration through Berkefeld "V" and "N" filters, and through gradocol membranes of pore size 0.65 μ . It is abolished by filtration through Seitz filters, through gradocol membranes of pore size 0.15 μ and by centrifugation at high speeds. In the high-speed centrifugation experiments the antigenic activity is retained in the deposits.

The B virus from acute ascending myelitis in man. Dr. A. B. Sabin (now of the Rockefeller Institute, New York) continued his study of this interesting virus, giving special attention to certain affinities it appears to manifest with other neurotropic viruses such as herpes and pseudorabies. The infection is easily transmitted to rhesus monkeys and rabbits. In the latter the course of the human infection is fairly faithfully reproduced; from an inflammatory and necrotic skin lesion the virus ascends by the peripheral nerves to the cord, where it gives rise to an ascending myelitis terminating in involvement of the vital medullary centres. In the monkey, while intracerebral inoculation is fatal, peripheral lesions show little tendency to originate an ascending infection of the central nervous system. In spite of these differences in the clinical course of the disease in the two species, the underlying cellular lesions are almost identical and bear close resemblance to those of pseudorabies and herpes. The guinea-pig is much less susceptible to infection, reacting only with a rise of temperature and subsequent immunity, or occasionally showing a myelitis of limited extent and severity.

The B virus is probably indigenous to the rhesus monkey, and occasional "normal" animals show a high titre of neutralising antibodies in their blood. The results of quantitative serum-virus neutralisation tests suggest that while this virus is an immunological entity, it has a partial (group) relationship with herpes and pseudorabies, but none with Virus III or vaccinia. Incidentally there appears to exist a similar partial relationship between pseudorabies and herpes.

The B virus readily passes Berkefeld "V" and "N" and Chamberland L3 filters, but is held back by a two-disc Seitz filter.

The virus of equine encephalo-myelitis. Dr. E. W. Hurst has studied in monkeys and guinea-pigs the course of infection with equine encephalo-myelitis virus. Rapid multiplication follows intradermal, intramuscular or intravenous inoculation, and the virus may attain considerable concentration in the blood. Unexpectedly, this multiplication cannot be attributed to growth in the tissues as a whole: either the virus multiplies actually in the blood, or else in some tissue (endothelium) closely connected with the circulation and forming only a small fraction of the total weight of the organs. Unlike other neurotropic viruses, the present contagium reaches the nervous system by way of the blood stream and not by the peripheral nerves.

Other experiments to ascertain the relative ease with which infection is brought about by intravenous inoculation of various neurotropic viruses, and to study their fate on intramuscular inoculation are still in the initial stages. It is hoped ultimately to establish a basis of classification of these viruses.

Poliomyelitis: estimation of potency of human convalescent sera. In collaboration with the Danish State Serum Institute, Copenhagen, an investigation is being made by Dr. Eagles into the neutralising value of a number of samples of serum from Danish cases of poliomyelitis at different periods following recovery from the disease. For purposes of quantitative comparison a serum obtained by immunisation of horses at Elstree with the virus is being used. When completed and in conjunction with the epidemiological data the results of these tests, for which monkeys are necessary, should be of interest and value.

The mechanism of action of antiviral serum. The nature of the interaction, if any, that takes place *in vitro* between the neutralising antibodies in a serum and the virus and of the immunity that antiviral serum obviously confers when introduced with the virus into the body, has long been a matter of controversy. A renewed intensive study of this important question has been made by Dr. Sabin. With the aid of a high-speed centrifuge (14,000 r.p.m.), which rendered possible the complete sedimentation and quantitative recovery of vaccinia, pseudorabies, and B virus, it was found that the protective substance in antiviral sera neither combined with nor inactivated these viruses even after prolonged periods of incubation. In cultures containing susceptible tissue, immune serum, and virus, the protective effect was exerted without any demonstrable direct effect upon the virus; the protective substance apparently acted upon the tissue by rendering it refractory to "infection," and unsuitable for the multiplication of the virus. By treating normal, susceptible tissue with antiviral serum and then separating the tissue from the serum, it was possible to show that it had been rendered refractory to infection even though it was surrounded by and had "fixed" active virus. It was also found that the protective substance is fixed by the tissue, and that both the fixation and the effect are reversible by washing. Experiments with leucocytes revealed that while they take up or fix virus, they become highly infectious thereby and thus play no part in preventing infection; no opsonic effect of immune serum on virus was demonstrable. Dr. Sabin's study suggests that the virus itself may not be the direct antigenic stimulus, but that some substance upon which it acts and which becomes antigenic when liberated from infected cells may be the factor responsible for the formation of the immune protective substance.

It is possible, however, that other interpretations of the experimental data set forth by Dr. Sabin may be forthcoming and also that other experimental conditions may reveal a state of affairs not so dissimilar to that shown to exist between antigens and antibodies commonly studied. The problem is being again attacked on somewhat different lines from those of Dr. Sabin.

Avian Sarcoma: The nature of the tumour-exciting agent in filtrates of avian sarcomata. During the past eighteen months Professor J. C. G. Ledingham, in collaboration with Dr. W. E. Gye of the

National Institute for Medical Research, has made a preliminary study of these fowl tumours on lines which have already yielded fruitful results when applied to virus problems. It has now been shown that the agent responsible for tumour-production can be thrown down from cell-free filtrates by high speed centrifugalisation and that in the deposit it exists in the form of elementary bodies of considerably smaller size than those of vaccinia. Suspensions of these elementary bodies recovered from filtrate-deposits have been prepared and it has been possible to demonstrate in the serum of tumour-bearing fowls antibodies (agglutinins) for these elementary bodies. A new approach is thus open to further enquiry into the aetiology of these avian tumours and possibly also of the mammalian tumours which hitherto have not proved filterable.

Dr. C. R. Amies (formerly Research Fellow in Bacteriology) is continuing Professor Ledingham's work on this subject at the Institute under the auspices of the Scientific Advisory Committee of the British Empire Cancer Campaign.

Acute Rheumatism in Children. Dr. Amies, during the latter part of his tenure of a Research Fellowship in Bacteriology, made a tentative enquiry into this disease from the standpoint of its being possibly due to a virus. Some very interesting data have been collected which, at any rate, point in the direction indicated and it is proposed to publish a note on the work at an early date. Material for investigation was supplied by Dr. Bernard Schlesinger of the Hospital for Sick Children, Great Ormond Street.

Pleuropneumonia-like organisms as symbionts of certain bacteria. Dr. E. Klieneberger, in the course of her work on the growth phases of pleuropneumonia and agalactia, had her attention drawn to certain peculiar swollen elements of doubtful nature in cultures of *Streptobacillus moniliformis*, an organism pathogenic to man and also to mice, but occurring saprophytically in the nasopharynx of rats. It has now emerged, from morphological investigations carried out by the fixing and staining technique previously used in Miss Klieneberger's study of the morphology of pleuropneumonia and agalactia, that in these cultures a filamentous network producing swollen bodies and small granules develops independently from the bacillary chains of the *Streptobacillus*. The presence of these formations, which resemble those of the organisms of pleuropneumonia and agalactia, in all strains of *Streptobacillus moniliformis* investigated led to the conclusion that these strains are composed of two components, a true bacterium and a pleuropneumonia-like germ. This was proved by cultural experiments; it was possible to isolate this pleuropneumonia-like organism and to subculture it without the bacillus in 50 passages up to date. This L.1 organism, culturally and morphologically resembling the organism of pleuropneumonia shows also in respect of filterability a close similarity with pleuropneumonia and agalactia.

Study of a streptococcus from the nasopharynx of the guinea-pig revealed similar facts. A very delicate pleuropneumonia-like organism (L.2) was found to be living in symbiosis with this coccus. Pure cultures of the L.2 germ have not yet been obtained.

It is to be expected that similar symbionts may also be found in association with other bacterial cultures. It may, indeed, emerge that polymorphic elements described by their observers as belonging to sexual phases or cycles of bacteria, may prove in reality to be attributable to symbionts contaminating these cultures.

Serological Studies : Antigenic Constitution, Virulence and Immunising Properties of Bacteria.

***B. pestis* and its envelope antigen.** Dr. H. Schütze's examination of anti-plague therapeutic sera has shown that the antibody for the envelope antigen is contained chiefly in the pseudoglobulin fraction of serum prepared in the buffalo and in the euglobulin fraction of serum prepared in the sheep. The protective values of such sera when tested on rats are correlated with their content of envelope antibody as recorded by the precipitin test. It would thus appear that a similar importance attaches to envelope antibody in therapeutic passive protection and to envelope antigen in prophylactic experiments on rats with anti-plague vaccine.

The Pasteurella Group. Dr. H. S. Yusef (Cairo) completed his investigation of the use of the precipitin test as a method for the classification of strains belonging to the Pasteurella group. Using a specially prepared bacterial antigen and the technique of the absorption test, he was able to classify 14 of the 21 strains examined. Three serological types were, in this way, established, consisting of 7, 4 and 3 strains respectively, the remaining 7 strains not being classifiable, in all probability because of the lack of any distinctive antigen. When these results were contrasted with a previous attempt by Dr. Cornelius to classify the same set of strains by the agglutination-absorption method, it was seen that there was agreement between the two methods in 8 cases and disagreement in 2, that 3 strains previously classified were now unclassifiable and that of 8 strains previously found unclassifiable, 4 were now classified by the precipitin method.

Spore-bearing Anaerobes : *C. oedematis maligni* : histology of response in protected and unprotected animals. Dr. D. Henderson (Beit Memorial Research Fellow) has completed his study of the antibacterial mechanism of protection against experimental infection in guinea-pigs. Histological examinations were made of the progressive dermal lesions in immunised and control animals subjected to an intradermal injection of an activated spore suspension. Lesions in normal animals were characterised by

lack of polynuclear response with extensive bacterial proliferation and death of the animal in from 14-18 hours. The outstanding feature in protected animals was the massive polynuclear response which immediately restricted the area of bacterial proliferation. At 12-14 hours after infection there was evidence of clumping of organisms and also direct lysis. The barrier of polymorphs was extended until about the 50th hour when the first evidence of histiocytic activity appeared. Thereafter dense granulation tissue was laid down and complete healing was effected within 14 days.

The bearing of guinea-pig and rabbit protection tests on the practical application of anti-bacterial prophylaxis (e.g. in sheep). A new development in the practical application of antibacterial methods has followed Dr. Henderson's experiments on the active immunisation of rabbits against the intramuscular injection of activated suspensions of *C. oedematis maligni*. It was first shown that rabbits were more readily immunised than guinea-pigs and that quantities of "O" antigen insufficient to protect guinea-pigs afforded complete protection to rabbits. Subsequently, experiments were made on the comparative value of formolised whole-culture and the bacterial substance and formolised filtered toxin respectively, which could be obtained from the same volume of whole-culture. The results showed that 10 c.c. of whole-culture or the bacterial substance from this volume protected rabbits against multiple infective doses of spores, while 10 c.c. of formolised filtered toxin gave no protection against a minimal infecting dose. It was also shown that the sera prepared with the first two antigens gave passive protection to mice while the antitoxic sera were valueless. Previous conclusions, therefore, drawn from the results of guinea-pig experiments in relation to the practical application of antibacterial prophylaxis require modification. It is clear that the results of experiments with one animal species cannot be interpreted in terms of another and no claim can be made as to the impracticable nature of pure antibacterial prophylaxis until field experiments have been conducted with the animal species involved in the naturally-occurring disease.

An intradermal technique for the more accurate titration of activated spore suspensions and of sera in protection tests has proved very satisfactory.

***C. tetani*.** Dr. Henderson has completed his studies on prophylaxis of *C. tetani* infection. The experiments have been mainly concerned with the passive protection of mice against activated spore-suspensions, using pure "O" horse sera and to a lesser extent pure antitoxic sera.

Antibacterial methods similar to those used in protection against experimental gas-gangrene infection have been shown to be less effective against tetanus. Where the calcium chloride technique was used for initiating infection no protection could be afforded either to guinea-pigs or mice by the use of high-titre pure "O" sera. When less severe methods were used specific protection was demonstrated, although a particularly careful balance in the degree of activation of spores was necessary in order to repeat the results with success. Non-toxic tetanus cultures and strains of *B. subtilis* were used as activators in these latter experiments.

The "Vi" antigen of *B. typhosus*. Dr. A. Felix, in collaboration with Miss R. M. Pitt, has continued the study of the relationship between virulence and susceptibility to the action of the "O" antibody. The experiments reported previously had indicated the presence in virulent strains of *B. typhosus* of some obscure factor, that renders the smooth "O" antigen resistant to the action of the "O" antibody. Further experiments revealed the fact that the unknown factor is itself an antigen, separate and distinct from the long-established "O" and "H" antigens of *B. typhosus*. Because of its close association with virulence the symbol "Vi" has been adopted for the new antigen and its corresponding antibody.

Demonstration of the "Vi" antigen by *in vitro* methods. The "Vi" antibody is capable of clumping the inagglutinable strains, whereas the "O" antibody is unable to produce this effect. The "Vi" antigen can also be demonstrated by absorption tests with serum containing "Vi" antibody. This procedure at present would appear to be the most reliable method of quantitative estimation of the "Vi" antigen. Saline extracts of cultures of virulent strains of *B. typhosus* contain "Vi" antigen precipitable by pure "Vi" antiserum.

Active and passive immunisation of mice. Mice can be protected against infection with a highly virulent strain of *B. typhosus* only by using for immunisation vaccines from strains containing "Vi" antigen. Vaccines prepared from smooth cultures or strains devoid of "Vi" antigen are efficient in protection against infection with this type of *B. typhosus* of low virulence, but of no value in protection against the virulent type which possesses "Vi" antigen.

Passive protection experiments in mice showed that rabbit immune sera containing "Vi" antibody exert a powerful protective action against infection with highly virulent strains of *B. typhosus*, whereas sera containing high-titre "O" antibody but no demonstrable "Vi" antibody are of no protective value whatever.

Neutralisation of endotoxin. The toxic effect on mice of a dead vaccine of *B. typhosus* is neutralised by immune sera with high content of "O" antibody. The "Vi" antibody does not contribute towards the endotoxin-neutralising power of the serum, just as the "Vi" antigen present in virulent strains does not cause any increase in toxicity.

Serum therapy of typhoid fever. The results of the experiments in mice indicated that the efficacy of a therapeutic anti-typhoid serum would depend on the presence in it of both the "Vi" and "O" antibodies. Serum of this type has been prepared from horses in collaboration with Dr. Petrie, Dr. McClean and Dr. Morgan. Thanks to facilities granted by the Colonial Office and by the Government of Palestine, Dr. Felix has had the opportunity of testing the serum on a number of typhoid patients in Palestine. In the course of these clinical trials 43 patients were treated with two batches of the new anti-typhoid serum and 17 control cases were treated with normal horse serum. The results of the treatment with the two anti-typhoid sera were definitely encouraging and have been published.

During his stay in Palestine Dr. Felix, in collaboration with Dr. K. S. Krikorian and Dr. R. Reitler of the Government Department of Health, tested 90 strains of *B. typhosus* freshly isolated from typhoid patients and found only two strains entirely devoid of "Vi" antigen, whereas 88 strains were of the intermediate type containing this antigen. From this observation two conclusions of practical importance seem to be quite obvious: (a) to prevent infection with the most common type of *B. typhosus* the vaccine used must contain "Vi" antigen; (b) to be efficient in the treatment of the most common type of typhoid case an anti-typhoid serum is required containing "Vi" antibody.

The blood serum from 113 typhoid patients and convalescents was also tested for the presence of "Vi" antibody. Only 11 of these patients were found to have formed "Vi" antibodies in their blood serum, indicating that this antibody is not readily elaborated as a result of an attack of typhoid fever. This observation further strengthens the conclusion that the "Vi" antibody is an essential component of an efficient anti-typhoid serum.

Phagocytosis of *B. typhosus*. Captain S. S. Bhatnagar (Indian Medical Service, Karachi), working with Dr. Felix, has studied phagocytosis of *B. typhosus* in relation to the antigenic structure of the organism and the antibody components of the sensitising serum. Normal and immune sera from rabbits were used and they were tested against the three types of *B. typhosus*: inagglutinable (virulent), agglutinable (avirulent) and of intermediate agglutinability (and virulence).

When a single normal serum was tested against a number of strains of the three types, the degree of phagocytosis was invariably found to depend on the susceptibility of the organisms to the action of the "O" antibody. On the other hand, when a single strain was tested against a number of normal sera, the degree of phagocytosis invariably depended on the titre of the natural "O" agglutinin contained in the normal serum. The natural opsonin can, therefore, justifiably be identified with the natural "O" antibody acting in conjunction with the complement.

In immune sera containing "O" and "H" agglutinins it is the "O" antibody alone that is responsible for sensitisation for phagocytosis. The "H" antibody does not take part in this reaction.

The resistance to the opsonising effect of the "O" antibody of virulent strains of *B. typhosus* is due to the "Vi" antigen present in this type of strain. Immune sera containing "Vi" antibody, either alone or in the presence of "O" antibody, exert a strikingly powerful opsonising effect on strains possessing "Vi" antigen. The presence of complement is indispensable for this reaction taking place.

***Micrococcus gonorrhoeae*.** Dr. V. B. Abdoosh (Cairo) is investigating problems concerned with gonococcal immunity, natural and acquired, and for this purpose he has been carrying out tests of the bactericidal power of serum, human and animal, normal and immune. He has been able to show that while nearly all animal sera tested possess a high content of natural bactericidins for the gonococcus, normal human serum is practically devoid of them.

The biological reactions of Gram-positive and Gram-negative organisms.

Dr. M. Gutstein (Berlin) is continuing his studies in the Institute on the Gram-positive and the Gram-negative groups of micro-organisms. He had already shown that the addition of various dyes to nutrient medium resulted in the production of cultures with colour reactions corresponding to a pH of about 6.4 in the case of Gram-positive organisms and in the case of Gram-negative organisms to a pH of 7.2-7.6. He considers that these facts indicate that Gram-positive organisms possess a more acid cell content than do Gram-negative ones. He is now attempting to test this hypothesis by the varying ability of the two ferments, trypsin and pepsin, to suppress the growth of members of the two groups of organisms. It would appear from preliminary experiments that trypsin has a greater inhibitory power upon the Gram-negative, that is to say, the supposedly more alkaline group of bacteria, while pepsin has a converse effect.

Resistance to Infection and Genetic Constitution.

Resistance to infection of pure lines and selected races of mice. Dr. Schütze is continuing with Dr. P. A. Gorer the work which he began with Dr. Finkelstein on the resistance to infection of two selected and two pure-line strains of mice. They have demonstrated that those strains which are more resistant to infection with *S. typhi murium* are also more resistant to infection with *S. enteritidis*; on the other hand, no significant difference in the resistances of the strains of mice has been observed when they are infected with the pneumococcus or the virus of louping-ill.

Blood groups in mice. Using a human Group II serum, Dr. Gorer has found it possible to distinguish two types of mouse erythrocytes. A strong reaction (as measured by ability to absorb agglutinin and by direct agglutination) is determined by a single gene, dominant to a weak reaction. Individual differences between the bloods of different mice have been detected by means of experiments with immune sera.

Studies on Corynebacteria.

C. ovis (Preisz-Nocard) and *C. ulcerans*: **Fermentation of Starch.** Dr. M. M. Barratt had noted that the aberrant corynebacteria from the human nasopharynx (*C. ulcerans*) which exhibited many affinities with *C. ovis* fermented starch. This *C. ovis* failed to do (1 equine and 9 ovine strains tested). Dr. H. R. Carne, D.V.Sc. (Sydney), in the course of his work here examined 150 strains of *C. ovis* mainly from Australian ovine sources and all failed to ferment starch.

Another point of difference has emerged. While all the *C. ulcerans* strains and the equine strain of *C. ovis* with which Mrs. Barratt worked failed to produce chronic caseous adenitis in guinea-pigs, even the smallest doses of the ovine strains investigated by Dr. Carne invariably produced such lesions both in Australian guinea-pigs and in guinea-pigs at this Institute. An elaborate investigation was carried out by Dr. Carne with the object of ascertaining whether the production of such lesions in guinea-pigs (as in the naturally occurring ovine disease) could be prevented by active immunisation in various ways (toxoids, toxin-free bacillary "O" antigen) or by passive immunisation with the corresponding antibodies prepared in goats. The results were, on the whole, rather disappointing in so far as the complete prevention of caseous lesions was concerned. It appeared, however, that antitoxin played an important part in the protective mechanism. The failure of the antibacterial sera was probably due to too drastic treatment of the "O" antigen and improvement in the preparation of the latter may give more satisfactory results. These further experiments and other studies commenced here will be undertaken in Australia.

In the last report the isolation of 6 additional aberrant strains of Corynebacteria at the City Laboratory, Nottingham, St. Bartholomew's Hospital and at the Institute was recorded. Examination of broth cultures in the guinea-pig, with the use of a high titre *C. ovis* (Preisz-Nocard) antitoxin prepared by Dr. Carne, in addition to diphtheria antitoxin, has shown that the 11 aberrant corynebacteria isolated in this country fall into 2 almost equal groups. The first, including Mair's strain "2255," and 5 of the additional strains, cause changes in the suprarenals resembling those produced by *C. diphtheriae* and the lives of animals inoculated with multiples of the lethal dose are saved by diphtheria antitoxin but not by *C. ovis* antitoxin. The second group, including one of the additional strains and all the first series, "Stead," "Quitman," etc., with the exception of Mair's strain, resemble *C. ovis* in having little, if any, effect on the suprarenals and the high titre *C. ovis* antitoxin saves the lives of animals inoculated with multiple lethal doses whilst diphtheria antitoxin does not protect, if at all, against more than 1 lethal dose. In neither group does the protecting antitoxin prevent a lesion at the site of inoculation.

In the second group the protection given against the living culture by the *C. ovis* antitoxin was of particular interest in confirming the close relationship of the "Stead-Quitman" type of aberrant strain to *C. ovis*, which had already been demonstrated in their cultural and other characters, and by Dr. Petrie and Dr. McClean in their toxin-antitoxin relationships.

Brucella abortus.

In the examination for *Brucella abortus* of a series of milk samples produced in Norfolk, Dr. Barratt found that 25% of the 36 strains isolated did not require CO₂ for growth in primary culture or the first subculture. No other series of milk samples has been examined but the isolation of similar strains from a few random samples tends to show that such strains occur more commonly in the milk of this country than was at one time supposed.

European Foul Brood of Bees.

Dr. H. A. L. Tarr, seconded from Rothamsted Experimental Station, was granted facilities for the cultural and serological examination of a variety of strains of the spore-bearing organism *B. alvei* isolated from bee larvae affected with European Foul Brood and considered to be the causative organism of that disease. The work is in progress.

The Physical Nature of Ultramicroscopic Particles.

The problem of investigating the physical properties of very small particles of animate or inanimate matter, is becoming increasingly important in many biological fields. It appears to be unlikely on theoretical grounds that the existing methods of studying individual particles, for example, by means of the ultramicroscope, will be capable of extending our knowledge to particles smaller than the largest viruses. There is, however, another method of attack which depends on the investigation of physical properties of large collections of particles which represent statistical mean properties of all the particles. In this category

there stands out the investigation of moving boundaries in high centrifugal fields or in strong electric fields. These two methods are capable of giving valuable information regarding the size, shape, and charge of the particles, provided these are all approximately alike.

Svedberg and collaborators in Upsala have developed the technique of these experiments to a high standard of perfection and have applied it to the investigation of the very small particles which constitute protein molecules. Their results have introduced a precision and order into our conception of these important substances where formerly only the vaguest ideas existed. Dr. A. S. McFarlane (Beit Memorial Research Fellow) working in Svedberg's laboratory, has brought to light certain phenomena relating to the state of the proteins in untreated serum in health and disease which promise to open up new possibilities in the fields of diagnosis and prognosis. It has been found also that the urinary proteins in nephritis are not always identical with the serum proteins, a fact which has certain implications with regard to the pathology of this disease.

During the past six months Dr. McFarlane has continued his researches here along theoretical lines and in the preliminary setting up of the complicated physical apparatus which is required for the measurement of the rate of migration of charged proteins in the electric field.

When the Svedberg centrifuges, to which we have already referred, have been installed in the Institute, it will, we hope, be possible for Dr. McFarlane to continue the line of investigation on which he embarked in Upsala.

Endocrinology.

Dr. V. Korenchevsky with the assistance of Mrs. M. H. Dennison and in some experiments with the collaboration of Dr. S. Levy Simpson, investigated the effect on male rats of the sexual hormones (testicular hormone and oestrin) and of adrenalectomy. At the request of Professor Ruzicka of Zürich he undertook to co-operate with him in the investigation of the male sexual hormones and allied substances prepared synthetically by Professor Ruzicka.

On the method of Assay. An alteration was introduced in the definition of the rat unit in the method of assay of male sexual hormone, since, while only with testicular hormone was there a marked change in the weight of the prostate, the weight of the seminal vesicles is increased both by testicular hormone and by oestrin. Two rat units were, therefore, suggested for the male sexual hormones :

- (i) a rat unit of the "comb-growth promoting" factor of the testicular hormone preparations (as judged by changes in the weight of the prostate) ;
- (ii) a rat unit of the "whole male sexual activity" of the sexual hormone preparations (as judged by the changes in the weight of the prostate and seminal vesicles taken together).

Assay of androsteron, the synthetic testicular hormone, prepared by Ruzicka. With the Korenchevsky method of assay it was found that the effect of androsteron in increasing the weight of the atrophied prostate of castrated rats is nearly directly proportional to the dose, with doses of 0.2 mgm. to 0.9 mgm., after which dose the curve flattens.

Androsteron contains both one rat unit of "comb-growth" activity and one rat unit of "whole male sexual activity" in about 170%, the rat unit approximating to, if not being equal to, the capon unit.

The effects of prolonged injections of androsteron. After the injection for three weeks of a nearly saturated solution of androsteron in olive oil (about 11 rat units per day) the return towards normal obtained in the weight of the atrophied secondary sexual organs was only to the extent of about one third. To obtain a complete return to normal the large doses used should be given for a much longer period, or, as is indicated by the results mentioned below, the simultaneous action of at least two hormones is necessary.

In castrated males and in ovariectomised females, androsteron also caused the following changes (1) a return towards or to normal in weight of the adrenals (hypertrophied after castration) ; (2) an increase in the weight of the liver, kidney and, in most males to a slight extent, the thyroid (which decreases after castration) ; (3) the restoration of the normal rate of involution of the thymus ; and (4) an increase in the appetite and gain in body weight. Injections into castrated males also increased the weight of the heart (which is slightly decreased by castration). In addition to these favourable results on the weight of the organs, histological investigation showed the absence of any toxic effect of androsteron in those organs of castrated rats, the structure of which is not considerably changed by castration (liver, kidney and heart) and a return towards or to normal in those organs, the microscopical structure of which undergoes severe changes after castration (sexual organs, adrenals).

Androsteron did not cause a return to normal (in weight or histologically) of the hypertrophied hypophysis of castrated males nor did it produce oestrus in ovariectomised females, even when injected in doses of 1.8 mgm. for 21 days. It also had no "rejuvenating" effect on old male rats in the doses used.

Effect of Oestrin on males. Both testicular hormone and oestrin are produced, often in approximately equal quantities, in males and in females. It is, therefore, important to study the effect on both sexes of the administration of these hormones alone and simultaneously. Up to the present only the investigation of the effects of these hormones on males has been completed.

In normal rats large daily doses of oestrin decreased the appetite and gain in body weight, without any definite changes in fat deposition, depressed the development of the sexual organs and produced hypertrophy of the adrenals and hypophysis.

In castrated rats, oestrin decreased the gain in body weight of most animals but did not produce definite changes in fat deposition; greatly increased the weight of the seminal vesicles, slightly increased that of the prostate and less constantly that of the penis and preputial glands and produced hypertrophy of the hypophysis. With larger doses, or after a prolonged period of injection, the rate of involution of the thymus was increased in most cases. It may, therefore, be concluded that the presence of oestrin in the male organism has a definite effect on some of the organs, which are also influenced by testicular hormone.

The effect on male rats of the simultaneous administration of male and female sexual hormones. The addition of oestrin to testicular hormone injections greatly increased the weight of the seminal vesicles and caused only a slight increase, if any, in the weight of the prostate, penis and preputial glands. Besides the effect on the sexual organs, the addition of oestrin to testicular hormone injections was followed by a decreased gain in body weight and fat deposition, an acceleration in the speed of involution of the thymus and increased hypertrophy of the hypophysis and a slight increase in the weight of the kidneys.

The histological investigation of all the organs is not completed, but it is possible to state now that oestrin in some doses, in addition to the physiological effect mentioned above, has a pathological effect on the secondary sexual organs of the male rat.

Adrenalectomy in male rats. A study was made of the symptoms of chronic adrenal deficiency in adrenalectomised rats, some of which are similar to those associated with Addison's disease.

It is not possible to make an accurate study of any of the effects of adrenalectomy unless the control litter mates are paired-fed, i.e., the same amount of food is given to the control rats as was consumed the previous day by the adrenalectomised litter mates. Paired feeding was used in order to distinguish the effects of adrenal deficiency on the body weight, fat deposition and weights of adrenalectomised rats from those due to the decreased appetite always present in such rats.

One or both of these factors were found to be responsible for the various changes obtained in nearly all the organs investigated. Both were concerned in the impaired growth, decreased gain in body weight and poor fat deposition of the experimental rats.

Adrenal deficiency alone seemed to be responsible for the increase in the weight of the secondary sex organs and the delayed involution of the thymus.

The small decrease in the actual weight of the heart and the somewhat larger loss in weight of the liver were chiefly due to the decreased food intake.

The gain in body weight, when calculated per unit of food intake was much less in the adrenalectomised rats than in their paired control litter mates. This suggests that cortical hormone may have, in addition to an "appetite producing property," an "anabolic" function on metabolism and that both are important for the building of tissues and for the growth of the animal.

It is interesting to note that rats which, as judged by their external appearance, behaviour, gain in weight and normal appetite, were considered to have recovered from the effects of adrenalectomy, showed the changes in the organs which have been mentioned above and were, therefore, actually in a state of latent adrenal insufficiency.

Coagulation of the Blood.

Dr. J. O. W. Barratt, in continuation of previous work, has been engaged in a comparative study of the anti-coagulant action of sodium chloride, heparin and chlorazol. Experiments have been made as far as possible under strictly similar conditions with the largest range of concentrations which could be usefully employed and have been illustrated by numerous graphs. These have led to an attempt to find a mathematical expression applicable to the observed coagulation periods which would enable the anti-coagulant effect to be exhibited in quantitative form.

Investigation of Kidney Function.

Dr. P. Ellinger has extended his method of intravital microscopy by direct photography of the living tissue. Photographs have been obtained showing the distribution of the lyochromes in the urine of frogs and rats.

The investigation of the mechanism of urine production has been continued. In normal urine excretion, both in frogs and mammals, the urine is produced by filtration through the glomerular epithelium and is concentrated by selective re-absorption in the tubules. After inundation of the body with substances excreted in the urine, or after a strong lowering of the blood pressure in the glomerular capillaries, the kidneys of both types begin to secrete urine through the epithelial cells of the proximal tubules. Urine produced in this way is always very acid while normal urine is only slightly acid or nearly neutral. These results have provided an interpretation of the classical experiments of Nussbaum and of Ghiron. If this

secretion of urine through the proximal tubules is prolonged, the epithelium of the proximal tubules loses the property of secreting the urine and becomes irreversibly destroyed, a fact which may explain some symptoms of kidney disease. The result suggests a new theory of urine production which may reconcile the antagonistic theories of Ludwig and Cushman and of Bowman and Heidenhain, respectively.

DIVISION OF PROTOZOOLOGY.

The study of the effect of gamma ray irradiation of actively growing cultures of the protozoon *Bodo caudatus* have been continued by Dr. Muriel Robertson and brought to a conclusion. The main results can be summarised as follows :—

The development of the maximum reduction in growth in irradiated cultures takes place during the life of the first generation of cells exposed and, if the irradiation is continued, there is a recovery in the rate of multiplication while still in the presence of the radium, so that, after the 10th hour, coinciding with the evolution of the 2nd generation of cells in the exposed plate, the bodos are multiplying at about the normal rate. There is, however, no overtaking of the normal by the irradiated and the discrepancy of about 40% in the total numbers remains constant to the end of the growth period of 22 hours, which was the limit of the experiments.

The bodos whose prolonged delay in entering division in the presence of the radium causes the maximum discrepancy in multiplication, occupy a certain position in the age and size groups of the cells at the time of the inauguration of the irradiation. This was found to indicate a period of particular sensitiveness to gamma rays in the life of the cell.

The cells during the period of maximum suppression of division grow to an enhanced size above that of the normal and a larger size is maintained during the subsequent development of the culture in the presence of the radium. It is this process of temporary morphological and physiological adaptation that appears to permit of the resumption of the growth rate and may be termed the "radium conditioning" of the cells.

Upon removal from the radium, an effectively irradiated culture, after a period of time varying with the effectiveness of the irradiation, begins to show an acceleration in the rate of multiplication above the normal until, if sufficient time is allowed, the numbers of cells on the released plate come to equal those on the normal control. During this compensatory process the enhanced size produced by growth in the presence of radium gradually returned to the normal.

There was no sign of radium fastness and re-irradiations were always effective in some degree.

Certain aspects of the metabolism of these irradiated cultures have also been worked out in collaboration with Mr. Norman Lawrie (Beit Memorial Research Fellow, Department of Biochemistry, Cambridge).

The thanks of the Institute are due to the Medical Research Council for the loan of the radium used in these researches.

DIVISION OF NUTRITION.

Vitamin Standards and Units.

Second International Conference on Vitamin Standardisation—London, June, 1934.

During 1934 much of the time and attention of the workers in this Department was devoted to special studies connected with the above Conference, which met in June, in London, at the invitation of the Health Section of the League of Nations. For this Conference, held under the Chairmanship of Dr. E. Mellanby, Dr. W. R. Aykroyd and Dr. H. Chick acted as technical secretaries, assisted by Miss E. M. Hume.

Special investigations of the Vitamin standards provisionally adopted at the 1931 Conference, as well as of pure Vitamin preparations since discovered, had been carried on in the intervening years by workers in this and other Institutes. These researches included studies of the stability of the standards under various conditions, and of the effect of variation in the technique of manipulation and in the solvents used, as well as a scrutiny of new methods of vitamin assay for trustworthiness and applicability. These researches were carried out in connection with separate Sub-committees set up by the Accessory Food Factors Committee of the Lister Institute and Medical Research Council, to deal with the problems connected with the Standardisation of the several Vitamins.

The information thus obtained contributed greatly to the success of the Conference by enabling it to reach important decisions marking further advances in Vitamin Standardisation. The previous standards for Vitamins D and B, had proved entirely satisfactory and were retained; in the case of Vitamin A and Vitamin C, pure substances were adopted as Standards, viz. β -carotene and L-ascorbic acid, respectively, in place of the previous ones. In one instance (for Vitamin A in oils and concentrates) a physical method was adopted as a permissible method of estimation, in addition to the usual biological methods.

These collective investigations, more especially those relating to Vitamin A, are considered to contain material of permanent value to Vitamin workers. The results are, therefore, being collected by Miss Hume and Dr. Chick for publication in a special Report of the Medical Research Council.

Following the decisions of the 1934 Conference the British Pharmacopœia Commission have appointed a Committee to consider the desirability of adding various Vitamin preparations to the British Pharmacopœia, and to draw up the appropriate descriptive monographs. Dr. H. Chick and Miss Hume are serving on this Committee.

Vitamin A.—Biological value of carotene as a source of vitamin A.

Miss Hume and Miss Henderson Smith, in co-operation with Dr. I. Smedley MacLean, have continued their work on the international carotene standard for vitamin A, adopted in 1931.

Relationship between the former international carotene standard and pure β -carotene. It seemed desirable to change the vitamin A standard from the impure mixture of carotenes previously adopted to a pure substance, such as pure β -carotene. In the event of the 1934 Conference deciding to make this change, accurate knowledge of the relative biological values of the old, and proposed new, standards was required. This test was made in several laboratories. Miss Hume and Miss Henderson Smith found 1.0 γ (1 unit) of the 1931 international standard to be equal in biological effect to 0.66 γ of β -carotene. The figure adopted by the 1934 conference was 0.6 γ , which, therefore, represented 1 unit of Vitamin A in terms of the new standard.

Solvents for carotene. The work of Miss Hume and Miss Henderson Smith agreed with that of workers in other laboratories making the same tests in preparation for the Conference, in finding that some samples of coconut oil provided a solvent in which carotene was reasonably stable, for the purpose of administration to the test animals.

Some months before the Conference met, it became apparent that it might be more desirable to distribute the standard in solution in a natural oil, rather than in crystalline form, as had previously been done. If distributed in solution, the standard would need to remain stable in the solvent for a much longer time and in much more varying conditions of temperature, than under the previous conditions of use. Fresh colorimeter and biological tests were instituted, the results of which made possible the selection as solvent, of a sample of coconut oil, in which there was reasonable expectation that the standard would retain its stability even in these more exacting conditions. This oil is being used as solvent for the distribution of the new β -carotene vitamin A international standard. It was previously reported that no protective influence of hydroquinone on carotene in coconut oil solution, could be detected; in the more drastic conditions of this later series of experiments, a very marked protective action was revealed, and an addition of hydroquinone to the standard solution of β -carotene is to be made before distribution.

Spectrophotometric determination of vitamin A. For the conversion of values obtained by the spectrophotometric method into units based on the international standard, it was necessary to ascertain as accurately as possible the biological relationship between the international standard β -carotene and a rich concentrate of vitamin A, the value of which for $E_{1\%}^{1\text{cm}}$ 328 m μ was known. Miss Hume and Miss Henderson Smith carried out this determination and the value fixed for the conversion factor was based on their result, together with those of other workers. The different results varied somewhat widely, however, and the value adopted for the conversion factor is to be regarded as provisional.

Effect of Vitamin A deficiency on the Eye. Dr. K. Tansley Lythgoe has confirmed her previous results on the effect of vitamin A deficiency on the development of the retina, and the research is now nearly completed.

It has been found that the development of the rat retina during foetal life is unaffected by even a severe condition of vitamin A deficiency in the mother. If, however, the deficiency continues during the suckling period visual purple is not produced and the typical staining reactions and structural defects of the deficient retina are found present at weaning. Visual purple and the staining reactions return to normal if the young rats are given cod-liver oil after weaning, but, if any structural damage is present, this cannot be repaired.

A condition of uterine inertia with prolonged labour which may end in death has been found to be a feature of vitamin A deficiency in the pregnant rat. This condition is now being investigated.

Work on the Vitamin B Complex.

Further investigations on Vitamin B₂ (anti-dermatitis, ? anti-pellagra) have been carried out by Dr. H. Chick, Miss A. M. Copping (Medical Research Council Grantee) and Miss C. E. Edgar (Francis Maitland Balfour Scholar, Newnham College).

The results have proved that vitamin B₂, formerly regarded as a single entity, is composed of two constituents:

- (1) heat-stable lyochrome pigment (such as lactoflavin in whey or hepatoflavin in liver);
- (2) a supplementary substance, water soluble, heat- and alkali-stable, which occurs in yeast extract.

These results are in accord with those contained in the more recently published paper of Kuhn, Wagner-Jauregg and György, from Heidelberg and Cambridge. The above work in this Division has been rendered possible by generous gifts of pure lactoflavin by Professor Ellinger and of pure hepatoflavin by Dr. Sydney Smith of the Burroughs Wellcome Laboratories.

The effects of these two constituents of vitamin B₂ have been studied separately and, although an association of both has been found necessary for maintenance of growth and health, yet the lyochrome constituent

appears to be connected with the prevention and cure of the generalised skin affection seen in young rats deprived of vitamin B₂, while the supplementary substance, called "Vitamin B₆," by György, appears to be specially required for prevention of the more florid, symmetrical dermatitis which has been called "rat pellagra" and regarded as the analogue of the human disease.

Miss Edgar is making a study of the chemical and physical properties of the supplementary substance "Vitamin B₆". The results so far obtained show it to be heat and alkali-stable and capable of dialysis through a cellophane membrane; it is not absorbed on fuller's earth in acid solution nor precipitated by lead acetate at pH 4.0 - 8.0.

The so-called "rat pellagra," or the symmetrical florid skin inflammation in rats deprived of vitamin B₂, has in the past shown great irregularity in its occurrence, only a small proportion of the rats showing this characteristic syndrome, and this fact has been a hindrance to its study. Recent work carried out by Dr. Chick and Miss Copping has shown that an important determining factor is the amount of the reserve store of vitamin B₂ possessed by the young rats before receiving the experimental diet. The incidence of this characteristic dermatitis has become more regular since care has been taken to give no excess of vitamin B₂ to the mothers of litters destined for this work during the lactation period, and to withhold it altogether during the last week, before beginning the experiment proper. The type of carbohydrate in the experimental diet also seems to be a factor of importance and substitution of an impure maize sugar for the starch previously used seems to be conducive to the development of this condition.

Vitamin B₄ Work has been carried out by Dr. Chick and Miss Copping to investigate "Vitamin B₄" the heat-labile dietary factor described by Reader, as contained in yeast, in addition to the antineuritic, (antiberiberi) vitamin B₁, and stated to be necessary for maintenance of health and prevention of skin and nervous diseases in the rat. Concentrates of vitamin B₁ and vitamin B₄ respectively were prepared by Mr. Prunty from yeast extract, following the methods prescribed by Reader, Peters and others and the effect of these concentrates in the diet of young rats was studied. No clear or consistent evidence was obtained of any difference in action between these two concentrates except variation in potency nor could the nervous symptoms developed in absence of one be distinguished from those developed in absence of the other. This work did not, therefore, provide any evidence of the existence of an antineuritic "Vitamin B₄" apart from vitamin B₁.

Presence of Porphyrin in the urine of rats deprived of Vitamin B₂. Professor Ellinger, in the course of his studies on excretion of lyochromes (fluorescent compounds) in the urine of rats, has demonstrated the presence of a pink fluorescent substance in the urine of rats deprived of vitamin B₂. This substance has been separated from the urine and, from a study of its properties and absorption spectrum (carried out with the assistance of Dr. N. S. Lucas), is judged to be coproporphyrin. Sufficient material is not yet available for complete analysis and identification. This porphyrin is found present in traces or small quantities in the urine of some rats receiving a normal stock diet or a complete synthetic diet. When, however, the latter is deprived of vitamin B₂, the amount is greatly increased and disappears again when vitamin B₂ or its two constituents are restored to the diet. If either flavin or the supplementary substance "Vitamin B₆" are given separately, the porphyrin excretion in the urine remains unaltered, showing that these two constituents of what formerly was considered vitamin B₂, have a combined physiological action on the metabolism.

A second abnormal substance was found in the urine of rats deprived of vitamin B₂. This substance is not fluorescent, is yellowish brown in colour, has one absorption band only, at 485 mμ and does not give the reactions of urobilin.

The presence of coproporphyrin in the urine of rats deprived of vitamin B₂ may be of significance in connection with the theory (held especially by the Goldberger school in the United States) that human pellagra, in which the skin shows a pathological sensitisation to sunlight, is caused by a lack of vitamin B₂ in the diet.

Biological Value of Proteins.

The investigation of the scope, applicability and trustworthiness of the balance sheet method for estimating the biological value of proteins, as carried out in this Division, with male adult rats as experimental animals, has been completed.

With this method (in which measurement is made of the power to maintain nitrogenous equilibrium over short periods) the influence on biological value of altering the proportion of protein in the diet has been studied on the proteins of wheat, maize and milk. In all cases an increase in the proportion of the protein in the diet has been found to lower the biological value, i.e., its utilisation has been less economical.

A special study has also been made of caseinogen, both before and after undergoing the processes of heating and purification, as ordinarily carried out before inclusion in diets used in vitamin work. Purification or prolonged heating at 120° was found to have no significant effect on the biological value; heating for long periods at 150°, however, lowered both the digestibility and the biological value.

The above work which has extended over several years, in which Mrs. Fixsen, Dr. Chick, Mr. Hutchinson and Miss Jackson have all collaborated, has just been prepared for publication.

Biological Value of Fats.

A study of the syndrome arising in rats fed on a diet devoid of fat, and particularly of highly unsaturated fatty acids, has led Miss Hume and Miss Henderson Smith to the conclusion that a sporadically occurring dermatitis with scaly tail, which is often seen among rats in nutritional experiments is a partial deficiency, due to this cause.

It even seems probable that this deficiency may also be the cause of the failure to produce and rear living young, which is widely found when attempts are made to breed rats on synthetic diets containing all the generally recognised dietary essentials. This point is being investigated further.

The exact nature of the chemical compounds which will make good the deficiency is also being investigated in co-operation with Dr. I. Smedley-MacLean.

Researches on the Antiscorbutic Principle (Vitamin C).

The urinary excretion of ascorbic and dehydroascorbic acids in man. This problem was studied in detail by Dr. S. S. Zilva and Dr. S. W. Johnson, the experiments being carried out on the observers themselves and some of their colleagues. It was found that dehydroascorbic acid (the reversible oxidised form) is reduced in the body since it appeared in the urine as ascorbic acid, the conditions under which it was excreted being the same as those of ascorbic acid. The major part of ascorbic acid was voided in the urine a few hours after ingestion. The urinary output of the vitamin, however, depended on the amount stored in the body as well as on the quantity consumed. Dr. Zilva and Dr. Johnson observed further that when the store of ascorbic acid was complete a more or less constant level of excretion could be achieved — a level which varied with the magnitude of the dose. The store of vitamin C can, under so called normal conditions, be so low that it may take as long as seventeen days on a daily dose of about 120 mg. of ascorbic acid (about 4 oranges) to reach saturation. In spite of this the subjects were not only free at the beginning of the experiment from scurvy but enjoyed full bodily and mental vigour. Once saturation was reached, the condition continued for some time although the urinary excretion dropped to a very low level. The amount of ascorbic acid not accounted for in the urine varied with the magnitude of the dose consumed and could not, therefore, be taken as an index of the daily requirement of vitamin C.

The catalytic oxidation of Vitamin C. L-Ascorbic acid which is stable in solid condition, when dissolved in ordinary distilled water, slowly deteriorates. The cause of this inactivation has been traced by Dr. Zilva and Mr. A. E. Kellie to the catalytic action of metals. Thus, when quartz apparatus was used throughout the experiments, minimising the solution of traces of metals, the ascorbic acid was found to be very much more stable even when the water was previously saturated with pure oxygen. On the other hand, the addition of extremely small quantities of metals to quartz-distilled water caused a marked increase in the rate of inactivation of vitamin C. It has been further shown by them that in the presence of tissues such as liver, kidney, etc. the catalytic action of the metals was greatly diminished—an observation which partly explains the stabilising influence of tissues on vitamin C in solution.

Vitamin C in the intestine of the guinea-pig. It is now known that certain organs and glands such as the anterior lobe of the pituitary, the adrenals, the liver, etc., of animals whether susceptible to scurvy or not, contain ascorbic acid in higher concentration than most of the other tissues. Recent observation on the mouse and on the rat showed that the vitamin C is concentrated in their intestinal tract, especially in the small intestine. In connection with a major research, Dr. Zilva had the opportunity of investigating the guinea-pig in this respect. This animal although unlike the rat and the mouse unable to synthesise vitamin C, showed a content of ascorbic acid in the small intestine when on a diet containing vitamin C similar to that found in the mouse by other observers. That the concentration was not due to the passage of the vitamin of the food through the gut, was demonstrated by the fact that this selective concentration in the intestinal tissue could be brought about also by injecting ascorbic acid into the blood stream of the guinea-pig.

The influence of ascorbic acid on the activity of cathepsin. Dr. Johnson has been engaged in an investigation of this problem with the ultimate aim of discovering whether the presence of vitamin C was necessary for the formation of intercellular supporting tissue. Cathepsin, being the most widely distributed intracellular proteinase in the animal body, was employed in this investigation. The following facts have so far been established in this connection. With gelatin as a substrate, it was found that ascorbic acid had little activating influence on the hydrolytic activity of cathepsin when free from the usual activators such as glutathione, cysteine, etc., but it enhanced its proteolytic activity in the presence of cysteine. On adjusting the reaction in hydrolyzed gelatin to pH 7.2, when synthesis might be expected to occur, the addition of ascorbic acid did not result in any synthesis as had been observed in autolysed liver and other tissues.

The respiration of animal tissues in the presence of *l*-ascorbic acid. It has been claimed that in the presence of *l*-ascorbic acid liver tissue from scorbutic guinea-pigs absorbed more oxygen in a Barcroft apparatus than in the absence of the vitamin. Mr. Kellie repeated and extended this work without being able to substantiate this claim.

Ascorbic acid, its homologues, analogues and derivatives. The following compounds synthesised in the Chemistry Department of the University of Birmingham were tested prophylactically for their antiscorbutic activity:—*l*-ascorbic acid, *d*-arabo-ascorbic acid, *d*-gluco-ascorbic acid (10mg.), *d*-galacto-ascorbic acid (10 mg.), *l*-arabo-ascorbic acid (10 mg.), *d*-ascorbic acid (10mg.), *l*-dimethyl ascorbic acid (3 mg.), imino-*l*-ascorbic acid (3 mg.). The synthetic *l*-ascorbic acid was found to be active in daily doses of 0.5 mg.—1 mg., i.e., the same potency as the natural compound, whilst *d*-arabo-ascorbic acid showed 1/20th–1/40th of this activity. The remaining compounds failed to offer any antiscorbutic protection in the daily doses given above.

Vitamin C and physiological changes in the apple. This work was carried out by Dr. Zilva and Miss E. O. V. Perry in collaboration with Drs. F. Kidd and C. West of the Low Temperature Research Station, Cambridge. In an inquiry on the possible connection of vitamin C and the respiratory process of the apple it was revealed that the vitamin content of the fruit stored in nitrogen and in oxygen at 0°C. and 15°C. showed no marked difference.

Mention was made in previous reports of the high concentration of vitamin C near the skin of the apple as compared with that in the remaining tissues of the fruit. It is now found that this concentration is even higher in portions of the apple where the skin possesses a marked red flush. In the same apple the red portion of the skin may be twice as potent as the green portion. Furthermore in "rosy" Bramley's Seedling apples, i.e. apples which are almost entirely red, and in the entirely green fruits, the same disparity in antiscorbutic potency in the skin holds true. The activity of the flesh of apple is not affected by the colour of skin. It may be mentioned in this connection that the red skin of the Bramley's Seedling apple has been found to contain about 40 international units, a fact which places it with the Alphonso Mango, the Paprika and the anterior lobe of the pituitary gland amongst the richest natural sources of vitamin C so far known.

The addition of synthetic ascorbic acid in the canning of fruits and vegetables. The identical physiological activity of synthetic ascorbic acid with the natural product and the probability of the manufacture of the vitamin in the near future at an economic price invited an inquiry into its possible application in the preparation of certain foods for hygienic reasons. With this end in view, exploratory experiments were carried out in collaboration with the Low Temperature Research Station, Cambridge. In this preliminary work the following facts were ascertained. By adding the synthetic vitamin to vegetables before canning, the major part of the original activity was found in the final product. In the case of runner beans which naturally contain very little if any vitamin C, the loss incurred in the process of canning was about 25%: the vitamin had been taken up by the solids, the liquid being practically without activity. In canned spinach the loss was of a similar order but in this case the added vitamin was distributed between the solids and the liquid. The preservation of the antiscorbutic activity to the extent of 70–80% of the ascorbic acid introduced before the treatment, was also recorded with apples and apple jelly.

DEPARTMENT OF BIOCHEMISTRY.

Phosphate Metabolism and the Calcification of Animal Tissues.

Phosphoric esters of blood. The continued investigation, by Professor R. Robison and Dr. M. G. Macfarlane, of the phosphoric esters occurring in rabbit blood has failed to confirm the presence of hexosephosphates which can, at most, form but a very small proportion of the total esters. Diphosphoglycerate and derivatives of nucleotides account for nearly the whole of the acid-soluble organic phosphorus. The hydrolysis of these esters by acids and by bone phosphatase and their possible influence on the calcification of the skeleton have been further studied in collaboration with Dr. J. J. Rae (University of Toronto, 1851 Exhibition Research Scholar). The monophosphoglycerate formed by partial hydrolysis of the diphosphoric ester is an important intermediate product of carbohydrate metabolism in muscle and yeast and may well occur in small amounts in blood. Although readily hydrolysed by the phosphatase of bone it exerts a pronounced inhibitory action on the calcification of bone slices *in vitro*. This effect presents so marked a contrast to that of other phosphoric esters that its explanation should prove instructive. It may, perhaps, be due to the fixation of calcium ions by glyceric acid, but other interesting possibilities are being explored.

Deposition of strontium salts in hypertrophic cartilage. Professor Robison and Dr. A. H. Rosenheim have previously shown that deposits not only of calcium but also of barium, strontium and magnesium phosphates can be obtained, *in vitro*, in hypertrophic cartilage, with the aid of the phosphatase mechanism. Only strontium resembles calcium in forming such deposits in cartilage from supersaturated

inorganic solution, i.e. by the second mechanism alone. These experiments have been continued in collaboration with Miss Kathleen Law (Melbourne University). Deposits are regularly obtained by immersion of hypertrophic cartilage, from which all calcified tissue has first been removed, in Ca-free solutions of strontium salts. The solutions must be supersaturated and a high concentration of bicarbonate is necessary. With the co-operation of Mr. Hugh Ramage (Norwich) and of Dr. N. S. Lucas, it has been shown, by spectrum analysis, that these deposits consist of strontium phosphate or carbonatophosphate. The bearing of these results on the problem of strontium-rickets is being examined.

Development of the calcifying mechanism. During the past four years, Dr. Honor B. Fell (Strangeways Research Laboratory, Cambridge) and Dr. J. S. F. Niven (University of Glasgow) have collaborated with Professor Robison in experimental studies concerning the development of the calcifying mechanism in avian cartilage and osteoid tissue and in the long bones of rabbit embryos. The results of these investigations, which have now been published, are in accord with the view that a specialised mechanism is gradually developed during the differentiation of these tissues before they can be calcified in the body. They support the conclusion that phosphatase is an important component of this mechanism though not the sole factor concerned. The elaboration of this complex biochemical mechanism during the later stages of development of hypertrophic cartilage cannot be reconciled with the view expressed by some histologists, that hypertrophy of the cells represents a process of senescence.

The Phosphohexokinase of bone. The study of the hydrolysis of fructosediphosphate by bone phosphatase has been completed by Dr. Béla Tankó and Professor Robison. It was shown in the course of this work that crude preparations of bone phosphatase contain a phosphohexokinase which is also present in bone marrow. This enzyme is probably identical with that found by Lohmann in extracts of muscle and yeast. Dr. Rae has now shown that it is present also in hypertrophic cartilage. The association of this kinase with various glycolytic systems and with the phosphatase of bone provokes interest as to its possible role in calcification.

Alcoholic Fermentation.

Work on the isolation and characterisation of the phosphoric esters formed in alcoholic fermentation has been continued by Professor Robison and Dr. Macfarlane. The partial hydrolysis of fructosediphosphate by acids has been shown to yield, under certain conditions, an ester differing in properties from either of the known fructosemonophosphates. The possibility that migration of the phosphoric acid group may occur under such conditions is being investigated.

Dr. V. N. Patwardhan (Grocers' Company Research Scholar) has completed his study of phosphomannonic acid and its lactones. His experiments on the fermentation of mannose by dried yeast at high temperature suggests that the predominance of mannosemonophosphate in the phosphorylated products is due to inactivation of the yeast phosphohexokinase, which, under normal conditions, converts the monophosphoric esters into an equilibrium mixture of glucose, fructose and mannose derivatives.

Mr. C. J. Virden (Dublin) has studied the effect of fluoride in low concentration on fermentation of sugars by yeast preparations.

The Metabolism of Galactose.

Professor Harden and Mr. Taxner were unable to find any definite function of hexokinase in the fermentation of galactose by adapted yeasts. Work is being continued by the former on the general question of the function of hexokinase in alcoholic fermentation.

Dr. G. A. Grant (Beit Memorial Research Fellow) has continued his study of the fermentation of galactose by preparations of adapted yeast. No evidence of the formation of galactosephosphoric esters has been obtained; but fructosediphosphate and, in lesser amounts, trehalosemonophosphate constitute the major proportion of the phosphorylated products. Synthetic galactose-6-phosphate, moreover, is not fermented by these yeast preparations. The polysaccharide stored by this yeast, when grown on galactose as the sole sugar, has been shown to yield only glucose on hydrolysis. The possibility that phosphorylation plays an essential part in the interconversion of the sugars is being further studied and the investigation has been extended to the problem of the synthesis of lactose in the active mammary gland. Evidence of the conversion of glucose into lactose by tissue slices *in vitro* has been obtained and the mechanism of the process is being studied by means of this technique.

The Chemical Nature of Antibodies.

Dr. A. H. Rosenheim (Medical Research Council grantee) has been investigating the action of various enzymes on the agglutinins in the globulin fraction of anti-typhoid sera. The antibody groups were not destroyed by preparations of yeast dipeptidase and polypeptidase at pH 7.8 and 7.0 respectively over 24 hour periods at 37°, nor by a sample of non-activated papain. Progressive destruction of the flagellar and somatic agglutinins was, however, rapidly effected by activated papain, trypsin and pepsin. It has been found that the rate of inactivation of antibody by proteolytic enzymes does not run parallel to the rate of hydrolysis of the globulin, being practically complete before more than 15-20 per cent of the protein is destroyed.

The Oxytocic Hormone of the Posterior Lobe of the Pituitary Gland.

Dr. J. M. Gulland and Mr. S. S. Randall have observed that under suitable experimental conditions the oxytocic activity is decreased when the hormone undergoes chemical reduction and is largely restored by aeration or removal of the reagent. Changes of this nature, studied in some detail, indicated the presence in the hormone molecule of a group, or groups, which might enter into reversible oxidation-reduction processes. The accuracy of this view was demonstrated by an examination of the variations in oxytocic activity when the hormone was placed in solutions of dyestuffs of known oxidation-reduction potentials. The hormone undoubtedly contains one redox system, of which the E_0' value at pH 6 was defined, and the results of the investigation also suggested that there might be a second labile group of a similar nature. The interpretation of the facts was unfortunately complicated by the unavoidable difficulties of working with impure material—unavoidable, since the pure hormone is not known—by the approximations arising out of the method of biological assay, and by various technical problems in connection with the behaviour of redox systems under unusual conditions. The recognition of the hormone as a redox system invites speculation and research as to the mechanism by which it excites contraction of smooth muscle, since Johnson, McClosky and Voegtlin have shown that oxidation-reduction processes constitute an important factor in the regulation of the tone of smooth muscle. Further studies will be undertaken when purer preparations of the hormone become available.

In the hope of throwing light on the nature of the redox group, or groups, Dr. Gulland and Mr. Randall have examined the effect of various chemical reagents on the oxytocic activity of the hormone, and have concluded in the first place that some of their results indicate that the hormone may contain a disulphide linkage, and secondly that none of the evidence in the literature is opposed to this view.

Dr. Gulland, Miss M. Freeman (Walter and Eliza Hall Institute, Melbourne) and Mr. Randall, in collaboration with Dr. N. S. Lucas, have made an intensive attack on the problems offered by the isolation and purification of the hormone by chemical and physical methods, since the progress of future work in this field undoubtedly depends on the successful elimination of inactive, concomitant substances. This work is still in progress.

The examination of the effects of various enzymes on the hormone have been continued.

The Constitution of the Nucleic Acids.

Dr. Gulland's work in this field is still in progress, and he is continuing his collaboration with Dr. E. R. Holiday (London Hospital Medical School).

The Action of Chemical Substances on Cells.

Their work on this subject referred to last year has led Dr. Gulland and Mr. T. H. Mead to study in greater detail the equilibria existing between amino-acids and aromatic aldehydes; such equilibria are analogous to those occurring between formaldehyde and amino-acids and proteins in formol titrations.

Studies in the Amino-acid Group.

Dr. Gulland and Mr. C. J. O. R. Morris have isolated β -hydroxyglutamic acid from amongst the products of the acid hydrolysis of casein. This amino-acid was first isolated from protein hydrolysates by Dakin in 1918, but since then has eluded other workers who attempted to obtain it. The yield was small, but sufficient to allow characterisation of the compound and repetition of some of Dakin's observations. This work affords much-needed confirmation that such β -hydroxylated amino-acids—apart from serine—may occur in nature.

Dr. Gulland and Mr. Morris are engaged in other investigations in the amino-acid group.

Experiments bearing on the Metabolism of Carbohydrate and Fats.

Dr. Smedley-MacLean has continued the work begun with Miss D. Hoffert in 1924 which established the fact that incubation of yeast in a solution of maltose had a quite specific effect in producing an increased storage of carbohydrate in the yeast cell as compared with incubation in solutions of glucose, fructose or sucrose. With Miss R. McAnally, the question how far this increase is due to a rise in the glycogen content of the cell has been investigated. The glycogen content of yeast after incubation in glucose solution showed very little increase, though in a maltose medium the rise of glycogen content was very marked. This gives the first direct experimental support for the view that the formation of maltose is a stage in the synthesis of glycogen. The fact that 80% of starch could be converted to maltose had already led to the conception of a structural formula for the starch molecule built up of conjugated maltose units. The addition of phosphates to the glucose medium produced an increased glycogen content similar to that found when the yeast was incubated in maltose solution but the addition of phosphates to the maltose medium produces only a slight increase. These results are consistent with the view that glucose is converted into maltose through an intermediate stage of hexosephosphate, but further work will be necessary definitely to establish this.

With Mr. R. O. Jones (Morna Macleod Research Student), a quantitative study has been made of the oxidation of the fatty acids with hydrogen dioxide under constant conditions with and without the addition of a copper catalyst. The results show that β -hydroxybutyric acid is much more readily oxidised than acetoacetic acid and suggest that in the body it is more probable that acetoacetic acid is first reduced to β -hydroxybutyric acid which then undergoes oxidation but not through the intermediate stage of acetoacetic acid. The effect of the addition of glucose to the fatty acid solution before oxidation showed that the presence of the fatty acid had a very marked inhibiting effect on the oxidation of the glucose.

DEPARTMENT FOR THE STUDY AND PREPARATION OF THERAPEUTIC SERA.

Experimental work on the endotoxin of the Meningococcus.

The preparation of an efficacious anti-meningococcus serum and its standardization are dependent upon knowledge of the nature and mode of action of the protective substances that it contains. An important part of the curative effect is almost certainly due to the action of an antibacterial component but it has been maintained that the serum should also possess a neutralizing substance for the endotoxin. During the past year Dr. Petrie has carried out experiments with the endotoxin by utilizing the intracerebral route in the guinea-pig for tests of lethality and for estimating the neutralizing power of experimental sera. Two horses, in addition to rabbits and guinea-pigs, were specially immunized with preparations of the endotoxin obtained from the cocci by extraction methods. These proved to be lethal when introduced directly into the brain of the guinea-pig but it was doubtful whether the effects were specific since the same train of symptoms could be induced by extracts derived from unrelated bacteria. Numerous attempts to obtain evidence of neutralization by immune sera consistently failed, and it therefore appears that the intracellular lethal substance of the meningococcus is not a specific antigen which is capable of producing an anti-endotoxin in experimental animals.

Specific neutralization of neurotoxins in the cerebral tissues of the guinea-pig.

During the course of the above mentioned work it seemed desirable to control the possibility that the cerebral tissues of the guinea-pig are so sensitive to the action of the meningococcal toxin that neutralization by a specific serum cannot be expected. Accordingly, Dr. Petrie undertook a series of experiments with three well-known neurotoxins, namely, diphtheria toxin, tetanus toxin and scorpion venom. The ease with which many multiples of the intracerebral lethal dose of each of these toxins could be neutralized by the corresponding immune-serum, even when the serum-toxin mixtures were injected directly into the brain substance, formed a striking contrast to the difficulty of demonstrating by the same technique a specific antibody for the endotoxin of the meningococcus. Thus 50 intracerebral doses of a freshly prepared potent diphtheria toxin were neutralized without the appearance of any toxic symptoms; the minimum amount of this toxin which produced the typical symptoms of diphtheritic paralysis, when introduced directly into the brain of the guinea-pig, was 1/200,000 c.c.; the test dose of toxin that was used in this experiment contained 1250 paralyzing doses.

The results obtained with scorpion venom were even more demonstrative, because this venom, like others of its class, has a powerful neurotoxic action with scarcely any latent period when injected by the usual routes. The intracerebral introduction of the venom causes immediate symptoms leading to a rapidly fatal issue. Nevertheless, it was possible to neutralize 20 intracerebral doses when they were mixed with a sample of antiscorpion serum obtained from a horse undergoing immunization at Elstree.

These experiments confirm the idea that the failure to obtain evidence of neutralization of meningococcus toxin by "immune" serum is due to the lack of a specific neutralizing antibody. Apart from this conclusion, the experiments with neurotoxins were impressive as indicating the remarkable efficiency of the neutralizing mechanism. The animals were kept sufficiently long for the foreign horse serum which carried the antibody to be excreted, and yet there was no evidence of dissociation of the toxin-antitoxin complex, for the animals remained perfectly well.

Production of an antitoxin against the exotoxin of the Meningococcus.

Ferry in Chicago has recently published an account of work which has led him to conclude that the Meningococcus produces an exotoxin in an appropriate culture medium, and that this can be neutralized by an antitoxin prepared in the horse. This work, which does not seem to have been confirmed up to the present, is undergoing examination by Dr. B. G. Maegraith of the Department of Pathology, Oxford. Two horses have been specially immunized with the exotoxin and samples of the serum at different stages of the immunization have been supplied to Dr. Maegraith for testing purposes.

Production of an anti-typhoid serum in the horse.

Reference to this work has already been made but it may be mentioned here that steps have been taken to issue a concentrated preparation of the serum for general distribution through the usual channels.

The occurrence of a typhoid bacilluria in a highly immune horse.

One of the horses which had received a course of intravenous immunization with killed and living smooth typhoid bacilli containing the "Vi" antigen fell ill soon afterwards with a continuous fever and was found to be excreting typhoid bacilli in the urine. Since there seemed to be no likelihood of recovery the horse was anaesthetized to death after an illness of 25 days. *Post-mortem* examination showed no gross changes in the organs but cultures taken from the kidneys yielded a growth of a highly virulent strain of *B. typhosus*, and the urine in the bladder gave a count of 500,000,000 bacilli per cubic centimeter. The pathogenesis of the condition is evidently similar to that which occurs in man, namely, the interception by the tissues of the kidney of the bacilli that are circulating in the bloodstream and their elimination in the urine. The preservation of full virulence by the infective strain in spite of its growth in highly immune tissues is of considerable theoretical interest in view of the fact that growth *in vitro* of a smooth culture in a homologous immune serum is a recognized method of obtaining a rough variant.

An attempt to produce an antibacterial serum in the horse against the "gravis," "intermediate" and "mitis" varieties of *C. diphtheriae*.

The recent work initiated by McLeod and his colleagues in Leeds on varieties of *C. diphtheriae* that appear to be correlated with a severe or, on the other hand, a mild type of the disease has helped to throw light on the incidence of malignant diphtheria. Research work on the subject has been carried on for some time by Dr. D. T. Robinson in the laboratories of Professor H. B. Maitland in Manchester. In the autumn of last year, Dr. Petrie was approached by Prof. Maitland with the suggestion that sera might be prepared from horses and these are now being tested by Dr. Robinson prior to their employment in a clinical trial. It is hoped by this investigation to ascertain whether the addition of an antibacterial element reinforces the therapeutic action of diphtheria antitoxin and if this is so, whether the therapeutic effect is specifically related to the strain of bacillus employed for the immunization.

The Antigenic nature of the Specific Polysaccharide of *B. dysenteriae* (Shiga).

At the present time it is still undecided whether pure protein-free bacterial polysaccharides are capable of stimulating the production of specific immune substances that are demonstrable in *in vitro* experiments. Dr. Morgan's work on the isolation and purification of the specific polysaccharide of Shiga's bacillus has provided an opportunity for investigating the problem by carrying out a number of carefully controlled animal experiments. Groups of rabbits were immunized intravenously with a series of doses of the specific substance: the individual animals in each group received a total amount which varied from 0.00015 to 1.5 milligramme. The antigenic response was estimated by means of agglutination tests with suspensions of *B. dysenteriae* (Shiga) and the results showed that the response was greater in the groups to which smaller amounts of the polysaccharide were administered. When, however, the total amount of the material injected intravenously was less than 0.007 milligramme a smaller amount of antibody was formed and the individuals of the groups, each of which received a total amount of 0.00015 milligramme, failed to respond with the formation of antibodies demonstrable by the agglutination technique. The titre of the sera examined was quite low; a maximum titre of 1 in 16 being recorded for complete agglutination. The results of these experiments indicate that under certain conditions of dosage the purified specific polysaccharide, when given intravenously to rabbits, is able to induce the formation of specific agglutinins of low titre.

Standardisation of Gas-Gangrene (Oedematiens) Antitoxin.

Dr. Morgan has carried out experiments in connection with a co-operative inquiry which was organized with the purpose of establishing a standard preparation of gas-gangrene (oedematiens) antitoxin; this has now been accepted for International use. The results of the work were communicated to the Permanent Commission on Biological Standardisation which met last August in Copenhagen.

A serum preparation for the Control of Measles.

The statement that globulin preparations from human placentae contain immune substances which have a neutralising action on the virus of measles is being examined by Dr. Morgan. He has carried out experiments in order to determine the best method of preparing potent antiviral globulin solutions from the placentae of healthy women for use in the control of measles. A clinical trial of two batches of human globulin solution, each of which has been prepared from a large number of placentae in order to equalize the varying antibody content, will be undertaken in the autumn, when a recrudescence of the disease is expected to occur.

The Chemical Nature of the Heterophile Antigen of *B. dysenteriae* (Shiga).

Dr. Morgan, in collaboration with Dr. Kurt Meyer (France), has inquired into the nature of the heterophile antigen in Shiga's bacillus. The heterophile hæmolytic antibody produced in rabbits as the result of a few intravenous doses of heat-killed *B. dysenteriae* (Shiga) was found to be completely and specifically neutralised by very small amounts of the purified specific polysaccharide hapten— $[\alpha]_D^{+97}$, $N=1.6\%$ —which has been isolated from Shiga's bacillus by Dr. Morgan. Treatment of this hapten with dilute acid was found to destroy its ability (1) to neutralise the hæmolytic action of the Shiga heterophile antibody on sheep red-cells and (2) to form a specific precipitate with an homologous antibacterial rabbit serum. Hydrolysis of the purest available preparation of hapten with dilute alkali caused an immediate and significant fall in its power to neutralize the lytic action of the heterophile antibody but, on the other hand, left largely unimpaired its capacity to bring about precipitation with an antibacterial serum. If the preliminary experiments should be confirmed it would seem probable that there are at least two distinct chemical groupings in the polysaccharide molecule each of which is associated with a specific immunological function. The power of forming a precipitate with immune serum and of neutralising the Shiga heterophile antibody is completely lost when the specific polysaccharide hapten is acted on by the species of myxobacterium, the isolation of which was mentioned in last year's report.

Production of Staphylococcus Toxin and Toxoid.

The addition of agar to the fluid culture-medium used for the production of staphylococcus toxin is generally regarded as being advantageous, but the mode of action of the agar is not understood and its presence complicates the filtration of the toxin, whether this is intended for the production of antitoxin in the horse or for the toxoid preparation which is coming into use for the treatment of staphylococcal infections in man. Dr. McClean has found that, when normal saline solution contained in a cellophane bag is dialysed against a suitable broth medium, the contents of the bag form a favourable culture medium for the production of staphylococcus toxin, whereas the broth outside the bag is quite unsuitable. The difference between the results given by the internal and the external medium was at first ascribed to a process of differential osmosis, whereby the cellophane membrane had permitted nutritive materials to pass through into the saline but had prevented the passage of a substance possessing an inhibitory action on the formation of toxin. Further experiments, however, indicated that this explanation is not the true one, but that the cellophane acts as a surface which adsorbs the inhibitory substance from the medium. This view is supported by the fact that a number of other materials which provide an adsorbing surface will act in the same way. It is probable, therefore, that the incorporation of agar favours toxin production by furnishing the requisite adsorbing surface.

The toxic filtrate obtained by the method of cultivation in a cellophane bag is relatively more free from non-specific constituents than that which is prepared from the broth-agar medium. Dr. McClean has, therefore, adopted this technique in the preparation of staphylococcus toxoid for the immunization of patients. Experimental tests have proved that the product thus obtained is an efficient immunizing reagent, and it is hoped that clinical trial will show that its use reduces the incidence and severity of the local reactions that may follow the injection of staphylococcus toxoid.

During the past year the Department has co-operated with the Therapeutic Trials Committee of the Medical Research Council by supplying staphylococcus toxoid and antitoxin for use in clinical tests to determine the therapeutic value of these specific remedies.

Standardization of Staphylococcus Antitoxin.

Dr. McClean has collaborated with serologists in other countries on the methods of standardizing Staphylococcus antitoxin. This work was begun and organized in the Biological Standards Department of the Medical Research Council. The results have shown that a satisfactory degree of accuracy can be obtained by the employment of various methods of titration, both *in vitro* and *in vivo*.

Experiments on Tissue Permeability.

The action of certain Bacterial Extracts. When carrying out intracutaneous tests with bacteria filtrates in the course of routine work Dr. McClean observed that the toxin of *Cl. welchii* diffused in the dermis in a manner comparable with the diffusion of testicular extract. The permeability of the tissues showed an immediate and dramatic increase, and the resulting lesion was spread over a considerable area and tracked downwards under the influence of gravity. The action of this toxin on tissue permeability is similar to that of culture filtrates of invasive strains of the staphylococcus, as described in 1933 by Duran Reynals.

Further observations have shown that culture filtrates prepared from all the members of the gas-gangrene group contain variable amounts of the diffusing substance, and that filtrates from some strains may be potent in extremely high dilutions. Other bacteria have been similarly tested and have yielded

interesting results ; for example, a virulent strain of Type I pneumococcus showed considerable diffusing activity, whereas a rough variant obtained by cultivating the strain in immune-serum did not influence the permeability of the tissues.

Preliminary investigations into the nature of the diffusing substance in bacterial filtrates have been made. The method elaborated by Dr. Morgan and Dr. McClean for the purification of testicular extract has proved useful in a slightly modified form. Solutions with a total weight of dry solids of 0.15 mgm. per c.c. and a diffusing activity in a dilution of 1/75,000 have been obtained. Thus the degree of activity of the substance is comparable with that obtained from extracts of testis.

The action of Tumour Extracts. In co-operation with Dr. E. Boyland of the Cancer Hospital, London, Dr. McClean has investigated the presence of a substance with a similar influence on tissue permeability which is present in aqueous extracts of transplantable mammalian tumours. Extracts of many varieties of tumour, of embryos, and of placentas show considerably greater diffusing activity than any of the "normal" tissues that have been examined.

The action on tissue permeability of extracts from the testis, malignant tumours and cultures of certain invasive bacteria may prove to possess considerable significance in the associated physiological and pathological processes. Work on the question whether the diffusing substances are antigenic is in progress.

Local immunity and Tissue Permeability. Dr. Favilli (University of Florence) has again collaborated with Dr. McClean in further observations on the relation between tissue permeability and local immunity. A modification in the technique of their previous experiments enabled them to eliminate the influence of the inflammatory cellular response in the dermis to the materials they selected for injection. In this way they succeeded in observing the direct reaction of the tissues to substances which increase or decrease local permeability. The results they obtained support the contention of Favilli and his co-workers in Italy that the reaction of the dermis to infective agents and the spread of the resulting lesions are influenced by the state of permeability of the tissues or by any substance that is capable of altering the tissues in this respect.

Dr. T. Lumsden, of the Cancer Department of the London Hospital, has again, during the past year, been given facilities for his work on the production of an anti-cancer serum by the immunization of sheep.

Laboratories in the Department have been placed at the disposal of the Committee of the Medical Research Council that is investigating the relation between iodine deficiency and thyroid disease, and have been occupied for this purpose during the past year by Miss M. G. Crabtree, M.Sc.

The hospitality of the Department has been extended to Mr. A. T. Dann, M.Sc., an assistant Research Officer on the Staff of the Commonwealth Council for Scientific and Industrial Research. He has arranged to work under the guidance of Dr. Morgan on the methods of isolating and purifying bacterial polysaccharides and intends to apply the knowledge thus gained to problems concerned with the diagnosis of animal diseases in Australia.

DEPARTMENT FOR THE STUDY AND PREPARATION OF VACCINE LYMPH.

This Department, which has been maintained at Acton Castle, Marazion, Cornwall, since the war years will be transferred to Elstree before the close of 1935 when Dr. A. B. Green retires from the post of Bacteriologist-in-Charge.

NATIONAL COLLECTION OF TYPE CULTURES.

The number of cultures of bacteria and micro-fungi distributed to workers at home and abroad again exceeded five thousand in the year under review, and over two hundred new strains were deposited for maintenance in the collection.

Arrangements have been made with the Director and staff of the Forest Products Research Laboratory, Princes Risborough, Bucks, by which its extensive collection of lignicolous fungi will in future be listed in the publications of the National Collection. The types conserved comprise some 180 species of wood-destroying fungi, and fungi causing discoloration in timber. Applications received by the staff of the National Collection for these cultures will be referred to the Forest Products Research Laboratory, which will deal with them direct.

The revised list of fungi and bacteria of economic importance maintained in the collection, referred to in the last report, is now in the Press and will be published in an early number of the *Transactions of the British Mycological Society*. A new edition of the catalogue of the collection is also in course of preparation.

The report of the Sub-Committee of the Nomenclature Committee of the International Society for Microbiology appointed to advise on the classification of the *Salmonella* group of organisms and on which sub-committee the Curator acted as Secretary, was published in a recent number of the *Journal of Hygiene*. The report embodies the views of the sub-committee on this subject, but does not necessarily represent the opinions of the members of the Nomenclature Committee of the Society.

Miss Dagny Erikson, with a personal grant from the Medical Research Council, made a taxonomic study of the considerable number of types of aerobic *Actinomyces* species isolated from man and animals maintained in the collection, and gave valuable assistance in the mycological work of the Department.

GENERAL AND FINANCIAL.

The Accounts and Balance Sheet for the year ending December 31st, 1934, show balances to the credit of the Pension Fund of £27,311 2s. 0d., of the Contingency Fund of £22,000 0s. 0d., of the Sinking Fund of £26,039 5s. 8d., and of the Capital Fund of £529,550 1s. 10d. The balance of income over expenditure of the Pension Fund, viz., £798 1s. 0d., has again been transferred to the General Account as a contribution towards the payment of premiums on the Federated Superannuation System policies of the staff. Of the excess of income over expenditure of the General Account, viz., £10,384 2s. 0d., £2,000 0s. 0d., has been utilised in increasing the Contingency Fund and the Balance, £8,384 2s. 0d., added to the Capital Fund.

The following changes in investments have taken place during the year, viz. :—

General Fund : £8,000 4½% Conversion Stock, 1940/44 has been sold ; £5,000 4% Funding Stock 1960/90 and £8,000 3½% War Stock purchased and £3,000 Port of London B. Stock has been converted into £3,000 Port of London 3½% Registered Stock 1965/75.

Sinking Fund : £1,450 3½% Conversion Stock 1961 has been purchased.

The income from the sale of the Institute's products during the year 1934 was £31,381 11s. 3d. After adjustment of the stock of Sera on hand at the beginning and end of the year, income from this source appears in the account as £32,739 10s. 3d. Sales of Sera, Vaccines, &c., and Investigation, Diagnosis and Analysis Fees both show an increase compared with the previous year.

The expenditure for the year was £44,718 16s. 2d., against £40,995 6s. 7d., in 1933. Salaries and Wages, Serum and Vaccine Lymph Laboratories Expenses and Animal House Expenses and Forage mainly accounted for the increased expenditure compared with last year.

The Governing Body gratefully acknowledge the receipt of legacies totalling £550 during 1934, viz :—

£50 from Dr. C. A. Rayne, of Lancaster, for whom in the past the Institute had carried out some personal investigations, and £500 from Miss Sophie Auguste Graf, of Queensdown Road, Clapton, in memory of her brother, Mr. Frederick Graf, an importer of drugs whose family came to England from Germany in 1862.

In conclusion, the Governing Body desires to express its appreciation of the devoted co-operation of the Director and all members of the staff in carrying out the work of the Institute.

WILLIAM BULLOCH.

Chairman of the Governing Body.

BALANCE SHEET

AND

ACCOUNTS.

Dr.

The Lister Institute

BALANCE SHEET

	£	s.	d.	£	s.	d.
To CREDITORS				2,002	6	9
To PENSION FUND—						
As per Account at 31st December, 1930.. .. .				27,311	2	0
To JENNER MEMORIAL RESEARCH STUDENTSHIP FUND—						
As per Account at 31st December 1933	8,269	13	7			
Add Amount transferred from Income and Expenditure Account, 1934	156	18	6			
				8,426	12	1
To CONTINGENCY FUND—						
As per Account at 31st December 1933	20,000	0	0			
Add Amount transferred from Income and Expenditure Account, 1934	2,000	0	0			
				22,000	0	0
To SINKING FUND to 31st December 1934				26,039	5	8
To CAPITAL FUND to 31st December 1934—						
Donations, &c., received to date from the following:—						
Dr. Ludwig Mond (1893)	2,000	0	0			
The Berridge Trustees (1893/98)	46,879	10	1			
The Grocers' Company (1894)	10,000	0	0			
Lord Iveagh (1900)	250,000	0	0			
Lord Lister's Bequest (1913) as per Account at 31st December, 1923	18,904	5	8			
William Henry Clarke Bequest (1923/6)	7,114	5	7			
Other Donations (1891-1926)	20,421	18	3			
Legacies, 1934	550	0	0			
Income and Expenditure Account:—						
As per Account at 31st December, 1933.. ..	165,796	0	3			
Add Balance for the year ending 31st December, 1934	8,384	2	0			
				174,180	2	3
				529,550	1	10

WILLIAM BULLOCH, *Chairman.*G. W. ADDISON, *Hon. Treasurer.*

£615,320 8 4

REPORT OF THE AUDITORS

We have audited the above Balance Sheet. We have obtained all the information and explanations we have required, being held by the Institute on their behalf. In our opinion, such Balance Sheet is full and fair, and properly drawn and the explanations given to us and as shown by the books of the Institute.

London, 17th April, 1935.

of Preventive Medicine.

31st DECEMBER, 1934.

Cr.

By Cash—						£	s.	d.	£	s.	d.
At Bankers: Deposit Account						2,000	0	0			
Current Accounts						9,175	16	8			
In hand						109	10	10	11,285	7	6
By INVESTMENTS, GENERAL FUND (at cost, less amounts written off)—											
£70,000 4 per cent. Consolidated Stock, 1957 or after						62,740	19	0			
£35,000 4½ per cent. Conversion Stock, 1940-44						32,808	14	9			
£17,000 5 per cent. Conversion Stock, 1944-64						15,997	0	7			
£52,000 4 per cent. Funding Stock, 1960-1990						45,661	13	9			
£44,000 3½ per cent. War Stock, 1962 or after						42,098	2	11			
£37,000 Local Loans 3 per cent. Stock						20,829	1	7			
£3,000 Port of London 3½ per cent. Registered Stock, 1965-75						2,686	17	7			
£1,000 Dominion of Canada 4 per cent. Registered Stock, 1940-1960						928	4	6			
£2,000 Cape of Good Hope 3½ per cent. Consolidated Stock, 1929-49						1,902	12	0			
£23,000 Cape of Good Hope 3 per cent. Consolidated Stock, 1933-1943						23,850	0	0			
£25,000 Natal 3 per cent. Consolidated Stock, 1929-1949						21,400	0	0			
£25,000 New South Wales 3 per cent. Inscribed Stock, 1935						22,900	0	0			
£2,000 New South Wales 4 per cent. Inscribed Stock, 1942-62						1,882	3	4			
£23,000 New Zealand Government 3 per cent. Inscribed Stock, 1945						22,114	0	0			
£26,100 South Australian Government 3 per cent. Consolidated Stock, 1916 or after						16,800	0	0			
£2,900 Commonwealth of Australia 3½ per cent. Registered Stock, 1936-37						2,925	6	0			
£800 Union of South Africa 4 per cent. Consolidated Stock, 1943-1963						594	2	0			
£23,000 Victorian Government 3 per cent. Consolidated Inscribed Stock, 1929-1949						19,800	0	0			
£700 Western Australia Government 4 per cent. Inscribed Stock, 1942-1962						698	7	0			
£20,000 Southern Railway Preferred Ordinary Stock						13,500	0	0			
£6,200 London & North Eastern Railway 3 per cent. Debenture Stock						3,961	0	0			
£3,000 Great Central and Midland Railway Joint Committee 3½ per cent. Guaranteed Stock						3,623	0	0			
£353 London & North Eastern Railway 4 per cent. First Guaranteed Stock						499	11	0			
£6,690 London, Midland & Scottish Railway 4 per cent. Preference Stock						7,960	0	0			
£15,625 London, Midland & Scottish Railway 4 per cent. Preference Stock, 1923						11,300	0	0			
£18,750 London & North Eastern Railway 4 per cent. First Preference Stock						13,028	6	7			
£25,000 East Indian Railway 3 per cent. New Debenture Stock						13,890	0	0			
£661 Madras & Southern Mahratta Railway 4 per cent. Debenture Stock, 1938						656	19	7			
£800 Grand Trunk Railway Company of Canada Great Western Borrowed Capital 5 per cent. Perpetual Debenture Stock						936	0	0			
£1,937 Grand Trunk Railway Company of Canada 4 per cent. Guaranteed Stock						1,733	0	0			
£800 Ontario and Quebec Railway 5 per cent. Permanent Debenture Stock						984	0	0			
£3,400 Gas Light and Coke Company Ordinary Stock						3,638	0	0	431,127	2	2
By INVESTMENTS, SINKING FUND (at cost)—											
£9,800 4½ per cent. Conversion Stock, 1940-44						8,806	16	7			
£7,450 4 per cent. Funding Stock, 1960-1990						5,860	5	3			
£13,150 3½ per cent. Conversion Stock, 1961 or after						11,337	16	2			
Balance uninvested						44	7	8	26,039	5	8
By INVESTMENTS, JENNER MEMORIAL RESEARCH STUDENTSHIP FUND (at cost)—											
£2,650 Southwark and Vauxhall Water Co. 3 per cent. Debenture Stock "B"						2,756	10	0			
£1,596 Southern Railway 5 per cent. Preference Stock						2,740	5	0			
£726 11s. 4d. Liverpool Corporation 3 per cent. Stock, 1942, or after						556	15	6			
£2,000 4 per cent. Funding Stock, 1960-1990						1,797	14	0			
Balance uninvested						575	7	7	8,426	12	1
By INVESTMENTS, PENSION FUND (at cost)—											
£22,000 4 per cent. Funding Stock, 1960-1990						17,165	3	5			
£13,000 3½ per cent. Conversion Stock, 1961 or after						10,038	1	5			
Balance uninvested						107	17	2	27,311	2	0
(The book value of the above Investments is, in the aggregate, less than their market value at 31st December 1934.)											
By DEBTORS											
By FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS—											
At cost less depreciation as per account 31st December 1920											
By EXPENDITURE ON INSTITUTE BUILDINGS AT CHELSEA—											
As per account 31st December 1910, including purchase of freehold site, £6,000											
By PURCHASE OF FREEHOLD LAND ADJOINING THE "STUDIOS" CHELSEA—											
As per account 31st December 1912											
By LEASE OF THE "STUDIOS" CHELSEA, as per last account						1,262	10	9			
Less Amount written off for the year						65	2	0	1,197	8	9
By QUEENSBERRY LODGE FARM, ELSTREE—											
Purchase of freehold land and buildings and Expenditure on new buildings—											
As per account 31st December 1912											
By STOCK OF ANIMALS						468	0	0	20,455	10	0
By STOCK OF ANTITOXINS						5,551	13	0	6,019	13	0
* Nothing has been charged for depreciation of Furniture, &c. since new purchases made during the year to a greater amount than the estimated depreciation (10%) have been written off.										£615,329	8 4

TO THE MEMBERS.

The Superannuation Scheme for certain of the Staff provides for the Life Policies for the time being in existence up so as to exhibit a true and correct view of the state of the Institute's affairs, according to the best of our information

COOPER BROTHERS & CO.

Chartered Accountants. } Auditors.

The Lister Institute of

Dr. INCOME AND EXPENDITURE ACCOUNTS

INCOME.								£	s.	d.
To Interest and Dividends on General Fund Investments	...	...	...	...	...	...	...	17,797	17	2
To Interest on Sinking Fund Investments	...	...	...	...	...	...	...	1,139	10	0
To Investigation, Diagnosis and Analysis Fees, &c. ...	...	...	...	...	...	...	...	2,542	8	0
To Sales of Sera, Vaccines, &c., and Stock at 31st December 1934, less Stock at 1st January, 1934	...	...	...	...	...	...	...	32,739	10	3
To Rent of Rooms	...	...	...	...	...	...	...	85	11	9
To Pension Fund—excess of Income over Expenditure transferred	...	...	...	...	...	...	...	798	1	0

£55,102 18 2

Dr.

Pension

	£	s.	d.
To Interest on Investments	1,335	0	0

£1,335 0 0

Dr.

Jenner Memorial Research

	£	s.	d.
To Interest and Dividends on Investments	261	1	10

£261 1 10

Dr.

Morna Macleod Research

	£	s.	d.
To Balance from last account	183	15	0

£183 15 0

Preventive Medicine.

for the year ending 31st December, 1934.

Cr.

EXPENDITURE.

	£	s.	d.
By Rent, Rates, Taxes and Insurance	1,357	19	6
By Salaries and Wages of Staff	26,250	7	9
By Premiums on Federated Superannuation Policies	1,509	9	5
By Stationery, Printing and Postage	389	7	1
By Printing of Collected Papers	172	2	0
By Office Expenses, Auditors' Fee and Sundries	280	4	11
By Travelling Expenses	9	15	8
By Gas, Water and Fuel	1,320	10	0
By Electric Light and Power	374	2	1
By Nutrition, Protozoological and Experimental Pathology Expenses, including Apparatus	625	8	5
By Bacteriological Laboratory Expenses, including Apparatus	458	0	3
By Vaccine Laboratory Expenses, including Bottles	58	19	1
By Water and Bio-chemical Laboratory Expenses, including Apparatus	605	13	11
By Serum and Vaccine Lymph Laboratories Expenses, including Apparatus and Bottles	4,735	4	9
By Culture Media	149	13	10
By Animals	1,149	5	4
By Animal House Expenses and Forage	2,250	0	10
By Alterations, Repairs and Renewals, including Workshop Expenses	879	10	11
By Library Expenses	342	11	8
By General Stores	185	8	2
By Amount written off Lease of the "Studios," Chelsea	65	2	0
By Sinking Fund (½% per annum on Cost of Buildings and Interest on Investments)	1,550	3	7
By Amount transferred to Contingency Fund	2,000	0	0
By Balance, transferred to Capital Fund	8,384	2	0
	10,384	2	0
	£55,102	18	2

Fund.

Cr.

	£	s.	d.
By Pensions	536	19	0
By Balance, transferred to General Income and Expenditure Account	798	1	0
	£1,335	0	0

Studentship Fund.

Cr.

	£	s.	d.
By Salary of Student	104	3	4
By Balance, transferred to Balance Sheet	156	18	6
	£261	1	10

Studentship Account.

Cr.

	£	s.	d.
By Salary of Student	105	0	0
By Cash in hand	78	15	0
	£183	15	0

SCIENTIFIC PAPERS PUBLISHED FROM THE LABORATORIES OF THE INSTITUTE DURING THE YEAR.

AMIES, C. RUSSELL ...	...	...	THE INFLUENCE OF TEMPERATURE ON THE SURVIVAL OF PURE SUSPENSIONS OF THE ELEMENTARY BODIES OF VACCINIA. <i>British Journal of Experimental Pathology</i> , Vol. XV., 1934.
" " ...	...	...	THE ELEMENTARY BODIES OF ZOSTER AND THEIR SEROLOGICAL RELATIONSHIP TO THOSE OF VARICELLA. <i>British Journal of Experimental Pathology</i> , Vol. XV., 1934.
" " ...	...	...	(See SCHLESINGER, B.)
BARRATT, MARY M. ...	...	...	SOME OBSERVATIONS ON THE ISOLATION OF BRUCELLA ORGANISMS FROM NEW MILK. <i>Journal of Comparative Pathology and Therapeutics</i> . Vol. XLVIII., 1935.
BOAS-FIXSEN, MARGARET A. ...	...	...	(See CHICK, HARRIETTE).
BHATNAGAR, S. S. ...	...	...	(See FELIX, A.)
CHICK, HARRIETTE ...	...	...	DIET AND CLIMATE. (CANTOR LECTURES, 1935). <i>Journal of the Royal Society of Arts</i> , Vol. 83, 1934-5.
CHICK, HARRIETTE, COPPING, ALICE M. AND EDGAR, CONSTANCE E.	...	...	THE WATER-SOLUBLE B-VITAMINS. IV. THE COMPONENTS OF VITAMIN B ₂ . <i>Biochemical Journal</i> , Vol. XXIX., 1935.
CHICK, HARRIETTE, HUTCHINSON, J. C. D. AND JACKSON, HESTER M.	...	...	THE BIOLOGICAL VALUE OF PROTEINS. VI. FURTHER INVESTIGATION OF THE BALANCE SHEET METHOD. <i>Biochemical Journal</i> , Vol. XXIX., 1935.
CHICK, HARRIETTE, BOAS-FIXSEN, MARGARET A., HUTCHINSON, J. C. D. AND JACKSON, HESTER M.	...	...	THE BIOLOGICAL VALUE OF PROTEINS. VII. THE INFLUENCE OF VARIATION IN THE LEVEL OF PROTEIN IN THE DIET AND OF HEATING THE PROTEIN, ON ITS BIOLOGICAL VALUE. <i>Biochemical Journal</i> , Vol. XXIX., 1935.
COPPING, ALICE MARY ...	...	...	ORIGIN OF VITAMIN D IN COD-LIVER OIL: VITAMIN D CONTENT OF ZOOPLANKTON. <i>Biochemical Journal</i> , Vol. XXVIII., 1934.
" " ...	...	...	(See CHICK, HARRIETTE).
DENNISON, MARJORIE ...	...	...	(See KORENCHESKY, V., SIMPSON, S. LEVY)
EAGLES, G. H. ...	...	...	THE PREPARATION AND TESTING OF ELEMENTARY BODY SUSPENSIONS FROM VACCINIA FILTRATES AND THEIR POSSIBLE USE IN SMALLPOX PREVENTION.
" " ...	...	...	THE CULTIVATION OF VACCINIA VIRUS: FURTHER EXPERIMENTS WITH CELL-FREE MEDIUM. <i>British Journal of Experimental Pathology</i> , Vol. XVI., 1935.

- EDGAR, CONSTANCE E. ... (See CHICK, HARRIETTE).
- ELSON, L. A. ... (See MORGAN, W. T. J.)
- FAVILLI, G. ... (See MCCLEAN, D.)
- FELL, HONOR B. AND ROBISON, R. THE DEVELOPMENT OF THE CALCIFYING MECHANISM IN AVIAN CARTILAGE AND OSTEOID TISSUE. *Biochemical Journal*, Vol. XXVIII, 1934.
- FELIX, A. ... CLINICAL TRIALS WITH A NEW ANTITYPHOID SERUM. *Lancet*, Vol. I., 1935.
- FELIX, A., BHATNAGAR, S. S. AND PITT, R. MARGARET OBSERVATIONS ON THE PROPERTIES OF THE VI ANTIGEN OF *B. typhosus*. *British Journal of Experimental Pathology*, Vol. XV, 1934.
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- GULLAND, J. M. AND MEAD, T. H. CORRELATION OF THE SPERMICIDAL EFFICIENCIES OF AROMATIC ALDEHYDES WITH THEIR CHEMICAL REACTIVITIES, AND ELECTROMETRIC ALKALINE TITRATIONS OF GELATIN IN PRESENCE OF AROMATIC ALDEHYDES. *Biochemical Journal*, Vol. XXIX., 1935.
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- " " " " VI. FURTHER STUDIES OF THE ACTION OF OXIDISING AND REDUCING AGENTS. *Biochemical Journal*, Vol. XXIX., 1935.
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- HENDERSON, D. W. ... EXPERIMENTS WITH THE "O" ANTIGEN OF *Clostridium oedematis maligni* (*Vibrio septique*). *British Journal of Experimental Pathology*, Vol. XV., 1934.
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- HURST, E. W. ... (See SABIN, A. B.)
- HUTCHINSON, J. C. D. ... (See CHICK, HARRIETTE).
- JACKSON, HESTER M. ... (See CHICK, HARRIETTE).
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- KLIENEBERGER, EMMY ... THE COLONIAL DEVELOPMENT OF THE ORGANISMS OF PLEUROPNEUMONIA AND AGALACTIA ON SERUM AGAR AND VARIATIONS OF THE MORPHOLOGY UNDER DIFFERENT CONDITIONS OF GROWTH. *Journal of Pathology and Bacteriology*, Vol. XXXIX., 1934.
- " " " ... THE NATURAL OCCURRENCE OF PLEUROPNEUMONIA-LIKE ORGANISMS IN APPARENT SYMBIOSIS WITH *Streptobacillus moniliformis* AND OTHER BACTERIA. *Journal of Pathology and Bacteriology*, Vol. XL., 1935.
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- MEAD, T. II. ... (See GULLAND, J. M.)
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- PATWARDHAN, V. N. MANNOSEMONOPHOSPHATE. III. PHOSPHOMANNONIC ACID AND ITS LACTONES. *Biochemical Journal*, Vol. XXVIII., 1934.
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- PETRIE, G. F. AND McCLEAN, D. ... THE INTER-RELATIONS OF *Corynebacterium ovis*, *Corynebacterium diphtheriae*, AND CERTAIN DIPHTHERIOD STRAINS DERIVED FROM THE HUMAN NASO-PHARYNX. *Journal of Pathology and Bacteriology*, Vol. XXXIX., 1934.
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THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE

Report of the Governing Body,
1936.

CHELSEA BRIDGE ROAD,
LONDON, S.W. 1.

June 9th. 1936.

The Lister Institute of Preventive Medicine,

CHELSEA BRIDGE ROAD, LONDON, S.W. 1.
ELSTREE, HERTS.

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ANNUAL GENERAL MEETING
OF
The Lister Institute of Preventive Medicine,
June 9th. 1936.

REPORT OF THE GOVERNING BODY.

The Governing Body has the honour to present the Institute's 42nd Annual Report.

GOVERNING BODY.

No change in the personnel of the Governing Body has occurred during the year. At the meeting held last year, the Council re-elected Professor W. Bulloch, Professor A. E. Boycott and Professor A. Harden as its representatives on the Board until December 31st, 1936.

The Governing Body has pleasure in reporting that the honour of Knighthood was conferred on Professor Harden, in January last.

COUNCIL.

At the Annual General Meeting last year the three members of Council retiring were Professor R. T. Hewlett, Dr. Louis C. Parkes and Dr. Harriette Chick, three representatives of the Members. Each of these representatives was re-appointed a member of the Council. Vacancies created in the representation of the Members of the Institute by the deaths of Sir John Rose Bradford and Sir James Kingston Fowler were filled by the appointment of Dr. E. Mellanby and Lord Horder respectively.

The three members of Council, who in accordance with the Articles of Association retire this year by rotation, but who are eligible for re-election are Lt.-Col. G. W. Addison and Lord Moyne, two representatives of the Members of the Institute and Colonel Ralph Key Harvey, one of the representatives of the Worshipful Company of Grocers.

MEMBERS.

The Governing Body regrets to report the deaths during the year of Capt. S. R. Douglas, a member since 1931, of Professor J. S. Haldane whose connection with the Institute had extended over thirty years and of Sir Wilmot Herringham, a member since 1918.

STAFF.

Dr. Alan B. Green, Bacteriologist-in-charge of the Vaccine Lymph Department since its inception in 1904, retired from the service of the Institute on December 31st, 1935 having reached the age of 65 years. As his successor the Governing Body has appointed Dr. Douglas McClean of the Bacteriological staff at the Serum Department and the Vaccine Lymph Department has now been removed from Cornwall to the Institute's premises at Elstree.

Dr. J. M. Gulland, first assistant in the Biochemical Department, has been elected to the Chair of Chemistry at University College, Nottingham, and Dr. E. Weston Hurst, Reader in Experimental Pathology, has been appointed Director of the new Institute for Medical Research in Adelaide, South Australia. They are vacating their appointments here in the Autumn of this year.

In October last Mr. D. W. Henderson, B.Sc., Ph.D., late Beit Memorial Research Fellow, was appointed to the bacteriological staff of the Serum Department; Dr. A. S. McFarlane, late Beit Memorial Research Fellow was appointed an Institute Research Fellow in Biophysics; Mr. T. F. Macrae, Ph.D., late of The London Hospital, was appointed an Institute Research Fellow in the Division of Nutrition, and Miss Emmy Klieneberger, Ph.D., was appointed Jenner Memorial Research Student and has been attached to the Bacteriological Department.

Mr. R. O. Jones, Morna Macleod Research Student, and Mr. S. S. Randall, Research Student in Biochemistry, completed their appointments in September, 1935 and April, 1936 respectively. In October Mr. L. D. MacLeod, B.Sc., was appointed Morna Macleod Research Student and attached to the department of Biochemistry.

Mr. Eric Downing has continued his tenure of the Grocers' Company Research Studentship during the year.

Dr. E. Weston Hurst, at the invitation of the University Authorities, delivered the William Withering Lectures in the University of Birmingham on the neurotropic virus diseases in May, 1935.

RESEARCH WORK.

Before surveying the scientific work carried out during the year in the various departments, the Governing Body desires again to record its appreciation of the continued co-operation it has enjoyed with the Medical Research Council. Salaries of the staff of the National Collection of Type Cultures, of Miss Hume and Dr. Zilva and his assistants and part salaries of Professor Korenchevsky and Miss Hume's assistant have been furnished by the Council, the Institute providing, as hitherto, accommodation for each of these workers and the necessary materials for their researches.

The velocity and equilibrium centrifuges with their accessories manufactured for the Institute by Professor Th. Svedberg in Upsala are being installed in a new building specially designed to accommodate them at Chelsea. As mentioned in last year's report the centrifuges have been purchased out of a grant of £3,400 made for this purpose by the Rockefeller Foundation of New York through its European Assistant Director of Medical Sciences, Dr. Daniel P. O'Brien.

The hospitality of the Institute's laboratories has again this year been extended to foreign guests displaced from scientific posts in Germany, viz., Professor Ph. Ellinger and Dr. M. Gutstein.

DEPARTMENT OF BACTERIOLOGY, SEROLOGY AND EXPERIMENTAL PATHOLOGY.

Studies on Viruses.

During the past five years considerable attention has been paid by workers on virus problems at this Institute to fundamental studies on the elementary bodies which are generally believed to constitute the sole effective etiological agents in all virus diseases. The fact that these bodies can now, by various procedures, be separated from crude virus-containing material in a state of comparative purity has greatly facilitated the study of their etiological relationships and even in those virus diseases such as chickenpox and herpes zoster, that so far have defied attempts to secure their transmission to laboratory animals, it has been possible by serological analysis, particularly agglutination experiments with highly concentrated suspensions, to demonstrate not only an etiological connection between the elementary body and the virus disease but also in some cases to explore the nature of the affinities, sometimes quite unexpected, between one virus and another.

Vaccinia. The antigenic structure of the virus of vaccinia has been under investigation by Dr. M. H. Salaman (Beit Memorial Research Fellow) since October, 1935. An *in vitro* technique has been devised for the detection of serological affinities between the viruses of vaccinia, variola and the various animal pox diseases. For this purpose it is necessary to secure large quantities both of the flocculable soluble constituents of the virus of vaccinia that pass those filters which retain the elementary bodies, and also of the pure washed bodies in sufficient concentration to provide material for adsorption experiments. So far, it has been possible to show that by the use of an optimum flocculation technique, the flocculating power and the antiviral or neutralising power of a potent antivaccinial serum can be removed by absorption with a V filtrate of rabbit dermal vaccinia. Absorption experiments with the washed elementary bodies are also in progress with the object of demonstrating the nature of the union between the body antigen and the antivaccinial substances in the immune serum—a problem that is still under discussion.

Avian Sarcoma: The nature of the tumour-exciting agent in filtrates and cell-free extracts of Rous No. 1 sarcoma and Fujinami myxosarcoma. Professor J. C. G. Ledingham, in collaboration with Dr. W. E. Gye of the National Institute for Medical Research, reported in 1935 the results of certain experiments which indicated that the filterable tumour-exciting agents of the Rous and Fujinami fowl sarcomata were probably particulate in nature. Dr. C. R. Amies, working on behalf of the British Empire Cancer Campaign, has continued these investigations and has obtained further evidence that points to the same conclusion. Improvements in the design of the high-speed centrifuge have enabled speeds of 15,000 r.p.m., equal to a centrifugal force 20,200 times that of gravity, to be obtained without appreciable increase in the temperature of the rotor. The application of this centrifugal force for one hour has been sufficient in most cases to remove completely the infective agent from potent cell-free tumour extracts. From the deposits thus obtained, the agent has been recovered quantitatively. In some cases, indeed, the resuspended deposit has been found to be more potent than the original tumour extract. This finding is probably explained by the fact that the agent is in this manner removed from the influence of inhibiting substances which remain in the supernatant fluid. By repeated fractional centrifugation it has been possible to obtain highly infective suspensions which, according to the serological tests employed, were free from fowl protein.

Dr. A. S. McFarlane and Dr. Amies have constructed an air-driven centrifuge similar to that designed by Professor McIntosh of the Bland Sutton Institute, Middlesex Hospital. This instrument works on the principle of the Henriot-Huguénard spinning top, and with it a centrifugal force of 36,000–39,000 times gravity has been obtained. Under these conditions the tumour agents of the Rous and Fujinami sarcomata have been completely sedimented from potent cell-free extracts in 20–30 minutes.

Microscopical examination of actively tumour-exciting, fowl protein-free suspensions by the method of dark-ground illumination reveals the presence of numberless particles of slightly varying size. These particles are below the limit of resolution with visible light but their constant presence in infective suspensions, their absence from the non-infective supernatant fluids, and the knowledge already available regarding similar particles (elementary bodies) in virus diseases leads almost inevitably to the conclusion that these bodies represent the actual causal agent. By depositing silver or a powerful stain on these bodies it is possible to increase their apparent size sufficiently to enable them to be seen by transmitted light.

The fact that these "elementary bodies" are agglutinated in specific fashion by the serum of fowls bearing actively growing or regressing tumours has also been confirmed. Agglutinins, however, are often not demonstrable and high titres have seldom been obtained.

Further investigations of the properties of these purified tumour-exciting suspensions are in progress.

The filterable viruses of rabbit myxomatosis (Sanarelli, 1898) and rabbit fibroma (Shope, 1932). In the course of his preliminary study of the nature of the rabbit fibroma discovered by him in 1932, Dr. Shope made the interesting observation that rabbits which had recovered from the fibroma were to a high degree protected against the fatal issue that almost invariably follows the inoculation of the virus of myxomatosis. Since that time the cross immunity relationships of these two viruses have been the subject of extensive study, and there would seem to be little doubt that the antigens concerned possess elements in common that would account for the immunological affinities.

Professor Ledingham has for some time been engaged in a study of this relationship by methods which have proved of value in elucidating problems of this nature. He has been able to prepare concentrated suspensions of the elementary bodies from these two virus diseases and by their aid to explore their affinities serologically. Through the kindness of Sir Charles Martin who, at Cambridge, has been studying the communicability of myxomatosis to wild rabbits in nature, he has obtained a few myxomatosis-recovered rabbits, the study of whose sera has greatly aided this enquiry.

The investigation has so far yielded promising results and it is hoped that a preliminary communication on the affinities between these viruses elicited by this direct serological comparison of antigens may soon be possible. A study of the detailed histology of these two virus diseases has been commenced by Dr. E. Weston Hurst.

Studies in the etiology of acute rheumatism and acute rheumatoid arthritis. In 1935 the published report of an investigation by Dr. Amies and his colleagues, Drs. Schlesinger and Signy, pointed very strongly to a virus agent in acute rheumatic fever. These studies on acute rheumatism are now being carried on by Dr. G. H. Eagles with the assistance of Dr. P. R. Evans, Registrar for Medical Diseases of Children, and Medical Officer in Charge of the Rheumatism Clinic, King's College Hospital, and Mr. A. G. Timbrell Fisher, National Commission on Chronic Rheumatic Diseases, Royal College of Physicians, whose operative procedure in the treatment of rheumatoid arthritis gives access to abnormal synovial tissue. A number of physicians attached to hospitals and clinics have supplied material from cases for this research.

Pericardial and pleural exudates and joint fluids from acute rheumatic fever, and joint fluids and synovial membrane from acute rheumatoid arthritis have yielded suspensions of bodies which, in stained preparations and dark-ground examination, are indistinguishable from elementary bodies demonstrable in similar suspensions from recognised virus diseases. In view of the minute size of these bodies in many virus diseases serological studies to support mere observational evidence are imperative. Agglutination tests in hanging-drop preparations are being carried out with these suspensions using sera from cases in different stages of rheumatic infection, sera from normal persons, and from cases of infection not attributable to virus agents.

It is hoped in this way to confirm the findings in acute rheumatic fever, to establish a possible virus etiology in rheumatoid arthritis and to investigate the possibility of a relationship between acute rheumatic fever and acute and subacute rheumatoid arthritis by cross-agglutination tests.

Unfortunately, there is, so far, no known animal unquestionably susceptible to rheumatic infection, but the possibility of securing infection with freshly-prepared suspensions from rheumatic material is not being lost sight of.

Neurotropic Virus Diseases.—Equine encephalomyelitis. Dr. Hurst has completed a study of the course of infection with equine encephalomyelitis virus begun during the previous year. Unlike many neurotropic viruses, this virus does not reach the central nervous system by way of the peripheral nerves: during its circulation in the blood stream it may sometimes be detected on the nasal mucosa, whence, apparently, it reaches the brain by the perineural lymphatics of the olfactory nerve. Such a mode of infection of the nervous system was previously unsuspected, except, of course, when viruses are introduced directly into the nose; in nature the equine encephalomyelitis virus is one of a group transmitted by biting insects.

Pseudorabies. Carrying to completion an investigation begun at the Rockefeller Institute, Princeton, Dr. Hurst found that, in the monkey, infection with the virus of pseudorabies runs a very different course from that in most animal hosts. While in the rabbit, guinea-pig, etc., this virus has affinities for a wide range of tissues, in the rhesus monkey it is apparently purely neurotropic. Some evidence was obtained that in monkeys immune to the B virus (Sabin and Wright), infection with pseudorabies virus ran a more favourable course than in animals not so immune.

Rabies. During some experiments on the intravenous inoculation of neurotropic viruses, it was observed that while virulent rabbit brain given intravenously in moderate doses rarely produced infection, after passage in the guinea-pig brain even minute doses may be effective. The reason for this change in "virulence" is under investigation.

Poliomyelitis: estimation of potency of human convalescent sera. The investigation undertaken by Dr. Eagles in collaboration with the Danish State Serum Institute, Copenhagen, into the neutralising value of samples of serum from cases of poliomyelitis at different periods following recovery from the disease has been completed and the results obtained will shortly be published.

Samples of pooled serum from paralytic cases taken on an average of 29 days after recovery showed a low titre of protecting antibody. From non-paralytic cases taken about the same period a slightly higher value was obtained. In these cases sera from patients belonging to blood-group "B" were tested in one pool. This pool was shown to have a very high titre of protecting antibody. A pooled serum from abortive cases taken 13 days after recovery also showed a very high level of protective antibody.

