THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

Balance Sheet and Accounts December 31st 1956

FINANCIAL REPORT OF THE GOVERNING BODY

1. The Balance Sheet as at 31st December 1956 shows balances to the credit of the various funds as follows: Capital Fund £651,143; Specific Funds £136,800; Bequest Funds £13,912 and Contingency Reserve £49,069. The balance on the Sinking Fund for Freehold Buildings of £95,302 is after charging the loss on sales of Sinking Fund investments amounting to £4,691 and transferring from income and expenditure account £4,088.

2. The General Fund Income and Expenditure Account shows the income for the year as £168,669 compared with £125,000 in 1955. Expenditure amounted to £146,794 against £141,841 last year. The surplus for the year is £21,875 compared with a deficit of £16,841 in 1955.

3. The year's surplus of £21,875 shown by the General Fund Income and Expenditure account has been transferred to the Contingency Reserve.

4. Stocks of Sera, Vaccine Lymph and Horses on hand at December 31st have the nominal value of £8,754, £3,624 and £4,816 respectively.

5. MESSRS. COOPER BROTHERS & Co., the retiring Auditors will, subject to the provisions of the Companies' Act, 1948, be re-appointed.

H. H. DALE, *Chairman of Governing Body.*

WAVERLEY, *Hon. Treasurer.*

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

28th May, 1957.

BALANCE SHEET

(1955) £		£	£
	Capital Fund:—		
	Donations, &c., received to date from the following:—		
2,000	Dr. Ludwig Mond (1893)	2,000	
46,380	Berridge Trustees (1893/98)	46,380	
10,000	Worshipful Company of Grocers (1894)	10,000	
250,000	Lord Iveagh (1900)	250,000	
18,904	Lord Lister's Bequest (1913/23)	18,904	
7,114	William Henry Clarke Bequest (1923/6)	7,114	
3,400	Rockefeller Foundation (1935/6)	3,400	
22,669	Other Donations and Legacies (1891-1954)	22,669	
	General Fund Income and Expenditure Account Accumulated Surplus as at 31st December, 1955 324,655		
	Less Loss on sale of investments 33,979		
324,655		290,676	
685,122			651,143
	Specific Funds:—		
95,905	Sinking Fund for Freehold Buildings 95,302		
36,259	Pension Fund 36,478		
5,020	Re-endowment Fund 5,020		
		136,800	
	Bequest Funds:—		
10,465	Jenner Memorial Studentship Fund 7,932		
8,360	Morna Macleod Scholarship Fund 5,980	13,912	
156,009			150,712
	Specific Grants and Legacies Unexpended:—		
772	Cancer Research Legacies (1937-50)	772	
1,583	Royal Society Grant (1951)	1,033	
3,722	Nuffield Foundation Grants (1952-6)	5,008	
4,518	Guinness Lister Research Grant (1953-6)	8,307	
10,595			15,120
	Contingency Reserve:—		
	As at 31st December 1955	27,194	
	Add Surplus on General Fund Income and Expenditure Account, 1956	21,875	
27,194			49,069
	Current Liabilities:—		
21,806	Creditors and accrued charges		17,562
£900,726			£883 606

H. H. DALE, *Chairman of Governing Body.*

WAVERLEY, *Hon. Treasurer.*

REPORT OF THE AUDITORS

We have examined the above Balance Sheet and annexed Income and Expenditure Account which are in all the information and explanations which we considered necessary for our audit. In our opinion these accounts information required by the Companies Act, 1948, and show a true and fair view of the state of the Institute's

London, 30th May, 1957.

31st DECEMBER 1956.

(1955) £		£	£	£
	Fixed Assets :—			
	FREEHOLD PROPERTY at cost :			
73,548	Land and Buildings, Chelsea	73,548		
20,456	Queensberry Lodge Estate, Elstree	20,456		
2,049	House, Bushey	2,049		
			96,053	
	(*Note*: *Additions and replacements since 1912 at Elstree and 1935 at Chelsea have been charged to Revenue*).			
	FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS :—			
2,472	At cost *less* depreciation to 31st December 1920		2,472	
98,525	(*Note*: *Additions and replacements since 31st December, 1920 have been charged to Revenue*)			98,525
	General, Specific and Bequest Funds.			
	Quoted Investments at cost, less amounts written off and Uninvested Cash :—			

		Investments	Cash		
606,421	GENERAL	559,614	—	559,614	
	SPECIFIC :				
95,905	Sinking Fund for Freehold Buildings ..	91,238	4,064	95,302	
36,259	Pension Fund	34,799	1,679	36,478	
5,020	Re-endowment Fund	4,941	79	5,020	
	BEQUEST :				
10,465	Jenner Memorial Studentship Fund ..	6,530	1,402	7,932	
8,360	Morna Macleod Scholarship Fund ..	4,841	1,139	5,980	
762,430		701,963	8,363	710,326	710,326
	(*Market Value £715,045*)				
	Current Assets :—				
27,003	Debtors and Payments in advance			65,519	
2,161	*Bills Receivable*			—	
10,607	Balance at Bankers and Cash in hand			9,236	
39,771					74,755

(*Notes*: *See paragraph 4 Governing Body's Financial Report for nominal values of Sera, Vaccine Lymph and Horses which have not been brought into the accounts.*

There is a contingent liability of £12,360 in respect of investments not fully called up. There is an outstanding capital expenditure commitment of £9,230 in respect of building at Elstree).

£900,726	£883,606

TO THE MEMBERS.

agreement with the books of account. In our opinion proper books of account have been kept. We have obtained amplified by the information given in paragraphs 1 and 4 of the Financial Report of the Governing Body give the affairs at 31st December, 1956, and of the surplus for the year ended on that date.

COOPER BROTHERS & CO.
Chartered Accountants.

INCOME AND EXPENDITURE ACCOUNT

GENERAL

(1955) £		*Total Expenditure* £	*External Contributions* £	£
56,581	Salaries and Wages	105,978	40,726	65,252
6,471	Emoluments of two members of the Governing Body in an Executive Capacity	6,543	—	6,543
2,518	Premiums on Federated Superannuation Policies	4,164	1,501	2,663
3,071	Premium on Group Pension Policy	3,683	218	3,465
3,647	Rent, Rates and Insurance	3,821	182	3,639
9,656	Gas, Water, Fuel and Electricity	13,577	2,567	11,010
1,803	Office Expenses, Stationery and Printing ...	2,583	201	2,382
210	Auditors' Fee	242	—	242
1,392	Travelling Expenses	878	194	684
1,430	Biochemistry Expenses	3,086	880	2,206
1,144	Microbiology, Immunology and Experimental Pathology Expenses	3,007	1,932	1,075
435	Biophysics Expenses	843	374	469
2,128	Virology Expenses	940	517	423
15,543	Serum, Vaccine and Vaccine Lymph Expenses	12,816	1,302	11,514
5,168	Animals	8,442	1,370	7,072
8,415	Animal House Expenses and Forage	9,417	1,314	8,103
15,532	Buildings, Alterations, Repairs and Renewals ...	12,466	628	11,838
620	General Apparatus and New Installations ...	1,801	—	1,801
912	Library Expenses	1,028	—	1,028
931	General Stores	446	—	446
583	Staff Canteen Loss	1,010	159	851
—	Blood Products Laboratory Expenses	1,436	1,436	—
3,651	Amount transferred to Sinking Fund for Freehold Buildings (including £3,664 Interest on Investments)	4,088	—	4,088
—	Surplus transferred to Contingency Reserve after charging to expenditure £6,944 for additions to property and equipment (*1955 £14,694*) ...	21,875	—	21,875
£141,841		£224,170	£55,501	£168,669

NUFFIELD FOUNDATION GRANTS.

(1955) £		£	(1955) £		£
6,671	Salaries, Wages, Laboratory Expenses and Animals	4,714	4,393	Balance at 1st January, 1956...	3,722
3,722	Balance carried forward ...	5,008	6,000	Amounts received	6,000
£10,393		£9,722	£10,393		£9,722

for the year ended 31st December 1956.

Fund.

(1955) £		£	£
	Interest and Dividends on Investments:		
27,794	General Fund	29,755	
3,227	Sinking Fund	3,664	33,419
88 767	Sales of Sera, Vaccines, Vaccine Lymph, &c.		130,084
5,212	Rent		5,166
16,841	*Deficit transferred to Contingency Reserve*		—
£141,841			£168,669

Guinness - Lister Research Grant.

(1955) £		£	(1955) £		£
5,479	Salaries and Wages	6,630	2,000	Balance at 1st January, 1956...	4,518
2,003	Laboratory Expenses	2,581	10,000	Amount received	13,000
4,518	Balance carried forward ...	8,307			
£12,000		£17,518	£12,000		£17,518

Pension Fund.

(1955) £		£	(1955) £		£
1,461	Pensions	1,387	36,114	Fund as at 1st January, 1956 ...	36,259
36,259	Fund as at 31st December, 1956	36,478	1,606	Interest on Investments (gross) ...	1,606
£37,720		£37,865	£37.720		£37,865

Jenner Memorial Studentship Fund.

£		£	£		£
—	Loss on realisation of investments	2,429	10,529	Fund as at 1st January, 1956...	10,465
354	Stipend of Student... ...	383	290	Interest on Investments (gross)	279
10,465	Fund as at 31st December, 1956	7,932			
£10,819		£10,744	£10,819		£10,744

Morna Macleod Scholarship Fund.

£		£	£		£
—	Loss on realisation of investments	2,558	8,138	Fund as at 1st January, 1956 ...	8,360
8,360	Fund as at 31st December, 1956	5,980	222	Interest on Investments (gross) ...	178
£8,360		£8,538	£8,360		£8,538

INVESTMENTS AT 31st DECEMBER 1956.

GENERAL FUND.

		Balance Sheet Value		Market Value
£9,000	A.P.V. Co., Ltd., 5 per cent. First Mortgage Deb. Stock, 1980/85 ..	£8,698	..	£7,650
6,000	Albright & Wilson Ltd., Ordinary Stock Units of 5/-	5,168	..	5,550
£5,000	Allied Bakeries Ltd , 5 per cent. Unsecured Loan Stock, 1966/70 ..	4,819	..	4,500
1,500	Associated Electrical Industries Ltd., Ordinary £1 Shares ..	4,498	..	4,968
£3,900	Australia, Commonwealth of, 4½ per cent. Registered Stock, 1960/62	2,666	..	2,711
£12,000	Australia, Commonwealth of, 3 per cent. Registered Stock, 1972/74	12,121	..	8,460
£10,000	Australian Estates Co., Ltd., 6½ per cent. Secured Loan Stock, 1971/76	9,848	..	9,550
£4,000	Bankers Investment Trust Ltd., Deferred Stock	7,806	..	11,320
£5,000	Birfield Ltd., 6 per cent. Unsecured Loan Stock, 1976/81	4,976	..	4,975
£2,000	British Electricity 3 per cent. Guaranteed Stock, 1974/77	1,898	..	1,470
£15,000	,, ,, 3½ ,, ,, ,, 1976/79.. ..	14,925	..	11,625

GENERAL FUND—continued.

3,000	British Oxygen Co., Ltd., Ordinary Stock Units of £1	6,165 ..	5,325
£9,000	British Titan Products Co., Ltd., 5½ per cent. Unsecured Loan Stock, 1970/75	8,820 ..	8,505
£20,000	British Transport 3 per cent. Guaranteed Stock, 1967/72	20,259 ..	13,700
£15,000	,, ,, ,, ,, 1978/88	15,000 ..	10,275
£10,000	Caltex (U.K). Ltd., 6 per cent. Guaranteed Loan Stock, 1971/76 ..	9,800 ..	9,800
3,500	Cater Brightwen & Co., Ltd., Ordinary Stock Units of £1	10,872 ..	8,312
£5,000	George Cohen 600 Group Ltd., 6 per cent. Unsecured Loan, 1975/80	5,000 ..	5,050
3,250	Debenture & Capital Investment Trust Ltd., Ordinary Stock Units of £1	6,585 ..	13,243
4,000	Dorman Long & Co., Ltd., Ordinary Shares of £1	4,981 ..	5,050
£1,000	Esso Petroleum Co., Ltd., 5½ First Debenture Stock, 1974/78 (60 per cent. paid)	550 ..	600
£5,000	Flowers Breweries Ltd., 5½ per cent. First Mortgage Debenture Stock, 1970/72	4,850 ..	4,800
£1,350	General Consolidated Investment Trust Ltd., Ordinary Stock ..	2,855 ..	4,185
£1,500	General Electrical Co., Ltd., 6 per cent. Unsecured Loan Stock, 1976/81 (50 per cent. paid)	2,157 ..	2,407
300	Hadfields Ltd., Ordinary Shares of £1	392 ..	360
£200	Harrods Ltd., Ordinary Stock Units of £1	443 ..	475
£9,000	Hope & Anchor Breweries Ltd., 5½ per cent. Mortgage Debenture Stock, 1980/85	8,843 ..	8,730
£5,000	Kenning Ltd., 5½ per cent. Unsecured Loan Stock, 1970/75 ..	4,703 ..	4,225
£10,000	Kraft Foods Ltd., 3 per cent. Debenture Stock 1965/75	10,000 ..	8,900
4,000	Lancashire Steel Corporation Ltd., Ordinary Shares of £1	5,328 ..	6,750
£10,000	R. A. Lister Ltd., 5 per cent. Unsecured Loan Stock, 1960/65 ..	9,975 ..	9,337
15,000	London & Montrose Investment Trust Ltd., Ordinary 5/- Shares ..	7,893 ..	12,187
7,500	,, ,, ,, ,, ,, New Ordinary 5/- Shares (Nil Paid)	— ..	4,312
£3,125	London Scottish American Trust Ltd., Deferred Stock	6,942 ..	10,781
7,700	London Trust Co., Ltd., Deferred 5/- Stock Units	4,292 ..	6,930
£1,570	Lowland Investment Co. Ltd., Ordinary Stock	2,873 ..	4,239
22,500	Mercantile Investment & General Trust Co., Ltd., Ordinary 5/- Stock Units	13,401 ..	19,687
£9,000	Mitchell Cotts & Co., Ltd., 6 per cent. Unsecured Loan Stock, 1976/81	8,800 ..	9,000
£4,000	R. H. Neal & Co., Ltd., 6½ per cent. Unsecured Loan Stock, 1966/71	4,000 ..	4,000
£25,000	New Zealand 3½ per cent. Stock, 1962/65	21,989 ..	20,625
£10,000	Norvic Shoe Co., Ltd., 5 per cent. Unsecured Loan Stock, 1970/75 ..	9,800 ..	9,250
£10,000	Peninsular & Oriental Steam Navigation Co., 5 per cent. Debenture Stock, 1975/80	9,542 ..	9,250
£3,000	Port of London Authority—Port of London 3½ per cent. Registered Stock, 1965/75	2,687 ..	2,160
£5,000	Powers-Samas, Accounting Machines Ltd., 6 per cent. Unsecured Loan Stock, 1975 78	4,950 ..	4,950
20,000	Rio Claro Investment Trust Ltd., Ordinary Stock Units of 5/- ..	11,192 ..	17,250
20,000	River Plate & General Investment Trust Co., Ltd., Deferred Stock Units of 5/-	7,691 ..	11,000
£4,000	Ruston & Hornsby Ltd., 6 per cent. Unsecured Loan Stock, 1975/80	4,000 ..	4,040
£25,000	3 per cent. Savings Bonds 1955/65	25,000 ..	21,875
£66,300	,, ,, ,, 1960/70	66,417 ..	52,045
£38,300	,, ,, ,, 1965/75	33,857 ..	28,533
3,000	Secoombe Marshall & Campion Ltd., Ordinary Shares of £1 ..	5,257 ..	4,875
5,250	Sphere Investment Trust Ltd., Ordinary Shares of £1	7,830 ..	14,043
11,454	Standard Trust Ltd., Ordinary Stock Units of 5/-	3,992 ..	7,301
£7,500	Sterling Trust Ltd., Ordinary Stock	8,731 ..	18,000
£10,000	Tennant Brothers Ltd., 6 per cent. Redeemable Debenture Stock, 1971	10,089 ..	9,900
£6,750	Third Guardian Trust Ltd., Ordinary Stock	6,783 ..	11,137
11,250	Typhoo Tea (holdings) Ltd., Ordinary Shares of 5/-	7,505 ..	8,156
£10,000	United Gas Industries Ltd., 6 per cent. Unsecured Loan Stock, 1973/75	9,900 ..	9,800
£10,000	United States Debenture Corporation Ltd., Ordinary Stock ..	13,153 ..	17,800
1,250	Westinghouse Brake & Signal Co., Ltd., Ordinary Shares of £1 ..	4,952 ..	4,375
£10,000	Whitbread Investment Co., Ltd., 5¾ per cent. Guaranteed Debenture Stock, 1980/85	9,950 ..	9,900
£6,000	Witan Investment Co. Ltd., Ordinary Stock	11,145 ..	13,800
		£559,614	£584,009