These cases tested at a later date—approximately four months after the onset of the meningitic state—showed on the whole a considerably lower titre of protective antibody.

While it is impossible to generalise on a limited number of tests it would seem that considerable variation in antibody production occurs amongst individuals following recovery and that the highest titres are to be expected early in convalescence.

It is obvious from an investigation of this kind that it would be of the greatest value if there were available a dried standard convalescent serum for assessing comparative values of sera tested by neutralisation experiments in monkeys.

Pleuropneumonia-like organisms as symbionts of certain bacteria. Dr. E. Klieneberger (Jenner Memorial Research Student) has continued her work on pleuropneumonia-like organisms as symbionts of certain bacteria. She has separated a number of L. 1 strains from *Streptobacillus moniliformis* cultures which had been obtained from mice infected with material from the nasopharynx of rats. Her previous studies had shown that the L. 1 organism is regularly found in association with a particular streptobacillus; this combined culture, formerly regarded as a pure bacterial culture, is known in the literature under the name of *Streptobacillus moniliformis*. The pure L. 1 cultures obtained have been used for detailed investigations of the biology and morphology of this newly-discovered L. 1 organism. These studies have confirmed her view of a close relationship in systematic standing between the L. 1 germ and the organisms of pleuropneumonia and agalactia.

Recently light has been thrown on the significance of the *Streptobacillus moniliformis*, which occurs frequently in the nasopharynx of rats, by a new discovery. In a disease of hitherto unknown origin, often attacking the lungs of laboratory rats, pure cultures of the L. 1 organisms have been obtained from the glairy masses which the consolidated and bronchiectatic parts of the lungs discharge on incision. Elements of the L. 1 organism seem to be present in the tissue of diseased parts of the lung when the dark-ground illumination method is used. This means that the L. 1 organism is probably the cause of the destruction of the lung tissue and justifies the supposition that the *Streptobacillus moniliformis* is not a harmless saprophyte of the rat nasopharynx (as previous authors have alleged), but may be directly responsible for a disease of the rat lung which has long been known but whose etiology has hitherto remained obscure. This and other associated problems are the subject of animal experiments now in progress.

Further, the growth and morphology of some anaerobic bacilli of the fusiform group have been studied and indications have been found that some of these strains may contain symbionts of the L. 1 type.

Serological Studies: Antigenic Constitution, Virulence and Immunising Properties of Bacteria.

The "Vi" antigens of *S. typhi* and of other *Salmonella* species. Dr. A. Felix with the assistance of Miss R. M. Pitt and in some experiments with the collaboration of Captain S. S. Bhatnagar (Indian Medical Service), has continued the investigation of various aspects of this subject.

Properties of the "Vi" antigen of *S. typhi* and of the corresponding antibody. This antigen is in some respects even more labile than the "H" antigen. Its agglutinogenic activity is destroyed by exposure to temperatures lower than that required for the inactivation of the "H" antigen, though its capacity of absorbing the "Vi" antibody is not entirely destroyed even by heating to 100° C. Temperatures during growth which permit of abundant development of "H" antigen markedly inhibit the formation of "Vi" antigen in the cultures. Suspensions of "Vi"-containing bacilli, killed by exposure to 0.5 per cent. phenol, are still agglutinable by the "Vi" antibody, but do not induce formation of the "Vi" antibody when used for the immunisation of rabbits. Treatment with dilute hydrochloric acid inactivates both the "Vi" and "H" antigens, but does not affect the "O" antigen. Alcohol, on the other hand, has different effects on "Vi" and "H" antigens; the latter is entirely inactivated by treatment with alcohol, whereas the former is only deprived of its agglutinability by the corresponding antibody and retains its immunogenic activity unimpaired. These facts indicate that the chemical structure of the "Vi" antigen of *S. typhi* differs profoundly from those of the "O" and "H" antigens.

"Functional deficiency" of the "Vi" antibody resulting from immunisation with formalised "Vi" antigen. The surprising fact was established that the "Vi" antibody elaborated in response to immunisation with formalised "Vi" antigen is not in all respects identical with that resulting from immunisation with the "natural" "Vi" antigen contained in the living bacilli. The phagocytosis-promoting activity and the protective power of the former are much inferior to those of the latter, though there is no difference in the agglutinating properties of the two varieties of antibody. In cross-absorption tests with the "natural" and formalised "Vi" antigen and the corresponding antisera complete mutual absorption is obtained. The nature of the alteration of the "Vi" antigen by exposure to 0.2 per cent. formalin is still obscure.

"Rough" variants of *S. typhi* containing "Vi" antigen. When "Vi"-containing strains of *S. typhi* vary from the smooth to the rough variant, the "Vi" antigen is not necessarily lost simultaneously with the smooth "O" antigen. Such variants, which are non-virulent, are yet capable of inducing active and passive immunity. The virulence of *S. typhi* depends on the combined activity of the smooth "O" and the "Vi" antigen. Nevertheless, the "Vi" antibody alone is sufficient to protect against infection with strains of the highly virulent type.

The preparation of therapeutic antityphoid serum. The original method of immunising horses with suspensions of virulent bacilli in the living state was not free from certain difficulties and risks. It could not be replaced by one using suspensions of bacilli killed in the customary way by heating or by treatment with phenol or formalin, since these procedures either entirely destroy or greatly reduce the immunogenic value of the "Vi" antigen. For some time an avirulent, rough, but "Vi"-containing variant served as the source of the "Vi" antigen, while the "O" antibody, which is the other essential component of the therapeutic serum, was produced by injecting a dead vaccine containing the smooth "O" antigen. Recently the method could be simplified by the use of alcohol-treated suspensions containing both the "Vi" and the "O" antigens, since the "Vi" antibody elaborated in response to immunisation with alcohol-treated suspensions is as potent in protective action as that resulting from immunisation with the "natural" "Vi" antigen contained in the live virulent bacilli.

The "Vi" antigens of *S. paratyphi* A and B and *S. aertrycke*. The presence of antigens of the type of the "Vi" antigen of *S. typhi* has been demonstrated in *S. paratyphi* A and B and *S. aertrycke*. Numerous earlier attempts to demonstrate these antigens in other members of the genus *Salmonella* had failed and the methods which ultimately led to their demonstration together with the results of a preliminary investigation of the inter-relationships between the "Vi" antigens of the various *Salmonella* species have been published.

Antityphoid vaccine studies. With the recognition of the special importance of "Vi" antigen in antityphoid immunity, the study of methods of vaccine preparation which should leave this antigen intact and as effective as possible becomes desirable. Dr. H. Schütze has demonstrated by experiments with mice that for prophylactic purposes the old-established method of heat-killing and phenol-preservation is as efficient as any of the alternatives he has tested.

Spore-bearing anaerobes: *C. oedematis maligni*. Until now, the opinion has been held that in the prophylaxis of *C. oedematis maligni* infections the only antigens taking part in the production of protective immune bodies are the soluble exotoxin elaborated in culture and the "O" antigen of the bacillary body. Recently, however, in the course of Dr. D. W. Henderson's work the presence has been established in this organism of a second bacterial antigen, hitherto unrecognised, which is heat-labile and capable on injection of stimulating the production of highly potent antibacterial sera. The sera from rabbits immunised respectively with steamed suspensions ("O" antigen) and unheated but formalised suspensions exhibit a wide range of difference in protective value; the latter are at least 10-20 times more effective than the pure "O" sera. Absorption experiments have shown that complete removal of "O" antibody from sera prepared against formalised suspensions only reduced their protective value by 25-50 per cent., while pure "O" sera are rendered valueless. It has also been shown that the enhanced value of such sera bears no relationship to their usually low antitoxic content. Additional evidence for the bacterial origin of this antibody is found in its type-specific distribution among various strains of *C. oedematis maligni*. Had the increased protective value been due to antitoxic content cross protection against various strains would have occurred. The extreme potency of this antibody warrants an examination of the part it may play in the protective value of so-called "pure" antitoxic sera. So far, no *in vitro* evidence has been obtained of the presence of this antibody; a possible relationship with "H" antibody is not yet established.

***C. welchii*.** During the course of an investigation by Dr. Henderson concerning the "invasive" properties of certain members of the gas-gangrene group of spore-bearing anaerobes a new type of antigen has been demonstrated by *in vitro* methods in certain *C. welchii* strains. The relationship of this antigen to the "invasive" properties of the organism is not yet clear, but it has been found associated only with those strains which produce the "D" type toxin ("ovitoxic" types). Evidence, now accumulating, suggests, in fact, that this new bacterial antigen may actually be a prototype of the "D" toxin.

An *in vitro* analysis of the antigens of *C. welchii* now in progress shows a wide diversity of "O" antigen among the various types. One exception to this rule is the apparent identity of "O" antigen possessed by the "lamb dysentery" strains of this organism. This fact, together with identification of the new antigen, may later help in the classification of a group which appears to become increasingly complex in membership and structure.

Bactericidins for *M. gonorrhoeae* in normal mammalian sera. Dr. Y. B. Abdoosh (Cairo) has completed his study of the gonococcal bactericidins and has come to the conclusion that human sera possess no natural bactericidins for this organism but that there is a steady rise in the bactericidal potency of the sera of gonorrhoeal patients during the course of the disease. Normal sera from 11 mammalian species other than man, all showed the presence of natural bactericidins, some of them to a very high degree. The possible bearing of these facts on natural immunity is noted. The serological heterogeneity of the gonococcus is revealed by the bactericidal test carried out with immune rabbit serum; thus previous observations, made with the agglutination and complement fixation tests, are confirmed.

A biological differentiation of Gram-negative and Gram-positive organisms. Dr. M. Gutstein (Berlin) has pursued his examination of the effect of the two ferments, trypsin and pepsin, on gram-negative and gram-positive organisms. Both ferments inhibit the growth of gram-negative bacteria while permitting that of the gram-positive varieties, but though pepsin needs an acid medium for this differentiation, trypsin produces the effect in either an acid or an alkaline medium. So far as observations permit of an interpretation, the phenomenon seems to be due to the action of the ferments on the medium rather than on the bacteria.

Resistance to infection and genetic constitution. In pursuance of their investigation into the varying resistances to experimental infection of pure line and selected races of mice, Dr. Schütze and Dr. P. A. Gorer have been able to show that in either case the mouse strains which prove resistant or non-resistant to one type of *Salmonella* are likewise resistant or non-resistant to another type of that bacterial group. When, however, infection is carried out with the virus of louping-ill, a *Pasteurella* of attenuated virulence, or a highly virulent *Pneumococcus*, no certain difference in the mortality rates of the mouse lines can be detected. *In vitro* tests demonstrated that the sera of mouse lines resistant to infection with *Salmonella* types possess the power of inhibiting the growth of those organisms to a greater extent than do the sera of non-resistant mouse lines. Estimation of antibody production after inoculation with killed *Salmonella* vaccine reveals the fact that resistant lines give a better response, at any rate of "H" antibodies, than do non-resistant lines.

Analysis of blood and tissue antigens in pure line mice. By means of immune sera and the hæmagglutination test, Dr. Gorer has been able to establish three different antigens differentiating the erythrocytes of mice belonging to three pure lines. These antigens appear to be confined to the erythrocytes. Experiments are in progress to detect antigenic differences in tissues other than blood. It is hoped to investigate the genetic basis of such differences when found.

Endocrinology.

Dr. V. Korenchevsky, with the assistance of Mrs. M. H. Dennison and the voluntary co-operation of Dr. S. Levy-Simpson and Mrs. I. Brovsin, has continued to study the effects of the sexual hormones and of adrenalectomy. Joint experiments with Miss E. M. Hume on the effect of sexual hormones on vitamin deficient rats and with Dr. J. M. Gulland on the gonadotropic hormones of the suprarenal cortex are also in progress. The co-operation with Professor Ruzicka has been continued in the investigation of male sexual hormones.

Investigation of artificial male sexual hormones. The following preparations were investigated: (1) androsterone, (2) androsterone-diol, (3) the water-soluble salt of androsterone, (4) the water-soluble salt of androsterone-diol, and (5) testosterone.

Since both male and female hormones are now known to be excreted in the urine of both sexes, it became necessary to make an investigation of the effects of each hormone on the organism of both sexes. Experiments were, therefore, performed on castrated male and ovariectomised female rats.

Assay of artificial hormone preparations on castrated male rats. Using Korenchevsky's method of assay, curves were drawn for all the preparations to show the relation between the dose of the preparation and the effect on the sexual organs. The value of the rat unit was estimated for each preparation and compared with that as expressed in capon units.

The following amounts were found to contain one rat unit: (1) androsterone, 170 γ ; (2) diol, 21 γ ; (3) the lithium salt of androsterone, 1600 γ ; (4) the lithium salt of diol, 67 γ ; and (5) testosterone, 8 γ . Considerable differences, both qualitative and quantitative, were found between the rat and capon units, showing that the effect on mammals is different from that on capons. The curves for the seminal vesicles and prostate show (1) that androsterone-diol is similar to testosterone in activity and that both differ from androsterone; (2) that androsterone-diol and testosterone appear to bring about a normal development of the sexual organs, while androsterone fails to do so.

The effect of prolonged injections on castrated males. Sexual and other organs. In the doses used only androsterone-diol was able to bring about complete recovery to the normal condition of all

the sexual organs atrophied after castration, both androsterone and testosterone failing to do so. With all three hormones, however, a return to, or exceeding, the normal development was obtained in the case of the preputial glands. After castration the normal involution of the thymus is much delayed, the liver, kidneys and heart decrease in weight and the adrenals hypertrophy. Injections of all the three types of hormone investigated were found to bring about a return of these changes towards, or to, the normal condition.

Body weight and deposition of fat. After castration the gain in body weight decreases, but the injection of any of the three hormones investigated brings about a restoration of the normal rate of growth. Comparison of the changes in the gain in body weight with those in fat deposition in the case of testosterone injections suggests that this hormone has a favourable effect on the anabolic processes in the metabolism of the organism.

Effect of œstrone on male rats. Histological investigation has shown that the effect of œstrone on male rats is both physiological and pathological. The stimulation of the development of smooth muscle and the enhancement of the effect of testicular hormone on the seminal vesicles, coagulating gland and prostate can be considered to be a beneficial and physiological effect. Œstrone also brings about a partial return to the normal histological structure of the "castration hypophysis."

The most important of the pathological effects of œstrone are the fibrosis and epithelial metaplasia produced in some of the secondary sex organs in castrated rats and the hypertrophy of the adrenals and hypophysis in normal rats.

The co-operative activity of œstrone and the male hormones in male rats. The effect of œstrone in enhancing the stimulation of the secondary sexual organs by male hormones might be regarded as a demonstration of the co-operative activity of these hormones. From the point of view of therapy it is important that the administration of the male hormone simultaneously with that of œstrone completely prevents the development of the pathological changes produced by œstrone.

Female rats. Female prostatic gland. In female animals this gland was first found and described in the present experiments. The female prostatic glands are atrophic in normal and in pregnant female rats and in ovariectomised uninjected or œstrone-injected rats. In spayed rats injected with androsterone-diol alone or simultaneously with œstrone, however, these glands developed into comparatively large "female prostates" having the normal structure of the ventral lobe of the prostate of males.

The effect of artificial male hormones on ovariectomised rats. Sexual and other organs. The injection of androsterone, androsterone-diol and of testosterone brings about a partial recovery of the atrophied uterus and vagina and complete recovery of the female preputial glands of ovariectomised females. There is, however, one important feature in the histological changes produced in the vaginal epithelium by medium and large doses of androsterone-diol and of testosterone. After the injections the vaginal squamous epithelium is converted into columnar mucous epithelium. Such a transformation occurs naturally in the vaginal epithelium of the rat during pregnancy, when, however, the degree of change is more pronounced.

While the restorative effect on the thymus is similar with all three hormones, the increase in the weight of the liver, kidneys and in the gain in body weight was only obtained with androsterone and androsterone-diol. While the decrease in the weight of the adrenals was considerable and constant with these two hormones, the changes obtained with testosterone were not so definite.

Comparison of the changes produced by male hormones with those produced by œstrone. The restorative effect of œstrone on the vagina and more particularly on the uterus is greater than that of the male sexual hormones. Œstrone has no effect on the female preputial glands which are strongly influenced by male hormones. Both female and male hormones increase the speed of involution of the thymus, while the depressing effect on the appetite, on the gain in body weight and on the fat deposition, is produced only by œstrone.

The co-operative activity of male and female sexual hormones in females. The co-operative activity of these hormones is very pronounced in bringing about the restoration of the atrophied female sexual organs towards, or to, the normal condition. Histologically, however, androsterone-diol and testosterone, when injected simultaneously with œstrone, did not bring about a return to the normal squamous type of the vaginal epithelium but caused the development of mucous columnar cells typical for "pregnancy cells." Thus the male hormones investigated have some of the important properties of the female hormones, so that, if these are present in the female organism, it would be expected that they would act in co-operation with œstrone in controlling the condition and function of the sexual and some other important organs in the female.

Assay of gonadotropic hormone. Hitherto the assay of the gonadotropic hormones has been made on female rats, thus measuring the female gonadotropic activity of these substances. It is equally important, however, to assay the male gonadotropic activity. Therefore, a new method of assay of this hormone was worked out on immature male rats using the prostate, seminal vesicles and penis as the test organs. The male rat unit proposed for the gonadotropic hormone of pregnancy urine was found to be equal to about 5 female rat units.

Histological changes in the kidneys of adrenalectomised rats. In adrenalectomised rats suffering from deficiency of adrenal cortical hormones degeneration of the tubules was found in the kidneys. Daily subcutaneous injections of cortical suprarenal extract diminished or prevented the occurrence of these changes.

Investigation of Kidney Function.

Dr. P. Ellinger has continued his investigations on the function of the kidney. Frogs were repeatedly injected with acid sodium sulphate over a period of some weeks in order to cause repeated compulsory excretion through the epithelium of the proximal tubules. At the end of the period the function of the kidney was observed by Dr. Ellinger's method of intravital microscopy. The action of the proximal tubules was found to have been altered and they were unable, as are the normal tubules, to concentrate previously injected fluorescein solution by absorption of water or to absorb alkaline radicles. Some proximal tubules also appeared to have undergone calcification. Histological investigation of the affected kidneys showed that a destruction of the epithelial cells of the proximal tubules had taken place.

Coagulation of Blood.

Dr. J. O. W. Barratt has further studied the nature of anti-coagulant action. In this connection it has been found necessary in each series of observations to determine the extent of any deterioration of fibrinogen, contained in plasma employed for experiment, occurring subsequently to withdrawal of blood. The actual measurement of the anti-coagulant activity of sodium chloride, heparin and chlorazol has been attended with difficulties of technique which have only recently been overcome and has presented several problems, the elucidation of which has required extended observations over a large range of concentrations, with the result that it is now possible to express anti-coagulant action in quantitative terms. It is hoped shortly to publish details of the methods of estimation employed.

The Technique of Investigations with High Centrifugal Fields.

During the year Dr. McFarlane (Research Fellow in Biophysics) has been almost entirely occupied in supervising the work on the new building being erected to house the Svedberg centrifuges and in arranging for the installation and disposition of the necessary refrigerating, electrical and optical equipments. He has, however, found time to elaborate a method for the accurate estimation of high rotational speeds.

The accuracy with which particle size may be determined in the centrifugal field depends among other things on the accuracy with which the high rotational speeds of the rotor can be measured. In the method he has elaborated the rotor in each revolution gives rise to an electric impulse and the frequency with which these impulses recur is measured by means of an electron beam oscillograph using the alternating current main as the reference standard of frequency. The accuracy of speed measurement is as high as the accuracy of time measurement by means of a synchronous electric clock. In the course of this work it was necessary to improvise a source of the higher frequencies and for this purpose an air-driven top, constructed by Dr. McFarlane and Dr. Amies, was used. The top was fitted with a spindle of stainless steel having a mirror surface ground on it. A beam of light reflected from this surface flickered with the frequency of the top and was subsequently used to generate electrical impulses of the same frequency. This oscillographic method of speed measurement is being incorporated in the new plant.

DIVISION OF PROTOZOOLOGY.

The work by Dr. Muriel Robertson on the effect of gamma ray irradiation of actively growing cultures of *Bodo caudatus* has been completed and published.

Reactions of protozoa to drugs. Experiments are now in progress with the aim of elucidating some of the principles underlying the reactions of protozoa to various drugs and poisonous substances. As a test object for this purpose, a ciliate (a *Glaucoma*, probably *Glaucoma piriformis*) has, after much difficulty, been isolated in pure bacteria-free culture. This organism has been cultivated in standardised counted cultures and very regular results have been obtained.

The reactions of *Glaucoma* to inorganic arsenic (As_2O_3) have been investigated for some months with the result that acclimatisation has not been found to take place as a consequence of cultivation in sublethal concentrations of the substance. In any constant set of conditions, repeated culture, or/and discontinuous culture, in arsenic, have revealed a surprising degree of constancy in the check upon the numerical evolution of the arsenic-containing cultures as compared with the controls and no advantage in favour of those previously exposed has so far been observed.

In time-exposures to lethal concentrations there is a certain advantage in survival on the part of cultures previously exposed in this same type of experiment, but not on the part of strains which had previously been cultivated in non-lethal amounts of the substance. It is not clear, however, that acclimatisation is the correct interpretation of the somewhat greater viability. At present the facts seem to point to a simple selection of the more viable individuals.

Antibody production in response to the injection of protozoa. The investigation of the antibodies produced in the blood of rabbits by the injection of protozoa has been continued using these bacteria-free cultures of *Glaucoma*. Immune sera can readily be made in response to injection of living *Glaucoma* as the antigen. The tests which required special care in the preparation of the suspensions, showed an early agglutination with titres up to 1/2000 and 1/3000 dilution of the serum. In heated sera the protozoa were not killed and some hours later were once more found swimming freely at the top of the tubes, while an almost completely transparent deposit was alone left at the bottom of those tubes in which agglutination had been marked.

When the tests were followed under the microscope, the ciliates were observed to be immobilised at first and agglutinated in clumps held together by the ectoplasmic structures. A complex series of changes was now seen to take place by means of which the organisms shed the outer envelope of the body either in the form of fragments or in an almost intact state. The *Glaucoma* could actually be seen in many cases finding their way out of the enclosing periplast which had been separated from the body of the ciliates but which had not yet been split or sloughed off. These empty envelopes and also the fragmentary protoplasmic exudate remain agglutinated in easily distinguishable masses. Once the *Glaucoma* with their regenerated or recovered cilia had swum out of the clumps they did not reagglutinate in the tubes.

The ciliates were extremely sensitive to the action of "complement" in fresh sera so that unheated immune sera and normal sera were found to be lethal in suitable concentrations. Heated normal sera of rabbit, horse and guinea-pig exerted no influence upon the organism in concentrations from 1 in 10.

DIVISION OF NUTRITION.

Vitamin Standards and Units.

Further research on vitamin standards and units has been carried out on the lines recommended by the 1934 International Conference on Vitamin Standardisation. The work has been carried out at the request of the Accessory Food Factors Committee of the Lister Institute and the Medical Research Council.

Vitamin A. The monograph, edited by Miss Hume and Dr. H. Chick, containing the existing information on the properties of pure β -carotene, the new vitamin A standard, as well as the evidence for regarding the spectrographic method of vitamin A estimation as a trustworthy alternative to the biological test, has been published as a special report of the Medical Research Council. Further research is, however, necessary to fix the exact value of the conversion factor by which the results of the spectrophotometric test must be multiplied in order to express the result in International Vitamin A Units. For this purpose and in order to test further the error of the biological method, a collective investigation is being arranged by the Vitamin A Sub-Committee, by means of which the results of spectrophotometric examination of certain selected materials are to be compared with those derived from biological tests carried out in several different laboratories. Miss Hume is acting as secretary to this Committee and is also taking part in the investigation.

Vitamin D. The anomalous behaviour of certain forms of vitamin D, e.g., irradiated ergosterol, and cod liver oil, when administered as antirachitic agents to certain species (e.g. poultry) has introduced a complication into the interpretation of vitamin D standardisation, seeing that the vitamin D standard is irradiated ergosterol and the usual biological tests are carried out on rats. The most probable explanation lies in the discovery by Windaus and others of the existence of more than one form of vitamin D, that in cod liver oil being different from that present in irradiated ergosterol, and birds being relatively insensitive to the second form. At the request of the Vitamin D Sub-Committee, Miss Hume has collected the existing scattered information in the literature upon this subject with a view to its publication.

Vitamin B. The 1934 International Conference requested that those having access to a crystalline vitamin B₁ preparation should compare its potency with that of the present International Standard (the vitamin B₁ adsorbate on fuller's earth from rice polishings) with a view to its future adoption as international standard. Dr. F. Vivanco (Madrid) is carrying out this test with crystalline material received from Professor Suzuki's laboratory in Tokyo.

Work on the Vitamin B Complex.

Relation of the two constituents of vitamin B₂ (flavin and vitamin B₆) to the skin lesions occurring in rats deprived of this vitamin. Further work by Miss A. M. Copping (Medical Research Council grantee) has confirmed the conclusion of György that vitamin B₆ prevents the (a) symmetrical florid dermatitis ("rat pellagra") and flavin the (b) skin affection involving loss of hair without swelling, and an exudate of serous fluid from eyes and nostrils. Rats maintained from the time of weaning on complete synthetic diets containing flavin and lacking only vitamin B₆, developed the (a) type, while those containing vitamin B₆ and lacking flavin developed only the (b) type of skin lesions.

Purification and Properties of Vitamin B₆. Miss C. E. Edgar (Francis Maitland Balfour Research Fellow of Newnham College, Cambridge) and Dr. T. F. Macrae have continued the study of the chemical and physical properties of vitamin B₆, as derived either from alcoholic extracts of defatted wheat embryo or from autoclaved yeast extracts freed from flavin by adsorption on fuller's earth. Some advance has been made but this research is necessarily slow of progress since each test for vitamin activity involves an animal test of several weeks' duration.

NOTE.—In all this recent work on Vitamin B₂ and its constituents, the Division of Nutrition is greatly indebted to Dr. S. Smith of Burroughs Wellcome and Company for his repeated gifts of pure crystalline flavin. Without this generous help these researches would not have been possible.

Porphyrim excretion in rats deprived of Vitamin B₂. Professor Ellinger and Miss Edgar have continued the experiments of the previous year and have also studied the effect of visible and ultra-violet light on the porphyrim excretion of rats deprived of vitamin B₂. Visible light had no effect either on the skin of the shaved rats or on the porphyrim excretion. Ultra-violet rays produced no skin lesion, but there was a large diminution in the porphyrim excretion, a rapid growth of hair of the shaved skin and improvement in the general condition. This effect, however, lasted only a few days and was followed by an increase in porphyrim excretion and general change for the worse.

Porphyrim excretion in human pellagra. Professor Ellinger has examined the urine of pellagra cases for porphyrim content in parallel with samples from normal subjects and persons suffering from other nervous diseases as controls. In nearly all cases of the last two categories the urine contained no porphyrim or only traces, except following treatment with iron compounds. Tests were made on pellagra cases during a visit to Mostar, Yugoslavia, in April, 1935, arranged with the co-operation of Dr. Dojmi of the Yugoslavian Public Health Service, and a large number of specimens of urine have since been received from Mostar, from Roumania (with the help of Dr. Ciuca (Bucharest)), and from the United States (with the help of Dr. T. B. Spies (Cincinnati)). The cases included endemic, alcoholic and "secondary" pellagra and all showed strong excretion of porphyrim. In cases tested at intervals during treatment there was a diminution of the porphyrim excretion simultaneously with improvement in the condition. Excretion of porphyrim would thus appear to be characteristic of pellagra and may provide a clue to the metabolic disorders causing the disease.

Quantitative estimation of the lyochrome content of animal or plant tissues. The exact quantitative estimation of the lyochrome content of plant and animal tissue is important since a trustworthy method might replace the biological test for estimation of flavin in foodstuffs. Many different methods are described but none of these seems to give a sufficiently correct result. It can be shown that the method most used—the "fluorimetric" method—cannot give exact results and the reasons for these discrepancies have been determined. An exact method which allows a correct estimation is now being elaborated.

Vitamin B₂ Content of Cereals and the Etiology of Pellagra.

Previous work by Aykroyd in this Division showed that cereals must be regarded as relatively poor sources of vitamin B₂ and that while both whole wheat and whole maize contained small amounts, their respective endosperms contained even less. The discovery of the composite nature of vitamin B₂ has made a revision of this work necessary and Miss Copping has investigated wheat, maize and their milling products and has found them to be good sources of vitamin B₂, but to be poor in flavin. Whole wheat contained appreciably more flavin than whole maize and this conclusion was confirmed by experiments made by Dr. Ellinger in which the pigment was extracted; a small amount was identified in the extract from wheat but none in the extract from maize. Much of the vitamin B₂ in wheat and maize, and of the flavin in wheat, was contained in the germ and integuments of the whole grain, and the endosperm contained relatively much less.

These results, which in the main confirm the observations of György and his co-workers at Cambridge, indicate that lack of vitamin B₂ cannot be connected with the etiology of pellagra, since the maize diets on which populations suffering from endemic pellagra subsist are rich in this vitamin. There would seem to be a definite nutritive advantage in wheat over maize in the possession of a greater amount of flavin, seeing that the foodstuffs found by Goldberger and his colleagues to be preventive and curative of pellagra were those rich in flavin. Recent clinical trials reported from the United States have failed, however, to demonstrate any curative effect for pellagrins of administration of pure flavin.

Investigation of the Nutritive Value of Wheat and Bread.

Laboratory investigations have so far failed to explain the relative immunity from serious deficiency disease existing among populations whose staple diet is wheat and bread made from white flour, when these are compared with populations subsisting too exclusively on maize or rice, which are subject to pellagra and beriberi respectively.

The content in these cereals of the B vitamins has not shown differences large enough to account for the observed difference in nutritive value, although administration of these vitamins (in the form of yeast) has

proved curative and preventive for both pellagra and beriberi. It seemed possible that the use of yeast in the manufacture of bread from wheat might provide the clue. With this idea an investigation has been started by Miss M. H. Roscoe and Miss Copping, both working with grants from the Medical Research Council. With the kind and generous co-operation of Dr. Kent-Jones and of Messrs. Chitty, millers of Dover, special samples of wheat and white flour have been secured and bread has been baked from these by different standard methods using varying amounts of a standard yeast. The amount of the B vitamins (B_1 , flavin and B_6) in the various ingredients and in the finished breads, is now being estimated, the aim being to determine what proportion of the vitamins in the finished product is derived from the added yeast and whether any synthesis takes place during the fermentation process.

Effect on Reproduction of a Dietary Essential contained in certain Fats.

Miss Hume and Miss H. Henderson Smith have continued their investigation of the effect on reproduction in the rat of a dietary deficiency believed to be that of the unsaturated fatty acids as described by Burr and Burr. Animals reared from weaning on a diet partially deficient in this factor grow and develop normally and show no symptoms of deficiency, but, after mating, the young frequently die *in utero* just before term, or if born alive fail to survive, although lactation does not appear to be defective. The few young which have survived and been reared, have shown skin and tail symptoms indistinguishable from those shown in the milder degrees of Burr and Burr's syndrome.

Incorporation in the diet of linseed oil, which is rich in Burr and Burr's factor, while enabling some litters to survive, has not completely corrected the deficiency and the matter is still under investigation.

Vitamin A Deficiency and Hormone Imbalance.

In conjunction with Professor Korenchevsky, Miss Hume and Miss Henderson Smith are continuing the study of the effects of chronic vitamin A deficiency combined with hormonal imbalance. A series of male rats was maintained for some months on a diet partly deficient in vitamin A, and at the same time received oestrone orally and parenterally. The *post mortem* appearances of these animals showed abnormalities, but the histology of the tissues is not yet fully worked out. A further experiment on the same lines is being carried out on castrated male rats in order that the state of hormonal imbalance may be exaggerated.

Effect of Vitamin A Deficiency on the Eye.

The work by Dr. K. Tansley Lythgoe referred to in last year's report is now complete and is in the press.

Researches on the Antiscorbutic Principle (Vitamin C).

The relation of chemical structure and antiscorbutic activity of compounds to their retention in the animal body. In last year's report mention was made of the assessment of the antiscorbutic activity of a number of homologues, analogues and derivatives of *L*-ascorbic acid. The results revealed that they possessed a varying degree of activity and that among the substances investigated which were active, *L*-ascorbic acid, the only member of this class of compounds so far found in nature, was the most, and *L*-gluco-ascorbic acid the least active. A number were found to be inactive in very high dosage. Dr. S. S. Zilva investigated the reasons for this specificity with the ultimate object of throwing some light on the general mechanism of the biological action of antiscorbutic compounds. He found that when these compounds were injected into the blood stream of guinea-pigs, the tissues of which were previously exhausted of vitamin C, the amounts of these compounds fixed by the tissues, especially the selective tissues such as the liver, adrenals, anterior lobe of the pituitary, and intestine, were proportional to their antiscorbutic activity. The inactive compounds were not perceptibly retained by the tissues. This phenomenon was reflected further in the fact that the kidney excreted these substances in amounts which varied inversely as their potency. Another observation bearing on this issue was that when generalised anaesthesia was employed during the injection, the excretion of the compounds in the urine increased though their retention by the tissues was unaffected. This increase, interestingly enough, was found also to be proportional to the antiscorbutic activity.

Dehydroascorbic acid *in vivo*. A view that blood is capable of dehydrogenating ascorbic acid and storing it in the reversibly oxidised form is held by a number of workers. Indirect evidence such as the tendency of the animal body to reduce dehydroascorbic acid, and the protective action against the oxidation of the reduced form of the vitamin exerted by most tissues *in vitro*, obtained by Dr. Zilva and his co-workers, militated against this view. In collaboration with Mr. A. E. Kellie he, therefore, investigated blood and its constituents for the presence of the reversibly oxidised form of the vitamin. The presence of this compound can be established only by previous reduction of the medium with hydrogen sulphide and by titrating the reduced compound thus obtained with indophenol. They found that the reduction of the indicator brought about by this treatment was not due to the presence of ascorbic acid as was previously assumed, but to an artefact. They also found that the plasma contained a precursor which, on reduction with hydrogen sulphide, yielded a substance reducing indophenol so rapidly as to simulate the presence of vitamin C.

The spectrographic determination of vitamin C. With the object of obtaining a higher degree of accuracy in estimating small quantities of vitamin C than is at present possible in physiological material, attempts were made by Dr. S. W. Johnson to adapt the spectrographic method on account of its possibly greater specificity. The various conditions necessary for the preparation of the material, and the spectrographic procedure were worked out in great detail. A number of modifications have been introduced in consequence, which promise to endow the method with the desired specificity and accuracy. It is hoped that the method developed will be capable of clearing up a number of points on which conflicting views are at present held concerning the presence of the vitamin in certain tissues.

The effect of incomplete diets on the concentration of ascorbic acid in the organs of the rat. It was found by Hopkins that in rats on a carbohydrate diet the concentration of ascorbic acid in the liver rises above that of rats kept on stock diet. An analogous rise in the intestine takes place when the animals are previously fasted or kept on a protein-fat diet or on a fat diet alone. From this he concludes that synthesis of the vitamin takes place in the liver from carbohydrates as the ultimate source and that in the absence of this constituent from the diet ascorbic acid is formed in the intestine from protein as the proximate source. Dr. Zilva has repeated this work and has been unable to confirm the conclusions reached.

Dehydroascorbic acid in apples. Some years ago Dr. Zilva demonstrated the presence of an enzyme in the apple capable of reversibly oxidising ascorbic acid *in vitro*. With Dr. F. Kidd of the Low Temperature Research Station, Cambridge, he has given further attention to the investigation of this reaction in apples. They found that in very young apples gathered at the end of June the expressed juice contains (unlike that of mature apples) the vitamin almost entirely in the reversibly oxidised form. Furthermore, when such young apples are previously heated at a temperature capable of inactivating thermolabile enzymes the vitamin of the juice is still predominantly present as dehydroascorbic acid. This observation suggests that the vitamin is present also *in vivo* in the early stages of the development of the fruit in the oxidised form.

The addition of synthetic ascorbic acid in the canning of fruits and vegetables. It was demonstrated last year that it was possible to add the synthetic vitamin to fruits and vegetables before canning without incurring great losses in the vitamin during the processing. These experiments were carried out on a laboratory scale. In the course of a repetition of this work under conditions resembling those employed in large scale canning, results were obtained which were equally favourable.

DEPARTMENT OF BIOCHEMISTRY.

Phosphate Metabolism and the Calcification of Animal Tissues.

Calcifying mechanism of bone. In continuation of previous work Professor R. Robison, Miss K. Law (Melbourne University) and Miss J. Barnett (London University Research Student) have sought for further evidence that the specific calcifying mechanism of hypertrophic cartilage and osteoid tissue is a complex enzyme system of which phosphatase forms one essential component. The anomalous effect of phosphoglycerate on calcification *in vitro*, mentioned in last year's report, has been studied in greater detail and the influence of other intermediate products of glycolysis in muscle has been investigated. Extracts of bone, prepared in various ways, have been examined for enzymes which form part of the glycolytic systems. The loss in activity of the calcifying mechanism which occurs when bone slices are left for a few hours in salt solutions at 37° C., previously demonstrated by Robison and Rosenheim, may be due to the exhaustion of one component of the system; phosphatase is not seriously affected by such conditions. A study of the factors which accelerate or diminish this loss of activity has already thrown some light on the nature of the change and promises to yield more information regarding the calcifying mechanism.

Calcification of the Aorta. Professor Robison and Dr. A. H. Rosenheim had previously shown that the rat or rabbit aorta may also be calcified *in vitro* by immersion in the experimental solutions; the process is, however, slow and erratic differing markedly from the rapid calcification of hypertrophic cartilage. Deposition of calcium salts in the aorta may be due to physical factors rather than to any enzymic mechanism, but it is important to discover whether such abnormal calcification is, *in vivo*, preceded by changes in the organic tissue favouring precipitation of the solid calcium salt from the tissue fluids. Professor Robison and Miss Law have studied the influence of changes produced by feeding animals with cholesterol but have found no evidence that the presence of lipid deposits, characteristic of cholesterol arterio-sclerosis, is associated with any increased proneness of the aorta to become calcified *in vitro*. When calcification occurred it did not specially favour those regions of the vessel in which cholesterol lesions were found. The effect of subsequent necrotic changes in these regions is being studied.

A similar investigation of the aortae of animals which have received massive doses of calciferol is being carried out by Professor Robison and Dr. M. Laskowski (University of Warsaw), in order to learn whether changes in the organic tissue play any determining part in the calcification which occurs in hypervitaminosis D.

Calcification of Dentine. Professor Robison and Mrs. S. Hughes (Strangeways Research Laboratory, Cambridge) are investigating the process of calcification in dentine. It has been shown that at an early stage of embryonic development when calcification is just beginning, the dentine caps of rat teeth possess a very high phosphatase activity and can be further calcified by immersion in solutions similar to those which calcify hypertrophic cartilage.

Alcoholic Fermentation.

Three component esters of the mixed fermentation hexosemonophosphate have now been identified; the fourth has been further studied by Professor Robison and Dr. M. G. Macfarlane, who have shown that after removal of the phosphate group by bone phosphatase the residual carbohydrate is not fermentable by yeast. The properties of this ester distinguish it sharply from the other hexosemonophosphates and suggest that it may be related to the enzymic system of yeast.

Further evidence has been obtained of the migration of the phosphate group in fructose-1-phosphate on heating in acid solution; this observation gains interest from the fact that such migration has recently been shown by other workers to take place in phosphoglycerol and in phosphoglyceric acid by chemical and enzymic agency. The migration of phosphoric acid from one OH group to another appears, indeed, to play a most important part in the enzymic breakdown of carbohydrate.

Dr. Macfarlane has studied the distribution of phosphorus in living yeast in relation to the fermentation process. A large part of the phosphorus is not extracted by trichloroacetic acid and significant changes in this fraction have been observed.

The Metabolism of Galactose.

The synthesis of lactose by the lactating mammary gland has been further investigated by Dr. G. A. Grant (Beit Memorial Research Fellow). The amounts of this sugar formed by slices of the active gland *in vitro* from various hexoses and phosphoric esters have been determined, using for this purpose the lactose-fermenting yeast *S. fragilis*, Jörg. While glucose was readily converted into lactose by the tissue slices very little synthesis was observed from galactose (which represents the other half of the lactose molecule), or from fructose or mannose; nor was any lactose produced from various hexosephosphoric esters. It is, however, uncertain to what extent the phosphoric esters are able to diffuse into the living cells. They were partially hydrolysed by the active phosphatase of the gland, while under certain conditions synthesis of phosphoric esters from sugar and inorganic phosphate could be demonstrated. The effect of the lactogenic hormone, prolactin, on the lactose synthesis is being studied.

The Chemical Nature of Antibodies.

Dr. Rosenheim (Beit Memorial Research Fellow) has continued her investigation of the action of enzymes on antibodies. The resistance of the flagellar, but not of the somatic, typhoid agglutinin to destruction by pepsin at pH 4.6 and by trypsin at pH 8.6 has been found to vary markedly in samples of immune serum obtained at different times from the same horse.

Attempts are being made to determine whether differences in the antibody can be correlated with differences in the method of immunisation.

Bacterial Metabolism.

Sir Arthur Harden has been carrying out experiments on the early stages of fermentation of the polyhydric alcohols by *Bacillus coli communis* but has not yet obtained definite results.

The Oxytocic Hormone of the Posterior Lobe of the Pituitary Gland.

Dr. Gulland, Mr. S. S. Randall (Research Student in Biochemistry) and Miss M. Freeman (Walter and Eliza Hall Institute, Melbourne) have described a technique in which simple adsorption on norite charcoal replaces the concentration under diminished pressure of large volumes of aqueous solution and subsequent adsorption of active material on the protein-ammonium sulphate precipitate, commonly used in purifying whole aqueous extracts of posterior lobe. This modification simplifies the manipulation of large volumes of extracts of the posterior lobe.

In a study of the ultra-violet absorption spectra of posterior lobe extracts and of purified hormone solutions, Dr. Gulland and Dr. N. S. Lucas have shown that the spectra are not characteristic of the hormone molecule but correspond to the substances which accompany it. These are probably peptide in character, resembling proteoses.

Dr. Gulland, Mr. Randall and Miss Freeman have found that electro-dialysis of purified hormone solutions under very varied conditions was not successful in raising the ratio of activity to weight, since removal of the proteose-like substances by this means seemed to reduce the stability of the hormone towards the alkali which was needed in the procedure. These results also showed that the hormone is either purely basic in character or is associated with basic substances throughout the range of pH 1.6-12.5. Failure to separate the oxytocic and pressor hormones by electro-dialysis at pH 11 suggests that the latter may also be purely basic in nature.

Dr. Gulland and Mr. Randall have continued the purification of the hormone, working on a larger scale than has hitherto been possible. Their purer preparations are considerably more active than those previously described. The problem is also being attacked by studying the potency of synthetic substances which might be expected to exhibit oxytocic activity.

The Constitution of the Nucleic Acids and Nucleotides.

Dr. Gulland has continued his investigations on this subject. The method of ascertaining certain features in the constitutions of the nucleosides by a study of ultra-violet absorption spectra, to which reference was made in the Report of 1934, has been extended in collaboration with Dr. E. R. Holiday (London Hospital Medical Unit), in order to settle the remaining points of doubt in the constitutions of the nucleosides, adenosine and inosine, and in the two adenylic acids derived respectively from yeast nucleic acid and from muscle. These observations also throw light on the constitutions of the adenylic group of certain co-enzymes, for example, adenylypyrophosphate, co-enzyme and the hydrogen-transferring co-enzyme of Warburg's oxidation system.

Studies in the Amino-Acid Groups.

Dr. Gulland and Mr. C. J. O. R. Morris have studied the chemistry of canavanine, an amino-acid occurring in the jack bean, *Canavalia ensiformis*, which has recently been reported to be of nutritive value. An improved method of isolation has been devised, and the constitution $\text{NH}_2\text{C}(\text{NH})\text{NH}\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{CO}_2\text{H}$ has been proposed. Confirmation of this by synthetical experiments is in progress.

Gonadotropic Hormones.

Dr. Gulland and Dr. Korenchevsky are engaged in an examination of the gonadotropic action of extracts from the suprarenal cortex.

Metabolism of Carbohydrate and Fats.

The investigation of the carbohydrates stored by the yeast cell has been continued by Dr. I. Smedley-MacLean and Miss R. McAnally. When yeast is grown in solutions of glucose the addition of sodium fluoride in small concentration produces a marked inhibition of the synthesis of glycogen and a still greater inhibition of its breakdown by the cell. The nature of the insoluble carbohydrate stored by yeast which is at present the object of investigation is proving of considerable interest.

Dr. Smedley-MacLean and Mr. L. D. MacLeod (Morna Macleod Research Student) are investigating the synthesis of fatty acids by yeast. The only materials other than sugars which have so far served as material for the synthesis of the higher fatty acids and sterols are ethyl alcohol and acetic acid and the nature of the conditions affecting this synthesis is under examination.

The work of Burr and Burr and of other observers has shown that some special constituent of fats is necessary to ensure normal growth in the rat and that definite symptoms of malnutrition are occasioned by its absence. Linseed oil is most effective in curing the symptoms of this deficiency, olive oil being entirely ineffective. This special curative power has been ascribed to linoleic acid, termed by Evans vitamin F, but it is still open to dispute whether linoleic acid itself is the active or at any rate the only active constituent which will produce this effect. Experiments designed to test this hypothesis are being carried out in conjunction with Miss Hume. A study of the oxidation products of linseed oil has been undertaken which it is hoped may throw further light on the constituents of this oil. Isomeric oxidation products of widely varying stability are formed when hydrogen dioxide acts on linseed oil and these products are being examined in co-operation with Mr. L. C. A. Nunn.

DEPARTMENT FOR THE STUDY AND PREPARATION OF THERAPEUTIC SERA.

The therapeutic action of anti-meningococcus serum.

The pathogenic action of the *Meningococcus* is apparently due to the intracellular poison which is liberated in the course of the natural disease and which can be separated from the coccal bodies under experimental conditions. Work carried out by Dr. G. F. Petric has led to the conclusion that this toxic substance is not antigenic and that, therefore, the therapeutic efficacy of the serum cannot be ascribed to an anti-endotoxin and is probably wholly dependent upon an antibacterial mechanism. This view is consistent with the results of interesting work on the virulence of the *Meningococcus* which has been carried out within recent years in the United States. Formerly, in the experience of most investigators, very large intraperitoneal doses of cocci were necessary in order to produce a fatal infection in mice and consequently it was difficult to dissociate the pathogenic effects due to the endotoxin and to the coccal invasion. It now appears that some freshly isolated strains are highly virulent and that the virulence of other strains is enhanced by incorporating mucin prepared from the gastric mucus membrane of the pig in the dose of the coccal suspension which is injected into the mouse; the lethal dose may contain as few as 10-20 cocci. By this means it has been found possible to arrange protective experiments which show that immune-sera from the horse can neutralize the pathogenic effect of many multiples

of the lethal dose of living cocci. The mode of action of the mucin is not understood but it may be presumed to involve protection of the coccus from the phagocytic cells of the host. Dr. Petrie has begun work on the factors which influence the virulence of the *Meningococcus* while it is living under saprophytic conditions and while it is multiplying in the tissues of the experimental animal. Fuller knowledge of the conditions that are requisite for maintaining the virulence of this micro-organism at a high level will simplify the application of methods of assaying the potency of anti-meningococcus serum.

The protective substances in anti-streptococcus serum.

The production of a specific toxin by certain hæmolytic strains of *Streptococcus* is now well established and it is also agreed that the corresponding antitoxin is able to neutralize the toxin in the tissues of man and animals. The problem whether an anti-bacterial element should be taken into account in preparing anti-streptococcus sera for therapeutic use is still unsettled. The multiplicity of serological types in the streptococcal group is a complicating factor from the point of view of the large-scale production of sera of this kind. Dr. Petrie is engaged in examining whether it is possible to devise protection tests in mice, similar to those employed for the assay of anti-pneumococcus serum, which will permit an estimate of the potency of the anti-bacterial constituent in specific streptococcal immune sera. He has chosen two serological types and has enhanced their virulence in mice by passage to an extent which makes it possible to give a suitable test-dose in protection experiments. Horses have been immunized with a variety of streptococcal strains including the two test strains, and the course of immunization has now reached a stage when protective immune-bodies may be expected to be present in the blood. It is hoped soon to titrate samples of serum from these horses.

The stability of the protective substance in the "Felton" fraction of anti-pneumococcus serum, Type 1.

Dr. Petrie and Dr. W. T. J. Morgan are continuing their observations on the unitage of samples from a batch of concentrated anti-pneumococcus serum, Type 1, after storage for varying times at various temperatures. The results show that in this particular batch a considerable loss of potency occurred during the first year of storage even under optimum conditions but that after another period of twelve months no further loss had taken place. This work represents part of a general inquiry into the stability of therapeutic sera which is being organized on behalf of the Pharmacopœia Commission by Dr. P. Hartley, Director of the Biological Standards Department of the Medical Research Council.

The specific polysaccharide hapten of *B. dysenteriae* (Shiga).

Dr. Morgan has continued his investigation of the specific polysaccharide of Shiga's bacillus. The properties of the specific substance, so far as they are known at present, support the belief that the basic structural unit in the polysaccharide aggregate consists of four hexose molecules and one *N*-acetylaminohexose molecule. The reducing power of the unhydrolysed polysaccharide, as measured by the sodium hypoiodite method, is equivalent to 3.4 per cent. This figure corresponds to a polysaccharide that has one hexose end unit, which possesses a free aldehyde group, in each group of 30 of the hexoses that constitute the whole polysaccharide structure. The molecular weight calculated from the hypoiodite titration is about 5,100, a number which indicates that the basic hexose unit repeats itself six times in the polysaccharide molecule. The reducing end-unit is not an *N*-acetylhexosamine unit. Acid hydrolysis of the polysaccharide under conditions which do not remove the acetyl groups from the *N*-acetylaminohexose, rapidly breaks the glycosidic linkages that join the aldehyde groups of the *N*-acetylaminohexose molecules with their adjacent hexoses. When the reducing power of the products formed by partial hydrolysis reaches about one-third of the value that would be attained on complete hydrolysis, the whole of the *N*-acetylhexosamine molecules within the polysaccharide possess a reducing aldehyde group. The products formed by partial hydrolysis at this stage of the hydrolysis fail to precipitate when they are mixed with a *B. dysenteriae* (Shiga) immune-serum. They still possess some degree of serological activity, however, since they cause specific inhibition of the precipitation which normally occurs between the unchanged polysaccharide and an antibacterial "Shiga" serum. Furthermore, the hydrolysis products are able to desensitise guinea-pigs which have been passively sensitised and thus protect them against the anaphylactic shock that is induced by a subsequent intravenous injection of the intact polysaccharide.

The nature of the heterophile hapten of *B. dysenteriae* (Shiga).

Dr. Morgan has continued his collaboration with Dr. Kurt Meyer (France). They have established a relationship between the heterophile hapten and the specific polysaccharide hapten of the "smooth" form of *B. dysenteriae* (Shiga). They have shown that the pure specific polysaccharide, which readily combines with the homologous antibacterial immune-substance, is also able to neutralise specifically the hæmolytic action of "Shiga" heterophile antibody on sheep red-cells. The relation between the power of the specific polysaccharide hapten to neutralise the heterophile antibody and to combine with the antibacterial immune-substance is the same for crude as for purified preparations and it remains unchanged

during the destruction of these specific serological properties by the action of acid, alkali or a species of *Myxococcus*. The experimental data furnish strong evidence for believing that the heterophile receptors and the specific bacterial receptors co-exist in a single homogeneous polysaccharide molecule. The fact that the polysaccharide hapten is able to form a specific combination with two entirely different types of antibody leads to the conjecture that a pure polysaccharide hapten may contain in each of its molecules two kinds of determinant receptors of diverse specificity.

The Standardisation of Gas-Gangrene (*histolyticus*) Antitoxin.

During the year Dr. Morgan has co-operated in an inquiry that was instituted by the Permanent Commission of Biological Standardisation of the League of Nations with the purpose of establishing a standard preparation of gas-gangrene (*histolyticus*) antitoxin for International use. The results of the work have been communicated to the Permanent Commission.

Production of Staphylococcus Toxin.

Agar is usually added to fluid culture media employed for the preparation of staphylococcus toxin, and it exerts a favourable action by furnishing an adsorbing surface which removes an inhibitory substance from the culture medium. Dr. D. McClean has continued his work on the conditions which control the production of staphylococcus toxin in a fluid medium and has found that various adsorbing surfaces such as cellophane, kieselguhr, potato, or even filter paper will act in the same way as agar. These substances need not be present during the period of growth, since satisfactory toxins are obtainable if the adsorbent material is added to the broth and then removed after an hour or two before inoculation with the culture. Attempts to recover the inhibitory substance from the adsorbing surface and to identify it have so far been unsuccessful.

The ability to prepare staphylococcus toxin in a fluid medium has considerably simplified the technical processes of its production, and also as a consequence, the preparation of antitoxin from the horse and of toxoid solutions for human immunisation.

The production of staphylococcus anti-leucocidin in the horse. Recent work by Dr. F. C. O. Valentine has indicated that the α -haemolysin and the leucocidin in staphylococcus toxin are distinct entities and has confirmed the importance of the leucocidin in acute infections by this organism. Dr. Valentine has shown that the leucocidin is antigenic and that the serum of patients who are suffering from staphylococcal infections contains varying amount of anti-leucocidin.

A horse was immunised at Elstree with a toxin rich in leucocidin and as a result the anti-leucocidin content of the natural serum showed an eight-fold increase; after concentration a further three-fold increase was obtained. Some of the toxin preparations for the immunisation of this horse were supplied by Dr. Valentine; others were made in the Department according to the method he has described.

Experiments on Tissue Permeability.

The action of bacterial extracts. Dr. McClean has completed his investigation of the factor in culture filtrates of the gas-gangrene group and of certain other pathogenic bacteria which causes a marked and immediate increase in the permeability of the tissues.

Experiments have shown that the diffusing factor in bacterial filtrates is distinct from any specific toxin that may be present in these and that it may be separated from the toxin by a relatively simple process of purification; the details of the method have been published. Nevertheless, it appears that the diffusing factor is elaborated mainly by toxigenic organisms and that the most toxigenic strains produce the most potent diffusing filtrates.

Purified solutions of the diffusing factor derived from *Cl. welchii* have proved to be antigenic and "anti-diffusing" sera have been prepared by subcutaneous injection of these solutions into rabbits. Gas-gangrene antitoxin, when obtained by the injection of the culture filtrate into the horse, possesses a measurable anti-diffusing power. The demonstration that the diffusing factor of bacterial origin is antigenic suggests the feasibility of carrying out a serological inquiry into the relationship between diffusing substances derived from various sources, such as members of the gas-gangrene group, the *Staphylococcus*, the *Streptococcus* and the *Pneumococcus*.

Elucidation of the nature and mode of action of diffusing factors obtained from such diverse materials as the mammalian testis, malignant tumours, and pathogenic bacteria depends upon further progress in their chemical purification. The isolation of the active factors in a pure state may throw additional light, not only on the processes of inflammation of bacterial origin, but also on the nature of malignant invasion of the tissues and the physiology of fertilisation.

The action of tumour extracts. Dr. E. Boyland of the Cancer Hospital, London, and Dr. McClean have completed their investigation of the factor in extracts of rapidly growing transplantable mammalian

tumours which increases the permeability of the tissues. They have found that the factor is present in an amount which is approximately proportional to the rate of growth of the tumour. Extracts of fowl sarcoma No. 1 do not increase diffusion in the tissues. Extracts of the Fujinami myxosarcoma, on the other hand, produce an increased diffusion comparable with that caused by the more vigorous mammalian tumours. The inactivity of extracts prepared from the fowl sarcoma may be related to the poor yield of the diffusing factor obtainable from fowl testis. Further observations are being made to determine whether there is any seasonal variation in the yield of this factor from the testis of the fowl and other avian species and, if so, whether the variation can be correlated with the amount of the factor in avian tumours.

Dr. T. Lumsden of the Cancer Department of the London Hospital, has again, during the past year, been given facilities for his work on the production of an anti-cancer serum by the immunisation of sheep.

Laboratories in the Department which were placed at the disposal of the Committee of the Medical Research Council that is investigating the relation between iodine deficiency and thyroid disease were occupied for this purpose until the end of October last year, when it became necessary to utilise them for the work of the Department.

The hospitality of the biochemical laboratories of the Department has again been extended to Mr. A. T. Dann, M.Sc., an assistant Research Officer on the Staff of the Commonwealth Council for Scientific and Industrial Research and to Miss M. Freeman of the Walter and Eliza Hall Institute for Medical Research, Melbourne.

DEPARTMENT FOR THE PREPARATION AND STUDY OF VACCINE LYMPH.

This Department was transferred from Cornwall to Elstree at the close of 1935 and the Bacteriologist-in-Charge, Dr. D. McClean is making arrangements for an intensive study of the preparation and behaviour on storage of pure suspensions of elementary bodies recovered from vaccine lymph. Dr. Eagles has shown that, after prolonged storage in the cold in suitable fluid media, elementary bodies obtained from dermal lapine are highly active and it is hoped to obtain similar suspensions from vaccine lymph which may be used for Jennerian prophylaxis in man.

Dr. McClean hopes also to complete the observations made by himself and Dr. Favilli (University of Florence) on the relation between tissue permeability and local immunity to infection. The dramatic action of the culture filtrates of certain pathogenic organisms upon the permeability of the tissues (*vide supra*) has added to the interest of this aspect of the work of Favilli and his collaborators in Italy. It appears that the initial fixation of bacteria or other injurious substances at the site of inflammation by means of a local reduction of tissue permeability is a protective mechanism which plays a definite part in immunity. A study of the balance between this reaction by the tissues of the host and elaboration of a factor by bacteria, which diminishes the efficiency of this protective mechanism is of considerable interest.

NATIONAL COLLECTION OF TYPE CULTURES.

(Medical Research Council.)

The number of cultures of bacteria and fungi distributed during the year under review, to workers at home and abroad, again exceeded 5,000, and some 250 new types were deposited for maintenance.

Through the courtesy of the Director and staffs of the Lister Institute of Preventive Medicine, the National Institute for Medical Research, the Veterinary Laboratory, Ministry of Agriculture, New Haw, Weybridge and the Rothamsted Experimental Station, Harpenden, authority has been given for listing the various filterable viruses—at present or from time to time maintained at these institutions—in the catalogues of the National Collection. Requests for filterable viruses, or bacteriophages, received by the Curator will be forwarded to the appropriate institute, it being naturally understood that the issue of such material is at the absolute discretion of the officials of the institutes concerned.

The revised list of fungi and bacteria of economic importance maintained in the collection, referred to in the last report, was published during the year in the *Transactions of the British Mycological Society*: a few reprints are available to correspondents, free of charge. A new edition of the catalogue of the Collection is now in the press and will shortly be published. Following the recommendations of the Nomenclature Committee of the International Society for Microbiology, of which Dr. St. John Brooks is one of the permanent secretaries, the use of the specific name "*Bacillus*" has been restricted in this catalogue to spore-bearing organisms. A statement on this subject by the Nomenclature Committee has been published in the *Zentralblatt f. Bakteriologie*.

The taxonomic study of the Actinomyces group by Miss Dagny Erikson, working in the laboratory of the Collection with a personal grant from the Medical Research Council, has now been published as a Special Report by the Council.

GENERAL AND FINANCIAL.

The Accounts and Balance Sheet for the year ending December 31st, 1935, show balances to the credit of the Pension Fund of £27,311 2s. 0d., of the Contingency Fund of £22,000 0s. 0d., of the Sinking Fund of £27,640 4s. 3d. and of the Capital Fund of £532,027 0s. 0d. after adding to it the balance of income over expenditure for the current year, viz., £2,476 18s. 2d. The balance of income over expenditure of the Pension Fund, viz., £733 11s. 0d. has again been transferred to the General Fund account as a contribution towards the amount expended as premiums on staff policies under the Federated Superannuation System.

The following changes in investments have taken place during the year, viz. :—

General Fund : £5,000 4½% Conversion Stock, 1940/44 has been sold ; £25,000 New South Wales 3% Stock, 1935 has been redeemed, and £12,000 3½% War Stock, 1962, £6,000 New South Wales 4% Stock 1942/62, £3,000 Western Australia 4% Stock 1942/62 and £700 Union of South Africa 4% Stock, 1943/63 have been purchased.

Sinking Fund : £1,350 4% Funding Stock 1960/90 has been purchased.

Jenner Memorial Research Studentship Fund : £573 8s. 8d. Liverpool Corporation 3% Stock 1942, of which only £226 7s. 9d. had been transferred by December 31st, 1935, has been purchased.

Income for the year amounted to £52,601 10s. 6d. Compared with 1934 there was an increase in Interest and Dividends on General Fund Investments of £1,280 0s. 6d. and in Investigation Diagnosis and Analysis Fees of £239 1s. 0d. Sales of Sera, Vaccines, etc., amounted to £29,531 3s. 1d. and after adjusting stocks on hand at the beginning and end of the year this appears in the accounts as £26,646 7s. 10d.

Expenditure was £50,124 12s. 4d. against £44,718 16s. 2d. in 1934. Salaries and Wages, Gas, Water and Fuel and Repairs and Renewals, mainly at Elstree, accounted for the increased expenditure during the year. £2,632 0s. 0d. expended on the erection of a new building for the Svedberg ultra-centrifuges at Chelsea has been charged to capital expenditure.

The Governing Body gratefully acknowledge the receipt of a further 300 Guineas from Mr. W. A. Macleod in continuation of his endowment of the Morna Macleod Research Studentship.

In conclusion, the Governing Body desires to express its appreciation of the devoted co-operation of the Director and all members of the staff in carrying out the work of the Institute.

WILLIAM BULLOCH,

Chairman of the Governing Body.

BALANCE SHEET

AND

ACCOUNTS.

Dr.

The Lister Institute

BALANCE SHEET

	£	s.	d.	£	s.	d.
TO CREDITORS				5,102	11	6
TO PENSION FUND—						
As per Account at 31st December, 1930..				27,311	2	0
TO JENNER MEMORIAL RESEARCH STUDENTSHIP FUND—						
As per Account at 31st December 1934 ..	8,426	12	1			
Add Amount transferred from Income and Expenditure Account, 1935				198	11	10
						8,625 3 11
TO CONTINGENCY FUND—						
As per Account at 31st December 1934 ..				22,000	0	0
TO SINKING FUND to 31st December 1935 ..				27,640	4	3
TO CAPITAL FUND to 31st December 1935—						
Donations, &c., received to date from the following:—						
Dr. Ludwig Mond (1893)	2,000	0	0			
The Berridge Trustees (1893/98)	46,379	10	1			
The Grocers' Company (1894)	10,000	0	0			
Lord Iveagh (1900)	250,000	0	0			
Lord Lister's Bequest (1913) as per Account at 31st December, 1923	18,904	5	8			
William Henry Clarke Bequest (1923/6)	7,114	5	7			
Other Donations and Legacies (1891-1934)	20,971	18	3			
Income and Expenditure Account:—						
As per Account at 31st December, 1934..	174,180	2	3			
Add Balance for the year ending 31st December, 1935	2,476	18	2			
				176,657	0	5
						532,027 0 0

WILLIAM BULLOCH, *Chairman.*G. W. ADDISON, *Hon. Treasurer.*

£622,706 1 8

REPORT OF THE AUDITORS

We have audited the above Balance Sheet. We have obtained all the information and explanations we have required, being held by the Institute on their behalf. In our opinion, such Balance Sheet is full and fair, and properly drawn and the explanations given to us and as shown by the books of the Institute.

London, 7th May, 1936.

of Preventive Medicine.

31st DECEMBER, 1935.

Cr.

By Cash—		£	s.	d.	£	s.	d.
At Bankers:	Deposit Account	17,000	0	0			
"	Current Accounts	2,542	7	7			
In hand		106	6	10			
					19,648	14	5
By INVESTMENTS, GENERAL FUND (at cost, less amounts written off)—							
£70,000	4 per cent. Consolidated Stock, 1957 or after	62,740	19	0			
£30,000	4½ per cent. Conversion Stock, 1940-44	27,224	17	9			
£17,000	5 per cent. Conversion Stock, 1944-64	15,997	0	7			
£52,000	4 per cent. Funding Stock, 1960-1990	45,661	13	9			
£56,000	3½ per cent. War Stock, 1962 or after	54,872	1	5			
£37,000	Local Loans 3 per cent. Stock	20,829	1	7			
£3,000	Port of London 3½ per cent. Registered Stock, 1965-75	2,686	17	7			
£1,000	Dominion of Canada 4 per cent. Registered Stock, 1940-1960	928	4	6			
£2,000	Cape of Good Hope 3½ per cent. Consolidated Stock, 1929-49	1,802	12	0			
£25,000	Cape of Good Hope 3 per cent. Consolidated Stock, 1933-1943	23,850	0	0			
£25,000	Natal 3 per cent. Consolidated Stock, 1929-1949	21,400	0	0			
£8,000	New South Wales 4 per cent. Inscribed Stock, 1942-62	8,040	1	4			
£25,000	New Zealand Government 3 per cent. Inscribed Stock, 1945	22,114	0	0			
£26,100	South Australian Government 3 per cent. Consolidated Stock, 1916 or after	16,800	0	0			
£2,900	Commonwealth of Australia 3½ per cent. Registered Stock, 1936-37	2,825	6	0			
£1,300	Union of South Africa 4 per cent. Consolidated Stock, 1943-1963	1,327	9	0			
£25,000	Victorian Government 3 per cent. Consolidated Inscribed Stock, 1929-1949	19,800	0	0			
£4,000	Western Australia Government 4 per cent. Inscribed Stock, 1942-1962	4,081	3	0			
£20,000	Southern Railway Preferred Ordinary Stock	13,500	0	0			
£6,200	London & North Eastern Railway 3 per cent. Debenture Stock	3,961	0	0			
£5,000	Great Central and Midland Railway Joint Committee 3½ per cent. Guaranteed Stock	3,623	0	0			
£353	London & North Eastern Railway 4 per cent. First Guaranteed Stock	499	11	0			
£8,650	London, Midland & Scottish Railway 4 per cent. Preference Stock	7,360	0	0			
£15,625	London, Midland & Scottish Railway 4 per cent. Preference Stock, 1923	11,300	0	0			
£18,750	London & North Eastern Railway 4 per cent. First Preference Stock	13,028	6	7			
£25,000	East Indian Railway 3 per cent. New Debenture Stock	13,890	0	0			
£661	Madras & Southern Mahratta Railway 4 per cent. Debenture Stock, 1938	656	19	7			
£800	Grand Trunk Railway Company of Canada Great Western Borrowed Capital 5 per cent. Perpetual Debenture Stock	936	0	0			
£1,937	Grand Trunk Railway Company of Canada 4 per cent. Guaranteed Stock	1,733	0	0			
£800	Ontario and Quebec Railway 5 per cent. Permanent Debenture Stock	984	0	0			
£3,400	Gas Light and Coke Company Ordinary Stock	3,638	0	0			
					428,691	4	8
By INVESTMENTS, SINKING FUND (at cost)—							
£9,600	4½ per cent. Conversion Stock, 1940-44	8,806	16	7			
£8,800	4 per cent. Funding Stock, 1960-1990	7,444	6	1			
£13,130	3½ per cent. Conversion Stock, 1961 or after	11,337	16	2			
	Balance uninvested	51	5	5			
					27,640	4	3
By INVESTMENTS, JENNER MEMORIAL RESEARCH STUDENTSHIP FUND (at cost)—							
£2,650	Southwark and Vauxhall Water Co. 3 per cent. Debenture Stock "B"	2,756	10	0			
£1,596	Southern Railway 5 per cent. Preference Stock	2,740	5	0			
£953	9s. 1d. Liverpool Corporation 3 per cent. Stock, 1942, or after	770	3	1			
£2,000	4 per cent. Funding Stock, 1960-1990	1,797	14	0			
	Balance uninvested	560	11	10			
					8,625	3	11
By INVESTMENTS, PENSION FUND (at cost)—							
£22,000	4 per cent. Funding Stock, 1960-1990	17,165	3	5			
£13,000	3½ per cent. Conversion Stock, 1961 or after	10,038	1	5			
	Balance uninvested	107	17	2			
					27,311	2	0
(The book value of the above Investments is, in the aggregate, less than their market value at 31st December 1935.)							
By DEBTORS					9,829	11	0
*By FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS—							
At cost less depreciation as per account 31st December 1920					2,471	17	2
By EXPENDITURE ON INSTITUTE BUILDINGS AT CHELSEA—							
As per account 31st December 1910, including purchase of freehold site, £6,000		70,916	3	1			
Add Additions during 1935		2,632	0	0			
					73,548	3	1
By PURCHASE OF FREEHOLD LAND ADJOINING THE "STUDIOS" CHELSEA—							
As per account 31st December 1912					169	6	8
By LEASE OF THE "STUDIOS" CHELSEA, as per last account							
Less Amount written off for the year		1,197	8	9			
		65	2	0			
					1,132	6	9
By QUEENSBERRY LODGE FARM, ELSTREE—							
Purchase of freehold land and buildings and Expenditure on new buildings—							
As per account 31st December 1912					20,455	10	0
By STOCK OF ANIMALS		516	0	0			
By STOCK OF ANTITOXINS		2,666	17	9			
					3,182	17	9
					£622,706	1	8

* Nothing has been charged for depreciation of Furniture, &c. since new purchases made during the year to a greater amount than the estimated depreciation (10%) have been written off.

TO THE MEMBERS.

The Superannuation Scheme for certain of the Staff provides for the Life Policies for the time being in existence up so as to exhibit a true and correct view of the state of the Institute's affairs, according to the best of our information

COOPER BROTHERS & CO.

Chartered Accountants. } Auditors.

The Lister Institute of

INCOME AND EXPENDITURE ACCOUNTS

Dr.	<u>INCOME.</u>							General		
								£	s.	d.
To Interest and Dividends on General Fund Investments	...	...	...	...	...	...	...	19,077	17	8
To Interest on Sinking Fund Investments	...	...	...	...	...	...	...	1,190	5	0
To Investigation, Diagnosis and Analysis Fees, &c.	...	...	...	...	...	...	...	2,781	9	0
To Sales of Sera, Vaccines, &c., and Stock at 31st December 1935, less Stock at 1st January, 1935	...	...	...	...	...	...	...	28,646	7	10
To Rent of Rooms	...	...	...	...	...	...	...	72	0	0
To Profit on Redemption of General Fund Investment	...	...	...	...	...	...	...	2,100	0	0
To Pension Fund—excess of Income over Expenditure transferred	...	...	...	...	...	...	...	733	11	0

£52,601 10 6

Dr.								Pension		
								£	s.	d.
To Interest on Investments	...	...	...	...	...	...	...	1,335	0	0
								<u>£1,335</u>	<u>0</u>	<u>0</u>

Dr.								Jenner Memorial Research		
								£	s.	d.
To Interest and Dividends on Investments	...	...	...	...	...	...	...	261	1	10
								<u>£261</u>	<u>1</u>	<u>10</u>

Dr.								Morna Macleod Research		
								£	s.	d.
To Balance from last account	...	...	...	...	...	...	...	78	15	0
To Cash received from W. A. Macleod, Esq.	...	...	...	...	...	...	...	315	0	0
								<u>£393</u>	<u>15</u>	<u>0</u>

Preventive Medicine.

for the year ending 31st December, 1935.

EXPENDITURE.

Fund.									Cr.
								£ s. d.	
By Rent, Rates, Taxes and Insurance	...	...	...	...	...	...	...	1,389 17	4
By Salaries and Wages of Staff	...	...	...	...	...	...	...	27,761 11	2
By Premiums on Federated Superannuation Policies	...	...	...	...	...	...	...	1,669 16	0
By Stationery, Printing and Postage	...	...	...	...	...	...	...	385 8	5
By Printing of Collected Papers	...	...	...	...	...	...	...	276 11	1
By Office Expenses, Auditors' Fee and Sundries	...	...	...	...	...	...	...	336 0	8
By Travelling Expenses	...	...	...	...	...	...	...	115 18	8
By Gas, Water and Fuel	...	...	...	...	...	...	...	1,526 12	8
By Electric Light and Power	...	...	...	...	...	...	...	360 12	0
By Nutrition, Protozoological and Experimental Pathology Expenses, including Apparatus	...	...	...	...	...	...	...	674 2	1
By Bacteriological Laboratory Expenses, including Apparatus	...	...	...	...	...	...	...	408 10	1
By Vaccine Laboratory Expenses, including Bottles	...	...	...	...	...	...	...	80 17	5
By Water and Bio-chemical Laboratory Expenses, including Apparatus	...	...	...	...	...	...	...	563 18	10
By Bio-physics Equipment and Expenses	...	...	...	...	...	...	...	685 12	7
By Serum and Vaccine Lymph Laboratories Expenses, including Apparatus and Bottles	...	...	...	...	...	...	...	4,259 1	9
By Culture Media	...	...	...	...	...	...	...	161 18	11
By Animals	...	...	...	...	...	...	...	1,151 8	11
By Animal House Expenses and Forage	...	...	...	...	...	...	...	2,370 16	5
By Alterations, Repairs and Renewals, including Workshop Expenses	...	...	...	...	...	...	...	3,717 10	4
By Library Expenses	...	...	...	...	...	...	...	362 18	8
By General Stores	...	...	...	...	...	...	...	199 12	9
By Amount written off Lease of the "Studios," Chelsea	...	...	...	...	...	...	...	65 2	0
By Sinking Fund ($\frac{1}{2}$ % per annum on Cost of Buildings and Interest on Investments)	...	...	...	...	...	...	...	1,600 18	7
By Balance, transferred to Capital Fund	...	...	...	...	...	...	...	2 476 18	2
								<u>£52,601 10</u>	<u>6</u>

Fund.									Cr.
								£ s. d.	
By Pensions	...	...	...	...	...	...	...	601 9	0
By Balance, transferred to General Income and Expenditure Account	...	...	...	...	...	...	...	733 11	0
								<u>£1,335 0</u>	<u>0</u>

Studentship Fund.									Cr.
								£ s. d.	
By Salary of Student	...	...	...	...	...	...	...	62 10	0
By Balance, transferred to Balance Sheet	...	...	...	...	...	...	...	198 11	10
								<u>£261 1</u>	<u>10</u>

Studentship Account.									Cr.
								£ s. d.	
By Salary of Student	...	...	...	...	...	...	...	118 2	6
By Cash in hand	...	...	...	...	...	...	...	275 12	6
								<u>£393 15</u>	<u>0</u>

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THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE

Report of the Governing Body,
1937.

CHELSEA BRIDGE ROAD,
LONDON, S.W. 1.

June 2nd, 1937.

The Lister Institute of Preventive Medicine,

CHELSEA BRIDGE ROAD, LONDON, S.W. 1.

ELSTREE, HERTS.

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Assistant Curator :

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* Recognised Teacher of the University of London.

ANNUAL GENERAL MEETING
OF
The Lister Institute of Preventive Medicine,
June 2nd. 1937.

REPORT OF THE GOVERNING BODY.

The Governing Body has the honour to present the Institute's 43rd Annual Report.

GOVERNING BODY.

No change in the personnel of the Governing Body has occurred during the year. At the meeting held last year, the Council re-elected Professor W. Bulloch, Professor A. E. Boycott and Professor Sir Arthur Harden as its representatives on the Board until December 31st, 1937.

The Governing Body has pleasure in reporting that the honour of Knighthood was conferred on Dr. J. A. Arkwright and Professor J. C. G. Ledingham, in February last.

COUNCIL.

At the Annual General Meeting last year the three members of Council retiring were Lt.-Col. G. W. Addison and Lord Moyne, representatives of the Members of the Institute and Colonel Ralph Key Harvey, one of the representatives of the Worshipful Company of Grocers. Each of these representatives was re-appointed a member of Council.

The three members of Council, who in accordance with the Articles of Association retire this year by rotation, but who are eligible for re-election are Mr. J. R. Drake, one of the representatives of the Worshipful Company of Grocers, Professor T. G. Moorhead, the representative of the University of Dublin and The President of the Royal College of Physicians, London.

MEMBERS.

The Governing Body regrets to report the deaths during the year of Professor E. H. Kettle, a member since 1931, and Dr. F. W. Alexander whose connection with the Institute dated from 1899.

STAFF.

Professor Ledingham, the Chairman of the Executive Committee of the 2nd. International Congress for Microbiology, held in London last July, officiated as President of the Congress.

Dr. E. Weston Hurst left the Institute in September to take up his appointment as Director of the Institute for Medical Science, Adelaide.

Dr. C. R. Amies, grantee of the British Empire Cancer Campaign, and formerly Research Fellow in Bacteriology of the Institute, and Dr. A. S. McFarlane, Research Fellow in Biophysics, have joined the staff of the Institute.

On the departure of Dr. Gulland, in the Autumn, the Governing Body appointed Dr. A. R. Todd, B.Sc., Ph.D., Beit Memorial Research Fellow, to the Biochemical department staff, to which also Miss M. G. Macfarlane has now been attached as a permanent member and Miss A. Tazelaar as a Research assistant.

Miss C. Edgar, B.A., formerly Francis Maitland Balfour Student, was given a temporary research appointment on the staff of the Division of Nutrition in August, 1936.

Dr. Emmy Klieneberger, Jenner Memorial Research Student, Mr. E. Downing, Grocers' Company Research Student and Mr. L. D. Macleod, Morna Macleod Research Student have continued to hold their respective appointments during the year. Mr. T. S. Work, B.Sc., Ph.D., succeeded Mr. S. S. Randall as Research Student in Biochemistry in October.

Mr. S. A. White, formerly Accountant, was appointed Assistant-Secretary and Accountant in April last.

RESEARCH WORK.

The Governing Body, before surveying the scientific work done during the year, desires once again to record its appreciation of the continued co-operation the Institute has enjoyed with the Medical Research Council, which has continued to furnish the salaries of the staff of the National Collection of Type Cultures, of Miss Hume, Dr. Zilva and his assistants and part salaries of Dr. Korenchevsky and of Miss Hume's assistant. The Institute provided, as hitherto, accommodation and the materials for their researches for each of these workers.

The Governing Body gratefully acknowledge the receipt of a further 100 guineas from Mr. W. A. Macleod in March, 1937, for the continuation of his endowment of the Morna Macleod Research Studentship.

To Sir Henri Deterding the thanks of the Board are also due for a grant of £400 to defray the salary of a histological assistant to Dr. Korenchevsky for two years.

The grant of £3,400 generously provided by the Rockefeller Foundation has now been completely expended on the velocity and equilibrium centrifuges designed by Professor T. Svedberg. These are accommodated in the new biophysics building erected by the Institute at Chelsea, and are in constant use in connexion with various problems under investigation.

The hospitality of the Institute's laboratories has again this year been extended to foreign guests displaced from scientific posts in Germany, viz., Professor Ph. Ellinger, who now holds a Fellowship of the Society for the Protection of Science and Learning, and Dr. M. Gutstein.

DEPARTMENT OF BACTERIOLOGY, SEROLOGY AND EXPERIMENTAL PATHOLOGY.

Studies on Viruses.

During the year, studies on various aspects of the virus problem have been continued by workers in this Department. The departure of Dr. E. W. Hurst to Australia in the autumn of 1936 created an unfortunate break in the investigation of the neurotropic group of viruses to which he had devoted so much attention during his period of service here. Reference is made below to certain enquiries carried out by him on rabies and on the myxoma and fibroma viruses of rabbits which he was able to bring to a conclusion before his departure.

Vaccinia. Dr. M. H. Salaman (Beit Memorial Research Fellow) has continued his investigation into the antigenic structure of the vaccinia virus and a first report embodying his findings is being published.

He has compared the serological properties of the elementary bodies with those of the soluble antigen of the virus which can pass through filters that retain the bodies. It has emerged that the virus-neutralising power of an anti-vaccinial serum can be removed by absorption with a sufficient dose of washed elementary bodies, but not by absorption even with large doses of the soluble antigen. On the other hand, precipitins and agglutinins could be absorbed from such serum in varying degrees by the bodies and by the soluble antigen.

The results of this quantitative study on absorption of antibody, based on the method of optimal proportions, clarify considerably some hitherto highly disputed questions relating to the capacity of virus to combine with homologous antibody and particularly with the neutralising antibody.

Studies in the Etiology of Rheumatic Diseases. Dr. G. H. Eagles, with the assistance of Dr. P. R. Evans, Registrar for Medical Diseases of Children and Medical Officer in Charge of the Rheumatism Clinic, King's College Hospital, Dr. J. D. Keith, the Children's Hospital, Birmingham, Mr. A. G. Timbrell Fisher, F.R.C.S., National Commission in Chronic Rheumatic Diseases, Royal College of Physicians, and Dr. W. H. Bradley, working under a grant from the Medical Research Council in the Department of Medicine, The University, Cambridge, has continued the study of a possible virus agent in the causation of acute rheumatism, rheumatoid arthritis and chorea.

The enquiry has been conducted along three main lines: (1) specific agglutination tests with the sera of patients, (2) infection experiments in monkeys, and (3) electrocardiographic investigation of sick animals that appeared to react to experimental inoculations.

(1) Suspensions of elementary bodies recovered from rheumatic exudates (pericardial, pleural, and joint), joint fluids and synovial membrane from rheumatoid arthritis and, in a few instances, from spinal fluid in chorea have been specifically agglutinated by sera from homologous cases. Evidence of cross-agglutination within the rheumatic group suggests that these diseases have in all probability a common aetiological factor. Other factors such as concomitant infection by streptococci probably play a part and the interaction of these with the agglutination reaction is being studied throughout the clinical course of the disease in a number of selected cases. A series of about 200 cases has been studied.

(2) Infection experiments have not so far given unquestioned confirmation of serological findings. In two monkeys, however, grave cardiac involvement has followed intrapericardial injection of rheumatic virus-body suspension. In one instance the suspension alone was inoculated and in the second, in

conjunction with a small dose of a streptococcal toxin. In both cases myocardial damage was revealed by histological study. The typical Aschoff node was not demonstrated. Results of control experiments would indicate that toxin alone is probably not responsible for the myocarditis. The difficulty in securing an adequate supply of elementary-body suspensions from human sources for this type of experiment has of necessity somewhat retarded the work. The possibility of combined infection with streptococci is also being investigated in monkeys.

(3) In the attempt to overcome the difficulty in diagnosing the nature of cardiac involvement in monkeys by ordinary clinical procedure the electrocardiographic method is being tested. A complicating factor lies in the fact that practically nothing is known about the simian electrocardiogram either in health or disease. The present indications are that it is likely to prove of valuable assistance in assessing cardiac damage in these experimental animals. The kind co-operation of the staff of the Cardiological department of King's College Hospital and the valuable assistance of Miss J. Hunt have made it possible to conduct this line of enquiry. Experiments are under way to discover, if possible, the determining factors in the causation of the prolongation, in monkeys, of the P-R interval, which is considered to a large extent pathognomonic of early rheumatic carditis in the human subject.

Avian Sarcoma. Dr. C. R. Amies, working on behalf of the British Empire Cancer Campaign, and since January 1937 as a member of the Institute Staff, has continued his investigations on the nature of the tumour-producing agents in filtrates of avian sarcomata. Highly potent suspensions of the Rous No. 1 sarcoma agent, prepared by repeated high-speed centrifugation of cell-free tumour extracts, have been employed as antigen for various serological experiments. The results obtained fully confirm the fact that fowls bearing this tumour develop antibodies for the tumour agent. Similar antibodies have occasionally been demonstrated in the serum of apparently normal adult fowls but they have not been found in 6-10 weeks' old chickens. This immunity reaction can be demonstrated by neutralisation tests and also by agglutination and complement fixation reactions.

It has now been found that the sera of rabbits which have been hyperimmunised with normal fowl protein, exhibit strong neutralising activity against these purified suspensions of the Rous No. 1 agent. This neutralising effect cannot be attributed to the presence of fowl protein in the tumour agent suspensions since precipitin tests fail to show the presence of such material. The possibility, therefore, has to be borne in mind that the tumour agent may itself contain fowl protein as one of its antigenic constituents. These facts, so far as they go, confirm certain experiments carried out some years ago by Dr. W. E. Gye and Dr. W. J. Purdy, on which their conception of the dual nature of the infective complex is largely based. Further experiments are, however, necessary to clarify this problem.

Lipoid Substances Extracted from Rous Tumour Desiccates. Dr. Amies and Dr. A. Pollard of the Courtauld Institute of Biochemistry, Middlesex Hospital, have collaborated in an investigation of the claim recently put forward by Jobling and Sproul that the Rous tumour agent is a lipid substance which can be obtained from desiccates of the tumour by extraction with the usual lipid solvents. From the results of a long series of experiments they have been able to show that the tumour-producing properties of these lipid substances are due to contamination with traces of the tumour desiccate itself. This is shown by the fact that even large amounts of this lipid substance are devoid of carcinogenic action provided that all particulate material is removed from them by filtration or centrifugation. Prolonged extraction of the desiccates by benzene, in an atmosphere of nitrogen, does not inactivate the tumour agent, almost all of which remains in the benzene-insoluble residue.

The filterable viruses of rabbit myxomatosis (Sanarelli, 1898) and rabbit fibroma (Shope, 1932). These two viruses, the former causing an almost invariably fatal disease in experimental rabbits, the latter a purely local fibromatous skin tumour which invariably regresses, are of very great interest as objects of study both on account of the proliferative changes they induce and on account of the relationship found to exist between them. Recovery from fibroma gives protection against a fatal issue when myxoma virus is later introduced (Shope), while Berry and Dedrick have shown that inoculation of the "living" fibroma virus in combination with the myxoma virus previously rendered inactive by heat treatment, induces in the rabbit a myxomatous disease with fatal issue. These facts would at least indicate the presence of some common factor in their antigenic structure, if not some still closer relationship. For some time Sir John Ledingham has been investigating the problem of this relationship by serological experiments conducted with the elementary bodies recovered from the lesions associated with these viruses. It has been found that animals infected with the fibroma virus, and particularly with the "inflammatory" variant of this virus, develop agglutinins for myxoma but often little or none for fibroma until a dose of myxoma is given, when agglutinins for both appear in the serum. The serological affinities tested in this manner are obvious enough and together with the data from neutralisation tests would seem to account sufficiently for the protection conferred by fibroma against a fatal myxoma, but the precise nature of the relationship between the viruses and particularly of the mechanism of the Berry experiment must still be the subject of extended enquiry.

Attenuation of the myxoma virus by intracerebral passage. Dr. Hurst passed the myxoma virus for a considerable number of generations through the brains of rabbits and obtained a variant whose capacity to cause death after intracerebral inoculation was much reduced, and which, when inoculated into skin areas generally failed to produce death though local and generalised lesions persisted. The microscopic picture of the neuromyxoma lesions differs in several important particulars from those associated with the virulent form of the virus. The discovery of this variant should greatly facilitate enquiries into the nature of the relationship between myxoma and fibroma as it is now possible to study animals that have recovered from myxoma without having to give them the fibroma virus first. Dr. Hurst, using the typical fatal form of the myxoma virus, has confirmed the Berry-Dedrick phenomenon but has been unable to effect the "transformation" of fibroma to myxoma by combining the heated neuromyxoma variant with the "living" fibroma virus.

The histology of the myxoma lesions. A new study of this subject by Dr. Hurst has brought to light several previously unrecorded changes, particularly in the vascular endothelium of the affected tissues.

Rabies virus. In the course of work on the virus of rabies, Dr. Hurst made the interesting observation that in the rabbit and guinea-pig rabies follows an intravenous or intramuscular inoculation much more readily and constantly when the infecting material is guinea-pig brain than when it is rabbit brain. Analysis of the phenomenon has shown that the number of infective units of guinea-pig virus needed to infect by the intravenous route is much smaller than that of rabbit virus so that guinea-pig virus units can truly be said to be more virulent by peripheral inoculation than rabbit virus units. This effect of guinea-pig passage is obtained only with viruses some passages removed from street virus. No evidence was obtained of local multiplication of guinea-pig or rabbit virus at the site of intramuscular injection.

Staining of elementary bodies. Dr. M. Gutstein (Berlin) has elaborated a method for rapid staining of the larger virus bodies, based on the use of basic dyes in alkaline solution. No mordant is required and excellent microscopical pictures are obtained.

Serological Studies : Antigenic Constitution, Virulence and Immunising Properties of Bacteria.

Studies on the "Vi" antigen of *S. typhi*. Dr. A. Felix with the assistance of Miss R. M. Pitt has continued the investigation of the properties of the "Vi" antigen of *S. typhi*.

"Rough" "Vi"-containing variants showing characters of "smoothness." A variant was described previously which in all respects behaved like a typical "rough" variant and was avirulent, although it contained "Vi" antigen. From a culture of this strain, which Dr. Petrie had kept for ten months at 37° C. in horse serum containing "O" and "Vi" antibodies, another variant was isolated, which still was antigenically "rough," i.e., devoid of "O" antigen, but otherwise resembled the "smooth" type. The growth on agar plates and in broth showed the characters of "smoothness" and suspensions of this "rough" variant were not agglutinated by salt solutions up to 5 per cent. NaCl, or by heating to 100° C. The virulence to mice of the smooth-looking variant, which is devoid of "O" antigen, is of the same low order as that of the "O"-agglutinable, smooth type which is devoid of "Vi" antigen. The earlier conclusion that only strains containing both the "O" and "Vi" antigens possess the highest degree of virulence of which *S. typhi* is capable, is thus further strengthened.

Different physico-chemical properties of the "Vi" antigen contained in "smooth" and "rough" typhoid bacilli. Suspensions of "smooth" "Vi" strains heated at 100° C. are no longer agglutinable by the "Vi" antibody, whereas similarly treated suspensions of the "rough" "Vi" strain are still agglutinated. Treatment with 75 per cent. alcohol also has different effects on the "Vi" agglutinability of the two varieties of "Vi" strain, viz. ; the agglutinability of the "smooth" variants is very much reduced, for practical purposes almost annulled, whereas that of the "rough" variant is not impaired. On the other hand the two variants do not differ with regard to the effects of heat or alcohol on their capacities of absorbing the "Vi" antibody or stimulating its formation.

In view of these unexpected findings the resistance to chemical treatment of the "Vi" antigen contained in "smooth" and "rough" variants was carefully investigated. The suspensions were exposed to the action of HCl, NaOH, phenol and formalin and were examined for agglutinability, absorbing power, and capacity of inducing the formation of circulating antibody. No difference was found in any of these tests between the two varieties of "Vi" strain. It would appear that the physico-chemical behaviour of an antigenic substance may vary as the result of the presence or absence of some other substance, which itself may be either antigenic or non-antigenic in nature. This process is independent of whether the antigen is being "haptensised" or not. The difference noted in earlier experiments between the effects of alcohol on the "Vi" antigens of *S. paratyphi* A and B and *S. aertrycke*, on the one hand, and the "Vi" antigen of *S. typhi*, on the other hand, is, therefore, of no greater significance than that now established for the "Vi" antigen contained in "smooth" and "rough" typhoid bacilli.

Experiments on antityphoid vaccination. In spite of numerous attempts, a method of preparing antityphoid vaccine, which would enable the "Vi" antigen to be preserved in its most effective form, has not yet been devised. From Dr. Schütze's recently published work it appears that active protection experiments with mice do not disclose those great differences in the antigenic value of various preparations of the "Vi" antigen, which are so clearly demonstrated by passive protection experiments or by *in vitro* tests. Dr. Felix, in co-operation with Dr. W. D. Nicol, Medical Superintendent of the Horton Mental Hospital, tested the antibody response in fifty persons who had been given three doses of an alcohol-killed typhoid vaccine by the subcutaneous route. A marked increase in the "O" antibody was observed in 100 per cent. of those inoculated, and a significant increase in the "Vi" antibody in about 50 per cent. However, re-examination of the vaccine after storage for half a year, with or without the addition of phenol or tricresol, gave the disappointing result, that the vaccines had almost entirely lost their capacity of stimulating formation of "Vi" antibody in the rabbit.

Diagnosis of the typhoid-carrier state by "Vi" agglutination. Following an observation made in the course of earlier work, Dr. Felix has been investigating the possible value of "Vi" agglutination in the detection of typhoid carriers. Thanks to the co-operation of Dr. F. Golla, Director, Central Pathological Laboratory, Mental Hospitals Department, London County Council, and of Dr. J. H. MacDonald, Medical Superintendent, Hawkhead Mental Hospital, Glasgow, sera from 45 typhoid carriers have been examined. The results so far obtained suggest that "Vi" agglutination seems to have a strong claim to a definite place in the routine diagnosis of typhoid carriers. It is of interest also to note that all the 25 strains from chronic typhoid carriers examined were found to contain "Vi" antigen.

Antigens of *B. pestis*. The early work carried out by Dr. Schütze on the antigens of *B. pestis* and the importance of the envelope component in prophylactic inoculation of rats has recently been supplemented by similar work performed at the Haffkine Institute, Bombay, where, however, results obtained with the mouse as experimental animal diverged from those obtained by Dr. Schütze. To explain this discrepancy Dr. Schütze is now undertaking a comparison of the two types of immunity as developed in the mouse and in the rat. As investigations at Bandoeng, Dutch East Indies, have shown that living avirulent plague inoculations produce excellent immunity but only when the organism is in what is considered to be the smooth state, Dr. Schütze is including the so-called rough and smooth variants of *B. pestis* in his analysis.

Resistance to infection and genetic constitution. Dr. Schütze and Dr. P. A. Gorer are continuing their investigations into the influence exerted by genetic constitution upon immunity and immune processes. Working with two pure lines and two selected lines of mice, they have tested the varying resistances of these lines to infection with *Salmonella typhi murium* and *Salmonella enteritidis*, their response to immunisation and the facility with which "H" and "O" antibodies are produced. Significant genetic and sex differences have been observed in resistance to infection and in ability to produce antibodies, but there seems to be no simple relationship between such ability to produce antibodies and natural resistance to infection.

Antigens in mouse erythrocytes in relation to tumour transplantability. Dr. Gorer has investigated the genetic basis of antigenic differences demonstrable in the erythrocytes of mice with special reference to the antigenic basis of tumour transplantation. Two such antigens were found to depend upon single dominant genes. A sarcoma arising in a pure line of albino mice was found to be transferable to all other members of the line and certain hybrids derived from it but not to unrelated mice. It was found that all hybrids susceptible to grafts of the tumour possessed an antigen in their erythrocytes derived from the albino ancestors. If the antigen was absent the tumour invariably regressed. The sera of mice in which the tumour has recently regressed may frequently be shown to contain agglutinins for the erythrocytes of albinos. It was concluded that the fate of a transplanted tumour is largely dependent upon the existence of iso-antigenic differences between the transplant and tissues of the host.

General Bacteriological Studies.

The pleuropneumonia-like symbionts of *Streptobacillus moniliformis*, and their independent occurrence in lung disease of laboratory rats associated with bronchiectasis. Since the report by Dr. E. Klieneberger (Jenner Memorial Research Student) and Dr. D. B. Steabben of the isolation from diseased lungs of stock and experimental rats of pure cultures of the L.1 organism, known hitherto as an invariable symbiont of *Streptobacillus moniliformis*, extensive investigations have been made with a view to securing evidence pointing to causal relationship. Groups of rats of ages 1-2 months, 3 months, and 6-20 months have shown naked-eye lung lesions in 3%, 7% and 80% respectively and the L.1 organism has been recovered in 18%, 70%, and 70% from these same groups. The number of L.1 colonies on the plates would seem to be directly proportional to the progress of the lung disease and in the advanced stage when the bronchiectatic cavities are filled with pus, innumerable L.1 colonies are found to grow from a small loop of material.

The difficulty, so far not surmounted, has been to secure a non-infected stock with which infection experiments with the L.1 organism might be carried out. It is now possible, however, to prepare satisfactory suspensions of the L.1 organism for serological tests and it has been shown that some, at least, of the L.1 strains isolated as independent colonies from lung lesions are antigenically related to the original L.1 strains recovered from *Streptobacillus moniliformis* with which they were symbiotically associated. The sera of rats with lung disease have not been shown to react in serological tests with the L.1 antigen.

The histology of the lung lesions at different stages has been investigated by Dr. Steabben, who is also giving special attention to the occurrence in the lungs of a pasteurella-like organism originally isolated from nasal discharges of rats observed at certain stages in the development of the lung disease. Usually the glairy exudate or the caseous pus in the bronchiectatic cavities reveals, on cultivation, only the L.1 organism in enormous numbers.

Testing of disinfectants: modifications in Technique of the Chick-Martin test. Dr. M. M. Barratt has served, since its inception, on a sub-committee of the British Standards Institution concerned with the standardisation of methods for the testing of disinfectants. During the past three years improvements in the technique of the Chick-Martin test have been under investigation and Dr. Barratt has taken part in the experimental work involved. The most important modification is the substitution of dried yeast for dried faeces as the organic matter in the test. This was first suggested in 1934 by Dr. Garrod, a member of the Committee, and has been found of considerable value.

There is evidence that an important factor in obtaining concordant results in the test is the condition of the culture used and special attention has been paid not only to the method of maintaining the stock culture but also to the medium on which it is grown for use in the test.

A much criticised point in the technique of the test has been the addition of the disinfectant to the organic matter before the culture. It has been frequently stated that phenol coefficients so obtained would be lower than if the organic matter and culture were added to the disinfectant together. Evidence is now available showing that this criticism is not supported by experimental data. Tests made with a number of disinfectants have shown that the organic matter and disinfectant may be well mixed together and left in contact for at least 4 hours without any lowering of the phenol coefficient.

A number of comparative experiments have been made with yeast and faeces using different disinfectants. These show that even when different media are used for the two tests and the order of adding the constituents is different, the agreement in the results obtained is very close.

Endocrinology.

The investigation of the effects of the sex hormones has been continued by Dr. V. Korenchevsky with the assistance of Mrs. M. Dennison and Miss K. Hall. In addition to the hormones mentioned in previous reports the following compounds were examined both by the "method of weights" and histologically, viz.: (1) the male hormones transdehydroandrosterone and testosterone propionate, and (2) the probable intermediate compounds in the biological formation of sex hormones from cholesterol, androstenedione and androstenediol. A study was also made of the effect on both male and female rats of various combinations of these hormones with oestrone.

The Bisexual properties of the sex hormones investigated. The most remarkable property of the sex hormones investigated is their bisexual function, i.e., the property of stimulating the development of the sexual organs of both sexes in normal rats and bringing about a return of the atrophied organs of gonadectomised animals to, or towards, the normal condition.

The most powerful hormone in this respect proved to be testosterone propionate, which, in suitable doses, brings about a return to the normal condition of the atrophied sexual organs in castrated male rats and to a nearly normal weight of the sexual organs in ovariectomised rats.

There is, however, a special feature of the reaction which still places these hormones in the "male hormone group," viz., that the restorative effect on all the sexual organs of the male rat is complete, while the complete effect was only obtained in females when testosterone propionate was administered simultaneously with the female hormone (oestrone). It is also important to note that with oestrone alone it was found impossible to obtain (with the doses so far examined) a complete recovery of all the atrophied sexual organs.

The "male" hormones investigated may be arranged in descending order of activity as "female sexual stimulators" as follows:—

- (1) testosterone propionate, (2) testosterone, (3) androstenediol, (4) androstenediol, (5) androstenedione, (6) transdehydroandrosterone, (7) androsterone.

From these results showing the co-operative activity of the male and female sex hormones a possible reason for the simultaneous presence of "male" and "female" hormones in the organism of either sex becomes obvious.

Pregnancy changes produced by the male hormones alone or in combination with the female hormone. In ovariectomised rats, with a few exceptions, the male hormones produce changes chiefly in the vagina and in some cases (testosterone propionate) in both the vagina and uterus, similar to those

seen during pregnancy: i.e., mucification of the vaginal epithelium and the "lacy" appearance of the uterine mucosa. The changes are most pronounced when the male and female hormones are injected simultaneously. It would not be anticipated that the changes typical of pregnancy, i.e., those most closely associated with the purely female function, would be produced by, or with the co-operation of the male hormones, and that the changes, that were the most "pregnancy-like," would be produced by the most powerful of the male hormones, testosterone propionate.

The Development of a Clitoris-like organ. In the normal female rat there is no clitoris, only a thickening of the peripheral part of the urethra. After ovariectomy this thickening atrophies considerably. When, however, androstene-dione, testosterone or testosterone-propionate (alone or in combination with oestrone) is injected into these rats, this portion of the urethra greatly hypertrophies into a structure, which externally has the appearance of a clitoris or rudimentary penis. The preliminary histological investigation has shown that this structure has more of the features of a clitoris than of a rudimentary penis.

Female Prostate. This gland which exists only in a rudimentary form in normal rats has already been described by Dr. Korenchevsky and his collaborators. It was found to be much hypertrophied after the injection of androstane-diol and histologically showed features typical of the anterior lobe of the male prostate. In the present experiments it was found that testosterone, testosterone-propionate and androstene-dione also caused the development of a female prostate of the same kind as that described in ovariectomised rats, the largest being about 6 by 4 by 3 m.m. Trans-dehydroandrosterone, the weakest of the male hormones investigated, also brings about the hypertrophy of this gland although to a much smaller degree than the hormones mentioned above.

Male and female preputial glands. All the male hormones investigated bring about an abnormal degree of hypertrophy of these glands. Since, after the injection of the female hormones, these glands are unchanged, their hypertrophy in response to female hormones will always indicate the presence of male hormones in the material assayed.

Adrenals. All the male hormones investigated decrease the weight of the adrenals of both male and female gonadectomised rats. The preliminary histological investigation of the male glands has shown that the changes produced are considerable and concern chiefly the lipid content and the size of the cells.

Other organs and functions. The rate of involution of the thymus increases in both males and females with all the hormones investigated but especially so with testosterone propionate. In males, androstene-dione and testosterone propionate slightly increase the weights of the liver and kidneys (which decrease after castration). The gain in body weight increases when small doses of either of these hormones are used (anabolic effect), but large doses of testosterone propionate considerably depress the gain in body weight.

"Lasting effect" produced by testosterone propionate. Unlike the other male hormones this hormone continues to have a stimulating though slowly decreasing action on the sexual organs for as long as 9 days after the last injection. For this period it completely maintains its effect on the adrenals, while the catabolic effect on the metabolism, as shown by the changes in the body weight, even increases. This property of testosterone propionate gives it an especial therapeutic value, since it makes it possible for effective results to be obtained with injections as infrequent as once in 10 or even perhaps 14 days.

Investigation of Kidney Function.

Professor P. Ellinger and Dr. A. Lambrechts (Liège) have studied the localisation of the glycosuric action of phloridzin. Using azo-phloridzin compounds in their experiments on frogs and rats, they found, by means of the intravital microscope, that only those phloridzin derivatives produce a glycosuria which are fixed in the epithelial cells of the proximal tubules and conclude, therefore, that the point of attack of phloridzin is localized in that tissue. The conditions under which these derivatives of phloridzin are reabsorbed and fixed in the proximal tubules, or are completely excreted in the urine, have not yet been ascertained.

These workers, using the same method, have also studied the effect of fluorescein, trypanflavin and rhodamine B.S. on the kidneys of winter frogs poisoned with phloridzin. They observed (1) that the reabsorption of some substances other than glucose is interrupted, (2) that the staining of the epithelium of the proximal tubules from the lumen does not take place, and (3) that the staining of the epithelium of the proximal tubules from the lymph or the blood capillaries remains unaffected. It is thus shown that phloridzin interferes with the passage of certain substances from the lumen into the epithelium of the proximal tubules. The mechanism of this blockade has, however, not yet been ascertained.

The influence of the cerebro-spinal fluid in acromegaly on urinary excretion of chlorides. Professor Ellinger, in collaboration with Dr. Dorothy Hare and Dr. S. Levy Simpson, has studied the influence of the cerebro-spinal fluid of an acromegalic woman on the urinary chloride and water

excretion of rabbits, injected subcutaneously with the fluid, and given water *per os* simultaneously. A definite increased rate of chloride elimination resulted, but normal water diuresis was unaffected. On the other hand cerebro-spinal fluid from normal people and sera from the acromegalic patient and other control patients had no action either on chloride excretion or on diuresis. This effect was less after irradiation of the pituitary gland, which also produced clinical improvement. In control experiments, a standard solution of pitressin gave an anti-diuretic effect as well as an increased rate of chloride excretion. It is concluded that the spinal fluid of the acromegalic patient contained sufficient amounts of a hormone of the posterior lobe of the pituitary gland, but so far, it has not been possible to decide whether the anti-diuretic and chloride eliminating effects are due to different hormones or whether the anti-diuretic effect of the posterior lobe hormone was masked by that of some other substance in the spinal fluid of this patient.

Coagulation of Blood.

Dr. J. O. W. Barratt has completed his investigation of anti-coagulant action. It has been found that sodium chloride, chlorazol and heparin resemble hirudin in producing their anti-coagulant effect upon thrombin: no action upon fibrinogen could be observed. With the aid of improved technique it was found possible to obtain an index of anti-coagulative power and also, within the limits of concentration over which investigation was possible, to calculate the coagulation time corresponding to varying concentrations of anti-coagulant. A few minor outstanding problems are still awaiting solution.

Investigations with High Centrifugal Fields.

The two ultracentrifuges, provided by a grant from the Rockefeller Foundation, were completed in time for Professor Svedberg's visit in September, 1936. A number of technical modifications have been incorporated by Dr. A. S. McFarlane in the new plant and these have now been tested under the conditions of routine working. The new oscillographic method of speed recording, mentioned in last year's report, has proved very satisfactory and is used on both centrifuges. Modifications in the drive of the turbine oil system of the velocity centrifuge have enabled a much improved constancy of speed to be obtained throughout the run.

The smaller equilibrium centrifuge which runs at speeds up to 18,000 r.p.m. has been found to be adaptable to the study of certain viruses and to the recently discovered virus proteins. Through the courtesy of Dr. Henderson Smith (Rothamsted), a specimen of the tobacco mosaic virus protein has been made available. Excellent sedimentation photographs have been obtained of this protein and a study of the factors which determine its homogeneity in respect of molecular size is in progress. The behaviour of the blue respiratory protein (haemocyanin) of the blood of *Helix pomatia* has been exhaustively studied recently by Svedberg and may be used as a model of homogeneity in a substance of molecular weight of the order of millions. Experiments have been made with *Helix* haemocyanin and the results used for this purpose.

Preparations of elementary bodies from rabbit and sheep vaccinia virus, supplied by Dr. Amies, Dr. Salaman and Dr. McClean, have been investigated by means of the photographic technique. The virus bodies have a strong light absorption in the ultraviolet due to the protein which they contain. With the use of ultraviolet light, sedimentation photographs have been obtained. These reveal some unexpected facts which are to be the subject of further investigation. Preparations of the herpes virus made by Dr. Salaman have also been investigated by the photographic method.

A form of micropipette has been designed which enables fluid at different levels in the centrifuge cell to be accurately removed after the run. In certain circumstances difficulties have been encountered in doing this owing to convection currents appearing in the cell before it comes finally to rest. Experiments are in hand which, it is hoped, will enable these difficulties to be overcome.

The high speed ultracentrifuge was given exhaustive preliminary low speed trials in September during the course of which satisfactory testing of the vacuum machinery, electrical thermometers and controls was carried out. It was then accelerated and reached the maximum speed for routine use, viz., 60,000 r.p.m. without mishap. It has subsequently been used at this speed to study the sedimentation of haemoglobin, serum albumin and serum globulin. In collaboration with Dr. Kekwick and Dr. Lythgoe of University College, London, it is being used at lower speeds to investigate the behaviour of visual purple.

On the occasion of a visit of Professor Svedberg in February, the opportunity was taken to run the velocity centrifuge up to the maximum speed considered as safe, viz., 65,000 r.p.m. No trouble was experienced and this speed was maintained for four hours and the opportunity taken to analyse a specimen of horse serum. A new optical method of recording sedimentation was published recently in which the sedimenting boundary appears as a black band easily visible to the naked eye. This method was tested in the velocity centrifuge with satisfactory results and will be put into routine use. It should prove valuable in the rapid visual analysis of protein mixtures.

A modified technique for the determination of molecular weights by means of osmotic pressure measurements has been devised and used in preliminary experiments on sheep fibrinogen. It has also been used in joint work with Dr. Morgan on the molecular weight of his new specific polysaccharide from the *Shiga* bacillus.

DIVISION OF PROTOZOOLOGY.

Antibody production in response to the injection of protozoa. Work on the antibodies produced in the rabbit by the injection of *Glaucoma* has been continued. Five strains, in addition to the one isolated by Dr. M. Robertson have been studied and sera made with them. These strains were obtained by the kindness of Dr. A. Lwoff of the Pasteur Institute, Paris, and a sixth strain has been received in the last month from Professor Chatton of the Biological Station at Sète.

The serological relations of these strains are being investigated and the results promise incidentally to have an interesting bearing on the extremely difficult question of the specific relations of the strains. The Lister strain, for example, proves to be serologically identical with a strain from America, while all the others are serologically distinct, with a faint group reaction in certain cases.

Two strains have been selected for detailed study: *Glaucoma piriformis*, strain "R," and *Glaucoma piriformis*, strain "Lwoff." The study is directed towards an understanding of the nature of the antigen or antigens and to the investigation of the substance shed by the ciliates in the presence of immune serum and named "tektin" by Breslau. These experiments involved the preparation of sera *v. Glaucoma* treated with alcohol and acid and heated for 1 or 2 hours in the steamer at 100° C.

Sera were also made (1) from the exudate from the *Glaucoma* obtained by an ingenious method used by Breslau, (2) from the washed bodies of *Glaucoma* after having been induced to shed the exudate, and (3) from these two components before separating them by centrifugation. This work is not yet complete, but the indications at present are that brief heating (and fixing) by exposure to temperatures of 70° C. to 75° C. for 2 to 5 minutes does not destroy the antigens. Heating to 100° C. for 2 hours apparently does destroy the antigenic properties, at least, in *Glaucoma* strain "R." Exposure to alcohol very seriously reduces, and in *Glaucoma* strain "Lwoff" destroys, the antigenic quality of the ciliates, while exposure to acid (HCl) reduces, but does not destroy, the antigenic value of both these strains of *Glaucoma*.

The separated exudate or "tektin" has antigenic properties and can be used to prepare a serum which will agglutinate the living ciliates. Breslau was of opinion that the washed bodies, after the emission of the "tektin," were not antigenic, but that has not been confirmed in this work.

Studies on the biological effects of drugs on growing protozoa. Work on the effect of As_2O_3 on *Glaucoma piriformis* strain "R" has been continued, with the result that acclimatisation has not been observed to take place. Studies on the curiously regular proportional diminution of growth found to take place in various concentrations of the substance are still in progress.

Various species of *Herpetomonas* have been cultivated and one strain has been grown in counted cultures giving results of great regularity. This is being propagated in sterile culture and is an excellent object for study. Experiments have been started with acriflavine and the production of aparabasal forms has been noted. These forms appear with great regularity as to numbers in accordance with the concentration of the drug and the age of the culture. Their fate in the further exposure and in subculture is being followed and their behaviour in acclimatisation, or in failure to acclimatise, is being observed. The actual reaction of the organisms can be noted in this way and correlated with the rate of growth and final numbers in cultures exposed to the drug.

DIVISION OF NUTRITION.

Vitamin Standardisation.

Work undertaken on behalf of the Accessory Food Factors Committee (jointly appointed by the Lister Institute and Medical Research Council).

Vitamin A. A collective investigation has been organised by Miss Hume, as Secretary of the Vitamin A Sub-Committee, in which ten different laboratories have participated to compare the results of biological and spectrographic estimations of Vitamin A. The selected materials included a halibut liver oil and a concentrate prepared from it after saponification. The aim was to obtain trustworthy figures for the conversion factor required for relation of the results of the biological and spectrographic tests, and expression of the latter in International Units. It was also hoped to investigate the causes of alleged discrepancy in the values of the conversion factor obtained from different types of Vitamin A-containing materials.

The results showed a satisfactory degree of concordance and gave no support to any alteration of the value 1,600 adopted for the conversion factor at the 1934 International League of Nations Vitamin Standardisation Conference. The discrepancy observed in the case of some estimations of the concentrate was found to be due to instability of the material during the period of the biological test and this suggested that other Vitamin A concentrates might behave in a similar manner. This instability was discovered when the actual solutions fed to the experimental animals were returned to the issuing laboratory to be checked by the spectrographic method at the end of the biological test.

Further work is contemplated along these lines with a view to explaining other reported discrepancies. If these cannot be satisfactorily resolved there is the possibility that the spectrographic method must be abandoned and Vitamin A standardisation made to depend only on the biological test.

Vitamin B₁. The 1934 Vitamin Standardisation Conference made a request that laboratories having available supplies of crystalline Vitamin B₁ should make biological estimations in comparison with the present International Standard (adsorbate on fuller's earth from rice polishings), in view of the probable adoption of the pure crystalline material as the future International Standard, when supplies should be available.

Dr. F. Vivanco, working with a Research Fellowship from the University of Madrid, has investigated a specimen of "oryzanin," the crystalline Vitamin B₁ hydrochloride prepared from rice polishings kindly provided by Professor Suzuki. A curve of response to varying daily dosage was constructed, using the rat growth method. An average amount of 3.3 γ of the crystalline material was found to possess a potency equal to that of 10 mg. of the adsorbate, i.e., of 1 International unit of Vitamin B₁. A few preliminary tests, made with a specimen of the synthetic crystalline material prepared by Professor Hörlein of I.G. Farben-Industrie, indicated an equal potency.

Investigation of Cereals.

The Nutritive Value of Maize and the Aetiology of Pellagra.

Experiments with pigs. Dr. H. Chick has collaborated with Dr. T. A. Birch and Sir Charles Martin at the Department of Animal Pathology, Cambridge, in an experimental investigation of the nutritive defects of maize, in the hope of throwing light on the etiology of pellagra as it occurs among populations which consume maize as staple cereal. The expenses of this work are being met by a grant from the Medical Research Council.

Young pigs have been used as experimental animals. The diet given contained over 80% ground whole white maize supplemented with peameal and a small amount of purified casein to increase the protein content, extra salts and cod liver oil. Dogs maintained on this diet develop a disorder known as nutritional black tongue, characterised by anæmia, diarrhoea, and stomatitis with necrotic changes in gums and tongue, which is held by many to be the analogue of human pellagra. After about six weeks on this diet the young pigs ceased to grow, showed a loss of weight, anæmia and severe diarrhoea and died unless the diet were changed. They showed no skin sensitisation to sunlight. The disease was prevented if 4% yeast was included in the diet or if the maize was replaced by a mixture of wheat and barley; it was cured in a dramatic manner when yeast or an autoclaved protein-free yeast extract was added to the diet. These facts pointed to a deficiency in maize of some heat-stable constituent in the yeast extract, e.g., some constituent of Vitamin B₂. Preliminary tests with flavin gave negative results and trials will now be made of other heat-stable fractions and preparations from yeast extract.

This part of the investigation is being done in close connection with the work of Dr. Macrae and Miss Edgar on the chemical separation of the components of the B vitamin complex. The Division has also provided Dr. Ellinger with a similar series of preparations for therapeutic trial on suitable cases of human pellagra in Egypt.

The above "black tongue diet" has been found relatively satisfactory for growth and maintenance of rats so that the missing anti-black tongue (? anti-pellagra) factor would appear either to be non-essential for the rat or to be adequate for this species in small amount. The pig, therefore, appears to be a more suitable experimental animal.

Porphyria in non-endemic pellagra.

In continuation of his work in this field Professor P. Ellinger together with Dr. W. Beekh and Professor T. D. Spies (Cincinnati General Hospital) has studied porphyrin excretion in non-endemic pellagra. In 10 out of 14 cases of "alcoholic" pellagra observed at Lakeside Hospital, Cleveland, U.S.A. an increased porphyrin excretion (copro-porphyrin) was observed, the intensity of which showed a rough relation to the severity of the clinical symptoms. Out of 77 control subjects consisting of 18 healthy persons, 49 patients suffering from different diseases, and 10 healthy persons, receiving treatment with liver or yeast, 5 only showed an increased porphyrin output. Three of the latter were suffering from anaemia and had received an iron medication. The cases of non-endemic pellagra showed a greater variation in their porphyrin excretion than the endemic pellagrins among maize-eating populations studied in Yugo-Slavia by Ellinger and Dojmi (see previous Report). This may have been due to the fact that non-endemic cases could only be studied when the clinical symptoms were more advanced and the porphyrin output had decreased.

Nutritive value and light sensitising properties of Buckwheat (*Fagopyrum*).

Buckwheat was selected by Dr. Chick for this study for its well-known property of causing light sensitivity in unpigmented animals feeding upon it, in the hope that some light might be thrown upon the causes of the light sensitivity which is characteristic of human pellagra. Albino rats were fed with a diet containing 90% of the ground seed together with extra fat and salt mixture and cod liver oil to provide

Vitamins A and D. This diet, in which all the proteins and B vitamins were derived from the buckwheat was found to be adequate for growth and reproduction in the rat and superior in these respects to a similar one made with whole wheat.

If the husk of the buckwheat grain was included in diet and consumed, the animals became slightly light sensitive and developed marked erythema of ears, paws and tail (sometimes followed by convulsive fits), if exposed to sunshine for long periods in summer on successive days, with or without interposition of glass. Exposure to the rays from a mercury arc for 20 minutes daily over long periods, however, was followed by no reaction. This pointed to an effect due to the visible or the near ultra violet rays. Later trials with standard light filters have shown the sensitising rays to be of longer wavelength than 420 $m\mu$.

A crop of buckwheat was grown, and the inflorescence was found to be richest in the sensitising material which was diminished towards the fruiting stage, a small residuum remaining in the husk of the seed. A small daily ration (0.1 g.) of the dried flowers, when given to albino rats on diets of whole wheat or other cereals, caused a high degree of light sensitivity shown in marked erythema of one ear after only 5 minutes exposure to the rays of a carbon arc, whereas animals maintained on diets composed of buckwheat were found to be completely insensitive, if the husk were removed from the meal, as is usual in milling. Similar experiments with maize diets have failed to reveal any similar light sensitivity in albino rats.

Dr. Macrae is collaborating in attempts to isolate the active substance and to study its chemical and spectrographic properties.

Investigation of Wheat and Bread.

This investigation, begun by Dr. M. H. Roscoe in 1935, has been continued by Miss A. M. Copping, working with a part time grant from the Medical Research Council.

By the generosity of Messrs. Chitty, Flour Millers of Dover, samples of whole wheat flour and straight run white flour, as commonly used for breadmaking in this country, milled from the same sample of wheat, were provided for this research. From these flours, breads of standard composition were baked under controlled conditions in the experimental bakery of Dr. Kent-Jones, to whom thanks are due for his kind co-operation.

The wholemeal flour and the white flour, both unbleached and after bleaching, and the yeast used in the baking of the bread, have been investigated by means of growth tests on rats, for their content of vitamins B_1 and B_2 , also by separate tests for that of flavin and other constituents of the vitamin B_2 complex, and the results compared with those obtained from equivalent amounts of the baked bread. As was to be expected, the vitamin B_1 and B_2 contents of the wholemeal flour and bread were much greater than those of the white flour and bread. The last two, however, proved to be unexpectedly rich in vitamin B_1 , and the amount in the bread was not to be attributed to the added yeast, seeing that the values for the flour and the bread were equivalent. Control experiments with ground polished rice, in which the rats developed characteristic nervous symptoms of vitamin B_1 deficiency, emphasised the difference between polished rice and white flour in this respect and afforded an explanation of the rare occurrence of beriberi on one-sided diets which contain wheat as the staple cereal.

The content of flavin was low in both types of flour and breads, the white flour and bread being definitely inferior to the whole meal. The second constituent of the vitamin B_2 complex (so called "Vitamin B_6 ") was abundantly present in the wholemeal flour and bread and less so in the white flour and bread. The nutritive value of wheat flour and bread would, therefore, appear to be limited by their low content of flavin.

Work on the Vitamin B Complex.

New growth factors in aqueous yeast extracts. Dr. T. F. Macrae and Miss C. E. Edgar have shown that aqueous yeast extracts contain two factors, in addition to vitamin B_1 and lactoflavin, necessary for the growth of rats. One of these factors is removed with lactoflavin from the yeast extracts by adsorption with fuller's earth in acid solution, whilst the other remains in the fuller's earth filtrate. The factor adsorbed by fuller's earth may be eluted with barium hydroxide.

Our understanding of the vitamins of the B group other than vitamin B_1 and lactoflavin have recently been in a somewhat chaotic condition, but the definite establishment of two factors in addition to vitamin B_1 and lactoflavin clarifies the position.

Dr. Macrae and Miss Edgar have been engaged during the year on the chemistry and purification of the factor which is not adsorbed by fuller's earth. The results obtained suggest that this vitamin may be distinct from vitamin B_6 , as described by György.

Attempts to find a precipitant for the vitamin have at last proved successful, and the precipitate given by barium salts in 90% alcohol is being further investigated. The vitamin is not precipitated by basic lead acetate, mercuric sulphate, silver sulphate, copper acetate, phosphotungstic acid, picric acid, picrolonic acid, Reinecke acid or flavianic acid. A combination of precipitations by flavianic acid and basic lead acetate has removed much inactive material and has yielded a more purified solution of the vitamin. Attempts to adsorb the vitamin with aluminium hydroxide were unsuccessful, but adsorption on norite

charcoal and subsequent elution by alkalis or glacial acetic acid has yielded a somewhat purified preparation. The vitamin is soluble in ethyl alcohol but not in acetone or ether and remains in the water soluble portion after treatment with acetic anhydride; it does not sublime when heated in a high vacuum.

Respiration of tissues of rats deficient in the B₆ group of vitamins. Miss Edgar and Dr. Macrae are investigating by Warburg's method the respiration of tissue slices of rats deprived of the various constituents of the B₆ vitamin group. Much work has been done and several interesting results have been obtained, but the investigation has not yet reached a report stage.

Effect on Reproduction of a Dietary Essential contained in certain Fats.

Miss E. M. Hume and Miss H. Henderson Smith have continued their study of the dietary deficiency in rats which affects their breeding capacity, the full term young dying *in utero* or failing to survive after they are born. In the experiments of last year such young as survived usually showed skin lesions characteristic of lack of the essential unsaturated fatty acid (linoleic acid). During the present year, therefore, diets have been used which contained this factor abundantly in the form of linseed oil. The skin lesions of the young have been corrected, but the other features of the syndrome have been only partially improved and the suspicion has been aroused that these symptoms are due to a partial deficiency of vitamin E which, when totally lacking, gives rise, as is well known, to resorption gestations in the female. Addition of an unsaponifiable fraction of wheat germ oil, or in some cases, of wheat germ oil itself, has, therefore, been made and the effect is at present under observation.

Researches on the Antiscorbutic Principle (Vitamin C).

Vitamin C requirements of the guinea-pig. Dr. S. S. Zilva has studied the relationship of the intake of ascorbic acid to the vitamin C content of the "selective" organs and other tissues of guinea-pigs, to their susceptibility to scurvy, to the urinary excretion of ascorbic acid, and to their general well-being. He found that in order to attain the maximum concentration in the tissues ten times the protective dose had to be given. Only traces of the vitamin were established in the "selective" tissues when a daily dose 2-3 times as great as the protective dose was administered. Guinea-pigs, the tissues of which contained these minimum amounts of vitamin C, were, however, observed to live for a number of years, during which time they attained very high weights. Furthermore, the time taken by guinea-pigs to succumb to scurvy when placed on a scorbutic diet was not appreciably different, whether the tissues carried their maximum load of ascorbic acid or whether only traces of it were present in them, prior to the animals being deprived of the vitamin. The accumulated vitamin C in the body of the guinea-pig, therefore, does not act as a store in the true sense of the word. The results of this investigation are in accord with the general observation made by Dr. Zilva and Dr. S. W. Johnson some years ago on human beings and lend support to the view that there is a wide margin of "unsaturation" with vitamin C which has no obvious detrimental effect on the health of the individual.

The relation of chemical structure and antiscorbutic activity of compounds to their retention in the animal body. Following the investigation by Dr. Zilva on ascorbic acid and its analogues in which it was found that the retention of these compounds by the animal body was proportional to their antiscorbutic activity, a further series of substances prepared by Dr. Reichstein of Zurich has now been tested in this respect and the above relationship has been observed to hold true in all cases.

The enzymic dehydrogenation of ascorbic acid. As previously reported, Dr. Zilva found an enzyme in the apple capable of dehydrogenating ascorbic acid. This enzyme has now been found by him to oxidise also the inactive analogue, glucoascorbic acid, an observation from which it may be concluded that antiscorbutic activity and stereochemical structure are not determining factors in this reaction. A further inquiry by Dr. Zilva and Dr. Johnson on the oxidising enzymes of a number of plants revealed that one group, including the cabbage, cauliflower, cucumber and marrow, possesses an enzyme which is capable of dehydrogenating ascorbic acid and glucoascorbic acid directly. The oxidation of these compounds by the disrupted tissues of a second group, to which the apple and the potato belong, is due, on the other hand, to the phenolase they contain. The oxidation in this case is indirect; the hydroxyphenolic compounds of the tissue are previously oxidised to quinones, which in their turn dehydrogenate ascorbic acid. The enzymic mechanisms of the two classes of plants involved in the above reactions are, therefore, distinct.

Vitamin C in Jensen Rat Sarcoma. Jensen rat sarcoma contains a substance capable of reducing indophenol and opinions are divided as to the identity of this reducing substance with vitamin C. Dr. Zilva and Mr. A. E. Kellie have investigated this problem by adapting the biological and spectrographic procedures to the co-ordination of the L-ascorbic acid with the indophenol-reducing capacity of these tissues. Dr. Boyland of the Royal Cancer Hospital generously supplied the large quantity of material necessary for the investigation. It was found that the greater part, if not all, of the reducing substance of the tumour could be accounted for as ascorbic acid.

Vitamin C and Cataract. The presence of vitamin C in the aqueous humour and lens in relatively high quantities has attracted the attention of those interested in the aetiology of cataract and contradictory views are held concerning the significance of this fact. Dr. Johnson has devoted his attention to the fate of the ascorbic acid in the humours and lens of the eye of the guinea-pig in the process of depletion of vitamin C and to its occurrence in the eyes of the depleted animals following the administration of the vitamin. He found that the humours and lens behaved like the "selective tissues," i.e., liver, adrenals, intestine, etc., in this respect that the vitamin disappeared during dietetic depletion and reappeared in the original concentration when ascorbic acid was injected into the animals. No cataract was observed in the vitamin C-depleted animals even as late as the premortal stage. The results, therefore, suggest that the deprivation of guinea-pigs of vitamin C has no direct bearing on the aetiology of cataract in these animals. He also found that the lens, but not the humours, contains a substance other than ascorbic acid which reduces indophenol.

The addition of synthetic ascorbic acid in the canning of fruits and vegetables. It has been previously shown that it is possible to add ascorbic acid to fruits and vegetables before canning without incurring serious destruction of the acid during the process. Preliminary work by Dr. Zilva and Mr. T. N. Morris of the Low Temperature Research Station, Cambridge, has since been carried out on the stability of the acquired antiscorbutic potency of these final products. It has so far been performed on apples (King Edward) to which ascorbic acid was added before and after exhausting. The former would be the more convenient procedure if the above treatment were applied commercially. The apples were canned in January, 1935, and were first tested in March, 1935, again in November, 1935, and finally in September, 1936, by the biological method. In both preparations no perceptible loss in activity during storage was recorded.

Dehydroascorbic acid in apples. Dr. Zilva, in collaboration with Drs. F. Kidd and C. West of the Low Temperature Research Station, Cambridge, has been following up the equilibrium between ascorbic acid and its dehydrogenated form during growth, maturity and senescence of the apple. The results of this investigation are not yet complete.

DEPARTMENT OF BIOCHEMISTRY.

Phosphate Metabolism and the Calcification of Animal Tissues.

Calcifying Mechanism of Bone. Previous work has shown that the activity of the calcifying mechanism of the hypertrophic cartilage of the bones of rachitic rats is generally lower than in that of normal embryonic bones and tends to decrease as the period during which the rat is fed on the rachitogenic diet increases. It is not yet clear whether this decreased activity is merely a sequel to the failure of the cartilage to become calcified or is partly responsible for this failure. In continuation of these studies, Professor R. Robison and Miss J. Barnett (London University Research Student and Lister Institute Grantee) have observed a very striking increase in the activity of the calcifying mechanism in bones of rachitic rats which have received a single moderate dose of calciferol (5-30 International Units Vitamin D) within 24-48 hours of death. Calcification of these bones *in vitro* occurs at unusually low levels of calcium and phosphate concentrations, even lower than those required for normal bones. The effect is most pronounced when the dose of calciferol falls just short of that which produces calcification *in vivo* in the same period, and the deposits in the two cases are exactly similar in character and location.

It has been shown by other workers that the type of rickets induced by addition of beryllium salts to the diet does not respond to vitamin D therapy. In agreement with this it has now been found that the administration of very large doses of calciferol (up to 100,000 I.U.) shortly before death produces no increase in the very low activity of the calcifying mechanism of the bones. The effects on this mechanism of many other physiologically active substances have been investigated, but the results as a whole do not yet justify any increased definiteness in the conclusions previously stated regarding the nature of the mechanism.

Professor Robison and Mr. E. Downing (Grocers' Company Research Student) have extended the study of calcification to the skeletons of amphibia and fishes. In order to obtain material suitable for experiment the development of phosphatase activity and the progress of calcification were followed in skeletal tissues of *Rana temporaria* and *Scyllium canicula*. In dog-fish embryos, phosphatase activity increases during development, and at the stage of incipient calcification deposits of calcium salts can be produced *in vitro* in the cartilage of the neural arch. For comparison with these results, the calcification of embryonic rabbit vertebrae *in vivo* and *in vitro* has been studied.

The chemical composition of the salts spontaneously deposited from the experimental calcifying solutions, on long standing, has been further studied in conjunction with an investigation of the inorganic salts of calcified animal tissues.

Calcification of the Aorta. Professor Robison and Dr. M. Laskowski (University of Warsaw) have completed their experiments on changes in the aorta in hypervitaminosis D. Calcification of this vessel occurs very rapidly in rats receiving large doses of calciferol with a diet of high calcium content but may

remain absent if the calcium intake is sufficiently reduced. Experiments made with the aortae of these rats have failed to produce any evidence of changes in the organic tissue prior to deposition of calcium salts but facilitating such deposition, analogous to the effects observed in bone. The results must be taken as supporting the view that calcification of the aorta in hypervitaminosis D is primarily the result of supersaturation of the blood with calcium salts. Further experiments, in extension of those previously recorded, have also been carried out on rabbits suffering from cholesterol arteriosclerosis. Considerable deposits of calcium salts were found in the aortae of animals which had received cholesterol during periods of ten months; but these aortae were not more readily calcified *in vitro* than those of normal rabbits. In this type of arteriosclerosis the condition of the necrosed tissue cannot be excluded as a cause of the deposition of calcium salts *in vivo*; but it is evident from these results that there is no development of any mechanism comparable with that of bone.

Calcification of Dentine. Mrs. S. Hughes (Strangeways Research Laboratory) has continued to collaborate with Professor Robison in experiments on the calcification of dentine. By a modification of the diet of pregnant rats, embryos in which calcification of the dentine is considerably retarded have been obtained. The calcification of this dentine *in vitro*, under the same conditions as those required for bone, has provided further evidence of the similarity of the mechanisms in both tissues.

Rôle of Kidney Phosphatase. The suggestion of Lundsgaard that phloridzin glycosuria may be due to the inhibitory effect of phloridzin on the kidney phosphatase has been the subject of experiments by Professor Robison and Dr. A. Lambrechts (University of Liège). The calcification of kidney slices which occurs, *in vitro*, in presence of phosphoric esters by the agency of the phosphatase, was not found to be significantly reduced by phloridzin, so that no confirmation of Lundsgaard's hypothesis was obtained. The possibility that inhibition is confined to the synthetic action of the enzyme is not, however, entirely ruled out.

Alcoholic Fermentation.

Current theories of the intermediate stages of carbohydrate metabolism are based chiefly on the enzymic reactions shown to take place in dead or cell-free preparations of tissues and it is a legitimate criticism that such reactions may not truly represent the process in the living cell. Dr. M. G. Macfarlane has continued a study of alcoholic fermentation in yeast from this aspect. The results so far obtained indicate that the process of fermentation of sugar by the living cell is analogous to that shown to take place in cell-free fermentations in that inorganic orthophosphate is esterified, with formation of fructosediphosphate and phosphoglyceric acid, so long as fermentation continues. The study is being extended to the changes taking place in respiration. Metaphosphoric acid, which has usually been considered to be absent from biological material, has been identified as a normal constituent of the yeast-cell and may prove to have an interesting part in cell economy.

The still unknown esters of the hexosemonophosphate of fermentation have been the object of continued work by Professor Robison and Miss A. Tazelaar, who have effected a very considerable concentration of one of these but have not yet obtained it in pure condition or solved the question of its identity. With Dr. M. Laskowski, the preparation of a phospho-osone from hexosediphosphate has been attempted.

During the year, Dr. N. Nielsen spent some time as a guest of the Department and carried out experiments on the effects of sodium and potassium ions on fermentation by yeast juice.

Metabolism of Carbohydrate and Fats.

The nature of the combination in which magnesium is present in yeast is particularly important since magnesium plays an essential but quite unknown rôle in the carbohydrate metabolism of both yeast and muscle. Dr. Smedley-Maclean has now isolated a magnesium substance from yeast, containing carbon, hydrogen, oxygen and magnesium, in which the magnesium appears not to be in the ionic condition, and the constitution of which is being investigated.

Magnesium was also found by Dr. R. McAnally in the insoluble residue left when all other constituents of the yeast cell were dissolved by caustic potash solutions. This residue has hitherto been regarded as the "insoluble carbohydrate" of the yeast cell. Under certain conditions a substance very similar to glycogen may be split off from it, and at the same time the magnesium is dissolved. Evidence was, however, obtained that these two phenomena are only accidentally associated and that the conversion of insoluble carbohydrate to glycogen is more probably due to its liberation from a complex phosphate-containing substance.

A second insoluble carbohydrate is also present, from which glucose is split off by boiling with normal acid, and a residual carbohydrate, containing an acid group, left. This latter substance was tested by Dr. Morgan and gave a definite agglutinin reaction with Type II pneumococcus serum.

In the obscure field of the mechanism of fat synthesis Mr. L. D. Macleod (Morna Macleod Research Student) has obtained some evidence that the process is one of greater complexity than has been assumed and is endeavouring to differentiate the various factors concerned in the process. Evidence has now been obtained confirming the view that the formation of fat is closely linked with the respiratory process.

In order to throw light on the constituent of linseed oil, absence of which from the diet produced a definite deficiency disease in rats, Mr. L. C. A. Nunn has made a quantitative study of the oxidation of the acids of linseed oil by alkaline permanganate and has proved the existence of hitherto unknown lactic acids formed from linoleic and linolenic acids as intermediate products of oxidation.

The Metabolism of Galactose.

Research has been continued by Dr. G. A. Grant (Beit Memorial Research Fellow), on the nature of the mechanism of lactose synthesis by the mammary gland. It has been found that maltose is much less readily utilized than glucose by slices of the lactating gland, *in vitro*, and experiments are in progress on the availability of various other sugars. Dr. Grant has also investigated the influence of certain hormones in reinstating lactose synthesis *in vivo* in regressing mammary glands of female guinea-pigs which have just ceased lactation. The lactogenic factor, prolactin, induced these regressed glands to produce only a limited secretion of a milk-like fluid of very low lactose content (0.2-0.4 per cent.). However, active secretion of milk containing 2.5 per cent. lactose is produced by prolactin, if the secretory cells of the acinar tissue have undergone a re-conditioning process through treatment of the animals with oestradiol and progesterone. Certain substances, for example, diphenyl- α -naphthylcarbinol, which do not possess the phenanthrene nucleus characteristic of the sex hormones, also caused mammary growth in male animals, though the possibility of this being an indirect effect on the general endocrine system requires further examination.

The Chemical Nature of Antibodies.

Dr. A. H. Rosenheim (late Beit Memorial Research Fellow) investigated further the action of proteolytic enzymes on the "H" and "O" antibodies in the serum of horses immunised with a series of intravenous doses of suspensions of *B. typhosus*, living or killed by various methods.

The "O" agglutinins in all serum samples were rapidly destroyed by pepsin, trypsin and activated papain; but whereas the "H" agglutinins in serum samples obtained from each of three horses after a first immunising course were rapidly destroyed by pepsin and trypsin, those in samples obtained after several immunising courses were not appreciably destroyed under identical conditions. It is significant that while the globulin fractions of serum obtained after the first and subsequent immunising courses were hydrolysed to approximately the same extent by proteolytic enzymes, the "H" agglutinins in the former were completely destroyed when the increase in amino-N due to peptic digestion was less than 5% of the total N, and those in the latter were not appreciably destroyed when the increase in amino-N was as much as 17% of the total N.

The "H" agglutinins apparently resistant to pepsin and trypsin were not resistant to activated papain, an enzyme known to differ markedly from pepsin and trypsin in its mode of attack on the globulin molecule. An analysis of the results obtained with horse serum and those obtained in preliminary experiments with the serum of rabbits immunised with suspensions of different strains of *B. typhosus* prepared in various ways, suggests that the apparent change in the "H" antibody in the direction of increased resistance to proteolysis is related to the length of the immunisation period rather than to the source and method of preparation of the "H" antigen.

The Oxytocic Hormone of the Posterior Lobe of the Pituitary Gland.

Dr. J. M. Gulland and Mr. S. S. Randall have discussed the complicated oxidation-reduction systems of the hormone in the light of their own investigations and the results of other workers in the field.

Studies in the Amino-acid Groups.

Dr. Gulland and Mr. C. J. O. R. Morris concluded their work on canavanine when Mr. Morris left the Institute to engage in research at Heidelberg. Since then the structure which they proposed for the amino-acid has been confirmed synthetically by Kitagawa and his collaborators in Japan.

Constitution of the Nucleic Acids and Nucleotides.

Dr. Gulland extended his investigations on this subject to other nucleosides and initiated enzymic experiments which are being actively pursued at Nottingham.

Chemistry of Vitamin B₁.

Continuing their earlier work in Edinburgh Dr. A. R. Todd and Dr. F. Bergel have completed a synthesis of aneurin (vitamin B₁), using an extension of the method previously devised by them for pyrimidylthiazolium salts. The synthetic product was identified with the natural vitamin by chemical and biological methods. The synthesis of a number of related compounds has also been undertaken with a view to determination of the structural features essential for antineuritic activity.

Vitamin E.

Preliminary work by Dr. Todd and Dr. Bergel, having confirmed the value of rice-germ oil as a starting material for the isolation of the anti-sterility factor (vitamin E), the investigation of the unsaponifiable fraction of this oil as well as of the more commonly used wheat-germ oil, has been taken up in collaboration with Dr. H. Waldmann (Emil Borell Research Fellow) and Dr. T. S. Work (Research Student in Biochemistry). The essential feature of the technique employed has been fractionation of distilled concentrates by chromatographic adsorption. From rice-germ oil in this way several crystalline alcohols have been isolated and characterised by acyl derivatives; in similar fashion crystalline acyl derivatives of a number of alcohols have been isolated from wheat germ-oil. The various products are at present being tested biologically by the method of Evans, but owing to the protracted nature of the tests it is not yet possible to state definitely whether or not any one of them represents the pure vitamin.

Anthelmintics.

Dr. Todd and Dr. Bergel have investigated the leaves of *Erythroxylon lucidum*, an infusion of which is widely used in Ceylon as a mild anthelmintic. The activity of the leaves appears to depend solely on their content of cocaine (ca. 0.3%). Among inactive constituents β -amyrin palmitate and β -crotinone have been identified.

The Active Principle of Hashish.

The resin left after removal of cannabinol from *Cannabis indica* extracts has been found to contain a substance or substances of much greater physiological activity than pure cannabinol. Dr. Work has effected considerable concentration of this material and is investigating its nature.

Dr. Todd is investigating erythrophlein, the African arrow poison from *Erythrophleum guineense*, and with Dr. Work, gossypol, the toxic colouring matter of cottonseed. Dr. Bergel is also engaged on investigations relating to sterol derivatives of physiological interest.

DEPARTMENT FOR THE STUDY AND PREPARATION OF THERAPEUTIC SERA.

The Specific Antigen of *B. dysenteriae* (Shiga).

During the year Dr. W. T. J. Morgan has continued his investigations into the nature of the specific antigen of *B. dysenteriae* (Shiga). He has elaborated a method for the isolation of the antigen by utilising the solubility of the antigenic complex in certain organic solvents at a neutral reaction and at normal temperatures. By this means it has been possible to recover the bacterial antigen in a form which can be considered to be its natural state. The material consists of a polysaccharide-lipoid complex, which readily engenders in the rabbit specific agglutinins and precipitins for *B. dysenteriae* (Shiga). The polysaccharide represents about 40 per cent. of the antigenic complex; an account of its chemical structure was given in last year's report. The lipid fraction is under investigation; its properties indicate that it probably belongs to the phospholipin group since its molecule appears to contain both phosphorus and nitrogen.

The above-mentioned method of preparing antigen solutions has been applied to other bacteria and, in particular, to an antigenic analysis of the stroma of sheep erythrocytes with the object of obtaining an insight into the nature of the heterophile antigen that gives rise to the homologous heterophile antibody and of ascertaining the exact relationship of this antigen to the heterophile antigen of *B. dysenteriae* (Shiga).

The Action of Alkali on N-acetylglucosamine.

Dr. Morgan has carried out further experiments to ascertain the nature of the change undergone by N-acetylglucosamine and N-acetylchondrosamine during treatment with dilute aqueous alkali. There seems little doubt that the suggestion put forward by Dr. Morgan and Dr. Elson in 1933 is correct and that an oxazole or oxazoline ring system is formed. Dr. Morgan has found that by heating the oxazole derivative with ammonia it can be converted into a glyoxaline ring by the simple replacement of the ring oxygen by nitrogen; such a reaction would seem to supply the evidence for the oxazole ring structure.

With the collaboration of Mr. A. T. Dann (of Adelaide, S. Australia), attempts have been made to prepare synthetically the glyoxaline derivative which is formed by the action of ammonia on the oxazole, but they have so far been unsuccessful.

In order to obtain additional evidence in support of the oxazole structure several new derivatives of glucosamine have been prepared and their behaviour in the presence of alkali has been investigated. Furthermore it has been observed that the colour reaction given by authentic specimens of substituted oxazoles with Ehrlich's *p*-dimethylaminobenzaldehyde reagent is analogous to those given by the glucosamine derivatives after they have received a preliminary treatment with alkali.

Serological Analysis of certain Spore-bearing Anaerobes.

Cl. oedematis maligni. Dr. D. W. Henderson has continued his study of the protective substances in specific antibacterial sera which control experimental infection with this organism. He has found that a heat-labile antigen stimulates the production of a protective antibody in the rabbit. The experimental evidence points to the association and perhaps the identity of this antigen with the flagellar substance. On this theory the antigen is the so-called "H-antigen" and the protective substance the "H-antibody."

An examination of the relative protective value of an antitoxic and antibacterial serum suggests that antibacterial prophylaxis may find practical application in the control of infections associated with *Cl. oedematis maligni*.

Cl. welchii. An *in vitro* analysis of the antigens of this organism is still in progress. The work carried out by Dr. Henderson indicates that the complexity of the problem increases with the examination of each strain. The relationship of the new type of bacterial antigen mentioned in last year's report to the "D" type of toxin elaborated by *Cl. welchii* (*ovitoxicus*) has not yet been established.

Experimental Meningococcal Infection in Mice.

Dr. G. F. Petrie has confirmed the statement that an inoculum containing a comparatively small number of living meningococci suspended in a solution of mucin is lethal to mice by intraperitoneal injection. It is now possible to estimate without difficulty the relative virulence of meningococcal strains by means of the mucin technique. Thus a Group I culture maintained under routine conditions for the past three years has proved to be as virulent as several freshly isolated strains. Again the lethal dose of three Group I strains which were isolated in 1931-1932 was found to be 100 times greater than that of three strains isolated two months ago. It is intended soon to investigate the therapeutic action of anti-meningococcus sera in mice with the aid of mucin and to determine whether this technique affords a means of titrating the protective activity of Group I and Group II immune sera against multiple test-doses of living virulent cultures.

The Production of Type-Specific Anti-Streptococcus Sera in the Rabbit and the Horse.

Dr. Petrie and Dr. Henderson have immunised rabbits and horses with three serological types of streptococcus which are frequently present as pathogenic agents in a variety of streptococcal infections in man. The rabbits have responded satisfactorily, as judged by their agglutinin titres, but the horses have yielded sera of much lower titre. This result is probably attributable, in part at least, to the relative dosage in terms of body-weight. The rabbit can be given relatively larger doses than the horse because it is much less sensitive to the allergic effects of serial injections of bacterial antigens such, for example, as the pneumococcus and *S. typhi*. As a consequence it is much easier to produce high-titre anti-pneumococcus and anti-typhoid serum in the rabbit than in the horse.

It is intended to carry out experiments in mice in order to ascertain whether the protective action of the experimental antistreptococcus sera is strictly type-specific. Preliminary tests in mice indicate that the limits of error are wider than those of similar tests in use for titrating anti-pneumococcus serum, Type I.

The Stabilising Action of Glycerine on Toxins and Sera used as Laboratory Standards.

The experience of the past fifteen years at the Serum Department has proved that the addition of 50-60 per cent. glycerine to tetanus toxin exerts a remarkable stabilising action on it and thus facilitates its use as a laboratory standard in routine tests of samples of antitoxin. Within recent years the principle has been extended to the toxin of *Cl. welchii* and to dysentery toxin (Shiga). Technical difficulties have hitherto precluded the application of the method to diphtheria toxin but Dr. Petrie has now prepared a glycerinated toxin by a simple method which permits of some degree of purification and of a three-fold concentration of the lethal toxin. The L₅₀ dose of this preparation is 0.0133 c.c. and the average lethal dose is 0.0002 c.c. for guinea-pigs of 250 grammes. The addition of glycerine to antitoxic and antibacterial sera is known to have a stabilising influence on the specific antibody and the method has been applied to most of the laboratory serum standards in use in the Department. Glycerinated preparations of this kind possess the great advantage that they can be stored at -10° C., a temperature which is not sufficient to freeze them but which retards internal chemical and enzymic changes and thus inhibits destruction of the specific molecules.

The Stability of the Protective Substance in Anti-Pneumococcus Serum, Type I, and of the specific Antitoxin in Anti-Dysentery Serum (Shiga).

Dr. Petrie and Dr. Morgan have continued their observations on the rate of destruction of the protective antibody in batches of natural and concentrated anti-pneumococcus serum, Type I, after storage for varying times at various temperatures. The results show that the concentrated product loses potency

at a much greater rate than the natural serum, and that the loss takes place mainly during the first year of storage. Dr. Petrie has begun similar observations on the stability of the specific antitoxin in anti-dysentery serum (Shiga). This work represents part of a general inquiry into the stability of therapeutic sera which has been organised on behalf of the Pharmacopoeia Commission by Dr. P. Hartley, Director of the Biological Standards Department, Medical Research Council.

The Determination of the Error of the Methods of Assaying Tetanus Antitoxin.

The Department, at the instance of Dr. Hartley, is collaborating in an inquiry which is being conducted by the Permanent Commission on Biological Standardisation of the League of Nations, with the object of determining the limits of error of the routine methods of assaying tetanus antitoxin. Samples of antitoxin of unstated potency have been received and titration tests are being carried out on them in accordance with the routine procedures in use in the Department.

Dr. T. Lumsden of the Cancer Department of the London Hospital has again, during the past year, been given facilities for his work on the production of an anti-cancer serum by the immunisation of sheep.

During the visit, in July, 1936, of the International Society for Microbiology to the Congress held in London, 120 members accepted an invitation to inspect the stables and laboratories at Elstree.

DEPARTMENT FOR THE PREPARATION AND STUDY OF VACCINE LYMPH.

The Preparation and Preservation of Elementary Body Suspensions for Jennerian prophylaxis.

Behrens and Neilson (1935) described a method for the purification of vaccinia virus by iso-electric precipitation of the associated proteins in a suspension of vaccine pulp. Dr. D. McClean has studied this method and its application to the production of bacteria-free suspensions of elementary bodies suitable for intracutaneous injection in Jennerian prophylaxis. Following iso-electric precipitation, about 75 per cent. of the tissue proteins may be removed by centrifugation, whereas the major portion of the virus is retained in the supernatant fluid, which can then be passed through a Berkefeld "V" filter. With certain modifications, this method yields supernatant fluids, the infective titre of which is usually from 10^{-3} to 10^{-6} . Further concentration of the virus can be obtained, if desired, by employing a Sharples super-centrifuge in which the cylinder has been lined with a cellophane sleeve; the elementary bodies are deposited on the cellophane, from which they may readily be removed by washing in a small volume of fluid.

The factors controlling the successful filtration of vaccinia virus through bacteria-proof filters require further investigation. The commercial filters that are available show considerable individual variation, and it is a matter of some difficulty to select those which will allow the virus to pass without serious loss of titre and yet will retain all the contaminating bacteria. Various filtering surfaces and the influence on the passage of the virus through them of different suspending fluids are being investigated.

The prolonged viability under suitable conditions of storage of elementary body suspensions prepared from dermal lapine was reported by Dr. Amies and by Dr. Eagles; this has been confirmed for similar preparations obtained from vaccine pulp by the method described above. These suspensions showed remarkable activity after exposure at 22° C. and at 37° C. for considerable periods. The addition of 0.5 per cent. phenol as a preservative against bacterial contamination does not materially hasten the rate of inactivation of the virus. The protective effect on the virus of the addition of various colloids has been investigated. It appears that the storage of elementary body suspensions of vaccinia prepared for clinical use is unlikely to present serious difficulties.

Samples of elementary body suspensions that were considered suitable for laboratory and clinical trial have been sent to Professor Gins of the Robert Koch Institute, Berlin, and Dr. Ørskov of the State Serum Institute, Copenhagen, who have kindly consented to test them.

The Influence of Tissue Permeability on Local Immunity.

Dr. McClean has completed the observations begun by Dr. Favilli (University of Perugia) and himself on the relation between tissue permeability and local immunity to infection. The inhibition of the diffusion of crude testis extract in the dermis by Besredka's "antivirus," plain broth and various substances known to reduce the permeability of the cell, was reported by Favilli and his collaborators; this has been confirmed, using purified preparations of the diffusing factor from the testis. Culture filtrates from certain invasive bacteria cause a dramatic increase in the permeability of the tissues and it has been shown that the diffusion of the purified spreading factors obtained from these filtrates is similarly inhibited by the same agents. The mechanism of this inhibition has been investigated and it appears that it is the inflammatory cellular response provoked by the injection of "antivirus," plain broth and other substances, which renders the tissues less permeable to these diffusing factors, whether they are derived from the testis or from bacteria.

These observations supplement the work of Menkin on the local fixation of inflammatory processes, and they indicate that the phenomenon of "local immunity" may be explained, at least in part, by a non-specific reduction in the state of permeability of the tissues. These observations also indicate the importance of the balance between the diffusing activity of invasive bacteria and the reduction in local permeability that results from the inflammatory response by the host.

Apart from the purely local reaction, it is known that the diffusing factors of bacterial origin are antigenic and provoke the appearance of neutralising substances in the serum. Preparations are being made to carry out a serological investigation of the relation between purified diffusing factors derived from different bacterial species such as *Staphylococcus*, *Cl. welchii*, and *Cl. chauvoei*.

NATIONAL COLLECTION OF TYPE CULTURES.

(Medical Research Council.)

The number of cultures of bacteria and micro-fungi distributed to workers at home and abroad again exceeded 5,000 and some 200 new strains were deposited for maintenance in the Collection.

The new and revised edition of the Catalogue of the National Collection of Type Cultures, referred to in the last report, was published during the year under review and is now available.

The Curator acted as Honorary General Secretary of the Second International Congress for Microbiology, which took place in London last July, and as Editor-in-Chief, on behalf of the Executive Committee, of the Report of Proceedings of the Congress, which appeared in March, 1937.

GENERAL AND FINANCIAL.

The Accounts and Balance Sheet for the year ending December 31st, 1936, show balances to the credit of the Pension Fund of £27,311 2s. 0d., the Contingency Fund of £22,000 0s. 0d., the Sinking Fund of £29,308 6s. 0d., and the Capital Fund of £535,733 5s. 11d., after adding the Rockefeller Grant of £3,400 0s. 0d., and the balance of income over expenditure for current year, viz., £306 5s. 11d. The balance of income over expenditure of the Pension Fund, viz., £726 18s. 0d., has again been transferred to the General Fund Account as a contribution towards the amount expended as premiums on policies under the Federated Superannuation System for the staff.

The following changes in investments have taken place during the year, viz. :—

General Fund : £5,000 4½% Conversion Stock, 1940/44 has been sold ; £2,000 Cape of Good Hope 3½% Consolidated Stock, 1929/49 redeemed, and £10,000 4% Consolidated Stock, 1957, and £8,000 3½% War Stock, 1962, purchased.

Sinking Fund : £1,400 4% Funding Stock, 1960/90 has been purchased.

Income for the year amounted to £56,822 13s. 3d. Compared with 1935 there was an increase in Interest and Dividends on General Fund Investments of £341 1s. 7d., but a slight decrease in Investigation, Diagnosis and Analysis Fees. Sales of Sera, Vaccines, etc., amounted to £31,662 6s. 11d., and after adjusting stocks on hand at the beginning and end of the year this item appears in the Accounts as £32,410 3s. 8d.

Expenditure during the year was £56,516 7s. 4d. against £50,124 12s. 4d. in 1935. Apart from considerable increases in Serum and Vaccine Laboratories Expenses, and in Alterations, Repairs and Renewals, mainly at the Serum department, items of expenditure showed little change.

In conclusion, the Governing Body desires to express its appreciation of the devoted co-operation of the Director and all members of the staff, and assistants, in carrying out the work of the Institute.

WILLIAM BULLOCH.

Chairman of the Governing Body.

BALANCE SHEET

AND

ACCOUNTS.

Dr.

The Lister Institute

BALANCE SHEET

	£	s.	d.	£	s.	d.
TO CREDITORS				2,472	1	2
TO PENSION FUND—						
As per Account at 31st December, 1930.. .. .				27,311	2	0
TO JENNER MEMORIAL RESEARCH STUDENTSHIP FUND—						
As per Account at 31st December 1935	8,625	3	11			
Add Amount transferred from Income and Expenditure Account, 1936	28	6	0			
				8,653	9	11
TO CONTINGENCY FUND—						
As per Account at 31st December 1934				22,000	0	0
TO SINKING FUND to 31st December 1936				29,308	6	0
TO CAPITAL FUND to 31st December 1936—						
Donations, &c., received to date from the following:—						
Dr. Ludwig Mond (1893)	2,000	0	0			
The Berridge Trustees (1893/98)	46,379	10	1			
The Grocers' Company (1894)	10,000	0	0			
Lord Iveagh (1900)	250,000	0	0			
Lord Lister's Bequest (1913/23)	18,904	5	8			
William Henry Clarke Bequest (1923/6)	7,114	5	7			
Rockefeller Foundation (1935/6)	3,400	0	0			
Other Donations and Legacies (1891-1934)	20,971	18	3			
Income and Expenditure Account:—						
As per Account at 31st December, 1935.. .. .	176,657	0	5			
Add Balance for the year ending 31st December, 1936	306	5	11			
				176,963	6	4
				535,733	5	11

WILLIAM BULLOCH, *Chairman.*G. W. ADDISON, *Hon. Treasurer.*

£625,478 5 0

REPORT OF THE AUDITORS

We have audited the above Balance Sheet. We have obtained all the information and explanations we have required, being held by the Institute on their behalf. In our opinion, such Balance Sheet is full and fair, and properly drawn and the explanations given to us and as shown by the books of the Institute.

London, 7th May, 1937.

By CASH—		£	s.	d.	£	s.	d.
At Bankers: Current Accounts		5,438	1	7			
In hand		42	3	6	5,480	5	1
By INVESTMENTS, GENERAL FUND (at cost, less amounts written off)—							
£80,000 4 per cent. Consolidated Stock, 1957 or after		74,272	16	0			
£25,000 4½ per cent. Conversion Stock, 1940-44		21,669	3	3			
£17,000 5 per cent. Conversion Stock, 1941-64		15,997	0	7			
£52,000 4 per cent. Funding Stock, 1960-1990		45,661	13	9			
£64,000 3½ per cent. War Stock, 1962 or after		63,407	13	5			
£37,000 Local Loans 3 per cent. Stock		20,829	1	7			
£3,000 Port of London 3½ per cent. Registered Stock, 1965-75		2,686	17	7			
£1,000 Dominion of Canada 4 per cent. Registered Stock, 1940-1960		928	4	6			
£25,000 Cape of Good Hope 3 per cent. Consolidated Stock, 1933-1943		23,850	0	0			
£25,000 Natal 3 per cent. Consolidated Stock, 1920-1949		21,400	0	0			
£8,000 New South Wales 4 per cent. Inscribed Stock, 1942-62		8,040	1	4			
£25,000 New Zealand Government 3 per cent. Inscribed Stock, 1945		22,114	0	0			
£26,100 South Australian Government 3 per cent. Consolidated Stock, 1916 or after		16,800	0	0			
£2,900 Commonwealth of Australia 3½ per cent. Registered Stock, 1936-37		2,825	6	0			
£1,300 Union of South Africa 4 per cent. Consolidated Stock, 1943-1963		1,327	0	0			
£25,000 Victorian Government 3 per cent. Consolidated Inscribed Stock, 1929-1949		19,800	0	0			
£4,000 Western Australia Government 4 per cent. Inscribed Stock, 1942-1962		4,081	3	0			
£20,000 Southern Railway Preferred Ordinary Stock		13,500	0	0			
£6,200 London & North Eastern Railway 3 per cent. Debenture Stock		3,961	0	0			
£5,000 Great Central and Midland Railway Joint Committee 3½ per cent. Guaranteed Stock		3,623	0	0			
£253 London & North Eastern Railway 4 per cent. First Guaranteed Stock		499	11	0			
£8,650 London, Midland & Scottish Railway 4 per cent. Preference Stock		7,960	0	0			
£15,625 London, Midland & Scottish Railway 4 per cent. Preference Stock, 1923		11,300	0	0			
£18,750 London & North Eastern Railway 4 per cent. First Preference Stock		13,028	6	7			
£25,000 East Indian Railway 3 per cent. New Debenture Stock		13,890	0	0			
£661 Madras & Southern Mahratta Railway 4 per cent. Debenture Stock, 1938		656	19	7			
£800 Grand Trunk Railway Company of Canada Great Western Borrowed Capital 5 per cent. Perpetual Debenture Stock		936	0	0			
£1,937 Grand Trunk Railway Company of Canada 4 per cent. Guaranteed Stock		1,733	0	0			
£800 Ontario and Quebec Railway 5 per cent. Permanent Debenture Stock		984	0	0			
£3,400 Gas Light and Coke Company Ordinary Stock		3,638	0	0			
					441,400	7	2
By INVESTMENTS, SINKING FUND (at cost)—							
£9,600 4½ per cent. Conversion Stock, 1940-44		8,806	16	7			
£10,200 4 per cent. Funding Stock, 1960-1990		9,079	0	1			
£13,150 3½ per cent. Conversion Stock, 1961 or after		11,837	16	2			
Balance uninvested		84	13	2			
By INVESTMENTS, JENNER MEMORIAL RESEARCH STUDENTSHIP FUND (at cost)—							
£2,650 Southwark and Vauxhall Water Co. 3 per cent. Debenture Stock "B"		2,756	10	0			
£1,596 Southern Railway 5 per cent. Preference Stock		2,740	5	0			
£1,300 Liverpool Corporation 3 per cent. Stock, 1942, or after		1,097	6	9			
£2,000 4 per cent. Funding Stock, 1960-1990		1,797	14	0			
Balance uninvested		261	14	2			
					8,653	9	11
By INVESTMENTS, PENSION FUND (at cost)—							
£22,000 4 per cent. Funding Stock, 1960-1990		17,165	3	5			
£13,000 3½ per cent. Conversion Stock, 1961 or after		10,038	1	5			
Balance uninvested		107	17	2			
					27,311	2	0
(The book value of the above Investments is, in the aggregate, less than their market value at 31st December 1936.)							
By DEBTORS					7,702	18	8
* By FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS—							
At cost less depreciation as per account 31st December 1920		2,471	17	2			
Add Ultracentrifuges &c. purchased with Rockefeller Foundation Grant		3,400	0	0			
					5,871	17	2
By EXPENDITURE ON INSTITUTE BUILDINGS AT CHELSEA—							
As per account 31st December 1935, including purchase of freehold site, £6,000					73,548	3	1
By PURCHASE OF FREEHOLD LAND ADJOINING THE "STUDIOS" CHELSEA—							
As per account 31st December 1912					169	6	8
By LEASE OF THE "STUDIOS" CHELSEA, as per last account							
Less Amount written off for the year		1,132	6	9			
		65	2	0			
By QUEENSBERRY LODGE FARM, ELSTREE—							
Purchase of freehold land and buildings and Expenditure on new buildings—							
As per account 31st December 1912					20,455	10	0
By STOCK OF ANIMALS							
		1,095	0	0			
By STOCK OF ANTITOXINS							
		3,414	14	6			
					4,509	14	6
					£625,478	5	0

* Nothing has been charged for depreciation of Furniture, &c. since new purchases made during the year to a greater amount than the estimated depreciation (10%) have been written off.

TO THE MEMBERS.

The Superannuation Scheme for certain of the Staff provides for the Life Policies for the time being in existence up so as to exhibit a true and correct view of the state of the Institute's affairs, according to the best of our information

COOPER BROTHERS & CO.

Chartered Accountants. } Auditors.

The Lister Institute of

INCOME AND EXPENDITURE ACCOUNTS

Dr.	<u>INCOME.</u>	General
		£ s. d.
To Interest and Dividends on General Fund Investments		19,418 19 3
To Interest on Sinking Fund Investments		1,244 5 0
To Investigation, Diagnosis and Analysis Fees, &c.		2,754 19 4
To Sales of Sera, Vaccines, &c., and Stock at 31st December 1936, less Stock at 1st January, 1936		32,410 3 8
To Rent of Rooms		70 0 0
To Profit on Redemption of General Fund Investment		197 8 0
To Pension Fund—excess of Income over Expenditure transferred		726 18 0
		£56,822 13 3

Dr.	Pension
	£ s. d.
To Interest on Investments	 1,335 0 0
	£1,335 0 0

Dr.	Jenner Memorial Research
	£ s. d.
To Interest and Dividends on Investments	 278 6 0
	£278 6 0

Dr.	Morna Macleod Research
	£ s. d.
To Balance from last account	 275 12 6
	£275 12 6

Preventive Medicine.

for the year ending 31st December, 1936.

Fund.	EXPENDITURE.	Cr.
		£ s. d.
By Rent, Rates, Taxes and Insurance	...	1,425 6 11
By Salaries and Wages of Staff	...	28,007 10 8
By Premiums on Federated Superannuation Policies	...	1,628 5 2
By Stationery, Printing and Postage	...	422 0 4
By Printing of Collected Papers	...	177 4 8
By Office Expenses, Auditors' Fee and Sundries	...	335 10 4
By Travelling Expenses	...	80 14 2
By Gas, Water and Fuel	...	1,597 5 0
By Electric Light and Power	...	483 2 8
By Nutrition and Protozoological Expenses, including Apparatus	...	680 15 3
By Bacteriological and Experimental Pathology Expenses, including Apparatus	...	526 16 9
By Water and Bio-chemical Laboratory Expenses, including Apparatus	...	705 4 9
By Bio-physics Building, Equipment and Expenses	...	917 12 0
By Serum and Vaccine Lymph Laboratories Expenses, including Apparatus and Bottles	...	7,187 5 4
By Culture Media	...	50 9 2
By Animals	...	964 13 4
By Animal House Expenses and Forage	...	2,999 18 2
By Alterations, Repairs and Renewals, including Workshop Expenses	...	6,023 1 11
By Library Expenses	...	337 4 3
By General Stores	...	233 2 9
By Amount written off Lease of the "Studios," Chelsea	...	65 2 0
By Sinking Fund ($\frac{1}{2}\%$ per annum on Cost of Buildings and Interest on Investments)	...	1,668 1 9
By Balance, transferred to Capital Fund	...	306 5 11
		£56,822 13 3

Fund.	Cr.
	£ s. d.
By Pensions	608 2 0
By Balance, transferred to General Income and Expenditure Account	726 18 0
	£1,335 0 0

Studentship Fund.	Cr.
	£ s. d.
By Salary of Student	250 0 0
By Balance, transferred to Balance Sheet	28 6 0
	£278 6 0

Studentship Account.	Cr.
	£ s. d.
By Salary of Student	157 10 0
By Cash in hand	118 2 6
	£275 12 6

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EAGLES, G. HARDY...	ACUTE RHEUMATIC FEVER AND RHEUMATOID ARTHRITIS AS VIRUS DISEASES. 2nd International Congress for Microbiology, London, 1936. Report of Proceedings, p. 84.
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THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE

Report of the Governing Body,
1938.

CHELSEA BRIDGE ROAD,
LONDON, S.W. 1.

June 2nd, 1938.

The Lister Institute of Preventive Medicine,

CHELSEA BRIDGE ROAD, LONDON, S.W.1.

AND ELSTREE, HERTS.

THE GOVERNING BODY.

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 THE RT. HON. SIR JOHN ANDERSON, G.C.B., G.C.S.I., G.C.I.E., M.A.,
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HARRIETTE CHICK, C.B.E., D.Sc.	" "
(Vacancy)	" "
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THE STAFF.

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Staff.

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 *H. L. SCHÜTZE, M.D., B.S.
 *G. H. EAGLES, M.D., D.P.H.
 A. FELIX, D.Sc.
 C. R. AMES, M.D., B.S.
 A. S. MCFARLANE, M.A., B.Sc., M.B. (*Biophysics*).
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 MURIEL ROBERTSON, M.A., D.Sc. (*Protozoology*).
 V. KORENCHIEVSKY, M.D. (*Endocrinology*) (*Institute and Medical Research Council*).
 KATHLEEN HALL, Ph.D. (*Temporary*).
 R. C. BURBANK, M.A., B.Sc. (*Research Student in Endocrinology*).
 EMMY KLIENEBERGER, Ph.D. (*Jenner Memorial Research Student*).
 SIR JOSEPH A. ARKWRIGHT, M.D., F.R.C.P., F.R.S. (*Honorary*).

Attached Workers :

- M. H. SALAMAN, M.D. (*Beit Memorial Research Fellow*).
 J. G. CARR, B.Sc. (*British Empire Cancer Campaign*).
 P. A. GORE, B.Sc., M.R.C.S. (*British Empire Cancer Campaign*).
 R. A. KERKICK, M.Sc. (*Medical Research Council Grant*).
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Attached Workers :

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 *IDA SMEDLEY-MACLEAN, D.Sc.
 *A. R. TODD, D.Sc., Ph.D., *Reader in Biochemistry, University of London.*
 MARJORIE G. MCFARLANE, B.Sc., Ph.D.
 ALICE A. TAZELAAR.
 E. DOWNING, B.Sc. (*Grocers' Company Research Student*).
 T. S. WORK, Ph.D. (*Research Student*).
 L. D. MACLEOD, B.Sc. (*Morna Macleod Research Student*).
 J. L. MADINAVEITIA, Ph.D. (*Research Student*).
 SIR ARTHUR HARDEN, D.Sc., LL.D., F.R.S., *Emeritus Professor of Biochemistry, University of London (Honorary)*.

Attached Workers :

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 G. A. GRANT, M.Sc., Ph.D. (*Toronto*).
 M. LASKOWSKI, D.Ph. (*Warsaw*).
 ANNI JACOB, DR. PHIL. NAT. (*Frankfurt a/M*).
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 EVA GERHARD (*Graz*).
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DEPARTMENT FOR THE PREPARATION AND STUDY OF THERAPEUTIC SERA, ELSTREE.

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- D. W. HENDERSON, B.Sc., Ph.D.
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DEPARTMENT FOR THE PREPARATION AND STUDY OF VACCINE LYMPH, ELSTREE.

D. MCCLEAN, M.B., B.S., M.R.C.S., *Bacteriologist-in-Charge.*

F. K. FOX, *Secretary to the Elstree Departments.*

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(*Medical Research Council.*)

R. ST. JOHN-BROOKS, M.A., M.D., D.P.H. (*Curator*).

MABEL RHODES (*Assistant Curator*).

ROSAMUND BARNES, B.Sc.

ANNUAL GENERAL MEETING
OF
The Lister Institute of Preventive Medicine,
June 2nd, 1938.

REPORT OF THE GOVERNING BODY.

The Governing Body has the honour to present the Institute's 44th Annual Report.

GOVERNING BODY.

The Governing Body reports with great regret the death during the year of Lt.-Col. G. W. Addison, one of the representatives of Lord Moyne and Honorary Treasurer of the Institute since 1915. At its meeting on March 28th, 1938, the following resolution was passed :—

“That the members of the Governing Body desire to record their high appreciation of Colonel Addison's invaluable services to the Institute whilst acting as its Honorary Treasurer during the last twenty-two years and of their own personal regret at the loss of a dear and honoured colleague. They also desire to express their deepest sympathy with Mrs. Addison and her family in the great loss they have themselves sustained.”

At the same meeting the Right Honourable Sir John Anderson, G.C.B., G.C.S.I., G.C.I.E., M.A., B.Sc., LL.D., M.P., was nominated by Lord Moyne as a member of the Governing Body and he has accepted the appointment of Honorary Treasurer in succession to Lt.-Col. Addison.

At the meeting held last year the Council re-elected Professor W. Bulloch, Professor A. E. Boycott and Professor Sir Arthur Harden to represent them upon the Board until December 31st, 1938.

In March last the Governing Body learnt with sincere sorrow that Professor A. E. Boycott who had served on the Board since 1925 felt compelled, owing to ill-health, to resign his appointment from the date of the Annual General Meeting. Professor Boycott's services to the Institute, both as a member of the scientific staff in pre-war days and as a Governor, have been incalculable and his keen personal interest in the scientific activities of the Institute have been greatly appreciated. The Council will be called upon to elect a representative upon the Governing Body in his stead.

[Since the above was written our colleague, Professor Boycott, has passed away (12th May, 1938.)]

COUNCIL.

At the Annual General Meeting last year the three members of Council retiring were Mr. J. R. Drake, one of the representatives of the Worshipful Company of Grocers, Professor T. G. Moorhead, the representative of the University of Dublin and the President, representing the Royal College of Physicians, London. Mr. J. R. Drake was re-appointed a representative of the Worshipful Company of Grocers and the President of the Royal College of Physicians being *ex-officio* a member of the Council resumed his seat. To the vacancy created by the retirement of Professor Moorhead, Professor J. W. Bigger was appointed by the University of Dublin.

The three members of Council, who in accordance with the Articles of Association, retire this year by rotation, but who are eligible for re-election, are, Sir Charles J. Martin and Lord Horder, both representatives of the members of the Institute and Sir Joseph Arkwright, representing the Royal Society.

The vacancy in the representation of the Members occasioned by the death of Lt.-Col. Addison has also to be filled.

MEMBERS.

The Governing Body regrets to report the death during the year of Professor G. H. F. Nuttall, a member of the Institute since 1904.

STAFF.

Dr. T. F. Macrae, formerly Research Fellow in the Division of Nutrition, has been appointed a permanent member of the staff.

Dr. W. T. J. Morgan, Biochemist at the Serum Department received an offer of a Rockefeller Foundation Research Fellowship in Immuno-chemistry and was given eight months leave of absence to enable him to work with Professor T. Reichstein at Zürich.

During Dr. Morgan's absence Dr. S. W. Johnson, late grantee of the Medical Research Council, was appointed temporary Biochemist at the Serum Department.

Dr. A. R. Todd was also granted two months leave of absence to enable him to accept an invitation from the California Institute, Pasadena, to deliver a series of lectures at that Institute.

Dr. Emmy Klieneberger, Jenner Memorial Research Student, Dr. T. S. Work, Research Student in Biochemistry and Mr. L. D. Macleod, Morna Macleod Research Student, have continued to hold their respective appointments during the year. Mr. E. Downing completed his tenure of the Grocers' Company Research Studentship in March, when he left to take up an appointment in Freetown, Sierra Leone.

Mr. R. C. Burbank, M.A., B.Sc., was appointed a Research Student in Endocrinology in July to assist Professor Korenchevsky in his researches.

RESEARCH WORK.

The Governing Body before surveying the scientific work done during the year, desires once again to record its appreciation of the continued co-operation the Institute has enjoyed with the Medical Research Council, which has continued to furnish the salaries of the staff of the National Collection of Type Cultures, of Miss Hume, Dr. Zilva and his assistants and part salaries of Dr. Korenchevsky, of Miss Copping and of Miss Hume's assistant. The Institute provided, as hitherto, accommodation and the materials for their researches for each of these workers. Mr. Kekwick's salary, together with a grant for his expenses, is also furnished by the Council. In addition Dr. Eagles has carried out researches on rheumatism and Dr. Ellinger on Pellagra in Egypt under grants from the Council.

The Governing Body also acknowledges the co-operation of the British Empire Cancer Campaign in the work being undertaken on cancer. This organisation is furnishing the salaries of Dr. Gorer and Mr. Carr, together with grants towards the expenses incurred by these workers.

To Sir Henri Deterding the thanks of the Board are also due for the grant of £400 to defray the salary of Miss Kathleen Hall, Ph.D., histological assistant to Dr. Korenchevsky, for two years and also to an anonymous donor for funds to carry out a study of the factors which influence tissue permeability. This study is being undertaken by Dr. J. L. Madinaveitia.

The arrangement of the studies carried out in the various departments of the Institute and reported in the following pages has this year been made on a subject and not, as hitherto, on a departmental basis. Valuable and increasing co-operation between members of different departments in attacks on particular problems by a combination of disciplines has made this change desirable and in the interest of the reader.

STUDIES ON VIRUSES.

Acute Rheumatism, Rheumatoid Arthritis and Chorea. The problem of the aetiology of rheumatism has been further investigated by Dr. G. Hardy Eagles in collaboration with Dr. P. R. Evans, Registrar for Medical Diseases of Children and Medical Officer in Charge of the Rheumatism Clinic, King's College Hospital, Dr. J. D. Keith, Children's Hospital, Birmingham, Mr. A. G. Timbrell Fisher, F.R.C.S., National Commission on Chronic Rheumatic Diseases, Royal College of Physicians, and Dr. W. H. Bradley, working under a grant from the Medical Research Council in the Department of Medicine, the University, Cambridge. Dr. Evans and Dr. Keith have been associated with the work only during the earlier part of 1937. Work on the agglutination of suspensions of virus-like bodies from cases of rheumatic fever, rheumatoid arthritis and chorea by sera from the corresponding diseases has been continued and cross-agglutination studies carried out. Positive results have been obtained only with sera from the rheumatic group and sufficient cross-agglutination has occurred to warrant the postulation of a common group antigen suggesting some intimate relationship between the causative agents of members of the group. The relation of various phases in the clinical course of joint disease, both rheumatic and non-rheumatic, under treatment at Addenbrooke's Hospital, Cambridge, to the agglutination reaction, is being studied with Dr. Bradley. Positive results have occurred with considerably greater frequency in cases of rheumatic fever and rheumatoid arthritis than in patients with other joint infections, and a suggestively higher proportion of positive agglutinations has been recorded during a first attack. These cases are being tested over a sufficiently long period to discover the precise relationship to fluctuations in the clinical state. A considerable part of this study has been published during the year.

Attempts to infect animals with suspensions from pericardial and joint effusions of rheumatic patients have been continued. Suspensions alone or in combination with streptococci and streptococcal toxin have not produced the typical lesions of rheumatic disease, although some suggestive clinical states with abnormal electrocardiographic and histological features have been produced in monkeys. The precise relation of these clinical states to the particular experimental interferences is not yet clear. An account of these infection studies has been published. During the past year valuable assistance has been given in supplying clinical specimens for this work by Professor J. W. McNee and his colleagues, of the Department of Medicine, University of Glasgow.

Avian sarcoma. Mr. J. Carr (Research Student, British Empire Cancer Campaign) is collaborating with Dr. C. R. Amies in further experiments on the nature of the filterable agents of the Rous No. 1 and other fowl sarcomata. At present, attention is being concentrated mainly on the technical problem of obtaining pure suspensions of the tumour agent in sufficient quantities to allow detailed serological and other experiments to be carried out on lines similar to those which have been used in the study of the elementary bodies of vaccinia. The Sharples centrifuge, which has recently been installed, is proving of value in this connection since it permits large volumes of tumour extract to be processed.

The experiments relating to the neutralisation of the Rous No. 1 tumour agent by sera from rabbits hyperimmunised with normal fowl protein have been considerably amplified. The results fully confirmed the previous finding that such sera will inactivate purified suspensions of the agent irrespective of whether guinea-pig complement is added to the mixture injected or not. It has also been found that rabbits which have been repeatedly inoculated with large amounts of the tumour agent suspension develop antibodies which will inactivate the agent. These sera, unlike those obtained by immunisation with normal fowl proteins, are practically devoid of anti-fowl antibodies (agglutinins and precipitins); it is probable, therefore, that the antibodies produced are directed against the tumour agent as such and not against the "fowl component" alone, if the latter is to be regarded as a genuine component of the causative agent as the neutralisation experiments with anti-fowl sera might suggest.

Vaccinia: the production of monodisperse suspensions of the elementary bodies and the histology of the associated skin lesions. It has been found by experience that satisfactory elementary body suspensions cannot be obtained from rabbit skin lesions if glycerolised sheep pulp or infrequently passaged rabbit pulp is used as the seed virus. Such material when processed by the usual method of fractional centrifugation yields suspensions of low infectivity which, when examined microscopically are found to contain particles of various sizes and degrees of refractility in addition to the elementary bodies proper. Repeated serial passage, with these inhomogeneous suspensions as the seed virus, has the effect of reducing the amount of this finely particulate material while the yield of elementary bodies is greatly increased until finally an apparently pure suspension of the bodies is obtained. Dr. Amies has investigated the factors which are responsible for this transition and concludes from his experiments that the finely particulate material, although non-infective itself, is of viral origin. At present, however, it is not possible to say whether these finer particles are degenerate forms of the elementary bodies or whether they represent a stage in the development of the virus.

It has also been found that the histological appearance of the skin lesions produced by inoculation of pure elementary body suspensions differs considerably from those produced by vaccinia virus in the form of sheep lymph or infrequently passaged lapine. The former are characterised by a marked increase in the thickness of the epidermis due to proliferation and swelling of the cells of the Malpighian layer. Many of these cells contain acidophil inclusion bodies within their cytoplasm and it is presumably from these inclusions that the elementary bodies are liberated. The infiltration of the dermis, with cells of the reticulo-endothelial system, which is such a conspicuous feature of the lesions produced by sheep lymph, is always considerably reduced and may be absent altogether. This evidence suggests that the effect of frequent passage by the inoculation method is to increase the normal affinities of the virus for tissues of ectodermal origin until ultimately a strain is produced which can proliferate exclusively in the epidermal cells.

Antigenic structure of vaccinia virus. Dr. M. H. Salaman (Beit Memorial Research Fellow) has continued the investigation of the antigenic structure of vaccinia virus and its reactions with its antiserum. Craigie's finding that the elementary bodies contain a heat-stable and heat-labile antigen was confirmed. It was further found that E.Bs. (elementary bodies) heated to inactivate the labile antigen were incapable of absorbing virus-neutralising antibody. Alcohol-treated E.Bs. behaved similarly. Search has been made for an agent capable of destroying the infectivity of the virus without depriving it of its power of absorbing virus-neutralising antibody. E.Bs. treated in the cold with the least amount of formalin needed to render them non-infective were found to retain Craigie's heat-labile antigen as well as a part of their power to absorb virus-neutralising antibody. Further work on these lines is in progress.

Work published last year gave definite evidence of *in vitro* union between E.Bs. and virus-neutralising antibody. Since then an allied problem has been investigated, viz., the effect of treatment with antiserum on the infectivity of the virus. E.Bs. were treated with antiserum, separated from it by centrifugation, washed, and their infectivity tested by intradermal injections of serial dilutions in rabbits' skin. It was found that such E.Bs. had a definitely lowered infectivity as compared with normal-serum-treated controls. This reduction was always partial; complete suppression of infectivity was never observed however high the ratio of serum to virus.

Metabolism of vaccinia virus in the elementary body form. With the co-operation of Dr. Salaman, Dr. M. G. Macfarlane has undertaken a study of the enzymic activity of the elementary bodies of the vaccinia virus. There are obvious, and at present unavoidable, difficulties in using for this purpose material which can be obtained only from the tissues of a host organism; bacteriological, chemical and physical examinations of the suspensions of the elementary bodies which have been used indicate, however, that

they have not only a large content of virus but also such a high degree of homogeneity that the presence of particles other than virus is virtually excluded. Both positive and negative results will, therefore, have some significance in assessing the metabolic activity of the virus. The suspensions showed no dehydrogenase activity towards a number of substrates, including glucose, lactate, pyruvate, α -glycerophosphate, succinate, alanine, leucine and peptone, tested under conditions in which the dehydrogenase activities of comparable amounts of bacterial and tissue suspensions are readily demonstrable. All the suspensions tested, however, contained phosphatase and catalase, the activities per unit weight being relatively high compared with, for example, *B. coli* or yeast. The experiments are being extended to other enzymes.

Physical investigations on vaccinia elementary bodies: sedimentation constant, density and charge. Dr. A. S. McFarlane has carried out certain physical investigations on elementary body suspensions prepared by Dr. Amies and Dr. Salaman. The virus has a strong light absorption in the ultraviolet and by means of this property sedimentation photographs have been obtained using the Svedberg equilibrium centrifuge. The boundaries are not sharp, indicating some variation in size of the virus bodies. This was also true of a preparation which had been fractionally centrifuged in an attempt to obtain a small quantity of perfectly homogeneous virus. The sedimentation constant of the smallest bodies present in an ordinary preparation is $5,007 \times 10^{-13}$ cms. sec.⁻¹ dyne⁻¹ at 20° C., a value which agrees with that obtained by Wyckoff *et al.* The sedimentation constant does not appear to change with repeated passage of the virus.

The size of the virus (assumed spherical) may be calculated by means of the Stokes equation if the density is known. The density of the dried virus has been measured with a pycnometer and found to be 1.270. This has been confirmed in an approximate manner by studying the sedimentation of the virus in solutions of different density. In sucrose solutions of density greater than 1.30 the virus sediments upwards; in solutions of density less than 1.20 it sediments downwards. In the region 1.20 to 1.30 the results are erratic. Using 1.270 for the density in Stokes equation the value obtained for the diameter of the anhydrous particle is 190m μ .

In order to determine the density of the virus in the wet state Dr. McFarlane has employed a method based on the partition of salts in the virus suspension. A known amount of salt or other substance is added to the suspension and the concentration of this substance in the aqueous medium surrounding the virus particles is measured refractometrically. A simple calculation gives the amount of water associated with unit volume of dry matter in the particles. When suspended in a solution of 0.5% sucrose one volume of dry virus is associated with six and a half volumes of water into which the sucrose will not diffuse. On increasing the sucrose concentration to 1.5% the volume of bound water is reduced to five volumes, and further addition of sucrose to make 5% reduces the water to one and a half volumes. Similarly in a 0.5% solution of egg albumin one volume of dry virus matter is associated with seven and a half volumes of water and in 2% egg albumin the volume of water is reduced to 3 volumes. Moderate concentrations of sodium chloride also drive water out of the virus, and the flocculated virus is found to be associated with practically no water at all.

These results show that each virus particle has a water "atmosphere," the volume of which varies with the nature and concentration of substances in the suspending fluid. It does not seem possible that this water is enclosed in a form of semipermeable membrane since the mean density of such a particle with seven or eight volumes of water in it would be very little greater than unity, and the size of the particle would be such that it could be seen easily with the ordinary microscope.

Some experiments by Mr. R. A. Kekwick (working with a grant from the Medical Research Council) suggest a possible explanation of the unusually large amount of bound water. By photographing the migration of the virus in the Tiselius cataphoresis apparatus he has shown that at hydrogen ion concentrations between pH 6.5 and 8 the virus has a charge and mobility which are of a higher order than are shown by most proteins. It seems that the charged virus particles require a water envelope for their stabilisation and that salts lead to flocculation by removing this water.

Elementary body suspensions of vaccinia for Jennerian prophylaxis. Methods of preparation, filtration, and preservation. Dr. D. McClean (Elstree) has continued the comparative study of the production of bacteria-free suspensions of the elementary bodies of vaccinia by the method of iso-electric precipitation described in the last report, by simple dialysis of a suspension of vaccine pulp against distilled water and by the method of differential centrifugation. All three methods yield supernatant fluids which are rich in vaccine virus and may be passed through a Berkefeld "V" filter. When the vaccine pulp from the sheep is used it appears that the dialysis method gives somewhat better and more consistent results than iso-electric precipitation; differential centrifugation does not yield satisfactory results with this material owing to the relatively large amount of mucoid debris which is present in sheep pulp compared with that in lapine. A few similar experiments have been performed with lapine using a "homogeneous" strain of virus supplied by Dr. Salaman; with this material iso-electric precipitation is not satisfactory because deposition of the protein is too sudden and complete and most of the virus is carried down with the precipitate. The methods of dialysis and differential centrifugation have both given promising results with lapine and the latter procedure obviates the necessity of preliminary passage through a Berkefeld "V" filter.

The factors controlling successful filtration of the virus in order to render it completely free from all contaminating bacteria have been further investigated by Dr. McClean. It has been found that, if Berkefeld "V" filters are used as preliminary clarifying agents, the resulting fluid which contains 1% Difco Proteose Peptone can be passed through Elford's "Gradocol" membranes of pore size $0.65\ \mu$ to $0.75\ \mu$ with little further loss of virus. Culture tests for the presence of bacteria in the filtrates are consistently negative. The optimum state of the fluid to be passed through "Gradocol" membranes requires further investigation since successful filtration of the virus does not appear to bear any constant direct relation to the visible turbidity or opalescence of the fluid before filtration; high titres of virus may be obtained with relatively turbid fluids and *vice versa*.

Although it has been shown that elementary body suspensions remain viable for prolonged periods under reasonable conditions of storage, in view of the vicissitudes which this material is likely to face if it is issued for general prophylaxis, it has been thought better to issue it in the dried state. For this purpose a technique has been developed by Dr. McClean for drying the material in the frozen state in ampoules. This has given satisfactory results and an improved plant, which will deal with larger quantities, is under consideration.

The behaviour of "homogeneous" and "heterogeneous" strains of Lapine when transferred to sheep. In co-operation with Dr. Amies and Dr. Salaman, Dr. McClean has investigated the results of serial passage on the sheep of a strain of vaccinia virus which has been rendered "homogeneous" by previous serial passage on the rabbit. An ordinary "heterogeneous" lapine strain became "homogeneous" after serial passage through sheep, but did not show any marked increase in proliferation as measured by yield and potency of pulp. A "homogeneous" lapine strain retained its homogeneity, but showed a marked fall in yield of pulp during the first sheep passages with gradual recovery in successive animals. On the sheep there is no increase in the yield of virus when a homogeneous strain is used. Satisfactory elementary body suspensions could be obtained from these strains passed in the sheep and there was some evidence of an increase in the yield of virus in these suspensions. The final stage of filtration was, however, unsatisfactory for some reason which has not so far been explained, but which may be due to the amount or nature of the tissue debris in sheep pulp.

Prophylactic inoculation with elementary body suspensions by the intradermal method. In co-operation with Dr. R. G. Henderson, of the London County Council, and with the kind consent of Dr. Gunn and Dr. Stanley Banks, of the North Western and South Western Fever Hospitals respectively, immunisation of groups of nurses with elementary body suspensions has been undertaken by Dr. McClean. Local reactions have been slight and general reactions negligible. The resulting immunity has been tested a month to six weeks later by scarification with Government vaccine lymph. It appears that a single intracutaneous injection of an elementary body suspension active at a dilution of 1:10,000 in the rabbit skin is not quite sufficient to produce a solid immunity in all the nurses tested. At present, virus potent at 1:100,000 is being used; if the results of these tests are satisfactory, it is hoped to carry out a similar series of observations on children with suitably graded doses of virus.

The closely allied rabbit viruses, Myxomatosis and Fibroma. The results of serological experiments which Sir John Ledingham undertook with the elementary bodies extracted from the lesions of these two virus diseases and which were designed to throw light on the known cross-immunity relationships, have now been published. The conclusion was reached that the type and degree of resistance exhibited by fibroma-recovered animals to superposed infection with myxoma might be explained by the virus-localising action *in vivo* of circulating agglutinins for myxoma produced during the initial infection with fibroma. In response to infection with fibroma virus, both fibroma-agglutinating and myxoma-agglutinating antibodies are produced, the latter with greater regularity and intensity than the former.

The proliferative tissue reactions to these viruses and particularly to myxoma are of extreme interest for the light that their study may throw on primitive blood-vessel formation and the cell changes in inflammatory processes.

Other features of the myxoma picture such as the bone-marrow lesion and the massive local dermal response of immune animals to new infection are being studied.

SEROLOGICAL STUDIES: ANTIGENIC CONSTITUTION, VIRULENCE AND IMMUNISING PROPERTIES OF BACTERIA AND PROTOZOA.

Vi Antigen of *Bact. typhosum*: Action of phenol on Vi strains. The particular susceptibility to the action of phenol of the Vi antigen of typhoid bacilli, described in previous work by Dr. A. Felix and Miss R. Pitt, has been further investigated. It was found that the loss of the ability to induce the formation of circulating Vi antibody was not permanent and was due to definite destruction of the agglutinogenic activity. When suspensions of Vi strains were sterilised by treatment with 0.5% phenol during 48 hours at room-temperature and the phenol was subsequently removed and replaced by fresh saline solution, it was found that such phenol-treated vaccines invariably stimulated production of

circulating Vi antibody in the rabbit and this antibody was not deficient in protective power. The peculiar inactivation by phenol of the immunogenic function of the Vi antigen is apparently due to some kind of reversible reaction between phenol and the Vi antigen.

The preparation of antityphoid serum in the horse for therapeutic use in man. Numerous attempts were made during the past two or three years to secure some method of preparing a sterile antigen capable of replacing the living bacilli hitherto used for the immunisation of the horse. Dr. G. F. Petrie (Elstree) and Dr. Felix collaborated in these investigations. The procedure followed was first to examine the various antigenic materials for their capacity to stimulate the formation in the rabbit of Vi antibody of high titre in the agglutination test and of full efficacy in protective action, and subsequently to use the antigen for the immunisation of horses. Alcohol-treated vaccines from the virulent strain Ty2, which is particularly rich in Vi antigen, were tested in a considerable number of horses. The O titres of most of the horse sera were very high, but the Vi titres remained far below the accepted standard titre. Similar results were obtained in experiments with a phenol-killed suspension which was subsequently washed with saline, and also with Topley's purified antigenic fraction, a considerable quantity of which was very kindly supplied by Professor Topley.

These experiments showed clearly that the horse and the rabbit differ greatly in their ability to respond to immunisation with the Vi antigen. The "natural" Vi antigen, as contained in the living bacterial cells, stimulates the production of high-titre Vi antibody in both animal species, whereas the "denatured" Vi antigen, which is present in the whole bacterial cells after treatment with various chemicals or in the purified fraction of Topley, is capable of inducing the formation of abundant Vi antibody in the rabbit, but not in the horse. A comparable difference in immunity response between the two animal species is not known to exist in respect of the H and O antigens of the typhoid bacillus.

The position with regard to the preparation of potent antityphoid serum from the horse remains at present much the same as three years ago. The O antibody can be produced by injecting a dead vaccine or a purified antigenic fraction containing the O antigen, whereas the live bacilli remain the indispensable source of the "natural" Vi antigen required for the elaboration of the Vi antibody.

The protective value of Vi and O antibodies in relation to virulence of strains of *Bact. typhosum*. Dr. D. Henderson (Elstree) has confirmed the claim that mucin enhances the lethal action of the typhoid bacillus in the experimental infection of mice. When mucin is added, the number of organisms in an average lethal dose of a virulent strain may be reduced from 7.5 millions to 500. This method has been applied in assessing the value of Vi and O antibody against infection with both fully virulent and relatively avirulent strains. A small dose of either antibody affords complete protection against a multiple lethal dose of a fully virulent strain containing both Vi and O antigen. As the number of lethal doses increases, however, the O antibody becomes relatively less effective than the Vi antibody. These experiments have also shown that 20 to 50 times more antibody is required to protect against infection with relatively avirulent strains than is required for protection against fully virulent strains. This and other observations make it appear that the virulence of the test strain plays little, if any, part in determining the protective value of Vi or O antibody in the mouse. Indeed, the greater the virulence of the test strain, the smaller is the amount of immune-body required to protect against a constant number of lethal doses. The determining factor in the case of Vi antibody has proved to be the total amount of antigen which requires to be neutralised.

Plague vaccines. Dr. H. Schütze's investigations on the part played by the envelope antigen of *B. pestis* in the production of immunity have been continued. He finds that it is the determining factor in prophylactic vaccination in the white rat and probably also in the mouse. These two animals were chosen for their diverse reactions to the bacterium, the mouse succumbing to an infective dose of a few virulent organisms, the rat only after a much larger and more toxic dose. As envelope antigen can be developed equally well by both virulent and avirulent strains, by both rough and smooth varieties, if only growth takes place at a temperature approximating 37° C., these characters (virulence or smoothness) in a culture do not seem to affect the efficacy of a heat-killed vaccine prepared from them. The apparent contradiction in this work to that done elsewhere with smooth and rough forms of avirulent plague cultures, is probably to be explained by the fact that when living vaccines are employed, the rough variant is less antigenic and establishes itself less readily in the tissues of the inoculated animals, so that, as Dr. Schütze has been able to show, rabbits inoculated with a living avirulent *smooth* form develop higher titres for envelope antigen than do those that have been inoculated with a living avirulent *rough* form. This experiment demonstrates the difference between giving the important antigen preformed in a killed vaccine and relying upon its manufacture in the animal's own body after administration of a living vaccine.

The heterophile antigen of *Bact. dysenteriae* (Shiga). It is generally supposed that this organism possesses a heterophile antigen which has a specific affinity for "Shiga" heterophile antibody, but which is incapable of absorbing this type of antibody when produced by other antigens. During the past year, Dr. Henderson and Dr. W. T. J. Morgan (Elstree) have been engaged on experiments which may help to elucidate the restricted specificity of the substance in the "Shiga" bacillus which has hitherto been accepted as a heterophile antigen. Various types of rabbit immune-sera have been prepared by

means of a synthetic antigen built up from purified "Shiga" polysaccharide and a horse serum-globulin fraction. The work, although incomplete, indicates that the capacity of the specific antigen of *Bact. dysenteriae* (Shiga) to absorb heterophile antibody from homologous sera is referable to its affinity for the antibacterial receptor of the immune-globulin molecule and is not due to the presence of a specific heterophile grouping on the molecule of the antigen.

Serological analysis of the *Cl. welchii* group. Dr. Henderson has completed a preliminary examination of the bacterial antigens of members of this group. The classification adopted at the present time rests on a basis of toxin-production and for this reason representatives of each of the four toxin-producing "types" were included for examination. The results show that strains classified according to toxin-production can, with few exceptions, be placed in the same order when the specific affinities of their bacterial antigens are chosen as the test of relationship. The close parallelism between toxin production and antigenic structure offers scope for interesting speculation. Variants have been separated from the more complicated members of the group and it is hoped to examine their influence on the types of toxin elaborated in culture media.

Antibody-production in response to immunisation with Protozoa. Work by Dr. Muriel Robertson on the reaction of two *Glaucoma* varieties to sera prepared in rabbits, has been carried further and an analysis of the antigen has been attempted by preparing sera from *Glaucoma* treated in various ways. Absorption tests with *Glaucoma* appropriately treated have shown that the antigenic substance contains both a heat-stable and a heat-labile element. The antibody in sera prepared by injection of *Glaucoma* which had been steamed for one hour was very feeble, the only reaction noted being the formation and discarding of a sheath on the part of a certain number of the living ciliates. This sheath consisted of a substance secreted by the subcutaneous layer of the parasite. The phenomenon occurred as a rule after 10-20 hours exposure, but in spite of being so feeble it must be looked upon as a true serum reaction and as evidence of an element in some degree heat-stable.

Sera prepared from alcohol-treated *Glaucoma* were also illuminating. With the strain "Lwoff," it was found that such sera, when acting on living *Glaucoma*, produced practically no immobilisation and little or no agglutination, but apparently 100% of the organisms formed sheaths which were rapidly cast off. It, therefore, began to appear that the sheath-forming substance in *Glaucoma* was not only to some extent heat-stable but also alcohol-fast. In strain "Gl. R" alcohol treatment apparently altered the antigen less, for the serum made from it evoked some degree of immobilisation and agglutination together with a considerable amount of sheath substance.

Absorption with steamed *Glaucoma* was found to remove to a great extent the sheath-combining antibody from sera made with the living *Glaucoma*. Agglutination, however, and the formation of a very clear transparent exudate (not associated with the sheath) and immobilisation were little affected.

Absorption with alcohol-treated *Glaucoma* gave interesting results in that all the sheath-combining antibody was removed leaving the agglutinin effective if somewhat reduced.

A soluble exudate can be obtained from the living *Glaucoma* in saline by an appropriate method and this has been used in precipitin experiments. From these and other tests it was evident that the soluble exudate solution contained elements which reacted with the sheath-combining antibody and also with that derived from the heat-labile elements. This soluble exudate is being further investigated.

STUDIES ON PURE LINES.

Antibody-production and resistance. Dr. Schütze and Dr. P. A. Gorer have continued their search for a correlation between antibody-production and resistance to disease. The four mouse lines previously employed, two pure lines obtained by brother-sister inbreeding for over 30 generations, and two selected lines obtained by breeding selectively from more and from less resistant parents, were again used.

In all four lines females gave higher H and O titres for *S. typhi murium* and were more resistant to infection with that organism, but although there were inter-line differences for these characters, only H and not O antibody-formation could be correlated with resistance. With *S. enteritidis* genetic differences could be established but no correlation, sexually or interlineally, between resistance and antibody-production. Where significant correlation has been found, as with *S. typhi murium*, the two phenomena of resistance and antibody-formation are probably not causally related.

The experiments have shown that, with the highly inbred pure lines, a much greater uniformity in reaction to infection is obtained than with the lines which were selected but not intensively inbred. The value of such pure lines for the standardisation of biological products, apart from their use as more uniformly reacting animals in laboratory experiments of all kinds, is obvious. For example, to differentiate two vaccines, one of which is just capable of prolonging life in the immunised animals by one day more than the other, one hundred times more selected mice than pure line mice would have to be used.

The antigenic basis of tumour transplantation. It had previously been shown by Dr. Gorer that a sarcoma arising in a pure line of albino mice was specific for that line and would regress in about 14 days when inoculated into members of other strains. In hybrids derived from a cross with a resistant

strain of black mice the tumour would grow only if they inherited an antigen from their albino ancestors that was detectable in the erythrocytes by means of sera from immunised rabbits, the antigen being designated antigen II. It was further shown that iso-antibodies are present in the sera of animals in which the tumour has recently regressed and that iso-antibodies are also formed following the injection of whole blood (0.1-0.2 c.c.) and of suspensions of leukaemic cells from leukotic animals. Following the injection of blood or leukaemic cells iso-antibodies attain their maximal titres about 7 days after injection. Following tumour inoculation maximal titres occur about 14 days after inoculation. In all cases the titres drop considerably in the course of a week. Tumour grafting gives the best response and leukaemic cells would appear to be more effective antigens than erythrocytes. Experiments with hybrids have shown that the antibody formed is antibody II.

Absorption experiments indicate that the sarcoma and leukaemic cells contain greater quantities of antigen II than do the erythrocytes. Erythrocytes of new-born albinos appear to lack antigen II. It is concluded that iso-antigenic differences play a leading, and usually a decisive part in determining the fate of tumour transplants.

CHEMISTRY OF BACTERIAL ANTIGENS AND IMMUNOCHEMISTRY.

The extraction of antigenic substances from *Bact. typhosum*. Dr. Henderson and Dr. Morgan have obtained antigenic substances from *Bact. typhosum* by the method of extraction with diethyleneglycol previously described by Dr. Morgan. Substances corresponding to the Vi and O antigens have been extracted in a form apparently free from protein and capable of inducing antigenic response in the rabbit and the horse. Either one or both of these antigens may be found in the extract depending upon the strain chosen for extraction. A preliminary examination of these substances indicates that the O and Vi antigens exist in the intact bacterial cell as distinct and separate chemical entities with immunological properties which are strictly specific. The activity of the O antigen appears to be unaltered by the extraction process, but the Vi antigen is modified in at least one respect, since the immune-body produced by immunising with Vi extracts is much less effective in protection experiments than that produced by living suspensions of Vi bacilli. Dr. Henderson has begun an investigation into the nature of this deficiency.

Gram-positive organisms. Attempts to extract by similar methods antigenic substances from certain Gram-positive organisms including *Pneumococcus* Types I and II, *Vibrio septique* and *Cl. welchii*, were unsuccessful. Specific hapten-like fractions were obtained but they completely failed to stimulate the production of an antibody demonstrable *in vitro*. It may be, therefore, that protein forms an essential part of the antigenic complex of these Gram-positive organisms.

The action of alkali on acetyl-glucosamine. The results of investigations on this subject carried out by Dr. Morgan and arising out of his work on specific bacterial polysaccharides, indicated that acetyl-glucosamine lost one molecule of water during treatment with dilute alkali and gave rise to an oxazole derivative. Some of the properties of the resulting compound closely resemble the corresponding properties of authentic oxazole derivatives. However, certain properties of the compound resulting from the action of alkali on acetyl-glucosamine indicated that perhaps two molecules of the hexosamine had condensed together with the elimination of two molecules of water. In order to obtain further evidence in support of this suggestion the action of alkali on simple molecules that contain the essential and reactive grouping has been investigated. It has proved possible to isolate *N*-diacetylpyrazine by the action of alkali on *N*-acetylaminocetaldehyde, and *N*-dibenzoylpyrazine by similarly treating the corresponding benzoyl derivative. The pyrazine derivatives, when treated with Ehrlich's *p*-dimethylaminobenzaldehyde reagent, give coloured solutions identical with those arising when acetyl-glucosamine is similarly treated. Furthermore, the pyrazine derivatives show the same instability in the presence of dilute acid as has been observed for the acetyl-glucosamine product. The results of these experiments indicate the direction for future work in this field.

Action of proteolytic enzymes on antibodies. In her investigations at this Institute of the action of proteolytic enzymes on the H and O agglutinins to *Bact. typhosum* produced in horse immune serum, Dr. A. H. Rosenheim reported that whereas the antibodies formed during the early stages of immunisation were readily destroyed by pepsin and trypsin, those present in the serum at later stages of immunisation were resistant to the action of these enzymes. Dr. S. W. Johnson (Elstree) is carrying out further investigations in this field in order to ascertain whether the diphtheria antitoxin in horse serum can also be obtained resistant to peptic digestion. Using a serum of 1,000 Lf. units per c.c., it has been found that at pH 4.4, 35-40% of the serum proteins can be digested at 37° C. in 24 hours, involving a loss of only 10-15% of antitoxin, further periods of digestion causing little increase in proteolysis. Increasing the acidity to pH 3.0 allows digestion of protein to proceed to 90%, but results in the loss of 70% or more of antitoxin. At points intermediate between pH 4.4 and pH 3.0, as at higher temperatures and shorter periods, 70-75% of the protein can be digested involving losses of antibody no greater than 30%. These results show that the loss of antitoxin in this zone may equally well be due to denaturation as to peptic digestion. This possibility is being further investigated.

Toxoid-antitoxin floccules. It is generally conceded that this form of diphtheria prophylactic, though efficient as an immunising agent in man, does not pass the prescribed tests for antigenicity in the guinea-pig as easily as the other kinds of prophylactic reagent. The difficulties encountered when this work was first undertaken at the Serum Department seemed to be insuperable. During the past year Dr. Petrie has carried out experimental work which indicates that efficient floccule preparations can be made with constancy. It is not yet possible to state precisely what the underlying factors are which determine success or failure but it is probable that they will be traced to the influence of non-specific substances upon the specifically reacting molecules.

Physico-Chemical Investigations.

The Svedberg velocity ultracentrifuge has given very satisfactory service free from mechanical troubles.

Two new optical arrangements have been used with this machine. The scale-projection system to the optical design and construction of Adam Hilger Ltd., has been installed and the results obtained with it are unequalled in accuracy by any other system for the study of sedimentation. The second arrangement, known as the diagonal "schlieren" method, is less accurate but gives a more convenient visual method of observing sedimentation curves. Both methods have been used for serum analysis.

Proteins studied for various purposes by Dr. McFarlane and Mr. Kekwick include horse serum albumin and globulin, lactoglobulin, egg albumin, sheep haemoglobin, tobacco mosaic virus protein, helix haemocyanin, visual purple (in conjunction with Dr. R. J. Lythgoe), and normal and pathological animal and human sera. A special study has been made of normal and anti-vaccinial rabbit serum. Normal rabbit serum is shown in the undiluted state to contain practically no "apparent" globulin. On dilution the amount of globulin is still found to be remarkably small. After immunisation with vaccinia a small amount of globulin fraction appears coincident with the development of immunity. Mr. Kekwick has also observed a change in the appearance of the serum in the cataphoresis apparatus. He has been able to separate off the total globulin fraction in the U-tube and tests by Dr. Salaman have shown this fraction to carry a high proportion of the total antibody activity of the original serum.

STUDIES IN CONNECTION WITH THE STANDARDISATION OF THERAPEUTIC SERA, ETC.

Determination of the potency of a standard anti-pneumococcus serum (Type I) by comparison with a previous standard. This work was carried out, as part of a co-operative inquiry, by Dr. Petrie and Dr. Morgan on behalf of Dr. P. Hartley, Director of the Biological Standards Department, Medical Research Council. Comparative tests of the potency of each serum were made in mice according to a method which they have already described. Dr. J. O. Irwin, of the London School of Hygiene and Tropical Medicine, was good enough to advise as to the best way of planning the tests with a view to a statistical analysis of the results. He afterwards examined the data and found that they were adequate to define the relative potency of the standards.

The determination of the limits of error of the methods of assaying Tetanus antitoxin. This work formed part of an inquiry initiated by the Permanent Commission on Biological Standardization of the League of Nations, and was undertaken by Dr. Petrie. Five samples of antitoxin of unstated potency were received and tests were made on them in accordance with the routine procedures in use in the Department, the test animals being the guinea-pig and the mouse. The results show that the content of tetanus antitoxin in horse sera can be titrated with a high degree of accuracy, even when a group of not more than five animals is used for each dose of antitoxin. The titres reported from different laboratories for the same sample of antitoxin may, however, vary widely. The titres derived from tests in the mouse are, in general, lower than those from tests in the guinea-pig. Dr. Petrie has found that there is a considerable range of variation in the ratios of the two titres when different sera—including standard sera—are titrated against the test-toxin in use in his laboratory; the test-doses of this toxin are defined in relation to the British Standard Serum.

There is at present no clear explanation for these discrepancies and it is not certain whether differences in the test-toxins employed in each laboratory, or differences in the test-sera, such, for example, as the property of "avidity," or peculiarities in both reagents, play a part. Work on the problem is being continued.

A study of certain factors which may influence the formation and dissociation of the Tetanus toxin-antitoxin complex. While engaged on the work referred to above, Dr. Petrie felt it desirable to investigate such basic factors as the stability of tetanus toxin in various diluting fluids; the optimum time required for combination of the reacting molecules *in vitro*; the effect of varying the volume of saline in which the reaction takes place; the influence of certain non-specific substances in the reacting mixture; the significance of the route chosen for administering the toxin-antitoxin mixture to the test animals; the relation between the weight of the test animal and dissociation of the complex *in vivo*; and the degree of

dissociation which takes place on diluting the mixture after the period allowed for union of the reactign molecules. The results he has obtained cannot be dealt with in summary form but certain points of interest may be noted. The toxin, when diluted with phosphate buffer solutions within a range of pH 5.0 to pH 8.0 or with saline, is rendered inactive by shaking the solution in air for two hours at room temperature or keeping it at 37° C. for 6 hours; on the other hand, when diluted in broth or peptone water at pH 7.0-7.2 there is virtually no loss of toxicity from the same treatment. Again, the volume of saline in which the toxin and antitoxin molecules react is an important factor in determining the degree of neutralisation which takes place, as indicated by the lethal effect in test animals. The toxin-antitoxin complex has been found to undergo dissociation in proportion to the degree of dilution in saline of the mixture in which it is formed.

The curative action of tetanus antitoxin in experimental animals. The curative action of tetanus antitoxin in man is not regarded as being sufficiently potent to reduce the fatality-rate to any marked extent, although statistical evidence indicates that specific treatment is capable of saving life. The question of the most effective route by which the serum should be given is still a subject of controversy among clinicians, some of whom argue in favour of the intrathecal route whereas others prefer intravenous injections. In the course of work on the assay of tetanus antitoxin Dr. Petrie had occasion to test the efficacy of a representative pool of natural serum by injecting intravenously a single dose of 200 International units at varying times after 3 or 4 lethal doses of toxin had been administered subcutaneously to guinea-pigs and intraperitoneally or intramuscularly to mice. This amount of toxin represents at least 50 minimal doses capable of causing local tetanus when injected intramuscularly into the mouse. The results show that the therapeutic effect of the antitoxin is proportional to the time of its administration and that a neutralising action is apparent when it is given as late as one-third of the survival period of the control animals, all of which died within 90 hours.

The production of type-specific anti-streptococcus sera in the rabbit and the horse. Reference was made in last year's Report to experimental sera obtained by Dr. Petrie and Dr. Henderson from rabbits and horses after immunisation with three serological types of streptococcus. These have now been tested in mice and have proved to possess no protective value. It is intended to make a fresh attempt and to investigate the effect of modifying the immunising materials.

Standardisation of the Widal reaction for the diagnosis of Enteric fever. At the request of the Health Committee of the League of Nations, Dr. Felix collaborated with Professor A. D. Gardner, of the Standards Laboratory, Medical Research Council, Oxford, in the preparation of recommendations for the standardisation of the reagents and technical procedures employed in routine agglutination tests. These recommendations were approved by a Committee of Experts at a meeting held in London in November, 1937, and were recommended for adoption as the provisional International Standard method.

GENERAL BACTERIOLOGICAL STUDIES.

The systematic classification of the filterable pleuropneumonia-like organisms isolated from various sources, based on serological and other criteria. The investigation of pleuropneumonia-like organisms, occurring independently and in symbiosis with bacteria, has been continued by Dr. E. Klieneberger. Sixteen strains from different sources have been studied in detail and differential methods have been elaborated with a view to classification. Three of the pleuropneumonia-like cultures examined were isolated from the symbiotic association known as *Streptobacillus moniliformis*, 6 from lung lesions of rats and 1 from a swollen submaxillary gland of a rat. For comparison with these, 6 other strains, viz., 3 pleuropneumonia cultures, 1 agalactia culture and 2 of Shoetensack's organisms from lungs of dogs suffering from distemper, have been included in the inquiry.

Classification of the 16 strains has been achieved by a comparative study of type of growth on liquid and solid media, morphology, pathogenicity and serological affinities. The serological examination was of special importance for the whole problem of classification. For this purpose an agglutination test was devised which clearly revealed that the 16 strains belonged to seven different serological types. As the strains of the same antigenic structure showed the same growth, colony type and morphological appearance, seven species of pleuropneumonia-like organisms could be determined. Four of these are represented by pleuro-pneumonia, agalactia, *Asterococcus canis I* and *Asterococcus canis II* (Shoetensack). In addition to these, three new species, all isolated from rats, were set up: (1) the *L1* organism described as an invariable symbiont of *Streptobacillus moniliformis* and also isolated once independently from the lung of a rat; (2) the *L3* organism found in the lungs of rats suffering from pulmonary disease; (3) the *L4* organism only once isolated from the enlarged submaxillary gland of a rat.

New evidence of the symbiotic nature of the *Streptobacillus moniliformis* was given by agglutination test, in so far as all sera prepared by immunisation with *Streptobacillus moniliformis* cultures agglutinated to a fairly high titre all the *L1* strains.

The investigations on pleuropneumonia-like organisms have recently been extended to 9 saprophytic strains isolated by different workers from soil and sewage. Morphological and serological studies of these cultures are in progress.

A paper by Gerlach who claims to have isolated pleuropneumonia-like organisms from malignant tumours in men and animals has recently attracted attention. It was suggested by Sir John Ledingham that in animals such as rats which frequently harbour such organisms, transplanted tumours might be found invaded by them. As the rat is a carrier of three of these organisms a number of Jensen rat sarcomata were examined. From one of these sarcomata *Streptobacillus moniliformis* was isolated.

In experiments on the pulmonary disease of the rats continued by Dr. Klieneberger and Dr. D. B. Steabben it has not been possible to establish an aetiological relationship between the L3 organism and the pathological condition, because so large a percentage of the Institute stock rats harboured the organism and developed the characteristic lung condition. In the infection experiments carried out, the macroscopic appearance of the lungs, the cultural results, and the histological results were the same in both control and inoculated animals. A small number of rats from London stocks other than those bred at the Institute was examined, but the incidence of the condition was similar; the bronchiectasis, the progressive destruction of the lung tissue, and the degree of infection with the L3 organism depended, as with the Institute strain, on the age of the rats. Wild rats also were examined and from one of them the L3 organism was isolated.

Staining of Pleuropneumonia organisms. Dr. M. Gutstein applying new methods has continued his investigation of the staining properties of pleuropneumonia and pleuropneumonia-like organisms with a view to a study of their developmental processes.

Trichomonas as a cause of abortion and sterility in cattle. With Mr. W. R. Kerr, of the veterinary branch of the Ministry of Agriculture of Northern Ireland, who, during 1936-1937 had been paying attention to the occurrence of *Trichomonas* causing abortion and sterility in cattle, Dr. Muriel Robertson has been collaborating in the attempt to obtain cultures of this organism. Success was not achieved, but one incidental result was the isolation from the blood of a heifer of *Trypanosoma theileri* which has been maintained in culture for nearly a year. Recently living *Trichomonas* material has again been sent from Ireland by Mr. Kerr and further, apparently more hopeful attempts at isolation and sterile culture, are in progress at the present time.

Scorpion and Spider venoms and antivenins. In response to a suggestion that the serum prepared at Elstree against the venom of the Egyptian scorpions might be found to neutralise the venom of spiders belonging to the genus *Lutrodeus*, which are common in the Cape Province and whose bite is responsible for serious symptoms and occasional fatalities, Dr. Petrie has carried out cross-neutralisation tests with venoms and antivenins. These were obtained from Dr. M. H. Finlayson, of the Union Health Department, Capetown, who has investigated the toxicity of spider venoms and their neutralisation by specific sera. The method of intravenous injection of the venom-antivenin mixture into mice was used by Dr. Petrie in all the tests, and the following results were obtained: the venom of the Egyptian scorpions is neutralised by the serum prepared against it, but this serum does not neutralise the venom of the South African spiders, *Lutrodeus indistinctus* and *L. concinnus*, nor has evidence been obtained that it neutralises the venom of the South African scorpions, *Parabuthus capensis* and *Opisophthalmus pallipedes*. The venom of *L. indistinctus* is neutralised by the homologous serum.

Bee venom, procured through the kindness of Dr. Guggenheim of Messrs. Hoffman La Roche, is not neutralised by anti-venin prepared with the venom of *Lutrodeus indistinctus*.

Maintenance of the "smooth" character of *B. dysenteriae* (Sonne). The strong tendency of this organism to become "rough" as soon as it is isolated from the dejecta of an infected patient makes it extremely difficult to maintain strains in a suitable condition for the preparation of suspensions intended for immunising or serological purposes. The fact that the "S" form appears to be the predominant one in the blood and mucus of the dysenteric stool seemed to justify the conjecture that maintenance of the strain in a mixture of horse-blood and a mucoprotein of alimentary origin (gastric mucin from the pig) might conserve its "smooth" character. Dr. Petrie has confirmed this surmise but has found that horse blood is the essential ingredient. If this material, after a heavy bacillary inoculation and incubation for some days at 37°C., is plated on whole blood-agar or haemolysed blood-agar and a subculture made from a selected smooth colony on blood-agar slopes, stable suspensions are readily obtained, especially if the growths are not more than five hours old. A culture in whole blood when kept in the cold room or at 37°C., appears to remain for weeks in a satisfactory condition, although there is a progressive intrusion of the "rough" variant.

The occurrence of a cutaneous streptothrix infection in the horse. The subject of this condition was a horse which had been admitted to the stables at Elstree. The case is of interest because the disease has not hitherto been observed in this country. When the infective organism was recognized in the skin lesions, the animal was transferred to the Royal Veterinary College, where Dr. A. W. Stableforth examined the characters of the causal organism. An account of his observations has been published. The infective agent is closely related to the streptothrix that is associated with a chronic disease of the skin which affects cattle, sheep, goats and horses in the tropical colonies of British Africa; the disease of sheep in Australia, which is characterised by lumpy wool, has a similar aetiology.

Serum diagnosis of chronic typhoid carriers. Further evidence has been obtained by Dr. Felix of the value of Vi agglutination as an aid to the detection of typhoid carriers. The frequency of a significant Vi reaction in the serum from chronic typhoid carriers is very high, and is independent of the well-known intermittency of the excretion of bacilli. Though the serum test cannot serve as final proof of the carrier condition, it has been found in some recent typhoid outbreaks that the Vi reaction is of definite value in revealing the true carriers, whereas H and O agglutination are of little, if any, use.

FACTORS WHICH INCREASE TISSUE PERMEABILITY.

The influence of tissue permeability on bacterial invasion. *Vibrio septique* in common with other gas gangrene organisms elaborates a factor that increases the permeability of the tissues. Dr. Henderson and Dr. McClean have commenced a study of the influence of this factor on local infection by this organism. Sera which inhibit the activity of the diffusing factors of *Cl. welchii* and *Vibrio septique* have been produced in rabbits. By the use of these sera in conjunction with antibacterial and antitoxic sera it is hoped to elucidate the parts played by the anti-diffusing, antibacterial and antitoxic factors respectively in the resistance to infection by this organism. Highly invasive and relatively non-invasive strains of *Vibrio septique* are available and the effect of the addition or neutralisation of diffusing activity can be observed. One interesting fact has already emerged; the neutralisation of bacterial diffusing factors by anti-diffusing sera is strictly specific; a serum prepared against *Cl. welchii* diffusing factor neutralises that factor but not the similar factor elaborated by *Vibrio septique* or vice versa.

Chemical nature of the diffusing factor. Dr. J. Madinaveitia is engaged on an investigation of the chemical nature of the diffusing factor present in testicular extracts. Highly purified active preparations have been obtained which are stable to acid and alkali and whose aqueous solutions are not coagulated by heating to 100°C. The limitations of the biological test have created difficulties and it is not yet possible to draw any conclusions as to the chemical nature of the substance responsible for the biological action. The investigations are being continued.

OTHER PHYSIOLOGICAL STUDIES.

Coagulation of Blood. Dr. J. O. W. Barratt is extending his investigation of anticoagulant action. Using the technique already employed in studying the action of sodium chloride, chlorazol and heparin, the nature of the anticoagulant activity of trisodium citrate is being ascertained and an attempt is being made to determine the anticoagulant index of hirudin, which will thus be brought into line with the other anticoagulants so far dealt with. A re-determination of the constants of the equations by which coagulant and anticoagulant action are exhibited is also in progress.

Investigation of Kidney Function. The investigation of kidney function by aid of intravital microscopy has been continued by Dr. P. Ellinger, especially the function of the proximal tubules of frogs and rats. It has been shown that the proximal tubules (which normally reabsorb selectively water and solids from the glomerular filtrate) excrete, under special conditions, urine with properties differing from the normal. This excretion through the proximal tubules, when often repeated, leads to a destruction of the epithelium of the proximal tubules.

Estimation of Lyochromes in Animal and Plant Tissue. The distribution of lyochromes in the kidney of frogs and rats has been described by Dr. Ellinger. Two different kinds of lyochromes were demonstrated in the kidney by intravital microscopy: (1) green fluorescent, and apparently free lyochromes, excreted and present in the lumina of the active urinary tracts and in the epithelium of their proximal tubules, and (2) yellowish-fluorescent lyochromes found in the epithelium of the proximal tubules in the granules. Lactoflavin injected into the animals is quickly excreted through the glomeruli, partially reabsorbed and concentrated in the proximal tubules and further concentrated in the lower urinary tract.

The usual methods of quantitative estimation of lyochromes in animal and plant tissue have been carefully examined. It has been found that the methods of extraction generally used do not extract all the lyochromes contained in the tissue; that serious loss is involved in methods of purification and concentration by adsorption on fuller's earth and subsequent elution, and that the fluorimetric and colorimetric methods of estimation of the lyochrome contained in the eluate are inaccurate. The biological method of lyochrome estimation is considered to be the most reliable.

ENDOCRINOLOGY.

Dr. V. Korenchevsky, with the assistance of Miss K. Hall and Mr. R. Burbank (Research Student in Endocrinology) has continued his studies on the effects of pure male and female sex hormones and of different cyclic sexual periods on the sexual and other organs of rats.

Pro-oestrus changes. During pro-oestrus, the period of the sexual cycle preceding heat (oestrus), peculiar changes were found in the vagina. While the deeper rows of the vaginal epithelial layer were

already typical for oestrus (cornified), the upper rows were transformed into large lucid cells having a resemblance to mucous cells, but showing no mucous reaction. The name "pseudomucous metaplasia" has been suggested for these changes in the epithelium.

Prolonged dioestrus (resting period of sexual cycle). In rats, usually during the winter months, regular cyclic sexual changes sometimes cease and the resting period of dioestrus becomes abnormally long. During such periods histological investigation has shown the same changes in the uterus and vagina as were produced by small doses of progesterone (corpus luteum hormone) and oestrone and which are reminiscent of slight pregnancy changes. This fact suggests that the prolonged dioestrus is probably due to the maintenance in females of a somewhat similar ratio between oestrone and progesterone.

Pregnancy changes. In the sexual organs these changes were obtained only when the amount of progesterone injected was very large, and that of oestrone very small. Although histologically the changes were identical with those occurring at pregnancy, the size and weight of the sexual organs remained much less than during pregnancy. This suggests that the changes in the size and structure of the sexual organs during pregnancy are brought about by the co-operation of some other hormone or hormones with the oestrogenic hormones and progesterone.

Since male hormones are known to be present in females, and approximately in the same amounts as the female hormones, an attempt was made to produce better pregnancy-like development of the sexual organs by the addition of testosterone and some other male hormones to the combination of progesterone with oestrone. A much better development of the sexual organs was obtained, but still the condition produced was not quite identical with that during pregnancy. Further experiments for the elucidation of this problem are in progress.