SINKING FUND FOR FREEHOLD BUILDINGS.

		Balance Sheet Value		Market Value
£3,000	British Electricity 3 per cent. Guaranteed Stock, 1974/77	£2,934	..	£2,205
£3,000	,, ,, 3 ,, ,, ,, 1968/73	2,916	..	2,265
£5,000	Financial Times Ltd., 5½ per cent. Debenture Stock, 1980/85 ..	4,900	..	4,875
£4,500	3 per cent. Funding Loan, 1959/69	3,876	..	3,577
£9,000	Gallaher Ltd., 6 per cent. Unsecured Loan Stock, 1976/81 ..	8,884	..	8,910
£10,000	New Zealand Loan & Mercantile Agency Co., Ltd., 5½ per cent. 2nd Mortgage Debenture Stock, 1970/80	9,525	..	9,600
1,000	A. Reyrolle & Co., Ltd., New Ordinary Shares (Nil Paid)	963	..	1,125
£3,500	3 per cent. Savings Bonds, 1955/65	3,518	..	3,062
£10,000	,, ,, ,, 1960/70	9,622	..	7,850
£31,600	,, ,, ,, 1965/75	31,600	..	23,542
4,000	South Durham Steel & Iron Co., Ltd., Ordinary Shares of £1 ..	5,329	..	5,200
£3,000	Third Guardian Trust Ltd., Ordinary Stock	2,183	..	4,950
2,000	Union Discount Co. of London Ltd., Stock Units of £1	4,988	..	4,700
		£91,238		£81,861

PENSION FUND.

£3,000	British Oxygen Co., Ltd., 5¾ per cent. Debenture Stock, 1981/86 (50 per cent. paid)	£1,421	..	£1,605
£3,000	Dowty Group Ltd., 6 per cent. Unsecured Loan Stock, 1977/82 (25 per cent. paid)	757	..	757
£18,000	3½ per cent. Conversion Loan Stock, 1961 or after	15,173	..	13,690
£15,000	4 per cent. Funding Loan, 1960/90	11,243	..	19,125
£3,000	Ruston & Hornsby Ltd., 6 per cent. Unsecured Loan Stock, 1975/80	3,000	..	3,030
£2,200	3 per cent. Savings Bonds, 1960/70	2,205	..	1,727
£1,000	3 ,, ,, ,, 1965/75	1,000	..	745
		£34,799		£33,679

JENNER MEMORIAL STUDENTSHIP FUND.

£1,000	A.P.V. Co., Ltd., 5 per cent. First Mortgage Debenture Stock, 1980/85	£850	..	£850
£1000	British Titan Products Ltd., 5½ per cent. Unsecured Loan Stock, 1970/75	940	..	945
£1,000	Gallaher Ltd., 6 per cent. Unsecured Loan Stock, 1976/81 ..	1,000	..	990
£1,500	General Electrical Co., Ltd., 6 per cent. Unsecured Loan Stock, 1976/81 (50 per cent. paid)	750	..	802
£1,000	Hope & Anchor Breweries Ltd., 5½ per cent. Mortgage Debenture Stock, 1980/85	990	..	970
£1,000	Mitchell Cotts & Co., Ltd., 6 per cent. Unsecured Loan Stock, 1976/81	1,000	..	1,000
£1,000	R. H. Neal & Co., Ltd., 6½ per cent. Unsecured Loan Stock, 1966/71	1,000	..	1,000
		£6,530		£6,557

MORNA MACLEOD SCHOLARSHIP FUND.

£1,000	Agricultural Mortgage Corporation Ltd., 5 per cent. Debenture Stock, 1979/83	£958	..	£870
£1,000	British Guiana 5 per cent. Stock, 1980/85	928	..	925
£1,000	3½ per cent. Defence Bonds, Conversion Issue	1,000	..	1,000
£1,000	South Essex Waterworks Co., 5 per cent. Redeemable Debenture Stock, 1986/91	963	..	925
£1,000	Stockton-on-Tees Corporation 5½ per cent. Redeemable Debenture Stock, 1966	992	..	980
		£4,841		£4,700

RE-ENDOWMENT FUND.

£5,400	3 per cent. Savings Bonds, 1960/70	£4,941	..	£4,239

THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE

REPORT
OF THE
GOVERNING BODY
1957

CHELSEA BRIDGE ROAD, LONDON, S.W.1

THE GOVERNING BODY

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Deputy Director: Professor W. T. J. Morgan.

Superintendent of Elstree Laboratories: W. d'A. Maycock.

MICROBIOLOGY, IMMUNOLOGY and EXPERIMENTAL PATHOLOGY

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I. N. Asheshov, M.D. (*Medical Research Council External Scientific Staff*).

GUINNESS-LISTER RESEARCH UNIT

*B. A. D. Stocker, M.D., M.R.C.S., L.R.C.P.
C. J. Perret, M.A.
D. Kerridge, B.A.
Janet G. Campbell, B.Sc., (*Research Assistant*)
Helen L. Bernstein, A.B., M.A., (*Research Student*).
Helene de Margerie (*Honorary Research Staff*)

VIROLOGY

L. H. Collier, M.D.
W. A. Blyth, B.Sc., (*Research Student*).

BIOCHEMISTRY

†W. T. J. Morgan, D.Sc., Ph.D., F.R.I.C., F.R.S. (*Professor of Biochemistry in the University of London*). *Principal Biochemist, Elstree.*
*Marjorie G. Macfarlane, D.Sc., Ph.D.
*W. J. Whelan, D.Sc., Ph.D.
Winifred M. Watkins, B.Sc., Ph.D.
W. C. Crimmin, Ph.D., (*Research Assistant*).
J. Thomas, Ph.D. (*Research Assistant*).
Patricia M. Gibson, B.Sc. (*Research Assistant*).
F. W. Parrish, B.Sc. (*Research Assistant*).
M. Ruszkiewicz, M.Sc. (*Research Student*).
Valerie Lawton, M.Sc. (*Research Student*).
R. Côté, B.A., D.Sc., (*Beit Memorial Research Fellow*).
Gwen J. Walker, Ph.D. (*Agricultural Research Council Grantee*).
J. V. McLoughlin, M.Sc. (*Medical Research Council Grantee*).
G. M. A. Gray, B.Sc. (*Medical Research Council Grantee*).
S. Haq, M.Sc. (*Pakistan*).
S. A. Warsi, Ph.D. (*Pakistan*)
M. Abdullah, M.Sc. (*Pakistan*).
G. W. G. Bird, M.D. (*India*).

BIOPHYSICS

†R. A. Kekwick, D.Sc., (*Reader in Chemical Biophysics in the University of London*).
§Margaret E. Mackay, M.Sc., Ph.D. (*Medical Research Council External Scientific Staff*).
E. A. Caspary, M.Sc.
J. H. Pearce, B.Sc. (*Agricultural Research Council Grantee*).
Professor N. H. Martin, M.A., B.M., B.Ch., B.Sc. (*Honorary Research Associate*).

NUTRITION

§Dame Harriette Chick, D.B.E., D.Sc.
§E. Margaret Hume, M.A.

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†Appointed Teacher of the University of London.
*Recognised Teacher of the University of London.
§Honorary Member of Institute Staff.

PREPARATION and STUDY of THERAPEUTIC SERA (ELSTREE)

*B. G. F. Weitz, M.R.C.V.S.
I. Rodican, B.Sc.
Frances M. Lee-Jones, B.Sc.

BIOCHEMISTRY (ELSTREE)

*D. E. Dolby, B.Sc., Ph.D.
Sheila M. Lanham, B.Sc.

PREPARATION and STUDY of SMALLPOX VACCINE (ELSTREE)

*D. McClean, M.B., B.S., M.R.C.S.
C. Kaplan, M.Sc., M.B., Ch.B., Dip.Bact.
Lisel R. Thomas, B.A. (*Research Student*).

PREPARATION and STUDY of BACTERIAL VACCINES (ELSTREE)

*A. F. B. Standfast, M.A., Dip.Bact.
Jean M. Horton, M.A., Ph.D. (*Medical Research Council External Scientific Staff*).
M. Garay, B.Sc.

BLOOD PRODUCTS (ELSTREE)

*W. d'A. Maycock, M.B.E., M.D.
L. Vallet, M.A.
Constance Shaw, M.Sc., Dip.Bact.
R. H. Painter, B.Sc., Ph.D.
Shirley M. Evans, B.Sc.

RESEARCH UNITS HOUSED AT THE INSTITUTE

MEDICAL RESEARCH COUNCIL

Blood Group Research Unit.

§R. R. Race, Ph.D., M.R.C.S., L.R.C.P., F.R.S.
Ruth Sanger, B.Sc., Ph.D.
Phyllis P. Moores, B.Sc.
E. Hackel, M.S., Ph.D. (U.S.A.).

Blood Group Reference Laboratory

§*A. E. Mourant, M.A., D.Phil., D.M., M.R.C.P.
Dorothy M. Parkin, M.R.C.S., L.R.C.P.
Elizabeth W. Ikin, B.Sc.
Carolyn M. Giles, B.Sc.

ADMINISTRATION

Secretary and Accountant - - -	S. A. White, A.A.C.C.A.
Elstree Secretary and Estate Manager -	F. K. Fox

Solicitors:	**Auditors:**
Field, Roscoe & Co.	Cooper Brothers & Co.
52 Bedford Square, W.C.1.	14 George Street, Mansion House, E.C.4.

*Recognised Teacher of the University of London.
§Honorary Member of Institute Staff.

ANNUAL GENERAL MEETING

OF

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

June 27th, 1957

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the work of the Institute for the year 1956/57.

GOVERNING BODY

At its last meeting the Council re-appointed Sir Henry Dale and Sir Wilson Jameson as its representatives on the Governing Body until 31st December, 1957. The Council accepted with much regret the decision of Sir Paul Fildes, who had been a representative of the Council since 1941, not to offer himself for re-appointment. The Governing Body take this opportunity of expressing their gratitude to Sir Paul Fildes for his constant interest and wise counsel in the affairs of the Institute. The Governors welcome Sir Charles Dodds as the new representative of the Council.

Lord Balfour of Burleigh who, at the invitation of Lord Iveagh, became a Governor in 1950 has also felt obliged to resign. The Governing Body learned of this decision with regret and wish to place on record their appreciation of Lord Balfour's services and valuable advice, particularly in the financial affairs of the Institute, during his period of office. In Lord Balfour's place Lord Iveagh has appointed Mr. H. P. G. Channon as one of his representatives.

In accordance with the Articles of Association, Dr. D. McClean retired from the Governing Body and was succeeded by Professor W. T. J. Morgan as the Scientific Staff's representative.

COUNCIL

At last year's Annual General Meeting two of the three retiring members, Dame Harriette Chick and Sir Alan Drury, were re-appointed to the Council. The third retiring member, Professor A. V. Hill, did not wish to stand for re-appointment and Sir Charles Dodds was appointed to fill the vacancy. The Worshipful Company of Grocers appointed Mr. W. J. Thompson as one of their representatives in place of the late Professor S. R. K. Glanville.

The three members of the Council due to retire this year in accordance with the Articles of Association, but who are eligible for re-appointment, are Lord Iveagh and Lord Waverley, each a representative of the Members of the Institute, and Major L. M. E. Dent, a representative of the Grocers' Company.

The death of Sir Percival Hartley is recorded with regret. Sir Percival was a member of the Scientific Staff of the Institute from 1913 to 1919 and again worked at the Institute from 1949 to 1953.

MEMBERS

The Governing Body records with regret the deaths during the year of Dr. J. O. W. Barratt, Professor Winifred Cullis, Professor J. B. Leathes, Professor M. J. Stewart, Dr. J. Trevan, Mr. A. L. White and Dr. S. S. Zilva.

Dr. Zilva, who retired in 1950, had worked at the Institute since 1913. Mr. White first entered the service of the Institute in 1899 and was appointed Secretary in 1926, a post which he held until his retirement in 1949.

STAFF

Dr. J. Thomas, Miss P. M. Gibson and Mr. F. W. Parrish were appointed to the Biochemistry department, Miss C. Shaw to the Blood Products Laboratory and Miss J. Campbell and Mr. D. Kerridge to the Guinness-Lister Research Unit; Mrs. N. D. Easterbrook was awarded a research studentship in the same Unit.

The University of London has granted recognition to Mr. B. G. F. Weitz and Dr. W. J. Whelan as teachers of Pathology and Chemistry respectively.