Bisexual and co-operative activities of the sex hormones. These were studied in detail and the results obtained warranted the proposal of two new classifications, which characterise the activity of the sex hormones from two points of view.

From the point of view of *bisexuality* the hormones were divided and characterised as follows :

- I. Purely male or purely female hormones.
- II. Partially bisexual hormones.
 - (1.) Hormones with chiefly male properties.
 - (2.) Hormones with chiefly female properties (oestrogenic hormones).
- III. True bisexual hormones.

From the point of view of *co-operation* with oestrogenic hormones in the case of females, all the other sex hormones can be divided into 5 groups : (1) hormones having no co-operative activity with oestrogens ; (2) hormones having co-operative activity with oestrogens resulting in simple restoration to the normal dioestrus condition ; (3) hormones with pronounced male activity having co-operative activity with oestrogens, resulting not only in recovery of the sexual organs to normal size and weight but in addition producing development of pregnancy-like changes ; (4) co-operative activity of the two groups of female hormones, progesterone and the oestrogenic hormones, resulting in the production of typical pregnancy or pregnancy-like changes ; (5) co-operative activity of oestrogens with some other hormone or hormones, so far not identified, necessary for production of typical oestrus.

Antagonistic activity of sexual hormones. Besides this co-operative activity, an antagonistic activity was found between the sex hormones. For example, the cornifying effect of oestrogens can be suppressed by progesterone, testosterone and androstenedione. Similarly, while small doses of oestrogens co-operate with progesterone to produce pregnancy changes, large doses of oestrogens suppress the pregnancy action of progesterone.

Pathological changes produced by sex hormones. In addition to their physiological effects the hormones, especially when used in excessive doses, produce various pathological changes both in the sexual and some other organs. Amongst the most striking of these are :

- (1) *Gigantism*, produced by testosterone propionate in female and, to a lesser degree, in male sex organs.
- (2) Great development of the *clitoris* and of the *prostate* in normal and ovariectomized female rats injected with male hormones, indicative of masculinization.
- (3) Hypertrophy of the *female preputial glands*.
- (4) In female rats injected with large doses of oestrogens, a *metaplasia of the uterine epithelium* was produced, which, as the experiments of other workers have shown, might result in cancer-like or even true cancer changes, provided such injections are prolonged for several months. It was found that injections of progesterone simultaneously with oestrone are able to suppress these changes, and this fact indicates the importance of the addition of progesterone to oestrone treatment of females and might explain one of the causes predisposing to development of cancer in women possessing presumably some hereditary disposition to this disease, always provided oestrogens have the same carcinogenic properties in women as in certain lines of animals.

Adrenals. Many hormones, especially those with pronounced male activity and when used in large doses, also produce pathological changes in the adrenals of males (decreased size of zones, cells and of their vacuoles and depletion of lipoids). The changes are in some cases so pronounced that the tissue of some of the cortical zones becomes unrecognisable.

These facts provide a warning against the therapeutic application of large doses of sex hormones.

Changes in liver. Unlike the adrenals, no pathological changes were found in the liver after injections of sex hormones. On the contrary the effects of male sex hormones on the liver could be considered as physiologically stimulating, since after castration a slight atrophy of this organ develops. This could be completely cured and the organ returned to normal condition by injections of sex hormones.

THE ACCESSORY FOOD FACTORS.

On behalf of the Accessory Food Factor's Committee, work has been continued on standardisation problems.

Vitamin Standardisation.

Vitamin A. The collective investigation organised by Miss Hume as Secretary of the Vitamin A Sub-Committee on the spectrophotometric and biological methods of Vitamin A estimation, has been extended to an examination of the U.S.A. reference cod liver oil, which was reported to give discrepant values of the conversion factor connecting results obtained by these two methods of determining the Vitamin A content. As in the previous work on halibut liver oil and its concentrate, ten laboratories co-operated and precautions were taken to obviate any errors due to instability of the material during the period of the test. When these precautions were taken, the results, which were satisfactorily concordant, yielded a conversion factor in reasonable agreement with the figure 1,600 adopted by the 1934 International Conference (League of Nations) on Vitamin Standardisation. The result of this experiment confirmed the opinion already held by some workers in the United States that the value of 3,000 international units of vitamin A per gram, ascribed to the U.S.A. reference cod liver oil, is too high and that the exact figure is more nearly 2,600 I.U. per gram. It arises from this difference that any substance tested biologically against the U.S.A. oil, on the assumption that it has a value of 3,000 I.U. per gram, will also have too high a biological value and, consequently, too high a conversion factor. A great many oils and concentrates have been so tested against the U.S.A. reference oil, and one of the most serious discrepancies in connection with the conversion factor was that shown by a very large and consistent series of experiments on halibut liver oils tested in this way. This discrepancy now disappears, and the only outstanding difficulties at present seem to be connected with materials which have been subjected to drastic manufacturing processes. The whole position with regard to the conversion factor is now, therefore, distinctly more satisfactory.

It is proposed to publish these investigations on the relation of the spectrographic and biological method of vitamin A estimation in the Special Series Reports of the Medical Research Council.

Vitamin B₁. Through the good offices of Sir Henry Dale a relatively large amount of crystalline synthetic Vitamin B₁ was made available by the generosity of four commercial firms. This material was examined for its purity, was mixed, recrystallised and investigated for chemical and physical constants by Dr. A. R. Todd and Dr. F. Bergel. Samples of the 55 g. of available recrystallised material were distributed by Dr. T. F. Macrae, Secretary of the Vitamin B₁ Sub-Committee, to 22 laboratories in various parts of the world for biological investigation of its potency in comparison with that of the present International Vitamin B₁ standard (adsorbate on fuller's earth from an extract of rice polishings).

The results of these investigations have now been obtained and show good concordance in spite of the widely differing methods and different experimental animals with which the tests were made. An average of the values obtained shows 3 µg. of the crystalline material to be equivalent in potency to that of 10 mg. of the adsorbate, i.e. 1 international unit. The result obtained in this Institute by Dr. Macrae, Miss C. E. Edgar and Dr. M. M. El Sadr (Research Fellow of the Egyptian Government) was 3.0 µg.

Dr. H. Chick has served on the Technical Commission on Nutrition, appointed by the Health Organisation of the League of Nations, at the Conferences held in Geneva, June 1936, and in London, November, 1937. She has continued to act as Technical Secretary to the International Conference (League of Nations) on Vitamin Standardisation and Miss E. M. Hume has been appointed Co-Secretary.

VITAMIN STUDIES :

The B Group

Chemistry of vitamin B₁. Work on the chemistry of vitamin B₁ has been actively continued by Dr. Todd and Dr. Bergel. Subsequent to their synthesis of this vitamin mentioned in the last report, they have synthesised a number of its analogues with a view to determining the groups essential for physiological activity. Although the number of substances examined was relatively small it seems that

physiological activity is probable in a pyrimidylmethyl-thiazolium salt only if it contains (a) a 4-amino group on the pyrimidine ring, (b) a free 2-position in the thiazole nucleus, and (c) a hydroxyalkyl group in position 5 in the thiazole nucleus.

A new synthesis of thiochrome, the blue-fluorescent oxidation product of vitamin B₁, has been carried out which gives better yields than the original synthesis. It has also been established that *O*-acetyl-aneurin is an intermediate product in the vitamin synthesis carried out last year by Dr. Todd and Dr. Bergel. In the course of synthetic work on analogues of aneurin, derivatives of 1 : 3 : 6 : 8-benzotetrazine were obtained. Dr. Bergel has carried out certain investigations on the properties of compounds containing this theoretically interesting ring-system.

Nutritive Value of pyrimidine and thiazole derivatives. Miss Edgar and Dr. Macrae have investigated the nutritive value of pyrimidine and thiazole derivatives of which the molecule of Vitamin B₁ is now known to be composed. With certain bacteria, e.g. *Staphylococcus aureus*, these two constituent parts of the vitamin B₁ molecule when presented separately, as a mixture of 2-methyl-4 amino-5-amino-methyl pyrimidine and 4 methyl-5-hydroxy-ethyl thiazole, were found by Knight to have an effect in stimulating growth equal to that obtained when they were presented in a combined form as Vitamin B₁. A mixture of these two compounds, however, was unable to replace Vitamin B₁ in the diet of the rat.

Vitamin B₂ complex. The investigation of the essential dietary factors for the rat contained in an autoclaved yeast extract has been continued by Dr. Macrae, Miss Edgar and Dr. El Sadr. The corresponding factors contained in liver extracts have also been separated from each other by various methods and have been compared chemically and biologically with those obtained from autoclaved yeast extracts. Although, biologically, these materials appear to be identical, there are chemical differences between the corresponding products from the two sources.

Of the two dietary essentials other than lactoflavin contained in autoclaved yeast extract, the one which is adsorbed on fuller's earth (yeast eluate fraction) and subsequently eluted, has now been considerably purified and there are good prospects of its isolation in a pure state. Chemical work on the other factor (yeast filtrate fraction) contained in the filtrate after adsorption on fuller's earth has indicated methods of purification which should also simplify the isolation of this substance. Dr. Macrae and Dr. El Sadr have been assisted in this work by Dr. L. Gravel, Research Fellow of the Laval University of Quebec.

Nutritive value of nicotinic acid and nicotinic acid amide for rats. Dr. Macrae and Miss Edgar have shown that neither nicotinic acid nor nicotinic acid amide can replace either of the above two dietary factors separated from yeast extract, in the nutrition of the rat. Negative results were also obtained with co-dehydrogenase prepared from red blood cells (kindly supplied by Professor Warburg): this co-enzyme contains the nicotinic acid radical and co-operates with the yellow oxidation enzyme (lactoflavin) in important oxidation processes in living cells. It is uncertain, however, whether the above pyridine bases are essential for the nutrition of rats, as they are for that of dogs, pigs, and human beings (see below). In any case nicotinic acid amide was shown to be present in the preparations of the "eluate fraction" of the vitamin B₂ complex, as separated from yeast extract.

Role of B₂ vitamins in respiration. Miss Edgar and Dr. Macrae have investigated the respiration *in vitro* of various tissues of rats deprived of the several members of the Vitamin B₂ group and have found them to be considerably lower than those of corresponding tissues obtained from control animals normally fed. This would indicate that these vitamins have some concern with tissue oxidation processes.

Nutritive value of maize and etiology of pellagra. Effect of nicotinic acid: experiments with pigs. The investigation of the severe nutritional disease produced in pigs receiving a diet consisting mostly of maize with sufficient purified casein added to furnish an adequate supply of good protein for growing animals, has been continued at Cambridge in the Institute of Animal Pathology, supported by a grant from the Medical Research Council. In this work Dr. Chick and Dr. Macrae have collaborated with Sir Charles Martin and Dr. A. P. Martin.

Attempts have been made to determine which particular constituent of an autoclaved yeast extract has the power of the extract to supplement this diet. In this work the fractions isolated by Dr. Macrae and Miss Edgar have been fed as separate additions to the maize, pellagra-producing diet (containing 83% maize). It was found that when the material adsorbed on fuller's earth and subsequently eluted, was added to the diet in a small daily ration, the pigs remained healthy and developed normally, thus suggesting that the active constituent may be identical with the P-P factor of Goldberger and his colleagues, which they also found to be adsorbed on fuller's earth from acid solutions. The filtrate after fuller's earth adsorption of the yeast extract was found to be inactive. Riboflavin given in a dose of 5 mg. daily was also without effect.

While this work was in progress Elvehjem and his co-workers (Madison, U.S.A.) reported that nicotinic acid amide or nicotinic acid would cure dogs suffering from "black-tongue" which had

developed on similar maize diets. This result was at once applied to the experimental pigs and dramatic cures were obtained in animals which had become severely ill on the maize diet and had ceased to feed. At first the nicotinic acid (100 mg.) was injected intramuscularly; afterwards, when appetite was restored, it was given by mouth in doses of 60 mg. daily.

Simultaneously the curative effect of nicotinic acid amide upon human pellagra, including the mental, intestinal and skin symptoms, has been reported from various centres in U.S.A. where the disease is endemic, and from Egypt.

These facts afford additional evidence of the analogy existing between three symptom-complexes, viz., the disease produced in pigs, characterised by necrotic enteritis and dermatitis, the "black-tongue" of dogs, and human pellagra.

The maize diets used in these experiments, without any addition, were found to be adequate for the nutrition and reproduction of rats. When the amount of whole maize present was reduced and the necessary extra calories provided by a mixture of purified casein, rice starch, lard and salt mixture, evidence of deficiency became apparent, shown by a subnormal rate of growth. This could be remedied by giving small doses of autoclaved yeast extract. With addition of riboflavin the growth rate was also improved, but administration of nicotinic acid or nicotinic acid amide was without effect. It may be concluded, therefore, either that nicotinic acid is not an essential dietary constituent for the rat or that the requirements of this animal are relatively small and are more than satisfied by the small amount contained in maize diets on which pigs and dogs suffer from severe nutritional disturbance.

Pellagra Investigations. Dr. Ellinger, with the assistance of a grant from the Medical Research Council, spent the months March to May, 1937, in Egypt studying the conditions of Egyptian pellagra in collaboration with Professor Ali Hassan and Mr. M. M. Taha of the Biochemical Department of the Medical Faculty, University of Cairo. In Lower Egypt clinical examination of the population was made in pellagra districts in the Nile Delta. Of 204 people selected at random in the villages, 70 were found to be suffering from pellagra. Parasitic infection of the gastro-intestinal tract was found to be so frequent among these pellagrins that pellagra in Egypt was considered to be secondary to this parasitic infection. In Upper Egypt where parasitic infection is much less frequent, pellagra is very rare, but all pellagrins observed showed also parasitic infection of the gastro-intestinal tract. In Lower Egypt nearly all pellagrins showed an increased porphyrin excretion which, in fresh cases, became reduced when the clinical symptoms were fully developed. Pellagra occurred much more frequently among men doing hard manual work than among married women, while the increased porphyrin excretion was equally distributed among both sexes. The incidence of pellagra in Egypt appeared to result from two concomitant but independent factors: (1) mal-absorption and mal-nutrition accompanied by increased porphyrin circulation and excretion, i.e., a condition of latent pellagra, and (2) external factors, such as hard physical work by which in this condition active pellagra is precipitated.

Some therapeutic trials were carried out with yeast and fractions prepared from yeast, an egg-white concentrate and dried whey. The two last named gave no decisive results. Treatments with yeast and an autoclaved aqueous extract of yeast were successful. Trials with an eluate obtained from fuller's earth adsorbed with autoclaved aqueous yeast extract and with the filtrate after this treatment gave, when administered together with lactoflavin, promising results. The preparation of the yeast fractions was supervised by Dr. Macrae following methods employed in his work with Miss Edgar on the fractionation of the B₂ group of vitamins in yeast.

Vitamin C.

The relation between the rate of enzymic oxidation and the stereochemical structure of *l*-ascorbic acid and its analogues. Last year it was reported that Dr. Zilva and Dr. Johnson were able to differentiate between two plant enzymic systems capable of oxidising *l*-ascorbic acid and its analogues; one was found to be identical with a phenolase and the other was characterised by a direct and more specific action. Dr. Zilva and Dr. Johnson devoted their attention to the kinetics of the latter enzyme. They found that compounds of the ascorbic acid series, in which the oxygen ring engages a hydroxyl group to the right of the carbon chain (*l*-ascorbic acid, *d*-arabo-ascorbic acid, *l*-gluco-ascorbic acid, *l*-galacto-ascorbic acid), were oxidised at a much higher rate than their enantiomorphs. In the first group of compounds the oxidation proceeded at a linear rate, whilst in the second group the rate of dehydrogenation falls off after a time. In the six-carbon atom compounds of the slowly oxidizable group, i.e., with the oxygen ring to the left of the carbon chain, the oxidation proceeded more quickly than in the seven-carbon atom compounds of the same group. There is, therefore, a similarity, but only a partial one, between the influence of the stereochemical structure of these compounds on the rate of their enzymic oxidation on the one hand and on the other hand, on their antiscorbutic activity and their capacity to be retained in the animal organism, as previously established by Dr. Zilva. Further work on these lines is now in progress.

The mode of action of vitamin C. Experimenting with human beings and guinea-pigs, Dr. Zilva and Mr. A. E. Kellie have been investigating the mechanism of absorption of vitamin C by the animal organism. They have also been studying the influence of vitamin C on the respiratory capacity of tissues. Analysis of the results of these investigations has not yet been completed.

The alleged antitoxic properties of *l*-ascorbic acid in Diphtheria. When *l*-ascorbic acid is allowed to remain in contact with diphtheria toxin for some time *in vitro*, the latter loses its toxicity. Some workers claim further that guinea-pigs receiving an abundance of vitamin C in their diet are more resistant to the effect of diphtheria toxin than animals on a diet deficient in this vitamin. Dr. Zilva studied the effect of 1-2 M.L.D. of diphtheria toxin on guinea-pigs subsisting on different levels of vitamin C. The groups of animals which were investigated ranged from a condition of almost complete depletion of vitamin C to a condition of "supersaturation" brought about by the injection of very high doses of ascorbic acid before and after the introduction of the toxin. The degree of "supersaturation" thus attained can be judged from the fact that the ascorbic acid content of the plasma in such cases was, during certain periods of the experiment, about thirty-fold of that found in cases of guinea-pigs receiving, *per os*, vitamin C *ad lib* in the form of cabbage. Dr. Zilva, nevertheless, was unable to observe any difference in the response of the animals to the toxin doses employed. Both the course of the toxæmia and the length of time before death ensued were similar in all the various groups.

Dehydroascorbic acid in apples. This research, carried out by Dr. Zilva in collaboration with Dr. F. Kidd and Dr. C. West (Low Temperature Research Station, Cambridge), has now reached a stage at which the results can be conveniently summarised. The problem was first approached about five years ago and was the outcome of an observation made by Dr. Zilva that the apple contained an enzyme capable of reversibly oxidising ascorbic acid. This enzyme has since been found by Dr. Zilva and Dr. Johnson to be identical with the apple phenolase. Owing to the seasonal character of the experimental material the progress of this research was of necessity slow. Investigations on English apples in 1933, 1934, 1935 and on South African apples in the winter of 1935 showed that the extracts of the fruit in the early stages of development contained only about 50% of its vitamin C as *l*-ascorbic acid, the remainder being present as dehydroascorbic acid. In the mature fruit, on the other hand, the vitamin existed almost entirely in the reduced form. This change in the equilibrium of the two forms of *l*-ascorbic acid progressed with the development of the fruit. In subsequent investigations similar changes in the equilibrium were observed after heating the intact fruit, so as to destroy the oxidising enzyme before extraction. This fact suggests that the oxidation of the vitamin did not take place after the disintegration of the tissues in the process of analysis, but that the dehydroascorbic acid was actually present as such *in vivo* and that the change in the equilibrium of the two forms of the vitamin was a function of some metabolic process connected with the growth of the apple.

Vitamin C in canned apples. Owing to the high concentration of vitamin C in the peel of the apple, attempts were made by Dr. Zilva and Mr. T. N. Morris (Low Temperature Research Station, Cambridge), to increase the potency of canned apples by adding a cold-water extract of the peel to the pack before processing. This procedure, instead of enhancing the vitamin C content of the final product, proved on the contrary to have a deleterious effect on the potency of the canned fruit. It suggested a destruction of the vitamin by enzymic oxidation and experiments were therefore instituted in which the extract was made by boiling and pressing the peel. The exploratory results were promising and an investigation is now in progress in which the problem is being studied in greater detail.

Vitamin P. Szent-Györgyi and his colleagues have postulated the existence of a vitamin (vitamin P) which they claimed, regulated vascular permeability. This conclusion was based in the first instance on results obtained by its use in certain clinical conditions in man. Later, however, they alleged that the function of this vitamin could also be demonstrated in guinea-pigs. Vitamin P, according to Szent-Györgyi and his colleagues, was a crystalline compound "citrin" obtained from lemon juice; afterwards they found that "citrin" was a crystalline mixture of hesperidin and an eriodictyol glucoside. These compounds, when administered to guinea-pigs on a scorbutic diet, were found to prolong life and to diminish the intensity of the hæmorrhages in the animals. Dr. S. S. Zilva, using guinea-pigs, examined "citrin," prepared and generously placed at his disposal by the Wellcome Chemical Works, also a mixture of crude hesperidin and eriodictyol, and a purified sample of hesperidin. He found that these compounds did not influence either the onset of scurvy or the fatal termination of the disease. On the other hand, the administration of sub-optimal doses of *l*-ascorbic acid was found to be associated with a pathological condition in guinea-pigs, resembling that obtained with vitamin P by Szent-Györgyi and his collaborators. The results suggest, therefore, that the vitamin P activity observed by Szent-Györgyi and his co-workers was probably due to the contamination of the above compounds with traces of *l*-ascorbic acid.

Vitamin E.

Chemistry of vitamin E. Continuing the work described in the last report Dr. Todd, Dr. Bergel, Dr. T. S. Work (Research Student in Biochemistry) and (until his return to Switzerland in July) Dr. H. Waldmann (Emil Borell Research Fellow) have characterised three isomeric alcohols. α - β - and γ -orysterols $C_{26}H_{50}O$ from rice germ oil and β -amyrin, α -tritisterol and a third isomeric alcohol from wheat germ oil. These were isolated as their crystalline *p*-nitrobenzoates or β -naphthoates; none of the free alcohols showed vitamin E activity.

γ -Orysterol β -naphthoate appeared to be identical with a substance isolated by Kimm in 1935, which he claimed to derive from a biologically active alcohol. After removal of these inactive substances, the residual oils, both from rice and wheat, showed high vitamin E activity when tested on rats by the method of Evans. These oils, after treatment with cyanic acid, yielded products corresponding in properties to the α - and β -tocopheryl allophanates of Evans in the case of wheat and a product apparently β -tocopheryl allophanate in the case of rice.

On the basis of this preliminary work a concentration process has been devised for wheat-germ oil which regularly yields concentrates showing full vitamin E activity in a dosage of 15 mg. Allophanation of these concentrates followed by chromatographic analysis yields a series of compounds, one of which has been identified as phytyl allophanate while another, by further purification, has been separated into the allophanates of β -tocopherol and of β -amyrin. On hydrolysis of the former, pure β -tocopherol $C_{55}H_{105}O_2$, is obtained as a nearly colourless oil showing full vitamin E activity in rats in dosages of 3 to 5 mg.

Accompanying the crude β -tocopheryl allophanate is a varying amount of α -tocopheryl allophanate, also giving on hydrolysis a biologically active oil, α -tocopherol, of approximate formula $C_{55}H_{105}O_2$.

As a result of degradative and synthetic work conclusions have been drawn as to the structure of these biologically active compounds, especially of β -tocopherol, which is considered to possess the structure of a 5-hydroxycoumaran or a 6-hydroxychroman derivative bearing a long side chain, probably of the phytyl type, in the heterocyclic nucleus. Synthetic experiments already carried out tend to confirm this view. Further work towards the complete synthesis of the tocopherols is in progress.

Miss A. Jacob (Emil Borell Research Fellow) and Miss E. Gerhard (Graz) have also taken part in an investigation of synthetic methods for the preparation of coumarans and chromans derived from substituted quinols, with a view to their application in vitamin E synthesis.

Biological tests in connection with vitamin E investigation. Miss A. M. Copping, working with a part time grant from the Medical Research Council, has carried out biological testing of the vitamin E preparations (in connection with the work of Drs. Todd, Bergel and Work), using the Evans technique which involves the prevention of resorption gestation in female rats subsisting on a diet deficient in vitamin E. The colony of test animals has now been satisfactorily established and routine testing has been carried out since April, 1937. In addition to the routine testing a study is in progress of the pathological conditions produced in rats by prolonged deprivation of vitamin E. It is already clear that disturbances of the central nervous system occur in adult rats, producing symptoms which may be related to the paresis seen in young rats born of mothers receiving a single dose of vitamin E, and in young rats of litters from mothers incompletely deprived of the vitamin.

Mice on vitamin E-free diet and susceptibility to carcinogenic hydrocarbons. Dr. Work has commenced some experiments designed to test the susceptibility to carcinogenic hydrocarbons of mice reared on a vitamin E-free diet; the results of this work are not yet available.

Effect on reproduction of a dietary factor contained in certain fats. Miss Hume and Miss H. Henderson Smith have continued their study of a dietary deficiency in female rats which causes a failure to produce or rear living young. Addition of wheat germ oil or its unsaponifiable fraction was effective in correcting this deficiency except when the diet also contained hardened cottonseed oil, which appeared to exercise an antagonistic action. There seems, therefore, little doubt that the observed symptoms were due to a partial, but not total, deficiency of vitamin E, a conclusion which is in agreement with observations reported by other workers. It is remarkable that when as much as 20 or 30 per cent. of the diet consists of a natural vegetable oil such as linseed or raisin seed oil, insufficient vitamin E is supplied for normal reproduction and rearing.

The results have an added interest in that they contribute to the body of evidence which is growing up to show that vitamin E deficiency in rats is not a mere laboratory curiosity, as was long thought from study of the total E deficiency, but that when it exists as a partial deficiency there is much in common between the symptoms shown by rats and those shown by pregnant women with premature separation of placenta or with abortion.

The occurrence of pathological symptoms (flaccid paralysis followed by early death) in the young of mother rats insufficiently provided with vitamin E is also confirmed, but no connection between these symptoms and any condition in human infants has yet been recognised.

PHOSPHATE METABOLISM AND THE CALCIFICATION OF ANIMAL TISSUES.

Calcifying Mechanism of Bone.

In the last Report mention was made of experiments by Professor R. Robison and Miss J. Barnett showing a very striking increase in the activity of the calcifying mechanism in bones of rachitic rats which had been fed on diets deficient in phosphate and vitamin D but which had received a single dose of calciferol

24-48 hours before death. This effect could not be produced in rats suffering from the severe type of rickets caused by the addition of beryllium carbonate to an otherwise normal diet. These experiments have been continued by Professor Robison and Miss A. Tazelaar, who have shown that both types of rickets respond equally to sodium phosphate given to the animals by mouth two or three hours before death. In this short time, before any new calcification has occurred *in vivo*, the activity of the mechanism, as judged by calcification *in vitro*, is raised to a remarkable degree. The same result can also be brought about after death of the animal by immersing the excised bones for an hour or two in sodium phosphate solution. It is concluded that the effect of calciferol on the calcifying mechanism is indirect and is due to the raised level of inorganic phosphate in the plasma. In beryllium rickets the animal is so extremely deficient in phosphate that calciferol is unable to affect the calcifying mechanism in this way or to cause healing of the rickets *in vivo*. Experiments are in progress to discover the nature of the changes which occur within the bone and are manifested by this notable increase of activity towards calcifying solutions.

The studies of calcification in amphibia and in fishes have been continued by Professor Robison and Mr. E. Downing (Grocers' Company Research Student), whose experiments have shown that the mechanism of calcification in the cartilage of the frog and of the dog-fish exhibit marked similarities with that of mammalian cartilage and bone. Through the courtesy of the Director, Dr. Kemp, these workers spent a short time in the laboratory of the Marine Biological Association, Plymouth, where experiments were carried out on larval forms of various teleostean fishes, in which calcification occurs at very early stages of development. Owing to the small size and extreme fragility of these specimens, the experiments met with only partial success. The opportunity was taken of making preliminary experiments on the calcification of the crustacean exoskeleton, and some evidence was obtained that the chitinous integument of moulting crabs can be calcified *in vitro* in suitable solutions of high calcium content.

The work on the chemical nature of the bone salt has been continued.

Phosphoric Esters of Alcoholic Fermentation.

Reference has been made in several preceding reports to an unknown component of the mixed hexosemonophosphate of fermentation. The only property of this compound differentiating it from all the known components was its intense colour reaction with orcinol, by which its concentration has been followed. The investigation of this ester has been continued during the past year by Professor Robison, Dr. Macfarlane and Miss Tazelaar, who have isolated it in apparently pure condition and have studied its properties. Although proof of its chemical constitution is not yet complete there is little doubt that it is the phosphoric ester of a 7-carbon atom sugar, probably of manno-keto-heptose. This sugar has been found only in the free state in the Avocado pear (*Persea gratissima*) and its formation from sugars containing 6 carbon atoms would be a phenomenon of considerable biochemical importance. The phosphoric ester is produced during the fermentation of either glucose, fructose or mannose by yeast juice and the further study of the mechanism of its formation should be of great interest.

Professor Robison and Dr. Macfarlane have made further studies of the migration of the phosphate group in fructose-6-phosphate on heating in acid solution. With the collaboration of Miss D. Toten, the purification of the laevorotatory product, which is presumably a phosphoric ester of fructo-pyranose, has been successfully carried out and the investigation of its properties is in progress.

The investigation of the highly reactive phospho-osone prepared from hexosediphosphate has been continued by Professor Robison and Dr. M. Laskowski (Rockefeller Foundation Grant).

Fermentation in the Living Cell.

Dr. Macfarlane has continued her work on the intermediate changes occurring during the breakdown of carbohydrate by living cells, using as material, yeast and *B. coli*. It appears that though the process is in many respects analogous to that known to take place in cell-free fermentation the quantitative relationship between the observed esterification of phosphate and the evolution of CO₂ are very different in the living cell from that in cell-free fermentations; the results will form a useful basis for the investigation of similar processes in animal tissues.

MAGNESIUM IN YEAST.

The biological importance of magnesium has long been recognised from its presence in chlorophyll and is apparent also from the experiments of McCollum who showed that if mice were fed on a diet containing less than 2 parts per million of magnesium, nervous symptoms and emaciation developed and the skeleton became completely dephosphorylated. The presence of this element is known to be essential for the fermentation of yeast and for the carbohydrate metabolism of muscle. If unknown organic substances containing magnesium occur in yeast or animal cells, knowledge of the structure and function of these would be of very great interest.

Work on the organic substance containing magnesium isolated by Dr. I. Smedley-MacLean from yeast has been continued with the help of Miss M. Roberts; and the methods of isolation and purification have been improved. It occurs only in very small quantity, 1 kilogram of dried yeast giving about 25 milligrams of the substance, equivalent to 2 parts magnesium per million of yeast. This substance contains 8% magnesium and 4% nitrogen and progress is being made with the elucidation of its structure.

METABOLISM OF FAT.

The formation of fat by yeast has been further studied by Dr. Smedley-MacLean and Mr. L. D. Macleod (Morna Macleod Research Student). It has not hitherto been recorded that the cations present in the medium exert a marked influence on the storage of both carbohydrate and fat. Calcium added to a fermenting glucose solution produces a marked inhibition of storage products; sodium lowers the amount of carbohydrate stored. Evidence has been found suggesting that there is a direct path from acetic acid to fat without previous conversion of the acetate to carbohydrate.

The structure of the lactonic acids isolated as oxidation products of the acids of linseed oil has been further investigated by Dr. Smedley-MacLean and Mr. L. C. A. Num, and the structure of one of these has been established. A series of oxidation products of linoleic and linolenic acids has been prepared and these as well as pure linoleic and linolenic acids have been biologically tested by Miss Hume and Miss Henderson Smith. The findings of Burr and Burr that the rat is unable to synthesise linoleic acid and that its continued deficiency in the diet leads to failure of growth and development of epidermal symptoms has been confirmed.

OTHER BIOCHEMICAL STUDIES.

Active principles of Hashish. The fractionation of *Cannabis indica* extracts has been continued by Dr. Work. By chromatographic methods it has been possible to free the resin from cannabinol and to obtain a product which is of low toxicity and lacks the convulsant action of cannabinol, while showing a marked positive reaction (absence of corneal reflex) in the Gayer hashish test on rabbits.

The arrow poison Erythrophlein. Dr. Todd has continued the investigation of the arrow poison erythrophlein. The amorphous alkaloid yields on mild hydrolysis a crystalline nitrogen-free acid, erythrophleic acid, dehydrogenated by selenium to a phenanthrene derivative, a substance $C_{19}H_{16}Se$ being obtained as a by-product.

Derivatives of oestrone. Dr. Bergel and Dr. Todd have, in connection with other work, prepared the 3-diethylamino-ethyl ether and the 3- β -naphthoate of oestrone. The former is entirely devoid of oestrogenic activity, but the latter, a sparingly soluble substance, produces a prolonged oestrus in rats when administered in low doses. The effect of oestrone 3- β -naphthoate is similar to, but rather more intense than, that of the benzoate prepared by other workers.

Influence of Hormones on Mammary Secretion. Continuing his work on the metabolism of galactose and the secretory activity of the mammary gland, Dr. G. A. Grant (Beit Memorial Research Fellow) has shown that prolactin reinstates only a limited milk secretion in the regressing mammarys of female guinea-pigs. The acinar tissue of such glands must be reconditioned before it can enter on another cycle of secretion. This can be accomplished by administration of oestradiol together with progesterone, after which the gland becomes remarkably responsive to prolactin.

Dr. Grant returned to Canada in August, 1937.

NATIONAL COLLECTION OF TYPE CULTURES.

(Medical Research Council.)

During the year under review the work of the Collection has shown considerable expansion, the number of cultures distributed to workers at home and abroad, for the first time in the history of the organisation, exceeding 6,000. Over two hundred strains, including many new and interesting types, were deposited for maintenance.

The policy of preserving strains of bacteria by means of high vacuum desiccation has been actively pursued and all new types are, whenever possible, in addition to or in the place of routine subculture, conserved by this method. The results obtained so far have been very encouraging and it is hoped that by this technique the routine work of maintenance will be greatly facilitated.

GENERAL AND FINANCIAL.

The Accounts and Balance Sheet for the year ending December 31st, 1937, show balances to the credit of the Sinking Fund of £31,032 7s. 9d., the Jenner Memorial Research Studentship Fund of £8,681 15s. 11d., the Pension Fund of £27,311 2s. 0d., the Contingency Fund of £22,000 0s. 0d. and the Capital Fund of £540,790 6s. 10d., after adding the balance of income over expenditure for current year, viz., £5,057 0s. 11d.

The balance of income over expenditure of the Pension Fund, viz., £720 19s. 6d., has again been transferred to the General Fund Account as a contribution towards the amount expended as premiums on policies under the Federated Superannuation System for the Staff.

The following changes in investments have taken place during the year, viz. :—

General Fund :—£2,900 Commonwealth of Australia 3½% Registered Stock 1936/37 converted into £2,900 Commonwealth of Australia 3½% Registered Stock 1950/52.

Sinking Fund : £1,650 3½% Conversion Stock 1961 or after has been purchased.

Income for the year amounted to £53,867 2s. 2d. Compared with 1936 there was an increase in Interest and Dividends on General Fund Investments of £359 6s. 1d., but a decrease of £417 4s. 4d. in Investigation, Diagnosis and Analysis Fees. Sales of Sera, Vaccines, etc., amounted to £23,536 15s. 4d., but after adjusting stocks on hand at the beginning and end of the year this item appears in the Accounts as £20,675 7s. 4d.

Expenditure during the year was £48,810 1s. 3d. against £56,516 7s. 4d. in 1936. Apart from considerable decreases in Serum and Vaccine Laboratories Expenses, and in Alterations, Repairs and Renewals, mainly at the Serum Department, items of expenditure showed little change.

The Governing Body gratefully acknowledges a legacy amounting to £823 7s. 6d. from Mr. John George Mills of 4, Southern Road, Bournemouth. This has been funded and is being utilised specifically for cancer research in accordance with the terms of the bequest.

In conclusion the Governing Body desires to express its appreciation of the devoted co-operation of the Director and all members of the staff, and also of the assistants, in carrying out the work of the Institute.

WILLIAM BULLOCH,

Chairman of the Governing Body.

BALANCE SHEET

AND

ACCOUNTS

The Lister Institute

BALANCE SHEET

	£	s.	d.		£	s.	d.
CREDITORS					4,085	2	2
SINKING FUND to 31st December 1937					31,032	7	9
JENNER MEMORIAL RESEARCH STUDENTSHIP FUND:—							
As per Account at 31st December 1936				8,653	9	11	
Add Amount transferred from Income and Expenditure Account, 1937				28	6	0	
					8,681	15	11
PENSION FUND to 31st December, 1937					27,311	2	0
CONTINGENCY FUND—							
As per Account at 31st December 1934					22,000	0	0
CAPITAL FUND to 31st December 1937:—							
Donations, &c., received to date from the following:—							
Dr. Ludwig Mond (1893)				2,000	0	0	
The Berridge Trustees (1893/98)				46,379	10	1	
The Grocers' Company (1894)				10,000	0	0	
Lord Iveagh (1900)				250,000	0	0	
Lord Lister's Bequest (1913/23)				18,904	5	8	
William Henry Clarke Bequest (1923/6)				7,114	5	7	
Rockefeller Foundation (1935/6)				3,400	0	0	
Other Donations and Legacies (1891-1934)				20,971	18	3	
Income and Expenditure Account:—							
As per Account at 31st December, 1936.. .. .	176,963	6	4				
Add Balance for the year ending 31st December, 1937	5,057	0	11				
					182,020	7	3
					540,790	6	10

WILLIAM BULLOCH, *Chairman.*

JOHN ANDERSON, *Hon. Treasurer.*

£683,900 14 8

REPORT OF THE AUDITORS

We have audited the above Balance Sheet. We have obtained all the information and explanations we have required, being held by the Institute on their behalf. In our opinion, such Balance Sheet is full and fair, and properly drawn and the explanations given to us and as shown by the books of the Institute,
London, 29th April, 1938.

of Preventive Medicine.

31st DECEMBER, 1937.

CASH:—							£	s.	d.	£	s.	d.
At Bankers: On Deposit	..	..	..	..	..	..	3,000	0	0			
Current Accounts	..	..	..	..	..	..	4,534	18	3			
In hand	..	..	..	..	..	..	33	16	10			
INVESTMENTS, GENERAL FUND (at cost, less amounts written off):—												
£80,000 4 per cent. Consolidated Stock, 1957 or after	..	..	..	..	..	..	74,272	16	0			
£25,000 4½ per cent. Conversion Stock, 1940-44	..	..	..	..	..	..	21,669	3	3			
£17,000 5 per cent. Conversion Stock, 1944-64	..	..	..	..	..	..	15,997	0	7			
£52,000 4 per cent. Funding Stock, 1960-90	..	..	..	..	..	..	45,661	13	9			
£64,000 3½ per cent. War Stock, 1962 or after	..	..	..	..	..	..	63,407	13	5			
£37,000 Local Loans 3 per cent. Stock	..	..	..	..	..	..	20,820	1	7			
£3,000 Port of London 3½ per cent. Registered Stock, 1965-75	..	..	..	..	..	..	2,686	17	7			
£1,000 Dominion of Canada 4 per cent. Registered Stock, 1940-60	..	..	..	..	..	..	928	4	6			
£25,000 Cape of Good Hope 3 per cent. Consolidated Stock, 1933-43	..	..	..	..	..	..	23,850	0	0			
£25,000 Natal 3 per cent. Consolidated Stock, 1929-49	..	..	..	..	..	..	21,400	0	0			
£8,000 New South Wales 4 per cent. Inscribed Stock, 1942-62	..	..	..	..	..	..	8,040	1	4			
£25,000 New Zealand Government 3 per cent. Inscribed Stock, 1945	..	..	..	..	..	..	22,114	0	0			
£26,100 South Australian Government 3 per cent. Consolidated Stock, 1916 or after	..	..	..	..	..	..	16,800	0	0			
£2,900 Commonwealth of Australia 3½ per cent. Registered Stock, 1950-52	..	..	..	..	..	..	2,723	16	0			
£1,300 Union of South Africa 4 per cent. Consolidated Stock, 1943-63	..	..	..	..	..	..	1,327	9	0			
£25,000 Victorian Government 3 per cent. Consolidated Inscribed Stock, 1929-49	..	..	..	..	..	..	19,800	0	0			
£4,000 Western Australia Government 4 per cent. Inscribed Stock, 1942-62	..	..	..	..	..	..	4,081	3	0			
£20,000 Southern Railway Preferred Ordinary Stock	..	..	..	..	..	..	13,500	0	0			
£6,200 London & North Eastern Railway 3 per cent. Debenture Stock	..	..	..	..	..	..	3,961	0	0			
£5,000 Great Central and Midland Railway Joint Committee 3½ per cent. Guaranteed Stock	..	..	..	..	..	..	3,623	0	0			
£353 London & North Eastern Railway 4 per cent. First Guaranteed Stock	..	..	..	..	..	..	499	11	0			
£8,650 London, Midland & Scottish Railway 4 per cent. Preference Stock	..	..	..	..	..	..	7,960	0	0			
£15,625 London, Midland & Scottish Railway 4 per cent. Preference Stock, 1923	..	..	..	..	..	..	11,300	0	0			
£18,750 London & North Eastern Railway 4 per cent. First Preference Stock	..	..	..	..	..	..	13,028	6	7			
£25,000 East Indian Railway 3 per cent. New Debenture Stock	..	..	..	..	..	..	13,890	0	0			
£661 Madras & Southern Mahratta Railway 4 per cent. Debenture Stock, 1938	..	..	..	..	..	..	656	19	7			
£800 Grand Trunk Railway Company of Canada Great Western Borrowed Capital 3 per cent. Perpetual Debenture Stock	..	..	..	..	..	..	936	0	0			
£1,937 Grand Trunk Railway Company of Canada 4 per cent. Guaranteed Stock	..	..	..	..	..	..	1,733	9	0			
£800 Ontario and Quebec Railway 5 per cent. Permanent Debenture Stock	..	..	..	..	..	..	981	0	0			
£3,400 Gas Light and Coke Company Ordinary Stock	..	..	..	..	..	..	3,638	0	0			
							441,298 17 2					
INVESTMENTS, SINKING FUND (at cost):—												
£9,600 4½ per cent. Conversion Stock, 1940-44	..	..	..	..	..	..	8,806	16	7			
£10,200 4 per cent. Funding Stock, 1960-90	..	..	..	..	..	..	9,079	0	1			
£14,800 3½ per cent. Conversion Stock, 1961 or after	..	..	..	..	..	..	13,023	1	5			
Balance uninvested	..	..	..	..	..	..	123	9	8			
							31,032 7 9					
INVESTMENTS, JENNER MEMORIAL RESEARCH STUDENTSHIP FUND (at cost):—												
£2,650 Southwark and Vauxhall Water Co. 3 per cent. Debenture Stock "B"	..	..	..	..	..	..	2,756	10	0			
£1,596 Southern Railway 5 per cent. Preference Stock	..	..	..	..	..	..	2,740	5	0			
£1,300 Liverpool Corporation 3 per cent. Stock, 1942, or after	..	..	..	..	..	..	1,097	6	9			
£2,000 4 per cent. Funding Stock, 1960-90	..	..	..	..	..	..	1,797	14	0			
Balance uninvested	..	..	..	..	..	..	290	0	2			
							8,681 15 11					
INVESTMENTS, PENSION FUND (at cost):—												
£22,000 4 per cent. Funding Stock, 1960-90	..	..	..	..	..	..	17,165	3	5			
£13,000 3½ per cent. Conversion Stock, 1961 or after	..	..	..	..	..	..	10,038	1	5			
Cash Balance	..	..	..	..	..	..	107	17	2			
							27,311 2 0					
(The book value of the above Investments is, in the aggregate, less than their market value at 31st December 1937.)												
DEBTORS							7,104 10 7					
FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS:—												
*At cost less depreciation as per account 31st December 1920	..	..	..	..	..	..	2,471	17	2			
Cost of Ultracentrifuges, purchased in 1936, less amount written off	..	..	..	..	..	..	3,060	0	0			
							5,531 17 2					
EXPENDITURE ON INSTITUTE BUILDINGS AT CHELSEA:—												
As per account 31st December 1935, including purchase of freehold site, £6,000	..	..	..	..	..	..	73,548	3	1			
FREEHOLD LAND ADJOINING THE "STUDIOS" CHELSEA at cost (1912)	..	..	..	..	..	..	169	6	8			
LEASE OF THE "STUDIOS" CHELSEA, as per last account	..	..	..	..	..	..	1,067	4	9			
Less amount written off for the year	..	..	..	..	..	..	65	2	0			
							1,002 2 9					
QUEENSBERRY LODGE ESTATE, ELSTREE:—												
Freehold land and buildings (at cost) as per account 31st December 1912	..	..	..	..	..	..	20,455	10	0			
STOCK OF ANIMALS	..	..	..	..	..	..	643	0	0			
STOCK OF ANTITOXINS	..	..	..	..	..	..	9,553	6	6			
							10,196 6 6					
Nothing has been charged for depreciation of Furniture, &c. since new purchases made during the year to a greater amount than the estimated depreciation (10%) have been written off.												
							£633,900 14 3					

INCOME AND EXPENDITURE ACCOUNTS

INCOME.

General

	£	s.	d.
Interest and Dividends on General Fund Investments	19,778	5	4
Interest on Sinking Fund Investments	1,300	5	0
Investigation, Diagnosis and Analysis Fees, &c.	2,337	15	0
Sales of Sera, Vaccines, &c., and Stock at 31st December 1937, less Stock at 1st January, 1937	29,675	7	4
Rent of Rooms	54	10	0
Pension Fund—excess of Income over Expenditure transferred	720	19	6

£53,867 2 2

Pension

	£	s.	d.
Interest on Investments	1,335	0	0

£1,335 0 0

Jenner Memorial Research

	£	s.	d.
Interest and Dividends on Investments	278	6	0

£278 6 0

Morna Macleod Research

	£	s.	d.
Balance from last account	118	2	6
Cash received from W. A. Macleod, Esq.	105	0	0

£223 2 6

Cancer Research

	£	s.	d.
Legacy from John George Mills	823	7	6

£823 7 6

Preventive Medicine.

for the year ending 31st December, 1937.

EXPENDITURE.

Fund.

	£	s.	d.
Rent, Rates, Taxes and Insurance...	1,415	15	0
Salaries and Wages of Staff	28,776	1	5
Premiums on Federated Superannuation Policies	1,837	17	2
Stationery, Printing and Postage	373	14	8
Printing of Collected Papers	226	11	7
Office Expenses, Auditors' Fee and Sundries	293	12	4
Travelling Expenses	58	18	9
Gas, Water and Fuel	1,626	17	7
Electric Light and Power	437	4	5
Nutrition and Protozoological Expenses	570	5	4
Bacteriological and Experimental Pathology Expenses, including Apparatus	391	17	0
Water and Bio-chemical Laboratory Expenses	612	16	3
Bio-physics Expenses	185	18	7
Serum and Vaccine Lymph Laboratories Expenses, including Apparatus and Bottles	2,897	4	4
Culture Media	109	7	7
Animals	1,771	8	11
Animal House Expenses and Forage	3,839	10	1
Alterations, Repairs and Renewals, including Workshop Expenses	1,251	5	5
Library Expenses	295	17	1
General Stores	218	14	0
Amounts written off Lease of the "Studios," Chelsea and Ultracentrifuges	405	2	0
Sinking Fund ($\frac{1}{2}$ % per annum on Cost of Buildings and Interest on Investments)	1,724	1	9
Balance, transferred to Capital Fund	5,057	0	11
	<u>£53,867</u>	<u>2</u>	<u>2</u>

Fund.

	£	s.	d.
Pensions	614	0	6
Balance, transferred to General Income and Expenditure Account	720	19	6
	<u>£1,335</u>	<u>0</u>	<u>0</u>

Studentship Fund.

	£	s.	d.
Salary of Student	250	0	0
Balance, transferred to Balance Sheet	28	6	0
	<u>£278</u>	<u>6</u>	<u>0</u>

Studentship Account.

	£	s.	d.
Salary of Student	157	10	0
Balance unexpended	65	12	6
	<u>£223</u>	<u>2</u>	<u>6</u>

Account.

	£	s.	d.
Sundry Expenses	68	3	9
Balance unexpended	755	3	9
	<u>£823</u>	<u>7</u>	<u>6</u>

SCIENTIFIC PAPERS PUBLISHED FROM THE LABORATORIES OF THE INSTITUTE DURING THE YEAR.



AMIES, C. R.	...	...	VACCINIA ELEMENTARY BODIES: THE PRODUCTION OF HOMOGENEOUS SUSPENSIONS OF THE BODIES AND THE HISTOLOGY OF THE ASSOCIATED SKIN LESIONS. <i>Journal of Pathology and Bacteriology</i> , Vol. XLVI., 1938.
BARRATT, J. O. W.	...	...	THE ACTION OF ANTICOAGULANTS. <i>Journal of Physiology</i> , Vol. 90, 1937.
BERGEL, F. AND TODD, A. R.	...	...	ANEURIN. PART VIII. SOME ANALOGUES OF ANEURIN. <i>Journal of the Chemical Society</i> , 1937.
"	"	"	ANEURIN. PART IX. A NEW SYNTHESIS OF THIOCHROME. NOTE ON THE SYNTHESIS OF ANEURIN. <i>Journal of the Chemical Society</i> , 1938.
BERGEL, F., TODD, A. R. AND WORK, T. S.			STUDIES ON VITAMIN E. PART III. OBSERVATIONS ON THE STRUCTURE OF α - AND β -TOCOPHEROL. (<i>Ibid.</i>)
BERGEL, F., JACOB, ANNIE, TODD, A. R. AND WORK, T. S.			VITAMIN E. STRUCTURE OF β -TOCOPHEROL. <i>Nature</i> , Vol. 141, 1938.
BIRCH, T. W., CHICK, HARRIETTE AND MARTIN, C. J.			EXPERIMENTS WITH PIGS ON A PELLAGRA-PRODUCING DIET. <i>Biochemical Journal</i> , Vol. XXXI., 1937.
CHICK, HARRIETTE, MACRAE, T. F., MARTIN, A. J. P. AND MARTIN, C. J.			CURATIVE ACTION OF NICOTINIC ACID ON PIGS SUFFERING FROM THE EFFECTS OF A DIET CONSISTING LARGELY OF MAIZE. <i>Biochemical Journal</i> , Vol. XXXII., 1938.
"	"	"	EXPERIMENTS WITH PIGS ON A PELLAGRA-PRODUCING DIET. II. (<i>Ibid.</i>)
COPPING, ALICE M. AND ROSCOE, MARGARET H.			THE WATER-SOLUBLE B-VITAMINS IN YEAST, FLOUR AND BREAD. <i>Biochemical Journal</i> , Vol. XXXI., 1937.
EAGLES, G. H., EVANS, P. R., FISHER, A. G. T. AND KEITH, J. D.			A VIRUS IN THE AETIOLOGY OF RHEUMATIC DISEASES. <i>The Lancet</i> , Vol. II., 1937.
"	"	"	INFECTION EXPERIMENTS WITH VIRUS-LIKE BODIES FROM RHEUMATISM. <i>Journal of Pathology and Bacteriology</i> , Vol. XLVI., 1938.

- ELLINGER, P. ... LYOCHROMES IN THE KIDNEY, WITH A NOTE ON THE QUANTITATIVE ESTIMATION OF LYOCHROMES. *Biochemical Journal*, Vol. XXXII., 1938.
- ELLINGER, P., HASSAN, A. ... PELLAGRA IN EGYPT. *The Lancet*, Vol. II., 1937.
AND TAHA, M. M.
- " " " ... THERAPEUTIC TRIALS ON PELLAGRINS IN EGYPT. (*Ibid.*)
- FAVILLI, G. AND McCLEAN, D. ... THE INFLUENCE OF TISSUE PERMEABILITY ON LOCAL IMMUNITY. *Journal of Pathology and Bacteriology*, Vol. XLV., 1937.
- FELIX, A. AND GARDNER, A. D. ... THE SERUM DIAGNOSIS OF ENTERIC FEVER. *Bulletin of the Health Organisation of the League of Nations*, Vol. VI., 1937.
- FELIX, A. AND PETRIE, G. F. ... THE PREPARATION OF ANTITYPHOID SERUM, IN THE HORSE, FOR THERAPEUTIC USE IN MAN. *Journal of Hygiene*, Vol. XXXVIII., 1938.
- GORER, P. A. ... THE GENETICS OF CANCER IN THE MOUSE. *The Lancet*, Vol. II., 1937.
- GORER, P. A. AND SCHÜTZE, H. ... GENETICAL STUDIES ON IMMUNITY IN MICE. II. CORRELATION BETWEEN ANTI-BODY FORMATION AND RESISTANCE. *Journal of Hygiene*, Vol. XXXVIII., 1938.
- GRANT, G. A. ... THE INFLUENCE OF HORMONES ON THE SECRETORY ACTIVITY OF THE REGRESSING MAMMARY GLAND. *Biochemical Journal*, Vol. XXXI., 1937.
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THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE.

Report of the Governing Body,
1939.

CHELSEA BRIDGE ROAD,

LONDON, S.W.1.

June 16th. 1939.

The Lister Institute of Preventive Medicine,

CHELSEA BRIDGE ROAD, LONDON, S.W.1.

AND ELSTREE, HERTS.

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ANNUAL GENERAL MEETING
OF
The Lister Institute of Preventive Medicine,
June 16th. 1939.

REPORT OF THE GOVERNING BODY.

The Governing Body has the honour to present the Institute's 45th. Report.

GOVERNING BODY.

At its meeting on June 2nd last year the Council re-elected Professor W. Bulloch and Professor Sir Arthur Harden to represent it upon the Board until December 31st. 1939, and at the same time elected Professor H. R. Dean, M.D., F.R.C.P., LL.D., as its third representative in succession to the late Professor A. E. Boycott.

COUNCIL.

At the Annual General Meeting last year the three members of Council retiring were Sir Charles J. Martin and Lord Horder, representing the Members of the Institute, and Sir Joseph Arkwright, the representative of the Royal Society. Each of these representatives was re-appointed, while vacancies created in the representation of the Members of the Institute upon the Council by the deaths of Lt.-Col. G. W. Addison and Professor A. E. Boycott were respectively filled by the appointments of Sir John Anderson and Professor S. P. Bedson.

At the same meeting proposals by Sir Joseph Arkwright to increase the membership of the Council from 30 to 40 and to appoint three additional representatives of the Members of the Institute forthwith were approved and on his nominating Professor Major Greenwood, Professor C. R. Harington and Dr. P. Fildes, these were unanimously elected to the Council as representatives of the Members of the Institute.

The three members of Council, who in accordance with the Articles of Association, retire this year by rotation, but who are eligible for re-election, are Professor F. W. Rogers Brambell, the representative of the Royal Irish Academy, Professor S. P. Bedson, one of the representatives of the Members of the Institute and The President of the Royal College of Veterinary Surgeons, as the representative of that learned body.

MEMBERS.

The Governing Body regrets to report the deaths during the year of Sir E. Cooper Perry and Professor G. Barger, members of the Institute since 1918 and 1931 respectively.

STAFF.

Dr. G. F. Petrie, Bacteriologist-in-charge of the Serum Department since 1926 when he succeeded the late Dr. A. T. MacConkey, retires on attainment of the age limit, in June, 1939, after 37 years devoted service. He will be succeeded by Dr. C. R. Amies, a member of the Bacteriological Staff at Chelsea.

Dr. A. R. Todd, first assistant in the Department of Biochemistry resigned his post in September, 1938, on appointment as Sir Samuel Hall Professor of Chemistry and Director of the Chemical Laboratories in the University of Manchester. Dr. W. T. J. Morgan, who since 1929 had been Biochemist to the Serum Department was appointed to succeed him in January. The University of London Readership in Biochemistry held by Dr. Todd has been conferred on Dr. Morgan.

Dr. B. C. J. G. Knight, Halley Stewart Research Fellow and Senior Biochemist in the Research Unit directed by Dr. P. Fildes, F.R.S., at the Middlesex Hospital under the auspices of the Medical Research Council, was appointed Biochemist to the Serum Department in January.

Dr. Emmy Klieneberger previously Jenner Memorial Research Student was appointed to a Research Fellowship in Bacteriology, and Mr. L. C. A. Nunn, late grantee of the Department of Scientific and Industrial Research, to the Grocers' Company Research Scholarship in October.

Dr. T. S. Work, Research Student in Biochemistry resigned in August, on appointment to a post at the National Institute for Medical Research. Dr. S. M. Partridge, who had been engaged in research under Dr. J. Kenyon, F.R.S., at the Battersea Polytechnic, succeeded him in January, and Dr. D. E. Dolby, Demonstrator in the Department of Industrial Fermentation in the University of Birmingham was appointed Jenner Memorial Research Student on the same date.

Mr. L. D. Macleod, Morna Macleod Research Student and Mr. R. C. Burbank, Research Student in Endocrinology, completed the tenure of their respective studentships during the year.

RESEARCH WORK.

The Governing Body, before surveying the scientific work done during the year, desires once again to record its appreciation of the continued co-operation the Institute has enjoyed with the Medical Research Council, which has furnished the salaries of the staff of the National Collection of Type Cultures, Miss Hunne, Dr. Zilva and his assistants, and Miss K. Hall, assistant to Dr. Korenchevsky and part salaries of Dr. Korenchevsky, Miss Copping and Miss H. H. Smith. The Institute provided, as hitherto, accommodation and materials for the researches of each of these workers. Mr. Kekwick's salary and a grant for his expenses, and part-time grants to Dr. Eagles for research on rheumatism and to Dr. Ellinger for the investigation of Pellagra in Egypt have also been furnished by the Council.

Acknowledgment is also made to the British Empire Cancer Campaign for its co-operation in the researches being undertaken on cancer. This organisation furnishes the salaries of Dr. Gorer and Mr. Carr, together with expense grants for each of these workers.

The study of the factors which influence tissue permeability, the expenses for which have been provided by an anonymous donor, has been transferred to the University of Manchester where Dr. J. L. Madinaveitia is continuing his work on this problem under the supervision of Professor Todd.

The ultracentrifuge installed some years ago in the new Biophysics laboratory, together with other pieces of physical equipment since added, has been in constant use throughout the year and has given admirable service.

STUDIES ON VIRUSES.

Acute Rheumatism and Rheumatoid Arthritis. Dr. G. H. Eagles has continued his studies of a possible virus factor in the aetiology of rheumatic infections. Attempts to demonstrate the infective power of the virus-like particles procurable by high-speed centrifugation of exudates and pathological tissues in acute rheumatic fever, acute chorea and acute rheumatoid arthritis have not been successful. In certain instances monkeys under experiment have shown suggestive cardiac lesions but these on analysis could not be shown to be those of true rheumatic disease as encountered in man. This work, carried out in collaboration with Dr. P. R. Evans, Dr. J. D. Keith and Mr. A. G. Timbrell Fisher, F.R.C.S., has been published.

In collaboration with Dr. W. H. Bradley, working under a grant from the Medical Research Council in the Department of Medicine, the University, Cambridge, the relation of the agglutination of suspensions of virus-like particles from acute rheumatic exudates by rheumatic sera to the clinical course and concomitant streptococcal infection has been investigated. A study of 54 patients suffering from rheumatic fever, arthritis of the rheumatoid type (so-called unknown aetiology) or arthropathies not classified as true rheumatism, but with joint pain as a presenting symptom, has been made over several months. Although sera from human beings in normal health do not agglutinate rheumatic suspensions, agglutination has been elicited equally well by sera of the three groups under study. No consistent relation of agglutination to clinical phases was found nor any evidence that it runs parallel to antistreptolysin titre or erythrocyte sedimentation rate. The nature of the agglutination is, therefore, undetermined. It is possible that it may be related to the mutual precipitation occurring with rheumatic sera at certain phases of the rheumatic cycle, described recently by Coburn and Pauli. This is now under investigation.

Acute Rheumatism and the Pleuropneumonia group of organisms. Drs. Homer Swift and Brown of the Rockefeller Institute have recently announced the isolation of a pleuropneumonia-like organism from rheumatic fever exudates and it may be that the long sought for etiological agent of acute rheumatism has been found. In any case this finding opens up a new field of work and already Dr. Eagles and Dr. Helga Jahn are collaborating with Miss Klieneberger in the cultural study of rheumatic material, for the latter's experience during the past five years with representatives of this group of organisms (see later), will be invaluable. Should organisms of this group be recovered, steps will be taken to determine whether the "virus bodies" studied here by Dr. Amies and Dr. Eagles, which are agglutinated in the presence of sera from rheumatic fever and rheumatoid arthritis, are really the filterable elements of a pleuropneumonia-like organism or possibly break-down products antigenically related to such organism.

Avian Sarcoma. Dr. C. R. Amies and Mr. J. G. Carr have continued their studies on the nature of the filterable agents of the Rous I and other fowl sarcomata. Recent improvements in the methods of processing the tumour have made it possible to obtain adequate amounts of these agents in a highly concentrated and relatively pure form. Briefly, the method is as follows: A cell-free tumour extract is prepared and this is brought to a pH of 5.0 by the addition of a buffer solution. The effect of this is to cause a certain amount of aggregation of the tumour agent particles so that the latter are deposited when the extract is run through the clarifier bowl of a Sharples centrifuge. The deposit is then digested with trypsin at a pH of 9.0 and the agent, which is resistant to proteolysis, is recovered by high-speed centrifugation. By this procedure it is possible to obtain suspensions containing one million tumour-producing doses of the agent per cubic centimetre. The purity of the suspensions, however, is still below the standard required for physico-chemical analysis.

A new series of serological experiments carried out with these concentrated tumour agent suspensions has yielded still more evidence in support of the view that the agent is closely related antigenically to some constituent of normal fowl cells. It has been established, for example, that the serum of a rabbit which has been hyperimmunised with normal fowl protein will neutralise the tumour agent *in vitro*, and that these inhibiting properties can be removed entirely by absorption with a concentrated suspension of the tumour agent. The serum of a rabbit which has been repeatedly inoculated with concentrated tumour agent suspensions also contains antibodies capable of neutralising the agent *in vitro*; these are removed by absorption either with the tumour agent or normal fowl protein. In the fowl, the agent also behaves as a foreign protein; its presence in the malignant tissue and elsewhere eliciting the formation of neutralising antibodies and agglutinins. It is still too early to formulate any far reaching conclusions from these observations but it is believed that a full understanding of this phenomenon would constitute a big advance in our knowledge of the virus-induced tumours.

The Des Ligneris Sarcoma. A study has also been made of a filterable fowl tumour described by Dr. M. A. des Ligneris, of the South African Institute for Medical Research. This tumour originated in a fowl which had been inoculated with chicken fibroblasts grown in tissue cultures to which small amounts of dibenzanthracene had been added. Immunological experiments have shown that this tumour is not entirely distinct from the Rous I sarcoma, as has been claimed. The results obtained, in particular the fact that filtrates of the tumour will infect ducklings, have led to the view that this tumour is closely related to, if not identical with, the Fujinami sarcoma. Part of this investigation was carried out in collaboration with Dr. W. J. Purdy of the National Institute for Medical Research, Hampstead.

Cow Pox, Horse Pox and Swine Pox. By employing the methods used for vaccinia virus, Dr. Amies has obtained pure suspensions of elementary bodies from a strain of cow pox originally isolated from a case of "milker's dermatitis." As in the case of vaccinia, the gradual transition from inhomogeneous to homogeneous suspensions was secured by frequent serial passage with material obtained by fractional centrifugation. This change was accompanied by an alteration in the histology of the lesions. Originally the virus produced large compact eosinophil inclusions in the epidermal cells together with a pronounced reticulo-endothelial inflammatory response in the subjacent dermis. The homogeneous suspensions, however, give rise to multiple scattered inclusions similar to those produced by vaccinia elementary bodies and the reaction in the dermis is hardly noticeable.

A strain of variola vera, two strains of cow pox and one of horse pox were successfully passed by Dr. M. H. Salaman to the egg membrane, with the object of studying their serological relations in this medium. A strain of swine pox, received from Dr. Shope of Princetown, N.J., could not be passed to the egg membrane, or to mice, guinea-pigs or rats, but with the help of Professor T. Dalling of the Department of Animal Pathology, Cambridge, it was shown to be fully infective for swine.

Studies on the Virus of Measles. Undoubted successful propagation of the virus of measles experimentally in animals has never been achieved. More recently reports have appeared in support of the culture and propagation of the virus and have renewed interest in the successful experiments of Taniguchi in rabbits and guinea-pigs. During the past year Dr. Eagles has investigated the infective properties of fresh whole blood and filtered nasal washings from 21 cases of measles in the pre-eruptive and very early eruptive stages passed serially through rabbit and guinea-pig testes. In four cases acute orchitis appeared after a varying number of passages and could be carried on indefinitely. The appearance of the lesions was identical with that described by the Japanese workers. Virus III of rabbits was eliminated by the intense orchitis in the guinea-pig which is not susceptible to that virus. Agglutination and neutralisation tests showed that the infection was due to vaccinia virus accidentally contracted in spite of attempts to isolate the experimental animals and to avoid indiscriminate handling of animals in a laboratory where studies on vaccinia are in progress. The experiments are of interest only from this aspect and they may throw light on the claims of some earlier investigators. With a more rigid discipline it may be possible to avoid similar experiences in future when the problem is again attacked.

Antigenic structure of Vaccinia Virus. Following upon the work, reported last year by Dr. Salaman, on the effects of various agents on the antibody-absorbing power of vaccinia elementary bodies, rabbits were inoculated with large amounts of E.Bs. rendered non-infective by heat and formalin respectively. Precautions were taken against accidental infection with active virus.

Sera of rabbits which had received formalized E.Bs. all contained agglutinin in fairly high titre; virus-neutralizing power was variable but always present; precipitin was absent. Sera of rabbits which had received heated E.Bs. contained agglutinin in low titre, virus-neutralizing power was low or absent, and precipitin was absent. Resistance to subsequent infection with active virus was very variable in both groups, being definite in some animals, barely perceptible in others. In general the immune response of rabbits to these non-infective inocula differed from that of rabbits immunized with active virus chiefly by reason of its irregularity and the absence of precipitin-production.

Chemical nature and enzymic activity of Vaccinia Virus. Dr. M. G. Macfarlane has made a preliminary examination of the chemical nature of the fractions obtained by treatment of dried vaccinia virus with fat solvents. The first extraction with benzene or ether removes from 5% to 15% by weight of the dried virus, according to the number of preliminary clarifications of the suspensions of elementary bodies. This extract consists largely of cholesterol and acetone-soluble fat, with only traces of nitrogenous and phosphorus-containing substances. Extraction of the benzene-treated virus with a mixture of alcohol and ether removes a further fraction amounting to about 9% of the material. This fraction contains no cholesterol, no protein and only a trace of carbohydrate; judging from the phosphorus content it consists largely, though not entirely of lecithin-like substances. The residual virus material after extraction with alcohol-ether contains protein, carbohydrate and phosphorus. The material gives the Feulgen test characteristic of thymonucleic acid and the orcinol test for pentose; these reactions, together with the phosphorus content are consistent with the presence of 5-6% of nucleic acid. By comparison with a number of known sugars the carbohydrate content of the virus as estimated by Sørensen's orcinol method indicates the presence of some other carbohydrate in addition to that which may be present as thymonucleic acid.

Dr. Macfarlane in collaboration with Dr. Salaman, has continued the investigation of the enzymic activity of vaccinia virus suspensions. Homogeneous suspensions of the virus were previously shown to contain phosphatase and catalase; these enzymes have now been shown to be present in equal activity in the virus after extraction with benzene or ether. Phosphodiesterase is also present in this virus material, but no other positive results have been obtained; in particular the virus appears to lack the enzymes known to be concerned in the intermediate metabolism of glucose. This work is being continued in collaboration with Dr. D. E. Dolby.

Physical investigations on the elementary bodies of Vaccinia. The flocculation of this virus, hitherto regarded as an irreversible process, has been shown by Dr. A. S. McFarlane to be reversible. The dried virus is extracted with ether or benzene to remove non-specific lipoid, of which 5-15% is present, and is then ground in a close-fitting tissue grinder with a suitable salt solution. By repeating this process 60-70% of the virus is obtained in the form of a stable suspension of single virus particles. The infectivity is not affected by the treatment but the density is increased.

It has been shown that the virus particles depend for their stability on the presence of a well-marked Helmholtz double layer with which considerable amounts of water are associated. This layer on the surface of the particles forms an oriented ion atmosphere not ordinarily penetrable by substances soluble in water. It may be destroyed or reduced in thickness in various ways, notably by freezing, drying, high concentrations of salt, alcohol, acetone, etc. Destruction of the double layer leads at once to the formation of dense floccules and the layer may be reconstituted only by the expenditure of considerable amounts of mechanical work in the manner described.

Dry virus, which has been extracted exhaustively with benzene or ether, will give on treatment with alcohol a further 9% of lipoid which appears to be chemically bound in the virus. The residue now has the dry density and other physical characteristics of a single protein, but gives inhomogeneous boundaries in the Svedberg centrifuge.

The general physical behaviour of the virus particle suggests that it has a rigid type of internal structure as distinct from a fluid or protoplasmic constitution and its behaviour towards lipid solvents appears to preclude the existence of any familiar form of semi-permeable membrane.

Elementary body suspensions of Vaccinia for Jennerian prophylaxis. In co-operation with Dr. R. G. Henderson, London County Council, and other medical officers in that service, Dr. D. McClean (Elstree) has continued the experimental immunization by the intradermal route of groups of nurses and children with elementary body suspensions of vaccinia virus. The resulting immunity of all those who have been inoculated has been tested by re-vaccination by scarification with Government lymph after an interval of not less than six weeks. Although the nurses received an intracutaneous injection of a suspension of virus that was active in the rabbit skin at a dilution of 10^{-5} , it was found that a

small proportion were not immune to subsequent re-vaccination with lymph. Groups of children, who had not previously been vaccinated, were then immunized by the intracutaneous route with suspensions potent at a dilution of 10^{-3} and, later, at 10^{-4} . Local reactions were mild but definite and a small proportion of the children developed vesicles at the site of inoculation. The resulting immunity was, however, disappointing and there is some indication that the development of a firm immunity to further inoculation with the virus may depend upon the development of a typical vaccinal vesicle at the primary vaccination. This possibility is being investigated both in children and in laboratory animals. When applied by scarification, the suspension of elementary bodies produces typical vaccinal vesicles with general constitutional reactions that are indistinguishable from those produced by vaccine lymph. Groups of children and of animals have been vaccinated intracutaneously and by scarification with elementary body suspensions and, by scarification only, with ordinary lymph. The subsequent development of immunity in the different groups is being investigated. So far, the animal experiments have indicated that there is no significant difference in the degree of immunity attained following a single inoculation by either the intracutaneous route or by scarification. Observations are also being made on the smallest infective dose in rabbits that is followed by a satisfactory immune response to re-vaccination.

Preservation of elementary body suspensions of Vaccinia. Elementary body suspensions dried in the frozen state remain viable without appreciable loss of potency for at least a year at ordinary refrigerator temperatures. Dr. McClean has carried out observations on the resistance to heat of these dried suspensions. Exposure to 50°C . for 30 minutes does not appreciably reduce the potency of this material; a slight reduction follows a similar exposure to 75°C . Exposure to 100°C . causes a reduction of activity from a positive result at a dilution of 10^{-6} to a similar result at a dilution of 10^{-2} but, even at this temperature, the virus is not completely destroyed.

Elementary body suspensions in 1% peptone solution and in 50% glycerine have been kept in an incubator at 22°C . for several weeks. The fall in potency of the peptone suspension is very slow and the material would still be suitable for clinical use after several weeks at this temperature. The deterioration of the samples in glycerine is more rapid and is obvious after one week.

Cultivation of Vaccinia Virus on the chorio-allantoic membrane of the developing chick. Dr. McClean is carrying out serial passages on this membrane of (1) a strain of vaccinia virus which has been rendered physically "homogeneous" by previous serial passage on the rabbit and (2) the ordinary "heterogeneous" strain that is used in the routine production of vaccine lymph and of elementary body suspensions. It is hoped to observe whether any physical or immunological differences develop in either of these strains of virus as the result of cultivation in this membrane.

Plant Viruses. Dr. McFarlane and Mr. R. A. Kekwick have determined the molecular weight of the crystalline nucleo-protein causing Bushy Stunt disease. This virus has a symmetrical molecule whereas other plant viruses have rod-shaped molecules which are not amenable to exact methods of measuring particle size. For this reason more than usual significance attaches to the value for Bushy Stunt virus and to the correlation of it with estimates based on crystallographic data. The opportunity has also been taken to place on record certain other physical constants of the virus notably the dry density, isoelectric point, electrophoretic mobility and specific refraction increment. Dr. McFarlane has also completed a short study of the physical properties of the recently isolated virus of tobacco necrosis and is at present collaborating with Dr. Pirie and Dr. Bawden on an investigation of the disintegration products of Bushy Stunt virus.

SEROLOGICAL STUDIES: ANTIGENIC CONSTITUTION, VIRULENCE AND IMMUNISING PROPERTIES OF BACTERIA AND PROTOZOA.

Standardisation of therapeutic anti-typhoid serum. Dr. A. Felix with the assistance of Miss R. M. Pitt has continued his investigations on methods of titrating therapeutic anti-typhoid serum. The titration is based on the separate quantitative estimation of the two essential protective substances, the Vi and the O antibody. Each of the two antibodies is estimated by an *in vitro* and an *in vivo* method and in each of the tests a provisional standard serum forms the basis of the measurement of the Vi and the O antibody. The provisional standard serum had been prepared early in 1935 in co-operation with Dr. G. F. Petrie (Jelstree) and Dr. P. Hartley, Director of the Biological Standards Department, Medical Research Council. The serum was taken from the blood of the first horse that had been immunised with living bacilli of the rough avirulent Vi strain of *Bact. typhosum*, described by Felix and Pitt in 1935.

The Vi titres of the sera, as determined by the agglutination reaction, run parallel with their protective values as determined by tests in mice which received a test dose of living virulent typhoid bacilli. The O titres of the sera, as estimated by the agglutination reaction, run parallel with their protective values as determined by neutralisation tests in mice which received a lethal dose of killed typhoid

bacilli ("endotoxin"). Provided it is known that the Vi antibody contained in the sera does not belong to the variety characterised by the peculiar "functional deficiency" described by Felix and Bhatnagar in 1935, the Vi agglutinin titre as well as the O agglutinin titre can be accepted as the final measure of the therapeutic activities of the two antibodies. The routine titration of samples of serum taken in the course of preparation of therapeutic anti-typhoid sera consists, therefore, of agglutination tests alone, whereas for purposes of official control the mouse-protection test and the neutralisation test in the mouse are indispensable. The technical details of these tests are embodied in a paper.

At the request of Dr. Th. Madsen, Chairman of the Health Committee of the League of Nations, the paper by Dr. Felix was communicated, before publication, as a report to the Permanent Commission on Biological Standardisation. At a meeting of the Commission held in Paris, October 1938, it was decided that the report should be taken as the basis of an international comparative investigation, and that the anti-typhoid serum prepared by the Lister Institute and dried by Dr. Hartley should be used for this purpose.

Protective value of Vi and O antibodies in relation to virulence of *Bact. typhosum*. Dr. D. W. Henderson (Elstree) has obtained confirmation of his earlier observations and has shewn that the efficiency of the Vi or the O antibody in the prophylaxis of experimental infection of the mouse with *Bact. typhosum* is determined by the amount of bacterial antigen which accumulates in its tissues. The prophylactic dose of either of these antibodies is not determined by the virulence of the test strain but by the number of organisms in the test dose. Thus the Vi or the O antibody is relatively more efficient against infection with a small number of fatal doses prepared from a fully virulent strain than against the same number of fatal doses from a relatively avirulent strain. The Vi or the O antibody will protect mice against infection with a fully virulent Vi+O strain of *Bact. typhosum* but when a large number of fatal doses are given the Vi antibody is relatively more efficient than the O antibody.

Experiments with the Vi antigen and with two new antigenic substances extracted from strains of *Bact. typhosum*. In a previous report reference was made to certain immunological properties of the Vi and the O antigen obtained from *Bact. typhosum* by extraction with diethylene-glycol; the method originally introduced by Dr. W. T. J. Morgan for obtaining antigenic substances from *B. dysenteriae* (Shiga). Dr. Henderson has continued a study of the substances obtained from *Bact. typhosum* by this method and has shown that all the extracts examined so far contain two antigenic substances which are separate and distinct from the Vi and the O antigen. These substances which are obtained from rough as well as from smooth strains are apparently of a non-protein nature. One of them probably represents the surface antigen of a typical rough strain of *Bact. typhosum* and the source of the other may be the deeper layers of the bacterial substance. The earlier accounts of the functional deficiency of diethylene-glycol extracts containing Vi antigen have been confirmed. There is now evidence for believing that the portion of the Vi antigen molecule which is concerned in the production of complement-fixing antibody is structurally altered although extracts containing this antigen retain in part their power of stimulating the production of protective antibody. It is possible, therefore, that the groupings on the antigen molecule which are concerned in the production of complement-fixing antibody are not necessarily identical with those that stimulate the production of protective antibody.

Serological examination of the *Cl. welchii* group. In a previous report Dr. Henderson indicated the results of a preliminary examination of the bacterial antigens of members of this group. The close parallelism which was shown to exist between the complex toxin production and the antigenic structure of the complicated members of the group has been more fully investigated. A study of serological variants separated from certain strains indicates that this parallelism may be fortuitous; thus the loss of a particular antigenic component has been shown to occur independently of any alteration in the kinds of toxin elaborated. These researches which have progressed only slowly over a period of several years are now being collated and prepared for publication.

Plague prophylaxis. Dr. H. Schütze has completed his investigation of prophylactic plague vaccines. It has always been assumed that in the preparation of Haffkine vaccines (heat-killed, broth cultures) a highly virulent strain of *B. pestis* is essential. He has compared the respective merits of virulent and avirulent, smooth and rough variants in the manufacture of such vaccines and finds that they all give equally potent preparations. In the rat where the test dose was a large and toxic one, prophylaxis depended to a considerable degree upon the envelope antigen contained in the vaccine which should, therefore, be made from cultures grown at 37°C. In the mouse where the test dose was a small and invasive one, envelope antigen did not exert so marked an effect.

In the course of the experiments non-enveloped variants of *B. pestis* have been encountered for the first time. One of these variants was derived from a rough avirulent strain, but the other was smooth and virulent like the culture from which it originated.

Plague therapy. Dr. Schütze has reported the therapeutic effect on plague infection of some of the new chemical substances which have proved so successful in the treatment of a variety of other diseases. The drugs chosen to that end were 2-sulphanilyl-aminopyridine (M. and B. 693), Soluseptasine and a diamino-diphenylsulphone glucoside. Experiments were carried out on both the rat and the mouse and demonstrated a considerable curative effect on the part of all three compounds. M. and B. 693 prove most efficacious against plague in both series of animals, while Soluseptasine protected rats and not mice and the sulphone compound protected mice but not rats.

Prophylactic vaccination in the course of an epidemic. The desirability of carrying out anti-typhoid vaccination in a population which has been exposed to typhoid infection, has been questioned by certain authorities on the ground that undesirable reactions may rather precipitately intervene, followed perhaps by the appearance of the frank disease in persons who happen to have taken the causative organisms into their systems. Such persons may be supposed to be at the time of vaccination either genuinely incubating the disease in which case vaccination may, it is alleged, accelerate the onset of symptoms or they may be still mere temporary carriers, in which case the stimulus of vaccination may convert an otherwise innocent and temporary carrier-state to one of frank infection. Correspondence on this subject appeared in the medical press just over two years ago in the light of advice, tendered to authorities concerned with the control of a typhoid outbreak, that such possible happenings contra-indicated prophylactic vaccination. The opposite view was taken by the Director of the Institute but careful criticism of the evidence adduced, both pro and con, made it highly desirable that the problem should, if at all possible, be submitted to experimental test on animals.

In an attempt to find an answer to this problem Dr. Schütze has taken the mouse as the experimental animal and *S. typhi murium* as the infecting organism. It is impossible to immunise mice solidly against this infection, but valuable data can be obtained by noting the death rate in the course of an observation period of three weeks.

Though presumably vaccination during the incubation period has but little time in which to effect some increase of resistance, yet the results of a large series of experiments demonstrate not only that vaccination started after infection fails to damage the animal's normal resistance but, in a considerable proportion of cases, actually succeeds in enhancing it, when the survival rate is taken as criterion.

Antibody production in response to immunisation with Protozoa. During the past year the study by Dr. Muriel Robertson of the immune reactions *in vitro* of ciliates belonging to the species *Glaucoma* to antibodies produced against them in the blood of rabbits has been concluded and an analysis of some of the antigenic properties has been made. It was shown that these organisms possess at least two elements in the antigenic complex, viz., (1) the sheath antigen and (2) the heat-labile element in the exudate which the ciliates pour out under certain conditions. The sheath antigen is apparently alcohol- and acid-resistant and is to a certain extent heat-stable. The exudate which the organism can be induced to pour out without being injured contains some sheath substance but also a heat-labile antigen. This heat-labile substance evokes the antibody which brings about agglutination and also some degree of immobilisation when in contact with the living homologous organisms. The sheath substance calls forth an immune body causing the ciliates to secrete a clearly visible envelope or sheath which, in the highest concentration, imprisons the organism. In lower concentrations the sheaths are cast off and the animal swims freely again. The appropriate use of sera made from antigens treated in various ways in absorption experiments has demonstrated that the agglutinin produced by the heat-labile antigen combines with complement to produce death with lysis. The sheath-reacting antibody combines with complement to kill but not to lyse the ciliates. It interferes with the lytic action when both immune bodies are present.

STUDIES ON PURE LINES.

Immunity to tumour-transplantation. Dr. P. A. Gorer's previous work has shown that immunity to transplanted tumours depends upon the presence of genetically determined antigenic differences between transplant and host. It is possible, however, that antigenic differences between malignant and normal cells might also afford an efficient immunising stimulus under certain conditions. If this is so, it should be possible to immunise a genetically homogeneous population (pure line) against a growth which has spontaneously arisen in one of its own members.

Two strains of mouse leukaemia have been transmitted within two different pure lines (albinos and blacks) and have given 100% "takes" within the homologous line and 0% in the heterologous line.

Attempts have been made by Dr. Gorer to immunise the *homologous* pure line as follows:—

- (1) By means of previous injection of heterologous normal adult tissues, embryonic tissues and malignant tissues of heterologous origin.
- (2) By injecting homologous and heterologous malignant tissues together, in the hope that the latter would stimulate a reaction on the part of the reticulo-endothelial system which would result in the destruction of both types of malignant cell.

(3) By giving gradually increasing doses of homologous leukotic cells.

The first of these treatments appears to have been without effect, the second and third appear to accelerate death from the homologous leukaemia.

An interesting corollary has been found in connection with the incidence of *spontaneous* leukoses in the blacks; members of this line that have been hyperimmunised with "albino" sarcoma tissue (to which they are immune) develop spontaneous leukoses about 6 times as frequently as untreated controls.

Renal disease in pure lines of mice. During routine post-mortem examinations, performed by Dr. Gorer to determine the incidence of spontaneous neoplasms, interesting data have been obtained concerning the incidence of renal disease in 3 pure lines (Strong's A (albino), Little's C57 (black) and Strong's CBA (agouti)).

A peculiar type of glomerular lesion may be found in mice of any strain under a variety of conditions; this consists of a columnar-celled metaplasia of the glomerular capsule so that the tuft appears as though it were situated in a tubule.

Members of Strong's A strain that survive 12 months almost all develop a cystic condition of their kidneys that somewhat resembles congenital cystic kidney. The disease is usually fatal before the animals are 20 months old.

Members of Little's C57 strain develop a hyaline degeneration of the glomeruli at ages over 18 months which somewhat resembles amyloid but fails to give the typical staining reactions. Members of this strain frequently survive 2 years.

Neither of these lesions has been identified as yet in the relatively small number of CBA kidneys that have been examined to date. Members of this strain are long-lived and frequently survive over 2 years.

THE CHEMICAL NATURE OF BACTERIAL ANTIGENS.

B. dysenteriae (Shiga). Whilst working at Zürich Dr. Morgan continued his work on this subject and succeeded in isolating *D*-galactose and *L*-rhamnose from the specific polysaccharide component of the antigen of *B. dysenteriae* (Shiga). All attempts to isolate and identify the supposed *N*-acetylhexosamine failed. The phospholipin component of the antigen was saponified and palmitic acid and oleic acid were isolated from the hydrolysis products. As a result of further work which has been completed since Dr. Morgan's return, α -glycerophosphoric acid has also been isolated and the whole lipid material is considered to be a monoamino-monophosphatide possessing a nitrogen-phosphorus ratio of unity.

Dr. Morgan and Dr. S. M. Partridge are examining the acid and water-insoluble component that separates from solution during the hydrolysis of the antigenic complex with dilute acetic acid. The purified material contains about 11% nitrogen, part of which appears to be in the form of polypeptide and part as "purine" nitrogen; this fraction also contains phosphorus in organic combination and carbohydrate. These facts are consistent with the presence of a nucleotide in the antigenic complex.

In collaboration with Dr. E. Jolles, Dr. Morgan has prepared a number of derivatives of chondrosamine with the object of simplifying the isolation of this hexosamine from mixtures containing hexoses and pentoses. The experience gained with chondrosamine will be useful in designing methods whereby the hexosamine present in the hydrolysis products of the specific polysaccharide of *B. dysenteriae* (Shiga) may be isolated and identified.

PHYSICO-CHEMICAL INVESTIGATIONS.

Serum Proteins. Mr. Kekwick has continued to investigate the behaviour in the ultracentrifuge and electrophoresis apparatus of normal and pathological serum. The diagonal "schlieren" optical method introduced last year in the ultracentrifuge has been used consistently for this work and Mr. Kekwick has applied Lamm's scale method to the electrophoresis apparatus with considerable advantage. The electrophoretic constitution of normal human serum has been established and shown to differ fundamentally from the picture obtained with various pathological human sera. Pathological states for which a series of cases were available for study include Multiple Myeloma (in collaboration with Dr. G. Z. Walker, Edinburgh Royal Infirmary), Kala Azar (with Dr. E. S. Horgan, Khartoum) and Acute Rheumatism (with Dr. W. H. Bradley, Cambridge). The abnormalities found in the protein constitution of these sera were well marked and consistent for a given disease. It is not possible to say how specific they are until a wider pathological field has been investigated. The sensitivity of the method of investigation is increased by electrophoretic separation of new serum components and their chemical investigation. Mr. Kekwick has in this way obtained a globulin from human myeloma serum and is comparing its properties with those of the corresponding globulin isolated from normal human serum.

In the field of animal anti-sera Mr. Kekwick has collaborated with Dr. Amies, Dr. Salaman and Dr. Morgan in studies on anti-vaccinal and anti-Shiga serum. The serum changes produced by

immunisation in these two cases are very similar, but unfortunately quantitatively slight, in comparison with the gross changes which occur for example in anti-diphtheria and anti-pneumococcal sera. This raises a technical difficulty in isolating by electrophoretic methods quantities of pure antibody adequate for further investigation. Experiments are in progress to obtain an anti-Shiga serum which will be more suitable for the mass preparation of pure antibody by electrophoretic means. Fractions separated by electrophoresis from anti-vaccinia serum were tested by Dr. Salaman for antibody-content. The antibody was found to be in association with the globulin but further work will be required to determine the precise distribution of the antibody among the various globulins separated by electrophoretic methods.

The state of the proteins in native egg-white. Prof. E. G. Young has investigated the behaviour of native egg-white in the ultracentrifuge and electrophoresis apparatus and finds one predominant particle size but five well-marked electrophoretic fractions.

Molecular studies on polysaccharides. Dr. B. R. Record has continued investigations in this field on materials prepared in Prof. Haworth's laboratory, in the University of Birmingham. The pneumococcus polysaccharides, Types I, II and III behave similarly in giving extremely sharp boundaries in concentrations exceeding 0.25%. Below this concentration diffuse boundaries are obtained suggesting polydispersity. All three show also a marked but not linear decrease of sedimentation constant with increasing concentration. As would be expected this behaviour is accompanied by anomalous diffusion phenomena. By extrapolation of sedimentation and diffusion data the molecular weight of Type I is found to be 171,000 and of Type II, 504,000. Values of the frictional coefficient indicate that the length of these molecules is many times their width especially in the case of Type II. Similar studies on methylated lichinin have yielded results analogous to those obtained by Dr. Record in Upsala for methylated glycogen.

The brown pigment in the blood in Blackwater Fever. Dr. McFarlane has collaborated with Dr. N. Hamilton Fairley in work on the nature of the brown pigment occurring in the blood in blackwater fever. This pigment sediments at the same rate as serum albumin and migrates in the electrophoresis apparatus in a homogeneous manner but at a slightly different rate from serum albumin. The evidence supports the view that the pigment is formed by the union of serum albumin and haematin.

GENERAL BACTERIOLOGICAL STUDIES.

The Pleuropneumonia-like organisms. The work on pleuropneumonia-like organisms occurring in symbiosis with bacteria, and independently, has been continued by Dr. E. Klieneberger. Two new symbiotic cultures have been studied. One of them contains a gram-negative streptobacillus and a pleuropneumonia-like organism similar to the *Streptobacillus moniliformis*—but unlike the *Streptobacillus moniliformis* it is pathogenic for guinea-pigs and not for mice and rats. It has been isolated on several occasions from purulent lymph glands in the necks of guinea-pigs, and like the pleuropneumonia-like organisms it requires a serum-enriched special medium for growth. An L 1-like culture has been separated from the parent symbiotic strain. The second of the new symbiotic cultures was isolated from the skin of a pig. It is of special interest because in this case a gram-positive coccus is combined with a pleuropneumonia-like organism. It has, so far, not been possible to separate a pleuropneumonia-like organism from its coccus symbiont.

Three more species of pleuropneumonia-like organisms have been studied during the past year. The L 4 species, previously isolated from the swollen submaxillary gland of a rat, has been proved to be identical with Woglom's pyogenic "virus" in laboratory rats. It produces, if injected, oedema and abscesses in these animals. The infected rats acquire immunity against new infection by this organism.

A new pleuropneumonia-like organism, designated "L 5," distinguished by its specific growth and serological affinities has been isolated from the brains of mice suffering from "rolling disease," a syndrome reported by Findlay and Sabin. Its pathogenicity has been studied in collaboration with Dr. Findlay of the Wellcome Research Institution. The "L 5" organism when grown *in vitro* in a fluid medium is incapable of initiating symptoms in mice when injected intracerebrally alone; when, however, the culture is injected along with a neurotropic virus, or with agar, typical symptoms result. Cultures secured after a series of rapid intracerebral passages, are also effective. Thus evidence has been brought forward that the "L 5" organism is the agent causing this nervous disease in mice.

Recently another new pleuropneumonia-like organism, "L 6," has been isolated from brains of mice which had been injected intracerebrally with blood of splenectomized mice containing *Eperythrozoon coccoides*. In collaboration with Dr. Findlay experiments are being carried out to decide if there is a connection between the occurrence of this "L 6" organism and the blood parasite.

Another research conducted during the past year was an examination of Laidlaw's and Seiffert's water, sewage and soil strains of pleuropneumonia-like organisms. It was found that these strains were morphologically similar to the pathogenic members of the pleuropneumonia-like group, and that

Seiffert's strains could be classified serologically as members of Laidlaw's type A. These saprophytic organisms will grow at room temperature and do not require specially enriched media for their growth. They are serologically quite distinct from strains of the L series.

The resistance of desiccated bacteria to extraction with fat solvents. Experiments carried out at this Institute and elsewhere have demonstrated that vaccinia virus and the filterable agent of the Rous I sarcoma are not inactivated by treatment with organic solvents such as benzene, ether or carbon tetra-chloride provided that the infective material is in an anhydrous condition. This observation prompted an enquiry into the effect of such solvents on desiccated bacteria. Dr. Amies has now shown that cultures of *M. phlei*, *Bact. coli* and *Staph. aureus*, grown in bulk and subsequently dried *in vacuo* from the frozen state, can be extracted with benzene for periods of several hours without any apparent loss of activity. The extractions were carried out in a Soxhlet apparatus, the solvent being made to boil at 37°C. under reduced pressure. The amount of lipid removed by this procedure varied from 1 to 5 per cent. according to the particular organism used and the medium in which it was cultivated. The results so far obtained indicate that the removal of this fatty material does not influence the viability, morphology, cultural characters or suspension stability of the organism. Further experiments are being carried out with bacteria which are capable of growth in a completely synthetic medium and in these attention is being directed to the possibility that such extraction may influence the antigenic structure of the organism, though this appears unlikely.

Vi-agglutination in the detection of chronic typhoid-carriers. The investigation of this subject has been completed by Dr. Felix. Following the publication of the results obtained the test is now being tried in the routine examination for carriers, and cultures of Vi strains of *Bact. typhosum* suitable for the test are being asked for by workers in various parts of the world. Since a standard serum is an indispensable reagent for the exact estimation of the Vi agglutinin, the cultures are sent out together with a sample of a "Provisional standard serum for Vi agglutination." This serum has been standardised by comparison with the "Dried provisional standard anti-typhoid serum," previously referred to, which, for obvious reasons, must be reserved for work in connection with therapeutic anti-typhoid serum.

Trichomonas as a cause of abortion and sterility in cattle. A strain of *Trichomonas fetus* has been cultivated continuously for a year from material sent to Dr. Muriel Robertson by Mr. W. R. Kerr of the Veterinary Department of the Ministry of Agriculture of Northern Ireland. In addition a number of strains of this and some allied organisms have been obtained through the courtesy of workers in Amsterdam and Paris. A study of the serological relations of these organisms is being attempted. One of the strains, a *Trichomastix* derived from a Gecko and isolated by Prof. Chatton, is proving particularly useful for the theoretical working out of the antigenic properties of these organisms.

Further collaboration with the Veterinary Department of the Ministry of Agriculture for Northern Ireland is being undertaken in regard to the study of *Trichomonas fetus* as the cause of abortion and sterility in cattle.

FACTORS WHICH INCREASE TISSUE PERMEABILITY.

The influence of tissue permeability on bacterial invasion. Dr. Henderson and Dr. McClean have continued their observations on the influence of the diffusing factor that is elaborated by *Vibrio septique* on the development of infection with this organism. Anti-diffusing sera were prepared by immunising rabbits with a solution of the purified diffusing factor derived from culture-filtrates. Preliminary experiments indicate that whereas the anti-diffusing serum alone exerts no inhibitory influence on the development of the infection with *Vibrio septique*, the addition of this serum to minimal protective doses of antibacterial serum appears to increase the protection afforded by the antibacterial factor. The addition of the anti-diffusing serum produces a greater effect than the addition of small quantities of antitoxin. Unfortunately, further progress with these observations has been delayed by difficulties in the purification of the diffusing factor from *Vibrio septique* cultures; this factor appears to be much more labile than that derived from *Cl. welchii*. When this difficulty has been overcome it is hoped to complete the observations on the part played by these factors in infection.

Chemical nature of the diffusing factor. Dr. J. L. Madinaveitia continued his investigation of the testicular diffusing factor at the Institute until October, 1938, and then at the University of Manchester in association with Dr. Todd. A new technique for biological assay of diffusing factor preparations has been devised in which their effect on the rate of spread of haemoglobin through skin is measured. By this means much greater accuracy is obtained than by earlier methods. In addition, a new concentration method has been developed based on ammonium sulphate fractionation and subsequent adsorption on alumina; preparations have been obtained showing diffusing properties at a concentration of 0.001 µg. per c.c. The homogeneity of these preparations is being investigated, as also is the possibility of a more accurate biological test, since the very high activity of the materials necessitates accurate assay. The

purest preparations obtained have many of the properties of proteins and it is hoped that degradative studies now in progress will throw some light on the structure of the diffusing factor. It has been shown that while the action of the testicular diffusing factor is similar to that of diffusing factors from snake venom and bacterial filtrates, it is quite distinct from the effect produced by solutions of kallikrein, the non-insulin containing principle of Kraut and Frey recovered from pancreas and urine.

COAGULATION OF BLOOD.

Dr. J. O. W. Barratt has investigated the anticoagulant action of trisodium citrate and of hirudin, and has carried out a redetermination of the constants of the equation by means of which anticoagulant action is expressed. The experimental work is now completed, but the necessary calculations have been deferred in order that an estimation of the total anticoagulant action of citrated blood plasma might be made and thus greater accuracy of calculation secured. This latter work is still in progress.

ENDOCRINOLOGY.

Dr. V. Korenchevsky, with the assistance of Miss K. Hall, Mr. R. C. Burbank and Miss M. A. Ross has continued his investigation of different aspects of the activity of sex hormones using the rat as the experimental animal.

Effect on senility. The most active male sex hormone, testosterone propionate, can produce in senile rats even supernormal development of the sex organs, but it does not cause any noticeable improvement in the senile appearance and behaviour of these animals. Taking into consideration the fact that the senile rats had normal sex organs, and that these organs and sexual function may be well preserved in otherwise senile men, the absence of a "rejuvenating" effect of the sex hormones is not surprising.

The over-stimulation of the secondary sex organs of senile rats by testosterone propionate cannot be regarded as a rejuvenating effect, since, without simultaneous improvement of the general condition and other features of senility, this artificial sex stimulation is biologically unnatural and in human patients is medically undesirable. Moreover, the administration of testosterone propionate depressed the development of the sex glands of the senile rats, thus checking the secretion of the natural sex hormones by the testes.

Tumours, probably of cancerous nature, were found in the hypophyses of two of the injected senile rats and it is not inconceivable that their occurrence may have been connected with the injections.

Reverse activity of some male sex hormones in normal animals. All the male hormones produce more or less pronounced stimulation of the secondary sex organs. If, however, the weak male hormones are injected for long periods, the corresponding stimulating effect on the sex organs is overcome by the simultaneous strong depressing effect of these hormones on the hypophysis, which then secretes such a small amount of gonadotropic hormone that the natural secretion from the testes is not stimulated. This results in a "reverse" activity of the sex hormones, namely, depression of the development of the testes and secondary sex organs.

It follows, therefore, that the development of the sex organs may be stimulated or depressed by some of the male hormones according to the dose injected.

Sex hormones with prolonged activity. Recently enol and aliphatic esters of sex hormones have been prepared, chiefly by Swiss scientists. The most important feature of these esters is their prolonged activity after the cessation of the injections. This property, which is of great importance in therapy, is considered to be connected with a slow absorption of the injected esters from the subcutaneous tissue.

The following compounds have been investigated: testosterone dipropionate, oestradiol dipropionate and oestradiol benzoate-butyrate. With certain of the doses used the maximum effect of the first two hormones was maintained for at least 10 days, and that of oestradiol benzoate-butyrate for more than 106 days after the discontinuation of the injections.

"Pregnancy" effect of oestrogenic hormone. Oestradiol benzoate-butyrate may produce in ovariectomised rats, during the later part of the period after the cessation of the injections, progestational changes in the vagina (mucification) instead of the usual oestrus cornification. The most probable explanation of this paradoxical effect seems to be the co-operation of the slowly absorbed oestradiol compound with progesterone secreted by and naturally present in the adrenals.

Effect of ovariectomy on non-sexual organs. Castration in males causes changes in several of the non-sexual organs, while gonadectomy in females is followed only by an increase in the body-weight as compared with that of the normal control rats (the opposite effect following castration in males) and by delayed involution of the thymus as in males.

Manifold effects of the sex hormones during prolonged injections. (a) In castrated males.

Oestradiol dipropionate can produce great development (for a female hormone) of the secondary sex organs, including the penis, but as with other oestrogens this is not true restoration towards normal but is of pathological nature and caused chiefly by increased development of fibrous tissue.

Oestradiol dipropionate also produces stunted growth, decreased fat deposition, small dark liver, decreased thymus, spleen and heart, specific degenerative changes in adrenals and tumour-like hyperplasia in hypophysis.

Male hormones have the important property of being able to neutralise more or less completely most of the pathological effects of oestrogens. For example, the tumour-like hyperplasia of the hypophysis is decreased and in some cases almost completely prevented.

When injected alone male hormones also produce some changes in the non-sexual organs: the development of the liver, kidneys, heart and spleen is stimulated, the involution of the thymus increased, and the "castration" adrenals returned to, or towards, normal.

(b) In ovariectomised females.

Oestradiol dipropionate is able to restore the vagina to normal or even to produce super-normal weight and size, but even with very large doses of this hormone it is impossible to obtain a normal uterus, which indicates that some other hormone must co-operate in this respect.

In the non-sexual organs and functions, oestradiol produced stunted growth and a decrease in fat deposition, thymus, liver, spleen and heart. The hypophysis became hyperplastic and large doses led to tumour formation. In the adrenals, specific pathological changes in the cortex, similar to those in males, are produced.

Male hormones. These hormones also restore towards normal the size and structure of the female sex organs, the strongest effect being produced by the propionate or dipropionate of testosterone. The histological structure is reminiscent of that occurring during pregnancy. However, this progestational effect is followed by some pathological changes. The same non-sexual organs are affected in females as in males, but the liver, kidneys, heart and spleen are enlarged to more than their normal size and weight. Male hormones also decrease the weight and size of the adrenals and thymus, producing in them changes similar to those in males.

Neutralizing effect of male hormones. If the male hormones are injected simultaneously with oestradiol, the depression of growth, decrease of fat deposition and pathological effect of oestrogens on the liver, kidneys, heart, spleen, hypophysis and adrenals are more or less completely neutralised. Since in the female organism the existence of at least two male hormones (androsterone and *trans*-dehydroandrosterone) is already proved, a similar beneficial interaction between the male and female sex hormones can be suggested as a probable natural occurrence in the organism.

The stimulation or prevention of precancerous oestrogenic changes in the uterus by some sex hormones. Injections of oestradiol dipropionate are followed by development in the uterus of special metaplastic changes in the epithelium, which are the first stage of a precancerous condition in this organ. This condition develops more frequently or in a more severe form, when androsterone, *trans*-dehydroandrosterone and testosterone dipropionate are injected simultaneously with the oestrogens. On the other hand, progesterone and (when injected in a special ratio to oestradiol) testosterone propionate can prevent these precancerous changes.

The clitoris in rats treated with the male hormones. After injections of male hormones the clitoris develops into a much enlarged penis-like organ. Histologically, however, although some features of a penis are present, important changes, which do not occur in the normal penis or clitoris, are also developed.

THE ACCESSORY FOOD FACTORS.

On behalf of the Accessory Food Factors (Vitamins) Committee, work has been continued on standardisation problems.

Vitamin Standardisation.

Vitamin A. The problems of Vitamin A standardisation continue to occupy the attention of the Vitamin A Sub-Committee, of which Miss E. M. Hume is Secretary. The recent isolation of relatively pure preparations of Vitamin A and of its esters, has raised the question of the desirability of retaining pro-vitamin A, β -carotene, as the International Vitamin A standard. Vitamin A itself, the alcohol, is however, very unstable, but its esters have much greater stability. Certain of the latter have been offered for investigation to the Vitamin A Sub-Committee and a large co-operative experiment is now contemplated, in which this material should be used to determine the value of the conversion factor, the figure employed for converting the results of spectrophotometric estimation to

International Units (expressing biological potency). There has been much controversy about the value of this conversion factor and the figure 1,600, adopted at the 1934 International (League of Nations) Conference on Vitamin standardisation, has been criticised as not being applicable to Vitamin A as present in the liver oils of certain species. As the result of previous co-operative investigations organised by the Vitamin A Sub-Committee, some of these discrepancies have been traced to instability of the material during the time taken for the biological test. It is felt that a trustworthy estimation of the conversion factor carried out upon a pure preparation of Vitamin A would do much to clarify the position and to determine whether the spectrophotometric method of estimation is permissible.

The work is being done in preparation for the meeting of the International Conference on Vitamin Standardisation which is expected to meet in the late autumn of the present year. Dr. H. Chick and Miss Hume are acting as Secretaries to that Conference.

Vitamin B₁. The results of the collective investigation organised by Dr. T. F. Macrae (Secretary of the Vitamin B₁ Sub-Committee), in which estimations were made in 22 different laboratories of the biological activity of pure crystalline aneurin in comparison with that of the standard adsorption product, then serving as International Vitamin B₁ standard, were summarised in last year's report. These results were circulated by the Health Organisation of the League of Nations to the members of the International Conference on Vitamin Standardisation, and a unanimous decision was obtained that the sample of pure vitamin B₁ (aneurin) hydrochloride, which had been thus investigated, should in future be adopted as International Vitamin B₁ standard and that the International Vitamin B₁ unit be defined as the potency of 3.0 μ g. of the pure material.

Vitamin E. The isolation of the series of pure compounds, tocopherols, possessing Vitamin E activity, has made it possible to contemplate the standardisation of this Vitamin. A Sub-Committee has been formed for investigation of this and other matters connected with Vitamin E, with Miss Hume as Secretary. At its first meeting, a co-operative international research was planned, in which the vitamin E potency of a specimen of pure synthetic *dl*- α -tocopherol acetate, of which an amount of 100 g. has been generously presented by Messrs. Hoffmann-La Roche, Basle, should be tested for Vitamin E activity on suitably prepared female rats. Seventeen laboratories in Europe and America have been invited to co-operate; of these 14 have already signified their acceptance. Miss Copping will carry out a set of these investigations with her rat colony for Vitamin E testing, at this Institute.

VITAMIN STUDIES.

The B Group of Vitamins.

Vitamin B₁ Complex. Work on this subject has been continued by Dr. Macrae, Mrs. C. E. Work (née Edgar) and Dr. M. M. El Sadr.

Riboflavin (lactoflavin). Since considerable criticism has been directed against the chemical and physical methods employed in estimating riboflavin in foodstuffs and other materials, a reliable biological method is badly needed. The difficulty, hitherto, has been to prepare a basal diet devoid of riboflavin, but containing adequate amounts of the other dietary essentials of the vitamin B₂ complex required by the rat. Treatment of aqueous extracts of liver with norite charcoal has yielded a filtrate free, or nearly free, from riboflavin, but rich in all other vitamin B₂ factors, and therefore the biological estimation of riboflavin by rat growth method has become possible.

Riboflavin is an important constituent of coenzymes. One of these compounds, alloxazine-adenine-dinucleotide, of which a specimen was kindly supplied by Professor O. Warburg, was investigated for its nutritional value. It was found to have no demonstrable vitamin B₂ activity for rats, other than that possessed by virtue of the riboflavin it contains bound in its molecule. Equimolecular amounts of alloxazine-adenine-dinucleotide and riboflavin had similar vitamin potencies.

Other B₂ Vitamins. The existence of a further unidentified factor of the vitamin B₂ complex required by the rat has been proved by growth experiments. Rats receiving a complete synthetic diet lacking only B vitamins, when supplied with adequate amount of vitamin B₁ (aneurin), have been found to need in addition the following members of the heat-stable Vitamin B₂ complex: (a) riboflavin, (b) "yeast eluate factor" of Macrae and Edgar (=Vitamin B₂ of György, Factor 1 of Lepkovsky), (c) "yeast filtrate factor" (=Factor 2 of Lepkovsky), and in addition (d) a further unidentified factor. This additional unidentified nutrient is not adsorbed by fuller's earth nor extracted by amyl alcohol and is, therefore, present in the residue after separation of the eluate fraction by fuller's earth and of the "filtrate fraction" by amyl alcohol.

The identity of Macrae and Edgar's yeast eluate factor with vitamin B₂ (György) and with factor 1 (Lepkovsky) has been established. Crystalline factor 1 was found to replace yeast eluate factor completely in rat growth experiments, and a crystalline material identical with the hydrochloride of factor 1 was isolated from active concentrates of the yeast eluate factor.

Filtrate Factor. Experiments on the purification and chemical nature of the filtrate factor, as contained in autoclaved extracts of yeast and in extracts of liver, have been continued. The filtrate factor has been obtained from liver in such highly purified state that it seems probable that the pure vitamin will be isolated in the near future. Although the filtrate factor, as contained in extracts of yeast and liver, was found to be biologically identical in rat growth experiments, the forms in which it occurs in liver and yeast respectively were found to be chemically distinct. They have been found to differ in (1) stability to acid, (2) extractability by amyl alcohol, (3) solubility of the lead salts in water and (4) solubility of the barium salts in aqueous alcohol.

Concentrates of liver filtrate factor and liver eluate factor have been supplied to Dr. L. Wills who is examining their effects on the macrocytic nutritional anaemia of monkeys.

Histological examination of the tissues of rats deprived of one only of the various factors of the vitamin B₂ complex is being carried out; pathological changes in the liver, kidneys and thymus have been observed. The effect of deprivation of the vitamin B₂ factors on the blood picture of rats is also being examined.

The study of the effect of deprivation of the vitamin B₂ factors on the respiration rates of the isolated tissues of rats has been continued.

Study of the dermatitis and other skin disorders developed in rats deprived of one or more members of the Vitamin B₂ complex. This work, begun by Dr. Chick with Dr. L. Gravel, has been continued with the help of Mr. A. Worden. A special study has been made of the etiology of the typical symmetrical dermatitis, erroneously known as "rat pellagra," in which there is inflammation and oedema of the skin of the paws and of the edges of the ears, spreading to the limbs and trunk and leading to exfoliation of epidermis and loss of fur. With a basal diet in which vitamin B₁ is supplied as pure aneurin, carbohydrate as pure sucrose, and other B₂ vitamins as highly purified preparations, this condition has developed invariably in deficiency of eluate factor vitamin B₆ and has been cured or prevented when this vitamin is provided, whatever deficiency there may have been of other members of the vitamin B₂ complex.

Other skin affections, of an eczematous nature, marked by stained serous exudates from eyes and nostrils, and a matted condition of the fur leading to baldness, were found present in deficiency of riboflavin and of filtrate fraction and were not apparently connected specifically with deficiency of either. This work is still in progress.

Porphyrin excretion. Dr. Ellinger is examining the effect of vitamin B₂ deficiency on the excretion of abnormal pigments, especially porphyrins, in the urine of rats and the influence of the different members of the vitamin B₂ complex on this excretion. The effect of vitamin B₂ deficiency on the skin and the relation of the skin disorder to the porphyrin excretion is also being studied.

Nutritional work with pigs: Water soluble Vitamins other than Vitamin B₁ (aneurin) required for nutrition of the pig. These researches were carried on during 1938 by Dr. Chick and Dr. Macrae in collaboration with Sir Charles Martin and Dr. A. P. Martin at the Institute of Animal Pathology, Cambridge, with the help of a grant for expenses from the Medical Research Council. They form a continuation of previous studies, designed to throw light upon the etiology of human pellagra, in which pigs were fed upon diets composed largely of maize. The results of these studies, especially on the effect of nicotinic acid in correcting the deficiencies of maize diets for the pig (as for the human being and the dog) were summarised in last year's report.

For the present work, in which the fundamental nutritive requirements of the pig were studied, a synthetic diet was devised, consisting of purified maize starch, purified casein, cotton seed oil, an appropriate salt mixture and cod liver oil; this proved satisfactory for rearing young pigs from the period of weaning, provided 4% yeast were added to the diet. If the yeast were omitted and suitable daily doses provided of vitamin B₁ (aneurin), nicotinic acid and lactoflavin, all given as pure crystalline preparations, the pig soon ceased to grow. At least two other essential nutrients, contained in what is known as the heat-stable vitamin B₂ complex, were found to be necessary. If the synthetic diet was further supplemented by either (a) the fraction adsorbed on fuller's earth from yeast or liver extracts and subsequently eluted (vitamin B₆ or eluate fraction) or (b) the nutrient contained in the filtrate from such adsorption (filtrate factor), growth failed; when both fractions were given, good growth resulted. When vitamin B₆ was omitted, the pigs became severely anæmic and suffered from epileptic fits; when lacking the filtrate factor, they developed paralysis of the hind legs. Recovery took place in both cases when the missing nutrient was provided. The large amounts of these materials required for these trials were prepared at the Institute, following on the preliminary treatment of large quantities of liver extract, etc., kindly carried out by Glaxo Laboratories, Ltd.

It seems, therefore, that the pig, like the rat, requires in addition to vitamin B₁ and lactoflavin (riboflavin), at least two heat-stable nutrients. Both are contained in watery extracts of yeast and

liver; one, vitamin B₆, is adsorbed on fuller's earth at pH 1.0, and the second, the filtrate fraction of Macrae and Edgar, is contained in the filtrate after treatment with fuller's earth and is extracted from this filtrate with amyl alcohol.