Mrs. N. D. Easterbrook, Miss A. J. Thompson, Miss P. A. Turner and Mr. C. Quadling resigned during the year.

In October, 1956, Professor A. A. Miles served on the Expert Committee on Biological Standardisation of the World Health Organisation held in Geneva.

Professor W. T. J. Morgan and Dr. W. M. Watkins attended the Congress on Human Genetics held in Copenhagen, August, 1956.

At the invitation of the Pan American Sanitary Bureau, Dr. D. McClean attended, as a consultant, the Seminar on Smallpox held in Lima, Peru, in August, 1956.

Mr. B. G. F. Weitz attended the Conference on Zoonoses, organised by the East African Medical Research Scientific Advisory Committee in Kampala, Uganda, in January, 1956, and at the invitation of the Committee, opened the discussion on Trypanosomiasis. Mr. Weitz also visited the Reyfik Saydam Central Institute of Hygiene, Ankara, Turkey, as consultant on behalf of the World Health Organisation, in connection with the problems of antitoxin production in Turkey.

Dr. E. Nobel took part by invitation in a Symposium on L-forms held in Lausanne, May, 1956.

VISITORS

The following visitors worked for short periods in the Institute laboratories: Dr. R. Arevalo, Director, Public Health Laboratory, San Salvador; Dr. B. J. Bines and Mr. G. Jones, University College of North Wales, Bangor; Dr. S. Cmelik, Central Institute of Hygiene, Zagreb; Dr. J. P. Dempster, Imperial College of Science, London; Mr. J. S. Gale, Department of Genetics, Cambridge; Dr. S. Govindarajan, King Institute, Guindy, Madras; Dr. Y. Hamon, Institut Pasteur, Paris; Mr. O. W. Heal, Durham University; Dr. S. Iseki, The Gunma University, Japan; Dr. M. J. Naumovic, Institute of Hygiene, Belgrade. Dr. G. Pontier and Dr. F. Sgambati, Instituto di Microbiologia, Napoli; Dr. R. Pournaki, Institut Pasteur, Teheran; Dr. W. Sackman, Veterinär-bakteriologisches Institut der Universität, Zürich; Dr. H. S. Sodhi, Mr. J. L. Fitton and Mr. C. C. Worrill, Evans Medical Supplies for Burma; Dr. J. Spasojevic, Higijenski Institut; N. R. Srbije, Yugoslavia; Dr. N. Tulga, Reyfik Saydam Central Institute of Hygiene, Ankara, Turkey.

The Governing Body notes with satisfaction the successful continuance of the researches under Professor Miles and Professor Morgan, made possible by the benefaction of the Nuffield Foundation. Acknowledgment is also due to the Foundation for its generous action in renewing the yearly grant to Professor Morgan, for a further period of five years.

The Blood Group Research Unit and the Blood Group Reference Laboratories are still accommodated at the Institute, and Miss E. M. Hume continues to edit Nutrition Abstracts and Reviews on behalf of the Medical Research Council and the Commonwealth Bureau of Animal Nutrition, Aberdeen.

MICROBIOLOGY

PROTOZOOLOGY

Antigenic Structure of Trichomonas. Dr. Robertson has continued her study of the antigens of *Trichomonas* species of protozoa by the Ouchterlony precipitin technique in agar gels. The two serological varieties of *T.fœtus* distinguishable by the agglutination of the live organisms have been found in the gel diffusion plates to have many antigenic components in common. The polysaccharide antigens of the two varieties, isolated by the method of Dr. Feinberg and Dr. Morgan (1953), are however distinct. *T.fœtus* and *T.vaginalis* appear to have few antigens in common. The Ouchterlony method is useful in studying aberrant strains of doubtful specificity.

Cytology of Amœbo-flagellates. Mr. Pittam has continued his study of soil amœbæ, specially *Nægleria gruberi*, an organism whose flagellate phase can be induced at will by diluting laboratory cultures with weak sodium phosphate solutions. A glass-slide method of culture was devised which permits of the rapid fixation of the organisms *in situ* for histochemical or microanatomical study. The cell ribonucleates and desoxyribonucleates have been located. The changing distribution of the mitochondria during the transformation from amœboid to flagellate form, and the peculiarities of the nucleokaryosome are being studied.

He has also discovered a method of producing giant multinucleate *Nægleria*, which have certain advantages as objects of study.

WHOOPING COUGH BACILLUS

Assay and Identification of Protective Antigens. Mr. Standfast and Dr. Horton have continued their work on the two antigens of *Hæmophilus pertussis* protective for mice and the two antibodies they elicit (1955 Report). The two antigens can be distinguished by active and passive protection tests in mice as determined by the intracerebral and intranasal routes of challenge.

Assays of vaccines carried out using the intranasal and the intracerebral routes of challenge do not arrange protective vaccines in the same order of potency. It is now known from the Medical Research Council clinical trials (M.R.C. Report, British Medical Journal 1956), that protective potency by the intracerebral assay in mice corresponds to that determined by clinical trials in children.

Attempts have been made to isolate the two antigens and hence to obtain pure antisera, and to find an *in vitro* test that will distinguish them satisfactorily; so far without real success.

Distinction of protective antigen and other antigens. Histamine-sensitizing factor. The histamine-sensitizing antigen (HSF) has proved to be distinct from the antigen protecting mice against an intracerebral challenge (Dr. Horton, 1956 Report), but there remained the possibility that HSF was the antigen

protecting mice against an intranasal challenge. This is not the case; the HSF is distinct from both 'intranasal' and 'intracerebral' protective antigen.

Sublethal Intranasal Test. In addition to the 'Intracerebral Mouse Test' and the 'Intranasal Mouse Test', the 'Sublethal Intranasal Mouse Test' has been used in Australia and Denmark. In this test the mice are killed after a sublethal challenge and the numbers of viable *Hæmophilus pertussis* in the lung are counted. The work started with Dr. Thow has been continued. The results suggest that both the 'intracerebral protective antigen' and the 'intranasal protective antigen' may be involved in this test. The antibody to one brings about the sterilization of the lung, either by preventing the primary lodgement of the organism or by causing its total elimination—probably the latter, because prevention of primary lodgement appears to be a property of antitoxin. Antibody to the other antigen, though unable to stop the organism lodging and growing in the lung, in some way prevents its growth in the lung to the point where a pathological condition results.

TYPHOID BACILLUS

Assay of Protective Antigens. Mr. Standfast has continued the investigation of laboratory assays of potency of *Salmonella typhi* vaccines, as part of a collaborative study initiated by the World Health Organisation. Various methods of assay have been used, both active and passive, on two vaccines tried in the field. Although their behaviour in man was different (Ikic, Rep. 2nd Congr. int. Biol.Stand. Rome, 1956, p. 311), these vaccines could not be distinguished by any of the commoner laboratory tests. They were however distinguishable in the same sense as by the field trial, by a newly-devised test in which a Vi-negative strain of *S.typhi* was used for the challenge dose.

INHERITANCE IN BACTERIA

Inheritance of Flagellar Characters, and of Flagella. Dr. Quadling completed his investigation of the spontaneous appearance of rare motile cells in non-motile bacterial strains; he demonstrated the phenomenon in a considerable proportion of non-motile strains of species which are normally motile, but not in strains of species like *Salmonella gallinarum*, which are normally non-motile. He also showed that in flagellated bacteria induced to multiply without synthesizing new flagella, the existing flagella are handed on for many generations as active locomotor units. If they are amputated by violent mechanical agitation, they will regrow, provided they have not been handed on for more than 2-3 bacterial generations; i.e., the flagella-synthesizing granule has a relatively short synthetic life, compared with the active life of the locomotor unit as a whole.

Madame de Margerie and Dr. Stocker have continued their investigation of abortive transduction of motility to a non-flagellated *Salmonella* strain in which the mutated gene causing inability to synthesise flagella is linked to a gene determining flagellar antigenic character. The gene for flagellar synthesis introduced by a phage particle is not replicated, but passes down one line of descent to a single descendant, which, because it synthesises several flagella, has in consequence several motile offspring, only one of which receives the gene.

These motile offspring are susceptible to the immobilizing action, not only of antibody specific for the flagellar antigen latent in the recipient non-motile strain, but also of antibody specific for the flagellar antigen of the motile donor strain; showing that the donor genes for flagella synthesis and for antigenic specificity of the flagella, which are linked on the genic fragment carried by the transducing phage, are transmitted in the linked state for many generations.

Mating-types and Gene Recombination in Escherichia coli. Studies of gene recombination in *Escherichia coli* have hitherto been complicated by the influence of parental mating type. The mating type of a culture is characterised by its fertility with other cultures, by its possession or lack of a transmissible agent F (which makes its host fertile), and by its susceptibility or resistance to 'infection' by such an agent. Using these criteria, Mrs. Bernstein has studied nine strains of *E.coli* and identified at least one new mating type in each strain examined.

There are diverse mechanisms determining mating type, some being easily defined genetically and others being complicated by the presence of an F agent. Besides cell mating-type differences, Mrs. Bernstein found that F agents themselves differ in many respects (Report, 1956), not only determining the fertility of the host but also affecting frequencies of gene recombination. Mrs. Bernstein also found that the zygote, or fertilisation nucleus, can form in either parent in the mating pair but that often a majority will form in one parent rather than the other, depending on the mating types of the parents. In many crosses, one parent appears to contribute the 'lion's share' of genes to the recombinants, but there is no consistent correlation between the parent making the major genetic contribution and the parent in which the zygote usually forms.

Colicinogenesis in Salmonella *spp.* Many coliform bacteria produce colicines, substances which kill certain strains of coliform bacteria; such antibiotic producing strains are called colicinogenic. When a non-colicinogenic strain is grown in a mixture with a colicinogenic strain, some of its cells may acquire the colicinogenic property. The mechanism of this 'infective heredity' is uncertain. If the colicinogenic property is determined by a chromosomal gene, it should be possible to transduce it from one strain to another by means of phage. As a first step in such an experiment Dr. Hamon grew various *Salmonella* strains (known to be suitable as donors of genetic material by transduction) in mixtures with colicinogenic coliforms, and isolated colicinogenic *Salmonella* variants. Dr. Stocker and Mrs. Easterbrook find that the transducing phage (PLT 22) multiplied feebly in these colicinogenic *Salmonella*. That is, the ability to synthesise a colicine interferes with ability to support the growth of phage. It is hoped that further study may elucidate the mechanism of this interference; and perhaps give some information on the possible evolutionary relationship between colicines, which seem to be proteins, and phages, which can be regarded as living organisms.

BACTERIAL PHYSIOLOGY

Flagellar Structure. Preparations of purified flagella from normal and 'paralysed' (flagellated but non-motile) strains of *Salmonella*, prepared by Mr. Porter and Dr. Stocker, have been examined by Dr. Beighton, of the University of Leeds, by X-ray diffraction; there were no major differences in the patterns obtained. The paralysis therefore probably does not result from inability of the flagellar protein to exist in the 'super-contracted' state.

Flagella from bacteria of other genera have been prepared for X-ray diffraction and other investigations; about 1% of the dry weight of a bacterial crop may be recovered as flagella.

Antigenic and Toxic Properties of Flagella. Dr. Stocker is collaborating with Dr. N. Datta and Dr. C. M. P. Bradstreet, of the Central Public Health Laboratory, Colindale, in the investigation of the antigenic properties of purified flagella. Like flagellated bacteria they are potent immunising agents; a dose of only 3×10^{-5} micrograms of purified *Salmonella paratyphi B* flagella stimulated antibody production in more than half the rabbits tested; the equivalent

number of flagellated bacteria (c. 3000) was also effective. Attempts to produce paralysis of the immunological response failed, for injection of flagella in milligram amounts killed new-born rabbits; this toxic property is being investigated.

Biochemistry of Flagella. Bacterial flagella may be regarded as bacterial muscles, and by analogy with other forms of muscle might be expected to get their power from the break-down of high-energy adenosine triphosphate. Mr. Kerridge has tested *Salmonella* flagella for ability to catalyse the break-down of this substance, but so far with negative results; in this respect bacterial flagella differ both from myosin obtained from muscle in higher organisms, and from the flagella of algæ and of fish sperm. This lends weight to the contention that bacterial flagella represent an independent line of evolution.

Synthesis of Flagella. Bacterial flagella are known to consist of a protein of an unusual amino-acid composition. Flagella may be amputated in live bacteria, which become non-motile (see Report, 1956). The return of motility to deflagellated cultures indicates the synthesis of the specific flagellar protein. Mr. Kerridge, assisted by Miss Campbell, is using this index to test the ability of various nutritionally-exacting mutant strains of *Salmonella* to synthesise flagellar protein in media lacking the relevant essential growth-factors. A tyrosine-requiring mutant did not recover motility unless provided with tyrosine; and a leucine mutant behaved similarly. By contrast, both histidine- and tryptophan-requiring mutants regained motility even in media lacking these amino-acids (i.e., media which would not support their growth). This is consistent with the reported presence of leucine and tyrosine in flagellar protein, and the absence of histidine and tryptophan.

Bacterial Growth and Division. Mr. Perret has extended his studies of the relationship between bacterial growth and cell division. When *Escherichia coli* is grown in a synthetic medium with glucose as the growth-limiting nutrient, cell division continues after the optical density of the culture has reached its maximum. This 'stationary-phase-multiplication' can be observed directly under the microscope; it is also revealed indirectly by an increase in the number of bacteria per unit volume of culture. A similar phenomenon occurs when bacteria growing in broth are rapidly and thoroughly washed free of nutrients and resuspended in suitable solutions of inorganic salts; during a subsequent period of one hour at 30°C. the optical density of the suspension slowly falls, but the total count of bacteria may increase by more than 60%. These observations seem to support the hypothesis (Report, 1956, p. 9) that a bacterium can complete the later stages of cell division without drawing on external carbon or energy sources.

Continuous cultures at constant population density occasionally manifest behaviour contrary to that predicted by the accepted mathematical theory of the system. Mr. Perret has continued a critical analysis of the basis of that theory, and concludes that the anomalies arise from the use of an over-simplified kinetic model of growing bacteria. The total bacterial mass in a growing culture must be treated as behaving kinetically not as a one-stage chemical reaction, nor even as a linear open system capable of existing in the steady state, but as a reticular expanding system capable of existing in the 'exponential state'. This more complex model appears to have properties which are closer to experimental reality, but which also cast doubt on some common assumptions in bacterial physiology. For instance, there appear to be no grounds for assuming that the concentrations of all intracellular molecular species maintain constant mutual ratios during the logarithmic phase of batch culture; or for rejecting the concept of a type of 'master reaction'.

NON-SPECIFIC URETHRITIS IN MAN

Dr. Klieneberger-Nobel, with the assistance of Miss Card and Mr. Blyth, has continued the investigation of the ætiology of non-specific urethritis, undertaken on behalf of the U.S. Public Health Authority, in collaboration with Dr. G. W. Csonka of St. Mary's Hospital, and Dr. J. K. Oates and Dr. R. D. Catterall of the Whitechapel Clinic.

The Role of Pleuropneumonia-like Organisms (PPLO). The association of non-specific urethritis, in a substantial proportion of cases, with the presence in the genital tract of a peculiar type of pleuropneumonia-like organism (PPLO) has been more firmly established (*cf.* Report, 1955). Ninety-seven strains have so far been isolated, all of the same morphology and growth type in artificial culture; and all of the 50 odd strains so far tested belong to the same serological group (as indicated by a complement fixation test with selected rabbit antisera), which is distinct from that of PPLO of animal origin.

The PPLO was found in 32% of males, and 90% of females with acute gonorrhœa; and in 47% of males and 81% of females with acute non-gonococcal urethritis, vaginitis or cervicitis. It occurred only in a few of a large number of patients with other pathological conditions. Urinary sediments proved in some cases to be a more fruitful source of PPLO than urethritic discharge. Of 100 adult male controls tested in this way, only three were positive.

Susceptibility of Human PPLO to Antibiotics. Mr. Blyth is investigating the susceptibility of different strains of PPLO to antibiotics. All strains tested were susceptible to antibiotics of the tetracycline group in concentrations as low as 0.5 μg./ml. of medium. Streptomycin in concentrations of 16μg./ml. and above largely inhibited the growth of all strains except two. However, all the relatively sensitive strains showed a small proportion of resistant colonies, which, when tested separately, withstood concentrations of up to 250 μg./ml. of streptomycin.

Serology of Non-Specific Urethritis. Miss Card has made a survey of human sera for complement-fixing antibodies against the human PPLO. The incidence of positive sera among patients attending venereal clinics is 44% in women and 17% in men. Among children and healthy adult blood donors the incidence is only 2%. The distribution of antibody in general reflects the distribution of genital infection with PPLO; and the distribution of both is consistent with the view that this PPLO is at least associated with a venereal disease.

Cytopathogenicity of PPLO. The frequent recovery of PPLO from patients with non-specific urethritis raises the question of the action of PPLO on living cells *in vivo* and *in vitro*. Mr. Blyth is studying the action of PPLO on tissue cultures, and has shown that they can multiply in human amnion cells. He is also investigating their pathogenicity for rhesus monkeys. Dr. Collier found that some stock lines of human carcinoma cells (Hela strain) maintained in different laboratories are contaminated by PPLO, and that this contamination is transmitted with the Hela cells during repeated subculture of the latter, without, however, producing gross cytopathogenic effects.

VIROLOGY

The establishment of a full range of tissue cultures for the virus researches of the Institute has continued. Dr. Collier and Mr. Blyth have worked on the propagation and maintenance of various human and animal cells in tissue culture, including the adaptation of a line of human carcinoma cells (Hela strain) to growth in media containing horse serum.

TRACHOMA

In collaboration with Dr. C. Smith of the Institute of Ophthalmology, Dr. Collier has begun a study of the ætiological agent of this disease. Material from cases of trachoma is now being received by air from Gambia. Attempts are being made to determine the best method of maintaining the viability of the virus during transport by testing it in rhesus monkeys on arrival. Concurrently, the material is inoculated into various tissue cultures and laboratory animals in the hope of obtaining evidence of viral proliferation.

MEASLES

Dr. Collier has begun an examination of the behaviour of measles virus in tissue culture.

VIRUSES AND NON-SPECIFIC URETHRITIS

Dr. Collier has examined urethral smears from over 50 male cases for the presence of inclusion bodies, the material being provided by Dr. G. W. Csonka of St. Mary's Hospital, and Drs. J. K. Oates and R. D. Catterall of the Whitechapel Clinic. Although cytoplasmic inclusions were found in several cases, none showing the classical appearance of inclusion urethritis were encountered. This work will be continued, together with attempts to isolate infective agents by inoculation into tissue cultures.

VACCINIA VIRUS

Irradiated Vaccines. Work recorded in recent Reports on the inactivation of vaccinia virus by ultra-violet irradiation without destruction of its immunizing properties has been continued. Technical difficulties were encountered in freeze-drying batches of inactivated virus in a form suitable for human immunization. These difficulties have now been overcome and it is hoped in the near future to have sufficient material for trials in human volunteers.

Dr. McClean and Dr. Kaplan are continuing their collaboration with Mr. H. J. M. Bowen and Mr. T. Horne of the Atomic Energy Research Establishment, Harwell, on the antigenicity of dried vaccinia virus irradiated with gamma rays. Inactivation by gamma rays may be directly proportional to exposure, i.e., a first order reaction. Complete inactivation curves for this virus are being constructed as an essential preliminary to experiments on the antigenicity of the irradiated virus.

In contrast, the inactivation of vaccinia virus by β-propiolactone is not directly proportional to the exposure to this substance, and the degree of inactivation varies widely in successive experiments—a result similar to that predicted by Sven Gard from his studies of the inactivation of poliomyelitis virus by formaldehyde.

Dried Smallpox Vaccine. Dried smallpox vaccine that will resist the hazards of transport and storage in tropical temperatures and which can be used in such climates to maintain reserves of potent vaccine for use in sudden epidemics is now in general use.

The results of the combined laboratory and clinical trial of dried vaccines sponsored by the World Health Organisation have been published. The trial on the dried vaccine produced by the Lister Institute has been extended by Dr. McClean and Dr. Kaplan in collaboration with Wing Commander R. M. Cross, R.A.F., and Professor A. W. Downie and Dr. K. R. Dumbell of the Department of Bacteriology, Liverpool University. After two hours exposure at 100°C., or after two years storage at 45°C., this vaccine still produced 100 per cent. successful vaccinations in the field.

Vaccine Production in Tissue Culture. The successful development of tissue culture methods for the routine preparation of vaccine would result in considerable economy in production and a vaccine free from contaminating bacteria and the other products of tissue inflammation. The growth of vaccinia virus in cultures of bovine skin, by the methods developed in Sweden, has yielded concentrations of virus too low for bulk vaccine production. Moreover, the supply of bovine embryos in this country is too irregular to permit the use of this method for vaccine production. Dr. McClean, Dr. Kaplan and Miss Thomas have accordingly investigated the suitability of chick embryo cell cultures for this purpose. In simple stationary cultures of cells obtained by tryptic digestion of embryo tissues, virus titres of about 10^8 infectious units/ml. can be obtained using a rabbit-adapted strain of virus as seed. The optimum conditions for large yields from such cultures are under investigation.

Titration of Vaccinia Virus. With Dr. G. Belyavin of University College Hospital Medical School, Dr. Kaplan has completed a statistical study of the titration of the virus by pock counting in the chorio-allantois of the chick embryo. Although this is the most sensitive titration generally available and is more reliable than titration in the rabbit skin, it was found that counts did not conform to theoretical expectations and that no valid estimate of the overall coefficient of variation was possible. Nor was it possible to assess the intrinsic reproducibility of the test.

Dr. Kaplan is investigating the titration of vaccinia virus by intravenous injection of chick embryos and, with Miss Thomas, by plaque counts in monolayer cultures of chick embryo cells.

Reproductive Cycle of Vaccinia Virus. Adequately quantitative work is impossible in the chick chorio-allantoic membrane because the number of cells infected with virus cannot be determined. Dr. Kaplan has therefore transferred these studies to monolayer cultures of chick embryo cells. Results so far obtained have supplied information relevant to the problems of vaccine production in tissue culture and should provide information about the time relations of the appearance of various antigens in the reproductive cycle of the virus.

BACTERIOPHAGES

Antiviral Antibiotics. Dr. and Mrs. Asheshov continued their investigation on antiphage substances produced by actinomycetes, in the hope that they may prove useful in the study and control of animal viruses.

In the light of evidence (Report, 1956) that rutilantins, produced by actinomycete A220, have a significant action on influenza virus in the chick embryo, their production on a larger scale in a purer form, for more extensive trials, was undertaken in collaboration with the Antibiotic Research Station (Medical Research Council, Clevedon, Somerset). A close and continuous collaboration between the two units has been established, Clevedon carrying out the chemical processing. The actinomycete A855y, though different from A220, produces substances that have proved to be either identical with, or very closely related to, rutilantins. Further work on this mould has been deferred until the fuller investigations of rutilantins are concluded.

The actinomycete A803 has been studied in detail. This mould seems to produce at least three active substances—(1) an antiphage substance chemically related to rutilantins but having an entirely different range of antiphage activity; (2) a substance of different chemical character and biological activity with a limited but pronounced antiphage activity; and (3) a substance with a very wide range of antibacterial activity (including Gram-negative organisms).

The isolation of these substances for preliminary biological tests is under investigation.

Virulence of E. coli *Bacteriophages.* Mrs. Easterbrook is searching for a 'temperate' phage related to the T2,T4,T6 group of *E. coli* phages which, unlike their bacterial host, and unlike animals, plants and other viruses, contain hydroxymethyl-cytosine in their deoxyribonucleic acid (the substance which it is believed determines hereditary properties). The known phages of this group are all 'virulent'—always killing their bacterial host. A 'temperate' phage of this group, i.e., one capable of setting up a hereditary symbiotic relationship with its bacterial host (lysogenicity), would be of considerable interest, as a means of testing whether the possession of hydroxymethyl-cytosine is necessarily associated with virulence of bacteriophage. So far, 49 temperate phages have been isolated from 300 coliform strains tested; none of them, however, was serologically related to the T2,T4,T6 group.

IMMUNOLOGY AND SEROLOGY

DISTRIBUTION OF ANTIBODY IN PLASMA PROTEINS

Animal Sera. Dr. Mackay has completed the first part of her investigation into the identification of bacterial agglutinins with the electrophoretic components of sheep serum, which was described in a previous Report.

A Tiselius apparatus with a long centre section cell allowed a more critical appraisal of serum fractions produced by precipitation with ether from immunized sheep, and showed the presence of gamma globulin in the G2 which contained both 'natural' and 'induced' agglutinin.

Efforts were made to separate the components of G2 by sub-fractionation; both G2/1, electrophoretically 45% alpha globulin, 41% beta globulin and 13% gamma globulin, and G2/2, 44% beta globulin and 55% gamma globulin, were obtained. Agglutinin titration showed that the 'natural' antibody was equally distributed between the two fractions, but the ratio of 'induced' antibody in G2/1 to G2/2 was 1 to 4.

The results suggested that the 'natural' antibody was associated with beta globulin, and the 'induced' antibody with gamma globulins. This was confirmed by the presence of 'induced' agglutinin only in G3, which is 90% gamma globulin, and contains no beta globulin.

Human Sera. Dr. Maycock, Miss Turner and Mr. Rodican, have begun an investigation of the partition of staphylococcal *a*-hæmolysin antitoxin and diphtheria antitoxin which occurs during the small and large scale ether fractionation of human plasma.

SEROLOGICAL IDENTIFICATION OF INSECT BLOOD MEALS

Tsetse Flies. Mr. Weitz and Miss Lee-Jones have continued the study of natural feeding habits of tsetse flies, made in co-operation with the staff of the East Africa Tsetse and Trypanosomiasis Research and Reclamation Organisation. They have confirmed many of the results obtained in 1956. At that time samples were obtained from engorged flies caught by the standard methods of baiting and the catches contained only 3 per cent. females instead of the 50 per cent. expected. Dr. J. P. Glasgow and Dr. P. Isherwood at Shinyanga, Tanganyika, have developed a new method of catching engorged flies from their resting places very soon after feeding. This method regularly yields equal numbers of male and female flies. In order to determine the validity of the results derived from the old method of catching, tests were made of blood meals of monthly catches of *G. swynnertoni* from a specified area made by both the new and old

methods: the same feeding habits are indicated by both methods. There appear to be no seasonal differences in feeding habits.

In all, some 5,000 blood meals were identified.

Mr. Weitz is investigating the antigenic structure of trypanosomes. At present the work consists chiefly in devising suitable techniques for the study.

Mosquitoes. About 50,000 identification tests have been made on mosquito stomach contents, chiefly on behalf of malaria control schemes.

ANTITOXIN PRODUCTION

Refinement of Therapeutic Antitoxins. Dr. Dolby has completed his studies on the adsorption of inactive material from pepsin-refined antitoxic horse sera; and activated fullers' earth is now in routine use for this purpose in the large-scale production of antisera.

Miss Lanham has undertaken an investigation of the loss of antitoxin occurring during the various stages of the refining process. In this connection she has compared different methods of preparing pure samples of albumin and globulins from horse serum by fractionation; and has investigated the conditions for obtaining on filter paper a constant dye uptake by the various fractions with a view to making quantitative analyses of whole sera by the paper electrophoresis technique.

Proteolytic Enzymes for Refining Antisera. In the course of further attempts to separate fractions from preparations of the enzyme pepsin with optimal activities on hæmoglobin at pH 2 and 3.2, Dr. Dolby has tried the effect of partial inactivation of the enzyme by heating it, either alone or in the presence of sodium cyanate. Activity at both pH's is lost at the same rate. Adsorption on various materials under differing conditions of salt concentration and pH have also failed to separate the activities.

Experiments by Miss Lanham are in progress on the kinetics of peptic digestion of the albumin and globulins separated from horse serum.

EXPERIMENTAL PATHOLOGY

MECHANISMS OF INFLAMMATION

Dr. Wilhelm, Professor Miles, Miss Sparrow and Mr. Mill continued their work on substances that increase capillary permeability, particularly the globulin substances already identified in mammalian sera.

Permeability Factors in Mammalian Sera. The permeability factor, activable by dilution, and its inhibitor, present as such in guinea-pig serum, are not substantially changed during streptococcal bacteræmic infection or during shock induced by the histamine-liberator, compound 48/80.

Dextran sulphates, with molecular weights of 150,000-1,000,000 and sulphur contents of more than 10 per cent., induce anaphylactoid shock in guinea-pigs, and in the serum from such animals, the permeability factor cannot be activated by dilution. *In vitro*, dextran sulphate prevents the activation of the precursor of the permeability factor.

The permeability factor in guinea-pig and rat serum is an α-globulin, that in rabbit and human serum is a β-globulin. All are active in guinea-pigs, rats and rabbits, but their potency varies considerably according to the test animal. In general, the guinea-pig, rat and rabbit permeability factors are most potent in the homologous species. The intravenous injection of large, shocking doses of the guinea-pig permeability factor into guinea-pigs, induces no histological changes detectable 48 hours later.

Rat serum contains an inhibitor of the rat permeability factor, and like the corresponding guinea-pig inhibitor, is an *a*-globulin with low inhibitor potency. The rat serum inhibitor antagonises the rat and guinea-pig permeability factors in rats, but not in guinea-pigs, whereas the guinea-pig inhibitor antagonises both permeability factors in each species.