Investigations on Pellagra.

Dr. Ellinger spent the months of January to May, 1938, in Egypt with the intention of completing his former investigations on pellagra, in collaboration with Prof. Ali Hassan and Mr. M. M. Taha of the Medical Faculty of Cairo. He studied the effect of treatment with nicotinic acid, nicotinamide and preparations of the eluate and filtrate factors, made from yeast and liver according to the methods of Dr. Macrae and his collaborators, and previously tested by them on rats. Unfortunately this work on pellagra could not be completed owing to serious illness contracted by Dr. Ellinger in Cairo, as a result of which his work had to be abandoned.

Studies on Vitamin C.

The indophenol-reducing capacity of leucocytes. Dr. S. S. Zilva and Dr. A. E. Kellie have investigated the variation in the reducing capacity of the leucocytes from guinea-pigs receiving generous supplies of vitamin C and from animals existing for varying periods on a scorbutic diet. It was found that the ascorbic acid content of the white cells in guinea-pigs "saturated" with vitamin C is of the same order as that of muscle tissue. The injection of exceptionally high quantities of ascorbic acid into the blood stream of guinea-pigs did not raise the vitamin content of the white cells to any very appreciable extent. These experiments were carried out on leucocytes obtained by pathological stimulation, i.e., by intraperitoneal injection of broth. It has since been found that when these blood cells are obtained directly from the blood of "saturated" guinea-pigs their vitamin C content is considerably higher and may reach at times figures observed in the so-called "selective" tissues which are outstanding for their capacity of accumulating ascorbic acid. Unlike most guinea-pig tissues the leucocytes retain some of their capacity for reducing indophenol after the animals have been maintained on a scorbutic diet for some days. This observation suggests that the high reducing capacity of the leucocytes of leukaemic patients, as compared with that of the white cells of other subjects observed by Stephens and Hawley, may possibly be due to the presence in the cells of the former of a substance other than *l*-ascorbic acid. In order to settle this problem Dr. Zilva is examining the bloods from leukaemic and other human subjects.

The vitamin C requirements of man. Dr. Zilva and Dr. Kellie have finished an investigation in which the minimum daily dose required to maintain an adult "saturated" in respect to vitamin C was ascertained. At the same time they also studied the ascorbic acid content of the blood during the various stages of the inquiry. The experiments were carried out on themselves. It was found that on reaching "saturation" on a certain dose of ascorbic acid, the level of excretion at equilibrium varied with the condition of the subject at the beginning of the experiment. It was lower when the person was "unsaturated" or "saturated" on a lower dose at the beginning of the experiment than when the same dose was taken after previously attaining "saturation" with a higher dose. The equilibrium in the excretion was established on the following doses 100 mg., 50 mg. and 30 mg. and the minimum "saturation" dose was found to be in the neighbourhood of the last figure. Previous investigations on animals suggest however that a daily dose of about a half the magnitude of the "saturation" dose is sufficient to meet the theoretical requirements of man. The ascorbic acid content of the blood was determined during "saturation" and "unsaturation" at different intervals after the administration of the vitamin in various doses. The comparison of these figures with those of the urinary excretion suggests that there is no constant renal threshold for ascorbic acid but that there is a competition for the ascorbic acid of the blood by the absorptive capacity of the tissues and the excreting function of the kidney. Blood levels determined at random do not, therefore, indicate the degree of "saturation" of a subject.

The determination of glutathione in animal tissue. The accurate determination of glutathione in the tissues is of great importance in studying the physiological function of ascorbic acid in the metabolism of the animal organism. Dr. Zilva and Mr. G. A. Snow have re-examined Binet and Weller's method in which the reduced glutathione is precipitated by cadmium lactate at pH 6.8-7.0 and is estimated iodometrically in the precipitate. This method was found to be open to criticism since the precipitation was incomplete and the iodometric titration procedure employed gave high figures with pure glutathione. Very much better results were obtained when trichloroacetic acid extracts were titrated with iodine in the presence of KIO₃ and an excess of KI; corrections were made for ascorbic acid which was determined by indophenol titration. This method, however, calls for further development on account of lack of absolute specificity.

The photochemical decomposition of vitamin C. Dr. Zilva and Dr. Kellie have found that *l*-ascorbic acid but not dehydroascorbic acid undergoes photochemical decomposition when exposed to light of the ultraviolet region of the spectrum not only in the presence of sensitizers (as in the case of visible light), but also in their absence. An interesting point observed in this connection was that the decomposition of the vitamin by the action of ultraviolet light took place in the absence as well as in the presence of oxygen. Dr. Zilva and Dr. C. L. Arcus are carrying out experiments in order to obtain further information concerning the character of this reaction.

The mode of action of vitamin C. The investigation of the influence of vitamin C on the respiratory capacity of tissues begun by Dr. Zilva and Dr. Kellie is being continued in collaboration with Mr. Snow.

Dehydroascorbic acid in apples. Previous work has indicated that a change in the equilibrium between dehydroascorbic acid and ascorbic acid takes place progressively with the development of the apple. Dr. Zilva in collaboration with Dr. F. Kidd and Dr. C. West (Low Temperature Research Station, Cambridge) is now investigating the influence of various environmental factors on the relative content of the two forms of the vitamin in the apple in order to link up the change in equilibrium with a specific physiological change involved in the metabolism of the fruit.

The vitamin C of the potato. Dr. Zilva in collaboration with Dr. J. Barker (Low Temperature Research Station, Cambridge), is carrying out an investigation on the ascorbic acid content of King Edward VII potatoes lifted from the soil at various stages of their development, and of the subsequent decline in the vitamin C concentration of the tubers during storage at 10°C. The content of ascorbic acid reached a maximum at the third lifting in the early part of September. Between this date and the time of the final lifting in October there was a considerable drop in the ascorbic acid concentration. On storage at 10°C. the potatoes lifted in October continued to lose the vitamin rapidly at first but later the drop in the concentration was gradual. In contradistinction to this the fall in the ascorbic acid content on storage at 10°C. in samples lifted before October, i.e., the normal time of lifting, was strikingly more marked. The determinations of the ascorbic acid were carried out by titration with indophenol but a biological test on one of the samples showed that the greater part of the indophenol reduction was due to ascorbic acid, although the possibility of the presence of a small proportion of another reducing substance in these tubers could not be excluded.

Vitamin C in canned apples and potatoes. This problem is being investigated with Mr. T. N. Morris (Low Temperature Research Station, Cambridge). In order to ascertain the effect of prolonged storage on vitamin C in canned apples, samples canned early in 1935 and tested in 1936 were re-examined in 1938. A 50% loss incurred during the period of storage was recorded, previous intermediate tests having shown this loss to be progressive. Preliminary experiments have also been carried out on the effect of canning on the vitamin C content of King Edward VII potatoes lifted at a time of the year when their ascorbic acid content is at its highest.

Structure and Synthesis of Vitamin E.

Investigations on this subject have been continued by Dr. A. R. Todd, Dr. F. Bergel, Dr. T. S. Work (Research Student in Bio-chemistry), Dr. A. Jacob and Dr. M. Steiger. In the last report the active constituents of wheat germ oil, α and β -tocopherol were formulated as 5-hydroxy-coumaran or 6-hydroxy-chroman derivatives bearing in the heterocyclic nucleus a long side chain derived from phytol. These conclusions have now been justified by the synthesis of racemic α -tocopherol and of racemic β -tocopherol and two of its isomers all of which show the physiological action of vitamin E.

Condensation of ψ -coumiquinol with phytol in presence of zinc chloride gave racemic α -tocopherol ($C_{55}H_{100}O_2$) in good yield, the product being just as active as the natural material. The synthetic method left some slight doubt as to whether α -tocopherol contains a coumaran or chroman nucleus but experiments on synthetic analogues together with degradative evidence obtained by John make the chroman structure practically certain, i.e., α -tocopherol is 6-hydroxy-2:5:7:8-tetramethyl-2-[4':8':12'-trimethyl-tridecyl]-chroman.

The production of ψ -coumiquinol on pyrolysis of β -tocopherol ($C_{55}H_{100}O_2$) and the identity of the side chain in the latter substance with that in α -tocopherol indicate that β -tocopherol differs from the α -compound in having only two methyl groups on the aromatic ring. Clearly three isomers are possible which could satisfy these conditions; these might be obtained by methods analogous to that used for α -tocopherol, i.e., by the condensation of *o*-, *m*-, and *p*-xyloquinols with phytol. All three compounds have now been synthesised; this work, commenced in the Institute has been completed at the University of Manchester where the work was continued by Dr. Todd and his colleagues.

Condensation of *m*-xyloquinol with phytol yielded a tocopherol purified as its crystalline *p*-nitrophenylurethane; it shows vitamin E activity in doses as low as 3 mg. (i.e., its activity is of the same

order as that of α -tocopherol). The synthesis of tocopherols by direct condensation of *o*- and *p*-xyloquinols with phytol proved to be rather impracticable owing to the tendency to double ring closure, etc. These difficulties were surmounted by using in place of the free quinols, their monobenzyl ethers, or better their monobenzoates, the protecting groups being removed from the final products under mild conditions. After purification *via* crystalline *p*-nitrophenylurethanes, the tocopherols were obtained as nearly colourless oils having chemical properties exactly like β -tocopherol. In biological tests the product from *p*-xyloquinol monobenzoate is active in rats at a dose of 5 mg. (minimum dose for natural β -tocopherol=5 mg.); biological tests on the product from *o*-xyloquinol monobenzoate are not yet complete but it has already shown full activity at a dose of 10 mg. A final decision as to which of these three isomers is β -tocopherol must await their resolution into optically active constituents, but various properties suggest that the product from *p*-xyloquinol probably corresponds to the natural vitamin.

It would thus appear that elimination of one methyl group from the aromatic nucleus in α -tocopherol may cause little diminution in biological activity. The corresponding tocopherols containing one methyl group (from toluquinol) and no methyl groups (from quinol) have also been prepared in Manchester in connection with further examination of constitutional specificity but results of biological tests are not yet available.

Vitamin E colony. Miss A. M. Copping has maintained the "vitamin E colony" of rats during 1938/39 as in 1937, mainly for the purpose of the testing of synthetic and natural vitamin E preparations made by Dr. A. R. Todd and his assistants working on the chemical nature of vitamin E. Several isomeric synthetic tocopherols have been found almost equally active. During the past year the technique of testing has been somewhat modified and the direct testing method of Demole has been employed in preference to the older method of using only rats which had had previous resorptions. In addition to the above work, a study has been made of animals maintained on the vitamin E deficient diet for 8 to 12 months and the histology of their organs is now being worked out by Dr. Korenchevsky, in an attempt to investigate further the pathology of vitamin E deficiency in the rat.

Nutritive value of Buckwheat (*Fagopyrum esculentum*) and its sensitising action to light. Investigation of the light sensitising action of buckwheat has been continued by Dr. Chick. Albino rats were found to thrive for two generations on a diet consisting of 90% ground whole buckwheat with addition of salt mixture, cotton seed oil, lard and cod liver oil; the protein content of the diet was 9.6%. A similarly composed diet made with whole wheat was markedly inferior in supporting growth and reproduction, but was greatly improved when 2% casein was added, thus suggesting inferiority in the nutritive value of the proteins of wheat compared with those of buckwheat.

Albino rats fed on the buckwheat diet were sensitive to sunlight, showing discomfort with reddening and swelling of the ears, paws and tail when exposed to sunlight (behind window glass). The animals sometimes suffered from convulsive fits when exposed for longer periods, but recovered after removal into the shade. The animals were sensitive to the visible rays, but not ultra-violet rays, from artificial sources of light.

No sensitivity to light was present in albino rats fed on similar diets containing whole wheat or whole maize in place of buckwheat.

The buckwheat diet had no light sensitising action if the husks were removed from the ground buckwheat meal. The largest amount of the sensitising material was found in the flowers of the plant, especially when young, diminished in amount at the fruiting stage and to be absent altogether from the leaves. Albino rats fed on the wheat diet with a small dose, 0.05 to 0.2 g. daily, of dried buckwheat flowers became highly sensitive in about 3-4 days.

The sensitising material could be extracted from the dried flowers to a small extent by alcohol at low temperature (under 40°C.), or by dilute HCl, but not by water, chloroform, acetone or dilute alkali; it was destroyed by hot alcohol or by steam. It could, however, be extracted by digestion with pepsin at pH 2.0 but not by pepsin at pH 5.0 and it seems likely that in the plant it is combined with material of a protein-like nature.

The exact position on the spectrum of the activating rays is now being examined with the collaboration of Dr. Ellinger. They have so far been located between wave-lengths of 540 m μ and 620 m μ .

PHOSPHATE METABOLISM AND THE CALCIFICATION OF ANIMAL TISSUES.

Professor R. Robison's work on the calcification of bone was interrupted through illness during the past year, but the investigation of changes occurring within the bone and controlling the activity of the calcifying mechanism has been recommenced in collaboration with Miss A. Tazelaar. Dr. W. D. Armstrong (University of Minnesota) was also associated in this work for a short period.

Phosphoric Esters of Alcoholic Fermentation.

The heptose phosphoric ester formed during the fermentation of hexoses by yeast juice and described in last year's report, has been further studied by Professor Robison and Miss Tazelaar, who have simplified the methods for its isolation from the crude hexosemonophosphates. With the larger amounts of this ester now being prepared it is hoped that its constitution may be definitely established.

Miss D. Toten has further improved the methods for the preparation and isolation of the levorotatory fructosephosphate formed from fructose-6-phosphate by migration of the phosphate group. The identity of the new ester as a fructose derivative has been proved but the position of the phosphate group is not yet known.

The Role of Diphosphoglyceric Acid in Red Corpuscles.

The role of monophosphoglyceric acid as one of the intermediaries in tissue glycolysis is well established; but the significance of diphosphoglyceric acid, which forms a large proportion of the acid-soluble phosphoric esters of blood, is still obscure. The concentration of this ester can vary greatly and very rapidly in certain pathological conditions and the part which it plays in blood chemistry is obviously of great interest.

Dr. A. Lemmerstrand (Rockefeller Foundation Research Fellow) and Mrs. Lemmerstrand have commenced an investigation of the mechanism of the formation and breakdown of this ester. Meyerhof's discovery that the specific rotation of monophosphoglyceric acid is enormously increased by ammonium molybdate has provided a means for distinguishing this acid from the diphosphoric ester. With this method it has been possible to show that monophosphoglyceric acid is formed during aerobic glycolysis in haemolysed blood when pyocyanin is present as hydrogen acceptor. Diphosphoglyceric acid was not formed under these conditions.

CHEMISTRY OF SUGARS AND SUGAR DERIVATIVES.

Mr. W. S. Reich (Paris) has extended his previous studies of methods for the separation of mixed sugars and polysaccharides by the formation of coloured azobenzene derivatives and their subsequent treatment by chromatographic adsorption. It has been possible in this way, to separate fructose and glucose from a few milligrams of a mixture containing as little as 2% of one component. In collaboration with Miss D. Toten he has applied similar methods to the separation of the mixed hexosephosphoric acids of fermentation.

Mr. Reich has also elaborated a new method of phosphorylation by which it has been possible to synthesise monophosphoric esters of glucose.

During the early part of the year Dr. Morgan held a Rockefeller Foundation Fellowship and worked at the Eid. Technische Hochschule, Zürich with Professor T. Reichstein. During part of the time Dr. Morgan was engaged in the study of the 2-keto-methyl pentoses and their conversion into analogues of ascorbic acid (Vitamin C). A synthesis of *d*-fructo-methylose was developed utilizing *d*-fructose as starting material. Oxidation of this 2-keto-methylose to the corresponding methylosonic acid and subsequent hydrolysis gave 6-deoxy-*d*-arabo-ascorbic acid. This analogue showed only slight anti-scorbutic power.

Dr. Morgan has completed a preliminary examination of the action of dilute alkali on *N*-acetyl and *N*-benzoyl amino-acetaldehyde and has shown that *N*-substituted derivatives of pyrazine are readily formed. These heterocyclic compounds give an intense colouration with Ehrlich's *p*-dimethyl-aminobenzaldehyde reagent similar to that produced by the *N*-acetylhexosamines after treatment with alkali. These results indicate that by the action of alkali on the *N*-acetylhexosamines, *N*-substituted pyrazines are most probably formed together with *N*-substituted oxazoles and oxazolines as was previously suggested.

METABOLISM OF FAT.

Investigation of the part played by unsaturated fatty acids in the metabolism of the rat has been continued by Dr. I. Smedley-MacLean and Dr. L. C. A. Nunn, working in collaboration with Miss Hume and Miss Henderson Smith, who have been responsible for the biological part of the work. Burr and Burr found that young rats maintained for a long time on a fat-free diet cease to grow or to store fat, and develop a scaly tail and scurfy condition of the skin which can be cured by adding to the diet a small daily dose of linoleic or linolenic acid.

The biological technique now adopted has made it possible to compare quantitatively the potency of the materials tested, both with regard to their power of promoting increase of weight and of curing the skin lesions developed, when the rats were fed on the fat-free diet. In much of the work done in this field the criterion chiefly used has been increase in weight. In the most favourable experimental conditions this is an unsatisfactory criterion, being non-specific; in the present investigation it was particularly so, since it became increasingly clear that, after administration of certain supplements, weight increase might take place without cure of symptoms, though cure of symptoms never seemed to occur without weight increase.

The healing of the skin symptoms has in the past been neglected as a criterion, in estimation of this factor, undoubtedly because of the difficulty in using it. Most of the lesions, particularly the scaly tail condition, develop with great irregularity, varying widely in severity between animals of the same litter. Furthermore, the condition is cured only very slowly even when highly potent supplements are added. It has, however, been found in the course of the present study that a marked dryness and scurfiness of the skin of the ankles develops regularly in all animals, and is established to a uniform degree by the end of the 4 months' preparatory period, after which the tests for weight increase are due to begin. This state of the ankles responds well to treatment with a potent preparation of the essential unsaturated fatty acid, and is capable of complete cure in an experimental period of 5 weeks. By observing the behaviour of this symptom, three results, viz., no cure, partial cure, and complete cure could be differentiated, which made possible a rough quantitative assessment, capable of comparison with the associated degree of weight increase.

In contrast to the results of Burr and Burr, it has been found that linoleic acid (containing two double bonds) and linolenic acid (containing three double bonds) are not equally effective but that the former is at least six times as potent as the latter in promoting increase of weight and in healing the skin symptoms. In one or two cases, rats failing to grow when receiving a linolenic acid supplement at once renewed their growth when this acid was replaced by linoleic acid. Further, in rats receiving a daily dose of linolenic acid, growth ceased as soon as this supplement was removed from the diet; on the other hand, rats which had received linoleic acid continued to grow for some weeks after dosage had been stopped. This is interesting in view of the fact that when animals are fed with fats containing linoleic acid, this acid is found stored both in the tissues and in the fat deposits; on the other hand, unless excessive doses of linolenic acid are fed it is not found in the body fat, so that this acid must have undergone rapid transformation on entering the body.

Examination of the fatty acids of the liver showed that in rats maintained for a considerable time on the fat-free diet, there was complete absence of the highly unsaturated acids, arachidonic $C_{20}H_{32}O_2$ with four double bonds and elupandonic $C_{22}H_{34}O_2$ with five double bonds, these being the characteristic constituents of the lipin and neutral fat of normal livers. These acids were found however in the livers of rats which had been kept for many months on the fat-free diet but which, before killing, had received curative doses of linoleic and linolenic acids. From the livers of rats fed on the fat-deficient diet, an acid containing 20 carbon atoms and only three double bonds was isolated as its hexabromide; this acid was previously unknown.

Considerable discrepancies appear in the literature as to the curative potency of cod liver oil. The characteristic acid of this oil, containing 22 carbon atoms and six double bonds has recently been isolated by Dr. E. H. Farmer, who kindly supplied a specimen of its methyl ester for biological testing. A supplement of this ester, when fed to rats on a fat-free diet restored growth and enabled fat to be deposited in the tissues but had no effect on the skin symptoms. This result is similar to that obtained by Burr and Burr with cod liver oil.

It appears undesirable to retain the term "Vitamin F" introduced by Evans for linoleic and linolenic acids; these are rather to be regarded as building stones from which the higher unsaturated fatty acids, characteristic of the body lipins, are built up.

THE NATIONAL COLLECTION OF TYPE CULTURES OF MICRO-ORGANISMS.

(Medical Research Council.)

The National Collection of Type Cultures has continued to show expansion. Some 6,300 cultures of micro-organisms have been distributed during the year to workers at home and abroad and many new strains deposited for maintenance.

During the summer the Collection was visited by Dr. Mario Mollari, the recently appointed Curator of the American Type Culture Collection, and he and Dr. St. John-Brooks had the opportunity of discussing problems of mutual interest relating to the organisation of type culture collections and the conservation of micro-organisms.

A new and revised report on the genus *Salmonella* will be presented to the Nomenclature Committee of the International Association of Microbiologists at the 3rd International Congress for Microbiology to be held in New York this autumn. The report is being prepared by the *Salmonella* Subcommittee, of which the Curator is Secretary.

GENERAL AND FINANCIAL.

The Accounts and Balance Sheet for the year ending December 31st, 1938, show balances to the credit of the Capital Fund of £549,108 15s. 3d., the Contingency Fund of £22,000, the Sinking Fund of £32,814 4s. 6d., the Pension Fund of £28,009 16s. 0d. and the Jenner Memorial Research Studentship Fund of £8,772 11s. 11d.

The balance of income over expenditure of the Pension Fund has this year, on actuarial advice, been added to the capital of the Fund.

The following changes in investments have taken place during the year, viz.:

GENERAL FUND:—£661 Madras & Southern Mahratta Railway 4 per cent. Debenture Stock, 1938, redeemed at par.

£4,100 3½ per cent. Conversion Stock, 1961 or after, purchased.

SINKING FUND:—£1,900 3½ per cent. Conversion Stock, 1961 or after, purchased.

Income for the year amounted to £57,583 3s. 1d. Compared with 1937 there is a decrease in Interest and Dividends on General Fund investments of £1,076 6s. 9d. and an increase of £220 15s 7d. in Investigation, Diagnosis and Analysis Fees. Sales of Sera, Vaccines, &c., amounted to £37,163 15s. 0d. and after adjusting stocks on hand at the beginning and end of the year, this item appears in the Accounts as £34,903 13s. 6d., an increase of £5,228 6s. 2d. on the previous year.

Expenditure during the year was £49,889 14s. 8d. against £48,810 1s. 3d. in 1937. This difference is mainly accounted for by an increase of £1,694 5s. 0d. in Serum and Vaccine Laboratories Expenses and a decrease of £790 10s. 1d. in Animal House Expenses and Forage.

The balance of income over expenditure on the year's working, amounting to £7,693 8s. 5d. has been transferred to the Capital Fund.

The Governing Body gratefully acknowledges legacies of £500 from Lloyd's Bank Limited acting as Trustees of Mr. James Henry Stephens of London and of £125 from Dr. G. A. Davies of Newport, Mon. These have been added to the Capital Fund. In addition the late Mr. A. W. Bacot, who was Entomologist to the Institute from 1911 to 1922, and who died from Typhus fever while investigating that disease in Egypt, bequeathed his house at Loughton to the Institute with a suggestion that it might be used for the recreation of the staff. Unfortunately its distance from the Institute precluded its use for this purpose and the Governing Body have decided to dispose of it and utilise the proceeds in some other appropriate manner.

In conclusion the Governing Body desires to express its appreciation of the devoted co-operation of the Director and all members of the staff, and also of the assistants, in carrying out the work of the Institute.

WILLIAM BULLOCH.

Chairman of the Governing Body.

BALANCE SHEET
AND
ACCOUNTS

The Lister Institute BALANCE SHEET

CAPITAL FUND to 31st December, 1938 :—

Donations, &c., received to date from the following :—

	£	s.	d.	£	s.	d.
Dr. Ludwig Mound (1893)	2,000	0	0			
The Berridge Trustees (1893/98)	46,379	10	1			
The Grocers' Company (1894)	10,000	0	0			
Lord Iveagh (1900)	250,000	0	0			
Lord Lister's Bequest (1912/23)	18,004	5	8			
William Henry Clarke Bequest (1923/26)	7,114	5	7			
Rockefeller Foundation (1935/36)	3,400	0	0			
The James Henry Stephens Bequest (per Lloyd's Bank Limited)						
(1938)	500	0	0			
Dr. G. A. Davies Bequest (1938)	125	0	0			
Other Donations and Legacies (1891-1934)	20,971	18	3			

Income and Expenditure Account :—

As per Account at 31st December, 1937	182,020	7	3			
Add Balance for the year ending 31st December, 1938	7,693	8	5			
				189,713	15	8

549,108 15 3

CONTINGENCY FUND :—

As per Account at 31st December, 1931	22,000	0	0

SINKING FUND to 31st December, 1938	32,814	4	6

PENSION FUND to 31st December, 1937	27,311	2	0

Add Amount transferred from Income and Expenditure Account, 1938	698	14	0

28,009 16 0

JENNER MEMORIAL RESEARCH STUDENTSHIP FUND :—

As per Account at 31st December, 1937	8,681	15	11

Add Amount transferred from Income and Expenditure Account, 1938	90	16	0

8,772 11 11

CREDITORS	2,316	6	3

WILLIAM BULLOCH, *Chairman.*

JOHN ANDERSON, *Hon. Treasurer.*

4643,021 13 11

REPORT OF THE AUDITORS

We have audited the above Balance Sheet. We have obtained all the information and explanations we have required. are held by the Institute on their behalf. In our opinion, such Balance Sheet is full and fair, and properly drawn and the explanations given to us and as shown by the books of the Institute.

London. 19th May, 1939.

of Preventive Medicine.

31st DECEMBER, 1938.

EXPENDITURE ON INSTITUTE BUILDINGS AT CHELSEA:—

As per account 31st December, 1935, including purchase of freehold site, £6,000

FREEHOLD LAND ADJOINING THE "STUDIOS" CHELSEA at cost (1912) ...

LEASE OF THE "STUDIOS" CHELSEA, as per last account ...

Less Amount written off for the year ...

QUEENSBERRY LODGE ESTATE, ELSTREE:—

Freehold land and buildings as per account 31st December, 1912 ...

FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS:—

*At cost less depreciation as per account 31st December, 1920 ...

Cost of Ultracentrifuges, purchased in 1936, less amounts written off ...

GENERAL FUND INVESTMENTS (at cost, less amounts written off):—

£80,000 4 per cent. Consolidated Stock, 1957 or after ...

£4,100 3½ per cent. Conversion Stock, 1961 or after ...

£25,000 4½ per cent. Conversion Stock, 1940-44 ...

£17,000 5 per cent. Conversion Stock, 1944-64 ...

£52,000 4 per cent. Funding Stock, 1960-90 ...

£64,000 3½ per cent. War Stock, 1962 or after ...

£37,000 Local Loans 3 per cent. Stock ...

£3,000 Port of London 3½ per cent. Registered Stock, 1965-75 ...

£1,000 Dominion of Canada 4 per cent. Registered Stock, 1940-60 ...

£25,000 Cape of Good Hope 3 per cent. Consolidated Stock, 1933-43 ...

£25,000 Natal 3 per cent. Consolidated Stock, 1929-49 ...

£8,000 New South Wales 4 per cent. Inscribed Stock, 1942-62 ...

£25,000 New Zealand Government 3 per cent. Inscribed Stock, 1945 ...

£26,100 South Australian Government 3 per cent. Consolidated Stock, 1916

or after ...

£2,900 Commonwealth of Australia 3½ per cent. Registered Stock, 1950-52 ...

£1,300 Union of South Africa 4 per cent. Consolidated Stock, 1943-63 ...

£25,000 Victorian Government 3 per cent. Consolidated Inscribed Stock, 1929-49 ...

£4,000 Western Australia Government 4 per cent. Inscribed Stock, 1942-62 ...

£20,000 Southern Railway Preferred Ordinary Stock ...

£6,200 London & North Eastern Railway 3 per cent. Debenture Stock ...

£5,000 Great Central and Midland Railway Joint Committee 3½ per cent. Guaranteed Stock ...

£253 London & North Eastern Railway 4 per cent. First Guaranteed Stock ...

£8,650 London, Midland & Scottish Railway 4 per cent. Preference Stock ...

£15,625 London, Midland & Scottish Railway 4 per cent. Preference Stock, 1923 ...

£18,750 London & North Eastern Railway 4 per cent. First Preference Stock ...

£25,000 East Indian Railway 3 per cent. New Debenture Stock ...

£800 Grand Trunk Railway Company of Canada Great Western Borrowed Capital 5 per cent. Perpetual Debenture Stock ...

£1,937 Grand Trunk Railway Company of Canada 4 per cent. Guaranteed Stock ...

£800 Ontario and Quebec Railway 5 per cent. Permanent Debenture Stock ...

£3,400 Gas Light and Coke Company Ordinary Stock ...

SINKING FUND INVESTMENTS (at cost):—

£9,600 4½ per cent. Conversion Stock, 1940-44 ...

£10,200 4 per cent. Funding Stock, 1960-90 ...

£16,700 3½ per cent. Conversion Stock, 1961 or after ...

Balance uninvested ...

PENSION FUND INVESTMENTS (at cost):—

£22,000 4 per cent. Funding Stock, 1960-90 ...

£13,000 3½ per cent. Conversion Stock, 1961 or after ...

Balance uninvested ...

JENNER MEMORIAL RESEARCH STUDENTSHIP FUND INVESTMENTS (at cost):—

£2,650 Southwark and Vauxhall Water Co. 3 per cent. Debenture Stock "B" ...

£1,596 Southern Railway 5 per cent. Preference Stock ...

£1,300 Liverpool Corporation 3 per cent. Stock, 1942, or after ...

£2,000 4 per cent. Funding Stock, 1960-90 ...

Balance uninvested ...

(The book value of the above Investments is, in the aggregate, less than their market value at 31st December, 1938)

STOCK OF ANIMALS ...

STOCK OF ANTITOXINS ...

DEBTORS

CASH:—

At Bankers: On Deposit

Current Accounts

In hand

* Nothing has been charged for depreciation of Furniture, &c. since new purchases made during the year to a greater amount than the estimated depreciation (10%) have been written off.

TO THE MEMBERS.

In accordance with the provisions of the Superannuation Scheme for certain members of the Staff, the relative Life Policies up so as to exhibit a true and correct view of the state of the Institute's affairs, according to the best of our information

COOPER BROTHERS & Co.

Chartered Accountants.

Auditors.

INCOME AND EXPENDITURE ACCOUNTS

INCOME.

General

	£	s.	d.
Interest and Dividends on General Fund Investments	18,701	18	7
Profit on Investment redemption	4	0	5
Interest on Sinking Fund Investments	1,358	0	0
Investigation, Diagnosis and Analysis Fees, &c.	2,558	10	7
Sales of Sera, Vaccines, &c., and Stock at 31st December, 1938, less Stock at 1st January, 1938	34,903	13	6
Rent of Rooms	52	0	0
Donations	5	0	0

£57,583 3 1

Pension

	£	s.	d.
Interest on Investments	1,335	0	0

£1,335 0 0

Jenner Memorial Research

	£	s.	d.
Interest and Dividends on Investments	278	6	0

£278 6 0

Morna Macleod Research

	£	s.	d.
Balance from last account	65	12	6

£65 12 6

Cancer Research

	£	s.	d.
Balance of Legacy from John George Mills (1937)	755	3	9

£755 3 9

of Preventive Medicine.

for the year ending 31st December, 1938.

Fund.	EXPENDITURE.												
											£	s.	d.
Rent, Rates, Taxes and Insurance	...	...	...	...	...	...	...	...	...	...	1,455	3	11
Salaries and Wages of Staff	...	...	...	...	...	...	...	...	...	...	28,986	17	4
Premiums on Federated Superannuation Policies	...	...	...	...	...	...	...	...	...	...	1,649	9	5
Stationery, Printing and Postage	...	...	...	...	...	...	...	...	...	...	389	1	10
Printing of Collected Papers	...	...	...	...	...	...	...	...	...	...	200	7	8
Office Expenses, Auditors' Fee and Sundries	...	...	...	...	...	...	...	...	...	...	309	10	2
Travelling Expenses	...	...	...	...	...	...	...	...	...	...	47	16	5
Gas, Water and Fuel	...	...	...	...	...	...	...	...	...	...	1,494	16	1
Electric Light and Power	...	...	...	...	...	...	...	...	...	...	439	13	8
Nutrition and Protozoological Expenses	...	...	...	...	...	...	...	...	...	...	632	19	7
Bacteriological and Experimental Pathology Expenses	...	...	...	...	...	...	...	...	...	...	390	12	11
Water and Bio-chemical Laboratory Expenses	...	...	...	...	...	...	...	...	...	...	480	15	4
Bio-physics Expenses	...	...	...	...	...	...	...	...	...	...	91	15	6
Serum and Vaccine Lymph Laboratories Expenses	...	...	...	...	...	...	...	...	...	...	4,591	9	4
Culture Media	...	...	...	...	...	...	...	...	...	...	113	17	3
Animals	...	...	...	...	...	...	...	...	...	...	1,979	18	10
Animal House Expenses and Forage	...	...	...	...	...	...	...	...	...	...	2,539	0	0
Alterations, Repairs, Renewals, and Workshop Expenses	...	...	...	...	...	...	...	...	...	...	1,369	10	0
Library Expenses	...	...	...	...	...	...	...	...	...	...	313	8	9
General Stores	...	...	...	...	...	...	...	...	...	...	206	11	2
Amounts written off Lease of the "Studios," Cinema and Ultracentrifuges	...	...	...	...	...	...	...	...	...	...	405	2	0
Sinking Fund (3% per annum on Cost of Buildings and Interest on Investments)	...	...	...	...	...	...	...	...	...	...	1,781	16	9
Balance, transferred to Capital Fund	...	...	...	...	...	...	...	...	...	...	7,693	8	5
											<u>£57,583 3 1</u>		

Fund.													
											£	s.	d.
Pensions	...	...	...	...	...	...	...	...	...	...	636	6	0
Balance, transferred to Balance Sheet	...	...	...	...	...	...	...	...	...	...	698	14	0
											<u>£1,335 0 0</u>		

Studentship Fund.													
											£	s.	d.
Salary of Student	...	...	...	...	...	...	...	...	...	...	187	10	0
Balance, transferred to Balance Sheet	...	...	...	...	...	...	...	...	...	...	90	16	0
											<u>£278 6 0</u>		

Studentship Account.													
											£	s.	d.
Salary of Student	...	...	...	...	...	...	...	...	...	...	65	12	6
											<u>£65 12 6</u>		

Account.													
											£	s.	d.
Sundry Expenses	...	...	...	...	...	...	...	...	...	...	83	4	8
Balance unexpended	...	...	...	...	...	...	...	...	...	...	671	19	1
											<u>£755 3 9</u>		

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"	THE TITRATION OF THERAPEUTIC ANTI-TYPHOID SERUM. <i>Journal of Hygiene</i> , Vol. XXXVIII., 1938.
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" " " "	EPERYTHROZOON IN THE BLOOD OF MICE AND ITS POSSIBLE RELATIONSHIP TO PLEUROPNEUMONIA-LIKE ORGANISMS IN MICE. <i>Journal of the Royal Army Medical Corps</i> , Vol. 72, 1939.
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"	THE GENETIC INTERPRETATION OF STUDIES ON CANCER IN TWINS. <i>Annals of Eugenics</i> , Vol. VIII., 1938.
HALL, KATHLEEN	THE CLITORIS OF THE RAT AFTER OVARIECTOMY AND THE INJECTION OF SEX HORMONES. <i>Journal of Pathology and Bacteriology</i> , Vol. XLVII, 1938.
HENDERSON, D. W.	THE PROTECTIVE VALUE OF THE VI AND THE O ANTIBODY IN RELATION TO THE VIRULENCE OF STRAINS OF <i>Bact. typhosum</i> . <i>British Journal of Experimental Pathology</i> , Vol. XX., 1939.
"	EXPERIMENTS WITH THE VI ANTIGEN OF <i>Bact. typhosum</i> AND WITH TWO NEW ANTIGENIC SUBSTANCES EXTRACTED FROM STRAINS OF THIS ORGANISM. (<i>Ibid.</i>)
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" " ...	THE INDOPHENOL-REDUCING CAPACITY OF GUINEA-PIG LEUCOCYTES. <i>British Journal of Experimental Pathology</i> , Vol. XIX., 1938.
" " ...	THE VITAMIN C REQUIREMENTS OF MAN. <i>Biochemical Journal</i> , Vol. XXXIII., 1939.
KLIENEBERGER, EMMY	STUDIES ON PLEUROPNEUMONIA-LIKE ORGANISMS: THE L4 ORGANISM AS THE CAUSE OF WOGLOM'S "PYOGENIC VIRUS." <i>Journal of Hygiene</i> , Vol. XXXIX., 1939.
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THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE.

Report of the Governing Body,
1940.

CHELSEA BRIDGE ROAD,
LONDON, S.W. 1.

June 21st, 1940.

The Lister Institute of Preventive Medicine,

CHELSEA BRIDGE ROAD, LONDON, S.W.1.

AND ELSTREE, HERTS.

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* Recognised teacher of the University of London.

ANNUAL GENERAL MEETING
OF
The Lister Institute of Preventive Medicine,
June 21st, 1940.

REPORT OF THE GOVERNING BODY.

The Governing Body has the honour to present the 46th Report of the Institute.

GOVERNING BODY.

No change in the personnel of the Board has occurred since the last Report. At its meeting held on June 18th last year the Council re-elected Professor W. Bulloch, Sir Arthur Harden and Professor H. R. Dean to represent it upon the Board until December 31st, 1940.

COUNCIL.

At the Annual General Meeting last year the three retiring members of Council were Professor F. W. Rogers Brambell, Professor S. P. Bodson and the President of the Royal College of Veterinary Surgeons. Each of these members was duly re-elected.

The three members of Council, due to retire in accordance with the Articles of Association this year, but who are eligible for re-election, are Professor H. R. Dean, who represents the University of Cambridge, Professor T. J. Mackie, the representative of the University of Edinburgh and Sir Humphry Rolleston, representing the British Medical Association.

MEMBERS.

On the invitation of the Governing Body, Dr. C. R. Amies, Dr. G. H. Eagles, Professor L. J. Witts, Professor J. H. Dible, Professor J. R. Marrack and Miss Eleanor M. Hume were elected new members of the Institute during the year. The Governing Body reports with great regret the death of Sir Patrick Laidlaw, a member of the Institute, in April last.

STAFF.

Dr. Petric, Bacteriologist-in-charge of the Serum Department, retired in June, 1939, having attained the age of 65 years. He has been succeeded by Dr. C. R. Amies.

Dr. A. Felix and Dr. M. M. Barratt were seconded in September to the War Emergency Public Health Laboratory Service at Oxford and Sherborne respectively. The laboratory to which Dr. Barratt was attached closed down in April and she has returned to the Institute. Dr. Felix is continuing to work as a member of this Service.

The Director, together with eight other members of the staff attended the Third International Congress of Microbiology, held in New York in September. Sir John Ledingham, in addition to his representation of the Institute, was also appointed by the Prime Minister Chief Delegate of His Majesty's Government to the Congress as the representative of the Medical Research Council.

Dr. D. W. W. Henderson, a member of the Elstree staff since 1935, was transferred to the staff of the Bacteriological Department in September.

Dr. L. C. A. Nunn, Grocers' Company Research Student and Dr. D. E. Dolby, Jenner Memorial Research Student, resigned their appointments in March last on taking up new posts. To replace these, Mr. H. J. Rogers and Mrs. Dolby, B.Sc. (formerly Miss D. Toten), were appointed temporary research assistants in April last. Dr. S. M. Partridge continues his tenure of the Research Studentship in Biochemistry to which he was appointed in January, 1939.

At the outbreak of war, the Institute in common with many other institutions, conformed with the general scheme of evacuation from London and with the exception of the Biochemical and Biophysical Departments, the Secretary and general maintenance staff, arrangements were made for the continuance of work elsewhere.

The Bacteriological Department, including the Vaccine, Diagnosis and Culture Media laboratories, the National Collection of Type Cultures and the Assistant Secretary with books and records were found accommodation at the Serum Department, Elstree. The Library was transferred to Aldenham School, near the Elstree laboratories, where a large room was placed at the disposal of the Institute through the kindness of the Headmaster and the Governors of the School.

The Division of Nutrition, at the invitation of Sir Charles Martin, is continuing its activities at his house at Chesterton, Cambridge, and additional accommodation has also been provided for the Division by Sir Frederick Hopkins at the Biochemical Department of the University of Cambridge.

Dr. Muriel Robertson transferred her work to the Institute of Animal Pathology, Cambridge, where Professor T. Dalling most kindly provided her with laboratory accommodation.

Dr. Korenchevsky and Miss Hall were, by the courtesy of Professor Goodrich and the University authorities, given facilities for their work at the Department of Zoology and Comparative Anatomy of the University of Oxford.

Dr. Zilva and his staff were transferred to the East Malling Research Station, Maidstone, where Dr. Hatton kindly provided the necessary accommodation.

The Governing Body takes this opportunity of expressing its gratitude to all those who have so generously provided research facilities for evacuated members of its staff. The consequence has been that dislocation of research activity has been surprisingly little in evidence.

RESEARCH WORK

The Governing Body, before surveying the scientific work done during the year, desires once again to record its appreciation of the continued co-operation the Institute has enjoyed with the Medical Research Council, which has furnished the salaries of the staff of the National Collection of Type Cultures, Miss Hume, Dr. Zilva and his assistants, and Miss K. Hall, assistant to Dr. Korenchevsky and part salaries of Dr. Korenchevsky, Miss Copping and Miss H. H. Smith. The Institute provided, as hitherto, accommodation and materials for the researches of each of these workers. Mr. Kekwick's salary and a grant for his expenses, together with a research grant to Mr. H. G. Hind, have also been furnished by the Council.

Also, the British Empire Cancer Campaign provided the salaries of Dr. Gorer and Mr. Carr together with expense grants up to December and October, 1939, respectively.

VIRUS STUDIES.

Elementary body suspensions of Vaccinia for Jennerian prophylaxis. Dr. D. McClean and Dr. R. G. Henderson of the London County Council have completed their investigation of the immunity following intracutaneous and subcutaneous injection of elementary body suspensions of vaccinia virus. One hundred and sixty-four primary vaccinations were performed and the resulting immunity was tested by revaccination by scarification with Government vaccine lymph after an interval of not less than six weeks. Seventy-five of those inoculated developed typical vaccinal vesicles at the time of primary vaccination; of these, 72 were completely immune and 2 partially immune on revaccination. Eighty-nine patients did not develop vesicles as a result of the primary vaccination; of these only 31 were immune on revaccination and the remaining 58 developed typical vaccinal vesicles as a result of subsequent scarification with vaccine lymph. There is thus strong *prima facie* evidence that, in man, with the strains of vaccinia virus in common use, immunity as so tested is not established unless a typical cutaneous vesicle develops.

Comparable immunisation experiments were performed on groups of rabbits and it was found that intracutaneous vaccination in these animals produces an immunity comparable with that obtained by scarification and that vesicle production is not of importance. There would appear, therefore, to be a difference between the nature of the immune response of rabbits and of children. Some evidence was obtained that vaccinia virus proliferates more freely in the rabbit dermis than in that of children or adults.

Cultivation of various strains of Vaccinia Virus on the chorio-allantoic membrane of the developing chick. Strains of vaccinia virus derived from sheep produce thin plaques on the chorio-allantoic membrane and these plaques, although not actually haemorrhagic, have a very congested appearance. Strains of virus obtained from the calf, on the other hand, produce thick oedematous plaques without marked congestion of the blood vessels. The sheep strains usually produce miliary vaccinal pocks throughout the membrane whereas the calf strains usually remain localised at or near the point of inoculation. This difference must be due to repeated passage in the different species of animal since both strains were originally obtained from the same source. Moreover, if the calf strain is passed a few times on the skin of the sheep it acquires the typical characters of a sheep strain when re-inoculated on the chorio-allantoic membrane. Dr. McClean is investigating the differences between these strains and, for comparative purposes, has cultivated other pock virus strains on the same medium.

In view of the correlation between vesicle production and immunity to vaccinia, referred to above, an attempt has been made to adapt strains of virus to proliferation in the dermis by serial intracutaneous passage. These strains have also been propagated on the chorio-allantoic membrane and their characters are being studied. Further clinical trial of these strains in human immunisation experiments must be postponed until the normal social services are again available.

Contagious pustular dermatitis of sheep. Although the lesions of this disease bear a considerable resemblance to the pock diseases of sheep and other animals there are definite histological differences and no cross immunity has been demonstrated. The dermatitis virus has never been cultivated *in vitro*. In co-operation with Mr. Glover of the Medical Research Council Farm Laboratories, Dr. McClean has prepared

bacteria-free suspensions of this virus by the same methods as those employed for the preparation of vaccinia virus elementary body suspensions. These suspensions have been titrated in sheep, proved potent, and preserved by desiccation in the frozen state. Attempts have been made by both Mr. Glover and Dr. McClean to cultivate this virus on the chorio-allantoic membrane of the chick embryo. So far it has not been possible to maintain the virus on this medium.

Serial testicular passage of strains of Vaccinia Virus. Dr. Amies and Dr. McClean are studying the effect of rapid serial passage of (1) a neuro-testicular strain and (2) a dermal strain of vaccinia virus in the rabbit testicle. Changes in the histological picture as a result of repeated passage of both strains of virus and in the characters of the elementary body suspensions prepared from the infected testicle have been observed.

The soluble specific substance of Vaccinia Virus. Dr. B. R. Record has investigated the nature of the soluble specific substance obtained from the lesions on the backs of rabbits after removal of the elementary bodies by filtration. Ultracentrifugal and electrophoresis studies of the crude tissue extract have shown that for the most part it bears a close resemblance to normal rabbit serum but in contrast to this, on acidification to pH 4.5, it yields a flocculent precipitate which can be redissolved in phosphate buffer at pH 6.5. Purification of this material by repeated precipitation yielded a substance which, in the ultracentrifuge and in the electrophoresis apparatus, showed the presence of a single component only. The purified product gave a precipitin test with antivaccinial rabbit serum (diluted with its own volume of saline) in a dilution of 1 in 500,000, and contained practically the entire activity in this respect of the original pulp. The acid treatment had resulted in a 100-fold purification of the soluble specific substance.

The chemical nature and enzymic activity of Vaccinia Virus. Further work on the chemical nature of vaccinia virus has been carried out by Dr. M. G. Macfarlane. She had previously shown that the elementary bodies, after drying and removal of adventitious lipid material by treatment with ether, gave colour tests characteristic of nucleic acid and carbohydrates and still contained lipid material, which could be removed with alcohol. It has now been found that on treatment with weakly alkaline solutions part of the virus material passes rapidly into solution; the residue, which is free from nucleic acid, is gradually disintegrated on prolonged treatment with alkali. Attempts to separate the nucleic acid in pure condition from the alkaline extracts yielded various preparations containing by analysis 40 to 60% nucleic acid and giving an intense Feulgen reaction characteristic of thymonucleic acid; the presence of ribonucleic acid has not been excluded. Calculated on the dry weight, the composition of the infective particles is approximately: protein 85%; lipid material 9%; nucleic acid 3 to 4%; carbohydrate, including a small amount of glucosamine-residues, 3 to 4%.

Dr. Macfarlane and Dr. D. E. Dolby have continued their investigations of the enzymic activity of vaccinia virus suspensions. In experiments previously reported, tests for dehydrogenase activity towards various substrates, such as lactate and glucose, were found to be negative. It was thought possible that this might be due not to the absence of the protein components of the enzymes but to lack of the necessary co-enzymes, which might have been removed in the repeated washings necessary for the preparation of the virus suspensions. Tests for dehydrogenase activity have, therefore, been repeated with the addition of the appropriate co-enzymes and also in the presence of a hydrogen-carrying system such as flavoprotein; but no dehydrogenase activity towards triosephosphate, hexosemonophosphate, lactate or alanine could be demonstrated.

Further work on the phosphatase activity of the virus suspensions has shown that adenosine-5-phosphate is rapidly hydrolysed. This fact is of some interest in the pathology of the virus in view of the importance of this compound in cellular metabolism. Both ribo- and thymo-nucleic acids are hydrolysed with production of inorganic phosphate, but the virus is apparently unable to effect the further breakdown of the nucleosides.

Physical investigations on the elementary bodies of Vaccinia. Further work on the physical properties of this virus after drying and extraction with lipid solvents has been carried out by Dr. A. S. McFarlane in collaboration with Professor F. L. Hopwood and Dr. M. H. Salaman. It has been shown that more than 95% of the dried virus may be resuspended in the form of single particles in an aqueous medium, without loss of infectivity, by the action of ultrasonic vibrations of 500 kilo cycles per second applied for a few minutes. Treatment of the resuspended virus with 1% sodium carbonate over a period of days and with constant agitation produces a disintegration in which 70-80% of the virus substance goes into solution. The clarified solution has the bluish sheen characteristic of large molecules in solution and deposits a clear gel on centrifugation at 8,000 r.p.m. With increasing speed more dissolved material is regularly deposited until at 60,000 r.p.m. particles of the order of size of the smallest protein molecules are thrown down. No distinguishable sedimenting boundary is seen at any stage and it is evident that the solution consists of particles of a regular size distribution over a wide range extending up to the size of the original virus. In agreement with this the solution can be separated into arbitrary fractions by salt precipitation and Dr. Record has shown that these fractions are specifically precipitable in high dilutions by anti-vaccinial serum. These properties of the virus have implications with regard to its constitution which are discussed in full in a recent paper by Drs. A. S. McFarlane, M. G. Macfarlane, C. R. Amies and G. H. Eagles.

When vaccinia virus is examined in the electrophoresis U-tube, marked endosmotic streaming is visible, resulting in an anomalous distribution of virus throughout the various segments of the U-tube. Dr. McFarlane has described the phenomenon and discussed its implications in relation to the water atmosphere which has been shown by salt partition experiments to surround each virus particle.

SEROLOGICAL STUDIES, ANTIGENIC CONSTITUTION, VIRULENCE AND IMMUNISING PROPERTIES OF BACTERIA AND PROTOZOA, AND CHEMOTHERAPY.

Combined serum- and chemo-therapy in anaerobic infections. Dr. D. W. Henderson and Dr. P. Gorer have examined the therapeutic action of sulphapyridine and sulphanilamide for the control of experimental infection of mice with *Vibrio septique* or *Cl. welchii*. Both drugs showed activity but under the particular experimental conditions, sulphapyridine proved by far the more effective and most experiments were performed with this substance. The effectiveness of the drug was influenced by the time of administration and by the route of spore infection. Thus, against intradermal infection with *Vibrio septique*, sulphapyridine was highly efficient even if withheld until 4 or 5 hours after infection, whereas, against intramuscular infection, only a small proportion of animals could be saved by a prophylactic dose of the drug. The effectiveness of antitoxin or antibacterial serum was also found to vary according to the route of infection. Co-operative action between the antibacterial agent (drug or serum) and phagocyte may be more difficult in muscle tissue than in skin and also the potency of the toxin produced in muscle is probably greater. Experiments with *Cl. welchii* were less successful and a comparison of the effectiveness of the therapeutic agents against *Vibrio septique* and *Cl. welchii* led to the conclusion that their value was determined not only by the susceptibility of the organism to the drug but also by its ability to invade healthy tissue; the chemotherapeutic agent was found to be more effective against the more highly invasive species.

The combined action of two therapeutic agents may result in a survival rate significantly greater than that which would be expected if the reagents acted simultaneously but independently. Such synergic action was observed when infection with *Vibrio septique* was treated with sulphapyridine in combination with antitoxin or antibacterial serum but not with a combination of the two types of anti-sera. A synergic effect between antitoxin and drug probably occurs in the control of *Cl. welchii* infection but for technical reasons it is more difficult to detect.

This research gives grounds for believing that chemotherapy may prove a useful adjuvant in the control of certain anaerobic infections in man. It is clear, however, that chemotherapy used as the sole prophylactic or therapeutic interference cannot be relied upon to control gas-gangrene infection and an attempt should be made to elicit the synergic effect produced by the combined action of drug and serum.

Certain aspects of chemotherapy in relation to pathogenic anaerobes are also being investigated by Dr. Muriel Robertson at Cambridge.

Combined treatment with sulphapyridine and specific antiserum of experimental meningococcal infections. Sulphapyridine when given orally to mice will protect them against as many as one million lethal doses of meningococci provided that the administration of the drug is commenced immediately after the injection of the test dose of organisms. When the treatment is delayed for a few hours, however, a much lower degree of protection is obtained. It has now been shown that in the treatment of an established infection in mice the combined administration of sulphapyridine and anti-meningococcus serum gives better results than either drug or serum alone. These experiments, which have been carried out by Dr. Amies on a large scale, fully confirm the results of earlier work on the complementary action of sulphanilamide and anti-meningococcus serum reported by Branham and her colleagues in America. On the evidence of these experiments it is suggested that the recommendation to abandon serum treatment entirely in favour of sulphapyridine should not be adopted until further clinical experience of the results of combined treatment has been obtained.

Preparation of anti-typhoid serum in the horse. Two disadvantages attending recent methods for the preparation of anti-typhoid serum in the horse have been (1) the necessity for employing living bacilli for the preparation of Vi antibody and (2) the apparent necessity, because of the condition of the animals, of exsanguinating after only one course of immunisation. Dr. Henderson and Dr. Amies, with the co-operation of Dr. D. B. Steabben, have now found that acetone-dried and killed bacilli prepared from suitable strains of *S. typhi* are capable of stimulating the production of Vi antibody in the horse at least as readily as the living organisms. Acetone-dried organisms may also be used for the preparation of O antibody. Suitable blendings of rough Vi and of O organisms are injected intravenously and the quantity of each antigen is adjusted according to the antibody response. The Vi antibody thus produced is as protective for mice as that elaborated in response to living bacilli. An added advantage of this method is that the horses remain in good condition and can readily withstand at least three full courses of immunisation.

The antigens of *S. typhi*. Dr. Henderson in collaboration with Dr. W. T. J. Morgan has continued the study of the antigens extracted from *S. typhi* and other *Salmonella* types by the methods of Morgan, of Boivin and of Topley and Raistrick. It is now known that the "non-protein" antigens present in extracts of rough

types are in the nature of group substances. Active immunisation experiments with mice in which these "non-protein" antigens were used failed to show any evidence of cross protection against infection with smooth organisms. The functional deficiency of Vi antigen obtained by extracting with diethylene-glycol has not been observed in preparations obtained by the trichloroacetic acid process or by digestion with trypsin. *In vitro* methods have now been devised by which this functional deficiency of Vi antigen may be detected. Experiments now in progress indicate that certain properties of the Vi substance of *S. typhi* may be modified according to the conditions of growth of the organism but it is not yet possible to define the conditions that may lead to a modification of this molecular complex.

Virulence and toxicity of *S. typhi*. Since Arkwright's work on smooth-rough variation nearly twenty years ago a number of bacterial species have been investigated with the view of establishing the relationship between virulence and antigenic constitution. In some instances a close association between virulence and certain well defined antigenic components has been established, while in others the position is still obscure. In the case of the typhoid bacillus it is now recognised that only strains containing maximum amounts of the O and the Vi antigen possess the highest degree of virulence of which this organism is capable when tested in the mouse, guinea-pig or rabbit. The exact mechanism by means of which the two antigenic components exert their effect on its virulence is still, however, in question. According to Ørskov and Kauffmann and other workers, the Vi antigen in the living bacillus is acting as a toxin, and the so-called virulence test in the mouse is in reality a toxicity test. Dr. A. Felix, with the assistance of Miss R. M. Pitt, has tested this hypothesis, and concludes from experiments devised to throw light on this problem that it does not appear possible to ascribe the lethal effects of O + Vi bacilli in the mouse solely to the summation of the toxic actions of the two antigens. The two substances, when combined in the bacterial cell, obviously exert some other activities in addition to their direct toxic action. It has been shown in previous work that the Vi antigen protects the O antigen against the action of the natural or immune O antibody and thereby protects the bacterial cell as a whole against the bactericidal and opsonising activities of the serum. The present experiments indicate that this function of the Vi antigen is of greater consequence than its direct toxic action, so far as virulence to the mouse is concerned. Since experiments in man cannot be carried out it is impossible to state whether the different variants of *S. typhi* possess the same comparative virulence for man and for the mouse. The information accumulated during the past few years, however, clearly indicates that typhoid strains, when freshly isolated from the blood of typhoid patients, invariably contain the two antigens. Earlier reports on the isolation from the blood of typhoid patients of strains devoid of the Vi antigen were obviously due to inadequate methods of growing and testing the cultures.

The immunological unity of *S. typhi*. The most recent advance in the knowledge of the typhoid bacillus is due to the discovery by Craigie and Yen in 1938 of type-specific Vi bacteriophages. Typhoid strains from different sources, when subjected to the action of such phages, can be divided into a number of different types. In view of the striking stability of the phage types it appeared highly desirable to examine typhoid strains belonging to different phage types and to ascertain whether serological differences could be detected in the Vi antigen they contain. Dr. Felix, with the assistance of Miss Pitt, has studied the problem by means of the following serological methods: cross-agglutination and cross-absorption, phagocytosis, passive protection of mice and active immunisation of rabbits. None of the tests disclosed any difference in the reactions between homologous or heterologous phage type sera and bacilli. There seems, therefore, no need for the employment of more than one Vi strain in the preparation of typhoid vaccine or anti-typhoid serum—a result very reassuring from the point of view of anti-typhoid inoculation and serum therapy.

Prophylactic inoculation during the incubation period. The view has been held by some authorities that prophylactic anti-typhoid or other inoculation undertaken after an outbreak of the disease in question is a mistaken and even dangerous policy. They argue that, in consequence of an alleged negative phase resulting from the inoculation, certain individuals will develop the disease, who would otherwise have been only temporary hosts of the bacteria and so have escaped infection; they also consider it likely that those acquiring the disease will experience the illness in a more virulent form than they would have done, had they not been given the specific vaccine during the incubation period. Such arguments have, however, been supported by no valid evidence. Most field observations have lacked the necessary controls, while experiments on animals do not appear to have been performed. With a view to amplifying our knowledge on this matter, Dr. Schütze carried out a series of experiments in which mice were given *S. typhi-murium* vaccine subsequent to their infection with *S. typhi-murium*. The infecting dose was of such a size that during the period of observation (3 weeks) about 50% of the controls might be expected to die. In this way a harmful influence of the vaccine could declare itself by an increase in the mortality rate, a beneficial influence by a decrease. Not only was the usual laboratory white mouse used in these experiments but also a pure line strain (Little's C.57 blacks). The investigations afforded no evidence that vaccination during the incubation period exacerbated the course of the disease; 216 white mice thus treated had a mortality of 28%, while the mortality of the 120 untreated controls was 41%; the average length of life of the pure line mice was not decreased by vaccination in the incubation period. A similar result was obtained in an experiment in which *S. enteritidis* vaccine was administered after infection with *S. enteritidis*. These results indicate that it should not be considered dangerous to inoculate a community during the course of a typhoid or other similar epidemic.

Simultaneous immunisation against Whooping cough and Diphtheria. As the efficiency of whooping cough vaccine becomes more generally accepted and the practice of active immunisation against diphtheria more widely adopted, it will be of obvious convenience to combine the two prophylactic measures and administer both the immunising agents simultaneously. To demonstrate whether such a combination proves damaging to either constituent, Dr. Schütze has undertaken the immunisation of two groups of guinea-pigs with diphtheria toxoid, one set with and the other without the inclusion of *H. pertussis* vaccine in the inoculum. Similarly two groups of rabbits have been immunised against *H. pertussis*, the one set with and the other without the addition of diphtheria toxoid to the vaccine. The results of the former experiment are already available and demonstrate most clearly that not only does the addition of *H. pertussis* vaccine to diphtheria toxoid not interfere with the efficacy of the latter but that on the contrary the influence of the toxoid is enhanced and the guinea-pigs receiving the mixture are more highly immunised against diphtheria toxin than those receiving the toxoid alone.

The effect of temperature on the stability of antisera. In time of war, antisera may have to be stored at temperatures far removed from those that are optimal for the conservation of antibody. Supplies of serum in transit overseas may, for example, have to be accommodated in the ship's hold if cold storage is not available; or they may be held up at the quayside exposed to the rigours of winter or the high temperatures of summer. Similar conditions may be encountered at medical stations in the war zone. Full information on the effect of these conditions is not available and experiments have accordingly been undertaken by Dr. Amies to supply this. He has ascertained that tetanus antitoxin is not appreciably affected by being maintained for 3 weeks at -16°C ., or by repeated freezing and thawing. When the transition from the liquid to the solid phase takes place slowly, the different components of the serum separate into fairly well defined layers. The water then separates out in the form of ice leaving a relatively strong concentration of antiseptic in contact with the still fluid protein layers. It was thought that under these conditions a denaturation of the antibody-globulin might take place with a consequent diminution of potency. From the results of a number of experiments, however, it appears that no appreciable fall in antibody content occurs. These investigations are being continued.

Standardisation of Anti-meningococcus serum. A mouse protection test based upon that described by Branham, Pittman, Rake and Sherp in America has been employed by Dr. Amies for determining the potency of anti-meningococcus sera. This test has proved very satisfactory. The protective capacity of each batch of serum can in this manner be estimated in terms of a provisional laboratory standard. The test is also of value in assessing the relative merits of different methods of immunisation. It may be of interest to record that a dried preparation of anti-meningococcus globulin that has been kept in cold storage since 1932 was found to be fully potent against a number of recently isolated strains of *Group 1 meningococcus*.

An analysis of the variable interactions of Tetanus toxins and antitoxins. Recent work on the titration of tetanus antitoxins has shown that, despite the accuracy with which assays can be carried out in the mouse and the guinea-pig, the values assigned to samples of antitoxin in different laboratories may vary widely. A review of the subject has been published by the Permanent Commission on Biological Standardisation of the League of Nations. Dr. G. F. Petrie has carried out experiments with the object of throwing light on the problem and has obtained results which point to the conclusion that the toxin and toxoid molecules in crude toxins, derived from short-growth cultures, possess greater antigenic uniformity than those in toxins derived from long-growth cultures. It would seem that tetanus toxins, whether employed as immunising antigens or test-toxins, are to be regarded as mixtures in varying proportions of the primary undifferentiated toxin and of an antigenic variant of the primary toxin; preparations of tetanus antitoxin consist likewise of variable mixtures of the corresponding antitoxins. Differences in the neutralising affinities of these components in a reacting system account for the discrepancies in titre of tetanus sera when different test-toxins are used for the assays.

When samples of tetanus sera from various sources are titrated in both the mouse and the guinea-pig by the same test-toxin the potency ratios (M/G-P) vary within wide limits. These variations are probably referable to physical or chemical differences in the antitoxin component of the reacting system.

BIOCHEMICAL STUDIES ON ANTIGENS AND ANTIBODIES.

Bacterial antigens. Dr. Morgan and Dr. Partridge have continued their examination of the antigen of *Bact. dysenteriae* (Shiga). The antigenic material has been shown to consist of a complex of polysaccharide, phospholipin and polypeptide, and a method has been developed for the stepwise degradation of this complex depending on the dissociating action of the solvent. By means of this reagent the phospholipin component is easily removed and a complex consisting of polysaccharide and polypeptide is obtained. The latter is strongly antigenic and gives rise to antibacterial immune-bodies, qualitatively similar to those produced by the whole organism. More thorough treatment of the phospholipin-free complex with slightly acid formamide eliminates the polysaccharide component. The carefully purified polysaccharide and polypeptide are not antigenic. The prolonged action of trypsin on the original antigenic material removes the polypeptide and gives rise to a polysaccharide-phospholipin complex which is not antigenic.

Dr. Morgan and Dr. Partridge have attempted to reconstitute the original antigenic complex from the antigenically inactive components. It has been found that the polysaccharide and polypeptide can be re-combined by solution in formamide and the resulting complex gives rise to specific *Bact. dysenteriae* (Shiga) agglutinins and precipitins. The possibility of replacing the polypeptide component with polypeptide material derived from proteins such as gliadin, casein, etc., is being investigated.

The chemistry of amino sugars. In continuation of Dr. Morgan's investigation of the nature of the amino sugars present in certain bacterial antigens, Dr. Morgan and Dr. Jolles have elaborated a method for the isolation of small quantities (10-30 mg.) of glucosamine and chondrosamine. The method depends on the capacity of the amino-sugars to react with certain aromatic aldehydes with the formation of sparingly soluble Schiff bases. An examination of a large number of aldehydes led them to select 2-hydroxyl-1-naphthaldehyde as a suitable reagent. By means of this method, quantities of the naturally occurring amino-sugars as small as 10-20 mg. can be readily isolated in good yield from mixtures containing sugars and amino-acids. Thus, from the polysaccharide component of crystalline egg albumin, 80% of the glucosamine present has been isolated; while, from as little as 100 mg. of chondroitin sulphuric acid, 17 mg. of crystalline glucosamine hydrochloride has been obtained. Glucosamine has also been isolated in this way from the ecto-skeleton of the tail segments of the Egyptian scorpion, *Buthus quinquestratus*.

Immune sera. On the outbreak of war Professor Robison and his colleagues in the Biochemical Department offered their co-operation to Dr. Amies and the staff of the Serum Department in the investigation of certain problems concerned with the concentration of immune sera. Such problems are related in general to the nature of the serum proteins, and since it was desired to correlate the behaviour of different types of sera submitted to purification with the immunological and physical properties of the protein components present, the work was planned and discussed in close association with the members of the Biophysical Laboratory, whose investigations on this subject are reported below.

The method of purification of antitoxic sera by treatment with pepsin described by Pope had already been used by Dr. Morgan for the purification of antidiphtheritic serum with satisfactory results, and Dr. Knight has investigated its application also to other types of antitoxic sera, tetanus, gas gangrene group, Shiga and scorpion. The method consists essentially in treating the serum with pepsin at a suitable pH, followed by fractional heat denaturation, the non-antitoxic protein being separated in the coagulum while the major part of the antitoxin remains in solution. A series of experiments has been carried out by Dr. Knight, Dr. Macfarlane, Dr. Dolby and Dr. T. White, on the effect of variation in the different factors involved in this process, in order to find the optimum combination of conditions. The effect of antiseptics is also being investigated with the assistance of Mr. Rogers.

Dr. Macfarlane and Dr. Dolby have carried out experiments on the proteolytic action of pepsin on the proteins of normal and antitoxic sera. Under standard conditions, which conserve 80% of the antitoxic activity, serum albumin is very rapidly converted into a form not coagulated by heat; about 30-40% of the protein present in serum globulin fractions, obtained either from normal or antitoxic sera is also fairly readily hydrolysed but the remaining protein is very resistant to peptic action. The rapidity and extent of hydrolysis of both normal and antitoxic sera was such as was to be expected from the relative proportions of albumin and globulin present. Though the change in the total and relative concentrations of albumin and globulin in the serum which frequently accompanies immunisation may alter the rate and extent of hydrolysis of the total protein, no evidence was obtained that immunisation results in an alteration of the digestibility of the individual protein components.

PHYSICO-CHEMICAL INVESTIGATIONS.

Serum Proteins. Mr. Kekwick has continued his investigations on serum from cases of multiple myelomatosis and it appears that two types of divergence from the constitution of normal serum occur. The more frequent shows a great increase in the amount of γ -globulin, the more rare an increase in the β -globulin component. These two types differ in their appearance in the ultracentrifuge, the former showing merely an alteration in the quantities of the components observed in normal human sera, the latter showing many components of molecular dimensions not observed in normal human serum. The abnormal constituents from some of these sera have been isolated and studied electrophoretically, and by microchemical methods.

Mr. Kekwick in collaboration with Dr. Amies, Dr. Knight and Dr. Record is investigating antitoxic sera from the horse. Sera examined include those prepared from *C. diphtheriae*, *Cl. tetani* and *Cl. Welchii*, antidiphtheritic serum receiving the greatest attention. Each shows marked divergence in electrophoretic constitution from normal horse serum. The changes during the course of immunisation with diphtheria toxin have been followed with the ultracentrifuge and electrophoresis apparatus and the results from the latter correlated with Ramon flocculation studies carried out by Dr. Record. It has been established that both the β and γ globulins are associated with antitoxic activity and these components, isolated electrophoretically, show characteristic properties with respect to the Ramon test, and also with regard to the *in vivo-in vitro* ratio. It is probable that some antitoxic activity is also associated with the α -globulin of antidiphtheritic serum.

In an attempt to obtain pure antibody preparations in large quantity, experiments on the fractionation of antidiphtheritic globulin by salt and pH-fractionation are in progress, the electrophoretic technique being used to control fractionation. Dr. McFarlane and Mr. Kekwick are also studying the fractionation of normal human serum with a view to a better chemical characterisation of the fractions.

Technical Developments. An important improvement in the electrophoresis apparatus has resulted from the design and construction by Dr. McFarlane and Mr. R. A. Kekwick in collaboration with Mr. F. J. Perry of Adam Hilger, Ltd., of optical arrangements adapting the diagonal "schlieren" method of observation to this instrument. This method which shows the migration of the protein boundaries directly on a ground glass plate saves much labour and time and is now in routine use both in the electrophoresis apparatus and in the ultracentrifuge. Dr. McFarlane and Mr. Kekwick have designed a microform of U-tube which has certain advantages over the original Tiselius model; and Dr. McFarlane has also designed a photo-electric colorimeter and absorptiometer incorporating features of several existing models. All the accurately machined metal parts for the new application of the "schlieren" method, for the mounting of the U-tube and for the construction of the colorimeter have been made in our own workshops.

Studies on Flocculation tests with toxins and antitoxins. *Diphtheria Toxin and Antitoxin.* Special attention has been given by Dr. Record to the flocculation reaction and the flocculation time, Kf, in the optimal proportions mixture, under standard conditions. The Kf value appears to be a reproducible property of the different globulin fractions isolated electrophoretically by Mr. Kekwick from antidiphtheritic horse sera. The Kf of any serum may be accounted for on the basis of the relative amount of the active globulins present. Bleedings taken during the early stage of immunisation have a short Kf and the results suggest that the initial reaction to immunisation is the formation of antibody associated with γ -globulin.

The problem of obtaining pure diphtheria toxin for absorption studies on antitoxic sera and for further investigation of the toxin-antitoxin reaction is being studied by Dr. Record and Dr. Knight, and their attention is now centred on the growth of *C. diphtheriae* on simplified amino-acid media. The Kf values, with toxins obtained from such media are also being studied.

Tetanus toxin and antitoxin. The possibility of using the flocculation test as a means of supplementing the *in vivo* test for the preliminary measurement of units of toxin and antitoxin is being investigated by Dr. Eagles. According to the experiences of some workers the tendency to form multiple zones and the wide variation in the Lf/L+ ratio make the test of little practical value. Sufficient work has not yet been done with different samples of toxin, toxoid and antitoxin to give any definite opinion on these points, but there are some indications that the test is more satisfactory with sera of relatively high antitoxic value.

GENERAL BACTERIOLOGICAL STUDIES.

The Pleuropneumonia-like organisms. Dr. Klieneberger's pioneer studies have created a wide interest in this group of organisms which have now been found to be responsible for various types of lesion in rodents (rats, mice and guinea-pigs) including arthritis and at the 3rd International Congress for Microbiology, held in New York in September, 1939, a Section was devoted to the subject.

The first of this L series of organisms was isolated in the pure state by Miss Klieneberger from cultures of *Streptobacillus moniliformis* with which it appeared to be symbiotically associated. It is still, however, disputed whether the L 1 organism so recovered is a genuine symbiont or a peculiar variant of *S. moniliformis*. To the former view Miss Klieneberger still adheres but the matter will be settled only after further sustained efforts to overcome the great technical difficulties met with in following the morphological developmental stages in culture of these puzzling organisms. In this work Miss Klieneberger is still engaged and a new study, based on improved methods of fixing and staining the pleuropneumonia organism itself is in progress which promises to yield valuable results.

An interesting side issue has been opened up by work carried out in collaboration with Dr. Partridge. It has been shown that the L 1 culture as well as cultures of *S. moniliformis* contain large amounts of cholesterol which is apparently responsible for the myelin structures regularly found in colonies of these organisms. Recently Miss Klieneberger has published a fresh investigation of the serological relationships of the L series of organisms (L 1, L 3, L 4, L 5 and L 6) and of the saprophytic types of filterable organisms isolated by Laidlaw and Elford and by Seiffert from sewage and soil. These latter types are found to have no antigenic affinities with the parasitic types isolated from rodents.

Bronchiectatic Lesions in rats. In 1937, Dr. Klieneberger and Dr. Steabben recorded the isolation of a pleuropneumonia-like organism (L 3) from the purulent bronchopneumonia so common among laboratory rats. So large a percentage of the older stock rats in different laboratories harbour this organism in the affected lungs that direct evidence of its etiological importance is difficult to secure. Further observations by Miss Klieneberger and Miss Steabben during the past 3 years have recently been published which show that as the age of the rat increases, the percentage in which characteristic lung lesions occur rises as also does the percentage in which the lesions are found to harbour the L 3 organism. Direct experimental infection has not yet succeeded but it is probable that some adjuvant factor may yet be found which will

help to establish an experimental infection in the lung, for it is only recently that the pleuropneumonia organism itself when introduced along with agar into the lungs of cattle, has been found to induce lesions comparable with those of the naturally contracted disease.

Acute Rheumatism in man and the Pleuropneumonia Group of Organisms. Eighteen months ago considerable interest was evoked by the report that Drs. Homer Swift and Brown of the Rockefeller Institute had isolated a pleuropneumonia-like organism directly from human rheumatic fever exudates. This claim was withdrawn by Dr. Swift at the New York Congress in September, 1939. In the interval, however, Dr. Eagles and Dr. Helga Jahn with the co-operation of Miss Klieneberger carried out a series of isolations from various fluids and tissues (joint fluids, pericardial and pleural effusions, nodules, glands, synovial membranes, etc.). These specimens came from true rheumatic fever, rheumatoid arthritis and from control non-rheumatic conditions and the isolations were made on appropriate media, but completely negative results were obtained. Incidentally it was not possible to demonstrate either that rheumatic sera which agglutinated the granular suspensions from rheumatic exudates had any action on the granular suspensions prepared from cultures of true pleuropneumonia-like organisms or that high titre sera for members of the pleuropneumonia group had an agglutinative effect on the granular suspensions from rheumatic pericardial exudes previously studied by Dr. Amies and Dr. Eagles.

***Trichomonas foetus* as a cause of abortion in cattle.** Dr. Muriel Robertson has continued her study of this parasite and of an allied organism, *Eutrichomastix colabrorum*, mainly from a serological point of view and with the object of facilitating the diagnosis in cattle of this type of infection. Investigations of the reactions of *T. foetus* to immune sera prepared in rabbits and to sera from naturally and artificially infected cattle have been carried out in collaboration with Dr. W. R. Kerr of the Veterinary Department of the Ministry of Agriculture of Northern Ireland.

Variants of *B. pestis*. In the course of his plague investigations Dr. Schütze has encountered variants of *B. pestis* which grow without development of the characteristic envelope of normal strains. These unenveloped forms may be fully virulent or may lack that quality entirely. A comparison of the toxicity and virulence of such strains for white rats and for mice has helped to elucidate the reactions of these animals to plague infection. It has been found that, for mice, virulence is in no way dependent on the presence of an envelope; subcutaneous inoculation of a few organisms of a virulent strain kill as readily when the strain is unenveloped as when enveloped. On the other hand in rats there is a notable difference; the presence of envelope enhances virulence very considerably. Unlike virulence, toxicity is associated with envelope substance for both the rat and the mouse. In both cases an unenveloped strain will fail to kill in doses so high as $60,000 \times 10^6$ per 100 grammes animal weight, while $2,000 \times 10^6$ of an enveloped form will kill between 50 and 100% of the test animals.

FACTORS WHICH INCREASE TISSUE PERMEABILITY.

Chain and Duthie have recently reported that purified fractions of the diffusing factor from mammalian testis show marked mucolytic activity. The viscosity of mucin obtained from various sources is rapidly reduced by this enzyme with the liberation of reducing sugars. Dr. McClean has confirmed this observation and shown that purified diffusing factors obtained from various bacterial filtrates have a similar mucolytic activity. He has also been able to show that this activity is inhibited by anti-diffusing sera. These sera are strictly specific in their activity and neutralize only the enzyme derived from the same species of bacterium as the diffusing factor against which they were prepared; the anti-mucase and anti-diffusing activity of these sera have a similar specificity. The function of these enzymes in the metabolism of invasive bacteria, which also have the property of increasing the permeability of the tissues, is being investigated. It appears from the work of Miller and Kurzrok that semen contains a mucolytic enzyme by means of which the spermatozoa penetrate the plug of mucus normally present in the cervical canal of the uterus; thus we have an explanation of the physiological function of the diffusing factor obtained from the testis. Russell's viper venom which also diffuses in the tissues has similar mucolytic activity.

The evidence now available suggests that these mucolytic enzymes and the corresponding diffusing factors are identical. Final proof of this identity depends upon further work now in progress on the chemical purification of these factors and a study of the kinetics of the action of these enzymes from various sources on purified mucin and other substrates.

THE CHEMISTRY AND PHYSIOLOGICAL REACTIONS OF PHOSPHORIC ESTERS.

Further studies of the chemistry of carbohydrate-phosphoric esters and of the physiological processes in which they are concerned have been made by Professor R. Robison and Miss A. Tazelaar.

Dr. A. Lennerstrand (Rockefeller Foundation Research Fellow) and Mrs. Lennerstrand, who were obliged to return to Sweden at the outbreak of war, have published accounts of their work on the metabolism of diphosphoglyceric acid, the principal phosphoric ester of the red corpuscles, and on the oxidative breakdown of hexosediphosphoric acid in haemolysed blood. They find that dephosphorylation of the former ester occurs

not only in the intact red cells but also in haemolysed cells provided that adenylic acid and magnesium are added, these being essential factors in the process. In the aerobic decomposition of hexosediphosphate by haemolysed cells they find evidence of phosphorylation with formation of hexosemonophosphates, triosephosphates and monophosphoglyceric acid.

Mention was made in the last report of Dr. Reich's work on the separation of sugars by the formation of coloured azoyl derivatives to which the method of chromatographic analysis can be successfully applied. Since Dr. Reich's return to Paris in April 1939, Miss D. Toten, with the aid of a grant from the Institute, has spent some months in his laboratory continuing her study of the application of this method to hexose-phosphoric esters. Starting from the mixed fermentation hexosemonophosphates, tetraazoyl and hexaazoyl derivatives have been prepared and partially separated; but some difficulty has been found in a tendency for the phosphoric acid group to be eliminated during the chromatographic adsorption, a reaction which is itself of interest. The investigation has been temporarily interrupted by present conditions.

METABOLISM OF FAT.

In last year's report the changes produced in fat-starved rats when supplied with small doses of linoleic and linolenic acids were described. These highly unsaturated acids which occur in plant fat had been shown by Burr and Burr to cure the symptoms of fat deficiency disease in rats.

This year the work has been extended and the fat-starved rats have been given doses of the methyl ester of the highly unsaturated arachidonic acid characteristic of animal fat; it had previously been shown that this acid was absent in the liver of rats after a very long period of fat-starvation.

Dr. L. C. A. Nunn and Dr. Smedley-MacLean working in collaboration with Miss Hume and Miss Henderson Smith have found that arachidonic acid is about twice as effective as linoleic acid in restoring the growth rate of fat-starved rats and about equally effective in curing the dry and scurfy condition of the skin. The effect of giving minimal doses of arachidonic ester was investigated. The rats which received the smallest doses showed increased deposition of fat without formation of new tissue. As the doses increased the excessive fat disappeared and normal growth took place.

Two distinct processes in which arachidonic acid plays an essential part appear to be indicated by these results: (1) The filling up of the body fat depots especially in the abdominal region; and (2) The utilisation of the fat thus deposited in the formation of new tissue.

In collaboration with Professor G. Hevesy, of Copenhagen, Dr. Smedley-MacLean has examined the turnover of phospholipins in fat-starved rats which received injections of radioactive phosphate four hours before being killed. This turnover is measured by the proportion of radioactive phosphate which has been incorporated in the lipins of the various tissues. In the rat furnished with a plentiful supply of carbohydrate but with no fat, rapid formation of phospholipin takes place. In liver and kidney the formation of phospholipin takes place just as rapidly in the fat-starved rats in which growth has ceased as in those which are receiving the essential fatty acid and in which growth has been resumed. In muscle tissue the turnover of phospholipin has been found to be even 30 per cent. higher in the fat-starved rats than in those receiving the curative doses and growing actively. The formation of fatty acid from carbohydrate and its incorporation into phospholipin seem to be as efficient in the fat-starved as in the normal rat. The effect of the essential unsaturated acids seems therefore to be concerned not with the formation of the fatty acids but with their subsequent utilisation in the body.

STUDIES ON KIDNEY FUNCTION.

The method of intravital microscopy as used by Dr. P. Ellinger in his studies on the physiology of the kidney has been improved by the use of colour photography for recording the exact tints of the fluorescent microscopic images of living organs. These vary according to the pH of the fluid and thus it has been possible to make an estimation of the pH present in the cells and body fluid. By this device the mechanism of the acidification of urine in rats and frogs has been studied. Acidification is found to take place in the proximal as well as in the distal tubules if the urine is strongly acidified and only in the latter if the urine is less acid. The acidification occurs probably by absorption of alkali in both sets of convoluted tubules.

COAGULATION OF BLOOD.

Dr. J. O. W. Barratt has completed his estimation of the total anticoagulant action of citrated blood plasma. Owing to war conditions it has not yet been found possible to present the results obtained in a form suitable for publication but it is hoped that the work will shortly be resumed.

STUDIES ON PURE LINES.

Antigenic basis of tumour transplantation. Dr. P. A. Gorer has continued his studies on the antigenic basis of tumour transplantation. The susceptibility to inoculation of leukaemic cell suspensions originating from a case of myeloid leukaemia that arose spontaneously in a female mouse of Strong's A strain, has been

studied genetically and has given results essentially similar to those previously obtained with a sarcoma. The genetic experiment indicated that at least two factors were essential for the growth of the leukaemic cells. Serological tests showed that the leukaemic cells contained two distinct antigens and if either of these were absent from the normal tissues of any host, the cells were destroyed and antibodies directed against one or both of these antigens were usually demonstrable in the sera of immune animals. These experiments confirm the hypotheses concerning immunity to transplanted tumours dealt with in former reports.

Experiments have also been performed to ascertain whether the haemagglutinating sera that may be obtained from immunised mice of a heterologous pure line are able to influence the course of the disease in mice of the susceptible strain. Since it seemed probable that all antibodies would be absorbed by the normal tissues of susceptible mice, suspensions of leukaemic cells were suspended in immune, or normal serum prior to inoculation. Normal serum from the heterologous pure line was without any effect upon the course of the disease. The effect produced by the immune sera varied with the dose of cells employed; when this was of the order of one million, there was a very marked retardation in the onset of the initial lesions, but thereafter the disease ran a somewhat accelerated course and the animals died at about the same time as the controls. If the dose of cells is reduced about 10 times, lesions may fail to develop at all. The cells do not appear to be lysed to any appreciable extent prior to inoculation and it does not appear that any lengthy period of incubation is required to obtain positive results. It is interesting to note that in a single experiment in which sarcoma cells were suspended in immune serum, tumours developed more rapidly than in controls. It may be that the slight injury produced by the antibodies was insufficient to kill the sarcomatous cells but simply stimulated cell division.

The incidence of liver tumours in a pure line of mice. It has long been known that members of Strong's CBA strain are liable to tumours of the liver, but Dr. Gorer has shown that males of this strain are far more susceptible than females. Twenty-nine males came to autopsy, aged 14 months or over; of these 18 developed tumours, and in 14 of these one or more hepatomata were present. Thirty-eight females were examined during the same period; only 10 of these had tumours of any kind and only 3 had hepatomata.

ENDOCRINOLOGY.

Dr. V. Korenchevsky, with the assistance of Miss K. Hall, Miss M. A. Ross and Mr. R. Burbank, has continued the study of the co-operative and antagonistic activities of the sex hormones on different organs.

Changes which appear to be connected with tumour development. In females. In the last report the precancerous metaplastic changes produced by oestrogens in the uterus of the rat were described. These changes were prevented by the addition of progesterone but were stimulated by the simultaneous injections of male hormones with oestrogens. In the more recent experiments of long duration it has been possible to confirm these results and also to produce in rats, simultaneously injected with male hormones and oestrogens, adenoma-like overgrowth of the uterine glands, i.e., tumour-like development. In some cases these adenomatous structures also showed the "oestrogenic precancerous" changes already described. The results obtained were reminiscent of some pathological conditions which occur in the uterus of women, and it is therefore reasonable to suggest that a disturbance of the balance between male and female hormones may play a rôle in the development of some tumours in the human uterus.

In males. When oestrogens are injected for a prolonged period into males, an overgrowth of fibrous tissue occurs in the sex organs so that finally these organs have a chiefly fibrous structure. Since this overgrowth is confined to certain areas it is possible to suggest some similarity to the overgrowth of fibrous tissue in fibromata. When the weak male hormone, androsterone, is injected simultaneously with oestrogens into castrated rats, the adenoma-like changes develop in the epithelial tubules of the seminal vesicles.

In contrast to this pathological co-operative activity in males of strong oestrogenic hormones with the weak male hormone androsterone, simultaneous injections of oestrogens with the strong male hormone testosterone or its esters were followed by normal development of the sex organs. This demonstrates a neutralising and therefore physiological co-operative activity of these hormones.

The adenoma-like structures described above did not, however, in either sex, overgrow into a typical tumour, or infiltrate the fibro-muscular layers of the sex organs.

Cystic glandular hyperplasia of uterine mucosa ("Swiss cheese" mucosa) and formation of cysts in the ovaries. These two typical diseases, which occur in women, were found in the rats injected simultaneously with male and oestrogenic hormones. They did not occur when either of these hormones was injected separately. It is therefore suggested that these diseases in women might in some cases be produced by a disturbance of the natural balance between male and female hormones, which in both sexes are normally and simultaneously produced.

Protective rôle of corpora lutea. When male hormones or oestrogens are injected in excessive amounts, the ovaries react by the formation of numerous and hypertrophic corpora lutea. Since corpora lutea secrete progesterone, and progesterone can neutralise some of the toxic effects of oestrogens, the above reaction of the ovaries might be considered as a protective activity which neutralises the hyper-secretion of some toxic

hormones. If, however, toxic doses of the hormones are injected for a prolonged period, severe atrophy of the ovaries follows (ovarian collapse), the neutralising capacity being overwhelmed by too large and toxic doses.

Nephrotropic effect of male hormones. Amongst the male hormones, androsterone (weak sex hormone) and esters of testosterone (strong hormone), produce true hypertrophy of the kidneys with hypertrophy of the epithelial secretory elements.

Small Cystic degeneration of the kidneys of rats injected with oestrogens and its partial neutralisation by male hormones. In the kidneys of rats injected with oestrogenic hormones for a prolonged period, a peculiar degeneration occurs in the epithelial tubules, strictly confined to a special area between cortex and medulla. As the result of this degeneration, numerous minute cysts occur in this region. Similar degeneration has been observed in some kidney diseases of human beings, including Bright's disease. Simultaneous injections of androsterone or testosterone propionate in some cases prevented or decreased these pathological "oestrogenic" changes in the kidneys.

On the evidence of this nephrotropic activity of male hormones and their capacity to neutralise the toxic hormones, it is suggested that the male hormones may have therapeutic value as natural stimulants in suitable forms of renal disease. Androsterone is indicated for such clinical application, for this hormone possesses only a very weak sex effect, while its nephrotropic activity is as strong as that of testosterone esters.

Sex hormones and the Foà-Kurloff cell. The Foà-Kurloff cell was discovered in the blood of guinea-pigs in 1889 by Foà in Turin and independently by Kurloff working in Ehrlich's laboratory at Frankfurt. It has the morphological characters of the larger type of lymphocyte but contains in its cytoplasm a vacuole, which may be single or multiple, filled with material staining a dark brick red with the Giemsa stain. In the new born animals whether male or female, these cells are difficult to find and in the past many observers have believed that their presence in the blood cannot be detected with certainty until towards the end of the first month of life. Most observers have commented on the fact that these cells are invariably far more prevalent in the mature female and especially the pregnant female than in the male. Professor Ledingham, who published a study of these cells in 1906, returned to the subject some eighteen months ago, being attracted by certain reported claims, not however very well substantiated, that these cells disappeared from the blood after castration but could be made to reappear after administration of various sex hormones. He has recently placed the results of his new study on record and shown that the numbers of Foà-Kurloff cells in the blood are quite definitely regulated by hormonal impulses proceeding from the sex organs. After administration of oestradiol to the new born, whether male or female, Foà-Kurloff cells appear in the blood in a few days and within two or three weeks may reach large numbers even up to 40% of the total leucocyte population. Given to the pregnant female, oestradiol can induce the appearance of Foà-Kurloff cells in the foetal blood so that at birth a high content of these cells may be present. The urine of the pregnant human female exerts a very similar action.

The precise function of these cells remains obscure but there can be no doubt that this new experimental attack on the problem from the sex hormone standpoint is likely to throw light not only on this strange accessory of the sex hormone system of the guinea-pig but on the mechanism of action of oestradiol and other sex hormones generally. The great artificial increase of these cells by administration of oestradiol may also render the problem of the nature of the "inclusion" substance amenable to chemical attack. Of the many and strange theories put forward in the past 50 years with regard to the function of these cells and the nature of their "inclusions" this new theory based on their reaction to castration and to sex hormones is the only one that so far has lent itself to experimental verification.

THE ACCESSORY FOOD FACTORS.

On behalf of the Accessory Food Factors (Vitamins) Committee, work has been continued on standardisation problems.

Vitamin Standardisation.

The following co-operative work on standardisation problems was organised in preparation for a meeting of the League of Nations International Conference on Vitamin Standardisation, which was arranged for December 1939 with Dr. H. Chick and Miss Hume as Secretaries. In spite of the interruption caused by the outbreak of war, this work has been continued and much information is now available for consideration by the International Conference, when it is possible to have a meeting.

Vitamin A. A specimen of a pure preparation of the β -naphthoate, a solid ester, of Vitamin A, prepared by Dr. T. H. Mead of the British Drug Houses, was distributed from the Standards Department of the National Institute of Medical Research to 10 laboratories in Great Britain and U.S.A., in connection with an investigation, organised by Miss Hume (Secretary of the Vitamin A Sub-Committee) of its suitability as International Vitamin A standard to replace the existing β -carotene standard, which has certain disadvantages.

Tests of its biological activity for rats are being made for comparison with the results of spectrophotometric estimations. The results of all the tests, when fully worked out, should yield results of fundamental importance regarding the conversion factor relating biological and spectrophotometric values for Vitamin A, and the way in which β -carotene is changed into Vitamin A within the animal body. One series of biological tests was successfully carried out by Miss Hume without interruption in spite of the removal of the experimental animals from London to Cambridge which took place at the most critical period of the test.

The stability of the Vitamin A naphthoate has also been investigated and found to be reasonably satisfactory.

Vitamin B₁. Dr. Macrae, Secretary of the Vitamin B₁ Sub-Committee, has collected in a form suitable for publication the results of the co-operative investigation in 22 different laboratories which led to the adoption of pure crystalline aneurin hydrochloride as International Vitamin B₁ standard. The results have been statistically analysed by Dr. Irwin and the complete report will appear in a forthcoming number of the Bulletin of the Health Section of the League of Nations. In the same number will be published a report of the experimental work on the D vitamins described in the following paragraph, drawn up by Dr. Coward.

Vitamin D. A specimen of pure Vitamin D₃ (irradiated 7-dehydrocholesterol), the form in which the Vitamin is present in cod liver oil and other fish liver oils, was made available through Sir Henry Dale. Accordingly, quantitative tests with rats, of this material as compared with pure Vitamin D₂ (calciferol, irradiated ergosterol) were organised by Dr. K. Coward, Secretary of the Vitamin D Sub-Committee. In this co-operative investigation Dr. Zilva took part. The two forms of Vitamin D were found to be indistinguishable in activity for rats. Comparative clinical trials made elsewhere with the same specimens of Vitamins D₂ and D₃ showed these two forms to be equally efficacious in human rickets and osteomalacia. These results with rats and human beings are in contrast to the known greater activity for poultry of Vitamin D₃ as contained in cod liver oil, than that of calciferol.

The above investigations were undertaken to obtain information which might indicate the desirability of changing the present International Vitamin D Standard (solution of Vitamin D₂, irradiated ergosterol) for Vitamin D₃, the form of Vitamin D possessing more universal availability for different species.

Vitamin E. A co-operative test was also planned to investigate the suitability of a specimen of pure synthetic *dl*- α -tocopherol acetate as a possible International Standard for Vitamin E. Miss Hume, Secretary of the Vitamin E Sub-Committee, has organised co-operative biological and other tests of the suitability of this material, and these have been carried out by workers in 14 laboratories in Europe and America. Already Miss Copping has completed successful tests with her Vitamin E rat colony at this Institute.

Dr. J. O. Irwin has consented to make a statistical analysis of the results obtained in the co-operative tests described above in connection with the Standards for Vitamin A and E.

VITAMIN STUDIES.

Vitamin A.

Biological test for estimation of Vitamin A. In tests with rats carried out by Miss Hume as one participant in the co-operative investigation of the activity of Vitamin A β -naphthoate described above, a modification in method was adopted which has probably increased its accuracy. The biological test for Vitamin A is generally regarded as being subject to a very high degree of error. This is partly due to the possession by the rats of large and varying reserves of Vitamin A, of which they should be completely depleted before the test proper is begun. If completely depleted, however, the risk of epithelial sepsis is much increased and the accuracy of the test is impaired, owing to the interference with the growth response to dosage of Vitamin A, caused by the results of the infection suffered by the animal. It has been the usual habit to administer the test dose according to an adopted rule, e.g., when there has been no increase in weight at three successive weighings. In the present instance the time for giving doses was decided by the clinical condition and not by any rule. The results were eminently satisfactory and concordant, particularly in the avoidance of sepsis, though naturally this method could be used only by experienced workers.

The B Group of Vitamins.

Vitamin B₂ Complex. The researches on this subject have been carried on by Dr. Macrae, Mrs. C. E. Work, Mr. H. G. Hind and Dr. M. M. El-Sadr.

Riboflavin. Work on the biological estimation of riboflavin has been continued. Using the growth rate of young rats as the criterion of riboflavin potency, and employing a diet containing the filtrate prepared by treatment of aqueous extracts of liver with norite charcoal as source of B₂ vitamins other than riboflavin,

a method has been devised which is now being successfully used for the estimation of riboflavin in foodstuffs. Since the basal diet is apparently adequately supplied with all nutrients, other than riboflavin required by the rat, the values obtained are probably more reliable than those found by other workers who have invariably used diets deficient not only in riboflavin but in other B₂ vitamins.

Other B₂ Vitamins. Experiments on rats have shown that the Vitamin B₂ complex contains more essential nutrients than has been indicated hitherto. In addition to riboflavin and vitamin B₆ (previously called eluate factor), the rat requires for optimal growth at least three unknown factors; these are all contained in aqueous extracts of liver. One of these factors, which has been named liver filtrate factor (a), is present in filtrates obtained after treatment of liver extracts with fuller's earth and is readily extractable from acidified solutions by amyl alcohol or ether. A second factor (b) is also present in fuller's earth filtrates from liver but is not extractable by the above solvents. The third unidentified factor (c) is removed, together with vitamin B₆, from aqueous extracts of liver by treatment with fuller's earth; this unidentified factor is either absent from extracts of yeast or is present in these only in small amounts.

The chemical properties of the above unidentified nutrients (b) and (c) have been studied and attempts to secure more highly purified preparations have been made.

At present there are no satisfactory methods for the biological estimation of vitamin B₂ and the above unidentified nutrients in foodstuffs, since it has not been found possible to prepare basal diets deficient in one only of the individual factors but otherwise complete. Some knowledge has now been gathered which renders the preparation of such diets possible and it seems likely that satisfactory methods for the estimation of at least some of these factors will be forthcoming.

Liver Filtrate Factor. In collaboration with Professor A. R. Todd and Dr. B. Lythgoe of Manchester University, the work on liver filtrate factor (a) has been continued. It is now almost certain that this factor essential for rat growth, is identical with pantothenic acid, a factor required for the growth of certain micro-organisms and for the prevention of dermatitis in chickens. The following evidence indicates that the liver filtrate factor (a) and pantothenic acid are identical: (1) The chemical and physical properties of these two factors are strikingly similar; for example, they are inactivated at similar rates by acids and alkalis, both readily yield acetates and their solubilities and that of their various salts in water and other solvents are similar. (2) β -Alanine, a constituent part of the pantothenic acid molecule, has been isolated from the most potent concentrates of liver filtrate factor. (3) Dr. P. Fildes and his collaborators have kindly estimated the amount of pantothenic acid contained in four of the concentrates by determination of their growth-promoting properties for *Streptococcus haemolyticus*; in all cases the activity for rats ran parallel with the pantothenic acid potency.

In her investigations on the macrocytic nutritional anaemia of monkeys, Dr. L. Wills has examined the effects of concentrates of the liver filtrate factor (a). These experiments had reached an interesting stage when, at the outbreak of the war, they had to be discontinued because it became impossible to keep the monkeys on experiment.

In preliminary studies of the haematology of rats deprived of the B₂ vitamins, Dr. Chick and Dr. El Sadr have observed an abnormal condition in the blood after prolonged deprivation of liver filtrate factor, marked by a relatively high haemoglobin content with lowered count of red cells, of dimensions larger than the average. Prolonged deprivation of Vitamin B₂ on the other hand, produced severe anaemia, with low haemoglobin concentration and a relatively high red blood cell count.

Dr. El Sadr has continued his studies on the histological changes found in the tissues of rats deprived of various factors of the vitamin B₂ complex.

Mrs. Work has further investigated the effect of deprivation of vitamin B₆, riboflavin and other B₂ vitamins on the respiration rates of the isolated tissues of rats.

Dermatitis and other skin affections developed in rats deprived of different members of the Vitamin B₂ complex. Study of this subject has been continued by Dr. H. Chick, Dr. Macrae and Mr. A. N. Worden. Purified synthetic diets have been employed and the use of pure Vitamin B₂ (kindly provided by Dr. S. Lepkovsky of the University of California), of pure Vitamin B₁, riboflavin and nicotinic acid, and of more highly purified preparations of the filtrate factor, have permitted more precise conclusions to be drawn.

Vitamin B₆. Full confirmation has been obtained of the specific relation existing between Vitamin B₆ and the dermatitis known formerly as "rat pellagra," showing inflammation and oedema of the skin of the paws, nose and edges of the ears, spreading to that of the limbs and trunk if unchecked.

Nicotinic Acid. The rat, unlike the dog, pig and human being does not appear to need provision of nicotinic acid in its diet. It can either be synthesised by the rat or is needed in such small traces as may be present in the purified ration.

Riboflavin. Deficiency of riboflavin, which causes a skin affection of an eczematous nature affecting particularly the eyelids and eyes, has been the subject of special study by Dr. El Sadr. Conjunctivitis occurs, with inflammation of the eyelids which frequently become stuck together, the loss of hair causing

bald areas around the eyes, giving a spectacled appearance. Opacity of the cornea develops and in some cases, cataract. All the lesions except the cataract are promptly cured when riboflavin is given, the corneal opacity becoming lessened in a few hours. A histological study of the affected eyes has been made, and the results indicate that the opacity of cornea is due to infiltration of fluid in the layers of the epithelium, which drains away on the administration of riboflavin. Some damage to the cells of the retina was also observed.

Deprivation of filtrate factor was marked in some cases by greying of the fur and more particularly by staining with a serous reddish exudate from the nostrils and eyes. This contained no blood or blood pigments but owed its colour to a substance found by Mrs. Work to show the characteristic absorption band of a protoporphyrin. Sometimes the fur on the abdomen was stained, from soiling with the urine. The excretion of a porphyrin in the urine of rats deprived of B₂ vitamins and in that of human cases of pellagra was previously demonstrated by Dr. Ellinger.

The eczematous condition of the skin developed in rats deprived of riboflavin or filtrate factor did not appear to be specifically related to either of these two vitamins. It was also sometimes present in rats receiving both these essentials, but deprived of Vitamin B₆ for long periods, as was also secretion from eyes and nose of the serous fluid containing porphyrin. In order to maintain skin and fur in a healthy condition all the B₂ vitamins were found necessary.

Epileptiform Fits in rats deprived of Vitamin B₆. In the course of the above observations of Dr. Chick and Mr. Worden on the dermatitis produced in rats maintained on a synthetic diet designed to provide all the nutrient essentials but deprived of Vitamin B₆, a hypersensitive condition marked by fits occurred when the deficiency was prolonged over a period of about 4 months. The fits were usually induced by some outside stimulus, such as handling the animals for the purpose of giving the required daily doses. They showed well defined stages as follows: the rat suddenly became violently excited, rushed about the room, if not confined, leaping into the air and sometimes uttering cries. This was followed by a helpless stage in which there was twitching of the muscles and clonic spasms of the limbs, passing, in severe cases, to a stage of complete unconsciousness in which the heart beat was slow and weak and the corneal reflex absent. Consciousness returned gradually with a strengthened and accelerated heart beat, the forepart of the animal, head and forelimbs gaining power before the hind limbs. The duration of the fit varied from about 2-4 minutes and, after recovery, the animal remained in a very quiet but hypersensitive state for about an hour. If pure Vitamin B₆ was administered (15 µg. daily) the fits ceased to occur and neither dermatitis nor fits were observed when the diet was thus supplemented from the beginning.

Some of the rats suffering from the fits were sacrificed for bacteriological investigation, kindly undertaken by Dr. Emmy Klieneberger, in order to ascertain whether a pleuropneumonia-like organism might be present, such as that discovered in the "rolling disease" of mice; the results were entirely negative. In others, the central nervous system was examined histologically by Dr. C. E. Carmichael and Dr. J. G. Greenfield of the National Hospital, but no significant pathological lesions were detected.

The occurrence of these fits in rats deprived of Vitamin B₆ confirms the results of the observations made on pigs in 1938 by Dr. Chick and Dr. Macrae with Sir Charles Martin and Dr. A. P. Martin at the Institute of Animal Pathology at Cambridge. These pigs were maintained on a synthetic diet similar to that given to the rats and epileptic fits were produced in the group deprived of the "eluate fraction" of Macrae and Edgar. At that date pure Vitamin B₆ was not available, but the fits ceased on administration of the eluate fraction. Further proof is now available that this curative action was due to the Vitamin B₆ contained in the fraction.

Porphyrin excretion. The study of the effect of deficiency of B₂ vitamins on the excretion of abnormal pigments in the urine of the rat was continued by Dr. Ellinger up to the outbreak of the war. The study of these pigments required a method permitting colorimetric measurement of the concentration of a coloured substance in the presence of other coloured substances in the same solution. For this purpose an instrument called the Spectro-comparator has been devised. It consists mainly of a Duboscq colorimeter in which the eye-piece is replaced by a grating spectroscope. The slit of the latter is fixed so close to the division line of the Duboscq prism that both can be focussed together by the eye. This instrument can be used (1) as an ordinary colorimeter, (2) as an ordinary spectroscope and (3) for the purpose mentioned above. The estimation of the concentrations of more than one coloured substance can be carried out if the pigments have at least one absorption band in a region where the others do not absorb.

Fluorescence microscopic study of the localisation of Vitamins. Dr. Ellinger has examined the methods by which it is reported that the different Vitamins can be detected in animal tissues by means of their fluorescence. His results have shown that riboflavin can be detected in the liver and kidney of normally fed rats and frogs, and that probably Vitamin A also can be identified in living organs (liver and kidney) and their sections by its fluorescence and the behaviour of this fluorescence on irradiation or after the action of various solvents. The claims of some authors that members of the Vitamin B complex and Vitamin C are also to be detected by this method could not be confirmed.

Nutritive value of yeast as source of vitamins and protein. Yeast has long been recognised to be a very rich source of the B group of vitamins. Further, about half of its dry weight consists of nitrogenous substances of which about 80% are known to be of high nutritive value; there is some evidence that they have a supplementary value for the proteins of cereals comparable with that of the proteins of meat. The present scarcity of fish meal and other protein concentrates needed for feeding poultry, pigs and other stock animals makes it important to investigate further the value of yeast for this purpose and the possibility of its use in human diet. Dr. Macrae, with the assistance of Dr. El Sadr, is carrying out nutritive trials with pigs, in which yeast is used as (1) the sole source of protein, (2) as supplement to cereals in comparison with casein, which is taken as a type of protein of high biological value. These experiments, which are being made at the Institute of Animal Pathology, Cambridge, on the invitation of Professor Dalling, are designed to show to what extent the advantages of yeast are due to its protein, apart from its recognised value as a vehicle of B vitamins.

The Division of Nutrition is also attempting to fill the considerable gaps in our knowledge concerning the relative amounts of the different B vitamins, especially of the B₂ complex, contained in yeasts and the effect on these of differences of strain and of the medium in which the yeasts have been cultivated. Experiments with this aim in view have been undertaken by Dr. Macrae and Miss Copping.

Vitamin C.

Requirements of Vitamin C in fever. Dr. S. S. Zilva in investigating this problem has employed two modes of attack. In one set of experiments, compounds known to produce fever were administered to guinea-pigs receiving small doses of ascorbic acid and the clinical history as well as the *post mortem* findings were compared with those of guinea-pigs receiving similar doses of the vitamin only. In another set, animals "saturated" with vitamin C were treated with these compounds for several days in succession and the ascorbic acid contents of the tissues were compared with those of control animals. In the former set the guinea-pigs received *per os* daily doses of dinitro-o-cresol ranging from 5 to 20 mg. The animals were at the same time maintained on a scorbutic diet and a daily dose of 0.5 mg. or 1 mg. of ascorbic acid. Guinea-pigs on such doses, although unsaturated, are just protected from macroscopic signs of scurvy. The administration of the dinitro-o-cresol did not, however, in any way lessen the efficacy of these minimum doses of vitamin C. As no very significant rise of temperature in the animals was observed in this case, further experiments were performed in which the more drastic compound *p*-nitro- β -phenylethylamine hydrochloride was employed. Thirty mg. of this compound were injected subcutaneously twice daily. As a result of this treatment the animals became convulsed and the temperature usually rose by two degrees, the pyrexia lasting about half an hour. After several days of this treatment the animals, whose daily ascorbic acid intake was adjusted so as to maintain their tissues just "saturated," were killed and their tissues analysed for their ascorbic acid content. The action of the *p*-nitro- β -phenylethylamine did not in any way diminish the load of vitamin carried by the various tissues. The results of this investigation, therefore, do not suggest that the vitamin C requirement of the guinea-pig is higher in fever than under normal conditions.

The ascorbic acid content of the blood of leukaemic subjects. It has previously been reported by Dr. Zilva that the leucocytes of guinea-pigs contain reducing substances other than ascorbic acid. He has, therefore, examined the indophenol reducing capacity of the bloods of 7 leukaemic patients and 4 non-leukaemic patients all with a known vitamin C intake in order to ascertain whether the high reducing values claimed for the blood of leukaemic patients were due to the presence of ascorbic acid. The number of patients examined so far is too small to yield conclusive information and, unfortunately, this work is at the moment in abeyance.

The photochemical decomposition of Vitamin C. It was mentioned in last year's report that Dr. Zilva and Dr. Kellie found that *l*-ascorbic acid undergoes photochemical decomposition when exposed to light of the ultraviolet region of the spectrum in the absence of sensitisers and of oxygen. The investigation has since been continued by Dr. Zilva and Dr. Arcus. Employing a method for the determination of ascorbic acid based on the formation of furfuraldehyde they have established beyond doubt that during the exposure of the ascorbic acid to the ultraviolet light the vitamin is oxidised to dehydroascorbic acid in spite of the absence of oxygen in the medium. They have further found that the oxidation does not take place because of the formation of oxygen or hydrogen peroxide by actinic decomposition of the water in which the vitamin was dissolved. It is therefore concluded that the ascorbic acid was decomposed by the ultraviolet radiation with the formation of a compound capable of dehydrogenating some of the undecomposed ascorbic acid. Glutathione whether present in solution with the ascorbic acid or acting as a screen in a buffer solution exercises a protective action against this dehydrogenation. It is therefore improbable that any ultraviolet light penetrating superficial tissues containing ascorbic acid would bring about the oxidation of the vitamin *in vivo*. This photolytic decomposition of ascorbic acid is, however, of interest since it indicates the formation of degradation products not previously encountered in the decomposition of the vitamin molecule by chemical methods.

Influence of Vitamin C on the respiratory capacity of tissues. Many experiments have been carried out during the year on this subject by Dr. Zilva and Mr. Snow and the work has now reached a stage when, it is hoped, it will be ready for publication after the repetition of a few crucial experiments.

The preservation of ascorbic acid in urine. The inhibitory effect of 8-hydroxyquinoline on the oxidation of ascorbic acid in acidified urine was investigated by Dr. Zilva and Dr. Arcus at different temperatures, after various intervals of storage, and in the presence and absence of light. It was not found to be very effective in this respect and the claims made for this reagent by other workers could not be substantiated. It was, however, found that in an aqueous solution of ascorbic acid the oxidising effect of traces of copper at pH 7 was to a large extent neutralised by 8-hydroxyquinoline.

The enhancement of the vitamin content of meat and vegetable rations. Following up the observation previously made that a high concentration of vitamin C is reached in the King Edward potatoes in the month of September and that this diminishes particularly on storage, a trial canning experiment was carried out on a laboratory scale in order to ascertain whether by utilising this variety of potatoes at this stage of development, soon after lifting, the vitamin C of canned meat and vegetable rations could be enhanced. The promising information obtained from these trials justified further canning experiments on a large scale. Biological assay of the material indicated that less than 25% of the ascorbic acid was lost in the processing and that the inclusion of this variety of potatoes, soon after lifting, in canned meat and vegetable rations would be beneficial.

Vitamin E Colony.

The Vitamin E colony of rats was maintained during 1939 by Miss Copping, for testing the activity of the synthetic tocopherol derivatives prepared by Professor A. R. Todd of the University of Manchester. It was much increased in numbers to provide for tests of *dl*- α -tocopherol, the suggested possible International Vitamin E Standard (see above). These tests were satisfactorily completed shortly after the outbreak of war, just before removal of the Division of Nutrition to Cambridge. The results showed a striking sharpness between the results of administration of single doses of 1.8 mg. which induced successful gestations in the prepared depleted female rats, and doses of 1.3 mg., which failed to do so.

SENSITISING ACTION TO LIGHT OF BUCKWHEAT (*Fagopyrum esculentum*).

Further attempts have been made by Dr. Chick and Dr. Ellinger to extract the light sensitising substance from the flowers of this plant, in which the material is situated, the minimum daily sensitising dose being 0.02 g. dry weight for an albino rat of 100 g. weight. Partial success was obtained by digestion with pepsin at pH 2 and with normal human gastric juice. Trials were also made with trypsin at pH 8 and papain at pH 5, but negative results were obtained. Glacial acetic acid, diethylene glycol and dioxane in acetic acid were tried without success. A mixture of 90% glycol and 10% glacial acetic acid partly extracted the sensitising substance, and a mixture of 90% methyl alcohol and 10% glacial acetic acid was found to be entirely successful, the extracted material being completely inactive. Dr. Ellinger is now engaged in separating in a pure condition the pigments contained in this extract, three of which are found to be fluorescent, and in a study of their adsorption and emission spectra and light sensitising properties.

THE NATIONAL COLLECTION OF TYPE CULTURES OF MICRO-ORGANISMS. (Medical Research Council).