Mr. Mill prepared essentially homogeneous preparations of human permeability factor with a relatively high and consistent potency. Tested in guinea-pigs, the human permeability factor, like those from the guinea-pig, rat and rabbit, is highly susceptible to soya bean trypsin inhibitor, unaffected by the antihistamine drug mepyramine maleate, and like histamine, is moderately antagonised by sodium salicylate. An inhibitor fraction was separated from human plasma, but the active component was not identified.

The Pharmacology of the Serum Permeability Factors. Dr. Sparrow demonstrated that preparations of dialysed guinea-pig serum diluted in saline induce a delayed slow contraction of the isolated guinea-pig's ileum. The best response was obtained with dilutions of 1/90, and is substantially similar for dilutions held at 34°C. for 10-90 min. The contractor substance is antagonised by small doses of soya bean trypsin inhibitor, by high doses of the fraction containing the guinea-pig serum inhibitor, and partly antagonised by high doses of sodium salicylate. It is unaffected by mepyramine maleate and the protease inhibitor in ovomucoid.

The guinea-pig serum fraction, G2/1R, with high permeability-increasing potency, has little effect on the isolated guinea-pig's ileum, but induces a delayed slow contraction of the isolated rat's uterus; this effect also is antagonised by soya bean trypsin inhibitor and by the guinea-pig serum inhibitor.

Dr. Sparrow investigated the effects of these serum preparations and of histamine on the isolated mammalian heart. Small doses of histamine markedly increase the amplitude and slightly increase the rate of contraction; and moderately increase the volume of perfusate passing through the coronary vessels. Similar but less marked effects are induced by guinea-pig serum. The guinea-pig serum fraction, G2/1R, has no substantial effect; and even after incubation with guinea-pig serum, the mixture has less effect than guinea-pig serum alone.

The guinea-pig and rat serum fractions with high permeability-increasing potency induce hypotension in guinea-pigs, rats and rabbits, but not always in proportion to their permeability-increasing potency.

Other Permeability Factors. Other permeability factors, e.g., histamine, 5-hydroxytryptamine and compound 48/80, like the serum fractions, also vary considerably in potency according to the species of test animal. Histamine is highly active in guinea-pigs and rabbits, and 5-hydroxytryptamine and 48/80 in rats. Dr. Sparrow and Dr. Wilhelm demonstrated that, in the rat, the susceptibility of 5-hydroxytryptamine and compound 48/80 to inhibition by locally injected mepyramine maleate and lysergic acid, and the results of perfusing isolated rat hindquarters or treating pieces of excised rat skin with the permeability factors suggest that 5-hydroxytryptamine increases permeability, at least in part, by liberating histamine; and that 48/80 does so partly by liberating histamine and 5-hydroxytryptamine.

Mr. Mill found that the inflammation induced by chemical irritants develops more quickly than that induced by ultra-violet irradiation; and that the response to chemicals, unlike that to ultraviolet light, is inhibited by an antihistamine drug.

Permeability Factors in Therapeutic Antitoxins. Mr. Rodican has continued his investigations of substances in refined antitoxic horse sera that increase the permeability of blood capillaries in the rabbit. A method of assay with limits of error of $\pm 20\%$ has been developed. Chromatographic separation of the antitoxic preparations yielded several active fractions. Part of the active substance is isolable by precipitation with phosphotungstic acid. Virtually all the active material is contained in the non-protein portion of antitoxin and is produced during the pepsin treatment of the antitoxin.

MECHANISMS OF INFECTION AND DEFENCE

The Decisive Period in the Early Stage of Infection. The period of 4-5 hours, in which the fate of newly introduced bacteria is to a large extent decided, was deduced from the inability of substances damaging the local defences to affect the issue when the infection was more than four hours old. Professor Miles finds that local infections established for four hours or more are also insusceptible to augmentation of the defences by various non-specific stimuli, thus providing further evidence of the decisive nature of these early defences. Dr. Burke and Professor Miles completed their survey of vascular changes in the skin of the guinea-pig during the first few hours of local infection.

The Stimulation of Non-Specific Immunity. Increase in non-specific immunity is readily measured by titration of the challenge bacteria in the skin of the guinea-pig, in which several different bacteria can be tested simultaneously. Professor Miles finds that non-specific immunity rises with the injection of bacterial vaccines or endotoxins, with hyperthermia, and during the course of an ultimately fatal streptococcal bacteræmia. The immunity is largely indiscriminate, being effective against a variety of Gram-positive and Gram-negative bacteria, and therefore unlikely to be due exclusively to any one of the various humoral antibacterial factors that have been described as effective against either one or the other of these groups of pathogens. The analysis of this non-specific immunity continues.

Relation of Specific Antibacterial Immunity to Allergic Hypersensitivity. Dr. Cunliffe studied the effect on non-specific resistance to infection of the 'immediate' local anaphylactic reaction in the skin of guinea-pigs with a view to estimating the role of anaphylactic events in the defences of animals specifically sensitive to the invading pathogen. He also studied the changes in non-specific immunity, in specific immunity and in 'immediate' and 'delayed' hypersensitivity to intracutaneous *Brucella melitensis*, in guinea-pigs undergoing immunization with a Brucella vaccine.

PLASMA TRANSFUSION SUBSTITUTES

Dextran. Dr. Maycock and Miss Turner have continued the investigation of the antigenicity of dextran. Attempts to demonstrate the formation of antibodies in rabbits to small molecular weight dextran have not been successful. However, increases of nitrogen, precipitable by dextran, of the order of 20-50 μg./ml. have been observed in the sera of human volunteers injected subcutaneously with 1.0 mg. of highly purified dextran of molecular weight about one million. .

BIOCHEMISTRY

THE HUMAN BLOOD GROUP SUBSTANCES

The molecular nature of the water-soluble blood group substances. The demonstration by Professor Morgan and Dr. Watkins, that in the tissue fluids and secretions of group AB secretors a large proportion, if not all, of the macromolecules of blood group substances possess both A and B specificities (Report 1955), has been extended to the H and Le^a serological properties, when these occur in the secretions with A or B substances. By the use of animal and plant specific precipitating reagents there were found in group A_2 persons, whose secretions possess both A and H specificity, molecules which carry both these properties and others which carry H without A specificity. Similarly in ALe^a (or BLe^a) individuals some free Le^a molecules and others which have both A and Le^a (or B and Le^a) specificity appear to be present. These observations suggest that the blood group genes function at a late stage in the biosynthesis of the mucopolysaccharide molecules and compete for a common preformed substrate.

Inhibition of cross reactivity with anti-Type XIV pneumococcus serum. Inhibition reactions with simple substances have been widely used to study the relationship between chemical structure and serological specificity and, in general, the preferential inhibition of a precipitation reaction can be taken as an indication that a similar structure is present in the antigen under investigation. The A, B and H blood group substances, when slightly degraded, precipitate with anti-Type XIV pneumococcus horse serum and this cross reactivity was believed to be due to the presence of both galactose and *N*-acetyl glucosamine in the Type XIV polysaccharide and the blood group substances. Professor Morgan and Dr. Watkins therefore tested the capacity of three known β-linked disaccharides of galactose and *N*-acetyl glucosamine, and a number of other sugars, to inhibit cross precipitation. *O*-β-D-galactosyl-(1→4)-*N*-acetyl-D-glucosamine gave considerable inhibition whereas the other two disaccharides possessing 1:3 and 1:6 linkages respectively were scarcely more active than galactose alone. It was therefore inferred that cross reactivity is attributable, at least in part, to the presence of β-linked 1→4 galactosyl *N*-acetyl glucosamine units.

Microbial Enzymes and Blood Group Substances. In blood group studies known disaccharide structures are required for serological inhibition experiments and to serve as model substrates in enzyme reactions. Dr. Watkins has examined the capacity of the enzymes in extracts of the protozoan flagellate *T.fœtus* to synthesise disaccharides by transglycosylation.

By incubation of the extracts with melibiose and *N*-acetyl glucosamine a new disaccharide was produced which from its chromatographic behaviour appears to be *O*-α-D-galactosyl- (1→6)-*N*-acetyl glucosamine. Replacement of *N*-acetyl glucosamine by *N*-acetyl galactosamine leads to the formation of a second disaccharide. Attempts to isolate these compounds in sufficient quantities for chemical identification and serological examination are in progress.

Miss Gibson and Dr. Watkins are examining the conditions for the maximum separation, by zone electrophoresis on starch columns, of the enzymes in extracts of *T.fœtus* which destroy the serological activity of the blood group B and H substances.

A new method for the preparation of blood group substances. An alternative procedure which does not involve the use of phenol for the isolation of the specific blood group substances is long overdue, because with one exception, almost all the group specific mucopolysaccharides described in the literature

which have been thoroughly examined by chemical, physical and immunochemical methods have been treated with phenol at some stage in their preparation, and may all therefore to some extent have been modified. Miss Lawton and Professor Morgan have elaborated a method for the isolation of blood group substances from ovarian cyst fluids, saliva, etc., which uses the powerful proteolytic activity of the enzyme ficin at pH 7 to remove the non-specific protein, leaving group substances to be recovered by dialysis and simple fractionation from aqueous solution by inorganic salts or organic solvents. Blood group materials prepared by this method have the same qualitative and quantitative composition and specific activity as have those prepared earlier by the phenol procedure. However, the solubility of the ficin treated materials in 90% phenol, their low viscosity and their behaviour in the ultracentrifuge, reveal that the group specific materials after treatment with ficin are more polydisperse and of a smaller molecular size, although remaining electrophoretically homogeneous.

Enzymes Solubilizing water-insoluble mucopolysaccharides. Mr. McLoughlin and Professor Morgan have continued their studies on the solubilization of water-insoluble mucopolysaccharides at pH 7 by the action of the enzyme, ficin. Although the underlying process which brings about the solubilisation has not yet been established, much progress has been made in determining the best conditions for bringing into solution a number of biological systems such as gels of paramucin, stomach mucus and mucus epithelium, with the result that several large preparations of human blood group substances have been successfully obtained from materials which were completely intractable before enzymic treatment.

Analysis of Blood Group A Substance: The products of partial acid hydrolysis. In an attempt to learn more of the detailed chemical structure of human blood-group substances and the relationship of structure to serological specificity, the products of the partial hydrolysis of these important materials have been examined in some detail by Dr. Côté and Professor Morgan. Of the disaccharides liberated two, *O*-β-D-galactopyranosyl-(1$\rightarrow$3)-*N*-acetylglucosamine and the corresponding disaccharide containing a (1$\rightarrow$4) linkage, were readily identified as they had been isolated earlier from the carbohydrate components of human milk and meconium. Further disaccharide units were isolated and identified as *O*-β-*N*-acetyl-D-glucosaminoyl-(1$\rightarrow$3)-D-galactose and the corresponding *O*-α-*N*-acetyl-galactosaminoyl-galactose, and a material which is most probably L-fucosyl-(1$\rightarrow$6)-*N*-acetyl-D-galactosamine. Other disaccharide units remain to be isolated and identified. The examination of these hydrolysis products in agglutination inhibition tests showed that *O*-α-*N*-acetyl-D-galacto saminoyl-(1$\rightarrow$3)-galactose is a structure more closely associated with blood group A specificity than any that has been identified so far.

Dr. Crimmin has studied the rate of liberation of fucose from the blood group substances by the action of mild acid. The fucose was separated from the other products of hydrolysis by adsorption chromatography on charcoal-celite columns. The results indicated that there was a rapid release of fucose during the first 12 hours but that ultimately the whole of the methylpentose present was liberated.

Dr. Crimmin and Professor Morgan have examined the purest preparations of blood group substances for the presence of sialic acid and have found in most instances between 2 and 3% of this substance. The significance of the presence of sialic acid is not known but it is possible that a small amount of this material in the group mucopolysaccharides might be responsible for the virus inhibiting capacity of some preparations of blood group substances.

The quantitative determination of amino-sugars. In the course of investigations involving the quantitative paper chromatography of hexosamines present in the specific blood group substances, Mr. Ruszkiewicz observed that contrary to previous reports, the two hexosamines, glucosamine and galactosamine, give different colour intensities per unit weight when determined by the method of Elson and Morgan. Carefully purified galactosamine and a synthetic specimen of this material gave 90-92% of the colour intensity developed by an equal weight of glucosamine.

Plant seed hæmagglutinins. Lt.-Col. Bird and Professor Morgan have been attempting to purify the anti-A hæmagglutinin prepared from the seeds of *Dolichos biflorus* and first described by Colonel Bird. Fractional precipitation of the seed protein from aqueous solution with ethanol at low temperatures has given highly active and specific hæmagglutinin preparations, which are yet to be completely characterised. Lt.-Col. Bird is making a serological evaluation of these agglutinins which have already found application in routine blood grouping, and in blood group research. The purified preparation is also a powerful and specific precipitin, of potential value in the study of the blood group substances.

CARBOHYDRATE STUDIES

Starch-metabolising Enzymes. Dr. Whelan and Dr. Walker have investigated several of the starch-metabolising enzymes, using radioactive sugar substrates. The first such enzyme was D-enzyme, found in potatoes. This has the property of splitting starch-like molecules ("donor" substrate) into two portions, then joining one portion to an "acceptor" substrate which may be any of a number of sugars, including radioactive glucose. It was not known which portion of the donor substrate was joined to the acceptor, nor the nature of the linkage used in joining the fragments. The problem has now been solved by using radioactive glucose as the acceptor and small starch-like glucose polymers (maltodextrins) were prepared, in which all or only one of the glucose units is radioactive. The radioactive maltodextrins are now being used to determine how such molecules are split by enzymes and by acid and the pattern of breakdown by salivary α-amylase has been determined. In maltodextrin molecules containing more than one linkage, acid preferentially hydrolyses the linkage remote from the glucose unit carrying the free reducing group, i.e., the so-called non-reducing end linkage.

Although all linkages in a maltodextrin are of the same type, it is known that when a maltodextrin acts as a donor substrate for D-enzyme, certain of the linkages cannot be split. By using radioactive maltodextrins it was shown that there are two such resistant linkages, one at the non-reducing end and one penultimate to the reducing end of the maltodextrin.

Chemical Synthesis of Polysaccharides. Mr. Haq has developed a method whereby derivatives of glucose can be made to polymerize, yielding polymers containing up to 10 glucose units. The type of linkage can be controlled and a variety of polymers has been made, some previously unknown, others occurring in nature. These include β-1:6-linked glucoses, occurring naturally in a lichen polysaccharide; α-1:2 linked glucoses, a linkage that is also formed by the dextran-synthesizing enzyme; β-1:2-linked glucoses, occurring in a bacterial polysaccharide. The constitution of the α-1:3-linked diglucose, nigerose, has been established for the first time by chemical synthesis. This linkage occurs in dextran, in an *Aspergillus* polysaccharide and in a lichen polysaccharide.