Arrangements for the safety of the National Collection of Type Cultures had been worked out in detail many months before the actual outbreak of hostilities so that on the declaration of war last September the work of evacuation of the Collection to its predetermined centre at Elstree was able to be carried out with the least possible delay.

Requests for cultures continued to show expansion up to the outbreak of war, after which, as might have been expected, a considerable decline took place. While for the first seven months of the year under review over 4,200 cultures were despatched, during the period September-December, 1939, 1,500 cultures were distributed. In the circumstances these results may be considered satisfactory. During the year some 300 strains were added to the Collection.

The Curator, Dr. St. John-Brooks, attended the Third International Congress for Microbiology in New York last September in the capacity of President of Section 1 (General Biology: Variation and Taxonomy) of the Congress. He read a joint paper with Miss Rhodes before that Section entitled "Taxonomy of the *Proteus* Group." He also presented the revised report of the *Salmonella* Committee, referred to in the previous report of the Governing Body, to the Nomenclature Committee of the Association, of which he is one of the joint secretaries.

Before his return to England Dr. St. John-Brooks visited the American Type Culture Collection at Washington, D.C., and had the opportunity of further discussions with the Curator, Dr. Mario Mollari, and his staff on the subject of the organisation and maintenance of type culture collections.

GENERAL AND FINANCIAL.

The Accounts and Balance Sheet for the year ending December 31st., 1939, show balances to the credit of the Capital Fund of £557,883 8s. 4d., the Contingency Fund of £22,000, the Sinking Fund of £34,662 11s. 3d., the Pension Fund of £28,642 17s. 11d., and the Jenner Memorial Research Studentship Fund of £8,800 17s. 11d.

The balance of income over expenditure of the Pension Fund has again this year been added to the capital of the Fund.

The following changes in investments have taken place during the year, viz. :

GENERAL FUND :—£4,900 3½% Conversion Stock, 1961 or after, purchased.

SINKING FUND :—£1,900 3½% Conversion Stock, 1961 or after, purchased.

PENSION FUND :— £800 3½% Conversion Stock, 1961 or after, purchased.

Income for the year amounted to £66,843 15s. 11d. Compared with 1938 there is a decrease in Interest and Dividends on General Fund investments of £466 14s. 6d. and a decrease of £1,169 5s. 6d. in Investigation, Diagnosis and Analysis Fees. Sales of Sera, Vaccines, etc., amounted to £45,689 5s. 3d., but whereas in former years the value of sales has included stocks held at 31st, December no value has this year been assessed for stocks on hand at that date. This year's figure, therefore, represents actual sales, less stocks on hand at January 1st.

Expenditure during the year was £58,069 2s. 10d. against £49,889 14s. 8d. in 1938. This difference is mainly accounted for by increased Serum and Vaccine Laboratories Expenses owing to increased sales.

The balance of income over expenditure on the year's working, amounting to £8,774 13s. 1d. has been transferred to the Capital Fund.

The house at Loughton, which as reported last year was bequeathed to the Institute by Mr. A. W. Bacot, has been sold and the proceeds, viz., £597 17s. 3d. are shown in the Accounts as the "A. W. Bacot Bequest." This sum is being invested and it is proposed to use it in some appropriate manner befitting the terms of the bequest.

In conclusion the Governing Body desires to express its appreciation of the devoted co-operation of the Director and all members of the staff, and also of the assistants, in carrying out the work of the Institute.

WILLIAM BULLOCH,

Chairman of the Governing Body.

BALANCE SHEET

AND

ACCOUNTS

The Lister Institute

BALANCE SHEET

	£	s.	d.	£	s.	d.	
CAPITAL FUND to 31st December 1939 :—							
Donations, &c., received to date from the following:—							
Dr. Ludwig Mond (1893)	..	..	..	2,000	0	0	
The Berridge Trustees (1893/98)	..	..	..	46,379	10	1	
The Grocers' Company (1894)	..	..	..	10,000	0	0	
Lord Iveagh (1900)	..	..	..	250,000	0	0	
Lord Lister's Bequest (1913/23)	..	..	..	18,904	5	8	
William Henry Clarke Bequest (1923/6)	..	..	..	7,114	5	7	
Rockefeller Foundation (1935/6)	..	..	..	3,400	0	0	
The James Henry Stephens Bequest (per Lloyd's Bank Limited) (1938)				500	0	0	
Dr. G. A. Davies Bequest (1938)	..	..	..	125	0	0	
Other Donations and Legacies (1891-1934)	..	..	..	20,971	18	3	
Income and Expenditure Account :—							
As per Account at 31st December, 1938..	..	189,713	15	8			
Add Balance for the year ending 31st December, 1939		8,774	13	1			
				198,488	8	9	
					557,883	8	4
CONTINGENCY FUND—							
As per Account at 31st December 1934	..	..	..	22,000	0	0	
SINKING FUND to 31st December 1939	..	..	..	34,662	11	3	
PENSION FUND to 31st December, 1938	..	..	..	28,009	16	0	
Add Balance transferred from Income and Expenditure Account, 1939				633	1	10	
					28,642	17	10
JENNER MEMORIAL RESEARCH STUDENTSHIP FUND :—							
As per Account at 31st December 1938	..	..	..	8,772	11	11	
Add Balance transferred from Income and Expenditure Account, 1939				28	6	0	
					8,800	17	11
A. W. Bacot Bequest (1939)	..	..	..	597	17	3	
CREDITORS	..	..	..	4,241	6	5	

WILLIAM BULLOCH, *Chairman.*

JOHN ANDERSON, *Hon. Treasurer.*

£656,828 19 0

REPORT OF THE AUDITORS

We have audited the above Balance Sheet. We have obtained all the information and explanations we have required, are held by the Institute on their behalf. In our opinion, such Balance Sheet is full and fair, and properly drawn and the explanations given to us and as shown by the books of the Institute,
London, 31st. May, 1940.

of Preventive Medicine.

31st. DECEMBER. 1939.

	£	s.	d.	£	s.	d.
EXPENDITURE ON INSTITUTE BUILDINGS AT CHELSEA:—						
As per account 31st December 1935, including purchase of freehold site, £6,000				73,548	3	1
FREEHOLD LAND ADJOINING THE "STUDIOS" CHELSEA at cost (1912)				169	6	8
LEASE OF THE "STUDIOS" CHELSEA, as per last account	937	0	9			
Less Amount written off for the year	65	2	0			
				871	18	9
QUEENSBERRY LODGE ESTATE, ELSTREE—						
Freehold land and buildings as per account 31st December 1912				20,455	10	0
FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS:—						
*At cost less depreciation as per account 31st December 1920	2,471	17	2			
Cost of Ultracentrifuges, purchased in 1936, less amounts written off	2,380	0	0			
				4,851	17	2
GENERAL FUND INVESTMENTS (at cost, less amounts written off):—						
£80,000 4 per cent. Consolidated Stock, 1957 or after	74,272	16	0			
£9,000 3½ per cent. Conversion Stock, 1961, or after	8,773	19	0			
£25,000 4½ per cent. Conversion Stock, 1940-44	21,669	3	3			
£17,000 5 per cent. Conversion Stock, 1944-64	15,997	0	7			
£52,000 4 per cent. Funding Stock, 1960-90	45,661	13	9			
£64,000 3½ per cent. War Stock	63,407	13	5			
£37,000 Local Loans 3 per cent. Stock	20,829	1	7			
£3,000 Port of London 3½ per cent. Registered Stock, 1965-75	2,686	17	7			
£1,000 Dominion of Canada 4 per cent. Registered Stock, 1940-60	928	4	6			
£25,000 Cape of Good Hope 3 per cent. Consolidated Stock, 1933-43	23,850	0	0			
£25,000 Natal 3 per cent. Consolidated Stock, 1929-49	21,400	0	0			
£3,000 New South Wales 4 per cent. Inscribed Stock, 1942-62	8,040	1	4			
£25,000 New Zealand Government 3 per cent. Inscribed Stock, 1945	22,114	0	0			
£26,100 South Australian Government 3 per cent. Consolidated Stock, 1916 or after	16,800	0	0			
£2,900 Commonwealth of Australia 3½ per cent. Registered Stock, 1950-52	2,723	16	0			
£1,300 Union of South Africa 4 per cent. Consolidated Stock, 1943-63	1,327	9	0			
£25,000 Victorian Government 3 per cent. Consolidated Inscribed Stock, 1929-49	19,800	0	0			
£4,000 Western Australia Government 4 per cent. Inscribed Stock, 1942-62	4,081	3	0			
£20,000 Southern Railway Preferred Ordinary Stock	13,500	0	0			
£6,200 London & North Eastern Railway 3 per cent. Debenture Stock	3,961	0	0			
£5,000 Great Central and Midland Railway Joint Committee 3½ per cent. Guaranteed Stock	3,623	0	0			
£353 London & North Eastern Railway 4 per cent. First Guaranteed Stock	499	11	0			
£8,650 London, Midland & Scottish Railway 4 per cent. Preference Stock	7,960	0	0			
£15,625 London, Midland & Scottish Railway 4 per cent. Preference Stock, 1923	11,300	0	0			
£18,750 London & North Eastern Railway 4 per cent. First Preference Stock	13,028	6	7			
£25,000 East Indian Railway 3 per cent. New Debenture Stock	13,890	0	0			
£800 Grand Trunk Railway Company of Canada Great Western Borrowed Capital 5 per cent. Perpetual Debenture Stock	936	0	0			
£1,937 Grand Trunk Railway Company of Canada 4 per cent. Guaranteed Stock	1,733	0	0			
£800 Ontario and Quebec Railway 5 per cent. Permanent Debenture Stock	984	0	0			
£3,400 Gas Light and Coke Company Ordinary Stock	3,638	0	0			
				449,415	16	7
SINKING FUND INVESTMENTS (at cost):—						
£9,600 4½ per cent. Conversion Stock, 1940-44	8,806	16	7			
£10,200 4 per cent. Funding Stock, 1960-90	9,079	0	1			
£18,600 3½ per cent. Conversion Stock, 1961 or after	16,698	14	2			
Balance uninvested	78	0	5			
				34,662	11	3
PENSION FUND INVESTMENTS (at cost):—						
£22,000 4 per cent. Funding Stock, 1960-90	17,165	3	5			
£13,800 3½ per cent. Conversion Stock, 1961 or after	10,801	3	5			
Balance uninvested	676	11	0			
				28,642	17	10
JENNER MEMORIAL RESEARCH STUDENTSHIP FUND INVESTMENTS (at cost):—						
£2,650 Southwark and Vauxhall Water Co. 3 per cent. Debenture Stock "B"	2,756	10	0			
£1,585 Southern Railway 5 per cent. Preference Stock	2,740	5	0			
£1,300 Liverpool Corporation 3 per cent. Stock, 1942, or after	1,097	6	9			
£2,000 4 per cent. Funding Stock, 1960-90	1,797	14	0			
Balance uninvested	409	2	2			
				8,800	17	11
(The book value of the above investments is, in the aggregate, less than their market value at 31st December 1939.)						
STOCK OF ANIMALS (No value assigned)						
STOCK OF ANTITOXINS (No value assigned)						
DEBTORS				28,371	1	4
CASH:—						
At Bankers: On Deposit	3,000	0	0			
Current Accounts	3,980	8	1			
In hand	58	10	4			
				7,038	18	5
* Nothing has been charged for depreciation of Furniture, &c. since 1920 as new purchases made during each year to a greater amount than the estimated depreciation (10%) have been written off.				£656,828	19	0

TO THE MEMBERS.

In accordance with the provisions of the Superannuation Scheme for certain members of the Staff, the relative Life Policies up so as to exhibit a true and correct view of the state of the Institute's affairs, according to the best of our information

COOPER, BROTHERS & CO.,
Chartered Accountants.

Auditors.

The Lister Institute

INCOME AND EXPENDITURE ACCOUNTS

INCOME.

General

									£	s.	d.
Interest and Dividends on General Fund Investments	...	...	...	...	...	...	...	...	18,235	4	1
Interest on Sinking Fund Investments	...	...	...	...	...	...	...	...	1,424	10	0
Investigation, Diagnosis and Analysis Fees, &c.	...	...	...	...	...	...	...	...	1,389	5	5
Sales of Sera, Vaccines, &c., less Stock at 1st January, 1939	...	...	...	...	...	...	...	...	45,689	5	3
Rent of Rooms	...	...	...	...	...	...	...	...	105	11	2

£66,848 15 11

Pension

									£	s.	d.
Interest on Investments	...	...	...	...	...	...	...	...	1,349	0	0

£1,349 0 0

Jenner Memorial Research

									£	s.	d.
Interest and Dividends on Investments	...	...	...	...	...	...	...	...	278	6	0

£278 6 0

Cancer Research

									£	s.	d.
Balance of Legacy from John George Mills (1937)...	...	...	...	...	...	...	...	...	671	19	1

£671 19 1

of Preventive Medicine.

for the year ending 31st. December, 1939.

EXPENDITURE.

Fund.

	£	s.	d.
Rent, Rates, Taxes and Insurance	1,568	8	7
Salaries and Wages of Staff	29,778	15	2
Premiums on Federated Superannuation Policies	1,606	18	3
Stationery, Printing and Postage	349	9	1
Printing of Collected Papers	190	17	3
Office Expenses, Auditors' Fee and Sundries	310	3	7
Travelling Expenses	362	10	3
Gas, Water and Fuel	1,462	1	5
Electric Light and Power	466	7	11
Nutrition and Protozoological Expenses	933	10	0
Bacteriological and Experimental Pathology Expenses	243	13	8
Water and Bio-chemical Laboratory Expenses	424	5	10
Bio-physics Expenses	290	7	10
Serum and Vaccine Lymph Laboratories Expenses	8,012	16	0
Culture Media	104	13	11
Animals	3,378	17	0
Animal House Expenses and Forage	3,164	13	9
Alterations, Repairs, Renewals, and Workshop Expenses	2,652	5	3
Library Expenses	300	12	3
General Stores	214	7	1
Amounts written off Lease of the "Studios," Chelsea and Ultracentrifuges	405	2	0
Sinking Fund ($\frac{1}{4}\%$ per annum on Cost of Buildings and Interest on Investments)	1,848	6	9
Balance, transferred to Capital Fund	8,774	13	1
	<u>£66,843</u>	<u>15</u>	<u>11</u>

Fund.

	£	s.	d.
Pensions	715	18	2
Balance, transferred to Balance Sheet	633	1	10
	<u>£1,349</u>	<u>0</u>	<u>0</u>

Studentship Fund.

	£	s.	d.
Salary of Dr. D. E. Dolby	250	0	0
Balance, transferred to Balance Sheet	28	6	0
	<u>£278</u>	<u>6</u>	<u>0</u>

Account.

	£	s.	d.
Balance unexpended	671	19	1
	<u>£671</u>	<u>19</u>	<u>1</u>

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(*Ibid.*)
- CHICK, HARRIETTE, MACRAE, T. F. RELATION OF SKIN LESIONS IN THE RAT TO DEFICIENCY IN THE DIET
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- " " " " " XIV. NOTE ON THE YEAST ELUATE FACTOR OF THE VITAMIN B₂ COMPLEX. *Biochemical Journal*, Vol. XXXIII., 1939.
- " " " " " THE ESTIMATION OF RIBOFLAVIN. Part 1. A NEW BIOLOGICAL METHOD. *Biochemical Journal*, Vol. XXXIV., 1940.
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THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE.

Report of the Governing Body,
1941.

CHELSEA BRIDGE ROAD,
LONDON, S.W. 1.

June 27th. 1941.

The Lister Institute of Preventive Medicine,

CHELSEA BRIDGE ROAD, LONDON, S.W. 1
AND ELSTREE, HERTS.

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*Recognised Teacher of the University of London.

ANNUAL GENERAL MEETING
OF
The Lister Institute of Preventive Medicine,
June 27th. 1941.

REPORT OF THE GOVERNING BODY.

The Governing Body has the honour to present the 47th Report of the Institute. The first of these reports presented by the Council of the British Institute of Preventive Medicine to the members on June 29th, 1894, dealt with the research activities of the Institute for the year following the amalgamation with the College of State Medicine when the premises of the latter at 101, Gt. Russell Street, London, were secured for the Institute's work. Members of the Institute may be reminded that the Certificate of Incorporation of the British Institute of Preventive Medicine under the Companies Acts, 1862 to 1890 dates from 25th. July, 1891, and that, therefore, the present year, 1941, might appropriately be recognised as its Jubilee year. In view, however, of the fact that active work could not be commenced till 1893 and that 1914 was regarded by the then Governing Body as the year of the Institute's coming of age, it has been decided that the year 1943, which will mark the completion of fifty years of the Institute's activities will be the more suitable for such celebration of the event as may be thought fitting and practicable at that date.

GOVERNING BODY.

The Governing Body records with sincere sorrow the loss by death of the services of Emeritus Professor W. Bulloch, its Chairman since February, 1932, who died on February 11th, and of Emeritus Professor Sir Arthur Harden, a member of the Board from 1931 and a former distinguished ornament of the Institute's staff (1897-1930) who died on June 17th. 1940.

At a meeting held on June 21st. 1940, the Council re-elected Professor Bulloch and Professor H. R. Dean and elected Professor C. R. Harington as successor to Sir Arthur Harden, to represent it upon the Board until December 31st. 1941.

The vacancy on the Governing Body created by Professor Bulloch's death falls to be filled by the Council at its next meeting.

COUNCIL.

The Governing Body records with regret the loss, in addition to Professor Bulloch and Sir Arthur Harden, of another member of the Council, viz., Emeritus Professor R. T. Hewlett, who died on September 10th. 1940, and who served on the bacteriological staff of the Institute from 1894 until his translation to King's College in 1901.

At the Annual General Meeting last year the three retiring members of Council were Professor H. R. Dean, Professor T. J. Mackie and Sir Humphry Rolleston. Each of these members was duly re-elected.

The three members of Council, due to retire this year in accordance with the Articles of Association, but who are eligible for re-election, are Sir Thomas Barlow, representing the Members of the Institute, the President (*ex-officio*) of the Royal College of Surgeons of England and Professor W. W. C. Topley, who is also a representative of the Members. Three vacancies in the representation of the Members of the Institute, created by the above noted deaths have also to be filled.

MEMBERS.

At the invitation of the Governing Body, Professor E. D. Adrian, Professor G. R. Cameron, Dr. W. Allen Daley, Professor T. Dalling, Professor A. V. Hill, Dr. W. T. J. Morgan, Dr. A. Landesborough Thomson, Dr. J. W. Travan and Professor G. S. Wilson accepted membership of the Institute during the year. The death, by enemy action, of Dr. W. M. Scott, a member of the Institute since 1931, is recorded with deep regret.

STAFF.

Dr. C. R. Amies, Bacteriologist-in-charge of the Serum Department was released for service with the Royal Army Medical Corps in December and Dr. G. F. Petrie has resumed his former post for the duration of the war, assisted by Dr. G. H. Eagles of the Chelsea staff.

Dr. Felix remains with the Emergency Public Health Laboratory Service and is now accommodated at the National Institute of Medical Research, Hampstead.

Dr. D. W. W. Henderson was seconded to the Ministry of Supply in August and is engaged in government work at the Experimental Station, Porton.

Dr. T. F. Macrae has recently been lent to the Royal Air Force for special scientific duties.

Mr. R. A. Kekwick, previously a grantee of the Medical Research Council, was appointed a scientific member of the staff in September and attached to the Biophysics laboratory. Dr. C. L. Arcus, also a former grantee of the Council, was appointed a temporary research assistant in the Department of Biochemistry in October.

Mr. H. J. Rogers, a temporary research assistant in Biochemistry, has been transferred to the Serum department as a Biochemical Research Student.

Mrs. D. Dolby, Miss E. Knight, Mrs. C. E. Work and Dr. S. M. Partridge have resigned their appointments, the latter continuing to work at the Institute as a Beit Memorial Research Fellow.

The disposition of the remaining members of staff has not changed since the last report. The Division of Nutrition remains at Roebuck House, Old Chesterton, Cambridge, with some additional accommodation in the University Departments of Biochemistry and Anatomy. Dr. Muriel Robertson remains at the Institute of Animal Pathology, while Dr. I. Smedley-MacLean and Dr. Arcus are continuing their work at the Molteno Institute, Cambridge. Dr. Korenchevsky and Miss Hall continue to work in the Department of Zoology and Comparative Anatomy, Oxford, and Dr. Zilva and his assistants at the East Malling Research Station, Maidstone.

The Governing Body would again express its gratitude to Sir Charles Martin, Sir Frederick Hopkins, Professor T. Dalling, Professor D. Keilin, Professor Goodrich and Dr. Hatton for continuing their provision of research facilities to evacuated members of the Institute's staff.

The main building at Chelsea, where the staffs of the Biochemical Department and Biophysics laboratory and the Secretary continue to work, suffered damage, mostly to windows and doors, in September, 1940, and again in April, 1941; on the latter occasion an explosive bomb wrecked the Institute's adjoining premises, known as the "Studio," and the animal houses attached to it. The main structure of the Institute itself sustained only superficial damage. Incendiary bombs also fell on buildings at the Serum department, Elstree, in January, 1941, causing slight damage.

RESEARCH WORK.

The Governing Body, before surveying the scientific work done during the year, desires again to record its appreciation of the continued co-operation the Institute has enjoyed with the Medical Research Council, which has furnished the salaries of the staff of the National Collection of Type Cultures, Miss Hume, Dr. Zilva and his two assistants, Miss K. Hall and Mr. Bacon, and part salaries of Dr. Korenchevsky, Miss Copping and Miss H. H. Smith. Mr. Kekwick's salary and a grant for his expenses up to August were also furnished by the Council. The Institute provided, as hitherto, accommodation and materials for the researches of each of these workers.

SEROLOGICAL STUDIES, TOXIN PRODUCTION AND IMMUNISING PROPERTIES OF ANTIGENS.

Toxin production by *Cl. welchii* isolated from war wounds and air-raid casualties: An enquiry has been made by Dr. Muriel Robertson (Institute of Animal Pathology, Cambridge) assisted by Mr. J. Keppie (Ministry of Agriculture Research Scholar) into the toxicity for mice of *Cl. welchii* strains from war wounds and air-raid casualties, and placed at her disposal by bacteriologists of the Emergency Medical Service. The purpose of this study was to ascertain how far the capacity of the strain to produce toxin *in vitro*, given the most favourable conditions of growth, could be correlated with the clinical features of the human sources of origin. By observing the pH values of the medium at 3-4 hours, a period coinciding with the peak of fermentation, and again at 6-7 hours when toxin production had reached its height, it became evident that strains produced their best toxins at certain pH values. A pH value of about 6-6.2 at 4 hours, with a slight movement towards the alkaline side at 6-7 hours was nearly always the successful cycle. This favourable growth was obtained by varying the sugar content of the medium according to the needs of the different strains. Toxicity for the mouse ranged from 5-80 M.L.D.'s per c.c. and there was only one practically atoxic strain out of 31 examined which had less than 5 M.L.D.'s per c.c. A report of the associated clinical conditions has been submitted to the War Wounds Committee of the Medical Research Council and here it may be noted that, while there is little doubt that the more toxic strains are likely to exert a more harmful effect upon the resistance of the patient than the less toxic, it is equally clear that even the least toxic are capable of setting up gas gangrene. The main source of danger lies in the conditions obtaining in the wound and it is of particular interest that highly toxic strains were present among those isolated from wounds where they had caused no disturbance.

The comparative prophylactic values of antibacterial and antitoxic sera prepared from *Bact. dysenteriae* (Shiga). Dr. D. B. Steabben has continued a study commenced with Dr. D. W. Henderson, of the so-called entero- and neuro- toxins of *Bact. dysenteriae* (Shiga) with a view to determining the prophylactic values of the corresponding immune sera against the test infection of mice with the living organisms. The antibacterial serum was prepared by immunisation with 5 hour old smooth cultures which appeared to be free from the characteristic Shiga toxin. Its antitoxic content was less than 5 units per c.c. The antitoxic serum was obtained by immunisation with toxic filtrates of the "rough" variant of the same strain and contained 500 units of antitoxin per c.c. The antibacterial serum was found to possess high protective value against known infective doses of living bacilli while the antitoxic serum had no such property. The import-

ance of the antisomatic element in dysentery serum thus demonstrated may, it is hoped, lead to progress and improvement in the preparation of serum for use in human dysentery. At present such sera are officially assayed solely on their content of antitoxin.

Experiments on anti-typhoid vaccination. Dr. A. Felix, serving as a member of the Emergency Public Health Laboratory Service, has continued his experiments on anti-typhoid immunisation. Since the special importance of "Vi" antigen in anti-typhoid immunity was recognised some years ago, numerous attempts have been made with a view to devising a method of vaccine preparation which should leave this antigen intact and as effective as possible. These early attempts failed. The position was quite inconsistent, since the greatest care was taken to include in the vaccine only cultures that are particularly rich in the Vi antigen, but very little was done to ensure that this substance was being preserved in its most efficient form. The customary active-immunity test in mice is not sufficiently sensitive to disclose how far the immunising properties of variously prepared typhoid vaccines have been preserved or impaired. This test, therefore, should not be accepted as the sole test for assaying the efficacy of typhoid vaccines; it should be supplemented by tests of the Vi and O antibody responses in the rabbit and in man.

It is known from earlier work that ordinary typhoid vaccine killed by heat and preserved with phenol stimulates in the rabbit and in man only negligible amounts of demonstrable Vi antibody. Freshly prepared alcohol-killed vaccine, on the other hand, readily stimulates this antibody when injected intravenously into the rabbit, and induces its formation in a relatively high proportion of human beings after subcutaneous inoculation. This Vi antibody possesses its full opsonising and protecting power. However, when stored for some months in the cold, in saline alone, or with the addition of 0.5 per cent. phenol or 0.35 per cent. tricresol, the alcohol-killed vaccine loses almost entirely its capacity of stimulating formation of Vi antibody in the rabbit, though O antibody is invariably produced to high titre. It is now found that a vaccine that is not only killed with alcohol but preserved in it still contains the Vi antigen in its effective form after storage in the cold for at least nine months, and probably much longer.

The new type of typhoid-paratyphoid vaccine, killed and preserved with alcohol, was compared with ordinary heat-killed T.A.B. or T.A.B.C. vaccine from various sources. Surgeon-Commander S. G. Rainsford, Royal Naval Medical School, and Dr. E. Joan Stokes, University College Hospital, collaborated in the comparative trials which were arranged in groups of volunteers among naval recruits, male and female hospital staffs and mental patients. Air Vice-Marshal H. E. Whittingham, Director of Hygiene, Air Ministry, also arranged for a trial in volunteers among R.A.F. recruits. Two marked differences were found between groups of subjects inoculated with the two types of vaccine: (1) The alcohol-killed and alcohol-preserved vaccines stimulated demonstrable typhoid Vi antibodies in a relatively high proportion of cases, whereas the Vi antibody response to ordinary vaccines was almost negligible. No significant difference in O antibody response was observed. (2) The reactions produced by the alcoholised vaccine were much milder than those caused by ordinary vaccine. General reactions were as a rule either absent or quite trivial. The local reaction was of a modified type and hardly ever prevented free use of the arm. No correlation was found between Vi or O antibody response and severity of general or local reactions.

Field trials of the new type of T.A.B.C. vaccine are now being conducted on a larger scale by the Emergency Public Health Laboratory Service.

Simultaneous immunisation against diphtheria and pertussis. Dr. Schütze had as already reported, shown experimentally that the addition of pertussis vaccine to diphtheria toxoid very definitely enhanced the immunising effect of the latter. He has now demonstrated that rabbits and guinea-pigs which receive this combination produce antibodies to *H. pertussis* to as high a titre as that reached by animals receiving pertussis vaccine alone. The practice, therefore, of combining these two agents is to be recommended.

Optimal spacing for vaccine inoculations. With the addition of an antigen such as tetanus toxoid to a bacterial vaccine it becomes necessary to space the doses of the bacterial vaccine at the interval of 4-6 weeks which is imperative for securing active immunity against tetanus, and it had to be determined, therefore, whether this alteration in bacterial vaccine practice did or did not affect antigenic potency. An experiment was carried out by Dr. Schütze on mice with a *S. typhi murium* vaccine and living *S. typhi murium*, a natural parasite of mice, as the infecting agent in the test dose. Pure line "E" mice were employed and two doses of vaccine were given at an interval of one week in one case and in the other of four weeks. Previous to the administration of the test dose, samples of blood were taken and tested for the presence of O antibody. The titres obtained showed that the longer interval resulted in a greater production of antibody. On the other hand, the average length of life after injection of a test dose of living *S. typhi murium* was the same for both groups. An increase, therefore, of the interval between vaccine doses from one to four weeks neither lowers nor raises the active immunity that results and the alteration in technique required by the incorporation of tetanus toxoid in the vaccine preparation should have no damaging effect on the potency of the latter.

Flocculation tests with tetanus toxoid and antitoxin. The possibility of employing the flocculation test for measuring the antigenic content of culture filtrates of *Cl. tetani* and hence of deducing the antitoxic content of the serum, has been further studied by Dr. Eagles and a comparison of the Lf values so obtained with the *in vivo* values has been attempted. The lack of a standard toxin and antitoxin for flocculation tests with tetanus toxin made it necessary to adopt by trial and error a suitable toxoid and a

serum whose *in vitro* and *in vivo* values coincided. Flocculation was readily obtained but flocculation time tended to be considerably longer than that customary in diphtheria toxin flocculation tests. Tests have been made with sera from casual bleedings and also from periodical bleedings in the course of immunisation of horses from the start onwards. It has emerged that antitoxin units based on flocculation results have, on many occasions and at all stages of immunisation, diverged widely from the unitage based on the *in vivo* test. The reasons for the divergence have not yet been elucidated.

THE ORGANISM OF PLEUROPNEUMONIA AND ITS CONGENERS.

Miss Klieneberger's study of these organisms has made good progress in the past year and work has been pursued in two directions. The pleuropneumonia-like organism (designated L1) isolated some years ago from *S. moniliformis* and maintained in pure culture, has always been regarded by her as a symbiont, and not, as one or two American writers have recently claimed, as a culture variant. She has now obtained some quite cogent evidence from absorption tests carried out with immune sera for L1 and for the mother culture *S. moniliformis*, that antigenic differences exist. Such evidence certainly supports the "symbiont" concept based on her previous morphological and cultural studies. This serological study will be extended to L1 strains derived from other mother cultures of *S. moniliformis*.

The life cycle of the pleuropneumonia organism. Recent morphological observations by Miss Klieneberger, based on an improved fixing and staining technique, have revealed structures, the function of which may well afford a key to the secret of the sequence of events in culture from filterable granule to filterable granule. Extensive comparisons have been made between these newly revealed structures in the fixed and stained state and the same structures as seen in the living state, with the valuable help of Mr. J. Smiles of the National Institute for Medical Research, employing the method of annular oblique incident illumination. The new knowledge will, it is hoped, materially assist efforts to define the systematic status of pleuropneumonia and organisms allied to it in nature.

CATTLE ABORTION FROM *TRICHOMONAS FOETUS* INFECTION.

Dr. Muriel Robertson has had the opportunity of continuing her study of this condition by the chance occurrence of *Trichomonas* infection in a herd at Cambridge; and through the kindness of Mr. Blakemore of the Institute of Animal Pathology she has made full use of the new material. Work has been directed chiefly towards observations of the serum reaction in recently aborted cows, where evidence of *Trichomonas* was clear. It was possible also to follow up the subsequent history of the infected individual and of other cases of sterility in the herd. Many sera from widely separated districts have now been tested, including a series giving high titres for *B. abortus*. The results seem to show that definite abortion associated with *Trichomonas* infection is also associated with the development of a characteristic agglutination of *T. foetus* by the serum of the case. Work on the experimental infection of heifers with the organism is being carried out in collaboration with Mr. W. R. Kerr of the Ministry of Agriculture of Northern Ireland.

BIOCHEMICAL STUDIES ON ANTIGENS AND ANTIBODIES.

Bacterial antigens. Dr. Morgan and Dr. Partridge (Beit Memorial Fellow) have continued their investigations on the nature of the antigenic complex of *Bact. dysenteriae* (Shiga). Four independent methods have been elaborated for the isolation in an undegraded condition of the specific bacterial polysaccharide. The material thus recovered dissolves in water to yield solutions of high viscosity; in this property it differs from polysaccharide preparations made by treatment of the antigenic complex with hot acetic acid, antiformin, etc. The high viscosity polysaccharide preparation is, however, unable to induce the formation of demonstrable agglutinins or precipitins in the rabbit but can be converted into an antigenic complex by combination with the polypeptide component of the original antigen. This combination can be readily brought about by mixing formamide solutions of the two components and precipitating the active complex with alcohol. It seems doubtful whether the corresponding degraded or low-viscosity polysaccharide can be combined and converted into an antigen in this manner. The polypeptide alone has poor antigenic capacity but after combination with the polysaccharide the resulting complex is a powerful antigen and appears to be as active as the original antigenic complex. Dr. Morgan and Dr. Partridge have extended these experiments to include the combination of "Shiga" undegraded polysaccharide with the polypeptide component derived from the "O" antigen of *Bact. typhosum*. The resulting complex is a powerful antigen and gives rise to *Bact. dysenteriae* (Shiga) agglutinins and precipitins. Although this work is far from complete, it appears that combination of the polysaccharide can most probably be made to occur with other naturally occurring proteins and with non-antigenic polypeptide complexes derived from commercial proteins. Evidence has been obtained which indicates that the polypeptide component of the complex plays a definite, although minor, rôle in inducing the formation of specific polypeptide receptor sites on, at least, part of the immune bodies formed.

Owing to the difficulty of obtaining at this time sufficient material of bacterial origin for a thorough study of the formation and nature of this type of antigenic complex, the investigation has been extended to include polysaccharides that are not normally antigenic. For some time it has been known that organisms

grown on agar—a non-antigenic polysaccharide—induce the formation of anti-agar immune-body. In this way potent anti-agar horse sera arise during the intense immunization employed in the manufacture of anti-bacterial therapeutic sera for clinical use. No explanation for the conversion of agar into an antigenic complex has been forthcoming, but in view of the work of Dr. Morgan and Dr. Partridge it seems that the agar may combine with certain protein or polypeptide components of the bacterial cell and in this manner be transformed into a full antigen. Experiments based on this suggestion have now been completed and it has been shown that agar, which by itself is non-antigenic, can be rendered antigenic by combination with the polypeptide components isolated from the antigen of *Bact. dysenteriae* (Shiga) or the "O" form of *Bact. typhosum*. Anti-sera that contain abundant precipitins for agar and certain of its break-down products, such as kanten, can now readily be produced in the rabbit, and can be employed for the detection of these products in antigenic preparations derived from bacteria grown on ordinary nutrient agar media.

The chemistry of amino-sugars. Dr. Morgan and Dr. Jolles have completed their investigation on the amino-sugars and have published a method for the isolation of small quantities of glucosamine and chondrosamine from biological material. The method is now being used by Dr. Morgan to identify the amino-sugars that are present in bacterial and cellular antigens.

The concentration of antitoxic sera. Members of the Biochemical Department at Chelsea have continued their collaboration with the Staff of the Serum Department in the investigation of problems concerned with the concentration of immune sera, particularly in the method of purification by treatment with pepsin and heat-denaturation. This method depends on the disaggregation of the antitoxic globulin into smaller protein molecules, of which those carrying the antitoxic activity are less readily denatured by heat. Dr. M. G. Macfarlane and Mr. H. J. Rogers have studied the course of this reaction and consider that it is essentially an enzymic process in that the rate of disaggregation of the antitoxic globulin is dependent on the temperature and pH, at which the reaction is carried out. According to Pope, the peptic action is almost instantaneous at low temperatures and is inhibited by high electrolyte concentration; it appears, however, that during the routine process at pH 4.2, the necessary action takes place during the heating of the serum-pepsin-ammonium-sulphate mixture to 55°, the pepsin being still active under these conditions. Mr. Rogers has also investigated the effect of various antiseptics on the denaturation of the protein.

The purification of diphtheria antitoxic sera by this method always involves some loss of antitoxic units, varying from 10% to 30% with sera from different horses after full immunisation. A bleeding from a horse in an early stage of immunisation showed an even higher loss; this serum had been examined by Mr. Kekwick and was known to contain a high proportion of the antitoxin activity in the γ -globulin component. A protein fraction containing a very high proportion of antitoxic γ -globulin, isolated by Dr. Record from another serum, was also submitted to the process, and only about 15% to 30% of the total antitoxic activity was recovered. It appears probable, therefore, that a large part of the antitoxic γ -globulin of diphtheria antitoxic sera is lost by this method of purification. The γ -globulin has been shown by Mr. Kekwick and Dr. Record to have the short flocculation time and high *in vivo/in vitro* ratio characteristic of "avid" antitoxic sera and its loss from the concentrated antitoxin, though sometimes negligible in terms of antitoxic units, may be of considerable therapeutic importance.

The application of the pepsin purification process to anti-*Cl. welchii* sera is also being studied. It has recently been shown that *Cl. welchii* toxin causes the development of an opalescence in egg-yolk emulsions, and this reaction can be utilised for the *in vitro* titration of antisera.

Enzymic activity of *Cl. welchii* toxin. Dr. Macfarlane is investigating, in collaboration with Dr. Knight, the enzymic activity of *Cl. welchii* toxins, in the hope of correlating these with the antigenic factors involved. Various samples of *Cl. welchii* (Type A) toxins have been found to contain a nucleotidase which hydrolyses adenosine-5-phosphate, one of the coenzymes concerned in the carbohydrate metabolism of cells; neither phosphomono- nor phosphodi-esterase is present. All the samples of this toxin so far examined contain a lecithinase, which hydrolyses egg-yolk lecithin with production of a neutral fat and a water-soluble phosphorus compound. It seems probable that this is the enzymic reaction underlying the production of an opalescence by this toxin in egg-yolk emulsions; it has been confirmed by Dr. Knight that the latter reaction in turn runs parallel with the lethal dosage of the toxin.

CHEMISTRY AND PHYSIOLOGICAL REACTIONS OF PHOSPHORIC ESTERS.

Continuing their work on the mechanism of calcification, Professor Robison and Miss A. Tazelaar have made further studies of the changes taking place in the cartilage of rachitic rats during the earliest stages of healing, after the animals have received doses of calciferol or sodium phosphate. The results of these and of numerous other experiments carried out during the past few years remain contradictory. Some appear strongly to support the conception put forward by Robison and Rosenheim in 1934, of a cycle of phosphorylation taking place within the cartilage and osteoid tissue, analogous to those forming part of the metabolic changes in blood cells and muscle. Other experiments have failed to give any such positive evidence. It is clear that there is some still unrecognised factor to be sought for in future experiments.

During August and September, Dr. H. Weil-Malherbe of the Cancer Research Department, Royal Victoria Hospital, Newcastle-on-Tyne, was a guest in the Biochemical Department and collaborated with Dr. Macfarlane in some studies on the anaerobic metabolism of carbohydrate in brain slices. During lactic acid formation from glucose, the inorganic phosphorus content remained almost unchanged and hexosediphosphate was detected in the tissue. In the absence of glucose there was a rapid breakdown of the pyrophosphate fraction, with liberation of inorganic phosphorus; after this had taken place the tissue was found to have lost its capacity to form lactic acid on the subsequent addition of glucose. These phenomena are considered to be consistent with the existence of a phosphorylating glycolysis.

METABOLISM OF FAT.

Dr. Smedley-MacLean, in collaboration with Miss E. M. Hume, has continued work on the fat-deficiency disease of rats.

Rats fed for many months on a diet from which all fat has been extracted, are emaciated little animals, stunted in growth, with scurfy skin and scaly tails. It has generally been accepted that the fat-free diet causes some fault in the metabolism of the fat and that in consequence of this the fat stores are depleted. Experiments now carried out show that the proportion of fat in the carcass and subcutaneous tissue of these fat-starved rats is actually higher than in the rats which have received, in addition to the fat-free diet, the essential unsaturated fatty acids which the animal is unable to make for itself.

These essential highly unsaturated acids, such as arachidonic acid, can be synthesised by the rat only if it is provided with the unsaturated linoleic or linolenic acid prepared by the plant. Apparently, the fat-starved rat is still able to synthesise palmitic and oleic acids, to build them into phospholipins, to store them and to oxidise them. There appears to be no fault of fat metabolism; but the power that is lacking is that of making new cells, which constitutes growth. The highly unsaturated arachidonic acid characteristic of the animal body plays some essential part in building up elements of the new tissue other than fat.

Dr. Smedley-MacLean, in conjunction with Mrs. Dolby and Dr. L. C. A. Nunn, has determined the constitution of arachidonic acid and has shown it to bear a simple relation to that of its precursor, linoleic acid.

PHYSICO-CHEMICAL STUDIES.

Conditions governing the drying of human serum. Dr. A. S. McFarlane has been engaged in a study of the conditions governing the drying of human serum for transfusion purposes. The ultracentrifuge and electrophoresis apparatus show that a solution of the powder obtained by drying from the frozen state has all the physical properties of the native serum with one exception. This relates to the state of fat the presence of which in native serum cannot be detected by eye but in reconstituted serum is betrayed by a milky appearance. The fat particles responsible for this opalescence are coated with a layer of β -globulin which is impermeable to ether and which frustrates all attempts at ether-extraction. Dr. McFarlane has shown that the β -globulin film becomes disorientated at low temperatures in the presence of ether, all the fat responsible for the opalescence of reconstituted human serum being removed in the ether layer. In conjunction with Mr. R. A. Kekwick and at the request of Dr. A. N. Drury (Medical Research Council), a study has also been made of the physical effects of various antiseptics on human serum before and after drying.

New Serum-drying Plant. Dr. McFarlane has been engaged on the design and operation of a new serum drying plant of large capacity. This differs from existing designs in that the drying of the serum is carried out in open trays and the stringent sterility demands imposed by the large amounts of serum injected at a transfusion have necessitated meticulous care in the sterilisation of the plant and in the subsequent bottling of the dry powder.

Electrophoretic components of normal and antitoxic sera. Mr. Kekwick has continued his investigations on serum and, in particular, has studied normal human and horse serum and various antitoxic horse sera.

The fractionation of sera by salting out has been followed by electrophoresis experiments and it has been found possible to prepare pure human γ -globulin in large quantities by a simple salt fractionation process. In applying the method to normal and antitoxic horse sera, the yields of γ -globulin have not been so satisfactory. The quantitative distribution of certain chemical constituents, e.g., carbohydrate and phosphorus, in the serum protein fractions has been studied.

With Dr. B. R. Record (Beit Memorial Fellow) a large number of samples of diphtheria antitoxic horse sera have been investigated, and it has been established that the total number of units of antitoxin in a serum can be quantitatively accounted for in terms of the activities and amounts of the β - and γ -globulins present in the serum.

The flocculation time and *in vivo/in vitro* ratio of sera are dependent on the relative distribution of activity between the β - and γ -globulins, a short flocculation time and high *in vivo/in vitro* ratio being associated with a high relative activity in the γ -globulin.

The characteristics of the antitoxin associated with the γ -globulin of diphtheria antitoxin suggests that it may be of value in the therapeutic treatment of cases of hypertoxic diphtheria.

The methods developed in the study of diphtheria antitoxin have been applied by Mr. Kekwick to the study of tetanus and *Cl. welchii* antitoxins. In these it has been shown that both the β - and γ -globulins are

antitoxic, and this would therefore seem to be the general rule in antitoxic horse sera. The distribution of antitoxic activity in these latter sera differs from that found in diphtheria antitoxic sera in that a higher proportion of the total activity is associated with the γ -globulin.

Purified diphtheria toxin. Dr. Record with Dr. B. C. J. G. Knight has been able to obtain preparations of diphtheria toxin of high activity from cultures of *C. diphtheriae* grown on simplified amino acid media. The purification and concentration of such preparations by freezing, pressure dialysis and salt precipitation have been investigated, and the resulting material studied in the ultracentrifuge and electrophoresis apparatus.

Using such purified toxin preparations, some preliminary experiments have been made with mixtures of pure β - and γ -globulins isolated electrophoretically from diphtheria antitoxic horse sera, in order to determine the relationship between flocculation time on the one hand, and the proportion of activity due to β - and γ -globulins on the other, in the toxin-antitoxin mixtures. The relation is not a linear function of composition, a small amount of γ -globulin in a mixture reducing the flocculation time very markedly from the value characteristic of β -globulin.

The amount of antitoxin nitrogen per L+/unit precipitated in the floccules formed from β - and γ -globulin and toxin was shown to be different; the values for a number of sera of differing quality, have all been found to lie between those of β - and γ -globulin.

SUBSTANCES THAT INCREASE TISSUE PERMEABILITY.

The titration of hyaluronidase from various sources. Dr. McClean, with the assistance of C. W. Hale, has completed and published a study of the mucolytic enzymes (hyaluronidase) which are closely associated if not identical with the substances that cause diffusion in the dermis. In addition to the observations recorded in the last annual report, it has been found that the relative potency assigned to enzyme samples from various sources measured viscosimetrically, is profoundly modified by the salt concentration of the system. Testicular enzymes appear to differ in their behaviour from bacterial enzymes as the salt concentration is reduced. This observation has a bearing on the lack of correlation reported by other workers, between the viscosimetric and diffusing activities of these substances. Recently a further test for the titration of these enzymes has been developed. This test depends upon the fact that preparations of the muco-polysaccharide in the presence of protein form a "mucin clot" on the addition of acetic acid; this clotting property is destroyed by these enzymes. Observations so far made suggest that if the protein-polysaccharide substrate is suitably standardised and the time of reaction kept constant, these enzymes can be titrated with an error of 20 per cent. This reaction is more sensitive to small concentrations of enzyme than the viscosimetric method and moreover gives an endpoint which is comparable as to dilution of the enzyme with that obtained with tests of diffusion in the skin. The reaction is being studied and its relation to the other manifestations of the activity of these enzymes examined.

Artificially prepared compounds that reduce the viscosity of mucoprotein. Favilli reported that diazotised compounds which diffuse in the skin also reduce the viscosity of mucoprotein. The activity of these compounds has been studied and it has also been found that ascorbic acid and its oxidation products and several other compounds of known reducing capacity show both activities. None of these artificially prepared compounds liberate reducing substances from the mucoprotein.

"Adaptive enzyme" effect of bacterial hyaluronidase. It has been shown that both *Cl. welchii* and certain strains of *Streptococcus pyogenes* respond to the inclusion of hyaluronic acid in the culture medium by a greatly increased production of hyaluronidase. It appears likely that as soon as these organisms start to proliferate in the tissues the hyaluronic acid there is attacked by means of this enzyme. Thus the organisms are able to use this metabolite and, at the same time, by destroying the viscosity of this material, to increase the permeability of the tissues and render more of the specific substrate available. In addition, the presence of hyaluronic acid causes the organism to produce greatly increased amounts of the enzyme. Thus a vicious circle is set up that promotes the rapid extension of the infection.

The relation of hyaluronidase to the capsulation of streptococci. Some strains of *Streptococcus pyogenes* develop capsules in young culture; other strains produce hyaluronidase. Capsules and hyaluronidase cannot co-exist in the same Group A or Group C strains since the capsular substance in these groups is composed of hyaluronic acid and is destroyed by this enzyme. The inclusion of hyaluronidase in the culture medium prevents the appearance of capsules.

Virulent type 1 pneumococcus produces hyaluronidase and responds in the same way as streptococci to the inclusion of hyaluronic acid in the medium. Pneumococcal hyaluronidase destroys streptococcal capsular substance but the capsules of the pneumococcus are not attacked by its own hyaluronidase or that from other sources. Other types of pneumococcus have not, so far, been examined.

Infection experiments are being carried out to determine the respective parts played by capsules and by hyaluronidase in infection by streptococci. A clear distinction must be maintained between the property of virulence or killing power and that of local invasiveness, which may be the one primarily affected by hyaluronidase.

The reported association between hyaluronidase and "Substance B" of diphtheria toxin. O'Meara has recently formulated a conception of diphtheria toxæmia which depends upon the hypothesis that diphtheria toxin is composed of two substances, A and B. According to him, substance A is of high lethal value for the guinea-pig; substance B predominates in saline extracts made from growths of *gravis* strains on Loeffler's medium and promotes the penetration of the tissues by substance A. O'Meara states that substance B has properties in many respects similar to diffusing factor and that evidence of their identity is accumulating. Dr. McClean has investigated 50 strains of *gravis*, *intermediate* and *mitis* type, including two Dublin strains reputed to be good producers of substance B, for their production of diffusing factor. Cultures were made in broth, broth enriched with hyaluronic acid and on Loeffler's medium and the enzyme production compared. Neither of the Dublin strains produced amounts of enzyme detectable either by diffusion in the skin or by the test for the destruction of the clotting power of mucin. A very few strains of all types produce traces of this enzyme, but the amount is so small compared with that produced by members of the gas-gangrene group, streptococci, pneumococci and staphylococci, that it seems unlikely that this substance plays an important part in the pathology of diphtheria.

ENDOCRINOLOGY.

Dr. V. Korenchevsky (Oxford), with the assistance of Miss K. Hall, Miss M. A. Ross and Mr. R. C. Burbank, has continued the study of the manifold effects of sex hormones. Mr. J. Cohen assisted with the statistical analysis of the data described in some of the published papers.

Thymus and sex hormones. After gonadectomy, the thymus becomes larger owing to later commencement of the physiological involution, and also to hyperplasia of thymic tissue, shown especially by the greater development of the cortex as compared with the medulla.

Sex hormones cause a decrease in thymus due to atrophy of the thymic lobules, the thymocytes starting to disappear first, the epithelial cells persisting even with a high degree of thymic atrophy. Fat and connective tissue partly replace the disappearing thymic tissue.

Oestrogenic hormones have the same effect as male hormones; but, in addition, the number of plasma cells in the thymic tissue is in most cases increased and a special adenoma-like hyperplasia of the epithelial structures occurs. Although these epithelial formations in rats injected with oestrogens were similar to the first stage ("preadenomatous change") in the formation of adenoma, in no case were they found to grow into a tumour. The histological similarity, however, of epithelial structures in thymomas and in the thymus of rats treated with oestrogens suggests an oestrogenic hypersecretion as one of the possible factors in the production of thymomas.

Simultaneous injections of male and oestrogenic hormones in general produced the same changes as those typical for each hormone separately, but of a more severe degree in most cases.

Are oestrogenic hormones necessary in the male organism for the normal development of the sex organs? The question has both theoretical and practical interest. In the male organism such large quantities of this female hormone are produced that several workers now consider that for the commercial manufacture of oestrone the urine of the stallion is a better source than that of the mare. Because of the abundance of this female hormone in the organism of males, and its co-operative activity with male hormones, the therapeutic treatment with oestrogens of male patients suffering from prostatic hypertrophy has been advised by some investigators of the problem.

The results of previous and recent experiments, however, induce Korenchevsky and his co-workers to conclude that when administered simultaneously with male hormones, oestrogens produce a very small increase in the development of the sex organs as compared with that which can be produced by increasing the dose of male hormone alone. Taking into consideration the pathological (including precancerous) changes which oestrogens produce, one must conclude that the female hormone is not only unnecessary for the normal development of the sex organs in the male organism, but might be harmful, and in any case its therapeutic application in the treatment of diseases of the male sex organs is not warranted.

Hepatotropic and cardiotropic properties of sex hormones. The effects of gonadectomy and the administration of sex hormones on the liver and heart of rats were investigated by examination of the histological changes and the changes in weight of these organs with the application of statistical analysis to the results. The effects on heart were also studied in experiments on the isolated heart of the rat.

The results obtained were in good agreement, and taking also into consideration similar experiments on kidneys previously reported, appear to warrant the view that the male hormones, androsterone and testosterone esters, might be considered as important natural physiological stimulants of such vital organs as the liver, heart and kidneys. Androsterone seems to be preferable to the testosterone esters since it does not produce any significant stimulation of the sex organs and is physiologically present in both sexes.

There is also a possibility that small doses of some oestrogenic hormones with a mild but prolonged action (such as oestradiol benzoate-butyrate) may also be considered to produce stimulation of the liver. Because of the toxicity of oestrogens in overdose, however, great caution is needed in their clinical application. Perhaps in this case the simultaneous administration with oestrogens of male hormones to females might be advantageous since the latter are able to neutralise to some extent the depressing effects of oestrogens on liver, kidneys and heart.

Adrenals and sex hormones. These endocrine organs are strongly influenced by sex hormones and this must be taken into consideration in the therapeutic application of the sex hormones in human patients. The changes are of the same kind in both sexes. Definitely toxic changes are found to be produced by oestrogens, if the latter are applied for a prolonged period even in comparatively small doses. Cellular atrophy may be very severe, with resultant degeneration or destruction of the cells of the *zona reticularis* and development of fibrous tissue, replacing the destroyed cells. Hyperaemia also reaches a pathological level.

The male hormones cause a return to or towards normal of the size and histological structure of the adrenals changed after gonadectomy. The testosterone esters, however, caused lipid depletion and slight hyperaemia in some cases.

In both sexes, when male hormones were injected simultaneously with oestrogens the male hormones prevented, partially or even completely, the pathological changes produced by oestradiol, dehydroandrosterone having the weakest and testosterone the strongest power in this respect.

As compared with experiments of shorter duration, prolongation of the injections of male sex hormones did not produce greater, but rather less pronounced changes, as if some neutralising mechanism had developed.

Sex-Hormones and the Foa-Kurloff cell. Work on this subject has been continued by Professor Ledingham. The response of the immature and of the castrated animal to stilboestrol as compared with that to oestradiol dipropionate has been tested and found to be very definitely less strong. The effect of variation in the dose of oestradiol dipropionate has been investigated in immature males (litter mates) less than three weeks old. A four-fold difference shows itself not so much in the height of the maximum Foa-Kurloff-content reached as in the longer duration of the period taken to fall to minimal levels (< 10 per 1000 leucocytes). Castrated animals (males and females) are being periodically tested for their capacity to respond to sex hormones. Two of them, castrated over 16 months ago, still respond. As a preliminary to chemical attack on the nature of the substance in the vacuole of the Foa-Kurloff cell, immature animals have been injected with emulsions of spleens from oestradiol-treated animals killed at the height of the response and containing enormous numbers of these cells. No clear response has been obtained and in view of this result, the substance included in the vacuole, if of oestrogenic origin, probably represents some inactive break-down product.

THE ACCESSORY FOOD FACTORS.

Researches on Vitamin Standardisation carried out for the Accessory Food Factors' Committee of the Lister Institute and the Medical Research Council.

Vitamin A. The co-operative tests of Vitamin A β -naphthoate organised by Miss Hume have been completed and eight reports have been received. These are now in the hands of Dr. J. O. Irwin for statistical analysis.

Vitamin B₁. Publication has been held up of the results of the investigation, organised by Dr. Macrae and carried out in 22 laboratories in Europe and America, which led to the adoption of pure crystalline aneurin hydrochloride as International Standard for Vitamin B₁. The report was being printed and the corrected proofs had been prepared for return to Geneva in the spring of 1940, when communication with Switzerland was interrupted.

Vitamin D. The results of work on Vitamins D₂ and D₃, organised by Dr. K. H. Coward and prepared by her for publication, had reached a similar stage. Publication of the Bulletin of the Health Organisation of the League of Nations, in which the above papers were to have appeared, has been suspended for the present.

Vitamin E. The co-operative tests of pure synthetic *dl*- α -tocopheryl acetate for suitability as International Standard for Vitamin E, which were organised by Miss Hume as Secretary of the Vitamin E Sub-committee, have been completed and the results have been analysed statistically by Dr. J. O. Irwin and Mr. E. J. Williams. Their report shows that, in the hands of eight out of twelve participants in the biological experiment, a satisfactorily graded response was obtained to doses of the tocopheryl acetate, graded in an undisclosed series. Miss Copping was one of the eight who obtained such a satisfactory result. The error of these tests was no greater than is usual with the biological estimation of other vitamins, so that the biological technique can be regarded as satisfactory.

The stability of *dl*- α -tocopheryl acetate in the circumstances to which it was exposed was satisfactory, and its adoption as International Standard for Vitamin E is recommended. It is proposed to make arrangements without delay for its temporary adoption in the British Empire and the United States, since formal acceptance by the International Conference on Vitamin Standardisation is now impossible. Publication of a brief account of the results of the co-operative experiment is planned to take place immediately, and a full account at some later date.

VITAMIN STUDIES. (Work at Cambridge).

The B₂ group of Vitamins.

Researches on the Vitamin B₂ complex have been carried on by Dr. Macrae with the assistance of Mrs. C. E. Work, Mr. H. G. Hind and Mr. J. S. D. Bacon and the collaboration of Professor A. R. Todd and Dr. B. Lythgoe of Manchester University. Further investigation has been made of the three factors (now termed α , β , and γ , respectively) of the vitamin B₂ complex which are present in extracts of liver and are required by the rat in addition to riboflavin and vitamin B₆. The α factor has now been identified with pantothenic acid, the factor required for the prevention of dermatitis in chickens and for the growth of certain micro-organisms. As yet neither the β factor nor the γ factor has been identified, but much is now known of the chemical properties of these factors and potent concentrates of each, free from one another, have been prepared. The β factor is not absorbed from extracts of liver by Fuller's earth or charcoal; it is not extracted from aqueous solution by various organic solvents, and as yet no precipitant for it has been found. In contrast to the β factor, the γ factor is fairly readily removed from aqueous solution by adsorbents but less readily than riboflavin; amyl alcohol and certain other solvents remove it from aqueous solution whilst salts of lead and mercury precipitate it. Neither factor β nor factor γ can be replaced in the diet of the rat by inositol, nicotinic acid or choline, compounds which have been found essential for the nutrition of various animals.

The Estimation of the Various B₂ Vitamins by Rat Growth Methods. It is a matter of considerable importance to have reliable methods for the estimation of the various B₂ vitamins in foodstuffs. Dr. Macrae and Miss A. M. Copping have devised methods for the estimation of vitamin B₆ and of the "filtrate factor" complex; these methods are proving satisfactory. The rat growth method of estimation of riboflavin described in the last report has been found satisfactory for the determination of the riboflavin content of foodstuffs.

Riboflavin. Dr. El Sadr has completed his study of the ocular changes produced in the rat by deficiency of riboflavin in the diet. These changes, which were consistent, included conjunctivitis, blepharitis and corneal opacity, developed in the order given, and finally, cataract in a proportion of cases. All the changes except the cataract were cured promptly when riboflavin was given. The corneal opacity is believed to be caused by a reversible oedematous condition, a conclusion which was confirmed by histological study.

Blood changes caused by deprivation of B₂ Vitamins. Dr. H. Chick and Dr. El Sadr have continued their investigations of the different types of blood changes in rats after prolonged deprivation of vitamin B₂ and the "filtrate factor," respectively. In the former case the anaemia, which was sometimes severe, was marked by a very low haemoglobin figure and high red cell count and was rapidly cured when vitamin B₂ was given. After prolonged deprivation of "filtrate factor," the blood changes were of a different type, the concentration of haemoglobin in the blood being usually little changed while the red cell count was reduced, and the dimensions of the cells were greater than normal. The blood condition returned slowly to normal when "filtrate factor" was given.

The "filtrate factor" obtained from yeast or liver is now known, from the researches of Dr. Macrae and his colleagues and others, to contain at least three different substances of which pantothenic acid is one (see above). Pure pantothenic acid was found ineffective for prevention or cure of the macrocytic type of anaemia described in the preceding paragraph, but cures were obtained with the factor removed from extracts of whole liver by treatment with amyl alcohol; this fraction contains, in addition to pantothenic acid, the γ factor described above.

It is not clear what relation these nutritional anaemias in rats may bear to the various forms of human anaemia, including pernicious anaemia. Their etiology appears to be similar to that of the nutritional anaemias of monkeys and pigs caused by deprivation of B₂ vitamins. Nevertheless there is a prospect that the rat may in future be useful as a test animal in the study of human anaemias and in the evaluation of therapeutic preparations employed for their cure and prevention.

Investigations on Wheat Flour and the Composition of Bread.

The energies of all members of the Division of Nutrition, at Cambridge, have been to a greater or less extent directed to filling the gaps existing in our knowledge regarding the nutritive value of different portions of the wheat grain. This subject is of special importance at the present time not only because of the known higher nutritive value of wholemeal as compared with white bread, but also because of the economy in shipping space which might be effected by a change from the latter to the former. The flour generally used in this country for making white bread represents 73% of the wheat grain, the remaining 27% being discarded from bread making and used for animal feeding. Wholemeal flour has been shown to be richer in mineral constituents and to contain more vitamin B₁ and more of the vitamin B₂ complex, and proteins of higher nutritive value than those of white bread. Some of the previous investigations on the results of which these statements were based could, however, be criticised in the light of more modern methods. In view of the importance of the subject at the present time, it was thought desirable to investigate further the digestibility of bread made from wholemeal and white flour, the relative nutritive value of the proteins contained in them and their content of the different members of the vitamin B₂ complex.

Proteins. The nutritive value of the proteins of white flour (73% extraction) compared with that of wholemeal flour derived from the same grist, has been studied by Dr. Chick and Mrs. Work by comparing the growth of young litter mate rats maintained on similarly composed diets in which the protein was equal in amount (12%) derived from the respective flours, supplemented by fat and minerals, with B vitamins (as yeast extract) and vitamins A, D, and E in optimal amounts. As the proportion of protein was sub-optimal the growth was also sub-optimal in both cases, but the rats receiving the wholemeal diet had better appetites and put on more weight, the increase over the observation period of nine weeks being nearly twice that of rats on the white flour diet. The food intake was accurately measured and the weight increase in g. corresponding to the ingestion of 1 g. of protein was determined. The average figures were 1.8 and 1.4, respectively, for the wholemeal and white flour diets. This figure which indicates a 30% superiority in nutritive value for proteins of the whole wheat was confirmed by the results of a second series of experiments in which the wholemeal diet was arranged to contain 30% less protein than the white flour diet, the calorie value being maintained by addition of pure starch. The result showed that the growth of the rats on the wholemeal diet with the protein thus reduced was in no way inferior to that of rats receiving a larger amount of white flour protein.

B₁ Vitamins. To compare the value of white and wholemeal flours in respect of their contents of B₁ vitamins, experiments have been carried out by Dr. Chick in which young rats have been reared on diets consisting largely of white or wholemeal flour. These diets were made equal in other respects by addition to both of (i.) casein, to raise the amount and quality of protein to the optimal; (ii.) minerals; (iii.) fat; (iv.) vitamins A and D, and (v.) by the addition of vitamin B₁ to the white flour diet. The group of rats receiving the white flour diet fortified with vitamin B₁ showed a rate of growth which was only about one half that of the group receiving the wholemeal; moreover the diet was much less economically utilised. On the wholemeal diet 1 g. weight increase corresponded to the ingestion of about 2 g. dry food, but on the white flour diet it required from 3 to 9 g. dry food. When the diets of the two groups were changed over, the results were reversed. Since both diets alike contained an optimum provision of protein, minerals and vitamin B₁, the superiority of the wholemeal diet in this experiment is attributed to the higher content of B₁ vitamins in the wholemeal as compared with the white flour.

More precise experiments are now being made by Miss Copping to determine quantitatively the relative content in the flours of different degrees of extraction, of the different members of the vitamin B₁ complex, viz., riboflavin, vitamin B₂, pantothenic acid and other filtrate factors.

Digestibility. The digestibility by human subjects of breads made from white flour (73% extraction) and from wholemeal flours (100% extraction) ground to different degrees of fineness, has been studied by Dr. Macrae assisted by Mr. Bacon, Mr. J. C. D. Hutchinson of the Cambridge Department of Agriculture and Mr. I. McDougall of the Institute of Animal Pathology, Cambridge. The observations were made on six human volunteer subjects, who consumed for periods of 10—11 days, diets consisting almost entirely of bread made from the different types of flour. The degree of fineness to which the wholemeal flour was ground did not affect the digestibility coefficients of the energy, nitrogen, fat and fibre of the breads. Five per cent. of the energy and nine per cent. of the nitrogen in the white bread ingested was found in the faeces, whilst of the ingested wholemeal breads 13% of the energy and 14% of the nitrogen appeared in the faeces. It is calculated that in the manufacture of white flour (73% extraction) about 20% of the total energy available to man in the wheat berry is discarded, while of the nitrogen 27% goes into the offals. None of the six subjects was adversely affected by the wholemeal breads of which they consumed 2 to 2½ lbs. daily.

The National Loaf. The Accessory Food Factors Committee of the Lister Institute and the Medical Research Council have in the course of the past year issued two memoranda recommending the use of 85% extraction flour with added calcium for bread making. In the experimental preparatory work on which these recommendations are based, the Nutrition Division at Cambridge has taken its full share.

Fat Soluble Vitamins in Wheat Flour.

Miss Hume and Miss Henderson Smith have studied the fat soluble pigments of wheat flour. The wheat grain contains a small amount of fat of which the larger part is in the germ but, in the process of milling, a considerable part is disseminated throughout the flour. The fat soluble vitamins present in this oil are provitamin A (carotenoids) and vitamin E. It is not known whether these are uniformly present in the fat of flour derived from all parts of the grain but it is probable that they are.

Provitamin A. Flour, even white flour, contains a varying and sometimes quite large amount of yellow carotenoid pigments, but only a biological test with rats can distinguish with absolute certainty what proportion of these can function as forerunners of vitamin A in the animal organism and how much is inactive. It has been found that the biologically active fraction is very small, so small that rats living on a diet composed almost entirely of flour failed to obtain sufficient provitamin A to protect them from vitamin A deficiency. Wholemeal flour was no richer than white flour, the distribution of active carotenoid throughout the grain being uniform. No superiority of wholemeal over white flour in this respect, therefore, exists.

Effect of Bleaching. The creamy colour which unbleached flour possesses, due to its content of carotenoid pigments, is not liked by millers and bakers, and various processes of bleaching and "improving"

are applied to flour to whiten it and improve the baking quality. Most of these are processes of oxidation. Only one of them, a process by which the flour is mixed with air which has been exposed to a flaming arc, has so far been investigated. The biologically active carotenoid pigment was destroyed by this process, but the amount originally present is so small that this cannot be regarded as a serious nutritive loss. It is rather as an indication of possible damage to other nutrients that this destruction is to be regarded as serious. The possible damage to vitamin E is under investigation.

Nutritive Value of Yeast.

By virtue of its high content of B vitamins, yeast is a highly nutritious food. Its production from certain waste materials is a comparatively simple procedure and therefore the culture of yeast for human consumption might prove valuable in maintaining the nutritive value of the national diet during the war. Dr. A. C. Thaysen of the Department of Scientific and Industrial Research has been investigating the cultivation for the above purpose of a type of yeast, *Torula utilis*, and the following studies have been made with this yeast which Dr. Thaysen kindly provided.

B₂ Vitamins. Miss Copping (with help from Dr. Macrae) has estimated the amounts of riboflavin, vitamin B₆ and "filtrate factor complex" in various specimens of the *Torula utilis*. In particular the effect of the culture medium and of the method of drying on the vitamin B₂ content, have been studied. Four samples of *Torula utilis* grown on a molasses medium and roller dried, freeze dried, vacuum dried and spray dried, respectively, all contained about 60 μ g. riboflavin and 50 μ g. vitamin B₆ per g. dry weight and also equal amounts of filtrate factor complex; the method of drying had apparently no effect on the B₂ vitamins of the yeast. Similarly the culture medium employed did not materially influence the amount of B₂ vitamins in the yeast. Samples of *Torula utilis* grown in media prepared from molasses, potatoes, straw, apple pomace and banana waste, all contained about 60 μ g. riboflavin; and the yeasts grown on molasses and on potato media were equal in their contents of vitamin B₆. A sample of dried bakers' yeast prepared by the Distillers' Co., Ltd., and a sample of brewers' yeast dried in the laboratory, had about the same amounts of the B₂ vitamins as the samples of *Torula utilis*.

In experiments on rats Dr. Chick has studied the supplementary value of the B vitamins of *Torula utilis* for those in straight run white flour. The addition of 5% yeast to white flour made it a better source of B vitamins than wholemeal flour, while the addition of 2½% made white flour approximately equal to wholemeal as regards B vitamins.

Protein. In experiments on pigs carried out at the Institute of Animal Pathology, Cambridge, Dr. Macrae, with the assistance of Dr. El Sadr and Mr. K. Sellers of the above Institute, investigated the supplementary value of the proteins of *Torula utilis* for maize proteins. Both casein and yeast protein had striking supplementary effects when fed to pigs adequately supplied with all other known nutrients but having maize as the sole source of protein. At all levels of protein fed, the animals given yeast thrived just as well as those given casein, and therefore, as a supplement for the biologically poor proteins of maize, yeast protein is equal to casein, a first class animal protein. The effect of the addition to a maize diet, adequate in all other respects, except protein, of even 5% *Torula utilis* was striking, as this amount converted the diet from one quite unsuited for the rearing of pigs to one which yielded excellent results. The protein of *Torula utilis* is therefore of high nutritive value.

Tests on Human subjects. Dr. Macrae has investigated means whereby yeast could be included in foods and is now observing the effect of feeding yeast to human subjects. Five per cent. yeast may be added to white or wholemeal breads without seriously affecting their palatability. In soups, meat and vegetable stews, steamed puddings and many other dishes, yeast may be added in reasonable amounts with advantage. The results of the tests on human subjects will be reported later.

Nutritive Value of Nitrogenous Substances in the Potato.

The potato is usually regarded as a source of carbohydrate in the diet, and less attention is paid to the nutritive value of its protein and other nitrogenous constituents, which form about 2% of the fresh weight of the tuber. Experiments with pigs have been started by Dr. Macrae to investigate this point. In addition, Dr. A. Neuberger (Medical Research Council grant), is now engaged in the separation and identification of the non-protein nitrogenous substances with a view to later investigation of their nutritive value.

SENSITISING ACTION TO LIGHT OF BUCKWHEAT (*Fagopyrum esculentum*).

Dr. P. Ellinger and Dr. Chick have continued their study of the photosensitising action of buckwheat. The minimum activating dose of dried, powdered, young buckwheat flowers was found to be consistently 0.25 g. per 100 g. rat, irrespective of whether this total amount was administered in one single dose or in a series of intermittent, smaller, daily doses. The photosensitising pigment had thus a completely cumulative effect which was at the same time reversible. The animals were sensitive within 24 hours of receiving a single dose, whether of minimum or larger size, and the sensitivity lasted for 7 to 21 days according to the size of the dose.

Dr. Ellinger has continued his study of the fluorescent pigments, extracted from buckwheat flowers by a mixture of methyl alcohol and acetic acid. These show a general absorption of the violet and ultra-violet portions of the spectrum and absorption bands in the visible part between wave lengths of 540 and 610 $m\mu$, this region corresponding to that of the rays which excite the reaction to light of sensitised animals. At least two of these pigments have, so far, been found to have a photosensitising effect on the rat.

In some of the spectroscopic examinations carried out during this investigation, Dr. Ellinger had the assistance of Dr. J. J. Fox, Government Chemist, and his colleagues, for which grateful acknowledgment is made.

VITAMIN C. (Work at East Malling Research Station).

Vitamin C. in animal metabolism. Dr. S. S. Zilva* in collaboration with Dr. C. L. Arcus and afterwards with Mr. J. R. Penney has been engaged in the study of determining small quantities of the oxidation products of ascorbic acid—dehydroascorbic acid and diketogulonic acid—in tissues and tissue fluids. In connection with this work attempts are being made to prepare diketogulonic acid. Phenylhydrazine, *p*-nitrophenylhydrazine and 2:4 dinitrophenylhydrazine derivatives as well as barium and calcium salts have been obtained and these are now in process of investigation.

Studies in the oxidation of ascorbic acid. With Mr. G. A. Snow, Dr. Zilva has been devoting his attention to the chemical characterisation and the determination of a non-enzymic substance capable of catalysing the oxidation of ascorbic acid.

Scurvy and anæmia. The effect of vitamin C deficiency on the blood of guinea pigs has been studied by Dr. Zilva in conjunction with Dr. R. Bodley Scott of St. Bartholomew's Hospital and the results are awaiting analysis.

Vitamin C in fruits and vegetables. This investigation was carried out by Dr. Zilva and his collaborators with the purpose of obtaining information which could find immediate application in practice.

Tomatoes. In collaboration with Mr. M. B. Crane of the John Innes Horticultural Institution, 21 varieties, including heterosis types, grown in the open ground and under glass, were examined for their vitamin content. Such differences in antiscorbutic potency as were observed were not sufficiently marked to make any of the varieties outstanding in this respect. Tomatoes grown in the open were, however, markedly more potent than those grown under glass. Suggestive observations of a fundamental nature were also made and it is hoped to study these in greater detail should circumstances permit.

Strawberries. Strawberry jam is one of the most popular jams and the fruit is claimed to possess a high vitamin C content. Most, if not all, of the data available are those obtained by the titration method. In view of the fact that the jam can be made available at times of the year when natural vitamin C is not so plentiful as at others, *i.e.*, late winter and early spring, it seemed desirable to carry out some biological tests. A batch of ripe berries collected at the height of the season was submitted to prophylactic biological tests as well as to titration tests. The biological response of the test animals was found to correspond with the reducing value in this case. The variation of 51—91 mg., in content of 57 berries with a mean of 70.3 mg. of ascorbic acid per 100 g. of fruit was established. The potency was therefore found to be of the same order as that of citrus fruits.

Potatoes. The influence of locality on the vitamin C content of potatoes in the King Edward (high vitamin C content) and Majestic (lower vitamin C content) varieties originating from Cambridgeshire, Lincolnshire and Lancashire was studied in collaboration with Dr. J. Barker, of the Low Temperature Research Station, Cambridge. Although differences in potency were observed between potatoes of different origins it cannot be asserted yet that this fluctuation was entirely due to locality since other differential factors could not be eliminated in the investigation. Tests were also performed on dried potato powders prepared and stored under different conditions by Dr. Barker. In all the potato experiments the determinations were made titrimetrically and confirmatory biological tests are being carried out.

Vitamin C in plant metabolism. It was previously reported that there is a change in the equilibrium of the *l*-ascorbic acid and dehydroascorbic acid present in the apple as the fruit develops. In the early stages of development 50% of the vitamin C is found to be *l*-ascorbic acid, the remainder being present as dehydroascorbic acid, whilst in the mature fruit the vitamin exists almost entirely in the reduced form. In order to co-ordinate this phenomenon with some physiological factor Dr. Zilva, in collaboration with Dr. C. West, has attempted to alter the equilibrium in young apples by artificial changes of environmental factors, mainly temperature. After various endeavours they have succeeded in doing so. They hope that by this approach they may possibly be able to throw some light on the part played by the change in equilibrium of the two forms of vitamin C in the metabolism of the apple.

Ascorbic Acid and Army Rations. Dr. Zilva's advice and services in connection with the investigation of problems coming under the above heading have been at the disposal of Government Authorities throughout the year.

THE NATIONAL COLLECTION OF TYPE CULTURES OF MICRO-ORGANISMS.

(Medical Research Council.)

The National Collection of Type Cultures continues to be housed at the Serum Department, Elstree, as a necessary emergency measure.

Despite the fact that requests for cultures from European countries have almost ceased and that communication with correspondents overseas is difficult, over 3,700 cultures were sent out and some 150 strains, many representing new types, were added to the Collection during the year under review. In the abnormal circumstances prevailing this may be considered satisfactory.

A new and revised catalogue is in course of preparation and will be published as soon as circumstances permit.

GENERAL AND FINANCIAL.

The Accounts and Balance Sheet for the year ending December 31st, 1940, show balances to the credit of the Capital Fund of £578,790 18s. 4d., the Contingency Fund of £25,000, the Sinking Fund of £37,370 11s. 5d., the Pension Fund of £31,038 8s. 4d., the Jenner Memorial Research Studentship Fund of £9,016 13s. 11d. and the Bacot Bequest Fund of £608 7s. 3d.

The following changes in investments have taken place during the year, viz.:—

- GENERAL FUND:—£12,500 4½% Conversion Stock, 1940/44, repaid at par.
£12,500 4½% Conversion Stock, 1940/44 converted into £12,500 2% Conversion Stock, 1943/5.
£1,000 Dominion of Canada 4% Stock, repaid.
£26,000 3½% Conversion Stock, 1961, purchased.
- SINKING FUND:—£9,600 4½% Conversion Stock, converted into £9,600 2% Conversion Stock, 1943/5.
£1,900 3½% Conversion Stock, 1961, purchased.
- PENSION FUND:—£900 3½% Conversion Stock, 1961, purchased.
- BACOT BEQUEST FUND:—£600 3½% Conversion Stock, 1961, purchased.

Income for the year amounted to £83,017 15s. 9d. which includes £4,202 0s. 8d. profit on redemption and conversion of investments. Compared with 1939 there is an increase in Interest and Dividends on General Fund Investments of £2,820 18s. 7d., and Sales of Sera, Vaccines, etc., of £9,746 7s. 10d., the latter increase being due to increased demands from various government departments. Stocks of Sera and horses on hand at December 31st are valued at £8,687 and £1,224 respectively, but are not included in the accounts.

Expenditure during the year was £57,110 5s. 9d., against £58,069 2s. 10d. in 1939. Increases in Serum and Vaccine Lymph Laboratories Expenses and decreases in Animals and Alterations, Repairs, Renewals and Workshop Expenses mainly account for this difference.

Of the balance of income over expenditure on the year's working, amounting to £25,907 10s. 0d., £3,000 has been transferred to the Contingency Fund, £2,000 to the Pension Fund and the remainder to the Capital Fund.

In conclusion the Governing Body desires to express its appreciation of the devoted co-operation of the Director and all members of the staff, and also of the assistants, in carrying out, often under difficult conditions, the work of the Institute.

MOYNE,

On behalf of the Governing Body.

The Lister Institute of Preventive Medicine.

BALANCE SHEET

AND

ACCOUNTS.

DECEMBER 31ST, 1940.

BALANCE SHEET

CAPITAL FUND to 31st December 1940:—

Donations, &c., received to date from the following:—

Dr. Ludwig Mond (1893)	..	..	..	..	2,000	0	0
The Berridge Trustees (1893/98)	..	..	..	..	46,379	10	1
The Grocers' Company (1894)	..	..	..	..	10,000	0	0
Lord Iveagh (1900)	..	..	..	..	250,000	0	0
Lord Lister's Bequest (1913/23)	..	..	..	..	18,904	5	8
William Henry Clarke Bequest (1923/6)	..	..	..	..	7,114	5	7
Rockefeller Foundation (1935/6)	..	..	..	..	3,400	0	0
The James Henry Stephens Bequest (per Lloyd's Bank Limited) (1938)					500	0	0
Dr. G. A. Davies Bequest (1938)	..	..	..	..	125	0	0
Other Donations and Legacies (1891-1934)	..	..	..	..	20,971	18	3

General Fund Income and Expenditure Account:—

As per Account at 31st December, 1939..	..	198,488	8	9			
Add Balance for the year ending 31st December, 1940		20,907	10	0			
					219,395	18	9

CONTINGENCY FUND:—

As per Account at 31st December 1934	..	..	..	22,000	0	0	
Add Amount transferred from General Fund Income and Expenditure Account, 1940	..	..	..	3,000	0	0	
							25,000 0 0

SINKING FUND to 31st December 1940 37,370 11 5

PENSION FUND to 31st December, 1939 28,642 17 10

Add Balance transferred from Pension Fund Income and Expenditure Account, 1940 395 10 6

Add Amount transferred from General Fund Income and Expenditure Account, 1940 2,000 0 0

31,038 8 4

JENNER MEMORIAL RESEARCH STUDENTSHIP FUND:—

As per Account at 31st December 1939 8,800 17 11

Add Balance transferred from Jenner Memorial Research Studentship Fund Income and Expenditure Account, 1940 215 16 0

9,016 13 11

BACOT BEQUEST FUND:—

As per Account at 31st December, 1939 597 17 3

Add Balance transferred from Bacot Bequest Fund Income and Expenditure Account, 1940 10 10 0

608 7 3

CREDITORS 7,633 9 11

MOYNE, *Chairman of Meeting, 30th May, 1941.*

JOHN ANDERSON, *Hon. Treasurer.*

£689,458 9 2

REPORT OF THE AUDITORS

We have audited the above Balance Sheet. We have obtained all the information and explanations we have required. are held by the Institute on their behalf. In our opinion, such Balance Sheet is full and fair, and properly drawn and the explanations given to us and as shown by the books of the Institute.
London, 4th June, 1941.

	£	s.	d.	£	s.	d.
EXPENDITURE ON INSTITUTE BUILDINGS AT CHELSEA:—						
As per account 31st December 1935, including purchase of freehold site, £6,000				73,548	3	1
FREEHOLD LAND ADJOINING THE "STUDIOS" CHELSEA at cost (1912) ..				169	6	8
LEASE OF THE "STUDIOS" CHELSEA, as per last account	871	18	9			
Less Amount written off for the year	65	2	0			
				806	16	9
QUEENSBERRY LODGE ESTATE, ELSTREE—						
Freehold land and buildings as per account 31st December 1912 ..				20,455	10	0
FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS:—						
*At cost less depreciation as per account 31st December 1920 ..	2,471	17	2			
Cost of Ultracentrifuges, purchased in 1936, less amounts written off ..	2,040	0	0			
				4,511	17	2
GENERAL FUND INVESTMENTS (at cost, less amounts written off):—						
£80,000 4 per cent. Consolidated Stock, 1937 or after	74,272	16	0			
£35,000 3½ per cent. Conversion Stock, 1961, or after	34,632	15	0			
£12,500 2 per cent. Conversion Stock, 1943-45	12,500	0	0			
£17,000 5 per cent. Conversion Stock, 1944-64	15,997	0	7			
£52,000 4 per cent. Funding Stock, 1960-90	45,661	13	9			
£64,000 3½ per cent. War Stock	63,407	13	5			
£37,000 Local Loans 3 per cent. Stock	20,829	1	7			
£3,000 Port of London 3½ per cent. Registered Stock, 1965-75 ..	2,686	17	7			
£25,000 Cape of Good Hope 3 per cent. Consolidated Stock, 1933-43	23,850	0	0			
£25,000 Natal 3 per cent. Consolidated Stock, 1929-49	21,400	0	0			
£8,000 New South Wales 4 per cent. Inscribed Stock, 1942-62 ..	8,040	1	4			
£25,000 New Zealand Government 3 per cent. Inscribed Stock, 1945	22,114	0	0			
£26,100 South Australian Government 3 per cent. Consolidated Stock, 1916 or after	16,800	0	0			
£2,900 Commonwealth of Australia 3½ per cent. Registered Stock, 1950-52	2,723	16	0			
£1,300 Union of South Africa 4 per cent. Consolidated Stock, 1943-63	1,327	9	0			
£23,000 Victorian Government 3 per cent. Consolidated Inscribed Stock, 1929-49	19,800	0	0			
£4,000 Western Australia Government 4 per cent. Inscribed Stock, 1942-62	4,081	3	0			
£20,000 Southern Railway Preferred Ordinary Stock	13,500	0	0			
£6,200 London & North Eastern Railway 3 per cent. Debenture Stock ..	3,961	0	0			
£5,000 Great Central and Midland Railway Joint Committee 3½ per cent. Guaranteed Stock	3,623	0	0			
£353 London & North Eastern Railway 4 per cent. First Guaranteed Stock	499	11	0			
£8,650 London, Midland & Scottish Railway 4 per cent. Preference Stock	7,960	0	0			
£15,625 London, Midland & Scottish Railway 4 per cent. Preference Stock, 1923	11,300	0	0			
£18,750 London & North Eastern Railway 4 per cent. First Preference Stock	13,028	6	7			
£25,000 East Indian Railway 3 per cent. New Debenture Stock ..	13,890	0	0			
£800 Grand Trunk Railway Company of Canada Great Western Borrowed Capital 5 per cent. Perpetual Debenture Stock	936	0	0			
£1,937 Grand Trunk Railway Company of Canada 4 per cent. Guaranteed Stock	1,733	0	0			
£800 Ontario and Quebec Railway 5 per cent. Permanent Debenture Stock ..	984	0	0			
£3,400 Gas Light and Coke Company Ordinary Stock	3,638	0	0			
				465,177	4	10
SINKING FUND INVESTMENTS (at cost):—						
£9,600 2 per cent. Conversion Stock, 1943-45	9,600	0	0			
£10,200 4 per cent. Funding Stock, 1960-90	9,079	0	1			
£20,500 3½ per cent. Conversion Stock, 1961 or after	18,658	5	8			
Balance uninvested	39	5	8			
				37,370	11	5
PENSION FUND INVESTMENTS (at cost):—						
£22,000 4 per cent. Funding Stock, 1960-90	17,165	3	5			
£14,700 3½ per cent. Conversion Stock, 1961 or after	11,694	10	5			
Balance uninvested	2,178	14	6			
				31,038	8	4
JENNER MEMORIAL RESEARCH STUDENTSHIP FUND INVESTMENTS (at cost):—						
£2,650 Southwark and Vauxhall Water Co. 3 per cent. Debenture Stock "B"	2,756	10	0			
£1,596 Southern Railway 5 per cent. Preference Stock	2,740	5	0			
£1,300 Liverpool Corporation 3 per cent. Stock, 1942, or after ..	1,097	6	9			
£2,000 4 per cent. Funding Stock, 1960-90	1,797	14	0			
Balance uninvested	624	18	2			
				9,016	13	11
BACOT BEQUEST FUND INVESTMENT (at cost):—						
£600 3½ per cent. Conversion Stock, 1961 or after	595	10	0			
Balance uninvested	12	17	3			
				608	7	3
(The book value of the above Investments is, in the aggregate, less than their market value at 31st December 1940.)						
STOCK OF ANIMALS						
STOCK OF ANTITOXINS						
DEBTORS AND PAYMENTS IN ADVANCE						
CASH:—						
At Bankers: On Deposit	1,000	0	0			
Current Accounts	2,339	14	6			
In hand	112	16	3			
				3,352	10	9
Nothing has been charged for depreciation of Furniture, &c. since 1920 as new purchases made during each year to a greater amount than the estimated depreciation (10%) have been written off				£689,458	0	2

TO THE MEMBERS.

In accordance with the provisions of the Superannuation Scheme for certain members of the Staff, the relative Life Policies up so as to exhibit a true and correct view of the state of the Institute's affairs, according to the best of our information

COOPER BROTHERS & CO.,
Chartered Accountants.

Auditors.

INCOME AND EXPENDITURE ACCOUNTS

INCOME.

General

	£	s.	d.
Interest and Dividends on General Fund Investments	21,056	2	8
Profit on Redemption and Conversion of General Fund Investments	3,408	17	3
Interest on Sinking Fund Investments	1,491	0	0
Profit on Conversion of Sinking Fund Investments	793	3	5
Investigation, Diagnosis and Analysis Fees, &c.	715	4	9
Sales of Sera, Vaccines, &c., ...	55,435	13	1
Rent	117	14	7

£83,017 15 9

Pension

	£	s.	d.
Interest on Investments	1,378	15	0
	<u>£1,378</u>	<u>15</u>	<u>0</u>

Jenner Memorial Research

	£	s.	d.
Interest and Dividends on Investments	278	6	0
	<u>£278</u>	<u>6</u>	<u>0</u>

Bacot

	£	s.	d.
Interest on Investment	10	10	0
	<u>£10</u>	<u>10</u>	<u>0</u>

Cancer Research

	£	s.	d.
Balance of Legacy from John George Mills (1937)... ..	671	19	1
	<u>£671</u>	<u>19</u>	<u>1</u>

for the year ending 31st. December, 1940.

EXPENDITURE.

Fund.

	£	s.	d.
Rent, Rates, Taxes and Insurance	2,057	8	3
Salaries and Wages of Staff	28,295	1	11
Premiums on Federated Superannuation Policies	1,731	13	5
Stationery, Printing and Postage	299	10	1
Printing of Collected Papers	204	5	2
Office Expenses, Auditors' Fee, and Provision for Bad and Doubtful Debts	346	18	0
Travelling Expenses	214	5	9
Gas, Water and Fuel	1,685	19	10
Electric Light and Power	591	5	4
Nutrition and Protozoological Expenses	912	15	11
Bacteriological and Experimental Pathology Expenses	214	19	3
Water and Bio-chemical Laboratory Expenses	357	16	1
Bio-physics Expenses	687	6	5
Serum and Vaccine Lymph Laboratories Expenses	9,389	7	6
Culture Media	42	3	0
Animals	2,316	8	2
Animal House Expenses and Forage	3,557	9	2
Alterations, Repairs, Renewals, and Workshop Expenses	761	8	6
Library Expenses	200	4	0
General Stores	127	17	10
Amounts written off Lease of the " Studios," Chelsea and Ultracentrifuges	405	2	0
Sinking Fund ($\frac{1}{2}\%$ per annum on Cost of Buildings and Interest on, and Profit on Conversion of, Investments)	2,708	0	2
Amount transferred to Pension Fund 2,000 0 0			
" " Contingency Fund... .. 3,000 0 0			
Balance, transferred to Capital Fund 20,907 10 0			
	25,907	10	0
	£83,017	15	9

Fund.

	£	s.	d.
Pensions and Gratuity	983	4	6
Balance, transferred to Balance Sheet	395	10	6
	£1,378	15	0

Studentship Fund.

	£	s.	d.
Salary of Dr. D. Dolby	62	10	0
Balance, transferred to Balance Sheet	215	16	0
	£278	6	0

Bequest Fund.

	£	s.	d.
Balance transferred to Balance Sheet	10	10	0
	£10	10	0

Account.

	£	s.	d.
Balance unexpended	671	19	1
	£671	19	1

SCIENTIFIC PAPERS PUBLISHED FROM THE LABORATORIES OF THE INSTITUTE DURING THE YEAR.



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- CHICK, HARRIETTE ... NUTRITIVE VALUE OF WHITE FLOUR WITH VITAMIN B₁ ADDED AND OF WHOLEMEAL FLOUR. *The Lancet*, Vol. II., 1940.
- CHICK, HARRIETTE AND ELLINGER, P. THE PHOTO-SENSITISING ACTION OF BUCKWHEAT (*Fagopyrum esculentum*). *Journal of Physiology*, Vol. 99, 1941.
- DOLBY, DORIS E., NUNN, L. C. A. AND SMEDLEY-MACLEAN, IDA THE CONSTITUTION OF ARACHIDONIC ACID. *Biochemical Journal*, Vol. XXXIV., 1940.
- EL SADR, M. M., MACRAE, T. F., WORK, C. ELIZABETH, HENRY, K. M., HOUSTON, J., KON, S. K., AND IRWIN, J. O. THE ESTIMATION OF RIBOFLAVIN. PART I. A NEW BIOLOGICAL METHOD. PART 2. THE ESTIMATION OF RIBOFLAVIN IN MILK: COMPARISON OF FLUORIMETRIC AND BIOLOGICAL TESTS. PART 3. STATISTICAL ANALYSIS OF THE DATA. (*Ibid*).
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- HENDERSON, D. W. ... THE SOMATIC ANTIGENS OF THE *Cl. welchii* GROUP OF ORGANISMS. *Journal of Hygiene*, Vol. XL., 1940.
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- KEKWICK, R. A., MACFARLANE, M. G., KNIGHT, B. C. J. G. AND RECORD, B. R. COMPOSITION OF DIPHTHERIA ANTITOXIC SERA. *The Lancet*, Vol. I., 1941.
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- KORENCHEVSKY, V.... ... SOME EFFECTS OF SEX HORMONES ON THE SECONDARY SEX ORGANS OF CASTRATED MALE RATS. *Journal of Pathology and Bacteriology*, Vol. LII., 1941.
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THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE.

Report of the Governing Body,
1942.

CHELSEA BRIDGE ROAD,
LONDON, S.W. 1.

June 26th. 1942.

The Lister Institute of Preventive Medicine,

CHELSEA BRIDGE ROAD, LONDON, S.W. 1
AND ELSTREE, HERTS.

THE GOVERNING BODY.

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DR. PAUL FILDES, O.B.E., M.A., M.B., B.Ch., F.R.S.
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MARY M. BARRATT, M.B., CH.B.
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KATHLEEN HALL, Ph.D. (*Medical Research Council Grant*).

DIVISION OF NUTRITION.

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T. F. MACRAE, D.Sc., Ph.D. (*Seconded to Royal Air Force*).
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- G. A. SNOW, B.Sc. (*Medical Research Council Grant*).
ALICE M. COPPING, M.Sc. { " " " " " }
J. R. PENNEY, B.Sc. { " " " " " }
J. S. D. BACON, B.A. { " " " " " }
A. NEUBERGER, M.D., Ph.D. { " " " " " }

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*IDA SMEDLEY-MACLEAN, D.Sc., F.I.C.
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H. K. KING, M.A., Ph.D., A.I.C. (*Research Student in Biochemistry*).

Attached Workers.

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C. L. ARCUS, B.Sc., Ph.D. (*Institute Grant*).
S. M. PARTRIDGE, B.Sc., Ph.D., A.I.C. (*Beit Memorial Research Fellow*).

DEPARTMENT FOR THE PREPARATION AND STUDY OF THERAPEUTIC SERA, ELSTREE.

- C. R. AMIES, M.D., B.S., *Bacteriologist-in-Charge* (Captain R.A.M.C.)
G. F. PETRIE, M.D., *Acting Bacteriologist-in-Charge*.
B. C. J. G. KNIGHT, D.Sc., *Biochemist*.
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(Medical Research Council.)

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MABEL RHODES (*Assistant Curator*).

ROSAMUND BARNES, B.Sc.

*Recognised Teacher of the University of London.

ANNUAL GENERAL MEETING
OF
The Lister Institute of Preventive Medicine,
June 26th. 1942.

REPORT OF THE GOVERNING BODY.

The Governing Body has the honour to present the 48th. report of the Institute.

GOVERNING BODY.

The Council, at a meeting held on June 27th. last year, re-elected Professor H. R. Dean and Professor C. R. Harington and elected Dr. Paul Fildes as successor to Professor Bulloch, to represent it upon the Board until December 31st. 1942.

COUNCIL.

The Governing Body records with regret the death of Professor R. Robison after serving as a member of the Council for seven years.

At the Annual General Meeting last year the three retiring members of Council were Sir Thomas Barlow, The President of the Royal College of Surgeons of England and Professor W. W. C. Topley. Sir Thomas Barlow, who had represented the Members of the Institute since 1917, resigned on account of advancing age and the other two members were duly re-elected. To vacancies created by deaths of members and the resignation of Sir Thomas Barlow, Professor John A. Ryle, Professor A. Fleming, Sir Henry H. Dale, Sir William Wilson Jameson and Professor H. S. Raper, were appointed.

The three members of Council, due to retire this year in accordance with the Articles of Association, but who are eligible for re-election, are Professor H. B. Maitland, representing the Victoria University of Manchester, Professor A. Fleming and Sir Henry H. Dale, both of whom represent the Members of the Institute.

MEMBERS.

The Governing Body has heard with great regret of the death of Professor W. J. Young, Professor of Biochemistry in the University of Melbourne and a member of the Institute since 1936.

Professor Young commenced his research career at the Institute in 1901 as assistant to the late Sir Arthur Harden and became the latter's loyal collaborator in his early contributions to the chemistry of alcoholic fermentation of yeast juice.

STAFF.

The Governing Body records with sincere sorrow the death of Professor R. Robison at the age of 58 on June 18th. 1941 after the report for that year had left the press. Dr. Robison had acted as First Assistant in the Department of Biochemistry under the late Sir Arthur Harden from 1913 to 1931 when he succeeded him as Head of the Institute's department and received from the University of London the title of Professor of Biochemistry. Professor Robison was elected to the Fellowship of the Royal Society in 1930 and was awarded the Baly Medal of the Royal College of Physicians in 1933. To the subject of his life work—the significance of phosphoric esters in the calcification of bone and in metabolism generally—he devoted the Herter Lectures which he was invited to deliver at New York University. His early decease has deprived Biochemistry of a front rank investigator.

Dr. McFarlane and Dr. Kekwick have transferred their activities to the L.C.C. Serum Institute at Carshalton, Surrey, where accommodation has been placed at their disposal by the London County Council and its Chief Medical Officer, Dr. W. Allen Daley.

Dr. J. H. Humphrey was appointed Jenner Memorial Research Student in July 1941, which appointment he resigned in April last, while Dr. H. King was appointed to a research studentship in Biochemistry in October, 1941.

Mrs. M. Cutting and Miss A. S. Cole have been appointed temporary members of the staff and are attached to the Division of Nutrition.

The disposition of the remaining members of the staff has not changed since the last report. The Division of Nutrition remains at Roebuck House, Old Chesterton, Cambridge, with some additional accommodation in the University Departments of Biochemistry and Anatomy. Dr. Muriel Robertson remains at the Institute of Animal Pathology, while Dr. Smedley-MacLean and Dr. Arcus have continued their work at the Molteno Institute, Cambridge. Dr. Korenchevsky and Miss Hall continue to work in the Department of Zoology and Comparative Anatomy, Oxford, and Dr. Zilva and his assistants at the East Malling Research Station.

The Governing Body would again express its gratitude to Sir Charles Martin, Sir Frederick Hopkins, Professor T. Dalling, Professor D. Keilin, Professor Goodrich and Dr. Hatton for the continuance of their provision of research facilities to evacuated members of the staff. The Library remains at Aldenham School through the good offices of the Governors and the Headmaster.

RESEARCH WORK.

The Governing Body, before surveying the scientific work done during the year, desires again to record its appreciation of the continued co-operation and collaboration the Institute has enjoyed with the Medical Research Council, which has furnished the salaries of the staff of the National Collection of Type Cultures, Miss Hume, Dr. Zilva and his assistants, part salaries of Dr. Korenchevsky and Miss H. H. Smith and research grants to Miss Copping, Miss K. Hall, and Mr. Bacon. The Institute provided, as hitherto, accommodation and materials for the researches of each of these workers.

SEROLOGICAL STUDIES, TOXIN PRODUCTION AND IMMUNISING PROPERTIES OF ANTIGENS.

The Vi agglutination test and the employment of Vi phage typing in the epidemiological control of Paratyphoid B Fever. Dr. A. Felix, serving as a member of the Emergency Public Health Laboratory Service and working at the National Institute for Medical Research, has investigated the possibility of applying Vi agglutination and Vi bacteriophage action to the routine examination of paratyphoid B cases or carriers. The Vi agglutination test for the detection of chronic typhoid carriers and the typing of typhoid bacilli with the Vi bacteriophage, according to the technique of Craigie and Yen, have both proved to be indispensable in the epidemiological investigation of outbreaks of typhoid fever. In this country paratyphoid B fever has been much more prevalent than typhoid fever during the past few years. It appeared, therefore, highly desirable to ascertain whether these two laboratory methods could be applied also to the investigation of outbreaks of paratyphoid B fever.

Detection of chronic paratyphoid B carriers with the aid of Vi agglutination tests. The Vi test is more complicated in paratyphoid B than in typhoid, since a pure Vi variant of *Bact. paratyphosum* B has not yet been isolated nor has a strain of *Bact. paratyphosum* B yet been encountered possessing a degree of O-inagglutinability comparable to that met with in certain strains of the typhoid bacillus. When a serum is tested for Vi antibody to paratyphoid B bacilli its O antibody content must, therefore, first be completely removed by absorption with a pure O culture. Most of the chronic paratyphoid B carriers that have been examined so far, gave a positive BVi agglutination reaction, but it is too early to state whether its frequency in paratyphoid B is as high as that of the TVi reaction in typhoid carriers. Though the test cannot be expected to give a positive result in every chronic carrier, its practical value is enhanced by the fact that the reaction is independent of the intermittency of bacillary excretion.

The proportion of mild and ambulant cases is very much greater in paratyphoid B than in typhoid fever, hence the temporary excreter plays the main part in the spread of paratyphoid B infection. Two or three tests for BVi agglutination, carried out at intervals of about six weeks, will often give an indication as to the condition of the subject. A decreasing BVi titre will indicate temporary excretion, while a steady or rising BVi titre will arouse suspicion as to a possible chronic carrier condition.

Typing of paratyphoid B bacilli with the Vi bacteriophage. The few reports so far published on the typing of typhoid bacilli with the Vi bacteriophage, according to the technique of Craigie and Yen, have amply corroborated the observations of these workers. In the experience of Dr. Felix during the past two years the results of the phage typing of 400 cultures isolated from about 300 cases of typhoid fever were in each instance, without any exception, in accordance with the facts established by epidemiological enquiry.

During 1941 Dr. Felix, with the assistance of Miss B. R. Callow, Emergency Public Health Laboratory Service, examined over 600 paratyphoid B cultures that had been isolated in various parts of the country. Bacteriophages were grown from specimens of faeces of paratyphoid B patients or carriers, and from lysogenic "rough" variants derived from these cases. It was soon found that most of the phages isolated were O phages, acting on every strain of paratyphoid B or *aertrycke* bacilli and on many other members of the *Salmonella* group. On the other hand a number of Vi phages were found which acted selectively on certain strains of the paratyphoid B bacillus but failed to lyse other strains, in spite of the fact that the latter also contained the heat-labile, somatic Vi antigen. Some of the Vi phages have been trained to develop strain specificity, while this has not been accomplished in the case of any of the O phages so far examined. When such adapted Vi phages were tested against strains from different localities it was found that the strains could be divided according to their response to different phage preparations and that strains from the same outbreak reacted in an identical manner. Several carriers from whom cultures could be examined over periods up to two years invariably yielded cultures of one type.

So far only three distinct phage types have been identified and a fourth group which is composed of those strains that are resistant to the available Vi phages. There can be little doubt that the latter group comprises a number of further types which will be defined if and when a greater number of adapted specific phages become available. The technique of typing paratyphoid B bacilli is, of course, not yet as perfect as Craigie's method of typing typhoid bacilli, but it has already been successfully applied in the epidemiological investigation of several outbreaks of the disease.

Toxin production by *Cl. welchii*. Dr. Muriel Robertson and Mr. J. Keppie at the Institute of Animal Pathology, Cambridge, have published their survey of *Cl. welchii* strains from war wounds and air-raid casualties with reference to their capacity for toxin production. This work was carried out under considerable difficulties as methods had to be improvised and adapted from those in use for the quite different purpose of obtaining toxin from noted strains to a means of studying all comers. A general insight into the toxicity was obtained and interesting approaches to the conditions necessary for toxin production were found but the results afforded only a somewhat incomplete analysis of the toxin.

Since the publication of this work, methods based on the egg-yolk reaction have been evolved and these and other strains are being reviewed in the light of this newer knowledge. The practical application of van Heyningen's measurement of the egg-reacting factor, which corresponds to the α -toxin of Glenny *et al* together with the hæmolysin test, affords a rapid, economical and accurate means of assessing the most important properties, including the serum-combining figure or "test-dose" of any toxin. This study is in progress and, so far, the importance of the change in pH in the simple modification now made of the medium used in the earlier study is being confirmed and it may be noted that the virtual suppression of the theta hæmolysin can be brought about by the presence of normal horse serum in the toxigenic medium.

The growth requirements of *Cl. welchii* for toxin production. Mr. H. J. Rogers has worked on the concentration and purification of a substance (or substances) which enhances the production of α -toxin over and above any increase due to increased mass of growth. The substance is present in mammalian tissues, and has been obtained in protein-free extracts. It has not been found in vegetable extracts prepared in the manner which yielded active extracts from mammalian tissues. A considerable purification of the factor which promotes toxin production has already been achieved. The nutrition of *Cl. welchii* is also being studied. The organism can be grown in a simplified medium and some of the nutrient requirements are already known. It may soon be possible to grow the organism in a chemically defined medium. This will be a preliminary to a study of the mechanism of toxin formation.

Dysentery prophylaxis. Dr. H. L. Schütze has been concerned with attempts to produce a satisfactory Shiga vaccine which will evoke good immunity and yet not possess the highly toxic properties characteristic of Shiga growths. To this end he has tested on mice the immunising power of Dr. Morgan's diethyleneglycol extract of *Bact. dysenteriae* (Shiga). Shiga toxoid and a vaccine prepared from an almost atoxic variant of *Bact. dysenteriae* (Shiga) are being tested in parallel.

***S. typhi* murium: Strain variation with respect to pathogenicity for male and female mice of a pure line.** While investigating the question of optimal spacing in vaccine practice, Dr. Schütze had observed in the case of strain "6079" a notable degree of enhanced resistance among the females of the line. With another strain, however, "480/40", no difference in resistance could be demonstrated. Consequently a further strain "Uganda" was tested and found to behave like "6079," males of the line showing a survival time after injection of 6.2 days and females 9.7 days. The cultures used had identical antigenic structures and so far no explanation is forthcoming for the differences observed.

THE ORGANISM OF PLEUROPNEUMONIA AND ITS CONGENERS.

Dr. E. Klieneberger, in collaboration with Mr. J. Smiles of the National Institute of Medical Research, has completed a morphological study of the organism of pleuropneumonia and allied types, employing two new methods specially developed for this purpose, and a joint paper on the subject has been published. She has also completed a new morphological and serological study of *Streptobacillus moniliformis* and its associated pleuropneumonia-like organism known as L1. Evidence is adduced that the L1 organism, whether in pure culture or in combination with *S. moniliformis*, passes through a cycle closely resembling that of pleuropneumonia. Light has also been thrown on the mode of origin of the filterable granules which, as in pleuropneumonia, reproduce the growth cycle.

CATTLE ABORTION AND STERILITY IN COWS FROM *TRICHOMONAS FOETUS* INFECTION.

During the past year further work on the serology of Trichomonads has been carried out by Dr. Muriel Robertson and a paper dealing with the agglutination reaction of certain trichomonads in sera obtained from vaccinated rabbits has been published. This work could not be carried out in as great detail as was desired owing to present conditions, but its publication was opportune as it afforded the theoretical basis for a practical application of the serological test to the problem of *Trichomonas foetus* infection in cattle.

In collaboration with Mr. W. R. Kerr, of the Ministry of Agriculture of Northern Ireland, the serological study of this disease has been carried very considerably further and some results have been published in the Veterinary Journal on the agglutination of living *Tr. foetus* by the sera of cattle as a means of diagnosing the infection. The results of the test in a survey of 165 animals are given and the procedure is found to afford a clear diagnosis in certain types of case. It is specific in the sense that no other infection in cattle has been found to raise the normal titre for *Tr. foetus* and that the characteristic "positive" reaction is associated with clear cases of *Trichomonas* abortion. The test is, however, not diagnostic of the mere presence of *Trichomonas* and a "negative" result does not mean that the animal can be classed as free from infection.

The question of a raised blood titre, in what is a purely venereal protozoan disease localised in the genital tract, is of considerable immunological interest and the acquiring or not of a raised immune body content of the blood is receiving study at the present time.

The experimental approach to the whole problem has been made possible by much appreciated co-operation of the Veterinary Division of the Ministry of Agriculture of Northern Ireland and further work on problems concerning the latency of the disease, its serious repercussions in sterility and abortion and the means by which the antigen is absorbed, is in progress.

BIOCHEMICAL STUDIES ON BACTERIAL AND TISSUE ANTIGENS AND TOXINS.

Bacterial Antigens. Dr. W. T. J. Morgan and Dr. S. M. Partridge (Beit Fellow) have continued their investigations on the nature of the somatic antigenic complex of *Bact. dysenteriae* (Shiga). The complex has been found to be soluble in concentrated (90%) solutions of phenol and, in solution, is largely dissociated into its protein and polysaccharide components which can be isolated in a free state by dialysis of the phenol solution. Some evidence has been obtained for the view that the acidic protein component previously described as "polypeptide-like" is a conjugated protein. This component can be degraded to yield a simple amphoteric protein which, however, is unable to combine with the polysaccharide and form an antigenic complex. The extraction of a "smooth" culture of *Bact. dysenteriae* (Shiga) with 90% phenol yielded the characteristic water-soluble antigenic complex and a rather larger amount of the simple amphoteric protein. Similar extraction of the corresponding "rough" variant yields no antigenic material but a considerable quantity of simple protein.

The continued difficulty of obtaining sufficient material of bacterial origin for a thorough study of the formation and nature of this type of antigenic complex, has obliged Dr. Morgan and Dr. Partridge to turn their attention to a study of the formation of artificial antigens which contain, as the serological specific hapten, polysaccharides of vegetable origin. Antigenic complexes have been formed from the non-antigenic polysaccharides agar-agar, gum acacia and cherry-gum and the conjugated protein component of the specific somatic antigens of *Bact. dysenteriae* (Shiga) and *Bact. typhosum*. The artificial antigens thus formed induce the formation of immune body specific for the polysaccharide components of the artificial antigenic complexes. The anti-gum acacia and anti-cherry gum immune sera were considered suitable for studying the serological relationships that might exist between these sera and other heterologous haptens such as the specific polysaccharides of pneumococcus (Types II. and III.), thus contributing to the valuable observations of Professor Marrack in this field. Unfortunately there has been no opportunity to study further many interesting points arising out of this work.

During the year Dr. Morgan has commenced an immunochemical study of the human blood-groups and tissue fluids. With the help of Dr. H. K. King, Dr. Morgan is attempting to isolate the A, B, M and N agglutinogens from human red-cells. The work is as yet, however, in its earliest stages of development. With the help of Miss A. Tazelaar, Dr. Morgan has isolated the specific blood-group A and B haptens from human saliva and is attempting to obtain a chemical differentiation between the saliva of "secretors" and "non-secretors" of the same group. The "A" hapten, after purification, is not antigenic but can be rendered so by combination with the conjugated protein component of the somatic antigens of *Bact. dysenteriae* (Shiga) and *Bact. typhosum*. In this way "A" immune rabbit sera have been obtained and are being used to study the difference between the group-substance "A" in saliva and in erythrocytes.

Biochemistry of Bacterial Toxins. Dr. M. G. Macfarlane has continued, in collaboration with Dr. Knight, of Elstree, investigations on the biochemical action of certain bacterial toxins. *Cl. welchii* toxins contain an enzyme which decomposes lecithin into phosphocholine and a diglyceride. This type of lecithinase has not previously been described, though certain snake venoms are known to contain a lecithinase which decomposes lecithin with formation of lysolecithin. It is probable that this lecithinase is identical with the α -toxin which is the lethal, haemolytic and necrotic substance predominant in the culture filtrates of *Cl. welchii*, Type A, one of the causative organisms of gas gangrene in man. The lecithinase is inhibited by specific antitoxic sera, the inhibitory action running parallel in the samples of Type A antisera examined with the protective power of the serum *in vivo*. The biochemical action can be used for the assay of α -toxin and α -antitoxin. The enzyme is relatively heat-stable and its activity is not appreciably affected by certain antiseptics or by sulphanilamide, but it is readily inactivated by surface active agents such as sodium dodecyl sulphate. The rate and amount of hydrolysis by this lecithinase, both alone and in the presence of antitoxin, are greatly affected by the concentration of calcium ions present, a fact which is of considerable importance in estimations of the haemolytic titre of *Cl. welchii* toxins.

A similar lecithinase is present in *Cl. oedematiens* toxin, though in the samples examined the activity per unit weight is very much less than that of *Cl. welchii*, Type A, toxins, and is probably of little significance in the lethality of these samples. This lecithinase is specifically inhibited by *Cl. oedematiens* antitoxic sera, but is not inhibited by the anti-lecithinase present in the *Cl. welchii* antitoxic sera; the *Cl. welchii* lecithinase is likewise not inhibited by the heterologous antitoxin.

It seems probable that the toxic action of other bacterial culture filtrates is due to enzymic activity and further experiments on these lines are in progress.

METABOLISM OF FAT.

Dr. Smedley-MacLean has continued at the Molteno Institute, Cambridge, her investigation of the changes produced in rats maintained for long periods on a fat-free diet.