The chemical synthesis of glycogen-like polysaccharides is in progress. They are intended for enzymic studies and will be free from many of the disadvantages of natural glycogen.

Periodate Oxidation and Structural Studies. Dr. Warsi has completed his studies of the periodate oxidation of monosaccharides. The oxidation, leading finally to a mixture of formic acid and formaldehyde, is a stepwise process in which the constituent carbon atoms are removed one at a time, beginning with the reducing carbon atom. This discovery promises to aid in the analysis of radioactive monosaccharides when it is desired to determine the radioactivity associated with each carbon atom.

The fungus *Pachyma Hœlen* was shown to contain 95% of a polysaccharide consisting entirely of β-glucose units joined through 1:3-linkages.

Molecular Weights of Polysaccharides. The controlled periodate oxidation of polysaccharides promises to be a very sensitive and accurate means of determining molecular weight. Mr. Parrish is investigating this method using a series of oligo- and poly-saccharides of known molecular size, which were obtained by enzymic synthesis.

Specificity of Carbohydrases. A slight modification in the structure of a carbohydrate, which can be effected by chemical methods, often impairs or destroys its susceptibility to attack by a given enzyme. Mr. Abdullah is studying the relative effects on enzyme action of modifying the various groupings present in starch-like molecules, with a view to discovering the manner in which these substances are attacked by carbohydrases.

PHOSPHOLIPIDS OF HEART MUSCLE

Acetalphospholipids. Dr. Macfarlane and Mr. Gray have continued their studies on the phospholipids of ox heart muscle. It has for long been considered that the main phospholipids in muscle and in brain are the classical glycerophosphatides, kephalin and lecithin, in which two fatty acids are esterified with glycerol, the remaining hydroxyl of which is esterified with phosphoric acid to which a nitrogenous base is attached. In the last few years it has become apparent that a large part of the 'lecithin' and 'kephalin' has not the classical structure but consists of acetal phospholipids, in which one of the fatty acid chains is replaced by a fatty aldehyde. These compounds have not yet been obtained pure and the exact nature of the aldehydogenic linkage is unknown. The work on this subject was begun from a consideration of the possible biochemical action of bacterial toxins on these substances, but it is clear that the structure, physiological function and biosynthesis of the acetal phospholipids is a problem of great general interest.

The first step was to obtain the acetalphospholipids pure, a matter of great difficulty owing to the similarity of their properties with those of the 'classical' glycerophosphatides with which they are associated. Mr. Gray developed a method of partition chromatography on secondary cellulose acetate in which only organic solvents are used, a method which should be of general interest for lipid chemistry. In this way samples of choline-containing acetal-phospholipid (acetal-lecithin) of about 80% purity have been obtained; the efficiency of the separation of acetal and classical lecithin is limited by the fact that both components are really families containing a variety of fatty acid residues; it may be possible to overcome this by variation in the solvent mixtures. Mr. Gray is also carrying out degradation studies by enzymic and chemical means to show conclusively, by comparison of the lyso lecithin with that formed from lecithin whether the aldehydogenic fatty group is on the α- or β-position of the glycerol moiety.

Phosphatidic Acids. The constitution of cardiolipin, the complex phosphatidic acid used as an antigen in the Wasserman test, has also been studied.

This substance was considered by Pangborn to contain four glycerol, three phosphoric acid and six fatty acid residues, the fatty acids comprising approximately one oleic and five linoleic acid residues. Dr. Macfarlane and Mr. Gray have made several preparations of cardiolipin, whose serological potency was kindly determined by Dr. A. E. Wilkinson of the Venereal Diseases Reference Laboratory; the analysis of these preparations indicates the constitution as a complex containing three glycerol, two phosphoric acid and four fatty acid residues which would leave one free hydroxyl group on a glycerol residue. Moreover, examination of the mixed fatty acids by various techniques in this laboratory, and definitively by Dr. A. T. James of the National Institute for Medical Research, indicates that the fatty acid composition is more variable than suggested by Pangborn; linoleic acid is the principal component (60%), together with palmitoleic, oleic, linolenic and small amounts of C_{20} acids, making up at least 90% of unsaturated acids in the compound. Degradation studies to establish whether a free hydroxyl group is present are in progress.

During this work the inositol-containing glycerophosphatide described by Fauré was isolated from ox heart muscle, and its structure as a complex containing one molecule of glycerol, phosphoric acid and inositol and two fatty acid residues, one saturated and one unsaturated, was confirmed. This phosphatide, which constitutes about 5% of the total phospholipid of ox heart muscle, is also probably a family in which the unsaturated fatty acid residue may vary, and it is of peculiar interest in that these acids are highly unsaturated and include a considerable amount of arachidonic acid. As with the acetal-phosphatides, the function and biosynthesis of cardiolipin and the glycerophosphoinositide have yet to be elucidated.

BIOPHYSICS

HUMAN PLASMA PROTEINS

The antihæmophilic factor. Dr. Kekwick and Dr. Wolf succeeded in separating from human plasma a concentrate of anti-hæmophilic factor of sufficient potency to justify clinical trials. The method of separation involves a slight modification of the fractionation system using ether as a precipitant, devised by Drs. Kekwick and Mackay, by which at least 90% of the antihæmophilic activity of human plasma is now precipitated with the fibrinogen fraction. By simple ancillary procedures a further purification provides material having 20-25 times the potency of fresh plasma on a dry weight basis, which can be dried from the frozen state with negligible loss in activity. The dry residue retains its activity almost unimpaired for several months and dissolves readily after such a period giving 100 ml. of a solution equivalent in activity to 1,000 ml. of fresh citrate plasma. The possibilities for further purification are under examination.

With the co-operation of several clinical colleagues at hospitals in the London area, six patients were treated with the antihæmophilic factor preparation with considerable success up to Christmas 1956. The conditions encountered were hæmorrhages into the joints, muscles and stomach, prophylactic treatment preceeding single and multiple tooth extractions and a condition entailing radical surgery of the rectum and adjacent buttock tissue.

Christmas factor. Mr. Caspary is examining methods for the assay of Christmas factor. At present a modified thromboplastin generation test is being used, which has the advantage of requiring no plasma from patients with the disease, but which is accurate only to $\pm 25\%$. The prothrombin conversion ratio method can be used to assay this factor but requires plasma deficient in Christmas factor. A search for an artificial substrate is being made.

Normal plasma is being fractionated in the hopes of finding material suitable for therapeutic use. The prothrombin fraction and the globulin subfraction G2/1 have Christmas factor activity.

Mr. Caspary has also followed the level of Christmas factor in the plasma of patients with the disease during treatment by transfusion with normal plasma or serum, to obtain information about the persistence of the activity in the blood-stream and the effective dosage for the treatment during hæmorrhagic episodes.

Hypogammaglobulinæmia. During the year the Medical Research Council set up a sub-committee to arrange for the collection of information on cases of hypogammaglobulinæmia occurring throughout the country, with a view to establishing the authenticity of reported cases and to organising a clinical trial of prophylactic gamma globulin.

Dr. Kekwick, and later Mr. Vallet, undertook to establish by moving boundary electrophoresis the gamma globulin content of serum samples from suspected cases. These same samples were also assayed at two other centres by serological methods which if they correlate well with the necessarily definitive electrophoretic results, should provide a less time consuming method for the assay of gamma globulin. The serum gamma globulin levels in patients were also determined at specified intervals during the course of treatment.

Pathological sera. Professor Martin has examined sera containing abnormal amounts of proteins with a sedimentation cœfficent of 17S or more. The condition of patients with such sera is referred to by Waldenstrom as macroglobulinæmia and is usually associated with high levels of gamma globulin. It would appear at present that no clearly defined clinical syndrome is associated with this abnormality, though it occurs predominantly in elderly people.

ANIMAL SERUM PROTEINS

Serum globulins in young rats. More detailed electrophoretic examination of the sera from young rats (Report 1955), showed that in addition to the abrupt fall in the serum gamma globulin from 18 to 24 days after birth, there is a sharp but temporary increase in beta globulin. Taken with other evidence, this prompted a re-examination of the distribution of antibody after immunization of animals of various ages for differing periods with a *Salmonella pullorum* vaccine. Antibody in the rat may be associated with both the beta and gamma globulins, and it probably appears initially in the beta globulin. In this work Dr. Kekwick is collaborating with Professor Rogers Brambell and Dr. R. Halliday, University College of N. Wales.

BLOOD PRODUCTS LABORATORY

The installation of equipment in the new laboratory was almost completed during the year. Dried plasma and plasma fractions were prepared in gradually increasing amounts for the National Health Service.

The large scale fractionation of plasma by the method of Kekwick and Mackay has given rise to problems not encountered when handling smaller volumes. To improve the separation of protein precipitates in large-scale fractionation, methods of continuous flow centrifugation have been examined by Mr. Vallet, and a technique has been designed for the resuspension of precipitates without preliminary removal from the centrifuge bowl. The large-scale freeze-drying of plasma fractions was very slow because ether from the

fractions was difficult to remove from the pumping system. Experiments with air-ballasted pumps and a liquid nitrogen trap showed that pumps of low oil-capacity fed with an accurately controlled supply of air through a flowmeter, gave results comparable with those in which a liquid nitrogen trap in the pumping line was used to collect ether before it could contaminate the pump. The bore of the pumping line was found to be another limiting factor in the rate of ether removal.

Fibrinogen. Mr. Vallet and Dr. Painter succeeded in recovering useful quantities of fibrinogen from plasma separated from outdated blood. This fibrinogen can be used for the treatment of fibrinogenopenia associated with premature separation of the placenta, long standing intra-uterine fœtal death, and amniotic fluid embolism. Considerable quantities of this fibrinogen were prepared and used during the year.

Gamma Globulin. Mr. Vallet has investigated the removal, by treatment with kaolin, of the traces of fibinogen in gamma globulin. These traces are slowly converted to fibrin, and their removal should result in preparations of more stable solutions of gamma globulin.

Hypogammaglobulinæmia. At the end of 1956, the responsibility for the electrophoretic examination of sera from patients in the therapeutic trial organised by the Medical Research Council Working Party on Hypogammaglobulinæmia was taken over by Mr. Vallet from Dr. Kekwick.

Stabilizers of Serum Albumin. Mr. Vallet has continued to study the thermal stability of human serum albumin with special regard to the preservation of concentrated solutions of heated albumin. A sensitive test for heat-denatured albumin is being developed. It was observed that the protein of the slow-moving component which appears in the electrophoretic pattern of albumin heated without stabiliser is insoluble at pH 5.4. In albumin solutions of low ionic strength at this pH, heat-denatured material, at concentrations in excess of about 3 mg. per 100 ml., produces a turbidity which can be measured nephelometrically. Under standard conditions this gives a measure of heat denaturation.

Prothrombin. Dr. Painter has investigated the adsorption and elution characteristics of prothrombin with the object of introducing an adsorption step into the routine procedure for preparing thrombin. The purity of the thrombin is thus improved and it has been found that prothrombin, after adsorption and elution, remains stable in the dried state. Different adsorbents, mainly salts of barium, and the optimum concentration of citrate for elution from each of them, and other factors, such as pH, were examined.

Anti-hæmophilic Factor. Dr. Painter and Miss Evans have adapted Dr. Kekwick's method of preparing anti-hæmophilic factor for medium scale operation, and several batches of this material have now been used successfully for controlling bleeding during various surgical and dental operations on hæmophilic patients. The preparation of large quantities of A-H.F. presents many difficulties and it is unlikely that more than very small amounts can be made for some time to come. The responsibility for allotting available supplies will be assumed by the Medical Research Council Hæmophilia Committee.

BLOOD GROUP RESEARCH UNIT

This year, as in past years, the work of the Unit has been directed mainly by chance. Samples of blood presenting varied problems arrive by post from colleagues in Britain, the United States, and elsewhere: it so happens that this year the resulting investigations have been mostly confined to white people and to the ABO system and to the MNSs system.

A gene that modifies the expression of the blood group gene A. Dr. W. Weiner, of the Regional Blood Transfusion Service, Birmingham, sent two samples of blood that were peculiar because no anti-A was to be found in the serum of either though, judged by red cell tests, one donor belonged to group O and the other to group B. Judged, however, by their saliva one was group A and the other AB—which fitted with the antibodies present. The groups of their families fortunately disclosed the genotypes of the two donors: the first was *AO* and the second *AB*, nor was there anything peculiar about the *A* genes for in other members of the families their behaviour was normal. The findings can most simply be explained by postulating the existence of a pair of modifying genes, *Yy* unlinked to the *ABO* locus. The homozygote *yy* does not affect the activity of the *B* gene but it inhibits that function of the *A* gene which normally results in A antigent being present in the red cells; *yy* does not interfere with the other known function of *A* genes—that which results in A antigen being present in the saliva.

Another possible modifying gene. In collaboration with Dr. A. Cahan, of New York, a family with the very rare and weak form of A called A_x was studied. The father's group is A_x that of his son is A_2B and that of his son's daughter is A_x.

For an A_2B person to transmit A_x does not fit the pattern of the normal inheritance of the *ABO* genes. Some other gene (not *y*) must be modifying the expression of the gene *A*.

Chimera blood. In cattle dissimilar twins usually share a circulation *In utero*. One effect is that primordial red cells from one twin settle down in the other; for the rest of the life of the animal these immigrant cells produce red cells which are genetically alien to the host—so the blood is a mixture. Another effect, if the twins are of different sex, is that the cow becomes a freemartin, due, it is thought, to the hormones of her bull twin.

Four years ago the Unit was involved in identifying a human example of mixed blood. On enquiry, the owner of the blood replied that she had had a twin brother who had died 25 years before. Another example has now been found at the North London Blood Transfusion Service. This time both twins are alive aged 21, and of different sex. Samples of blood were kindly sent by Dr. J. D. James and his colleagues and the red cells were separated by a technique developed in this Unit. Of the red cells of the male twin 86% were A_1, *MS/Ms*, *CDe/cde*, Fy^b Fy^b, jk^a jk^b and 14% were O, *MS/MS*, *cDE/cde*, Fy^aFy^b, Jk^aJk^a : 99% of the cells of his twin sister belong to the O lineage and 1% to the A lineage. Tests on the saliva showed that the A series belong genetically to the male and the O to the female. The 1% of A cells in the female has prevented her producing the anti-A expected of ordinary group O people—an example of "acquired tolerance". Dr. W. M. Davidson showed that ancestors of white cells too must have been successfully grafted, for the male twin has some polymorphs with female "drumstick" nodules.