The skin lesion: Sections of the skin from the fat-starved rats were compared by Dr. Williamson (Department of Pathology, Cambridge), with those from rats on a normal diet. In the former the skin was much thickened, the Malpighian layer consisted of two rows of cells while the *stratum granulosum* was greatly developed and the *stratum corneum* formed a thick crust. Fat-starved rats, the skins of which had shown the characteristic rough scurfy appearance, particularly marked on the ankles, were cured by doses of linseed oil given daily for five weeks. The skins of these cured animals resembled those of normal rats though in some cases a slight increase in the depth of the *stratum granulosum* remained.

Mitoses in epithelia of skin: Dr. Glucksmann, of the Strangeways Laboratory, has made counts of these at different stages of the disease. Though the growth rate of the fat-starved rats after 9 weeks had been reduced to about half that of the controls, mitoses among the cutaneous epithelia were 4 to 8 times as numerous as in the controls. The work is still in progress and duodenal and oesophageal epithelia are also being examined.

Tumour grafting in fat-starved rats: In experiments carried out in co-operation with Miss E. M. Hume and with the help of Dr. Haddow of the Cancer Hospital Research Institute, Walker tumours originating from a mammary carcinoma, were implanted in rats which had been fed for 14 weeks on the fat-free diet. These developed well both in the fat-starved rats and in their controls; growth was accompanied by a large decrease in the fat stored in the subcutaneous tissue and in the carcass when compared with control rats in which tumours had not developed. The fall was most marked in the subcutaneous tissue; here too the proportion of highly unsaturated acid stores showed a significant reduction.

It is of interest that while the total growth rate had been much reduced in the fat-starved rats, the cutaneous epithelium was in a much more active state of division than in the controls and the tumours grew equally well in both. Various investigations carried out with the object of ascertaining whether tumour formation can be influenced by diet have been reported but no convincing evidence that this can happen has yet been brought forward.

Nature of the fatty acids: Investigations on the nature of the fatty acids formed showed that when the fat-free diet was supplemented by linseed oil, considerable stores of more highly unsaturated acids were found in the liver. Here the C_{18} acids of vegetable origin appear to have been converted into elupanodonic acid containing 22 carbon atoms and five double bonds, one of the two acids characteristic of animal fat. As this acid was absent in the animals which had not received the linseed oil, this oil must have supplied the material from which it had been synthesised. There appears to be a biological synthesis of elupanodonic from linoleic acid. The structure of these highly unsaturated acids had, however, not been conclusively established.

Dr. C. L. Arcus has investigated the structure of arachidonic acid $C_{20}H_{38}O_2$ and has confirmed the preliminary results put forward by Dolby, Nunn and Smedley-MacLean which disprove the formula originally suggested by Brown and Shinowara. The constitution of arachidonic acid has, therefore, been satisfactorily established.

Dr. Smedley-MacLean and Dr. Arcus have also completed an investigation on the purification of a synthetic glycerol, a problem suggested by the Chemical Research Advisory Board.

PHYSICO-CHEMICAL STUDIES.

Drying from the frozen state. Dr. A. S. McFarlane, at the L.C.C. Serum Institute, Carshalton, has continued to study the effect of drying conditions on human serum and plasma for transfusion. Arising out of experience with the vacuum pan type of drier an alternative method has been evolved for economical drying from the frozen state on the commercial scale. This is the subject of a provisional patent application and is embodied in a smaller drying plant which has been used so far mainly for experiments on drying milk. The milk is first frozen on a metal coil and dried *in vacuo* from the surface of the coil. The heat required to permit evaporation of the water from the surface of the coil is derived from the condensation of the hot gas of the refrigerator. These experiments on milk were carried out in collaboration with Prof. H. D. Kay (National Institute for Research in Dairying) and Dr. Norman Wright (Hannah Dairy Research Institute) and are being continued in collaboration with them and the staff of the Low Temperature Research Station, Cambridge. A discussion covering the work to 1st Jan., 1942, is reported in detail in bulletin No. 6169 of the Agricultural Research Council.

Removal of lipoids from transfusion plasma. The technique described in last year's report for removing lipoids from serum by freezing with ether has now been adopted for a trial period by the Medical Research Council Emergency Blood Transfusion Service and quantities of 50 litres of plasma per week are being processed. Dr. McFarlane and Dr. Kekwick are investigating the special conditions relating to the extraction of plasma and Dr. J. W. Trevan (Wellcome Physiological Research Laboratories) has reported that the extraction process also removes fibrin and that the processed plasma either cannot be made to clot or does so only very slowly. This means that troubles due to the post-filtration clotting of citrated human plasma are likely to disappear and that a method is available for obtaining higher yields of serum proteins from blood than result from simply allowing it to clot.

Electrophoresis Studies. The Tiselius apparatus was moved to Carshalton in August and Dr. Kekwick resumed there his work on the physical properties of animal antitoxic sera and human pathological sera, particularly cases of multiple myelomatosis showing proteinuria. He has also collaborated with Dr. C. Rimington (National Institute for Medical Research) in examining the urine in cases of "crush injury," with Mr. F. C. Bawden and Dr. N. W. Pirie (Rothamstead Experimental Station) in some further sedimentation measurements on Bushy Stunt Virus, with Dr. A. Neuberger (Medical Research Council) on the anti-pernicious anaemia principle of liver, with Dr. F. G. Young (National Institute for Medical Research) on the hormones of the anterior pituitary and with Dr. F. X. Aylward (Courtauld Institute of Biochemistry, Middlesex Hospital) on gonadotropic preparations from pregnant mare serum.

SUBSTANCES THAT INCREASE TISSUE PERMEABILITY.

The titration of hyaluronidase. Dr. D. McClean has completed a study of the reaction in which the clotting power of mucin in the presence of acetic acid is destroyed by hyaluronidase. By the use of purified hyaluronic acid mixed with a solution of crystalline serum albumin to form the protein-polysaccharide complex, which will clot in the presence of acetic acid, a method of assaying the potency of hyaluronidase from various sources has been developed. The method is simple and makes possible the titration of many enzyme samples in a short time with an error of 20 per cent. The influence of pH, salt concentration, time and temperature on the reaction has been studied and the relation of this reaction to the other manifestations of enzyme activity investigated. The reaction is inhibited in the presence of appropriate anti-sera.

Preparation of sera against purified streptococcal hyaluronidase. In order to prepare sera against streptococcal hyaluronidase similar to those already obtained against the enzymes from *Cl. welchii* and *Vibrio septique*, a method of purifying the culture-filtrates and separating the hyaluronidase from the haemolysin had to be developed. A modification of the method described by Madinaveitia for the purification of testicular enzyme which involves adsorption on to alumina "C-γ" was found to give satisfactory results. Specimens derived from both group A and group C streptococci were kindly examined by Dr. E. W. Todd (L.C.C. Antitoxin Laboratories) and were free from detectable streptolysin A. Groups of rabbits were immunized with these preparations and anti-hyaluronidase sera of quite high potency obtained. There is no cross-neutralization of group C hyaluronidase by group A anti-sera or *vice versa*; on the other hand, group C anti-serum prepared from one strain, neutralised enzymes prepared from other group C strains of different serological type. It is hoped to use these sera in the investigation of the part played by streptococcal hyaluronidase in infections.

Reduction of viscosity of hyaluronic acid by artificially prepared compounds. Further work on the progressive fall in viscosity caused by ascorbic acid, diazotised and other compounds has shown that this fall is not related to their reduction potential. During the reaction there is no detectable oxidation of ascorbic acid which is one of the substances acting in this way.

The *in vivo* decapsulation of streptococci by hyaluronidase. Experiments on mice which were designed to show the influence of hyaluronidase on infection by highly virulent capsulated strains of both group A and group C streptococci revealed the fact that doses of enzyme far in excess of those required completely to decapsulate the organisms *in vitro* failed to do so in the animal body and that injection of the enzyme was without effect on the death rate or survival time of the animals. Further work has shown that the activity of the enzymes is inhibited by the presence of normal serum and by some substances which, though structurally related to hyaluronic acid, are not themselves attacked by the enzyme. This suppression of activity in the body appears to be due, in part at least, to competitive inhibition of the enzyme. The substance in serum causing this inhibition has not so far been isolated, but there is evidence that it is associated with the pseudo-globulin fraction.

The determination of diffusing activity in the skin. It seems that the method of assaying diffusing activity in the skin described by Bacharach *et al* is not altogether satisfactory since the dose response curve is sigmoid and, even if a relatively flat part of the curve is selected for the dose to be injected, it is admitted that the total area of diffusion which is measured is not proportional either to the concentration of the enzyme or to the logarithm of the concentration. It seemed desirable, therefore, to find out whether the highest dilutions of enzyme could be determined which would produce a significantly increased diffusion in a fixed short time. Preliminary observations, taking the mean results on groups of six guinea-pigs, indicate that determinations can be made which distinguish between two-fold dilutions of the enzyme and that the increase in area produced by the minimal diffusing dose is three times the standard deviation for the group of animals.

Biochemical studies. Dr. J. H. Humphrey has been investigating the action of hyaluronidase from various sources upon a number of substrates from the biochemical point of view, with the aim of correlating these actions with possible pathological significance of the enzyme. Hyaluronidase from bull testes causes liberation of N-acetyl glucosamine and reducing sugar from hyaluronic acid prepared from umbilical cords, from skin, and from a similar substance prepared from fresh lung tissue. The same preparation also attacks chondroitin sulphuric acid, and powdered cartilage, though the action upon the latter is relatively slow.

Preparations from streptococcal and *Cl. welchii* filtrates behave similarly, but their action upon chondroitin sulphuric acid and cartilage is very much weaker than upon hyaluronic acid. When the kinetics of hydrolysis are followed, acetyl glucosamine being estimated by Morgan and Elson's colorimetric method, and reducing sugar by Somogyi's modification of the Schaffer-Hartmann method, it is found that the acetyl glucosamine apparently liberated is several times greater than the corresponding reducing sugar and greater even than the total content of acetyl glucosamine as estimated by chemical hydrolysis and measurement of the glucosamine content. This apparent excess of acetyl glucosamine occurs only when hyaluronic acid is the substrate, and the amount of apparent acetyl glucosamine released for a given amount of reducing sugar seems to be characteristic partly of the source of the enzyme and partly of the purification of the substrate. When the hydrolysis is carried out at a pH greater than 6.0 a substance is produced which gives a purple colour on addition of Ehrlich's reagent without any previous treatment with alkali, and the phenomenon suggests that hyaluronic acid may contain preformed ring structures (possibly of the pyrazine type) which are set free in the course of hydrolysis.

An investigation has been made of the β -glucosaminidase content of the different enzyme preparations. β -glucosaminidase is present in crude testis extracts, but purified preparations have been obtained from which it has been practically eliminated. It is present in some of the *Cl. welchii* filtrates examined, but not in all, and has not been found in streptococcal filtrates. There is no correlation between β -glucosaminidase content and hyaluronidase activity.

In an attempt to render hyaluronic acid antigenic and thereby to obtain an antiserum which might act on streptococci which possess capsules made of this substance, hyaluronic acid has been coupled to horse serum albumin by a diazo-benzyl ether linkage (*c.f.* the method used by Avery and Goebel to link the Type III. pneumococcal polysaccharide). Injections into rabbits have so far failed to induce antibodies against the coupled hapten group, thereby confirming previous failure by others to obtain antisera to hyaluronic acid by less complicated methods.

ENDOCRINOLOGY.

Dr. Korenchevsky, at the University of Oxford, assisted by Miss K. Hall, and with the co-operation of Dr. Cohen, Miss M. A. Ross and Mr. R. Burbank, has studied the effects of male and female sex hormones and their co-operative activity with thyroid and adrenal cortical hormones, chiefly on the non-sex organs, liver, heart, adrenals and thymus. A study has also been made of the effect of ageing on the weights of endocrine and certain other vital organs.

Correlation between sex, thyroid and adrenal cortical hormones. It was found that both co-operative and antagonistic correlations exist between these three groups of hormones.

A particularly strong co-operative activity between oestrogens and thyroid hormone was shown in the hypertrophy of adrenals, liver, kidneys and heart of females and males, and, to a slight degree, of female sex organs. In males a similar stimulating co-operative effect was noticed between thyroid hormones and desoxycorticosterone on the weight and size of liver, kidneys, heart and, less constantly, spleen. On the other hand, the hypertrophy of the adrenals and the retardation of body growth produced by thyroid hormones was decreased when male hormones were administered simultaneously, the latter exerting a partial neutralizing (antagonistic) action on these effects of thyroid hormones.

Sex hormones and basophilic granulation of the liver cells. The basophilic granules are amongst the most striking features in the structures of the liver cells of animals, and very probably they are closely connected with metabolism and the disposition of proteins in the liver. In most cases, as compared with normal intact rats, the granules were decreased in number and often in size in gonadectomized uninjected rats, and returned to normal in gonadectomized rats treated with sex hormones. These changes would appear to correspond to the changes in weight and size of the liver of these three groups of animals, since in uninjected gonadectomized rats the liver is smaller than in the intact animal or in gonadectomized rats injected with sex hormones, in actual or relative weight or both.

When does the process of ageing start in the living organism? There are many factors concerned in producing ageing. Hypoplasia or atrophy of the organs must be considered to be one of the most important and typical features of senility.

In the rat relative hypoplasia of endocrine and other organs is a feature recorded as early as two or three weeks after birth, probably even earlier, and developing with age. The human data at present available show that, although the relative hypoplasia of some organs appears to be less developed in man than in the rat, it still starts very early in most of the organs examined, develops with advancing age and finally results in true senile atrophy. Because of this, hypoplasia must be considered as a definite and important feature of ageing. Its full significance, however, in the process of senescence cannot be defined with certainty. It may be in part a physiological adjustment to the slowing of growth, or it may also be one of the secondary causes producing senescence, indicating latent wear and tear of the living hormones. In the latter case, the relative hypoplasia of organs provides, independently and on a different basis, additional support to the theory of Minot, that the process of ageing starts from the first days of life. Robertson and his co-workers, and others, found that the nucleo-cytoplasmic ratio falls with ageing, starting from birth. Since the nucleus is the most important and vital "organ" of the cell, these findings also substantially support Minot's theory.

THE ACCESSORY FOOD FACTORS.

Researches on Vitamin Standardisation carried out for the Accessory Food Factors Committee of the Lister Institute and the Medical Research Council.

The Health Organisation of the League of Nations has resumed publication of its Bulletin and an issue which has just appeared contains descriptions of: (1) The series of investigations organised by Dr. T. F. Macrae, as Secretary of the Vitamin B₁ Sub-Committee, which led to the adoption of pure crystalline aneurin hydrochloride as International Standard for Vitamin B₁; (2) The co-operative tests arranged by Miss E. M. Hume, as Secretary of the Vitamin E Sub-Committee, of pure synthetic racemic α -tocopheryl acetate for its suitability as International Vitamin E Standard. This substance has, during the past year, been provisionally adopted as such in Britain and the United States.

Dr. H. Chick, as Secretary of the Accessory Food Factors Committee, and more especially Miss Hume and Dr. Macrae, as Secretaries of various Sub-Committees, have devoted much time to the special subjects connected with war-time Nutrition with which the Sub-Committees have been concerned. The following are instances: Flour from which Bread is made: Methods of Vitamin Estimation and Content of Various Vitamins in Common British Foods: Methods of Preparation of Vegetables and other Foods for the Table to preserve the nutritive value, especially in large scale cooking processes: Value of Food Yeast as supplement to the diet of school-children.

VITAMIN STUDIES. (Work at Cambridge).

Vitamin B₁ and the Vitamin B₂ Complex. Wholemeal flour with its content of 1—2 International units of B₁ per g., has about 4 times, and National Wheatmeal about 3 times the B₁ content of white flour derived from the same wheat.

B₂ vitamins in different types of flour. Miss A. M. Copping has tested the content of the various components of the Vitamin B₂ complex in different flours:

Riboflavin. The highest concentration (15 μ g. per g.) was found in the germ; that of "middlings" and bran was intermediate and that of white flour (endosperm) lowest, so low indeed that accurate estimation was not possible. The contents, in white, National Wheatmeal and wholemeal flours were in the order of 1—3—4. The amount present in wholemeal (about 3 μ g. per g.) and National Wheatmeal is only about $\frac{1}{2}$ or $\frac{1}{3}$ that present in such foods as meat, milk and eggs, reckoned on the dry weight. Nevertheless, breads made from these flours may provide an important source of this vitamin in human diets, owing to the relatively large amount of bread that is eaten.

Vitamin B₆ (pyridoxin). The content in wheat is higher than that of riboflavin and is more evenly distributed in the grain. Concentrations in white flour, National Wheatmeal and wholemeal (5 μ g. per g.) were in the order of 3—4—5.

"Filtrate Factors." The distribution of "Filtrate Factors" was similar to that of Vitamin B₆, except in the case of the germ which was relatively rich in Vitamin B₆, but deficient in pantothenic acid.

National Wheatmeal Bread. In its content of B Vitamins as in the biological value of its proteins National Wheatmeal has been found intermediate between wholemeal and white flours, though nearer to the former. National Wheatmeal bread represents a compromise by which the nutritional advantages of wholemeal are retained to a large extent in a form more generally acceptable to the consumer.

Pantothenic Acid. Mr. J. S. D. Bacon, working with Dr. G. N. Jenkins at the Biochemical Laboratory, Cambridge, has developed a new biological method for estimation of pantothenic acid using the growth of young rats as criterion. Wheat embryo, while rich in the as yet unidentified growth factors known to exist in liver and yeast extract, is deficient in pantothenic acid, and the addition of 0.5 g. daily to a basal diet containing all known B vitamins in pure condition, has been found to contribute all the B vitamins present in a liver extract with the exception of pantothenic acid. The growth rate of young rats on this basal diet progressively increased with increased (sub-optimal) additions of pantothenic acid. It is, therefore, hoped that by the use of this diet a trustworthy method of estimating the pantothenic acid content of foods, extracts, etc., has been achieved.

Mr. Bacon, continuing the work of Dr. Macrae, Mrs. C. E. Work and others on the possible existence of further water-soluble B vitamins besides the 5 already isolated and chemically identified, has provided further evidence of the reality of the γ factor. Little further progress in this interesting field has been made during the past year as the need for work on more practical problems has made it necessary to curtail more academic work.

Single Value Vitamin Tables of British Foods. War time food problems have created an official demand for Tables of the composition of the common foods with an approximate Vitamin value for each item, so that calculations can be made on the large scale for agricultural and import programmes and on the small scale for the analysis of dietary surveys. Miss Hume has worked on the compilation of such Vitamin Tables in progressively modified forms to suit the developing requirements of the Ministries of Health and

Food respectively. From this work problems concerning Vitamin A and Vitamin B₁ have arisen and received the special attention of Miss Hume and Dr. Macrae as respective Secretaries of the Vitamin A and Vitamin B₁ Sub-Committees of the Accessory Food Factors Committee.

Vitamin A. There has been an urgent need for more biological tests of Vitamin A in important food-stuffs and Miss Hume has taken part in co-operative researches designed to ascertain the Vitamin A value of English summer butter and the stability of added Vitamin A in Vitaminised margarine, as well as to check the reliability of the spectrophotometric method of estimation which is greatly used at the present time. In this work Miss Hume has been assisted by Miss Henderson Smith.

Investigations of Wheat Flours.

Proteins. Dr. H. Chick, with the assistance at first of Mrs. Work, and later of Dr. M. Cutting, has continued her work on the biological value of the proteins contained in flours representing various proportions of the wheat grain. In addition to wholemeal (100%) and white flour (73-75%) a study has been made of National Wheatmeal (85%) milled according to the specification published from the Accessory Food Factors Committee to contain as much as possible of the aleurone layer and embryo (or germ) of the grain, and as little as possible of the bran. The flours compared were always derived from the same specimen of wheat and were kindly supplied by the Research Association of British Flour Millers, St. Albans.

The observations were made, by the methods previously described, on young rats shortly after weaning at their most sensitive growth period. The biological value of the mixture of proteins in National Wheatmeal (as judged by the g. weight increase corresponding to 1g. of protein ingested) was found to be about 15% higher, and that of the proteins in wholemeal about 20% higher, than that of white flour protein.

The digestibility of the protein in the three types of flour was determined by calculations based on a comparison of the amount of nitrogen ingested as protein with that lost in the faeces. For wholemeal the digestibility coefficients (or percentage adsorbed of the protein consumed) ranged from 82 to 86, for National Wheatmeal from 83.6 to 85.4 and for white flour from 87 to 92. The loss due to lower digestibility in the two former flours is more than compensated by the higher biological value of their proteins.

One series of the samples of wholemeal and white flour studied was the same as that used in the human trials of digestibility carried out by Dr. Macrae and his colleagues described in last year's report. The figures obtained for digestibility of proteins, as well as for total calories, were almost identical with those obtained in the corresponding rat tests, showing that figures derived from such observations may justifiably be applied to the case of human diets.

Nutritive value of Protein and other Nitrogenous Constituents in the Potato.

Biochemical Researches. Dr. A. Neuberger, working at the Biochemical Laboratory, Cambridge, with a grant from the Medical Research Council, has investigated the nitrogenous materials contained in the potato giving special attention to the non-protein substances which form about half the total. Methods have been worked out for estimating some of the latter, more especially glutamine and asparagine. The amino-acid composition of the proteins is also being worked out. In this work Dr. Neuberger has been assisted by Mr. F. Sanger.

A survey of different English varieties has shown great differences in total nitrogen content (range 1.2 to 1.9%) calculated on the dry weight and in its relative distribution among the different types of nitrogenous compounds. For two extreme varieties in this respect, Golden Wonder and Majestic, 64% and 35% of their total nitrogen (1.4 and 1.7%, respectively) were present as protein, 18% and 40% as amides, 6% and 8% as amino-acids and 21% and 20% as other bases respectively. These differences are likely to be genuine genetic variations, since for the most part the varieties studied have been grown under identical environmental conditions. The different types of nitrogenous compounds are not evenly distributed in the tuber. The skin is richest in nitrogen, most being present as protein and asparagine. In the central portion of the tuber the nitrogen is stored chiefly as glutamine.

Experiments with rats. With the co-operation of Dr. Chick, some preliminary trials have been made with young rats on diets in which the nitrogen was derived entirely from the potato. For this purpose concentrates were prepared containing the original mixture of nitrogenous substances. There was no significant weight increase when these diets contained as much as 3.2% nitrogen (on dry weight), of which about half was protein nitrogen. When a small supplement of casein was added growth took place immediately. These experiments will be continued.

Experiments with Pigs. A study of the biological value of the nitrogenous matter (crude protein) of the potato was also arranged by Dr. Macrae to be carried out on pigs at the Institute of Animal Pathology by kind permission of Professor T. Dalling. After Dr. Macrae's departure to the Royal Air Force the work was continued and completed by Mr. J. C. D. Hutchinson, seconded for the purpose by the Cambridge University School of Agriculture. He was assisted by Mr. Bacon and Mr. A. N. Worden (Research Student in Animal Health, Ministry of Agriculture).

Twelve-week's old pigs were reared on a diet adjusted to contain 1.5% nitrogen, of which potatoes were the only source and also on a series of diets in which the potatoes were supplemented by addition of 1, 3, and 6% casein. The growth of the animals was compared with that of controls on a similarly constituted series of rations in which the potatoes were replaced by an amount of barley equivalent in total nitrogen. The food intake of the barley pigs was restricted to that of their fellows on the potato diets, so that differences in palatability of the rations and in appetite were eliminated.

The addition of the casein supplements materially increased the rate of growth in both groups, but the pigs receiving the barley diets thrived consistently better than those on the corresponding potato diets. The weight increases per unit nitrogen ingested was about 12 units for the latter and 15.5 for the former. Addition of 6% casein to the potato diet was required to give a figure for energy utilisation (units dry food consumed per unit weight increase) about equal to that obtained when 3% casein was added to the barley diet. The practical conclusion to be drawn from this result is that *if potatoes are substituted for barley in the ration of growing pigs, about twice as much additional protein concentrate must be given to obtain the same efficiency for conversion of food into live weight.*

The average coefficients of digestibility determined for the potato, 81.4 and 93.6 for nitrogen and total calories, respectively, were higher than those previously accepted. The corresponding figures for barley were 79.0 and 85.9. The differences in rate of weight increase on the two rations were, therefore, due to differences in the nutritive quality of the protein and not to differences in the degree of absorption. It must, therefore, be remembered that only about one-half of the nitrogen in the potato is in the form of true protein and in all the above calculations total nitrogen has been used as the basis for calculating the protein content.

Experiments on the fattening of pigs.

Brewers' Yeast. In continuation of previous experimental work with pigs in which the biochemical value of cultivated yeast protein was found equal to that of casein in supplementing cereal protein, Dr. Macrae has helped to organise practical tests on two local farms in collaboration with workers from the Institute of Animal Pathology. It was found that inclusion in the feed of 5 to 10% dried brewers' yeast was equally beneficial to pigs kept under field conditions. The productivity was high, 1 lb. of live weight corresponding to the consumption of 3.4 lbs. of feed.

Household Scraps. Tests to determine the value of replacing part of the ordinary pig feed by cooked household scraps were also completed by Dr. Macrae at the Institute of Animal Pathology in collaboration with the Staff. It was found that a considerable saving could be effected in the amount of meal required; the weight needed to obtain 1 lb. live weight gain when the pigs received no household waste and amounts equivalent to 60% and 80% of the total dry matter of their ration, was 3.7 lbs., 2.2 lbs. and 1.4 lbs., respectively. Young pigs fed on boiled household scraps alone or supplemented with a small amount of brewers' yeast did not thrive.

Trials of a dried yeast supplement to milk in a rural school.

The dried yeast used was prepared by Dr. A. C. Thaysen of the Government Laboratory, Teddington, from cultivated *Torula utilis*. It has no beery flavour and Dr. Macrae who had organised a preliminary trial of this substance in some hundreds of subjects, had found it to be well tolerated, digestive disturbances being reported in only 3 cases. Some volunteers had consumed as much as 64 g. daily.

Trials in a rural elementary school in Oxfordshire, containing many children from poor families, were organised by Dr. Dagmar C. Wilson (with a grant from the Institute for expenses), in co-operation with the Education Authorities and the Division of Nutrition. During 10 weeks, from Oct. to Dec., 1941, 79 children aged 6 to 10 years received a biscuit daily for 5 days a week, containing 10 g. of the cultivated dried yeast, and an equal number (80) of similar children received a similar biscuit containing no yeast: the biscuits were given with the school milk. During the above period the children receiving the yeast biscuit showed an average weight increase of 1.7 lbs., while the control group put on an average of 1.2 lbs. In each group 40 children, mostly aged 8 to 10 years, completed a 5 months' trial from Oct., 1941 to March, 1942: those receiving the yeast biscuit showed a weight increase of 4.4 lbs. and the control group one of 3.1 lbs. The general additional weight increase in both groups in 1942 is attributed to a much more regular supply of school milk during this period. There was no significant difference between the two groups as regards increase in height.

The results of this observation confirm those of others carried out by Dr. Wilson in the summer of 1941 on school children at Chipping Norton and Burford, and indicate that a small daily ration of dried yeast improves the nutrition of children of this class, as shown by an enhanced increase in weight.

PHOTO-SENSITISING ACTION OF BUCKWHEAT (*Fagopyrum esculentum*).

Dr. P. Ellinger has continued his work on the isolation of the photo-sensitising principle of active extracts by chromatographic adsorption analysis. The purity of the serial eluates was ascertained with the help of a spectrocomparator, an instrument described in an earlier report. The photosensitising activity of each eluate was tested in rats. By this means a non-crystalline powder possessing about 90 times the activity of the original dry buckwheat flowers has been obtained.

THE FLUORIMETRIC ESTIMATION OF VITAMIN B₁ AND RIBOFLAVIN IN FOODSTUFFS, TISSUES AND BODY FLUIDS.

More or less exact estimations of the intensity of fluorescent light emitted by solutions containing either thiochrome, into which vitamin B₁ is converted, or riboflavin, are frequently used for measuring the two vitamins. The results found with these methods differ considerably among themselves and from those gained by biological methods. The physical conditions for measuring the intensity of fluorescent light emitted by a solution containing fluorescent molecules and the relation of the intensity measured to the concentration of the fluorescent molecules are not sufficiently clear. The chemical and physico-chemical factors governing the intensity of fluorescence emitted by thiochrome and riboflavin are almost unknown. In order to elucidate these conditions an instrument has been devised by Dr. Ellinger which enables the intensity of fluorescence emitted by fluorescent solutions to be measured and its dependence on the concentration of the fluorescent molecules to be determined under most varied physical, physico-chemical, and chemical conditions.

THE ESTIMATION OF NICOTINAMIDE IN FOOD-STUFFS.

Najjar and co-workers observed that in cases of nicotinamide deficiency a mauve fluorescent substance is excreted in the urine, whereas in the urine of healthy individuals another mauve fluorescent substance which behaves differently from the former, is present. Dr. Ellinger, in collaboration with Dr. B. S. Platt and Dr. G. E. Glock of the Medical Research Council, used these findings to calibrate dogs and ferrets for the estimation of nicotinamide, orally or subcutaneously administered, by making them deficient in nicotinamide.

VITAMIN C. (Work at East Malling Research Station).

The influence of intermittent consumption of Vitamin C on the development of scurvy. It had previously been shown by Dr. S. S. Zilva that guinea pigs fully saturated in respect to vitamin C succumbed to scurvy on a scorbutic diet as easily as animals which did not possess a store of the vitamin in their tissues. As a consequence he studied the effect on saturated and unsaturated guinea-pigs of low doses which approximated to the minimum requirements but which were administered intermittently in multiples of the daily dose, allowing for the days on which the administration was omitted. It was thus found that 2 mg., a dose capable of promoting good growth and protecting guinea-pigs fully from scurvy, when offered every other or third day protected unsaturated and saturated animals alike from macroscopic scurvy. Only when this dose was given sevenfold (14 mg.) once a week were somewhat lower growth and signs of scurvy in a few animals belonging to both groups recorded. However, a weekly multiple of the daily dose of 5 mg. of ascorbic acid, a dose constituting the lower limit of a range of doses (5-8 mg.), capable of depositing minute traces of the vitamin in the tissues, induced normal growth and protected all animals from scurvy. These experiments show beyond doubt that the same lasting effect can be obtained through the intermittent consumption of high doses of vitamin C by both saturated and unsaturated guinea-pigs. In the case of man, where the requirements are about double and where the time taken for the onset of scurvy on a scorbutic diet is about ten times that of the guinea-pig, it would appear according to these results that a quantity of the order of 100 mg. of ascorbic acid consumed at intervals of several days or even weeks would offer protection against scurvy. This consideration has a bearing on the consumption of vitamin C during the winter and early spring. During this time of the year the urban population of this country has largely to depend on the potato for a continuous but moderate supply of vitamin C. The additional and occasional intake of high quantities of ascorbic acid present in vegetables and fruit rich in it, acts in all probability, as a safeguard, if and when the consumption of vitamin C through the medium of the potato is not quite adequate.

The action of *l*-ascorbic acid on the *in vitro* respiration of liver tissue from guinea-pigs on a scorbutic diet. During the last few years this problem has been under investigation, at first by Dr. Zilva and Dr. A. E. Kellie, and later by Dr. Zilva in collaboration with Mr. G. A. Snow. It originated from Harrison's observation made in 1933, that the addition of *l*-ascorbic acid to liver tissue of scorbutic guinea-pigs increased the oxygen uptake. This observation could not be reproduced by some workers. The controversy has now been cleared up by Dr. Zilva and his collaborators who have shown that the phenomenon is reproducible if certain experimental conditions are observed. The cause of the increase in the tissue respiration is, however, not due to the scorbutic condition of the animals as Harrison supposed, but to another dietetic factor, since the addition of ascorbic acid or of inactive chemically related compounds of a similar reducing potential to liver slices from animals kept on a quantitatively restricted diet but receiving at the same time high supplies of vitamin C, also increases the oxygen uptake. It has been definitely established that this increase in the oxygen uptake was not due to the oxidation of the added ascorbic acid or of the other reducing substances *per se* but to the catalytic action of these compounds on the respiration of the liver tissue from the dietetically restricted animals. In view of the potentialities of this observation, the investigation has been extended to the study of the relationship of the observed facts to the carbohydrate metabolism of the guinea-pig. The

influence of a number of reagents, known to interfere with some of the stages postulated to occur in the scheme of carbohydrate degradation have been investigated and indications, which are being further developed, have been obtained.

Work on the general function of vitamin C in animal metabolism and the catalytic action of some organic compounds on the non-enzymic oxidation of ascorbic acid has been continued by Dr. Zilva in collaboration with Mr. Snow and Mr. J. R. Penney.

Vitamin C in Plant Metabolism. In this investigation in which apples, tomatoes and strawberries have been used, results have been obtained during the year which call for repetition before definite conclusions can be drawn.

Ascorbic Acid and Army Diets. This work has been continued during the year and the results have been reported to the authorities. A few minor problems have also been investigated at the request of the War Office.

NATIONAL COLLECTION OF TYPE CULTURES.

(Medical Research Council.)

As a necessary emergency measure the National Collection of Type Cultures continues to be housed at the Serum Department, Elstree.

During the year under review over 3,000 cultures were sent out and 180 cultures, including some new types, were deposited for maintenance.

For some months the Curator, Dr. St. John-Brooks, has been in Washington, D.C., where he has received the hospitality of the American Type Culture Collection.

GENERAL AND FINANCIAL.

The Accounts and Balance Sheet for the year ending December 31st, 1941, show balances to the credit of the Capital Fund of £615,284. 10. 3., the Contingency Fund of £25,000, the Sinking Fund of £39,111. 18. 2., the Pension Fund of £31,768. 19. 10., the Jenner Memorial Research Studentship Fund of £9,119. 19. 11., and the Bacot Bequest Fund £629. 7. 3.

The following changes in investments have taken place during the year, viz. :—

GENERAL FUND :—Acquired by H.M. Treasury :

£35,000 Cape of Good Hope 3% Consolidated Stock, 1933-43.

£25,000 Natal 3% Consolidated Stock, 1929-49 and £1,300 Union of South Africa 4% Consolidated Stock, 1943-63.

New purchases of £8,000 3½% Conversion Stock, 1961; £35,000 2½% National War Bonds, 1949-51 and £35,000 3% Saving Bonds, 1955-65, have been made.

SINKING FUND :—£1,700 3% Saving Bonds, 1955-65, purchased.

PENSION FUND :—£2,000 3½% Conversion Stock, 1961, purchased.

Income for the year amounted to £96,235. 12. 5., with an additional £5,117. 14. 5. from profit on investments. Compared with 1940, Interest and Dividends on General Fund Investments and Investigation, Diagnosis and Analysis Fees show a decrease, while Sales of Sera, Vaccines, etc., show an increase of £19,282. 4. 5., this being due to greater demands from various government departments.

Stocks of sera and horses on hand at December 31st, were valued at £15,518 and £2,001, respectively, but are not included in the accounts.

Expenditure during the year amounted to £64,859. 14. 11., against £57,110. 5. 9., in 1940. Increases in Serum, Vaccine and Vaccine Lymph Expenses, Animals, and Animal House Expenses and Forage mainly account for this higher figure.

The balance of income over expenditure on the year's working, viz., £31,375. 17. 6. has been placed in the Capital Fund.

In conclusion the Governing Body desires to express its appreciation of the devoted co-operation of the Director and all members of the staff, and also of the assistants, in carrying out, often under difficult conditions, the work of the Institute.

MOYNE.

On behalf of the Governing Body.

The Lister Institute of Preventive Medicine.

BALANCE SHEET

AND

ACCOUNTS.

DECEMBER 31ST. 1941.

BALANCE SHEET

	£	s.	d.		£	s.	d.
CAPITAL FUND to 31st December 1941 :—							
Donations, &c., received to date from the following :—							
Dr. Ludwig Mond (1893)	2,000	0	0				
The Berridge Trustees (1893/98)	46,379	10	1				
The Grocers' Company (1894)	10,000	0	0				
Lord Iveagh (1900)	250,000	0	0				
Lord Lister's Bequest (1913/23)	18,904	5	8				
William Henry Clarke Bequest (1923/6)	7,114	5	7				
Rockefeller Foundation (1935/6)	3,400	0	0				
The James Henry Stephens Bequest (per Lloyd's Bank Limited) (1938)	500	0	0				
Dr. G. A. Davies Bequest (1938)	125	0	0				
Other Donations and Legacies (1891-1934)	20,971	18	3				
General Fund Income and Expenditure Account :—							
As per Account at 31st December, 1940.. .. .	219,395	18	9				
Add Profit on Investments	5,117	14	5				
Add Balance for the year ending 31st December, 1941	31,375	17	6				
					255,889	10	8
							615,284 10 3
CONTINGENCY FUND :—							
As per Account at 31st December 1940	25,000	0	0				
SINKING FUND to 31st December 1941							
	39,111	18	2				
PENSION FUND to 31st December, 1940							
	31,038	8	4				
Add Balance transferred from Pension Fund Income and Expenditure Account, 1941	730	11	6				
							31,768 19 10
JENNER MEMORIAL RESEARCH STUDENTSHIP FUND :—							
As per Account at 31st December 1940	9,016	13	11				
Add Balance transferred from Jenner Memorial Research Studentship Fund Income and Expenditure Account, 1941	103	6	0				
							9,119 19 11
BACOT BEQUEST FUND :—							
As per Account at 31st December, 1940	608	7	3				
Add Balance transferred from Bacot Bequest Fund Income and Expenditure Account, 1941	21	0	0				
							629 7 3
CREDITORS							4,685 5 11

MOYNE, *Chairman of Meeting, 10th June, 1942.*

JOHN ANDERSON, *Hon. Treasurer.*

£725,600 1 4

REPORT OF THE AUDITORS

We have audited the above Balance Sheet. We have obtained all the information and explanations we have required. are held by the Institute on their behalf. In our opinion, such Balance Sheet is full and fair, and properly drawn and the explanations given to us and as shown by the books of the Institute.
London, June 11th. 1942.

	£	s.	d.	£	s.	d.
EXPENDITURE ON INSTITUTE BUILDINGS AT CHELSEA:—						
As per account 31st December 1935, including purchase of freehold site, £6,000				73,548	3	1
FREEHOLD LAND ADJOINING THE "STUDIOS" CHELSEA at cost (1912)				169	6	8
LEASE OF THE "STUDIOS" CHELSEA, as per last account	806	16	9			
Less Amount written off for the year	65	2	0			
				741	14	9
QUEENSBERRY LODGE ESTATE, ELSTREE—						
Freehold land and buildings as per account 31st December 1912				30,455	10	0
FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS:—						
*At cost less depreciation as per account 31st December 1920	2,471	17	2			
Cost of Ultracentrifuges, purchased in 1935, less amounts written off	1,700	0	0			
				4,171	17	2
GENERAL FUND INVESTMENTS (at cost, less amounts written off):—						
£20,000 4 per cent. Consolidated Stock, 1957 or after	74,272	16	0			
£43,000 3½ per cent. Conversion Stock, 1961, or after	42,918	5	0			
£12,500 2 per cent. Conversion Stock, 1943-45	12,500	0	0			
£17,000 5 per cent. Conversion Stock, 1944-64	15,997	0	7			
£32,000 4 per cent. Funding Stock, 1960-90	45,661	13	9			
£64,000 3½ per cent. War Stock	63,407	13	5			
£37,000 Local Loans 3 per cent. Stock	20,829	1	7			
£33,000 2½ per cent. National War Bonds, 1949-51	35,000	0	0			
£35,000 3 per cent. Savings Bonds, 1955-65	35,000	0	0			
£3,000 Port of London 3½ per cent. Registered Stock, 1965-75	2,686	17	7			
£8,000 New South Wales 4 per cent. Inscribed Stock, 1942-62	8,040	1	4			
£25,000 New Zealand Government 3 per cent. Inscribed Stock, 1945	22,114	0	0			
£26,100 South Australian Government 3 per cent. Consolidated Stock, 1916 or after	16,800	0	0			
£2,900 Commonwealth of Australia 3½ per cent. Registered Stock, 1950-52	2,723	16	0			
£25,000 Victorian Government 3 per cent. Consolidated Inscribed Stock, 1929-49	19,800	0	0			
£4,000 Western Australia Government 4 per cent. Inscribed Stock, 1942-62	4,081	3	0			
£20,000 Southern Railway Preferred Ordinary Stock	13,500	0	0			
£6,200 London & North Eastern Railway 3 per cent. Debenture Stock	3,961	0	0			
£5,000 Great Central and Midland Railway Joint Committee 2½ per cent. Guaranteed Stock	3,623	0	0			
£353 London & North Eastern Railway 4 per cent. First Guaranteed Stock	499	11	0			
£8,650 London, Midland & Scottish Railway 4 per cent. Preference Stock	7,960	0	0			
£15,625 London, Midland & Scottish Railway 4 per cent. Preference Stock, 1923	11,300	0	0			
£18,750 London & North Eastern Railway 4 per cent. First Preference Stock	13,028	6	7			
£25,000 East Indian Railway 3 per cent. New Debenture Stock	13,890	0	0			
£800 Grand Trunk Railway Company of Canada Great Western Borrowed Capital 8 per cent. Perpetual Debenture Stock	936	0	0			
£1,837 Grand Trunk Railway Company of Canada 4 per cent. Guaranteed Stock	1,733	0	0			
£300 Ontario and Quebec Railway 8 per cent. Permanent Debenture Stock	984	0	0			
£3,400 Gas Light and Coke Company Ordinary Stock	3,638	0	0			
				490,885	5	10
SINKING FUND INVESTMENTS (at cost):—						
£9,600 2 per cent. Conversion Stock, 1943-45	9,600	0	0			
£10,200 4 per cent. Funding Stock, 1960-90	9,079	0	1			
£20,800 3½ per cent. Conversion Stock, 1961 or after	18,658	5	8			
£1,700 3 per cent. Savings Bonds, 1955-65	1,700	0	0			
Balance uninvested	74	12	5			
				39,111	18	2
PENSION FUND INVESTMENTS (at cost):—						
£22,000 4 per cent. Funding Stock, 1960-90	17,165	3	5			
£16,700 3½ per cent. Conversion Stock, 1961 or after	13,765	19	5			
Balance uninvested	837	17	0			
				31,768	19	10
JENNER MEMORIAL RESEARCH STUDENTSHIP FUND INVESTMENTS (at cost):—						
£2,550 Southwark and Vauxhall Water Co. 3 per cent. Debenture Stock "B"	2,756	10	0			
£1,596 Southern Railway 5 per cent. Preference Stock	2,740	5	0			
£1,300 Liverpool Corporation 3 per cent. Stock, 1942, or after	1,097	6	9			
£2,000 4 per cent. Funding Stock, 1960-90	1,797	14	0			
Balance uninvested	728	4	2			
				9,119	19	11
BACOT BEQUEST FUND INVESTMENT (at cost):—						
£600 3½ per cent. Conversion Stock, 1961 or after	595	10	0			
Balance uninvested	93	17	3			
				629	7	3
(The book value of the above Investments is, in the aggregate, less than their market value as 31st December 1941.)						
STOCK OF ANIMALS						
STOCK OF ANTITOXINS						
DEBTORS AND PAYMENTS IN ADVANCE						
CASH:—						
At Bankers: On Deposit	1,000	0	0			
Current Accounts	12,509	17	5			
				13,509	17	5
Nothing has been charged for depreciation of Furniture, &c. since 1920 as new purchases made during each year to a greater amount than the estimated depreciation (10%) have been written off						
				£725,600	1	4

TO THE MEMBERS.

In accordance with the provisions of the Superannuation Scheme for certain members of the Staff, the relative Life Policies up so as to exhibit a true and correct view of the state of the Institute's affairs, according to the best of our information

COOPER BROTHERS & CO.,
Chartered Accountants.

Auditors.

INCOME AND EXPENDITURE ACCOUNTS

INCOME.

General

									£	s.	d.
Interest and Dividends on General Fund Investments	...	...	...	...	...	...	...	...	19,636	1	8
Interest on Sinking Fund Investments	...	...	...	...	...	...	...	...	1,817	10	0
Investigation, Diagnosis and Analysis Fees, &c.	...	...	...	...	...	...	...	...	489	12	3
Sales of Sera, Vaccines, &c.,	...	...	...	...	...	...	...	...	74,717	17	6
Rent	...	...	...	...	...	...	...	...	74	11	0

£96,285 12 6

Pension

									£	s.	d.
Interest on Investments	...	...	...	...	...	...	...	...	1,429	10	0
									<u>£1,429</u>	<u>10</u>	<u>0</u>

Jenner Memorial Research

									£	s.	d.
Interest and Dividends on Investments	...	...	...	...	...	...	...	...	278	6	0
									<u>£278</u>	<u>6</u>	<u>0</u>

Bacot

									£	s.	d.
Interest on Investment	...	...	...	...	...	...	...	...	21	0	0
									<u>£21</u>	<u>0</u>	<u>0</u>

Cancer Research

									£	s.	d.
Balance of Legacy from John George Mills (1937)...	...	...	...	...	...	...	...	...	671	19	1
									<u>£671</u>	<u>19</u>	<u>1</u>

for the year ending 31st. December, 1941.

EXPENDITURE.

Fund.

	£	s.	d.
Rent, Rates, Taxes and Insurance	2,898	11	9
Salaries and Wages of Staff	29,618	19	0
Premiums on Federated Superannuation Policies	1,672	7	4
Stationery, Printing and Postage	256	4	8
Printing of Collected Papers	155	12	6
Office Expenses, Auditors' Fee, and Provision for Bad and Doubtful Debts	1,352	13	11
Travelling Expenses	230	19	11
Gas, Water, Fuel and Electricity	2,313	8	8
Nutrition and Protozoological Expenses	911	10	3
Bacteriological Expenses	104	11	2
Biochemical Expenses	451	7	5
Bio-physics Laboratory Expenses	910	0	8
Serum, Vaccine and Vaccine Lymph Expenses	11,385	3	6
Animals	4,161	15	3
Animal House Expenses and Forage	5,387	18	5
Alterations, Repairs, Renewals, and Workshop Expenses	615	1	2
Library Expenses	146	4	4
General Stores	150	16	8
Amounts written off Lease of the "Studios," Chelsea and Ultracentrifuges	405	2	0
Sinking Fund ($\frac{1}{4}$ % per annum on Cost of Buildings and Interest on Investments)	1,741	6	9
Balance, transferred to Capital Fund	31,375	17	6
	<u>£96,235</u>	<u>12</u>	<u>5</u>

Fund.

	£	s.	d.
Pensions	698	18	6
Balance, transferred to Balance Sheet	730	11	6
	<u>£1,429</u>	<u>10</u>	<u>0</u>

Studentship Fund.

	£	s.	d.
Salary of Dr. J. H. Humphrey	175	0	0
Balance, transferred to Balance Sheet	103	6	0
	<u>£278</u>	<u>6</u>	<u>0</u>

Bequest Fund.

	£	s.	d.
Balance transferred to Balance Sheet	21	0	0
	<u>£21</u>	<u>0</u>	<u>0</u>

Account.

	£	s.	d.
Balance unexpended	671	19	1
	<u>£671</u>	<u>19</u>	<u>1</u>



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THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE.

Report of the Governing Body,
1943.

CHELSEA BRIDGE ROAD,
LONDON, S.W. 1.

June 24th. 1943.

The Lister Institute of Preventive Medicine,

CHELSEA BRIDGE ROAD, LONDON, S.W. 1

AND ELSTREE, HERTS.

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(*Medical Research Council*)

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MABEL RHODES (*Assistant Curator*).

ROSANUND BARNES, B.Sc.

*Recognised Teacher of the University of London.

ANNUAL GENERAL MEETING
OF
The Lister Institute of Preventive Medicine,
June 24th. 1943.

REPORT OF THE GOVERNING BODY.

The Governing Body has the honour to present its report on the work of the Institute for the year 1943, which year is particularly noteworthy as marking the 50th. anniversary of the commencement of the Institute's scientific activities, as although incorporated on July 25th. 1891, it was not until 1893 that sufficient funds were available for its active work to be commenced. Under happier circumstances the Governing Body would have desired that such an important occasion should have been celebrated in an appropriate manner but under present conditions it is felt that this must be postponed.

GOVERNING BODY.

The Council, at a meeting held on June 26th. last year, re-elected Professor H. R. Dean and Dr. P. Fildes, and to the vacancy created by the resignation of Professor C. R. Harington on being appointed Director of the National Institute for Medical Research, elected Sir Henry H. Dale, P.R.S., as its three representatives on the Board until December 31st. 1943.

At a subsequent meeting of the Board, Lord Moyné, who had acted as temporary Chairman since the death of Professor Bulloch, proposed the election of Sir Henry Dale as Chairman of the Governing Body, which proposal was cordially agreed to by the other members of the Board.

COUNCIL.

The Governing Body records with regret the deaths of Dr. Louis Parkes, a member of the Institute since 1894 and Mr. John Ramsay Drake, a representative of the Worshipful Company of Grocers since 1914, who has been succeeded by Major Leonard M. E. Dent.

At the Annual General Meeting last year the three retiring members of Council were Professor H. B. Maitland, the representative of the Victoria University of Manchester, Professor A. Fleming and Sir Henry Dale, both representatives of the Members of the Institute. Each of these representatives was duly re-elected.

The three members of Council, due to retire this year in accordance with the Articles of Association, but who are eligible for re-election, are Professor H. W. Florey, Dr. John Fawcett and Lord Mildmay of Flete who respectively represent the University of Oxford, the University of London and the Royal Agricultural Society.

MEMBERS.

The Governing Body also regrets to record the death during the year of two of the Institute's members, Professor Warrington Yorke and Mr. Geo. Cooper. Mr. Cooper, who died at the age of 86 was the late Secretary to the Institute and had retired in 1931 after 40 years' service.

STAFF.

The Governing Body announce with regret the termination of Sir John Ledingham's long association with the Institute on March 31st. 1943, and at a meeting held on March 22nd. minuted the following resolution:

"That the Governing Body of the Institute desire to record their sense of the high value of Sir John Ledingham's devoted work for the Institute during the 38 years of his service, first as an Assistant Bacteriologist, then as Head of the Department of Bacteriology, and finally, during the thirteen years since Sir Charles Martin's retirement in 1930, as Director of the Institute. They take pride on behalf of the Institute in the record of distinguished contributions to scientific knowledge for which Sir John Ledingham has been responsible, through his own researches in the fields of pathology and microbiology, and through the many other researches which have been carried out, under his friendly guidance and stimulus, by members of the staff of the Institute and others enjoying its scientific hospitality. The Governing Body have also taken note with great satisfaction, of the many directions in which Sir John Ledingham's wide knowledge of pathology and bacteriology, and his personal distinction as an investigator in those fields, have enabled him to render to the Government, and the nation, services of great importance, which have been recognized by the public honours he has received."

To succeed Sir John Ledingham as Director the Governing Body have appointed Dr. Alan N. Drury, F.R.S., who took up his duties on April 1st. Dr. Drury was Huddersfield Lecturer in Special Pathology in the University of Cambridge and a member of the Scientific Staff of the Medical Research Council.

The Governing Body reports with pleasure the election of Dr. Felix to the Fellowship of the Royal Society.

Dr. G. H. Eagles joined the R.A.M.C. in December last and is now in charge of a mobile laboratory.

Dr. I. Smedley-MacLean retired from the staff of the Biochemical department on attaining the age limit in June 1942. Her first association with the Institute was as a Beit Memorial Research Fellow in 1910 and for the past ten years she has been a member of the scientific staff. She is continuing her work at the Institute on a voluntary basis.

Dr. B. C. J. G. Knight resigned in February and has taken up an appointment at the Wellcome Physiological Research Laboratories.

Miss A. S. Cole, who was attached to the Division of Nutrition at Cambridge resigned her appointment in September and has been succeeded by Mrs. Joyce M. Witt. Miss A. A. Tazelaar also left the Institute after twenty years' service in September, when she married.

Dr. Emmy Klieneberger, formerly Bacteriological Research Fellow, was appointed a member of the scientific staff on April 1st. and Mrs. R. van Heyningen was given a temporary research appointment on the biochemical staff on March 1st.

Except for Dr. Zilva and his assistants, who in November returned to the Institute from the East Malling Research Station, the disposition of the remaining members of the staff is unchanged. The Division of Nutrition is still accommodated at Roebuck House, Chesterton, Cambridge and has the use of additional accommodation in the University Departments of Biochemistry and Anatomy. Dr. Muriel Robertson remains at the Institute of Animal Pathology, Cambridge, and Dr. Korenchevsky and Miss Hall at the Department of Zoology and Comparative Anatomy, Oxford.

Dr. McFarlane and Dr. Kekwick have continued their work at the L.C.C. Serum Institute at Carshalton, but as the rooms they have occupied are now required for work by the Wellcome Foundation on behalf of the Ministry of Supply, provision is being made for the re-transference of their activities to the Institute.

The Governing Body would again express its gratitude to Sir Charles Martin, Sir Frederick Hopkins, Professor T. Dalling, Professor D. Keilin, Professor E. S. Goodrich, Professor J. A. Gunn and Dr. Hatton for their kind provision of research facilities to evacuated members of the staff. The Library still remains at Aldenham School through the good offices of the Governors and the Headmaster.

RESEARCH WORK.

The Governing Body, before surveying the scientific work done during the year, desires again to record its appreciation of the continued co-operation and collaboration the Institute has enjoyed with the Medical Research Council, which has furnished the salaries of the staff of the National Collection of Type Cultures, of Miss Hume, Dr. Zilva and his assistants, part salaries of Dr. Korenchevsky and Miss H. H. Smith and grants to Miss Copping, Miss K. Hall, and Mr. Bacon. The Institute provided, as hitherto, accommodation and materials for the researches of each of these workers.

SEROLOGICAL STUDIES, TOXIN PRODUCTION AND IMMUNISING PROPERTIES OF ANTIGENS.

The employment of the Vi agglutination test and of Vi phage typing in the epidemiological control of typhoid and paratyphoid fevers. Dr. A. Felix, serving as a member of the Emergency Public Health Laboratory Service and now enjoying the hospitality of the Wellcome Research Institution, has continued his studies on this problem. The control of these diseases depends ultimately on the control of the chronic carrier. The detection of a chronic carrier is usually a difficult task, though the introduction of refined culture media and of the Vi agglutination test have made the problem less formidable than formerly.

The Vi agglutination test as an aid to the differentiation between a chronic carrier and a temporary excreter. One of the difficulties that often arise in the course of the epidemiological investigation of an outbreak, is the question whether a person who has been found excreting typhoid or paratyphoid B bacilli is a true chronic carrier or a temporary excreter following a mild or symptomless infection. The former is the original source of the outbreak, whereas the latter is merely one of its victims, though he, or she, may have been responsible for the spread of the infection. The final verdict depends on the result of the bacteriological examination of the excreta that must be continued for not less than a year, but useful information can be obtained much sooner from the examination of the blood serum. Two or three tests for Vi agglutination, carried out at intervals of four to six weeks, will often give a clue. A decreasing Vi titre will indicate temporary excretion, whereas a steady or rising Vi titre will arouse suspicion as to a probable chronic carrier state.

The Vi test originally applied to typhoid alone, but has now been extended to paratyphoid B fever. In all the recent outbreaks of paratyphoid B in this country the persons responsible for the spread of the infection were found to be temporary excretors. The true culprits, namely the chronic carriers, escaped detection.

Experiences with the typing of typhoid bacilli with the Vi bacteriophage. During the past three years typhoid strains isolated from 450 patients or carriers in England, Wales and Scotland were examined by the Vi bacteriophage method of Craigie and Yen. Most of the strains were found to fall within

the eight original phage types of Craigie. In addition, Dr. Felix identified four new Vi phage types, indigenous to this country. These were given provisional designations, pending revision after the war, when co-operation between workers in different countries will be possible again.

About 15 per cent. of the typhoid strains were untypable or "imperfect" Vi forms. More than half of these were derived from three outbreaks and might have been typed successfully had special efforts been made at the time. Eight strains (less than 2 per cent. of the total number), could not be typed because the cultures were devoid of the Vi antigen ("O" forms). It is noteworthy that six of these strains were isolated from chronic carriers of many years' standing.

From a practical point of view the results of the typing of this series of cases is very satisfactory. It indicates clearly that the value of the typing cannot be emphasised too strongly. Without this new laboratory aid there can be little hope of determining the source of infection of many of the sporadic cases, or small groups of cases, that occur throughout the country.

Typing of paratyphoid B bacilli with the Vi bacteriophage. The investigation into the possibility of typing paratyphoid B bacilli by means of specific Vi bacteriophages has been continued by Dr. Felix, with the assistance of Miss B. R. Callow of the Emergency Public Health Laboratory Service. Thanks to the co-operation of many pathologists, considerable progress has been made. So far four different Vi phage types of *Bact. paratyphosum* B have been identified among the strains isolated in this country. The corresponding type specific BVi phages have been obtained by a process of adaptation similar to that employed by Craigie and Yen in their study of typhoid Vi phages. On the other hand, anti-O phages could not be trained to develop strain specificity.

The epidemiological significance of the paratyphoid BVi phage types is much the same as that of the now well-established typhoid Vi types. In all the paratyphoid B outbreaks that have been investigated up to the present time the results of the typing were in accordance with the facts established by epidemiological enquiries. It is of interest to note that the proportion of strains that could not be typed with the four type phages so far available has been very small; of 490 strains from outbreaks that occurred in England, Wales and Scotland during 1940-41 only 4.7 per cent were untypable; of 71 strains from sporadic cases examined during 1942 15 per cent. could not be typed.

Toxin production by *Cl. welchii*. The research on *Cl. welchii* strains isolated from war wounds and air raid casualties and on some miscellaneous strains from other sources has been continued by Dr. Muriel Robertson at Cambridge in collaboration with Mr. J. Keppie. The strains were tested for toxicity by the egg-yolk precipitin method and the serum-combining figures of the toxin were also obtained. The nature of the different strains with regard to the type of capsule found in the organisms taken from the bottles which were being incubated for toxin, was also examined.

A very definite loss of capacity for toxin-production was registered in most strains with the passage of time, but by plating and picking off smooth colonies, the toxicity could in some cases be restored and in one or two instances a better production of toxin was obtained than when the strain was newly isolated. In no case could strains which had been initially poor toxin-producers be induced by colony-selection to improve in this respect.

The capsules which were always present varied remarkably and while very great attention was not paid to this feature they were measured under a high power of the microscope from Indian Ink methylene blue slides. The toxigenic strains were in general those which had relatively narrow capsules whereas the slightly toxigenic strains and those which produced minimal and non-registrable amounts of toxin were among those which showed the thick broad bolster-like capsules.

Growth requirements for production of toxin by *Cl. welchii*. The substance (or possibly substances) in muscle which promote toxin-production (a toxin) has been further studied by Mr. H. G. Rogers and some advance has been made in effecting its purification and in the determination of some of its properties. Dr. Knight and he have also been concerned with the controlled production of *Cl. welchii* and *Cl. oedematiens* toxins, particularly with a view to their purification and conversion to the toxoid state. Dr. Robertson has been testing these toxoids for their antigenic value in guinea-pigs.

A method of identifying the pathogenic *Clostridia* of gas gangrene. Dr. Dorothy B. Steabben and Dr. G. F. Petrie have found that, if the three chief pathogenic *Clostridia* of gas gangrene, *Cl. welchii*, *Cl. septicum* and *Cl. oedematiens* are grown on the surface of an appropriate agar medium to which an optimum amount of the corresponding antitoxin has been added, toxin will diffuse from the colony and will interact with the antitoxin to form a zone of specific precipitate round the colony. At a later stage there is a considerable tendency for the uniform deposit of precipitate to assume the form of concentric rings (Liesegang rings). This appearance is so characteristic as to leave no doubt as to the specificity of the reaction and identification of colony is thus rendered possible, even when it may not be altogether pure. The method may prove to be useful in the investigation of the anaerobic flora of wounds and in the rapid diagnosis of gas gangrene.

Dysentery prophylaxis. In an attempt to avoid the toxic reactions of a bacterial Shiga vaccine, Dr. H. L. Schütze has been investigating the immunizing properties of a diethyleneglycol extract of *Bact. Shiga*. In a series of experiments he has been able to show that mice inoculated with this

extract develop a considerable immunity, the test dose being living Shiga bacilli injected with mucin intraperitoneally; with a mortality of 90 to 95 per cent. among the untreated, a survival rate of 60 to 80 per cent. can be expected among the inoculated.

As he has himself already demonstrated in the case of Salmonella immunization, Dr. Schütze was able to show in the case of the Shiga bacillus that optimal spacing of vaccine doses is a matter of some importance in establishing maximal immunity to infection. A comparison of the protection afforded by two inoculations with intervals of 2, 4 or 6 weeks indicated a quite definite superiority in the immunity following a spacing of the longest period.

Another point investigated was the stability of the diethyleneglycol extract in the presence of preservative. Formalin, merthiolate and phenol in the usual bacteriostatic strengths were tested, the mixtures being allowed to stand at room temperature for 4 months before being used and compared with extract kept in the cold without preservative. Formalin and merthiolate did not damage the prophylactic potency of the extract, about 60 per cent. of the treated animals remaining alive as they did with unpreserved extract, while the phenol-preserved extract protected only 30 per cent. In one of these experiments, serological estimations were carried out and revealed that a progressively higher agglutination titre is achieved by increasing the spacing of the inoculating doses from 2 to 4 and 6 weeks, though as is so often the case, complete correlation between anti-body titre of an animal and its subsequent resistance to infection could not be established.

An interesting observation made in the course of these experiments was the racial and sexual differences exhibited in the two strains of mice (here called M and T) which were used. Members of the M strain always achieved higher titres than did those of the T strain, while in both strains the female produced agglutinins more abundantly than did the male. This pronounced variation in antibody response indicates how important it is to balance sex and strain in groups of animals for comparative purposes. Neither of the strains was a pure line but similar genetical differences were disclosed in a second confirmatory experiment performed three months after the first.

Inoculation of human beings was also carried out on a small scale, a group of some 12 individuals being inoculated with the extract. The reaction was minimal in some, in others more pronounced but of short duration, consisting of a rise in temperature and malaise, with swelling and tenderness, not excessive, at the site of inoculation. The general reaction had subsided by the next day and the local reaction by the day after. In the majority of the cases an increase of antibodies in the blood could be demonstrated as a result of the inoculation.

The antigens derived from *Bact. Shiga* and the protective action of their antisera against the living organisms. Dr. Steabben has concluded an investigation by bacteriological methods of the antigens present in the washed bacilli of smooth Shiga strains and certain derived variants, including the rough type. The washed formol-killed smooth bacilli kill mice only in massive doses of the order of 5,000 millions, suggesting death from protein shock; no protection against a fatal issue is given by anti-bacterial sera. This finding has been considered in relation to recently acquired information obtained by chemical methods on the occurrence of a specific enterotropic toxin in the smooth bacilli, as distinct from the enterotropic toxin present together with a neurotoxin in autolysates of smooth and rough bacilli. Anti-bacterial sera prepared by immunisation with the smooth antigen have high protective value against infection with the living smooth bacilli but not against pure toxin, whereas purely antitoxic sera have no value against infection with the living smooth bacilli. From experiments so far performed, protection against infection with the living rough bacilli has been obtained only by sera prepared from formol-killed rough bacilli and from acetone-killed rough bacilli. One inference with a very practical bearing emerges from the experimental findings, namely, that in the treatment of dysentery in man antitoxic serum containing an antisomatic or "anti-endotoxic" component may be of prime therapeutic importance as an adjunct to chemotherapy. Such a serum will not only neutralise the toxin but may also arrest bacterial invasion, if it is given at the earliest possible moment in the disease before toxic symptoms have appeared. In addition, as the serum possesses both antitoxic and antibacterial components it should also be of value in the prophylaxis of dysentery in man in the face of a prevailing epidemic. The presence of two distinct antigens in the Shiga bacillus has been clearly demonstrated, since the protective values of their corresponding antisera are sharply defined, and do not overlap. The existence of the neurotoxin as a separate entity, unrelated to the smooth "endotoxin" is shown by the fact that it can be derived from a completely rough strain. While the smooth somatic antigen gives rise on inoculation to a highly protective antiserum, it has not been shown to possess any marked toxic power.

Typhus fever. Prophylactic inoculation and specific serum treatment. Dr. Felix has published a survey of the experimental and clinical evidence concerning immunity to louse-borne typhus in relation to the known antigenic constituents of *Rickettsia prowazekii*. It is known from the work of Castaneda and Zia that *R. prowazekii* has a heat-labile and a heat-stable antigen and that the latter is common to *R. prowazekii* and *Proteus* OX19 (Weil and Felix). The antibody to the heat-labile rickettsial antigen is of outstanding importance in phagocytosis of *R. prowazekii* and in protection against experimental infection with this organism. The heat-stable rickettsial antigen, in common with O antigens generally, acts as an endotoxin and the corresponding antibody as anti-endotoxin. It would thus appear that the roles of the two rickettsial antigens in typhus immunity are very similar to those played by the Vi and O antigens in immunity to typhoid fever. Due attention should, therefore, be paid to the separate estimation of the two different

antigens when testing the efficacy of typhus vaccines, and of the two different antibodies when assessing the potency of therapeutic anti-typhus sera.

Tests of OX19 agglutinin response were carried out on members of selected hospital staffs and sanitary personnel who were being inoculated under a scheme organised by the Ministry of Health in consultation with the Medical Research Council. The best available typhus vaccines were employed, yet the OX19 agglutinin response varied widely in the different vaccine groups. The most potent vaccine stimulated demonstrable OX19 agglutinins in 49.6 per cent. of the inoculated, whereas the corresponding figures for the two least effective vaccines were 0 and 4.4 per cent. respectively. It is, therefore, not advisable to rely on any one of the customary tests as the sole method of assay. Two or three different types of test should be employed when differently prepared vaccines are compared.

The present position with regard to the specific treatment of typhus is admittedly very unsatisfactory. Specific serum treatment of recognised value has not yet been evolved, and attempts at chemotherapy so far have also been unsuccessful. Therapeutic anti-typhus sera, prepared by immunisation of horses or rabbits with living rickettsiae, possess neutralising antibodies and show a definite protective action in animal experiments, but such sera contain only negligible amounts of the O antibody. The difficulty may possibly be overcome by the use of *Proteus* OX19 immune serum in combination with anti-*R. Prowazeki* serum.

The preparation of anti-OX19 serum from horses was begun at Elstree, in collaboration with Dr. Petrie, and is now being continued at the Wellcome Physiological Research Laboratories, in co-operation with Dr. H. J. Parish, Dr. C. G. Pope and Miss A. M. Brown. It is hoped that a certain quantity of a potent anti-OX19 serum (concentrate) will soon be ready for clinical trials in cases of louse-borne typhus.

THE PLEUROPNEUMONIA-LIKE ORGANISMS.

Dr. E. Klieneberger continues her study of these organisms. Recently with the generous help of the Venereal Clinical and the Gynaecological Department of the London Hospital, she has had an opportunity of examining bacteriologically a large number of specimens from the vaginal mucous membrane and has been able to confirm reports of American workers that organisms of the pleuropneumonia group occur in that region in certain circumstances. In 50% of the venereal cases and in 30% of cases from the gynaecological clinic, these organisms were present but only in a few instances was cultivation successful in more or less healthy antenatal patients. Material from the nasopharynx of patients with respiratory troubles is also being examined but so far with negative results. The relationship of these new types of human provenance to other types of animal origin is now being studied.

CATTLE ABORTION AND STERILITY IN COWS FROM *TRICHOMONAS FOETUS* INFECTION.

Research into the infection of cattle with *Trichomonas foetus* has been continued by Dr. Muriel Robertson in collaboration with Mr. W. R. Kerr of the Ministry of Agriculture, Northern Ireland, and animals have again been generously placed at their disposal by the Ministry for experimental work.

The investigation into the means by which the raised agglutination titre arises in infected animals has made considerable progress. The absorption of the antigen by the uterine route has been demonstrated by the instillation of sterile saline extracts of *Trichomonas*. The passage of antibody, as shown by the agglutination titre, from the cow to her calf is at present being studied.

The close touch with field work both in England and in Ireland has been maintained and many sera have been tested in connection with the work of the Sterility Investigation Officers and veterinary practitioners dealing with outbreaks of the disease.

BIOCHEMICAL STUDIES ON BACTERIAL AND TISSUE ANTIGENS AND TOXINS.

Bacterial Antigens. Dr. W. T. J. Morgan has completed and published the results of an investigation on the O antigen of *Bact. typhosum* commenced several years ago in collaboration with Dr. S. M. Partridge. The antigen has been shown to consist of a polymolecular complex containing three main components, a specific polysaccharide, a protein and a phospholipin. The protein component is very similar to the conjugated protein prepared from the specific somatic antigen of *Bact. Shiga*. It is of interest to find that the toxicity of the "O" antigens of *Bact. Shiga* and *Bact. typhosum* is largely due to the undegraded polysaccharide component, whereas the degraded polysaccharides, as isolated by the usual techniques, are practically without toxicity. An extension of this work to the O antigen of *Bact. dysenteriae* (Flexner), VI., strain 88, has shown that this antigen is also a polymolecular complex of polysaccharide and protein molecules.

Blood-group Antigens. Dr. H. K. King and Dr. Morgan have continued their investigations on the chemical nature of the substance present in crude gastric mucin that possesses blood-group A specificity. Two methods for the isolation of the A substance have been elaborated, one of which involves the use of 90% phenol. Both methods enable the substance, which is a polysaccharide-amino-acid complex, to be isolated in an undegraded condition. The substance is readily degraded by heating in 1% NaCl or by exposure to dilute alkali or acid. It is of considerable interest that such treatment gives rise to loss of capacity to inhibit

A-iso-agglutination but that the power to inhibit the hæmolysis of sheep cells by an anti-A rabbit serum engendered by human erythrocytes, is not destroyed. The undegraded specific A substance is not antigenic, forms solutions of high viscosity in saline and combines with the protein component of the O antigen of *Bact. Shiga* to form an antigenic complex. Rabbits immunized with this artificial complex give rise to potent anti-A-sera. Dr. Morgan is investigating the possibility of using powerful anti-A sera of this type for the detection of weakly reacting erythrocytes belonging to blood-groups A₁B, A₂ and A₁B. In collaboration with Dr. P. L. Mollison (South-West London Blood Supply Depot) experiments have been made to determine the value of these anti-A sera in the technique of differential agglutination and it has been shown that the agglutination of all but 0.04-0.40 per cent. of total A cells is frequently attained.

Gastric mucin as a virulence-enhancing agent. The preparation of the A substance has also permitted the investigation of the nature of the constituent in commercial gastric mucin responsible for its "virulence-enhancing" power, a property which bacteriologists and immunologists have put to much practical use in recent years. In collaboration with Dr. G. H. Eagles, Dr. Morgan has shown that the purest and most active A substance is also the most potent "virulence enhancing" agent. Treatment of the A substance that brings about degradation, as measured by fall in viscosity or decrease in power to inhibit iso-agglutination, also destroys the "virulence enhancing" power of the A preparation.

Biochemistry of Bacterial Toxins. Dr. M. G. Macfarlane has examined the enzymic activity of the toxins of several species of bacteria, with a view to establishing the biochemical basis of their toxic action in animals. The action of the toxins of *Cl. septicum*, *Cl. edematiens*, *Cl. tetani* and *C. diphtheria* on lecithin, sphingomyelin, cephalin and galacto-lipins has been examined. With the exception of *Cl. edematiens* toxin, none of these toxins had any detectable effect on the lipins. *Cl. edematiens* toxins frequently contain small amounts of a lecithinase similar in action to that of *Cl. welchii* toxins, but immunologically distinct; this enzyme is not identical with the main lethal factor.

The specificity of the *Cl. welchii* lecithinase, which is probably identical with the lethal α -toxin, has been tested. This enzyme also decomposes sphingomyelin, but does not attack either the amino-ethanol or serine form of cephalin, or the galactolipins. Previously it has been shown that the specific inhibition of the action of this enzyme by *Cl. welchii* α -antitoxin can be used as an *in vitro* test for the titration of high titre *Cl. welchii* antitoxic horse sera. With proper precautions against non-specific interference this test is also found to be suitable for low titre sera (0.5 International Units per cc.) and can be used for following the antibody response to inoculation with *Cl. welchii* toxoid.

Dr. Macfarlane has also collaborated with Dr. Knight and with other workers on the production of toxoids for preventive inoculation against gas gangrene.

METABOLISM OF FAT.

Dr. Smedley-MacLean has continued her study of the fat-deficiency disease of rats and in collaboration Dr. Glucksmann (Strangeways Institute, Cambridge) has made an examination of the various organs at different stages of the disease. So far the results suggest that it is primarily the fibroblastic cells which are affected.

The relative effects of butter and margarine in preventing the condition when added to a completely fat-free diet were examined and the former was found to be rather less effective than the latter in preventing the condition. This disposes of a suggestion that the increased substitution of margarine for butter in our war-time diet was a responsible factor in producing dry and scurfy skins.

In co-operation with Dr. Mann of the Molteno Institute, Cambridge, the effects on fat metabolism of substances inhibiting carbohydrate metabolism at various stages of the breakdown process, have been investigated. So far only those inhibitory substances which diminish respiration have been found to produce any effect on the synthesis of fat from fructose.

BIOPHYSICAL AND PHYSICO-CHEMICAL STUDIES.

Drying from the Frozen State. Dr. A. S. McFarlane has continued to develop the coil drier referred to in last year's report, and studies on the properties of whole milk dried with this machine have been made by workers at the National Institute for Research in Dairying, the Hannah Dairy Research Institute and the Low Temperature Research Station, by the kindness of the Directors, Prof. H. D. Kay, Dr. Norman Wright and Dr. Franklin Kidd. The findings are embodied in reports to the Agricultural Research Council. Studies have also been carried out on the freezing and drying of gelatin, agar, rose-hip extract, apple pectin and rubber latex.

Electrophoresis Studies. Dr. R. A. Kekwick has demonstrated the absence of a fibrinogen component in ether-extracted plasma and a reduced β -globulin content. Alkalised and filtered human plasma showed, on electrophoresis, various changes affecting mainly the fibrinogen component.

In collaboration with Dr. J. Vaughan and Dr. O. v. d. Walt, serum protein changes occurring immediately after transfusion of ether-extracted serum have been examined. Where the patient's serum before transfusion showed an abnormal protein constitution, as for example in nephritis, the effect of transfusing normal serum proteins could be observed in the electrophoresis photographs. Urinary proteins in nephritis were found to resemble normal serum proteins. This work has contributed to the file of information on the electrophoresis of pathological sera generally.

Human Serum and Plasma Processing for Transfusion: Ether-extracted Serum. Dr. McFarlane and Dr. Kekwick have been joined by Miss M. E. Mackay (Medical Research Council) in supervising the processing of human transfusion serum and plasma by ether extraction. During the year, 1,000 litres of serum and 2,500 litres of plasma have been received from the London Blood Supply Depots, and processed, filtered, bottled and issued for transfusion purposes after physico-chemical and bacteriological tests have been passed. The following routine technique has gradually been established.

The serum or plasma in 2 litre bottles is agitated vigorously on a mechanical shaker with ether, and frozen at -30°C . in a metal cabinet with alcohol-filled pockets. The bottle of frozen material is inverted on a sloping rack over a similar bottle at 5°C . and fluid rich in protein drips into the lower bottle where it collects under a layer of ether containing fat. The thawing is discontinued when a block of almost pure ice, representing about one-third of the water in the original serum or plasma, remains in the upper bottle. The water-protein layer is siphoned from the ether-fat layer and re-extracted. The concentrate is delivered into stainless steel pans in a sterile chamber and the dissolved ether removed by blowing sterile air from a series of jets on to the surface of the serum or plasma which is maintained at 2°C . The blowing off is continued for 40-48 hours after which the ether content of a 30 litre batch is below 0.05 vol.-%.

In the course of blowing off, the protein concentration is further increased and is adjusted with sterile distilled water to a prescribed value which is checked refractometrically. Plasma, which is received with a variable protein content around 3.5%, is issued with 5%, and serum with 6 and 12% protein. The serum at 2°C . is finally passed through a Seitz filter in the cold room, and distributed into transfusion bottles, which are then subjected to the usual sterility tests. During the ether extraction the bulk of the fibrinogen in plasma is precipitated and held in the ice block in the upper bottle. Traces which survive are removed at the final cold filtration as is also an insoluble material of obscure nature which is deposited from extracted serum on freezing. There is a tendency for more of this material to precipitate from the serum after some months of storage and investigations are in hand with the object of preventing this. Plasma is issued "fibrinogen free" if it gives no deposit when thawed slowly after freezing. Recently protamine sulphate has been used to precipitate traces of fibrinogen and this appears to afford a more sensitive and quicker test than freezing.

Clinical observations on the use of ether extracted serum and plasma in various acute and chronic conditions and on the use of 12% serum in the treatment of burn shock, have been made by Dr. J. Vaughan and Dr. J. F. Loutit and are described in a report to the Medical Research Council's Blood Transfusion Research Committee.

SUBSTANCES THAT INCREASE TISSUE PERMEABILITY.

The Titration of Hyaluronidase. Dr. D. McClean has shown that both the apparent pH optimums of viscosity-reducing activity and the titre of this enzyme are markedly affected by the concentration and nature of the salts in the test system; its activity is very much greater in a dilute buffer system of approximately isotonic concentration and, in this system, the pH optimum is in the region of neutrality. A method of assaying the skin-diffusing activity of these enzymes from the mean results on groups of six guinea-pigs was developed by Dr. J. H. Humphrey and it appears that determinations can be made which distinguish between twofold dilutions of enzyme. A representative series of enzyme preparations from different sources and showing a wide range of activity were assayed by their skin-diffusing activity, by viscosimetry in a dilute buffer system at pH 7.0, and by the mucin clot prevention test. The coefficient of correlation for the results obtained by all three tests was so high that the conclusion was drawn that these tests all measure the activity of the same agent.

The Role of Hyaluronidase in Fertilization. In collaboration with Dr. I. W. Rowlands (Medical Research Council), Dr. McClean has shown that the transparent viscous fluid or gel in which the cumulus cells and corona radiata of the rat ovum are embedded, is dissolved by hyaluronidase from any source with a consequent disintegration of the cell mass. There is a close correlation between the viscosity-reducing activity of the enzyme and the rate of denudation of the egg, no matter whether the enzyme is obtained from testis, spermatozoa, bacteria or snake venom. This observation may explain the fact that a reduction in the sperm count rapidly leads to sterility since there would be a corresponding reduction in the concentration of hyaluronidase in the neighbourhood of the egg. Further work is being carried out by Dr. Rowlands on the influence of this enzyme on fertilization and sterility.

The Early Diagnosis of Wound Infection by Anaerobic Clostridia. In collaboration with Mr. Rogers and with Mr. B. W. Williams (St. Thomas's Hospital), Dr. McClean has carried out infection experiments with organisms of the gas-gangrene group. When the infecting organisms produce hyaluronidase, this enzyme can be detected in the oedema fluid as soon as sufficient can be collected for examination and in the muscle as soon as the earliest sign of infection appears. Lecithinase (α -toxin) can ordinarily be detected at a similar stage in infections due to *Cl. welchii*. A survey of representative strains indicates that a large proportion of *Cl. welchii* strains, associated with clinical gas-gangrene, produce hyaluronidase. All the strains of *Cl. septicum* examined produce this enzyme. The position with *Cl. oedematiens* is unsatisfactory since only a small proportion of the strains examined produce this enzyme.

The Viscosity Reducing Activity of Streptococcal Hyaluronidase. Meyer and his colleagues reported that the viscosity-reducing activity of streptococcal hyaluronidase differed from that of hyaluronidase from any other source; after an extremely rapid initial fall there was complete inhibition of any further activity. This phenomenon had already been observed by us and had rendered viscosimetric assay of this enzyme impossible. Further observations revealed the fact that this behaviour was due to the extremely rapid destruction of streptococcal hyaluronidase at pH 4.5 the reaction at which all the assays had been carried out. If the viscosimetric assays are carried out at pH 7.0 the streptococcal hyaluronidase produces an activity curve in every way comparable with that obtained with enzymes from other sources.

ENDOCRINOLOGY.

Dr. Korenchevsky assisted by Miss K. Hall, and with the co-operation of Mrs. B. Clapham has studied the effects of different combinations of hormones on old rats, and of combinations of vitamins and thyroid hormone on young and adult rats.

Thyroid hormone. In administering thyroid hormone, its katabolic properties in raising metabolism and especially in reducing body fat are most often taken into consideration. The results of experiments on young and old rats and also those of some previous workers emphasise the fact that thyroid hormone also possesses anabolic properties which promote better development of such vital organs as liver, kidneys, spleen, heart and adrenals in both sexes and of ovaries in females. Experiments have also shown that it is possible by using moderate doses of thyroid hormone to reduce the body fat to about $\frac{1}{2}$ without affecting appreciably the gain in body weight, and at the same time preserving the hypertrophying anabolic effects of this hormone on the vital organs mentioned above. Thus from small or even moderate doses of thyroid hormone favourable results can be expected. When, however, large doses are given, the useful effects disappear and the hormone becomes atypically toxic and dangerous, producing loss of body-weight, considerably greater atrophy of seminal vesicles and prostate and considerably less hypertrophy of kidneys, atrophy instead of hypertrophy of liver and spleen, decrease of testes and pronounced involution of thymus. At the same time, all the animals become weak and emaciated, their fur becomes dull, rough and dirty, most suffer from diarrhoea and begin to lose weight, while some die before the end of the experiment from thyrotoxicosis.

Thyroid hormone in combination with vitamins A, B and C. The favourable effects of thyroid hormone cannot be fully operative unless simultaneously liberal amounts of vitamins are given, because of the increased requirements in vitamins which arise during conditions accompanied by increased metabolism, such as those produced by thyroid hormone. Hence the most favourable results can be expected from simultaneous administration of thyroid hormone with extra amounts of vitamins.

Since both thyroid hormone and male sex hormones possess hepato-, nephro-, cardio- and splenotrophic properties, and thyroid hormone also produces adrenotrophic effects, the possibility of therapeutical application of thyroid hormone in cautious doses with vitamins, or together with androgenic hormones, should be investigated in suitable diseases of liver, kidneys and heart, or when an increased activity of adrenals or spleen is desirable.

The results of Dr. Korenchevsky's experiments do not suggest, as has been stated by some authors, any direct antagonistic effects of vitamins on thyroid hormone, but the data indicate that some beneficial results of the administration of vitamins in some cases of experimental clinical hyperthyroidism were probably due chiefly to removal of the relative vitamin deficiency present in these cases. For example, in his experiments on rats kept on a diet containing a *minimal normal* amount of vitamins B and injected with toxic doses of thyroxine, the administration of extra amounts of the B₁-vitamin-complex was followed by a gain instead of a loss in body weight and the partial restoration of specific hypertrophying effects. In such animals the atrophic changes were decreased in seminal vesicles, prostate and liver, and replaced by hypertrophy of spleen, while in kidneys hypertrophy became more pronounced.

THE ACCESSORY FOOD FACTORS.

Accessory Food Factors Committee of the Lister Institute and Medical Research Council.

Dr. H. Chick, Secretary of this Committee, and more especially Miss Hume, as Secretary of its Vitamin A Sub-Committee and the Sub-Committee on Food Composition (Vitamins), have devoted much time to special work in connection with the war time activities of these Committees. These have included:

1. The organisation by the Vitamin A Sub-Committee of an experiment with human volunteers now in progress at Sheffield, planned to ascertain the daily requirements of vitamin A when taken as carotene or as preformed vitamin A.

2. The construction of Tables of Composition and Vitamin Values of British Foods for the special use of the Ministry of Food.

3. An investigation of methods for estimating vitamin B₁ and fibre in National Flour and bread, in which Miss Copping and Mrs. Cutting have co-operated.

NUTRITIONAL STUDIES.

Estimation of Vitamins in Foods and Diets. Miss Copping has made biological estimations of vitamin B₁ and riboflavin in special foods at the request of the Ministry of Food. She has also continued her estimation of riboflavin and other B vitamins in special samples of Food Yeast (*Torula utilis*) provided by Dr. A. C. Thaysen (of the D.S.I.R.). In this and other work Miss Copping, Miss A. S. Cole and Mrs. J. M. Witt co-operated.

Miss Copping has also co-operated with Dr. T. F. Macrae in determining biologically the vitamin B₁, riboflavin, and in some cases vitamin B₆ (pyridoxine), value of the entire diet consumed by R.A.F. and W.A.A.F. personnel at eight different aerodromes and Depots. Sample portions were removed at the time of serving each meal for a period of one week; all was mixed together and "freeze-dried" at the Cambridge Low Temperature Station, ground to a uniform powder and the vitamin estimations made without delay.

Miss Hume assisted by Miss Henderson Smith has tested biologically the vitamin A potency in certain of the above diets and her results have agreed well with those of spectrophotometric tests carried out in other laboratories.

Miss Hume and Miss Henderson Smith also collaborated in the co-operative tests undertaken in six laboratories to determine the vitamin A value of the vitaminised margarine now in use. Here again also the results of spectrophotometric tests were in accord with those of biological estimations.

Digestibility of Different portions of Wheat Grain. In the previous work of Dr. Chick on flours containing different proportions of the wheat grain, the biological value of the mixture of proteins in whole meal (100% extraction of the grain) was found to be about 20% higher than that of the white flour protein (73 to 75% extraction). Of the extra 25% of the grain contained in whole meal about 8.5% is endosperm, 1.5% germ and 15% bran. The supplementary value of the proteins of the germ for those of white flour was found to be nearly equal to those of milk protein. The small proportion of germ in the grain, however, makes its contribution of protein relatively unimportant.

In the bran are present the outer integuments of the grain and the closely adherent aleurone layer, which contains as much as $\frac{1}{3}$ to $\frac{1}{2}$ of the total protein of the grain, enclosed in thick walled cells. The following work carried out by Sir Charles Martin, Dr. Chick and Mrs. Cutting was undertaken to ascertain the degree to which the contents of the aleurone cells were made available during digestion.

Rats fed on diets in which finely ground bran was the only source of protein were killed and the contents of the various portions of alimentary tract collected separately, examined microscopically and analysed for nitrogen content, the results being compared with those obtained with the original bran. The protein and fat of the aleurone layer were found to be utilised to a large extent by the rat, the cells losing their contents chiefly in the small intestine and caecum and occasionally also in the stomach. During passage through the gut the different layers of the pericarp became separated from one another and could be recognised in the faeces.

Corresponding tests were made *in vitro* with bran subjected to the action of ptyalin followed by pepsin and trypsin under aseptic conditions in an incubator. Some separation of the bran layers took place and some digestion of the aleurone layer cells but to a less extent than occurred under normal conditions in the gut. The proportion of nitrogen removed was about the same.

The nitrogen of the bran was found by long term metabolism experiments to have a coefficient (apparent) of digestibility of about 60% compared with about 83 and 89 for that of wholemeal (100% extraction), national flour, white flour (75% extraction), respectively.

In human trials the degree of disintegration of the bran observed in microscopical examination of the faeces, though variable, was similar to that which occurred in the rat.

Analyses of Faeces. Sir Charles Martin, Dr. Chick and Mrs. Cutting have made a study of the faeces of rats fed on diets in which the protein was derived respectively from wholemeal, white flour, wheat bran, potato and milk. A suspension of the bacterial fraction was obtained by selective centrifugation (Strassburger's method) and this was agglutinated by adjustment to pH 3.0, the isoelectric point of bacterial protein. The bacterial fraction of the faeces was lowest and contained the least percentage (36%) of the total faecal nitrogen when the diet was composed of bran; with a wholemeal flour diet the bacterial fraction contained 53% of the total faecal nitrogen. When the diet contained white flour or milk the dry weight of the faeces passed was much lower and the bacterial fraction contained about two-thirds of their nitrogen. On a potato diet it also contained about two-thirds of the total faecal nitrogen.

When the diet was "nitrogen-free" (containing 0.076% N. on the dry weight) the bacterial fraction contained 80% of the total faecal nitrogen. These bacteria can only be nourished on the endogenous nitrogen contributed by gastric and intestinal secretions. The actual amount of bacterial nitrogen eliminated daily on such a diet is much less than that excreted on the other diets and it is uncertain whether the excess bacteria in these cases are nourished on undigested nitrogen of the food or on additional endogenous nitrogen secreted during digestion of protein-containing diets. To what extent this relatively large development of bacteria in the gut serves any nutritive purpose for the benefit of the human host is also uncertain.

Nutritive Value of the Protein and Other Nitrogenous Compounds of the Potato. For some time it has been obvious that to save shipping we should have to replace some of our wheat by potato. This has now come to pass. Compared with wheat, most varieties of potato have a much lower nitrogen content (calculated on the dry weight). Since less than half of this nitrogen is in the form of protein it was important to determine the nature of the other nitrogenous compounds and their nutritive value.

In last year's report a description was given of researches by Dr. Neuberger in the course of which he determined the proportions of nitrogen present in different varieties of potato as protein, amino-acids, amides and other bases, respectively. A series of trials made by Dr. Macrae and Messrs. Hutchinson, Bacon and Worden, in which young pigs were fattened on diets in which the nitrogen was derived wholly or chiefly from the potato was also reported. During the past year these researches have been continued by experiments with rats by Dr. H. Chick with the assistance of Mr. J. S. D. Bacon and Mrs. Cutting.

Preliminary tests with young growing rats have shown that the protein (tuberin) separated from the juice squeezed from the raw potato, possesses a biological value (as measured by the g. weight increase per g. protein ingested), slightly superior to that of the proteins of whole wheat, notwithstanding the fact that the latter are more digestible and better absorbed.

Value of the Total Nitrogen. It has been found possible to rear young rats from weaning on a diet containing whole cooked potato, fresh or dried, as sole source of nitrogen. The variety used, "King Edward," was grown on the Fen lands and contained somewhat high nitrogen content, about 1.9% reckoned on the dry weight. Of this 37% was in the form of true protein, 10% as amino-acid, 25% as basic and 32% as amide nitrogen, the latter chiefly in asparagine and glutamine. The rate of weight increase was subnormal as was to be expected from the low proportion of nitrogen (1.6% on dry weight) in the diet, but only slightly less than that observed on diets containing a similar amount of nitrogen from whole wheat protein. Seeing that less than half the nitrogen in the potato is in the form of protein, this result could be explained, either (1) if the amide and basic nitrogen is nutritionally able to supply the animal's nitrogenous requirements to some extent or (2) if the alkaline nature of the ash of the potato enables the protein metabolism to be worked more economically than when the protein of the diet is derived from wheat which yields an acid ash. These two theories are now being investigated experimentally.

The above results show the nutritive value of the protein separated from the potato and of the total nitrogen as contained in the tuber, to be much greater than that of the whole nitrogenous concentrate prepared from raw potato juice, described in last year's report. This juice contains only the more easily extracted nitrogenous substances and it is possible that the residue may contain a greater proportion of the nitrogen of higher biological value. This point is being investigated.

Nutritional-Surveys. Dr. Dagmar C. Wilson, working with a grant for expenses from the Institute, has been investigating the nutritional state of women in industry compared with that of housewives of similar age and social class. In this work Dr. Wilson has received assistance and hospitality from the Oxford Nutrition Survey under the direction of Dr. H. M. Sinclair.

The nutritional state of 374 women employed in 7 factories in and near Oxford has been compared with that of 288 housewives. The most striking result of the survey was the relatively large incidence of goitre and of dorsal spinal curvature, the latter being diagnosed in about 7% of 553 women examined. The home towns of these women were chiefly in N. Oxfordshire situated on the Lower Lias in areas where Dr. Wilson had previously found high incidence of goitre among the adults and evidence of fluorosis among the children, as shown by high incidence of mottled dental enamel and dorsal spinal curvature. Among 981 school children examined in Oxfordshire 74 cases of dorsal spinal curvature were diagnosed, 69 of which were among 526 children from rural schools, many of which were in the home areas of the affected women. Of a group of 115 industrial women workers from homes in these regions, 37 showed dorsal spinal curvature and 34 goitre. This suggested connection between low iodine and high fluorine content of water and soil, on the one hand, and high incidence of goitre, mottled enamel and dorsal spinal curvature, on the other, is being further studied. In her work Dr. Wilson has had the collaboration of Dr. Margaret Murray of Bedford College who has done the biochemical work on fluorosis.

Organic substances capable of catalysing the oxidation of ascorbic acid. Ascorbic acid in solution owes its instability to oxidation either by the action of enzymes or of metallic catalysts. Dr. S. S. Zilva and Dr. G. A. Snow, in the course of an inquiry into the stability of certain articles of food as carriers of synthetic ascorbic acid, have discovered that the oxidation of vitamin C can also be promoted by a group of non-enzymic organic substances found in tea extracts. The chemical nature of these compounds, the mechanism of the ascorbic acid oxidation promoted by them and the kinetics of the reaction have been studied in some detail. Attempts at isolation have not yet been successful owing to the fact that the compounds themselves or their derivatives, so far obtained, could not be crystallised. Chemical evidence suggests that these catalysts are chemically related to, or at least associated with, the tannin fraction of tea extracts. The activity of the compounds is conditioned by the presence of a hydroxyl group or groups, since the benzoyl or acetyl derivatives are inactive but regain their activity on hydrolysis. The compounds also form inactive complexes with boric acid. *d*-Catechin, tannic acid, catechol and quinol were, however, found to be almost inactive. A commercial sample of gallic acid did show some activity but this was in all probability due to the presence of impurities. The catalytic action is progressively retarded with the fall in pH; milk egg-

albumen and cysteine inhibit the catalysis. Apart from tea, the organic catalysts have been found in aqueous acetone extracts of the root and stem of rhubarb and camellia. A detailed investigation of their distribution in other plants has not been made.

The determination of 2:3-diketo-*l*-gulonic acid. In the first stage of oxidation of *l*-ascorbic acid, dehydro-*l*-ascorbic acid undergoes mutarotation and chemical evidence suggests that this change is due to the gradual opening of the lactone ring with the formation of 2:3-diketo-*l*-gulonic acid. Dr. Zilva and Mr. J. R. Ponney have developed a method for the direct determination of this compound. The method is based on the fact that a colour is formed with 2:4-dinitrophenylhydrazine in alkaline solution which can be measured photometrically by being absorbed through a spectrum green colour filter and matched against standard neutral density filters. *l*-Ascorbic acid and dehydro-*l*-ascorbic acid yield a similar colouration with 2:4-dinitrophenylhydrazine in alkaline solution but by adjusting the conditions of treatment their interference, when present, can be eliminated. 1 part in 200,000 of diketogulonic acid in the presence of dehydro-ascorbic and ascorbic acids in pure solution can be determined by this method. The method was found to be sufficiently sensitive for the determination of very small quantities of diketogulonic acid in biological material (whole blood, plasma, liver, kidney, muscle and urine) with a standard error of percentage deviation of 3-4%.

The action of *l*-ascorbic acid on the *in vitro* respiration of liver tissue from guinea pigs on a deficient diet. The observation made by Dr. Zilva and Dr. A. E. Kellie that the livers of guinea pigs kept on a restricted diet respired *in vitro* more vigorously in the presence of ascorbic acid or compounds of a similar reducing potential has been investigated further. The effect of inhibitors, and of various substrates as well as the influence of ascorbic acid on the livers of rats maintained on a deficient diet have been studied by Dr. Zilva and Dr. Snow.

Miscellaneous researches. The investigations on vitamin C in plant metabolism have been continued, apples, strawberries and tomatoes being again used. The outstanding observation made in this connection, in collaboration with Mr. M. B. Crane of the John Innes Horticultural Institute, was that the vitamin C content of unripe green tomatoes, apparently present mainly as dehydro-ascorbic acid, was considerably lower than that found in red tomatoes ripened in store after the season was over. The tomatoes ripened in store, however, attained a vitamin C content similar to that of fruits ripened on the plant. Murphy has previously observed a higher content in ripe than in unripe fruit immediately after picking at the height of the season but the disparity observed by her was of a much lower order. It is hoped that further work may throw more light on the formation of vitamin C with the ripening of the fruit.

The possibility of determining ascorbic acid chemically in the presence of other reducing substances is being investigated.

War Office investigations. This work has been continued and the results are being communicated directly to the authorities.

Fluorimetric Assay. The physical conditions governing the measurement of the intensity of fluorescent light have been examined by Dr. Ellinger, assisted by Miss Holden, with a view to designing a fluorimeter for routine work. The problem proved to be one of photometry of a luminous volume instead of an illuminated surface.

A study was made of the most suitable positions of light source, container for fluorescent solution, and analyser and of the combinations of light filters which would provide the proper wave-lengths for exciting the fluorescence of the various fluorescent pigments. Other filter combinations were investigated which would transmit the fluorescent beam and at the same time prevent the primary light from reaching the analyser.

Reliable results and high sensitivity were obtained by measuring directly the galvanometer deflection of one photocell together with simultaneous control of the intensity of the primary light source, but this method was found too laborious for routine work. Fluctuation of the primary source of light could be eliminated by using two photocells, and by comparing the intensity of the fluorescence of the solution to be examined with that of a stable standard. The measurement can be done either by altering the aperture of the primary beam or the size of the illuminated area of the photocell or by a potentiometric method. The relative merits of these methods are under examination.

The phenomenon of a maximal fluorescence intensity in solutions so concentrated that further concentration interfered with fluorimetry, was studied. It could be shown that this interference was mainly due to absorption of the primary and partial absorption of the secondary beam; in some substances, but to a lesser degree, to the formation of non-fluorescent polymers or to self-quenching. With narrow containers linear proportionality between fluorescence intensity and concentration could be obtained over a dilution range of a thousand-fold.

The Elimination of Fluorescent Pigments in the Urine caused by intake and lack of Nicotinamide and Related Compounds. The assay experiments carried out by Dr. Ellinger in collaboration with Dr. B. S. Platt and Dr. G. E. Glock of the Medical Research Council on the content of nicotinamide and related compounds in food by estimation of urinary fluorescent pigments have been continued. In collaboration with Mr. R. A. Coulson a number of human volunteers were calibrated after a dose of nicotinamide and the curve of the excretion of the fluorescent pigments studied. The influence of the intake

of nicotinamide, nicotinic acid and nikethamide on these pigments was examined and the difference observed provided some information about the probable mechanism of these compounds.

Attempts were made to determine the nature of the fluorescent pigments eliminated in the urine after nicotinamide intake, and it was shown that the silver blue fluorescence of an extract of normal urine is due to the simultaneous presence of at least two fluorescent pigments, one of which shows similar properties to thiochrome; both substances are under further investigation in collaboration with Dr. Morgan.

The relation of elimination of these pigments to certain diseases and other conditions are under investigation in collaboration with the Royal Air Force and the Medical Research Council.

NATIONAL COLLECTION OF TYPE CULTURES.

(Medical Research Council.)

The National Collection of Type Cultures continues to be housed at the Serum Department, Elstree, as a necessary war emergency measure.

During the year under review over 3,900 cultures were sent out and some 150 new strains were added to the collection. In view of the fact that the collection is still out of touch with the great majority of its correspondents abroad, this may be considered satisfactory.

The Curator resumed his duties during the summer of 1942 on his return from the United States. While there he visited the Iowa State College of Agriculture as the guest of Professor R. E. Buchanan and collaborated with him in the preparation of a revised edition of the Proposed Rules of Bacteriological Nomenclature the sanction for which, under the ægis of the Nomenclature Committee of the International Association of Microbiologists, was given at the Plenary Session of the Third International Congress for Microbiology, New York, 1939.

GENERAL AND FINANCIAL.

The Accounts and Balance Sheet for the year ending December 31st. 1942 show balances to the credit of various funds as follows: Capital Fund £626,124 17s. 5d., Contingency Fund £25,000, Sinking Fund £40,889 4s. 7d., Pension Fund £32,589 16s. 4d., Jenner Memorial Research Studentship Fund £9,281 12s. 7d. and the Bacot Bequest Fund £625 7s. 3d.

Changes in investments during the year have been :—

GENERAL FUND :—Acquired by H.M. Treasury :

£800 Grand Trunk Railway Company of Canada Great Western Borrowed Capital
5 per cent. Perpetual Debenture Stock and £1,937 Grand Trunk Railway Company
of Canada 4 per cent. Permanent Debenture Stock.

SINKING FUND :—£1,800 3 per cent. Savings Bonds, 1955-65, purchased.

PENSION FUND :—£1,300 3½ per cent. Conversion Stock, 1961 or after, purchased.

Income for the year amounted to £71,974 4s. 1d. with an additional £316 10s. 2d. from profit on investments. Compared with 1941, there was a considerable decrease from sales of Sera, Vaccines, &c., and Investigation, Diagnosis and analysis fees, the latter being due to the suspension of this branch of the Institute's activities in the early part of the year.

Expenditure during the year amounted to £61,450 7s. 1d. compared with £64,859 14s. 11d. in 1941, the decrease being mainly accounted for by reduced expenditure on Serum, Vaccine and Vaccine Lymph Expenses and Animals consequent on lower sales.

Stocks of Sera and horses on hand at December 31st. were valued at £15,825 18s. 9d. and £2,834 0s. 0d. respectively, but are not included in the accounts.

The balance of income over expenditure on the year's working, viz., £10,523 17s. 0d. has been placed in the Capital Fund.

In conclusion the Governing Body again desires to express its appreciation of all members of the staff, and also of the assistants, in carrying out, often under difficult conditions, the work of the Institute.

H. H. DALE,

Chairman of the Governing Body.

The Lister Institute of Preventive Medicine.

BALANCE SHEET

AND

ACCOUNTS.

DECEMBER 31ST. 1942.

BALANCE SHEET

CAPITAL FUND to 31st December 1942:—

Donations, &c., received to date from the following:—

Dr. Ludwig Mond (1893)	..	..	..	..	2,000	0	0
The Berridge Trustees (1893/98)	..	..	..	..	46,379	10	1
The Grocers' Company (1894)	..	..	..	..	10,000	0	0
Lord Iveagh (1900)	..	..	..	..	250,000	0	0
Lord Liater's Bequest (1913/23)	..	..	..	..	18,904	5	8
William Henry Clarke Bequest (1923/6)	..	..	..	..	7,114	5	7
Rockefeller Foundation (1935/6)	..	..	..	..	3,400	0	0
The James Henry Stephens Bequest (per Lloyd's Bank Limited) (1938)					500	0	0
Dr. G. A. Davies Bequest (1938)	..	..	..	..	125	0	0
Other Donations and Legacies (1891-1934)	..	..	..	..	20,971	18	3

General Fund Income and Expenditure Account:—

As per Account at 31st December, 1941..	..	255,889	10	8			
Add Profit on Investments	..	..	316	10	2		
Add Balance for the year ending 31st December, 1942		10,523	17	0			
					266,729	17	10

CONTINGENCY RESERVE:—

As per Account at 31st December 1941	..	..	..	25,000	0	0
SINKING FUND to 31st December 1942	..	..	..	40,889	4	7
PENSION FUND to 31st December, 1941	..	..	..	31,768	19	10
Add Balance transferred from Pension Fund Income and Expenditure Account, 1942	..	..	..	820	16	6
				32,589	16	4

JENNER MEMORIAL RESEARCH STUDENTSHIP FUND:—

As per Account at 31st December 1941	..	..	..	9,119	19	11
Add Balance transferred from Jenner Memorial Research Studentship Fund Income and Expenditure Account, 1942	..	..	..	161	12	8
				9,281	12	7

BACOT BEQUEST FUND:—

As per Account at 31st December, 1941	..	..	..	629	7	3
Deduct Balance transferred from Bacot Bequest Fund Income and Expenditure Account, 1942	..	..	..	4	0	0
				625	7	3
CREDITORS	..	..	..	4,958	14	7

H. H. DALE, *Chairman of Governing Body.*

JOHN ANDERSON, *Hon. Treasurer.*

£739,460 12 0

REPORT OF THE AUDITORS

We have audited the above Balance Sheet. We have obtained all the information and explanations we have required. are held by the Institute on their behalf. In our opinion, such Balance Sheet is full and fair, and properly drawn and the explanations given to us and as shown by the books of the Institute.
London, May 24th, 1943.

	£	s.	d.	£	s.	d.
EXPENDITURE ON INSTITUTE BUILDINGS AT CHELSEA:—						
As per account 31st December 1935, including purchase of freehold site, £6,000				73,548	3	1
FREEHOLD LAND ADJOINING THE "STUDIOS" CHELSEA at cost (1912)				169	6	8
LEASE OF THE "STUDIOS" CHELSEA, as per last account	741	14	9			
Less Amount written off for the year	65	2	0			
				676	12	9
QUEENSBERRY LODGE ESTATE, ELSTREE—						
Freehold land and buildings as per account 31st December 1912				20,455	10	0
FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS:—						
*At cost less depreciation as per account 31st December 1920	2,471	17	2			
Cost of Ultracentrifuges, purchased in 1936, less amounts written off	1,360	0	0			
				3,831	17	2
GENERAL FUND INVESTMENTS (at cost, less amounts written off):—						
£80,000 4 per cent. Consolidated Stock, 1957 or after	74,272	16	0			
£43,000 3½ per cent. Conversion Stock, 1961, or after	42,918	5	0			
£12,500 2 per cent. Conversion Stock, 1943-45	12,500	0	0			
£17,000 3 per cent. Conversion Stock, 1944-64	15,997	0	7			
£52,000 4 per cent. Funding Stock, 1960-90	45,661	13	9			
£64,000 3½ per cent. War Stock	63,407	13	5			
£37,000 Local Loans 3 per cent. Stock	20,829	1	7			
£35,000 2½ per cent. National War Bonds, 1949-51	35,000	0	0			
£35,000 3 per cent. Savings Bonds, 1955-65	35,000	0	0			
£3,000 Port of London 3½ per cent. Registered Stock, 1965-75	2,686	17	7			
£8,000 New South Wales 4 per cent. Inscribed Stock, 1942-62	8,040	1	4			
£25,000 New Zealand Government 3 per cent. Inscribed Stock, 1945	22,114	0	0			
£28,100 South Australian Government 3 per cent. Consolidated Stock, 1916 or after	16,800	0	0			
£2,900 Commonwealth of Australia 3½ per cent. Registered Stock, 1950-52	2,723	16	0			
£25,000 Victorian Government 3 per cent. Consolidated Inscribed Stock, 1920-49	19,800	0	0			
£4,000 Western Australia Government 4 per cent. Inscribed Stock, 1942-62	4,081	3	0			
£20,000 Southern Railway Preferred Ordinary Stock	13,500	0	0			
£6,200 London & North Eastern Railway 3 per cent. Debenture Stock	3,961	0	0			
£5,000 Great Central and Midland Railway Joint Committee 3½ per cent. Guaranteed Stock	3,623	0	0			
£353 London & North Eastern Railway 4 per cent. First Guaranteed Stock	499	11	0			
£8,650 London, Midland & Scottish Railway 4 per cent. Preference Stock	7,960	0	0			
£15,625 London, Midland & Scottish Railway 4 per cent. Preference Stock, 1923	11,900	0	0			
£18,750 London & North Eastern Railway 4 per cent. First Preference Stock	13,028	6	7			
£25,000 East Indian Railway 3 per cent. New Debenture Stock	13,890	0	0			
£800 Ontario and Quebec Railway 5 per cent. Permanent Debenture Stock	984	0	0			
£3,400 Gas Light and Coke Company Ordinary Stock	3,638	0	0			
				494,216	5	10
SINKING FUND INVESTMENTS (at cost):—						
£9,600 2 per cent. Conversion Stock, 1943-45	9,600	0	0			
£10,200 4 per cent. Funding Stock, 1960-90	9,079	0	1			
£20,800 3½ per cent. Conversion Stock, 1961 or after	18,658	5	8			
£3,500 3 per cent. Savings Bonds, 1955-65	3,518	4	0			
Balance uninvested	33	14	10			
				40,889	4	7
PENSION FUND INVESTMENTS (at cost):—						
£22,000 4 per cent. Funding Stock, 1960-90	17,165	3	5			
£18,000 3½ per cent. Conversion Stock, 1961 or after	15,179	7	5			
Balance uninvested	251	5	6			
				32,595	16	4
JENNER MEMORIAL RESEARCH STUDENTSHIP FUND INVESTMENTS (at cost):—						
£2,650 Southwark and Vauxhall Water Co. 3 per cent. Debenture Stock "B"	2,756	10	0			
£1,596 Southern Railway 5 per cent. Preference Stock	2,740	5	0			
£1,300 Liverpool Corporation 3 per cent. Stock, 1942, or after	1,097	6	9			
£2,000 4 per cent. Funding Stock, 1960-90	1,797	14	0			
Balance uninvested	889	16	10			
				9,281	12	7
BACOT BEQUEST FUND INVESTMENT (at cost):—						
£600 3½ per cent. Conversion Stock, 1961 or after	595	10	0			
Balance uninvested	29	17	3			
				625	7	3
(The book value of the above Investments is, in the aggregate, less than their market value at 31st December 1942.)						
STOCK OF ANIMALS				(No value assigned)		
STOCK OF ANTITOXINS				(No value assigned)		
DEBTORS AND PAYMENTS IN ADVANCE						
				44,955	10	8
CASH:—						
At Bankers: On Deposit				1,000	0	0
Current Accounts				17,150	11	6
In hand				79	14	4
				18,230	5	10
*Nothing has been charged for depreciation of Furniture, &c. since 1920 as new purchases made during each year to a greater amount than the estimated depreciation (10%) have been written off						
				£739,469	12	9

TO THE MEMBERS.

In accordance with the provisions of the Superannuation Scheme for certain members of the Staff, the relative Life Policies up so as to exhibit a true and correct view of the state of the Institute's affairs, according to the best of our information

COOPER BROTHERS & CO.,

Chartered Accountants.

Auditors.

INCOME AND EXPENDITURE ACCOUNTS

INCOME.

General

										£	s.	d.
Interest and Dividends on General Fund Investments	...	...	...	...	...	...	...	...	...	19,478	16	7
Interest on Sinking Fund Investments	...	...	...	...	...	...	...	...	...	1,353	9	8
Investigation, Diagnosis and Analysis Fees, &c.	...	...	...	...	...	...	...	...	...	188	8	6
Sales of Sera, Vaccines, &c.,	...	...	...	...	...	...	...	...	...	50,881	9	4
Rent	...	...	...	...	...	...	...	...	...	72	0	0

£71,974 4 1

Pension

										£	s.	d.
Interest on Investments	...	...	...	...	...	...	...	...	...	1,487	5	0
										<u>£1,487</u>	<u>5</u>	<u>0</u>

Jenner Memorial Research

										£	s.	d.
Interest and Dividends on Investments	...	...	...	...	...	...	...	...	...	278	6	0
										<u>£278</u>	<u>6</u>	<u>0</u>

Bacot

										£	s.	d.
Interest on Investment	...	...	...	...	...	...	...	...	...	21	0	0
Balance transferred to Balance Sheet	...	...	...	...	...	...	...	...	...	4	0	0
										<u>£25</u>	<u>0</u>	<u>0</u>

Cancer Research

										£	s.	d.
Balance of Legacy from John George Mills (1987)...	...	...	...	...	...	...	...	...	...	671	19	1
										<u>£671</u>	<u>19</u>	<u>1</u>

for the year ending 31st. December, 1942.

EXPENDITURE.

Fund.

	£	s.	d.
Rent, Rates, Taxes and Insurance	2,997	4	8
Salaries and Wages of Staff	29,217	2	0
Premiums on Federated Superannuation Policies	1,676	5	11
Stationery, Printing and Postage	237	19	5
Printing of Collected Papers	151	14	6
Office Expenses, Auditors' Fee	421	8	2
Travelling Expenses	140	19	4
Gas, Water, Fuel and Electricity	2,203	4	9
Nutrition and Protozoological Expenses	959	8	7
Bacteriological Expenses	225	18	11
Biochemical Expenses	266	12	9
Bio-physics Expenses	291	9	9
Serum, Vaccine and Vaccine Lymph Expenses	10,788	13	9
Animals	3,464	12	11
Animal House Expenses and Forage	5,867	14	2
Alterations, Repairs, Renewals, and Workshop Expenses	279	15	4
Library Expenses	129	7	8
General Stores	148	11	1
Amounts written off Lease of the "Studios," Chelsea and Ultracentrifuges	405	2	0
Sinking Fund ($\frac{1}{2}\%$ per annum on Cost of Buildings and Interest on Investments)	1,777	6	5
Balance, transferred to Capital Fund	10,523	17	0
	<u>£71,974</u>	<u>4</u>	<u>1</u>

Fund.