Hermaphrodite blood. Though the females of male-female chimera pairs are not freemartins it seemed worth testing the blood of intersexes whenever possible. In collaboration with Dr. C. N. Armstrong of Durham University, we have been able to test five pseudo-hermaphrodites and one true hermaphrodite. The true hermaphrodite was of an ABO group not previously described (red cells O, saliva B, serum anti-A only). It is difficult to believe that such an extraordinary ABO group finds itself merely by chance in a body so extraordinary in other ways. It seems reasonable to expect that in time the two conditions will be seen to be connected.

Pernicious anæmia. The blood grouping of families with pernicious anæmia (in collaboration with Dr. Sheila Callender of the Nuffield Department of Clinical Medicine, Oxford), mentioned in the last report, showed a non-significant preponderance of group A amongst the sufferers. The excess of A was seen to be significant when the results of several centres were pooled and analysed by Dr. Fraser Roberts.

Anti-M and anti-N sera. This investigation was precipitated by the arrival of a particularly powerful human anti-N serum sent by Dr. W. Hirsch of Haifa. Human anti-M sera did not react with N cells in any observable way. Anti-N sera on the other hand, though specific for N at certain temperatures, did agglutinate M cells when the temperature was reduced or when the cells were treated with enzyme; M cells absorbed anti-N and gave it up on elution. The simplest explanation seems to be that the anti-N molecule will fit the M molecule when assisted by temperature or by enzymes. It is curious that this behaviour of human anti-N appears to be paralleled by rabbit anti-N and even by the anti-N present in the extract of the seeds of a Brazilian plant *Vicia graminea.*

The antigens Mi^a and Vw. These two rare antigens are part of the MNSs system. They were thought to be identical but recent work, in collaboration with Dr. J. Wallace of the Blood Transfusion Service, Glasgow, and Dr. J. Mohn of the University of Buffalo, has shown them to be different. About half Mi(a+) people are Vw+ (and the genes responsible accompany the genes *Ns*) and about half Mi(a+) are Vw— (and the genes responsible accompany the genes *MS*). It seems that the MNSs system is at least as complicated as the Rh system.

Rh antibodies. Dr. Hackel has been analysing by absorption and elution methods the complicated Rh antibodies found in the serum of two persons of the genotype *-D-/-D-*. In both sera the antibodies anti-C, anti-c, anti-E and anti-e and anti-f have been demonstrated—the most complicated mixture of Rh antibodies yet analysed.

The Unit has continued to collaborate with Dr. Eliot Slater and Mr. James Shields of the Genetics Unit, the Maudsley Hospital, in testing the blood of twins taking part in a psychiatric investigation. For the supply of routine but often very scarce antisera the Unit is indebted to Dr. Mourant and the Blood Group Reference Laboratory. Innumerable samples of blood, without which the work would be impossible, are very kindly given by the staff of the Institute.

BLOOD GROUP REFERENCE LABORATORY

The Laboratory serves as reference centre for blood grouping problems and supply centre for grouping sera for the United Kingdom. It also acts under the auspices of the World Health Organisation as the world reference centre.

The continued increase in the demands of clinicians in the United Kingdom for blood for transfusion has been reflected in the demands for grouping serum, which are greater every year than the one before. The growing use of transfusion in the British Colonies and in other countries overseas had led to a further increase in demands for ampoules of dried serum. Laboratories overseas have been helped to start their own services by the supply of sera and the testing of red cells of staff members. Large numbers of specimens from laboratories in Great Britain and overseas have been examined for red cell antigens and for antibodies, both for clinical reasons and in order to select and identify sera suitable for use as diagnostic reagents.

Dr. Parkin has continued work on the effects of reconstituted dried plasma on red cells. Clinical trials have been planned in conjunction with the Medical Research Council Burns Unit at Birmingham. Her previous discovery of a family in England and Ireland with the exceedingly rare "Bombay" blood group (apparent red-cell group O, with anti-O in serum) made it possible for the laboratory to arrange for the transfusion of a baby in Holland with blood from one of the only two known potential donors in Europe.

Miss Ikin has carried out grouping tests for anthropological purposes on blood from persons in Jersey, Spain (Basques), Malta, Arabia, Socotra, India (Malayalis), Ceylon (Veddahs), Sarawak (Land Dyaks), and Uganda (various tribes), and from Burmese residents in England.

In conclusion the Governing Body desires to record its great appreciation of the manner in which the scientific, administrative and technical staffs have worked together during the period under review, and to congratulate them on the interest and range of their scientific activities.

H. H. DALE,
Chairman.

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THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

Balance Sheet

and

Accounts

31st December 1957

FINANCIAL REPORT OF THE GOVERNING BODY

1. The Balance Sheet as at 31st December, 1957, shows balances to the credit of the various funds as follows: Capital Fund £645,706; Specific Funds £132,718; Bequest Funds £14,279 and Contingency Reserve £73,494. The balance on the Sinking Fund for Freehold Buildings of £93,044 is after charging the loss on sales of Sinking Fund investments amounting to £7,074 and transferring from income and expenditure account £4,816. The Re-endowment Fund has been increased by donations during the year amounting to £1,690.

2. The General Fund Income and Expenditure Account shows the income for the year as £193,274 compared with £168,669 in 1956. Expenditure amounted to £168,849 against £146,794 last year. The surplus for the year is £24,425 compared with £21,875 in 1956.

3. The year's surplus of £24,425 shown by the General Fund Income and Expenditure account has been transferred to the Contingency Reserve.

4. Stocks of Sera, Vaccine Lymph and Horses on hand at December 31st have the nominal value of £6,378, £1,613 and £4,984 respectively.

5. Messrs. Cooper Brothers & Co., the retiring Auditors will, subject to the provision of the Companies' Act, 1948, be re-appointed.

H. H. DALE, *Chairman of Governing Body.*

HUGH BEAVER, *Hon. Treasurer.*

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

21st May, 1958.

BALANCE SHEET

(1956) £		£	£	£
	Capital Fund:—			
	Donations, &c., received to date from the following:—			
2,000	Dr. Ludwig Mond (1893)		2,000	
46,380	Berridge Trustees (1893/98)		46,380	
10,000	Worshipful Company of Grocers (1894)		10,000	
250,000	Lord Iveagh (1900)		250,000	
18,904	Lord Lister's Bequest (1913/23)		18,904	
7,114	William Henry Clarke Bequest (1923/6)		7,114	
3,400	Rockefeller Foundation (1935/6)		3,400	
22,669	Other Donations and Legacies (1891-1954)		22,669	
	General Fund Income and Expenditure Account Accumulated Surplus as at 31st December, 1956	290,676		
	Less Loss on sale of investments	5,437		
290,676			285,239	
651,143				645,706
	Specific Funds:—			
95,302	Sinking Fund for Freehold Buildings	93,044		
36,478	Pension Fund	32,964		
5,020	Re-endowment Fund	6,710		
			132,718	
	Bequest Funds:—			
7,932	Jenner Memorial Studentship Fund	8,038		
5,980	Morna Macleod Scholarship Fund	6,241	14,279	
150,712				146,997
	Specific Grants and Legacies Unexpended:—			
772	Cancer Research Legacies (1937-50)		772	
1,033	Royal Society Grant (1951)		347	
5,008	Nuffield Foundation Grants (1952-7)		5,489	
8,307	Guinness Lister Research Grant (1953-7)		9,812	
15,120				16,420
	Contingency Reserve:—			
	As at 31st December, 1956		49,069	
	Add Surplus on General Fund Income and Expenditure Account, 1957		24,425	
49,069				73,494
	Current Liabilities:—			
17,562	Creditors and accrued charges			22,810
£883,606				£905,427

H. H. DALE, *Chairman of Governing Body.*

HUGH BEAVER, *Hon. Treasurer.*

REPORT OF THE AUDITORS

We have examined the above Balance Sheet and annexed Income and Expenditure Account which are in all the information and explanations which we considered necessary for our audit. In our opinion these accounts information required by the Companies Act, 1948, and show a true and fair view of the state of the Institute's

London, 23rd May, 1958.

31st DECEMBER 1957

(1956) £					£	£	£
	Fixed Assets:—						
	Freehold Property at cost:—						
73,548	Land and Buildings, Chelsea				73,548		
20,456	Queensberry Lodge Estate, Elstree				20,456		
2,049	House, Bushey				2,049		
						96,053	
	(Note: Additions and replacements since 1912 at Elstree and 1935 at Chelsea have been charged to Revenue.)						
	Furniture, Fittings, Scientific Apparatus and Books:—						
2,472	At cost less depreciation to 31st December, 1920 ...					2,472	
98,525	*(Note: Additions and replacements since 31st December, 1920 have been charged to Revenue)* ...						98,525
	General, Specific and Bequest Funds.						
	Investments and Uninvested Cash:—						
		Quoted at cost, less amounts written off	Unquoted at cost	Cash			
	General—						
	Quoted in Great Britain	469,275	—	—			
	Quoted Elsewhere	39,523	—	—			
559,614	Unquoted	—	49,545	—	558,343		
	Specific—						
95,302	Sinking Fund for Freehold Buildings	87,601	—	5,443	93,044		
36,478	Pension Fund	31,746	—	1,218	32,964		
5,020	Re-endowment Fund	6,590	—	120	6,710		
	Bequest—						
7,932	Jenner Memorial Studentship Fund	5,340	1,940	758	8,038		
5,980	Morna Macleod Scholarship Fund...	5,060	—	1,181	6,241		
710,326		645,135	51,485	8,720	705,340		705,340
	(Market Value of Quoted Investments— £656,016)						
	Current Assets:—						
65,519	Debtors and Payments in advance					76,966	
9,236	Balance at Bankers and Cash in hand					24,596	
74,755							101,562
	(Notes: See paragraph 4 Governing Body's Financial Report for nominal values of Sera, Vaccine Lymph and Horses which have not been brought into the accounts.						
	There is a contingent liability of £19,931 in respect of investments not fully called up. There is an outstanding capital expenditure commitment of £2,460.)						
£883,606							£905,427

TO THE MEMBERS.

agreement with the books of account. In our opinion proper books of account have been kept. We have obtained amplified by the information given in paragraphs 1 and 4 of the Financial Report of the Governing Body give the affairs at 31st December, 1957, and of the surplus for the year ended on that date.

COOPER BROTHERS & CO.,
Chartered Accountants.

INCOME AND EXPENDITURE ACCOUNT

GENERAL

(1956) £		Total Expenditure £	External Contributions £	£
65,252	Salaries and Wages	116,637	48,037	68,600
6,543	Emoluments of two members of the Governing Body in an Executive Capacity	7,074	—	7,074
2,663	Premiums on Federated Superannuation Policies	4,189	1,721	2,468
3,465	Premium on Group Pension Policy	3,430	318	3,112
3,639	Rent, Rates and Insurance	4,092	270	3,822
11,010	Gas, Water, Fuel and Electricity	13,534	2,340	11,194
2,382	Office Expenses, Stationery and Printing	3,059	257	2,802
242	Auditor's Fee	326	—	326
684	Travelling Expenses	899	190	709
2,206	Biochemistry Expenses	2,690	398	2,292
1,075	Microbiology, Immunology and Experimental Pathology Expenses	3,671	2,517	1,154
469	Biophysics Expenses	455	101	354
423	Virology Expenses	1,347	919	428
11,514	Serum, Vaccine and Vaccine Lymph Expenses	15,956	987	14,969
7,072	Animals	8,608	963	7,645
8,103	Animal House Expenses and Forage	10,294	1,156	9,138
11,838	Buildings, Alterations, Repairs and Renewals	23,290	446	22,844
1,801	General Apparatus and New Installations	2,432	—	2,432
1,028	Library Expenses	790	—	790
446	General Stores	722	—	722
851	Staff Canteen Loss	1,365	207	1,158
—	Blood Products Laboratory Expenses	2,155	2,155	—
4,088	Amount transferred to Sinking Fund for Freehold Buildings (including £4,392 Interest on Investments and Underwriting Commission)	4,816	—	4,816
21,875	Surplus transferred to Contingency Reserve after charging to expenditure £18,698 for additions to property and equipment *(1956 £6,944)*	24,425	—	24,425
£168,669		£256,256	£62,982	£193,274

NUFFIELD FOUNDATION GRANTS

(1956) £		£	(1956) £		£
4,714	Salaries, Wages, Laboratory Expenses and Animals...	5,519	3,722	Balance at 1st January, 1957...	5,008
5,008	Balance carried forward ...	5,489	6,000	Amounts received	6,000
£9,722		£11,008	£9,722		£11,008

for the year ended 31st December 1957

FUND

(1956) £		£	£
	Interest and Dividends on Investments:—		
29,755	General Fund	32,435	
3,664	Sinking Fund	4,236	36,671
	Underwriting Commission:—		
—	General Fund	995	
—	Sinking Fund	156	1,151
130,084	Sales of Sera, Vaccines, Vaccine Lymph, &c.		149,925
5,166	Rent		5,527
£168,669			£193,274

GUINNESS-LISTER RESEARCH GRANT

(1956) £		£	(1956) £		£
6,630	Salaries and Wages	8,697	4,518	Balance at 1st January, 1957...	8,307
2,581	Laboratory Expenses	3,798	13,000	Amount received	14,000
8,307	Balance carried forward ...	9,812			
£17,518		£22,307	£17,518		£22,307

PENSION FUND

(1956) £		£	(1956) £		£
—	Loss on realisation of investments	3,858	36,259	Fund as at 1st January, 1957	36,478
1,387	Pensions	1,380	1,606	Interest on Investments (gross)	1,663
36,478	Fund as at 31st Dec., 1957 ...	32,964	—	Underwriting Commission ...	61
£37,865		£38,202	£37,865		£38,202

JENNER MEMORIAL STUDENTSHIP FUND

£		£	£		£
2,429	Loss on realisation of investments	—	10,465	Fund as at 1st January, 1957	7,932
383	Stipend of Student	354	279	Interest on Investments (gross)	460
7,932	Fund as at 31st Dec., 1957	8,038			
£10,744		£8,392	£10,744		£8,392

MORNA MACLEOD SCHOLARSHIP FUND

£		£	£		£
2,558	Loss on realisation of investments	—	8,360	Fund as at 1st January, 1957	5,980
5,980	Fund as at 31st Dec., 1957	6,241	178	Interest on Investments (gross)	261
£8,538		£6,241	£8,538		£6,241

INVESTMENTS AT 31st DECEMBER 1957

GENERAL FUND

	Quoted:—	Balance Sheet Value		Market Value
£9,000	A.P.V. Co. Ltd. 5% First Deb. Stock 1880/85	£8,698	...	£6,975
£500	Associated Commercial Vehicles Ltd. 5¼% Unsecd. Loan Stock 1977/82	501	...	450
6,000	Albright & Wilson Ltd. Ordy Stock Units 5/-	5,168	...	5,175
£5,000	Allied Bakeries Ltd. 5% Unsecd. Loan Stock 1966/70	4,820	...	4,375
1,500	Associated Electrical Industries Ltd. Ordy. Stock Units £1 ...	4,498	...	3,562
£2,900	Australia, Commonwealth of, 4½% Reg. Stock 1960/62	2,666	...	2,740
£12,000	Australia, Commonwealth of, 3% Reg. Stock 1972/74	12,121	...	8,220
£10,000	Australian Estates Co. Ltd. 6½% Secd. Loan Stock 1971/76 ...	9,848	...	9,450
£5,000	Birfield Ltd. 6% Unsecured Loan Stock 1976/81	4,977	...	4,800
£500	Bowater Paper Corpn. 5¼% Conv. Unsecd. Loan Stock 1978/82 ...	531	...	445
£15,000	British Electricity 3½% Guaranteed Stock 1976/79	14,925	...	10,800
3,000	British Oxygen Co. Ordinary £1 Stock Units	6,165	...	4,875
£5,000	British Petroleum Co. Ltd. 6% Convertible Debenture Stock 1976/80 (£20 paid)	1,229	...	1,250
2,500	British Tabulating Machine Co. Ltd. Ordinary £1 Shares	5,391	...	5,156
£20,000	British Transport 3% Guaranteed Stock 1967/72	20,259	...	13,500
3,500	Cater Brightwen & Co. Ltd. Ordy. £1 Stock Units	10,872	...	7,087
£5,000	George Cohen 600 Group Ltd. 6% Unsecured Loan 1975/80 ...	5,000	...	4,825
22,750	Debenture & Capital Investment Trust Ltd. Ordinary £1 Stock Units	6,585	...	13,081
4,000	Dorman Long & Co. Ltd. Ordinary £1 Shares	4,981	...	4,550

GENERAL FUND—continued.