	£	s.	d.
Pensions	666	8	6
Balance, transferred to Balance Sheet	820	16	6
	<u>£1,487</u>	<u>5</u>	<u>0</u>

Studentship Fund.

	£	s.	d.
Salary of Dr. J. H. Humphrey	116	13	4
Balance, transferred to Balance Sheet	161	12	8
	<u>£278</u>	<u>6</u>	<u>0</u>

Bequest Fund.

	£	s.	d.
Honorarium to Lecturer... ..	25	0	0
	<u>£25</u>	<u>0</u>	<u>0</u>

Account.

	£	s.	d.
Balance unexpended	671	19	1
	<u>£671</u>	<u>19</u>	<u>1</u>

SCIENTIFIC PAPERS PUBLISHED FROM THE LABORATORIES OF THE INSTITUTE DURING THE YEAR.



- COULSON, R. A., ELLINGER, P., GLOCK, G. E. AND PLATT, B. S. THE ROUTINE CHROMATOGRAPHY OF FLUORESCENT PIGMENTS IN URINE. *Biochemical Journal*, Vol. XXXVI., 1942.
- COULSON, R. A., ELLINGER, P. AND PLATT, B. S. THE COMPARATIVE EFFECT ON THE ELIMINATION OF FLUORESCENT PIGMENTS IN HUMAN URINE OF NICOTINAMIDE AND RELATED COMPOUNDS. (*Ibid*).
- ELLINGER, P. ... THE SPECTROCOMPARATOR. (*Ibid*)
- " ... GENERAL PRINCIPLES OF FLUORIMETRY OF BIOLOGICAL MATERIAL. (*Ibid*).
- " ... THE ESTABLISHMENT OF THE IDENTITY OR NON-IDENTITY OF CONSECUTIVE ELUATES IN CHROMATOGRAPHIC ADSORPTION ANALYSIS. (*Ibid*).
- ELLINGER, P., GLOCK, G. E. AND PLATT, B. S. THE URINARY ELIMINATION OF FLUORESCENT PIGMENTS IN ANIMALS AS A BASIS FOR THE ASSAY OF NICOTINAMIDE AND RELATED COMPOUNDS. (*Ibid*).
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1943.

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

LONDON, S.W. 1.

BALANCE SHEET AND ACCOUNTS. DECEMBER 31ST, 1943.

FINANCIAL REPORT.

The Accounts and Balance Sheet for the year ending December 31st, 1943 show balances to the credit of various funds as follows: Capital Fund £851,267 18s. 4d., Contingency Fund £25,000, Sinking Fund £42,735 11s. 4d., Pension Fund £33,621 1s. 4d., Jenner Memorial Research Studentship Fund £9,500 18s. 7d. and the Bacot Bequest Fund £646 7s. 3d.

Changes in investments during the year have been:—

GENERAL FUND:—Acquired by H.M. Treasury:

£25,000 East Indian Railway 3 per cent. New Debenture Stock. Purchased
£29,000 2½ per cent. National War Bonds and £29,000 3 per cent. Savings
Bonds.

SINKING FUND:—£1,800 3 per cent. Savings Bonds, purchased.

PENSION FUND:—£1,200 3 per cent. Savings Bonds, purchased.

JENNER MEMORIAL RESEARCH STUDENTSHIP FUND:—£800 4 per cent. Funding Stock, purchased.

Income for the year amounted to £89,550 7s. 2d. In addition £9,261 0s. 10d. was received from profit on investments. Compared with 1942, Sales of Sera, Vaccines, &c., showed an increase of £16,852 5s. 4d.

Expenditure amounted to £73,668 7s. 1d. compared with £61,450 7s. 1d. in 1942, the increase being mainly accounted for by the extended activities of the Serum department owing to the War and the purchase of new apparatus and installations at Chelsea.

Stocks of Sera and Horses on hand at December 31st. were valued at £18,622 6s. 9d. and £3,900 0s. 0d. respectively, but are not included in the accounts.

The balance of income over expenditure on the year's working, viz., £15,882 0s. 1d. together with £9,261 0s. 10d. profit on investments, have been placed in the Capital Fund.

BALANCE SHEET

CAPITAL FUND to 31st December 1943:—

Donations, &c., received to date from the following:—

Dr. Ludwig Mond (1893)	..	..	..	..	2,000	0	0
The Berridge Trustees (1893/98)	..	..	..	..	46,379	10	1
The Grocers' Company (1894)	..	..	..	..	10,000	0	0
Lord Iveagh (1900)	..	..	..	..	250,000	0	0
Lord Lister's Bequest (1913/23)	..	..	..	..	18,904	5	8
William Henry Clarke Bequest (1923/6)	..	..	..	..	7,114	5	7
Rockefeller Foundation (1935/6)	..	..	..	..	3,400	0	0
The James Henry Stephens Bequest (per Lloyd's Bank Limited) (1938)					500	0	0
Dr. G. A. Davies Bequest (1938)	..	..	..	..	125	0	0
Other Donations and Legacies (1891-1934)	..	..	..	..	20,971	18	3

General Fund Income and Expenditure Account:—

As per Account at 31st December, 1942..	..	266,729	17	10			
Add Profit on Investments, 1943	..	9,261	0	10			
Add Balance for the year ending 31st December, 1943		15,882	0	1			
					291,872	18	9

651,267 18 4

CONTINGENCY RESERVE:—

As per Account at 31st December 1941	..	..	..	25,000	0	0

SINKING FUND to 31st December 1943	..	..	..	42,735	11	4

PENSION FUND to 31st December, 1942	..	..	..	32,589	16	4

Add Balance transferred from Pension Fund Income and Expenditure												
Account, 1943	..	..	..	..	..	..	1,031	5	0			
							<u> </u>	<u> </u>	<u> </u>	33,621	1	4

JENNER MEMORIAL RESEARCH STUDENTSHIP FUND:—

As per Account at 31st December 1942	..	..	..	9,281	12	7

Add Balance transferred from Jenner Memorial Research Student- ship Fund Income and Expenditure Account, 1943	219	6	0

BACOT BEQUEST FUND:—

As per Account at 31st December, 1942	..	..	..	625	7	3

Add Balance transferred from Bacot Bequest Fund Income and Expenditure Account, 1943	..	..	..	..	21	0	0

CREDITORS	..	..	..	..	..	7,264	7	9

H. H. DALE, *Chairman of Governing Body.*

JOHN ANDERSON, *Hon. Treasurer.*

£770 086 4 7

REPORT OF THE AUDITORS

We have audited the above Balance Sheet. We have obtained all the information and explanations we have required. are held by the Institute on their behalf. In our opinion, such Balance Sheet is full and fair, and properly drawn and the explanations given to us and as shown by the books of the Institute.
London, 31st May. 1944.

	£	s.	d.	£	s.	d.
EXPENDITURE ON INSTITUTE BUILDINGS AT CHELSEA:—						
As per account 31st December 1935, including purchase of freehold site, £6,000				73,548	3	1
FREEHOLD LAND ADJOINING THE "STUDIOS" CHELSEA at cost (1912) ..				169	6	8
LEASE OF THE "STUDIOS" CHELSEA, as per last account	676	12	9			
Less Amount written off for the year	65	2	0			
				611	10	9
QUEENSBERRY LODGE ESTATE, ELSTREE—						
Freehold land and buildings as per account 31st December 1912 ..				20,455	10	0
FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS:—						
*At cost less depreciation as per account 31st December 1930 ..	2,471	17	2			
Cost of Ultracentrifuges, purchased in 1935, less amounts written off ..	1,020	0	0			
				3,491	17	2
GENERAL FUND INVESTMENTS (at cost, less amounts written off):—						
£80,000 4 per cent. Consolidated Stock, 1957 or after	71,272	16	0			
£43,000 3½ per cent. Conversion Stock, 1961, or after	42,918	5	0			
£12,500 2 per cent. Conversion Stock, 1913-45	12,500	0	0			
£17,000 5 per cent. Conversion Stock, 1914-61	15,937	0	7			
£52,000 4 per cent. Funding Stock, 1960-90	45,061	13	9			
£64,000 3½ per cent. War Stock	63,407	13	5			
£37,000 Local Loans 3 per cent. Stock	20,929	1	7			
£64,000 2½ per cent. National War Bonds	64,000	0	0			
£64,000 3 per cent. Savings Bonds	64,000	0	0			
£3,000 Port of London 3½ per cent. Registered Stock, 1965-75 ..	2,686	17	7			
£8,000 New South Wales 4 per cent. Inscribed Stock, 1942-62 ..	8,040	1	4			
£25,000 New Zealand Government 3 per cent. Inscribed Stock, 1945 ..	22,114	0	0			
£26,100 South Australian Government 3 per cent. Consolidated Stock, 1916 or after	16,800	0	0			
£2,900 Commonwealth of Australia 3½ per cent. Registered Stock, 1950-52 ..	2,723	16	0			
£25,000 Victorian Government 3 per cent. Consolidated Inscribed Stock, 1929-49 ..	19,800	0	0			
£4,000 Western Australia Government 4 per cent. Inscribed Stock, 1912-62	4,081	3	0			
£20,000 Southern Railway Preferred Ordinary Stock	13,500	0	0			
£6,200 London & North Eastern Railway 3 per cent. Debenture Stock ..	3,961	0	0			
£5,000 Great Central and Midland Railway Joint Committee 3½ per cent. Guaranteed Stock	3,623	0	0			
£353 London & North Eastern Railway 4 per cent. First Guaranteed Stock	499	11	0			
£8,650 London, Midland & Scottish Railway 4 per cent. Preference Stock	7,960	0	0			
£15,625 London, Midland & Scottish Railway 4 per cent. Preference Stock, 1923	11,300	0	0			
£18,750 London & North Eastern Railway 4 per cent. First Preference Stock	13,028	6	7			
£800 Ontario and Quebec Railway 5 per cent. Permanent Debenture Stock ..	984	0	0			
£3,400 Gas Light and Coke Company Ordinary Stock	3,638	0	0			
				538,326	5	10
SINKING FUND INVESTMENTS (at cost):—						
£9,800 2 per cent. Conversion Stock, 1913-45	9,600	0	0			
£10,200 4 per cent. Funding Stock, 1960-90	9,079	0	1			
£20,500 3½ per cent. Conversion Stock, 1961 or after	18,658	5	8			
£5,300 3 per cent. Savings Bonds	5,318	4	0			
Balance uninvested	80	1	7			
				42,735	11	4
PENSION FUND INVESTMENTS (at cost):—						
£22,000 4 per cent. Funding Stock, 1960-90	17,165	3	5			
£18,000 3½ per cent. Conversion Stock, 1961 or after	15,173	7	5			
£1,200 3 per cent. Savings Bonds	1,200	0	0			
Balance uninvested	82	10	6			
				33,621	1	4
JENNER MEMORIAL RESEARCH STUDENTSHIP FUND INVESTMENTS (at cost):—						
£2,650 Southwark and Vauxhall Water Co. 3 per cent. Debenture Stock "B" ..	2,756	10	0			
£1,595 Southern Railway 5 per cent. Preference Stock	2,740	5	0			
£1,300 Liverpool Corporation 3 per cent. Stock, 1912, or after	1,097	6	9			
£2,800 4 per cent. Funding Stock, 1960-90	2,705	6	0			
Balance uninvested	201	10	10			
				9,500	18	7
BACOT BEQUEST FUND INVESTMENT (at cost):—						
£500 3½ per cent. Conversion Stock, 1961 or after	595	10	0			
Balance uninvested	50	17	3			
				646	7	3
(The book value of the above Investments is, in the aggregate, less than their market value at 31st December 1943.)						
STOCK OF ANIMALS						
STOCK OF ANTITOXINS						
DEBTORS AND PAYMENTS IN ADVANCE						
CASH:—						
At Bankers: On Deposit	1,000	0	0			
Current Accounts	6,187	16	7			
In hand	21	4	2			
				7,509	0	9
*Nothing has been charged for depreciation of Furniture, &c. since 1930 as new purchases made during each year to a greater amount than the estimated depreciation (10%) have been written off				£770,036	4	7

TO THE MEMBERS.

In accordance with the provisions of the Superannuation Scheme for certain members of the Staff, the relative Life Policies up so as to exhibit a true and correct view of the state of the Institute's affairs, according to the best of our information

COOPER BROTHERS & CO.,
Chartered Accountants.

Auditors.

INCOME AND EXPENDITURE ACCOUNTS

INCOME.

General

									£	s.	d.
Dividends on General Fund Investments	...	...	...	...	...	...	...	...	20,008	14	6
Dividends on Sinking Fund Investments	...	...	...	...	...	...	...	...	1,422	10	0
Sales of Sera, Vaccines, &c.,	...	...	...	...	...	...	...	...	67,733	14	8
Rent	...	...	...	...	...	...	...	...	382	5	0
Donations	...	...	...	...	...	...	...	...	3	3	0

£89,550 7 2

Pension

									£	s.	d.
Dividends on Investments	...	...	...	...	...	...	...	...	1,510	0	0
									<u>£1,510</u>	<u>0</u>	<u>0</u>

Jenner Memorial Research

									£	s.	d.
Dividends on Investments	...	...	...	...	...	...	...	...	294	6	0
									<u>£294</u>	<u>6</u>	<u>0</u>

Bacor

									£	s.	d.
Dividends on Investment	...	...	...	...	...	...	...	...	21	0	0
									<u>£21</u>	<u>0</u>	<u>0</u>

Cancer Research

									£	s.	d.
Balance of Legacy from John George Mills (1987)...	...	...	...	...	...	...	...	...	671	19	1
									<u>£671</u>	<u>19</u>	<u>1</u>

for the year ending 31st. December, 1943.

EXPENDITURE.

Fund.

	£	s.	d.
Rent, Rates, Taxes and Insurance	2,774	12	3
Salaries and Wages of Staff	30,129	12	9
Premiums on Federated Superannuation Policies	1,069	5	3
Stationery, Printing and Postage	306	4	6
Printing of Collected Papers	111	1	2
Office Expenses, Auditors' Fee, Donations and Provision for Bad and Doubtful Debts	1,185	7	3
Travelling Expenses	242	1	1
Gas, Water, Fuel and Electricity	2,242	16	1
Nutrition and Protozoological Expenses	859	9	5
Bacteriological Expenses	241	14	4
Biochemical Expenses	489	17	10
Bio-physics Expenses	554	9	0
Serum, Vaccine and Vaccine Lymph Expenses	12,501	13	8
Animals	5,009	8	3
Animal House Expenses and Forage	8,471	8	1
Alterations, Repairs, Renewals, and Workshop Expenses	1,690	1	0
General Apparatus, New Installations and Furniture	2,326	12	10
Library Expenses	145	4	0
General Stores	162	18	10
Amounts written off Lease of the "Studios," Chelsea and Ultracentrifuges	405	2	0
Sinking Fund (½% per annum on Cost of Buildings and Dividends on Investments)	1,846	6	9
Balance, transferred to Capital Fund	15,882	0	1
	<u>£89,550</u>	<u>7</u>	<u>2</u>

Fund.

	£	s.	d.
Pensions	478	15	0
Balance, transferred to Balance Sheet	1,081	5	0
	<u>£1,510</u>	<u>0</u>	<u>0</u>

Studentship Fund.

	£	s.	d.
Salary of Dr. Rogers	75	0	0
Balance, transferred to Balance Sheet	219	6	0
	<u>£294</u>	<u>6</u>	<u>0</u>

Bequest Fund.

	£	s.	d.
Balance transferred to Balance Sheet	21	0	0
	<u>£21</u>	<u>0</u>	<u>0</u>

Account.

	£	s.	d.
Balance unexpended	671	19	1
	<u>£671</u>	<u>19</u>	<u>1</u>



THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE.

Report of the Governing Body,
1944.

CHELSEA BRIDGE ROAD,
LONDON, S.W. 1.

June 19th. 1944.

The Lister Institute of Preventive Medicine,

CHELSEA BRIDGE ROAD, LONDON, S.W. 1

AND ELSTREE, HERTS.

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DEPARTMENT FOR THE PREPARATION AND STUDY OF VACCINE LYMPH, ELSTREE.

D. McCLEAN, M.B., B.S., M.R.C.S., *Bacteriologist-in-Charge.*
H. J. ROGERS, B.Sc. Ph.D. (*Jenner Memorial Research Student.*)

ADMINISTRATION.

A. L. WHITE (*Secretary.*)
S. A. WHITE, A.L.A.A. (*Royal Corps of Signals.*)
(*Assistant Secretary and Accountant.*)

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9, New Square, Lincoln's Inn, W.C. 2.

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14, George Street, Mansion House, E.C. 4.

NATIONAL COLLECTION OF TYPE CULTURES.

(*Medical Research Council.*)

R. ST. JOHN-BROOKS, M.A., M.D., D.P.H. (*Curator.*)

MABEL RHODES (*Assistant Curator.*)

ROSAMUND BARNES, B.Sc.

*Recognised Teacher of the University of London.

ANNUAL GENERAL MEETING
OF
The Lister Institute of Preventive Medicine,
June 19th. 1944.

REPORT OF THE GOVERNING BODY.

The Governing Body has the honour to present its report on the work of the Institute for the year 1944.

GOVERNING BODY.

The Council, at a meeting held on June 24th. last, re-elected Professor H. R. Dean, Dr. P. Fildes and Sir Henry Dale as its three representatives on the Board until December 31st. 1944.

On January 1st. the Council of the Royal Society under its new standing Orders limiting the appointment of representatives on outside bodies appointed Professor S. P. Bedson its representative on the Board in succession to Sir Joseph Arkwright, whose representation of the Royal Society had extended over twelve years. The Governing Body recorded its appreciation of Sir Joseph Arkwright's services by the following resolution:

"That the Governing Body learns with regret of the retirement of Sir Joseph Arkwright. It desires
"to record its high appreciation of his keen and able interest in the work and affairs of the Institute;
"of his wise and valuable counsel while serving as a member of the Board since 1932 as the representative
"of the Royal Society and of his many valuable contributions to scientific knowledge during the course of
"his service with the Institute."

COUNCIL.

The Governing Body records with regret the death of Professor W. W. C. Topley who first became a member of the Council when appointed to represent the Victoria University of Manchester in 1922 and later as a representative of the Members of the Institute.

At the Annual General Meeting last year, of the three retiring members, Professor Florey, the representative of the University of Oxford, and Lord Mildmay of Flete, representing the Royal Agricultural Society, were re-elected and Professor G. S. Wilson was appointed by the University of London as its representative in place of Dr. John Fawcett.

The vacancy created in the representation of the Members by the death of Dr. Louis Parkes was filled at the same meeting by the appointment of Dr. A. N. Drury.

The three members of Council, due to retire this year in accordance with the Articles of Association, but who are eligible for re-election are, Sir Wilson Jameson, Professor Sir John Ledingham and Professor H. S. Raper, each representing the Members of the Institute.

MEMBERS.

The Governing Body also regrets to record the deaths of Professor J. W. H. Eyre and Sir David Prain, whose connection with the Institute had dated from 1906 and 1918, respectively, and of Dr. I. Smedley-McLean, a member since 1931 who had first worked in the Institute as a Beit Memorial Research Scholar in 1910 and later became a member of its permanent scientific staff until her retirement in 1942.

STAFF.

The Governing Body takes pleasure in announcing that the University of London has conferred the title of "Emeritus Professor of Bacteriology" upon Sir John Ledingham, the late Director of the Institute.

Dr. Amies, who joined the R.A.M.C., in 1941, returned from Egypt and resumed his duties as Bacteriologist-in-charge of the Serum Department in November last, when Dr. Petrie, who had been acting as his deputy, again retired.

Dr. A. S. McFarlane, who joined the Biophysics staff in 1935 resigned in February on taking up an appointment at the National Institute for Medical Research under the Medical Research Council.

Dr. Margery Cutting and Mrs. Joyce M. Witt resigned their appointments in the Division of Nutrition and have been succeeded by Miss V. R. G. Pond and Mr. E. B. Slack.

Mrs. van Heyningen (Biochemical Research Assistant) and Dr. H. K. King (Research Student in Biochemistry) have also resigned.

Dr. R. L. M. Synge, formerly with the Wool Industries Research Association, and Dr. E. L. McCarthy, late Lecturer in Physiology, St. Thomas's Hospital, have joined the Biochemical and Biophysical staffs respectively.

Dr. H. J. Rogers, formerly Research Student in Biochemistry, has now been appointed Jenner Memorial Research Scholar and attached to the Vaccine Lymph Department at Elstree.

Dr. D. W. W. Henderson remains seconded to the Ministry of Supply and Dr. Eagles and Mr. S. A. White are still on active service abroad.

The disposition of the remaining members of the staff is unchanged. The Nutritional staff continue at Roebuck House, Chesterton, Cambridge, with the use of additional accommodation in the University Departments of Biochemistry and Anatomy. Dr. Muriel Robertson is still at the Institute of Animal Pathology, Cambridge, and Dr. Korenchevsky and Miss Hall at the Department of Zoology and Comparative Anatomy, Oxford.

The Governing Body again desires to express its gratitude to Sir Charles Martin, Sir Frederick Hopkins and Professor A. C. Chibnall, Professor T. Dalling, Professor D. Keilin, Professor E. S. Goodrich and Professor J. A. Gunn for their kind provision of research facilities to evacuated members of the staff. The main Library still remains at Aldenham School through the good offices of the Governors and the Headmaster, Mr. G. A. Riding.

Various members of the staff have devoted time to the different Committees set up by the Medical Research Council. The Director has continued as Chairman of the Blood Transfusion Research Committee, the Committee on Hæmoglobin Surveys and on the Drying of Human Milk and as a member of the Industrial Health Research Board, the Shock Committee, the Committee on Jaundice and the Penicillin Therapeutic Trials Committee. He has also maintained close contact with the Emergency Blood Supply Depots administered by the Medical Research Council on behalf of the Ministry of Health. In addition he is a member of the Colonial Advisory Medical Committee of the Colonial Office and of the Ministry of Supply's General Penicillin Committee.

Dr. Zilva represents the Institute on the Accessory Food Factors' Committee, appointed jointly by the Institute and the Medical Research Council and is a member of its Vitamin A and Vitamin D sub-committees. Dr. H. Chick still acts as Secretary of the main Committee and its sub-committee on Opportunities for Post-War Nutritional Research in Occupied Europe. Miss E. M. Hume is Secretary of the Vitamin A sub-committee, and Dr. M. Robertson a member, and Dr. M. G. Macfarlane Secretary, of the sub-committee on Anaerobic Wound Infections of the Medical Research Council's War Wounds Committee.

The Medical Research Council, which is administering the London Blood Supply Depots on behalf of the Ministry of Health, has established a Unit at the Institute for research into, and filtration of, blood plasma and serum for transfusion.

The Governing Body, before surveying the scientific work done during the year, desires again to record its appreciation of the continued co-operation and collaboration the Institute has enjoyed with the Medical Research Council, which has furnished the salaries of the staff of the National Collection of Type Cultures, of Miss Hume, Dr. Zilva and his assistants, part salaries of Dr. Korenchevsky and Miss H. H. Smith and grants to Miss Copping and Miss K. Hall. The Institute provided, as hitherto, accommodation and materials for the researches of each of these workers.

BACTERIOLOGICAL, IMMUNOLOGICAL AND SEROLOGICAL STUDIES.

Anti-Proteus OX₁₀ serum in the treatment of louse-borne typhus. Dr. Felix, serving as a member of the Emergency Public Health Laboratory Service and enjoying the hospitality of the Wellcome Research Institution, has continued the work on the preparation of anti-OX₁₀ serum from the horse for use as a therapeutic agent in louse-borne typhus. In co-operation with Dr. H. J. Parish and Mr. A. J. Harms a concentrated serum has been prepared at the Wellcome Physiological Research Laboratories, the potency of which is such that a dose of 100 ml. contains approximately ten times the total quantity of OX₁₀ antibody present in the circulating blood of an adult patient whose serum has an OX₁₀ titre of 1:100. A few preliminary trials of the serum were carried out in North Africa in collaboration with Captain Theodore E. Woodward, Medical Corps, U.S. Army, of the United States of America Typhus Commission. Six selected severe cases were treated; three patients received the serum intravenously and three intramuscularly, the dose being 300 ml. in each case. One of the patients had been in a comatose state for several days but recovered, as did also the other five patients. These trials showed that the large doses of serum were well tolerated and that, as a result of the injections, an initial low-titre OX₁₀ reaction in the patient's serum (titre approximately 1:100) could be increased 20-30 times.

It is known that the most severe cases of louse-borne typhus, including the cases that succumb to uncomplicated typhus infection, show a low-titre OX₁₀ reaction. In such cases the maximum titre may never exceed or even reach the level of 1:100. The concentrated anti-OX₁₀ serum is intended for use in cases of this type, to supplement the anti-rickettsia serum which contains only negligible amounts of the OX₁₀ antibody. A quantity of anti-OX₁₀ serum is now available and it is hoped to arrange for a strictly controlled clinical trial.

Typing of typhoid, paratyphoid and food poisoning bacilli with the Vi bacteriophage. The investigation of this problem has been continued by Dr. A. Felix, with the assistance of Miss B. R. Callow of the Emergency Public Health Laboratory Service. One hitherto unknown Vi-phage type of the typhoid bacillus was identified during the past year as being responsible for a small outbreak of typhoid fever. This type does not seem to be indigenous to the British Isles, as it has been traced to a chronic carrier who acquired the infection during the war in South Africa more than forty years ago. On the other hand, a Vi-phage type of *Bact. paratyphosum B* was found which must be considered as indigenous to this country. The number of known Vi-types and subtypes of the paratyphoid B bacillus is now five.

The investigation into the possibility of typing *Bact. typhi-murium* has made considerable progress. Five different Vi-phage types and sub-types of this organism have been identified so far, and the method can now be usefully employed in the epidemiological investigation of outbreaks or sporadic cases of food poisoning due to *Bact. typhi-murium*.

Dysentery Prophylaxis. Dr. Schütze has investigated the conditions which govern the agglutination of *Bact. Shiga* and has ascertained that a very considerable range of agglutinability is normally found among members of this species. The titre of an anti-Shiga serum may vary from less than 1:25 up to 1:6400 according to the strain agglutinated. Strains which are hypo-agglutinable can be rendered more agglutinable by heating the suspension at 100°C., treating it with phenol or by growing the culture at a lower temperature, e.g., 20°-26°C. It has not been possible to demonstrate a labile antigen as the cause of this hypo-agglutinability. It has been shown, however, that the less agglutinable strains possess a greater content of specific antigen as evidenced by their diethylene-glycol extracts yielding considerably more precipitinogen and by the capacity of these extracts to induce higher antibody titres in the rabbit.

In collaboration with Dr. Morgan, the examination of diethylene-glycol extracts derived from strains of *Bact. Shiga* of high and low agglutinability has been undertaken. Although the investigation is as yet incomplete, it is already clear that there are profound chemical and serological differences in the antigenic material isolated from these two types of this species.

Twelve adult volunteers were immunized with the purified O antigenic complex of *Bact. Shiga*, and by means of a mouse protection test it was shown that in each person a definite degree of immunity had been induced.

Gas Gangrene. Dr. M. Robertson's work upon the anaerobes responsible for gas gangrene infections in wounds has consisted in the investigation of the response of guinea pigs to immunisation with toxoid. This research has been carried out in collaboration with Dr. James Keppie (Institute of Animal Pathology, Cambridge).

The difficulties which have delayed active immunisation against the pathogenic spore bearing anaerobes have lain in the biochemical field. Dr. van Heyningen and Dr. Knight (Wellcome Physiological Research Laboratories) have placed three concentrated toxoids at Miss Robertson's disposal, viz., those of *Cl. welchii*, *Cl. edematiens* and *Cl. septicum*.

Protection of guinea pigs against living organisms was found to be possible. It depended upon the presence of circulating antibody being evoked in the animals and as long as this was present in sufficient quantity excellent protection was achieved. With suitable immunisation the protection against *Cl. welchii* seems to persist for at least five months.

Further studies are now in progress to obtain more precise quantitative information and to see if multiple infections of two anaerobes alters the conditions of successful protection.

Dr. D. B. Steabben has continued her examination of the method by which *Cl. welchii*, *Cl. edematiens* and *Cl. septicum* can be identified when a specific toxin-antitoxin precipitate is formed round colonies on agar plates containing the homologous antitoxin. Strains of the three anaerobes recently isolated from clinical material were tested.

Cl. welchii: The majority of strains isolated from recognised cases of clinical gas gangrene gave good positive reactions; in the cases where gas gangrene was not present, most of the strains isolated gave no reaction.

Cl. edematiens: Twelve strains, six old (up to 25 years) and six new, of Type A (human), all reacted with Type B (animal) antitoxin.

Cl. septicum: Strains which had been long in culture (up to 25 years) produced glossy opaque colonies and all reacted well. Some recently isolated strains spread very rapidly over the surface of the medium; when this spreading was inhibited by the use of an "H" serum the colonies were discrete, flat, mottled and dry, but did not react with the antitoxin. Four out of five such strains were derived from cases which were not clinically gas gangrene. Four out of five strains from clinical gas gangrene gave good reactions, but of these, three produced the glossy opaque type of colony.

Dr. M. M. Barratt has been engaged in testing *Cl. welchii* antitoxins and toxins by the "egg" method—the action of the lecithinase of the toxin on a solution of egg yolk—and comparing the results with those obtained by animal or hæmolytic test, or both. Some discrepancies have been noted but agreement on the whole has been fairly uniform.

Nuclear Structures in Bacteria. Transformations of nuclear structures in bacteria have been studied by Dr. E. Klieneberger-Nobel by means of the hydrochloric acid-Giemsa method of Robinow. The micro-organisms chosen for this study were *Cl. welchii*, *Cl. oedematis*, *Cl. septicum*, var. *gigas*, *Bac. mycoides* and *Spharotilus natans* Kützinger.

Nuclear fusion (autogamy?) has been shown to occur in all five organisms. In *Spharotilus natans* this is of a special type. In the four spore-bearing organisms the dumbbell bodies, which are dispersed in the cells of the young growth, become aligned in the long axis of the cell where they eventually fuse into an axial nuclear cylinder. The fusion of the chromatinic material is the first step in the process of sporulation in the spore-bearers examined. During this development the chromatinic central cylinder of the fusion cell divides in two steps, with the result that it is segregated into four structures which often assume a dumbbell shape. Of these four structures one functions as the spore "chromosome" whereas the other three disintegrate. The ripe spore representing, as it does, the smallest cell unit, contains one nuclear structure or "chromosome." Therefore the two main features in spore formation of bacteria appear to be firstly, a fusion of nuclear material and secondly, a reduction partition which is reminiscent of, though not similar to, the more complicated corresponding phenomenon in the higher organisms.

Fusion of nuclear material occurs in the course of development in all the spore-bearing organisms so far examined. The fact that by exposure to air a fusion of the "chromosomes" can be induced suddenly in all the anaerobic spore-bearers tested seems of interest. The speed at which the induced fusion takes place varies according to the oxygen sensitivity of the organisms in question.

Substances that increase Tissue Permeability. Gas Gangrene Infection. Dr. McClean and Dr. Rogers have continued their infection experiments with organisms of the gas-gangrene group in order to elucidate some of the observations made during the work on the diagnosis of wound infection. Infection with non-hyaluronidase-producing strains results in intensely "sticky" (leptogenic) oedema fluid due to hyaluronic acid. It was found that similar leptogenic fluid could be obtained *in vitro* by the cultivation of non-hyaluronidase-producing strains in fresh muscle removed aseptically from guinea-pigs, the character of which is immediately destroyed by adding hyaluronidase. Similar fluid containing approximately the same amount of polysaccharide can be obtained by digesting fresh muscle with trypsin. The hyaluronic acid appears to be liberated by the digestion of the muscle during infection and is not actively secreted by either the infecting organisms or the host's tissues. It has been found that the leptogenic quality of the fluid varies independently of the viscosity and clot formation by acetone or acetic acid and that it rapidly disappears on standing.

During the progress of experimental *Cl. welchii* infection there is a steadily mounting titre of lecithinase and hyaluronidase in the oedema fluid and muscle and yet neither of these enzymes can ever be detected in the blood stream. Moreover if large doses of lecithinase are injected intravenously into mice this enzyme cannot be detected in the blood a few minutes later although the animals are obviously dying with marked intravascular haemolysis. Purified hyaluronidase disappears in the same way but crude hyaluronidase can be recovered quantitatively from the blood stream. This difference explains the failure to achieve the *in vivo* decapsulation of streptococci reported last year. In this work a purified enzyme from the testis was used, whereas Hirst, who was successful, used a crude enzyme preparation from leeches.

A preliminary report by MacLennan on his failure to detect hyaluronidase in the tissues of wounds infected with gas-gangrene suggested that this might be due either to the neutralisation of the enzyme by the antitoxin which the patients had received or by the digestion of the enzyme due to the activity of concomitant proteolytic organisms. If guinea-pigs are given 200 units of antitoxin 2 or 4 hours after injection with a large dose of *Cl. welchii* the appearance of free lecithinase or hyaluronidase in the tissues is markedly inhibited up to 24 hours later. As a diagnostic measure the detection of these enzymes is only suitable for those cases that have not received antitoxin; a positive result in a case that had received antitoxin would indicate that more antitoxin was urgently required. Mixed infection with proteolytic organisms does not inhibit the appearance of lecithinase or hyaluronidase. It would appear that a mixed infection with *Cl. histolyticum* and a relatively avirulent hyaluronidase-producing organism is more lethal than infection with either *Cl. histolyticum* or the other organism alone.

Biochemistry of Hyaluronidase Production. Dr. Rogers has commenced a comparative study of the biochemistry of hyaluronidase production by a variety of micro-organisms and of the properties of the purified enzymes. Each of these enzymes appears to have a similar chemical function in hydrolysing hyaluronic acid and yet is neutralised only by its homologous anti-serum. It is hoped that these studies will illuminate the general problem of the group involved in the enzymic action and in the antigenic specificity of proteins. The necessary purification of the enzymes for this study is greatly facilitated by cultivating the organisms in simplified media. Uracil is an important addition to a casein hydrolysate growth factor medium for rapid and optimal growth of Group C streptococci. The conditions for the production of potent hyaluronidase by a strain of Group C streptococcus in the simplified medium have been worked out. Chromatographic analysis has given promising results in the purification of *Cl. welchii* hyaluronidase.

The Action of Reducing Substances on Hyaluronic Acid. Mr. C. W. Hale, laboratory technician working with Dr. McClean, has investigated the loss of viscosity which occurs when certain polysaccharides

such as hyaluronic acid and starch are incubated with reducing substances such as ascorbic acid. The reaction is catalysed by copper and inhibited by sodium diethyldithiocarbamate and occurs only in the presence of oxygen. In the presence of catalytically active palladium, hydrogen will degrade hyaluronate or starch provided molecular oxygen is also present. It appears that hyaluronate does not catalyse the oxidation of ascorbic acid and it is suggested that this is yet another example of the breakdown of certain chemical bonds by the action of a reductant that is being actively oxidised.

Hyaluronidase is not readily activated by molecular oxygen nor does it require oxygen for its activity. Oxidising or reducing conditions are not developed during the activity of the enzyme and the presence of copper has no influence on the reaction.

Histology of Hyaluronic Acid. In common with other acid polysaccharides, hyaluronic acid forms a water insoluble stained complex with basic aniline dyes. The formation of this complex is inhibited by previous exposure of hyaluronic acid to hyaluronidase. Mr. Hale is developing a histo-chemical method depending upon these observations for the demonstration of hyaluronic acid in the tissues and the action of hyaluronidase on this substance *in situ*.

Trichomonas foetus. Further studies by Dr. Muriel Robertson in collaboration with Mr. W. R. Kerr (Ministry of Agriculture of Northern Ireland) have been made upon the immune response of cattle to infection with *Trichomonas foetus* and allied matters.

The investigation has been made by means of the agglutination test and by the observation of the degree of reaction produced by intradermal injection of extract of *Trichomonas*. The co-ordination of these results in animals whose state in regard to infection or vaccination is known has yielded a great deal of information.

The direct absorption of *Trichomonas* antibody by the newborn calf by the ingestion of the maternal colostrum has been observed in considerable detail. The calf can absorb antibody for about the first 24 hours of life. If, however, the calf is first fed on boiled milk for this period and then on the colostrum the antibody is not absorbed directly into the circulation. The absorption of antibody by the alimentary canal in the newborn animal is very rapid and in some cases a high titre was recorded in three hours from the time of feeding, whereas the serum of the calf at birth before feeding was completely negative.

It has become clear that the skin test is due to fixed antibody in the cells of the skin and this has been shown to occur secondarily to the presence of circulating antibody. Under certain conditions highly infected animals with a high blood titre are skin negative, because the balance of antigen absorption from the uterus is so high that full antibody is not present in sufficient quantity to admit of fixation of the skin. The removal of the focus is followed in a few days by the skin test becoming positive, indicating that in the absence of further absorption of antigen the excess of antibody has now become cell-fixed.

This work is still in progress but the diagnosis of the infection has been made much clearer by the co-ordination of the serological test with the skin test.

BIOCHEMICAL STUDIES.

Specific Blood-group substances. Dr. Morgan and Mrs. R. van Heyningen have completed an examination of 50 pseudo-mucinous ovarian cyst fluids for the presence of the specific blood-group factors A, B, O and Rh. It would appear that the cyst fluids obtained from women who possess the ability to secrete their blood group factor in a water-soluble form, are a convenient and potent source of the group substances A, B and O. In no instance was the blood-group character of the ovarian cyst fluid at variance with the specificity of the water-soluble group factor in the patient's saliva. It was observed that whereas the great majority of salivas obtained from secretors belonging to groups A and B inhibit the agglutination of O erythrocytes by absorbed anti-O cattle serum, only one of ten specimens of active A or B cyst fluid was found to give rise to any significant inhibition of iso-agglutination. Evidence was obtained which showed that a water-soluble Rh factor is not present in cyst fluids obtained from secretors and non-secretors belonging to groups ARh, BRh and ORh.

In collaboration with Dr. H. K. King, Dr. Morgan has isolated the A substance from ovarian cyst fluid and the chemical, physical and immunological properties of this substance have been compared with those of the purified A substance obtained from hog-gastric mucin. The two A substances are similar in chemical composition and physical properties and both are devoid of antigenic properties. By combination with the conjugated protein component of the O antigenic complex of *Bact. Shiga* or *Bact. typhosum* these two A haptens are converted into full antigens that will give rise to potent and specific anti-A agglutinins in the rabbit.

Dr. Morgan and Miss Steabben have recently started experiments designed to suppress and control the natural and immune anti-A (α)- and anti-B (β)-agglutinins in the rabbit. It is hoped that the results of this investigation will indicate a means whereby it is possible to prevent iso-immunisation of the mother by the foetus with resulting foetal death.

Bacterial Toxins. Dr. M. G. Macfarlane has continued work on the biochemical action of various bacterial toxins. Culture filtrates of various strains of *Cl. sordelli* (or *Cl. bifermentans*) contain small

amounts of a lecithinase which is similar in biochemical action to the lecithinase of *Cl. welchii* and *Cl. edematiens* toxins; it is, however, immunologically distinct, as it is partially but not specifically inhibited either by *Cl. welchii* antitoxin or by the anti-lecithinase present in *Cl. edematiens* antitoxic sera. The possibility of the presence of a similar lecithinase in culture filtrates of other species of bacteria is being investigated.

Further experiments have been made on the effect of *Cl. edematiens* and *Cl. septicum* toxins on various cell constituents, particularly on the enzymic systems concerned in the intermediate metabolism of sugars, but so far no clue to the biochemical basis of the pathological action of the toxins has been obtained.

Gramicidin. Dr. Synge has commenced studies aimed at relating the chemical nature with the biological activities of the antibiotic gramicidin. Work so far done includes a check of amino-acid composition, preliminary bacteriological and serological experiments and attempts to isolate and identify products of partial hydrolysis. For this last purpose partition chromatography with starch as supporting medium for the more polar phase has been developed and promises to yield good results. The applicability of this technique to other aspects of protein chemistry is being studied.

METABOLISM OF FAT.

Until her death in March, Dr. I. Smedley-Maclean in association with Mr. Eric Reid, was engaged in an investigation into vegetable oils and fats under the auspices of the Colonial Products Research Council.

BIOPHYSICAL AND PHYSICO-CHEMICAL STUDIES.

At the request of the Medical Research Council Dr. A. S. McFarlane was seconded for part time work at the National Institute for Medical Research during the year.

Low Temperature Drying. The programme of work on the low-temperature drying of biological materials was interrupted for some months while the plant at Carshalton was dismantled and re-erected at the Institute. Prior to the move two new lines of development were initiated, viz., the use of ethane as a refrigerant to obtain lower condensing temperatures and of diffusion pumps to achieve higher vacua in the drying chamber. A small standard methyl alcohol compressor charged with ethane easily maintained the condenser temperature at -75°C . while correspondingly higher rates of drying were obtained. While the optimum temperature for condensing has still to be determined in relation to a variety of conditions in the chamber it can now be assumed with confidence that ethane will be used in future to realise it. Further work must be done, however, to assess the value of the higher vacua obtained by the use of diffusion pumps.

Processing of Human Serum and Plasma for Transfusion. Dr. R. A. Kekwick and Dr. M. E. Mackay (Medical Research Council) have been studying problems arising in the large scale processing of human serum and plasma for transfusion.

The low temperature ether extraction process has been discontinued. The adjustment of the reaction of plasma to pH 5.4 with sterile citric acid prior to ether-extraction provides a method for the quantitative removal of fibrinogen, as well as removing the unstable lipid complexes. After readjustment of the product to pH 7 followed by Seitz filtration, a transfusion fluid is obtained which remains stable for at least 12 months. Extracted serum tends to form a precipitate after about six weeks, but if freeze-dried reconstitutes to a water-clear fluid.

The effect of ether-extracting serum experimentally contaminated with a variety of bacteria and viruses, is under investigation.

The filtration of plasma after acidification to pH 5.2-5.4 was also studied, with a view to the production of a material suitable for freeze-drying.

Problems arising in the kaolin processing of plasma have been surmounted and the Serum Unit has been filtering the entire output of the London Blood Transfusion Depots prior to despatch to Cambridge for freeze-drying.

Some physiological properties of transfusion fluids are under investigation with a view to correlation with biophysical and biochemical characteristics.

Foetal and Maternal Haemoglobin. Since joining the staff in October last, Dr. E. F. McCarthy has continued his experiments on the influence of the corpuscle on the oxygen dissociation curves of foetal and maternal haemoglobin.

Osmotic Pressure of Foetal Serum. Foetal sheep serum exerts a significantly higher osmotic pressure per gram of protein than the maternal serum. The difference cannot be explained by different albumin-globulin ratios of the two sera. Dr. McCarthy is now extending work on this subject to human foetal and maternal sera.

Osmotic Pressure of "defatted" Serum. In collaboration with Dr. Popják (St. Thomas's Hospital) Dr. McCarthy has made osmotic pressure measurements on normal human serum before and after "defatting" by low temperature ether extraction. The results indicate that the fat removed did not affect the serum protein osmotic pressure. Further experiments are being undertaken with the serum protein from cases of lipid nephrosis.

CULTIVATION OF VACCINIA VIRUS.

Work on the cultivation of Vaccinia virus on the chorio-allantoic membrane of the developing chick which was suspended shortly after the outbreak of war has been resumed by Dr. McClean with a view to preparing sufficient quantities of suitable virus for field trials and for clinical use in vaccination. The differences in the lesions produced on the chorio-allantoic membrane by strains of virus derived from sheep and those derived from the calf described in 1940 are the subject of further investigation and endeavour is being made to determine which strain is the more suitable for Jennerian prophylaxis. Observations are being made also to determine the best method of preserving stocks of cultivated virus and a convenient form in which it can be issued for clinical use.

ENDOCRINOLOGY.

Dr. Korenchevsky, assisted by Miss K. Hall and with the statistical co-operation of Mr. J. L. Nicholson (University of Oxford) has continued his studies on the effects of different combinations of hormones and vitamins on adult and old rats.

Effect of Sex Hormones on Blood of Rats. In senile rats mild hypochromia was observed. After treatment with androgens, the red cells, haemoglobin content and volume of packed cells were increased in normal senile and castrated adult male rats, the lowered colour index of the senile male rats being raised. Although small doses of oestrogens when injected alone had no effect, when given simultaneously with androgens they decreased their effect. When progesterone and oestrogens were injected simultaneously into female rats, the blood values were decreased.

Co-operative Hypertrophying activity of Sex, Thyroid and Adrenal Hormones on some Organs of Adult Male Rats. Although desoxycorticosterone acetate and thyroid hormones, when injected alone in small doses, had little or no effect on the liver, heart and kidneys, they produced considerable hypertrophy when injected simultaneously. The same small dose of desoxycorticosterone acetate definitely increased the hypertrophying effect produced by androgens.

Co-operative Hypertrophying activity of Thyroid and Female Sex Hormones on some Organs of Adult Female Rats. Combined administration of oestrogenic and thyroid hormones was followed by considerable hypertrophy of adrenals, liver and kidneys although when injected alone they had little or no effect. Combined with progesterone (inactive alone) much greater hypertrophy of liver and kidneys and also hypertrophy of spleen were obtained.

Peculiar Progestation-like Changes Produced by Oestrogens and Vitamin C. Progesterone and oestradiol combined produce progestational changes in the uterus and vagina similar to those of pregnancy. If oestradiol benzoate butyrate was combined with ascorbic acid, the development of folds of the uterine mucosa was similar to that in pregnancy, but the uterine and vaginal epithelium was the same as with oestrogens alone.

Thyroid Hormone and Vitamins A, B and C. Vitamins A, B or C administered alone produced only slight changes which were not in most cases statistically significant: increased number of large cells and nuclei in liver, and of large nuclei in kidneys.

Striking and typical changes were produced by thyroid hormone when administered in non-toxic doses: very pronounced hyperplasia (but not hypertrophy) of hepatic cells, hypertrophy of kidney tubules, hypertrophy of nuclei in liver and kidneys, basophilic staining of cytoplasm and disappearance of basophil granules in liver cells. The simultaneous administration of vitamins with thyroid hormone did not significantly alter the effect of thyroid hormone. The increased nucleocytoplasmic ratio and numerous mitoses indicated a reversal of the cells of adult or senile rats towards the state found in young animals.

Large doses of thyroid hormone, however, produced a pronounced atrophy of cells with only slight hypertrophy of nuclei in the liver, and in most cases some increase in size of convoluted tubules and their nuclei in the kidneys. A beneficial effect of vitamin B on these effects of thyroid was shown by a greatly increased number of mitoses, i.e., regenerative hyperplasia of the cells.

Effect of Pregnene-inonol (anhydro-oxy-progesterone). The effects of this hormone on various non-sex organs, including heart, were similar to, but weaker than, those of the testosterone group.

NUTRITIONAL STUDIES.

Vitamins in Foods and Diets. Accurate information of the human requirement for various vitamins is of great importance at the present time and the Ministry of Food was anxious to ascertain more accurately the human requirement for vitamin A as derived from preformed Vitamin A and from the provitamin carotene. This could only be done by a human experiment which has been carried out on conscientious objectors at the Sorby Institute at Sheffield, by a team of workers organised by Miss Hume, and financed by the Medical Research Council.

Since July 1942 the volunteers have received a diet made as deficient as possible in vitamin A and carotene; a small number have served as positive controls receiving supplements of carotene or vitamin A. Observation was specially directed to measurement of capacity for dark adaptation and of plasma values for vitamin A and carotene. At the end of a year none showed any definite signs of deterioration of the visual threshold. After 14 months one subject began to show definite deterioration of the visual threshold, slight skin symptoms and a very low value for plasma vitamin A, all of which signs progressed rapidly till he was given a small daily dose of vitamin A when the symptoms slowly regressed. After about 15 months the same signs became definite in a second subject. The experiment is still in progress. It has indicated how large are the vitamin A reserves in the liver of the healthy human adult and that a daily dose of vitamin A smaller than that usually recognised as the daily requirement is sufficient to stop depletion and restore the organism to health, though whether to perfect health is still uncertain.

Miss Copping, assisted by Mrs. J. M. Witt and later by Miss Vanda Pond, has continued her biological estimations of the vitamin B₁, riboflavin and vitamin B₆ (pyridoxine) values of the total diets consumed by R.A.F. and W.A.A.F. personnel. This work has been carried out in collaboration with Dr. Macrae. Miss Copping has also studied the practical application to food testing of the method for estimation of pantothenic acid worked out by Bacon and Jenkins.

Vitamin A Potency of Potatoes. The resistance to depletion of vitamin A in the Sheffield experiment suggested that some important foodstuff in their diet, though generally considered to have a negligible content of carotene, might yet be making a significant contribution.

Miss Hume and Miss Henderson Smith therefore examined in rats the effect of a diet largely made up of potatoes and containing no other source of vitamin A potency. Rats receiving a synthetic diet devoid of vitamin A were completely depleted of their reserves after about 70 days, while those of the same litter receiving the potato diet showed scarcely any sign of deficiency after 150 days.

Researches on the Potato: Nutritive Value of its Nitrogenous Substances. Dr. H. Chick, with Miss Cutting and later with Mr. E. B. Slack, has continued the experiments with young growing rats on the nutritive value of the nitrogen—both protein and non-protein—in the potato tuber. Weight for weight, this nitrogen was found to have a biological value at least equal to that of the proteins of whole wheat, although only about one half is in the form of protein. Diets have been compared therefore which contain optimum amounts of all other essential nutrients but a constant sub-optimal amount of nitrogen derived respectively from (a) the whole potato, (b) its separated protein, (c) the expressed juice which contains a mixture of nitrogenous substances resembling those of (a) but with a lower proportion of protein, and (d) protein-free juice. In the last case no increase of weight occurred even when the nitrogen in the diet was doubled. Nevertheless, the total nitrogen of the potato, of which about half was non-protein, was found to have at least as high a nutritive value for growth as that of an equivalent amount given as potato protein.

The only explanation is that a supplementary action occurs between the protein- and non-protein nitrogenous substances in the tuber, probably attributable to the amino acids present among the latter, though it is possible that the amides and basic nitrogenous compounds also take part.

The nutritive value of proteins from other foods was also found to be enhanced by addition of protein-free potato juice. This was demonstrated with diets containing their nitrogen in the form of casein or wheat gluten. Whether this property of supplementing protein is peculiar to the non-protein nitrogenous substances of the potato or is common to those of other tubers and root vegetables, is unknown, but it is an important nutritional point and it is hoped to continue investigations along this line.

Nutritional Surveys. From the Autumn of 1942 to December, 1943 Dr. Dagmar Wilson, supported with a grant for expenses from the Institute, examined the nutritional state of women employed in Oxford factories and of local housewives of similar age and social position. On the whole the nutritional state had deteriorated very slightly over this period but more so among workers than among housewives. No clinical signs of specific nutritional defects have been seen except that, among factory workers, 25% showed enlargement of the thyroid and 36% signs of dental fluorosis. The incidence of these conditions is high in the villages of N. Oxfordshire and at Hooknorton, where it is unusually high, therapeutic administration of iodine has been adopted.

Dr. Wilson has also carried out surveys in goitrous regions in Somerset and Devon to ascertain how in general occurrence of fluorosis accompanies that of goitre. Waters from goitrous and non-goitrous districts have been examined for content of fluorine, calcium and iodine, with the co-operation, respectively, of Dr. Margaret Murray, Miss E. M. Widdowson and Miss B. Simpson of the Rowett Institute. The work of Drs. Wilson and Murray on the incidence of endemic goitre and fluorosis in this country led to the formation

in 1943 of a Goitre Sub-Committee of the Medical Research Council. The incidence of endemic goitre, its causes and possible means for its prevention and cure, has also been adopted as a subject for study by the recently formed Institute of Social Medicine at Oxford, the hospitality of which and the support and co-operation of Professor J. A. Ryle, Dr. Wilson has enjoyed during the progress of her work.

Transformation products of vitamin C in vivo and in vitro. Utilising their method for the determination of diketo-*l*-gulonic acid Dr. S. S. Zilva and Mr. J. R. Penney have studied the conversion of dehydro-*l*-ascorbic acid into diketo-*l*-gulonic acid, two primary transformation products of *l*-ascorbic acid, *in vitro* and *in vivo*. In the latter case the conversion was studied by examining the tissues and the excretion products of the guinea pig after oral and parenteral administration. The chief deduction was that, if in the metabolism of the animal organism the vitamin was a link in a co-ordinated chain of reactions in which it was alternately oxidised to dehydroascorbic acid and reduced to its original form, some of the reversibly oxidised ascorbic acid would in all probability be irreversibly converted to the inactive diketogulonic acid. Since according to this hypothesis the quantities of dehydroascorbic acid formed can at any time be only minute, the rate of the ultimate disappearance of the ascorbic acid involved in this reaction would therefore be extremely slow. This fact would explain the long delay in the onset of scurvy in man and animals, not capable of synthesising the vitamin and not receiving it in their diet, after their tissues have been depleted of ascorbic acid.

Lugg's method for the determination of vitamin C. Dr. Zilva and Dr. G. A. Snow have carried out a critical examination of this method. The kinetics of the combination of ascorbic acid, and other indophenol reducing substances with formaldehyde, on which the procedure is based, and the structure of the ascorbic acid-formalin complex have been studied. The limitations of the method and the conditions under which the utmost accuracy could be obtained have been defined. Although this method is more accurate than direct titration with indophenol, it was not found to be entirely specific for *l*-ascorbic acid.

Synthesis of vitamin C in stored apples. The capacity for synthesis of vitamin C by young apples on storage has been established by Dr. C. West and Dr. Zilva. The interest of this observation lies in the fact that, as in the case of the diminution of the ratio of dehydroascorbic acid to ascorbic acid, the capacity for synthesis diminishes with the development of the fruit.

Miscellaneous researches. In collaboration with East Malling Research Station and the John Innes Horticultural Institute, work on the influence of vitamin C on the physiological processes of the tomato and strawberry has been in progress.

The investigation of practical problems for the Fighting Services has been continued by Dr. Zilva and his co-workers and, as in previous years, the results have been communicated to the respective Services through the Medical Research Council.

FLUORIMETRIC ASSAY.

Dr. Ellinger and Miss Holden have completed their investigations on the physical conditions governing the measurement of the intensity of fluorescent light. The use of the fluorimeter as a photo-electric potentiometric absorptiometer by these workers was finally achieved by the use of more selective light filters and potentiometers of lower resistance. It has been shown that with the aid of fluorimetric concentration curves it is possible to detect and measure, with increasing concentrations, the formation of non-fluorescent dimers or polymers of fluorescent pigments. The quenching effect of a number of electrolytes and organic compounds and of changes of pH on the fluorescent intensity of thiochrome, riboflavin and nicotinamide-metho-chloride was determined.

NICOTINAMIDE-METHO-CHLORIDE IN URINE FOLLOWING THE INGESTION OF NICOTINAMIDE.

Dr. Ellinger and Mr. Coulson have shown that these pigments are eliminated in the urine as a non-fluorescent precursor, the fluorescence being developed *in vitro* by alkalisation and transfer to isobutanol. The precursor was identified by mixed melting points and absorption spectrum as nicotinamide-metho-chloride. The non-fluorescent nicotinamide-metho-chloride gives rise to a mixture of two fluorescent pigments when treated with alkaline isobutanol. These pigments are most probably pseudo bases of nicotinamide-metho-hydroxide with p- and o- quinoid structure. This knowledge has enabled Dr. Ellinger, Mr. Coulson and Miss Holden to devise a method for the estimation of nicotinamide-metho-chloride in urine in amounts from 0.5 μ g./ml. upward with an error of 5 or 10%. By means of this method, Dr. Ellinger and Mr. Coulson have shown that the height of the nicotinamide-metho-chloride output was dependent on the intake and utilisation of nicotinamide and related compounds, the presence of methyl-donators and the efficiency of the methylating mechanism. The average daily urinary output of the nicotinamide-metho-chloride was found to be between 2 and 8mg. which represents 10-15% of ingested nicotinamide. The results obtained indicate the presence of an extra-dietary source of nicotinamide in man.

On the initiation of the Air Ministry an investigation was made by Dr. Ellinger and Mr. Coulson, in collaboration with Flight Lieutenant G. A. Smart, R.A.F.V.R., into the relationship of nicotinamide deficiency

and acute and chronic ulcero-membrano-gingivo-stomatitis. For this purpose the urinary elimination of nicotinamide-metho-chloride was determined in numerous cases of acute and chronic stomatitis and of healthy controls, before and after the ingestion of nicotinamide. The results indicated that in this country there is no correlation between gingivo-stomatitis and nicotinamide deficiency. Acute and chronic gingivitis may be slightly more frequent among populations with a low nicotinamide-metho-chloride elimination but the connection need not be ætiological.

NATIONAL COLLECTION OF TYPE CULTURES. (*Medical Research Council*).

The National Collection of Type Cultures continues to be housed at Elstree where more extensive accommodation has now been provided.

During the year under review over 4,000 cultures were distributed and some 200 strains were deposited for maintenance. In the abnormal circumstances prevailing the increase in the demand for cultures may be considered satisfactory.

In conclusion the Governing Body again desires to record its appreciation of the wholehearted service rendered by all members of the staff and also the assistants, in carrying out, often under difficult conditions, the work of the Institute.

H. H. DALE,

Chairman of the Governing Body.

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THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

LONDON, S.W. 1.

BALANCE SHEET AND ACCOUNTS. DECEMBER 31ST, 1944.

FINANCIAL REPORT.

The Accounts and Balance Sheet for the year ending December 31st, 1944 show balances to the credit of the various funds as follows: Capital Fund £679,404 2s. 0d., Contingency Reserve £45,000 0s. 0d., Sinking Fund £64,620 6s. 1d., Pension Fund £34,678 14s. 8d., Jenner Memorial Research Studentship Fund £9,548 14s. 7d. and the Bacot Bequest Fund £667 7s. 3d.

Changes in investments during the year have been:—

GENERAL FUND:—£17,000 5 per cent. Conversion Stock, 1944/64 repaid.

£49,000 3 per cent. Savings Bonds and £22,000 2½ per cent. National War Bonds, purchased.

SINKING (BUILDINGS DEPRECIATION) FUND:—£1,900 3 per cent. Savings Bonds, purchased.

PENSION FUND:—£1,000 3 per cent. Savings Bonds, purchased.

Income for the year amounted to £136,393 4s. 10d. In addition £1,002 19s. 5d. was received from profit on investments. Compared with 1943, Sales of Sera, Vaccines, etc., showed an increase of £45,664 7s. 7d.

Expenditure amounted to £69,260 0s. 7d. against £73,668 7s. 1d. in 1943, the decrease being mainly due to there having been no major purchases of new apparatus or installations and a reduction in Serum, Vaccine and Vaccine Lymph expenses.

The stock of sera on hand at January 1st. was largely drawn upon to meet the heavy demands during the year. The stock on hand at December 31st. was valued at £6,025 4s. 5d. Horses on hand at that date were valued at £3,920. Stocks of sera and horses are not included in the accounts.

Of the balance of income over expenditure on the year's working, viz., £67,133 4s. 3d., £20,000 has been allocated to the Contingency Reserve and a like amount to the Sinking (Buildings Depreciation) Fund. The remainder, £27,133 4s. 3d., together with £1,002 19s. 5d. profit on investments, has been placed in the Capital Fund.

BALANCE SHEET

£ s. d.

£ s. d.

CAPITAL FUND to 31st December 1944 :—

Donations, &c., received to date from the following :—

Dr. Ludwig Mond (1893)	..	..	..	..	2,000	0	0
The Berridge Trustees (1893/98)	..	..	..	..	46,379	10	1
The Grocers' Company (1894)	..	..	..	..	10,000	0	0
Lord Iveagh (1900)	..	..	..	..	250,000	0	0
Lord Lister's Bequest (1913/23)	..	..	..	..	18,904	5	8
William Henry Clarke Bequest (1923/6)	..	..	..	..	7,114	5	7
Rockefeller Foundation (1935/6)	..	..	..	..	3,400	0	0
The James Henry Stephens Bequest (per Lloyd's Bank Limited) (1938)					500	0	0
Dr. G. A. Davies Bequest (1938)	..	..	..	..	125	0	0
Other Donations and Legacies (1891-1934)	..	..	..	..	20,971	18	3

General Fund Income and Expenditure Account :—

As per Account at 31st December, 1943..	..	291,872	18	9			
Add Profit on Investments, 1944	..	1,002	19	5			
Add Balance for the year ending 31st December, 1944		27,138	4	3			
					320,009	2	5

679,404 2 0

CONTINGENCY RESERVE :—

As per Account at 31st December 1941	..	..	..	25,000	0	0
Add Transfer from Income and Expenditure Account, 1944	..			20,000	0	0
					45,000	0 0

SINKING FUND to 31st December 1944	..	..	..	44,620	6	1
Add Transfer from Income and Expenditure Account, 1944	..			20,000	0	0
					64,620	6 1

PENSION FUND to 31st December, 1943	..	..	..	33,621	1	4
Add Balance transferred from Pension Fund Income and Expenditure Account, 1944	..	..	..	1,057	13	4
					34,678	14 8

JENNER MEMORIAL RESEARCH STUDENTSHIP FUND :—

As per Account at 31st December 1943	..	..	..	9,500	18	7
Add Balance transferred from Jenner Memorial Research Studentship Fund Income and Expenditure Account, 1944	..	..	..	47	16	0
					9,548	14 7

BACOT BEQUEST FUND :—

As per Account at 31st December, 1943	..	..	..	646	7	3
Add Balance transferred from Bacot Bequest Fund Income and Expenditure Account, 1944	..	..	..	21	0	0
					667	7 3

CREDITORS	..	..	..	..	..	7,108 10 2

H. H. DALE, *Chairman of Governing Body.*

JOHN ANDERSON, *Hon. Treasurer.*

£841,027 14 9

REPORT OF THE AUDITORS

We have audited the above Balance Sheet. We have obtained all the information and explanations we have required. are held by the Institute on their behalf. In our opinion, such Balance Sheet is full and fair, and properly drawn and the explanations given to us and as shown by the books of the Institute.
London, 1st June. 1945.

	£	s.	d.	£	s.	d.
EXPENDITURE ON INSTITUTE BUILDINGS AT CHELSEA:—						
As per account 31st December 1935, including purchase of freehold site, £6,000						73,548 3 1
FREEHOLD LAND ADJOINING THE "STUDIOS" CHELSEA at cost (1912)						169 6 8
LEASE OF THE "STUDIOS" CHELSEA, as per last account	611	10	9			
Less Amount written off for the year	65	2	0			
						546 8 9
QUEENSDERRY LODGE ESTATE, ELSTREE—						
Freehold land and buildings as per account 31st December 1912						20,455 10 0
FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS:—						
*At cost less depreciation as per account 31st December 1920	2,471	17	2			
Cost of Ultracentrifuges, purchased in 1935, less amounts written off	680	0	0			
						3,151 17 2
GENERAL FUND INVESTMENTS (at cost, less amounts written off):—						
£80,000 4 per cent. Consolidated Stock, 1957 or after	74,272	16	0			
£43,000 3½ per cent. Conversion Stock, 1961, or after	42,918	6	0			
£12,500 2 per cent. Conversion Stock, 1943-45	12,500	0	0			
£52,000 4 per cent. Funding Stock, 1960-90	45,661	13	9			
£64,000 3½ per cent. War Stock	63,407	13	5			
£37,000 Local Loans 3 per cent. Stock	20,829	1	7			
£86,000 2½ per cent. National War Bonds	86,000	0	0			
£113,000 3 per cent. Savings Bonds	113,122	12	6			
£3,000 Port of London 3½ per cent. Registered Stock, 1965-75	2,686	17	7			
£8,000 New South Wales 4 per cent. Inscribed Stock, 1942-62	8,040	1	4			
£23,000 New Zealand Government 3 per cent. Inscribed Stock, 1945	22,114	0	0			
£26,100 South Australian Government 3 per cent. Consolidated Stock, 1916 or after	16,800	0	0			
£2,900 Commonwealth of Australia 3½ per cent. Registered Stock, 1950-52	2,723	16	0			
£25,000 Victorian Government 3 per cent. Consolidated Inscribed Stock, 1929-49	19,800	0	0			
£4,000 Western Australia Government 4 per cent. Inscribed Stock, 1912-52	4,081	3	0			
£20,000 Southern Railway Preferred Ordinary Stock	13,500	0	0			
£6,200 London & North Eastern Railway 3 per cent. Debenture Stock	3,961	0	0			
£5,000 Great Central and Midland Railway Joint Committee 3½ per cent. Guaranteed Stock	3,623	0	0			
£353 London & North Eastern Railway 4 per cent. First Guaranteed Stock	499	11	0			
£3,650 London, Midland & Scottish Railway 4 per cent. Preference Stock	7,060	0	0			
£15,625 London, Midland & Scottish Railway 4 per cent. Preference Stock, 1923	11,300	0	0			
£18,750 London & North Eastern Railway 4 per cent. First Preference Stock	13,028	6	7			
£300 Ontario and Quebec Railway 5 per cent. Permanent Debenture Stock	984	0	0			
£3,400 Gas Light and Coke Company Ordinary Stock	3,638	0	0			
						593,451 17 9
SINKING FUND INVESTMENTS (at cost):—						
£9,600 2 per cent. Conversion Stock, 1943-45	9,600	0	0			
£10,200 4 per cent. Funding Stock, 1960-90	9,079	0	1			
£20,500 3½ per cent. Conversion Stock, 1961 or after	18,658	5	8			
£7,200 3 per cent. Savings Bonds	7,227	7	2			
						44,564 12 11
PENSION FUND INVESTMENTS (at cost):—						
£22,000 4 per cent. Funding Stock, 1960-90	17,165	3	5			
£18,000 3½ per cent. Conversion Stock, 1961 or after	15,173	7	5			
£2,200 3 per cent. Savings Bonds	2,204	16	9			
Balance uninvested	135	7	1			
						34,678 14 8
JENNER MEMORIAL RESEARCH STUDENTSHIP FUND INVESTMENTS (at cost):—						
£2,650 Southwark and Vauxhall Water Co. 3 per cent. Debenture Stock "B"	2,756	10	0			
£1,596 Southern Railway 5 per cent. Preference Stock	2,740	5	0			
£1,300 Liverpool Corporation 3 per cent. Stock, 1912, or after	1,097	6	9			
£2,800 4 per cent. Funding Stock, 1960-90	2,705	6	0			
Balance uninvested	249	6	10			
						9,548 14 7
BACOT REQUEST FUND INVESTMENT (at cost):—						
£600 3½ per cent. Conversion Stock, 1961 or after	595	10	0			
Balance uninvested	71	17	3			
						667 7 3
(The book value of the above Investments is, in the aggregate, less than their market value at 31st December 1944.)						
STOCK OF ANIMALS				(No value assigned)		
STOCK OF ANTITOXINS				(No value assigned)		
DEBTORS AND PAYMENTS IN ADVANCE						39,435 8 0
CASH:—						
At Bankers: On Deposit	1,000	0	0			
Current Accounts	19,686	0	2			
In hand	123	13	9			
						20,809 13 11
*Nothing has been charged for depreciation of Furniture, &c. since 1920 as new purchases made during each year to a greater amount than the estimated depreciation (10%) have been written off						
						£841,027 14 9

TO THE MEMBERS.

In accordance with the provisions of the Superannuation Scheme for certain members of the Staff, the relative Life Policies up so as to exhibit a true and correct view of the state of the Institute's affairs, according to the best of our information

COOPER BROTHERS & CO.,
Chartered Accountants.

Auditors.

INCOME AND EXPENDITURE ACCOUNTS

INCOME.

General

									£	s.	d.
Dividends on General Fund Investments	...	...	...	...	...	...	...	...	20,779	6	7
Dividends on Sinking Fund Investments	...	...	...	...	...	...	...	...	1,460	18	0
Sales of Sera, Vaccines, &c.,	...	...	...	...	...	...	...	...	113,898	2	3
Rent	...	...	...	...	...	...	...	...	651	15	0
Donations	...	...	...	...	...	...	...	...	108	3	0

£136,893 4 10

Pension

									£	s.	d.
Dividends on Investments	...	...	...	...	...	...	...	...	1,535	11	10

£1,535 11 10

Jenner Memorial Research

									£	s.	d.
Dividends on Investments	...	...	...	...	...	...	...	...	310	6	0

£310 6 0

Bacot

									£	s.	d.
Dividends on Investment	...	...	...	...	...	...	...	...	21	0	0

£21 0 0

Cancer Research

									£	s.	d.
Balance of Legacy from John George Mills (1987)...	...	...	...	...	...	...	...	...	671	19	1

£671 19 1

for the year ending 31st. December, 1944.

EXPENDITURE.

Fund.

	£	s.	d.
Rent, Rates, Taxes and Insurance	2,390	19	7
Salaries and Wages of Staff	31,735	1	7
Premiums on Federated Superannuation Policies	1,631	10	2
Stationery, Printing and Postage	290	19	4
Printing of Collected Papers	57	14	8
Office Expenses, Auditors' Fee, and Donations	372	5	9
Travelling Expenses	214	5	10
Gas, Water, Fuel and Electricity	2,166	19	5
Nutrition and Protozoological Expenses	700	11	7
Bacteriological Expenses	92	3	10
Biochemical Expenses	294	4	7
Bio-physics Expenses and Apparatus	1,167	2	8
Serum, Vaccine and Vaccine Lymph Expenses	8,808	8	1
Animals	6,685	4	6
Animal House Expenses and Forage	9,448	17	10
Alterations, Repairs, Renewals, and Workshop Expenses	987	5	7
General Apparatus, New Installations and Furniture	63	12	2
Library Expenses	151	17	2
General Stores	204	19	6
Amounts written off Lease of the "Studios," Chelsea and Ultracentrifuges	405	2	0
Sinking Fund (¼% per annum on Cost of Buildings and Dividends on Investments)	1,984	14	9
Transfer to Contingency Reserve	20,000	0	0
Transfer to Sinking Fund	20,000	0	0
	40,000	0	0
Balance, transferred to Capital Fund	27,133	4	3
	<u>£136,393</u>	<u>4</u>	<u>10</u>

Fund.

	£	s.	d.
Pensions	477	18	6
Balance, transferred to Balance Sheet	1,057	13	4
	<u>£1,535</u>	<u>11</u>	<u>10</u>

Studentship Fund.

	£	s.	d.
Salary of Dr. Rogers	262	10	0
Balance, transferred to Balance Sheet	47	16	0
	<u>£310</u>	<u>6</u>	<u>0</u>

Bequest Fund.

	£	s.	d.
Balance transferred to Balance Sheet	21	0	0
	<u>£21</u>	<u>0</u>	<u>0</u>

Account.

	£	s.	d.
Balance unexpended	671	19	1
	<u>£671</u>	<u>19</u>	<u>1</u>

THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE.

Report of the Governing Body,
1945.

CHELSEA BRIDGE ROAD,
LONDON, S.W. 1.

June 18th. 1945.

The Lister Institute of Preventive Medicine,

CHELSEA BRIDGE ROAD, LONDON, S.W. 1

AND ELSTREE, HERTS.

THE GOVERNING BODY.

SIR HENRY H. DALE, O.M., G.B.E., M.D., F.R.C.P., P.R.S., *Chairman.*

SIR JOHN ANDERSON, P.C., G.C.B., G.C.S.I., G.C.I.E., M.A., B.Sc.,
LL.D., M.P., *Hon. Treasurer.*

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PROFESSOR H. R. DEAN, M.D., F.R.C.P., LL.D.

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LORD HORDER, G.C.V.O., M.D., B.Sc., F.R.C.P.

THE EARL OF IVEAGH, C.B., C.M.G.

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(Vacancy)	" "
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THE STAFF.

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*ALAN N. DRURY, C.B.E., M.A., M.D., F.R.S.

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*G. H. EAGLES, M.D., D.P.H. (*Major, R.A.M.C.*)
 A. FELIX, D.Sc., F.R.S. (*Seconded to Emergency Public Health Laboratory Service*).
 D. W. HENDERSON, D.Sc., Ph.D. (*Seconded to Ministry of Supply*).
 MURIEL ROBERTSON, M.A., D.Sc. (*Protozoology*).
 EMMY KLIENEBERGER-NOBEL, Ph.D., D.Sc.

EXPERIMENTAL PATHOLOGY.

*A. N. DRURY, C.B.E., M.A., M.D., F.R.S.
 SHIRLEY J. L. SPOONER, B.Sc. (*Research Assistant*).

NUTRITION.

*HARRIETTE CHICK, C.B.E., D.Sc. (*Honorary*).
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 E. MARGARET HUME, M.A. (*Honorary*). (*Medical Research Council External Scientific Staff*).
 ALICE M. COPPING, M.Sc.
 VANDA R. G. POND, B.Sc. (*Research Assistant*).
 E. B. SLACK, B.A. (*Research Assistant*).

HANNAH HENDERSON SMITH.
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 J. R. PENNEY, B.Sc., Ph.D., A.R.I.C.
 H. A. PAINTER, B.Sc., A.R.I.C.

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*W. T. J. MORGAN, D.Sc., Ph.D., F.R.I.C., (*Reader in Biochemistry in the University of London. Principal Biochemist, Elstree*).
 MARJORIE G. MACFARLANE, B.Sc., Ph.D.

R. L. M. SYNGE, B.A., Ph.D.
 MARION R. B. WADDELL, B.Sc. (*Research Assistant*).
 P. ELLINGER, DR. PHIL. AND MED. (*Grantee*).
 H. LAURELL, M.D. (*Sweden*). (*British Council Student*).

BIOPHYSICS.

*R. A. KERWICK, D.Sc.
 E. F. MCCARTHY, M.B., B. Ch., M.Sc.
 B. R. RECORD, Ph.D. (*Medical Research Council Grantee*).

C. J. B. BRADISH, B.Sc. (*Research Student*).
 B. CINADER, B.Sc. (*Research Assistant*).

PREPARATION AND STUDY OF THERAPEUTIC SERA and BACTERIAL VACCINES.

C. R. AMES, M.D., B.S.
 *H. L. SCHÜTZER, M.D., B.S.

J. KEPPIE, Ph.D., M.R.C.V.S.
 M. ZORRIASSATEIN, M.D. (*Persia*) (*British Council Student*).

PREPARATION AND STUDY OF VACCINE LYMPH.

D. McCLEAN, M.B., B.S., M.R.C.S.
 H. J. ROGERS, B.Sc. Ph.D. (*Beit Memorial Research Fellow*).

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Elstree Secretary and Estate Manager	F. K. FOX.
Assistant Secretary and Accountant	S. A. WHITE, A.L.A.A. (<i>Royal Corps of Signals</i>).
Librarian	PATRICIA J. DOWNMAN.

NATIONAL COLLECTION OF TYPE CULTURES.

(*Medical Research Council*.)

R. ST. JOHN-BROOKS, M.A., M.D., D.P.H. (*Curator*). MABEL RHODES (*Assistant Curator*). ROSAMUND BARNES, B.Sc.

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*Recognised Teacher of the University of London

ANNUAL GENERAL MEETING
OF
The Lister Institute of Preventive Medicine,
June 18th. 1945.

REPORT OF THE GOVERNING BODY.

The Governing Body has the honour to present its report on the work of the Institute for the year 1944/45.

GOVERNING BODY.

The Governing Body have noted with pleasure that their Chairman, Sir Henry Dale, has received the Order of Merit from His Majesty the King.

The Council, at a meeting held on June 18th last, re-elected Professor H. R. Dean, Dr. P. Fildes and Sir Henry Dale as its three representatives on the Governing Body until December 31st, 1945.

It is with great regret that the Governing Body report the loss of a valued colleague by the tragic death of Lord Moyne in Cairo on November 6th, 1944. At the time of his death Lord Moyne was the senior member of the Board and had acted as Chairman from 1941 to 1942. An appreciation of his interest and services to the Institute has been recorded in the Minutes of the Governing Body by the following resolution:

"That the members of the Governing Body learn with very deep regret of the tragic death of their colleague, Lord Moyne. As the son and successor on the Governing Body of the Institute's great benefactor, the first Earl of Iveagh, Lord Moyne had continued the association of his family with the Institute, maintaining a keen interest and full participation in the conduct of its affairs and giving wise and valuable counsel during the past twenty-five years."

The rights of nomination to seats upon the Governing Body passed on Lord Moyne's death to his brother, the Rt. Hon. The Earl of Iveagh. Lord Iveagh's decision to maintain the connexion of his family with the affairs of the Institute by joining the Board himself has been heartily welcomed by the other members of the Governing Body.

The death on November 22nd, 1944 of Sir Joseph Arkwright who retired from the Governing Body on December 31st, 1943 and an appreciation of whose services to the Institute was recorded in last year's report is also regretfully announced.

COUNCIL.

At the Annual General Meeting last year, the three retiring members, Sir Wilson Jameson, Sir John Ledingham and Professor H. S. Raper, each a representative of the Members, were re-elected, and the vacancy created by the death of Professor W. W. C. Topley was filled by the appointment of Sir Joseph Arkwright.

The three members of Council, due to retire this year in accordance with the Articles of Association, but who are eligible for re-election are, Dr. A. N. Drury, Sir Edward Mellanby and Dr. Harriette Chick, each representing the Members of the Institute.

It is with regret that the deaths of Sir John Ledingham, the late Director of the Institute, who had represented the Members since 1931, and of Sir Humphry Rolleston, the representative of the British Medical Association also since 1931, are announced. An appreciation of Sir John Ledingham's services and devotion to the work of the Institute was recorded by the Governing Body in its report for 1943.

MEMBERS.

The Governing Body noted with pleasure that four Members of the Institute, Dr. W. A. Daley, Professor A. Fleming, Professor H. W. Florey and Dr. P. Hartley were recipients of the honour of Knighthood in the King's Birthday Honours of 1944. It offers its congratulations on these well-merited honours.

The deaths of three Members have occurred during the year, *viz.*, Sir Thomas Barlow, a member since 1917, Dr. A. B. Green, who had been Bacteriologist-in-charge of the Institute's Vaccine Lymph department from 1905 till 1935 when he retired, and of Sir Thomas Lewis, a member since 1931.

STAFF.

The Governing Body takes pleasure in announcing the award of the C.B.E. to Dr. Drury and of the O.B.E. to Dr. Macrae in recognition of their services to the Government.

Dr. Harriette Chick, whose appointment had been extended beyond the normal period as her activities were closely related to nutritional problems arising out of the war, retired on pension on January 5th last, but is continuing her control of the nutritional work at Cambridge in an honorary capacity in the meantime. Dr. Chick was first appointed to a Jenner Memorial Research Studentship of the Institute in 1905 and became a permanent member of the staff of the Experimental Pathology Department under Sir Charles Martin in 1907. Her activities during her service with the Institute have covered a wide field, amongst which may be mentioned her researches on disinfection, coagulation of proteins and nutrition. At the conclusion of the last war she went to Vienna, where with her co-workers she investigated the ætiology of rickets, in collaboration with the late Professor von Pirquet, at the University Kinderklinik.

Dr. V. Korenchevsky, who had worked at the Institute from 1920 with a grant from the Medical Research Council, and from 1934 with a grant furnished jointly by the Institute and the Council, retired on June 30th, 1944. He is now Head of the Gerontological Research Unit which has been established at Oxford University as a result of a generous donation by Lord Nuffield. Towards the equipment of this Unit, the Institute supplied on generous terms most of the scientific apparatus previously used by Dr. Korenchevsky.

Dr. M. M. Barratt and Dr. D. B. Steabben, members of the Bacteriological staff, resigned their posts for domestic reasons in May. Dr. Barratt's appointment had dated from 1916 and that of Dr. Steabben from 1928, prior to which she had worked as a Beit Memorial Research Fellow.

Miss K. Hall, who had assisted Dr. Korenchevsky since 1936 as a grantee of the Medical Research Council, left the Institute in August having received an appointment as Lecturer in Physiology in the University of Liverpool.

Dr. J. Keppie joined the Elstree staff as Veterinary Bacteriologist in August 1944 and Miss A. M. Copping received a part-time appointment to the Nutrition staff on April 1st, 1945. Miss W. B. R. Waddell, Miss S. J. L. Spooner and Mr. B. Cinader have received temporary research appointments at Chelsea.

Mr. H. M. Green, Foreman Carpenter, after nearly forty years' service with the Institute retired on pension in September.

Dr. A. Felix is still serving as a member of the Emergency Public Health Laboratory Service and enjoying the hospitality of the Wellcome Research Institution. Dr. D. W. Henderson remains seconded to the Ministry of Supply. Dr. Eagles and Mr. S. A. White are still on active service abroad.

The disposition of the remaining members of the staff is unchanged. As mentioned above Dr. Chick and her co-workers continue at Roebuck House, Chesterton, Cambridge, with the use of additional accommodation in the University Departments of Biochemistry and Anatomy and Dr. Muriel Robertson is still at the Institute of Animal Pathology, Cambridge.

The Governing Body once again desires to express its gratitude to Sir Charles Martin, Professor A. C. Chibnall, Professor E. S. Goodrich and Professor J. A. Gunn for their continued kind provision of research facilities to evacuated members of the staff. The main Library has now returned to Chelsea from Aldenham School, where through the kindness of the Governors and the Headmaster, Mr. G. A. Riding, it has been housed during the war.

Various members of the staff have devoted time to the different Committees set up by the Medical Research Council, of which body Dr. Drury is now a member. He has continued as Chairman of the Blood Transfusion Research Committee, the Committee on Hæmoglobin Surveys and on the Drying of Human Milk and as a member of the Industrial Health Research Board, the Shock Committee, the Committee on Jaundice and the Penicillin Therapeutic Trials Committee. Also he has maintained close contact with the Emergency Blood Supply Depots administered by the Medical Research Council on behalf of the Ministry of Health. In addition he is a member of the newly appointed Colonial Medical Research Committee and of the Colonial Advisory Medical Committee of the Colonial Office and of the Ministry of Supply's General Penicillin Committee.

Dr. Zilva represents the Institute on the Accessory Food Factors' Committee appointed jointly by the Institute and the Medical Research Council and is a member of its Vitamin A and Vitamin D sub-committees. Dr. H. Chick is still Secretary of the main Committee and temporarily, of its sub-committee on Opportunities for Nutritional Research in Post-war Europe. Miss E. M. Hume is Secretary of the Vitamin A sub-committee, and Dr. M. Robertson a member and Dr. M. G. Macfarlane Secretary, of the sub-committee on Anaerobic Wound Infections of the Council's War Wounds Committee.

Dr. Felix is a member of the Typhus Committee, appointed jointly by the Medical Research Council and the War Office.

The Medical Research Council, which is administering the London Blood Supply Depots on behalf of the Ministry of Health, is continuing a Unit at the Institute for research into, and filtration of, blood plasma and serum for transfusion. The work of this Unit is controlled by Dr. Margaret Mackay with the assistance of Miss E. M. Richardson.

The Governing Body, before surveying the scientific work carried out during the year, desires again to record its appreciation of the continued co-operation and collaboration the Institute has enjoyed with the Medical Research Council. It has furnished the salaries of members of its External Scientific staff working in the Institute, *viz.*, the staff of the National Collection of Type Cultures, Dr. Zilva and his assistants and Miss Hume, half the salaries of Dr. Korenchevsky and Miss H. H. Smith and given grants to Dr. Chick and Dr. Korenchevsky for assistance by Miss Copping and Miss Hall respectively. The Institute provided, as hitherto, accommodation and expenses for the researches of each of these workers.

BACTERIOLOGICAL, IMMUNOLOGICAL AND PATHOLOGICAL STUDIES.

Typing of typhoid, paratyphoid and food poisoning bacilli with the Vi bacteriophage. Dr. Felix, with the assistance of Miss B. R. Callow (Emergency Public Health Laboratory Service), has continued work on this problem, mainly with a view to the practical application of the method. During the past year one further Vi-phage type of the typhoid bacillus has been added to the list of new types or subtypes previously identified in the British Isles. On the other hand, the number of known Vi-types and subtypes of the paratyphoid B bacillus indigenous to Britain remains five. It is, however, becoming apparent that hitherto unknown phage-types of typhoid and paratyphoid B bacilli are being introduced from abroad.

Further progress has been made with the typing of *Bact. typhi-murium*, one of the most common causes of food poisoning in this and in many other countries. Nine different Vi-phage types and subtypes of this organism have been identified so far, and their epidemiological significance has been confirmed in all those outbreaks that have been investigated up to the present time. The practical value of the bacteriophage typing of *Bact. typhi-murium* is however not as great as that of the typing of typhoid and paratyphoid B bacilli. Freshly isolated cultures of *Bact. typhosum* and *Bact. paratyphosum B* nearly always contain the Vi antigen and are therefore typable by the Vi-phage technique. Strains of *Bact. typhi-murium*, on the other hand, are often found to be devoid of the Vi antigen and cannot be typed by the bacteriophage method. It is too early to state what the incidence of such Vi-negative strains is, but the available evidence indicates that a considerable proportion of strains of *Bact. typhi-murium* occurring in this country belongs to the untypable Vi-negative variety.

Bact. newport and *Bact. oranienburg*, two quite common salmonellas of Group C of the Kauffman-White scheme, have also yielded strictly specific bacteriophages, but the experiments designed to prove by serological tests that these are also genuine anti-Vi phages have not yet been carried out.

Control of Typhoid Fever Convalescents. Reference was made in previous reports to observations by Dr. Felix on the employment of the Vi-agglutination test as an aid to the differentiation between a chronic carrier and a temporary excreter. A decreasing Vi titre indicates temporary excretion, whereas a steady or rising Vi titre will arouse suspicion that the person has been or is likely to become a chronic carrier of the infection. The Emergency Public Health Laboratory Service, in co-operation with the Ministry of Health, the Department of Health for Scotland and the three fighting services, has now prepared a scheme for the routine application of the Vi test to future cases of typhoid fever. It is hoped by this means to keep a check, as far as possible, on those persons, whether in service or civilian life, who are likely to become persistent carriers of typhoid bacilli.

Dysentery Prophylaxis. Dr. H. L. Schütze and Dr. W. T. J. Morgan have continued the examination of diethylene-glycol extracts of *Bact. Shigae*. With certain strains it is possible to separate fractions whose antigen and specific-substance content varies considerably and not in parallel. Serological investigation also indicates a quantitative difference in the surface and somatic reactions of such fractions. Information thus obtained may influence the choice of antigen most suitable for immunizing purposes.

Gas Gangrene. Dr. Muriel Robertson has carried out further experiments in collaboration with Dr. J. Keppie, in testing the active immunising value of *Cl. welchii* and *Cl. oedematiens* toxoids in guinea-pigs against attacks by the living organisms. The toxoids used were those which were prepared for possible human application. The toxoid of *Cl. oedematiens* evoked a solid immunity against the infection. The toxoid of *Cl. welchii* however, failed to immunise guinea-pigs and seemed to be much less antigenic than the samples tested during the previous year.

Dr. M. G. Macfarlane has continued work on various aspects of gas gangrene. Chemical examination of muscle specimens from cases of the disease and from patients with other clinical conditions such as cellulitis or vascular gangrene showed that in the pathological specimens there had been a loss of diffusible constituents, such as creatine, phosphate, adenylic compounds and potassium, similar to that seen in cases of crush syndrome; the gas gangrene specimens in particular showed an early and marked loss of contractility of the muscle clinically. The specimens from the gas gangrene cases tended also to have a decreased phospholipin content, consistent with the action of *Cl. welchii* lecithinase and an increase in the ratio of fat to protein which suggested that there had been a preferential loss of protein from the muscle *in vivo*.

A statistical analysis of the case reports of gas gangrene among casualties in the Central Mediterranean Force showed that though the present method of treatment with surgery, antitoxin and drugs was in general very effective.

tive, the death rate was still high among cases in which excision of the affected muscle was limited, thus indicating the possibility that some toxic factor might be liberated from the necrotic muscle. Experimental work on the nature and biological effect of various fractions, particularly protein fractions, from normal muscle and from muscle subjected to the action of Clostridial toxins is now being carried out by Dr. Macfarlane. Preliminary results indicate that soluble proteins are liberated from the stroma of muscle by the action either of *Cl. welchii* or *Cl. oedematiens* toxin; the stroma appears to be a lipoprotein complex, and apart from any pathological significance in this reaction, *Cl. welchii* lecithinase may be a useful biochemical agent for the further investigation of lipoprotein complexes.

Dr. Amies and Dr. J. Keppie have continued research to improve the quality of the antigens used for immunising horses, with particular regard to the cultural conditions necessary for the production of high-grade toxins by the gas-gangrene group of Clostridia. In the case of *Cl. welchii*, an extensive trial was made of culture media prepared from papain digests of horse meat. This type of medium, however, was abandoned because it was found to favour the formation of β -toxin as well as α -toxin; a partial suppression of antibody formation for the α -toxin resulted when the two antigenic components were injected into horses. Culture filtrates with a uniformly high content of α -toxin and very little β -toxin are now being produced in a specially designed infusion peptone medium containing cooked meat. From these and other studies it appears that variation in the metabolic activities of the pathogenic Clostridia are of frequent occurrence irrespective of the type of medium in which they are grown. Until it become possible to cultivate these organisms in simple chemically defined media it seems unlikely that any fundamental advances in our knowledge of this group will be made.

Hyaluronidase Production. Dr. H. J. Rogers (Beit Memorial Research Fellow), has continued the biochemical and immunological studies of hyaluronidase production by bacteria and has devised buffered simplified casein hydrolysate media which will allow the production of high titres of enzyme by a variety of micro-organisms. Interesting results have been obtained on the comparative conditions controlling the production of bacterial hyaluronidase. The synthesis of enzyme by streptococci and *Cl. welchii* is directly proportional to the concentration of potassium hyaluronate present in the growth medium. Purified potassium hyaluronate was necessary to prove this and a method for purification of the polysaccharide has been devised. The product obtained contains about 95% of the theoretical quantity of glucosamine after acid hydrolysis and 97-100% theoretical N. Its viscosity is very little changed by the procedure employed. Staphylococci show no adaptation to the addition of hyaluronate to any of the basal media used. *Cl. septicum* produces only low titres of hyaluronidase when grown upon the acid hydrolysed casein medium suitable for the production of enzyme by streptococci and *Cl. welchii*. The addition of a diffusible substance present in Evans peptone to the simplified medium will increase the amount of enzyme produced by *Cl. septicum*. This organism also shows no adaption to the inclusion of potassium hyaluronate in either the buffered simplified medium or peptone media.

Further studies have been made upon the chromatographic purification of hyaluronidase using alumina and activated charcoal as absorbents.

Whooping Cough. Dr. Schütze has undertaken an antigenic analysis of *B. pertussis* in the hope that further light thrown on the immunological processes of this organism may result in the achievement of a vaccine superior to those now current.

Nuclear Structures in Bacteria. Dr. Klieneberger-Nobel is studying the group of Actinomycetes on similar lines to her previous work on spore-forming bacilli. By means of two new staining methods (a) for the demonstration of the nuclear structures in bacteria and (b) for the demonstration of septation, the various stages of the life cycle of these organisms are being examined. So far mainly the group of spore-forming Actinomycetes (Oerskov's and Erikson's group I) has been studied with the result that characteristic and regular transformations during development, and particularly when spores are being formed, were observed.

It is planned to extend the work and to examine type-species of the various groups. It is hoped that these morphological studies will gradually reveal the development of the organisms in question more closely than has so far been possible, and that some new means for a natural classification of them will emerge.

Cardiac Hypertrophy. Dr. A. N. Drury, assisted by Miss S. J. L. Spooner, has continued his observations on the cardiac hypertrophy in the rabbit produced by an arterio-venous anastomosis. Such an anastomosis leads to a considerable degree of hypertrophy, the weight of the heart in many instances being doubled. If the anastomosis is removed, when the hypertrophy has been established for about two months, the weight of the heart quickly returns to a normal value. This indicates that the hypertrophy produced by such an anastomosis is physiological in type. In certain animals, however, the hypertrophy does not disappear, suggesting that in certain circumstances the hypertrophy may be pathological. This matter is being investigated further.

Trichomonas Foetus. Further work has been carried out by Dr. Robertson on trichomoniasis in cattle in collaboration with Mr. W. R. Kerr of the Ministry of Agriculture Northern Ireland.

The study of the passively acquired antibody of very young calves has been carried further. The relation of circulating antibody to immunity against the disease in heifers is also being investigated and it has been shown that animals may have a high agglutination titre and a positive skin reaction as a sequel to vaccination and yet be

fully susceptible to infection; the disease even culminating in frank abortion. This is being further studied. The appearance of two strains which differ to some degree in their antigenic make up from the typical and identical strains of the Belfast and Cambridge outbreaks have been noted and are being studied.

VACCINIA VIRUS STUDIES.

Antigenicity of Virus inactivated with alcohol. Dr. D. McClean has found that the injection of large doses of vaccinia virus inactivated with 50 per cent. ethanol induces a slight degree of immunity in rabbits and provokes the appearance of neutralising antibody in their serum. Since the inactivated virus produces some antibody the poverty of the immunity reactions may be due to the small amount of antigen injected rather than its partial destruction by the ethanol. A total dose of 24 ml. of a concentrated suspension with a minimal infective dose of 0.2×10^{-7} ml. contains only 0.05 mg. of inactivated virus and it is not known how much of this is composed of the antigen responsible for the production of virus neutralising antibody.

Virus neutralising activity of serum from recently vaccinated persons. In collaboration with Dr. J. F. Loutit (South West London Blood Supply Depot), Dr. McClean has examined pooled samples of serum from recently vaccinated persons in order to determine the time after vaccination at which the highest titres of virus neutralising antibody can be obtained. These tests indicate that the most potent sera are likely to be obtained during the third and fourth weeks after vaccination but that no marked fall in potency occurs up to the end of the sixth week. The observations were undertaken as a preliminary to the collection of such serum and its storage in the dried state for therapeutic trial in the complications following vaccination and possibly, in smallpox.

The Storage of vaccine lymph dried from the frozen state. Vaccine lymph (without glycerine), the bacterial content of which has been reduced with ether chloroform vapour or 1 per cent. phenol, can be dried from the frozen state without any apparent loss of potency. Elementary body suspensions or virus cultivated in the chorio-allantoic membrane of the developing chick can be similarly dried and they retain their activity for years when stored in the cold at $\pm 4^{\circ}\text{C}$. When either the lymph or purified material are stored at 37°C , however, the titre of virus activity drops more rapidly than that of a control of ordinary glycerinated lymph maintained at the same temperature. Preliminary observations suggest that viruses, and probably bacteria, rapidly lose viability when stored at relatively high temperatures in the dry state. This contrasts with the fact that those sera which have been tested have shown no loss of activity after comparable storage at 37°C . At present, it is thought inadvisable to prepare dried vaccine lymph for storage without refrigeration, but it is hoped that further experiment will provide the means of preventing this inactivation.

Cultivation of vaccinia virus. The cultivation of virus in the chorio-allantoic membrane of the developing chick has been continued with a view to field trials of this material in ordinary Jennerian vaccination and the selection of suitable strains of virus for further work on intracutaneous vaccination.

BIOCHEMICAL STUDIES.

Specific Blood-group Substances. Dr. Morgan and Miss M. R. B. Waddell have examined in considerable detail the chemical properties of the products of acid hydrolysis of the A-substances derived from hog gastric mucin and pseudomucinous ovarian cyst fluids.

The B-substance has been isolated from ovarian cyst fluid and, after purification, has been converted into a full antigen and used to immunize rabbits. The artificial antigenic complex has given rise to specific anti-B (β) agglutinins and the sera thus obtained employed in routine blood grouping tests.

Dr. Morgan and Dr. D. B. Steabben have continued their experiments designed to suppress and control the natural anti-A agglutinin and have investigated more particularly the effect of the injection of A-hapten into rabbits whose serum shows a high anti-A agglutinin titre. The primary result of such injection is the reduction of the initial anti-A agglutinin content by antigen-antibody combination. The agglutinin content of the circulation can be kept at a low level by closely-spaced serial injections of the A-hapten. The ultimate result of such injections is, however, to increase the titre of both anti-A and anti-B agglutinins. The final titre may reach a high level, persisting for at least a month, though the effect is not comparable with the response elicited when a very small quantity of the complete artificial A-antigen is injected. Further research is needed to discover whether a hapten preparation can be rendered sufficiently non-antigenic to make its use possible in the reduction of maternal iso-agglutinins below the pathological level.

Similar experiments in man have been carried out by Dr. Morgan and Dr. Steabben in collaboration with Dr. J. F. Loutit (South West London Blood Supply Depot). Hospital patients suffering from inoperable carcinoma were the first subjects used for the administration of A-substance to human beings. Subsequently patients with other clinical conditions and normal volunteers have been injected. Both the intravenous and intramuscular routes have been used on subjects of groups O, A and B. At present the intramuscular route is preferred as reactions attributable to the A-substance have been met with after intravenous administrations. Minor and

occasionally major pyrogenic reactions have been seen, also symptoms and signs similar to those seen during incompatible blood transfusions. In the majority of instances there has been a marked rise in the anti-A titre of subjects of group O and B one week or more after the injection. No rise of the anti-B titre has been observed in subjects of group A, though a rise in anti-B titre is not uncommon in group O subjects.

Gramicidin. Analysis by Dr. R. L. M. Synge of a partial acid hydrolysate of gramicidin, employing partition chromatography with starch, has so far led to the conclusion that both the glycine residues in the molecule occur in the sequence *-l-valylglycyl-*, and that the valine residues not so linked probably belong to the *d-* series. Parallel studies have been conducted of the kinetics of acid hydrolysis of gramicidin and of relevant synthetic dipeptides under the same conditions.

The hydroxyamino component of gramicidin has been found to be ethanolamine. This has been isolated after such mild hydrolytic treatment as to suggest that ethanolamine residues occur as such in the molecule. From what is known of the mechanism of the antibacterial action of gramicidin the possibility of the ethanolamine residues playing a special role in this, is suggested.

Working with Dr. Morgan, Dr. Synge has found that rabbit antisera specifically precipitating gramicidin can be obtained after injection of gramicidin together with the conjugated protein component of the O antigen complex of *Shigella*, but not after the injection of gramicidin alone. This observation shows that the action of the *Shigella* material in rendering non-antigens antigenic, hitherto only observed with polysaccharide-like substances, is not confined to this class of compounds.

"Gramicidin S". Analytical data so far obtained by Dr. Synge, indicate that this substance is a polypeptide hydrochloride, the stoichiometric minimum molecule consisting of one residue each of *d*-phenylalanine, *l*-leucine, *l*-valine, *l*-proline and *l*-ornithine, together with one residue of chloride and possessing one free basic (amino) group and no free acidic groups. "Gramicidin S" is thus seen to be very closely related to tyrocidine hydrochloride. Dr. R. D. Hotchkiss (New York), states that the nature of their antibacterial action is likewise similar.

Interaction of Antibacterial Agents. Working in Sir Alexander Fleming's laboratory at St. Mary's Hospital, Dr. Synge has studied the combined bacteriostatic action of pairs of the three antibacterial agents, gramicidin, "gramicidin S" and penicillin against Staphylococci and Streptococci. There was reason for expecting synergism, since it is likely that all three agents act by different chemical mechanisms. However, no considerable deviations were noticed from simple summation of the bacteriostatic actions.

Amino-acid analysis. Dr. Synge, working with Dr. A. J. P. Martin (Leeds) and Dr. O. Rosenheim (National Institute for Medical Research), has shown that the preparations of "glycoleucine" made by Thudichum in 1882 from ox-brain, often considered to have been *norleucine*, consist in fact of *dl*-leucine. It further proved impossible to detect, by a sensitive and specific method, the presence of *norleucine* in a hydrolysate of ox spinal cord.

In collaboration with Dr. A. J. P. Martin, Dr. Synge has made a review of the present condition of the analysis of proteins for their constituent amino-acids for publication in Vol. II of "Advances in Protein Chemistry".

BIOPHYSICAL AND PHYSIO-CHEMICAL STUDIES.

Human Plasma Products. Dr. R. A. Kekwick, Dr. M. E. Mackay (Medical Research Council) and Dr. B. R. Record (Medical Research Council) have developed new methods for the separation of fibrinogen and prothrombin from human plasma. Human fibrinogen, thrombin and materials prepared from them such as fibrin foam are being produced, freeze-dried, and issued for clinical use.

The separation of fibrinogen and prothrombin from plasma has been achieved by the use of ether as a precipitant at low temperature. The remaining plasma proteins are subjected to low temperature ether extraction, so providing a stable transfusion fluid.

Electrophoretic studies on the serum protein changes in various forms of hepatitis have been made by Dr. Kekwick. A large scale Tiselius U-tube for use in preparative work has recently been acquired.

Freeze Drying. In collaboration with Dr. A. S. McFarlane (National Institute for Medical Research), and Mr. C. M. Brain of Messrs. J. & E. Hall Ltd., Mr. C. Bradish has been concerned with the development of a tray type drier capable of reducing the solid content of 5 litres of fluid to a powder of the same apparent volume and a residual moisture content of 2%, in 15 hours. An indication of the optimum operating conditions for this type of plant has been obtained by a study of the drying of calcium penicillin, human plasma and human milk. On the basis of the experience obtained a large scale plant has been erected by Imperial Chemical Industries at Manchester, for the drying of penicillin.

Mr. Bradish has supervised the construction of two small centrifuge drying chambers capable of drying, under sterile conditions, up to 600 ml. of material in ampoules. By the use of a series of interchangeable heads ampoules containing from less than 1 ml. to 10 ml. quantities may be accommodated. The apparatus has been used for the freeze drying of vaccine lymph, human milk and human plasma products.

Fœtal and Maternal Hæmoglobin. Investigations by Dr. E. L. McCarthy in the osmotic pressure of serum protein prepared from human fœtal and maternal blood have not shown the significant differences previously demonstrated in the corresponding sheep sera.

Dr. Popjak (St. Thomas's Hospital) and Dr. McCarthy have extended their work on normal and "defatted" human sera to lipæmic nephrotic sera. The osmotic pressure of the serum protein seems unaffected by the high lipid concentration.

Dr. McCarthy, in collaboration with Sir Joseph Barcroft (Cambridge), is continuing his investigations of the high oxygen affinity of fœtal hæmoglobin. Preparations for isoelectric point measurements on fœtal hæmoglobin are being made.

ENDOCRINOLOGY.

Dr. V. Korenchevsky, assisted by Miss K. Hall and with the statistical co-operation of Mrs. B. Clapham (Nuffield College, Oxford), has continued his studies on the effects of different combinations of sex, thyroid and adrenal hormones and vitamins, A, B and C on adult and old rats. Part of the results obtained in some organs has been described in the previous report. The histological investigation of other organs is in progress.

NUTRITIONAL STUDIES.

Nutritive Value of Different Food Proteins. Dr. H. Chick and Mr. E. B. Slack have continued work on the growth-promoting value of the nitrogenous substances in potato and wheat and have also investigated the supplementary action existing between these and nitrogenous materials in yeast and an aqueous beef extract. The criterion adopted has been the rate of weight increase of young rats receiving a diet complete in other essentials but containing a sub-optimal amount of the proteins to be compared.

Potato. A definite deterioration in the nutritive value of the nitrogenous constituents was found to occur when potatoes were stored for several months in a clamp; this was less marked when storage took place in a chamber with temperature controlled at 5°C.

In a comparison between the value of the nitrogenous materials contained in the skin and that portion immediately underneath it and those present in the inner part of the tuber, no evidence was found that the former possessed any advantage.

Study has been continued of the potato protein tuberin and of the non-protein nitrogenous materials in the expressed juice. Evidence has been obtained of the existence of an additional unrecognised protein, but so far separation has not been effected.

Supplementary values in mixtures of nitrogenous substances from different foods. The non-protein nitrogen of the potato, alone incapable of supporting growth, had a marked supplementary action for wheat gluten. The rate of weight increase of young rats on a diet containing 20 per cent. of the latter was little more than one-half that of litter mates receiving a diet of equal nitrogen content, derived from a mixture of the two.

The proteins in dried yeast were found to be of much inferior value to those of whole wheat, but a mixture of the two was superior to the latter. A mixture of yeast protein with the non-protein nitrogen also had advantages over those of yeast protein given alone. The nitrogenous substances in domestic "beef-tea" possessed no growth-promoting value but when combined with those of white flour, provided a mixture whose nutritive value was much superior to that of white flour proteins fed alone. The supplementary action for cereal proteins of the nitrogenous substances in beef extract and yeast is probably due to the high content in these of the essential amino-acid lysine, in which cereal proteins are relatively deficient.

Deprivation of Vitamin A in the human subject. The human experiment on deprivation of Vitamin A began in July 1942 and carried out by a team of workers organised by Miss E. M. M. Hume, at the Sorby Institute, Sheffield, was concluded in September, 1944, the subjects being then transferred to an experiment on deprivation of Vitamin C, with which also Miss Hume is concerned though not primarily.

In the vitamin A experiment it was found that some of the subjects still showed little evidence of depletion after two years of the deficient diet, though others had well marked signs. It was hoped to publish an early preliminary report of this experiment but it was not possible to draw reliable conclusions until the whole of the results of blood tests, food and faeces tests and tests for capacity for dark adaption had been tabulated and analysed. Miss Hume, as Secretary of the Vitamin A. sub-committee has collected the available data and is preparing it for publication.

Vitamin A in Potatoes. Miss Hume and Miss Henderson Smith have completed a study of the potato as source of vitamin potency. It has been found that rats could survive indefinitely with no, or very slight, skin lesions of vitamin A deficiency, on a diet composed almost wholly of potato and with no other source of this vitamin.

Vitamin A deficiency and experimental gastric ulceration in rats. Miss Hume and Miss Henderson Smith have begun studies on the production of the gastric ulceration known to occur in rats deprived of vitamin A. Very severe lesions have already been produced.

Biological estimations of Vitamins. Miss A. M. Copping, with the assistance of Miss Vanda Pond has continued to carry out biological estimations of vitamins of the B-complex in diets and foodstuffs for the Royal Air Force, the Medical Research Council and the Ministry of Food.

An extensive study of the vitamin content of Royal Air Force diets has been completed and some of the results have been published in collaboration with Dr. Macrae and Mr. Barton-Wright (Cereals Research Station, St. Albans).

Biological estimations of vitamin B₆ and of riboflavin were made on materials provided by the Vitamin B₁ sub-committee of the Advisory Food Factors' Committee as part of a collaborative study of biological, microbiological and chemical methods of estimating vitamins of the B-complex.

Further investigations of the biological method of estimating vitamin B₆ (pyridoxin) are in progress.

Influence of Iodine Deficiency on Human Nutrition. Dr. Dagmar C. Wilson, supported by a grant for expenses from the Institute, has continued her work on the incidence of endemic goitre in relation to the iodine, fluorine and calcium content of drinking water. The work has been carried out in collaboration with Miss B. Simpson (Rowett Institute), Dr. M. M. Murray (Bedford College) and Miss E. M. Widdowson (Medical Research Council) and has been extended to certain selected Scottish localities.

In collaboration with Dr. Murray, Dr. Wilson has also investigated the incidence of congenital deaf-mutism in relation to low iodine content of drinking water.

Dr. Chick, as Secretary of the Accessory Food Factors' Committee, has drawn up a memorandum on the "Nutritive Value of Food Yeast" and Miss Hume has collaborated in the preparation of the tables of food composition contained in the "Nutritive Value of War-time Foods". These publications are being issued from the Committee as War Memoranda of the Medical Research Council.

Miss Hume, Miss Copping and Dr. Chick have continued their usual work in the production of "Nutrition Abstracts and Reviews", in co-operation with the Imperial Bureau of Animal Nutrition, Aberdeen.

Miss Henderson Smith has continued to breed a satisfactory supply of rats for the experimental work even during the most difficult winter months. Up to late Autumn the stock animals receive large amounts of lettuce. This being particularly rich in Vitamin E is suggested as being a contributory factor to successful winter breeding.

Nicotinamide and related compounds. The discrepancy between dietary nicotinamide intake and nicotinamide methochloride output in man referred to last year has been examined by Dr. Ellinger and Mr. R. Benesch (London County Council Central Pathological Laboratory, Epsom) and the extradietary source of nicotinamide found in the intestinal bacterial flora which can be responsible for up to 100 per cent. of the intake. This fact elucidates a number of hitherto unexplained features in the etiology and cure of pellagra and other nicotinamide-deficiency diseases.

Dr. Ellinger and Mr. Benesch has investigated the nicotinamide saturation in healthy human beings and in a number of nicotinamide deficiency diseases from severe cases of acute pellagra to cases with light single deficiency symptoms. Characteristic changes in the saturation curves were observed. An investigation into the nicotinamide methochloride elimination in patients suffering from liver diseases also indicating certain characteristic differences from the normal has been commenced.

Dr. Ellinger has investigated the chemical nature of the metabolite of nicotinamide and related compounds eliminated in the urine. A number of nicotinamide derivatives were examined for their toxicity, pharmacological properties and particularly for their ability to be eliminated as nicotinamide methochloride.

MEDICAL RESEARCH COUNCIL EXTERNAL SCIENTIFIC STAFF.

Dr. S. S. Zilva has continued his observations on vitamin C.

The isolation of 2:3-diketogulonic acid. The existence of 2:3-diketogulonic acid has been postulated by Hirst and his collaborators as an intermediate product in the oxidative degradation of *l*-ascorbic acid. This compound has not, however, been hitherto isolated. Several biological points bearing on the part played by *l*-ascorbic acid in nutrition could not be settled in a satisfactory fashion until this compound was obtained in pure form and fully identified. Dr. Zilva and Dr. J. R. Penney have succeeded in isolating the barium and calcium salts of this acid which has enabled them to clarify with some degree of finality the behaviour of diketogulonic acid in the animal body.

Lugg's method for the determination of vitamin C. Dr. Zilva and Dr. G. A. Snow have been investigating the behaviour of reducing substances, formed on heating solutions of glucose in the presence of alkali and of pectin in the presence of acid, on treatment with HCHO and on oxidation and subsequent reduction with

H₂S. The behaviour of these substances in this respect was found to be different from that of pure reductone and reductic acid previously reported. The bearing of these results on the accuracy of Lugg's method has been ascertained and a procedure based on this method which yields the most satisfactory results has been worked out.

Miscellaneous researches. The investigation of practical problems for the Fighting Services has been continued by Dr. Zilva and his co-workers and, as in previous years, the results have been communicated to the respective Services through the Medical Research Council.

National Collection of Type Cultures. The Collection continues to be accommodated at the Institute's laboratories at Elstree. Distribution of cultures is still almost exclusively confined to workers in this country and the Dominions but relations with Continental workers are gradually being re-established. During the year under review over 4,500 cultures have been distributed—a substantial increase as compared with the previous year. Some 200 strains were lodged for maintenance or investigation.

The Governing Body, in conclusion, again desire to record their appreciation of the wholehearted service rendered by all members of the staff and also the assistants, in carrying out, under difficult war conditions, the work of the Institute. They look forward now with interest and confidence to the devotion of the same qualities to the readjustment of the Institute's activities, to a programme in which problems related to conditions of peace and reconstruction may be expected to figure henceforward with a growing prominence.

H. H. DALE.

Chairman of the Governing Body.

$\frac{10}{1}$ Sister Jones (2)
6/10/41