400	First Bank Stock Corporation Common Shares of $10	4,623	...	4,469
200	First National City Bank of New York Common Capital Shares of $20	4,603	...	4,589
£5,000	Flowers Breweries Ltd. 5½% First Mtge. Debenture Stock 1970/72	4,850	...	4,600
£4,500	General Electric Co. Ltd. 6% Unsecured Loan Stock 1976/81	4,408	...	4,477
6,280	Greenfriars Investment Co. Ltd. Ordinary 5/- Stock Units	2,873	...	4,239
300	Hadfields Ltd. Ordinary £1 Shares	393	...	296
£9,000	Hope & Anchor Breweries Ltd. 5½% Mtge. Debenture Stock 1980/85	8,843	...	8,100
£5,000	Imperial Chemical Industries Ltd. 5½% Conv. Unsec. Loan Stock 1977/79	4,962	...	4,975
£5,000	Kennings 5½% Unsecured Loan Stock 1970/75	4,703	...	4,125
4,000	Lancashire Steel Corpn. Ltd. Ordy. £1 Shares	5,328	...	5,000
12,500	London & Montrose Investment Trust Ltd. Ordinary 5/- Shares	1,394	...	10,625
£10,000	London & Overseas Freighters Ltd. 6% First Mtge. Deb. Stock 1963/82 (20% Paid)	2,025	...	1,100
£2,000	London Scottish American Trust Ltd. Deferred Stock	2,778	...	7,500
7,700	London Trust Co. Ltd. Deferred 5/- Stock Units	4,292	...	7,700
700	Marine Midland Corporation Common Shares of $5	4,953	...	4,989
300	Montana Power Co. Common Shares of no par value	5,421	...	5,345
30,000	Mercantile Investment & General Trust Co. Ltd. Ordinary 5/- Stock Units	13,401	...	21,000
2,000	Metal Box Ltd. Ordinary £1 Stock Units	5,189	...	4,325
£9,000	Mitchell Cotts & Co. Ltd. 6% Unsecured Loan Stock 1976/81	8,800	...	8,415
£25,000	New Zealand 3½% Stock 1962/65	21,989	...	20,875
£10,000	Norvic Shoe Co. Ltd. 5% Unsec. Loan 1970/75	9,800	...	8,500
300	Oklahoma Gas & Electric Co. Common Shares of $10	4,808	...	4,738
400	Pacific Power & Light Co. Common Shares of $6.50	4,662	...	4,584
£10,000	P. & O. Steam Navigation Co. 5% Debenture Stock 1975/80	9,542	...	8,450
£3,000	Port of London Authority—Port of London 3½% Reg. Stock 1965/75	2,687	...	2,040
40,000	Rio Claro Investment Trust Ltd. Ordinary 5/- Stock Units	13,692	...	20,500
22,000	River Plate & General Investment Trust Ltd. Deferred 5/- Stock Units	8,691	...	13,200
£4,000	Ruston & Hornsby Ltd. 6% Unsecured Loan Stock 1975/80	4,000	...	3,840
£12,000	3% Savings Bonds 1955/65	12,000	...	10,200
£66,300	3% Savings Bonds 1960/70	66,417	...	50,056
£12,000	3% Savings Bonds 1965/75	10,608	...	8,460
6,000	Seccombe Marshall & Campion Ltd. Ordy. £1 Shares	10,162	...	9,450
4,000	Sphere Investment Trust Ltd. Ordy. £1 Shares	4,432	...	11,200
12,000	Standard Trust Ltd. Ordinary 5/- Stock Units	3,832	...	8,400
£7,500	Sterling Trust Ltd. Ordinary Stock	8,731	...	17,250
£10,000	Tennant Brothers Ltd. 6% Redeemable Debenture Stock 1971	10,089	...	9,550
£6,750	Third Guardian Trust Ltd. Ordinary £1 Stock Units	6,783	...	11,475
450	Tri-Continental Corpn. Common Shares of $1	5,023	...	4,767
11,250	Typhoo Tea (Holdings) Ltd. Ordy. 5/- Shares	7,505	...	9,984
£10,000	United Gas Industries Ltd. 6% Unsecured Loan 1973/75	9,900	...	9,500
£10,000	United States Debenture Corpn. Ltd. Ordy. Stock	7,391	...	13,500
5,534	Vickers Ltd. Ordinary £1 Shares (15/- paid)	4,012	...	3,597
400	Washington Water Power Co. Common Shares of no par value	5,429	...	5,278
10,000	Allen West & Co. Ltd. Ordy. 5/- Stock Units	6,126	...	4,875
3,000	Westinghouse Brake & Signal Co. Ltd. Ordinary £1 Stock Units	5,718	...	4,875
£10,000	Whitbread Investment Co. Ltd. 5¾% Guaranteed Debenture Stock 1980/85	9,950	...	9,600
£9,450	Witan Investment Co. Ltd. Ordinary Stock	11,745	...	14,931
		£508,798		£530,860
	Unquoted:—			
£9,000	British Titan Products Co. Ltd. 5½% Unsecured Loan Stock 1970/75	£8,820		
£10,000	Caltex U.K. Ltd. 6% Guaranteed Loan Stock 1971/76	9,800		
£10,000	Kraft Foods Inc. 5% Debenture Stock 1965/75	10,000		
£10,000	R. A. Lister 5% Unsecured Loan Stock 1960/65	9,975		
£4,000	R. H. Neal & Co. Ltd. 6½% Unsecured Loan Stock 1966/71	4,000		
£5,000	Powers Samas Accounting Machines Ltd. 6% Unsecured Loan Stock 1975/78	4,950		
£5,000	Tanker Charter Co. Ltd. 6½% "B" Secured Loan Stock (40% Paid)	2,000		
		£49,545		

SINKING FUND FOR FREEHOLD BUILDINGS

		Balance Sheet Value	Market Value
2,000	British Aluminium Co. Ltd. Ordinary £1 Stock Units	£5,830	£4,125
£3,000	British Electricity 3% Guaranteed Stock 1968/73	2,916	2,160
2,000	English Electric Co. Ltd. Ordinary £1 Stock Units	6,194	4,750
£5,000	Financial Times Ltd. 5½% Debenture Stock 1980/85	4,900	4,525
£4,500	3% Funding Loan 1959/69	3,876	3,510
£9,000	Gallaher Ltd. 6% Unsecured Loan Stock 1976/81	8,884	8,325
2,000	Guest, Keen & Nettlefolds Ltd. Ordinary £1 Stock Units	5,373	4,700
£10,000	New Zealand Loan & Mercantile Agency Co. Ltd. 5½% Second Mtge. Deb. Stock 1970/80	9,525	8,750
1,000	A. Reyrolle & Co. Ltd. Ordinary £1 Stock Units	4,463	4,000
£3,500	3% Savings Bonds 1955/65	3,518	2,975
£10,000	3% Savings Bonds 1960/70	9,622	7,550
£10,000	3% Savings Bonds 1965/75	10,000	7,050
4,000	South Durham Steel & Iron Co. Ltd. Ordinary £1 Shares	5,329	4,900
£3,000	Third Guardian Trust Ltd. Ordinary Stock £1 Units	2,183	5,100
2,000	Union Discount Co. of London Ltd. £1 Stock Units	4,988	4,600
		£87,601	£77,020

PENSION FUND

£3,000	Lewis Berger & Sons Ltd. 5¾% Debenture Stock 1977/82	£2,977	£2,865
£12,416	British Electricity 4½% Gtd. Stock 1967/69	11,322	11,484
£3,000	British Oxygen Co. Ltd. 5¼% Deb. Stock 1981/86	2,861	2,880
£3,000	Dowty Group Ltd. 6% Unsecured Loan Stock 1977/82	2,932	2,865
£5,800	4% Funding Loan 1960/90	3,050	5,017
£2,500	Metal Box Co. Ltd. 5½% Unsecured Loan Stock 1977/80	2,399	2,312
£3,000	Ruston & Hornsby Ltd. 6% Unsecured Loan Stock 1975/80	3,000	2,880
£2,200	3% Savings Bonds 1960/70	2,205	1,661
£1,000	3% Savings Bonds 1965/75	1,000	705
		£31,746	£32,669

RE-ENDOWMENT FUND

£7,600	3% Savings Bonds 1960/70	£6,590	£5,738

JENNER MEMORIAL STUDENTSHIP FUND

	Quoted:—		
£1,000	A.P.V. Co. Ltd. 5% Mtge. Debenture Stock 1980/85	£850	£775
£1,000	Gallaher Ltd. 6% Unsecured Loan Stock 1976/81	1,000	925
£1,500	General Electric Co. Ltd. 6% Unsecured Loan Stock 1976/81	1,500	1,492
£1,000	Hope & Anchor Breweries Ltd. 5½% Mtge. Deb. Stock 1980/85	990	900
£1,000	Mitchell Cotts & Co. Ltd. 6% Unsecured Loan Stock 1976/81	1,000	935
		£5,340	£5,027
	Unquoted:—		
£1,000	British Titan Products Ltd. 5½% Unsecured Loan Stock 1970/75	940	
£1,000	R. H. Neal & Co. Ltd. 6½% Unsecured Loan Stock 1966/71	1,000	
		£1,940	

MORNA MACLEOD SCHOLARSHIP FUND

£1,000	Agricultural Mortgage Corpn. Ltd. 5% Debenture Stock 1979/83	£958	£900
£1,000	British Guiana 5% Stock 1980/85	928	865
£1,000	South Essex Waterworks Co. 5% Redl. Debenture Stock 1986/91	963	855
£1,000	Stockton-on-Tees Corpn. 5½% Red. Debenture Stock 1966	992	960
£1,200	Tanganyika Government 5¾% Stock 1978/82	1,219	1,122
		£5,060	£4,702

THE LISTER INSTITUTE

OF

PREVENTIVE MEDICINE

REPORT

OF THE

GOVERNING BODY

1958

CHELSEA BRIDGE ROAD, LONDON, S.W.1

THE GOVERNING BODY

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The President of the ROYAL COLLEGE OF VETERINARY SURGEONS	Royal College of Veterinary Surgeons.
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Deputy Director: Professor W. T. J. Morgan.

Superintendent of Elstree Laboratories: W. d'A. Maycock.

MICROBIOLOGY, IMMUNOLOGY and EXPERIMENTAL PATHOLOGY

†A. A. Miles, C.B.E., M.A., M.D., F.R.C.P. *(Professor of Experimental Pathology in the University of London).*
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Brenda Mason, B.Sc.
D. D. Smith, M.B., Ch.B. *(Carnegie Senior Scholar).*
I. N. Asheshov, M.D. *(Medical Research Council External Scientific Staff).*
M. D. Pittam, B.A. *(Agricultural Research Council Grantee).*

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*B. A. D. Stocker, M.D., M.R.C.S., L.R.C.P.
D. Kerridge, M.A., Ph.D.
Janice Edgar, M.Sc.
Sylvia Smith, M.Sc.
H. Ozeki, B.Sc.
J. Joys, B.Sc. *(Research Student).*
Helene de Margarie *(Honorary Research Staff)*

VIROLOGY

L. H. Collier, M.D.
J. Janes Stocker, B.Sc.
Lilias G. MacGregor, B.Sc.
W. A. Blyth, B.Sc. *(Research Student).*

BIOCHEMISTRY

†W. T. J. Morgan, D.Sc., Ph.D., F.R.I.C., F.R.S. *(Professor of Biochemistry in the University of London) Principal Biochemist, Elstree.*
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M. Abdullah, M.Sc. *(Pakistan).*
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†R. A. Kekwick, D.Sc. *(Reader in Chemical Biophysics in the University of London).*
Sara Derechin, B.Sc.
M. Derechin, M.D. *(British Council Scholar).*
Professor N. H. Martin, M.A., B.M., B.Ch., B.Sc *(Honorary Research Associate).*

NUTRITION

§Dame Harriette Chick, D.B.E., D.Sc
§E. Margaret Hume, M.A

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†Appointed Teacher of the University of London.
*Recognised Teacher of the University of London.
§Honorary Member of Institute Staff.

PREPARATION and STUDY of THERAPEUTIC SERA (ELSTREE)

*B. G. F. Weitz, M.R.C.V.S.
J. Rodican, B.Sc.
Frances M. Lee-Jones, B.Sc.

BIOCHEMISTRY (ELSTREE)

*D. E. Dolby, B.Sc., Ph.D.
Sheila M. Lanham, B.Sc.

PREPARATION and STUDY of SMALLPOX VACCINE (ELSTREE)

*D. McClean, M.B., B.S., M.R.C.S.
C. Kaplan, M.Sc., M.B., Ch.B., Dip.Bact.
Lisel R. Thomas, B.A. *(Rsearch Student).*

PREPARATION and STUDY of BACTERIAL VACCINES (ELSTREE)

*A. F. B. Standfast, M.A., Dip.Bact.
Jean M. Dolby, M.A., Ph.D. *(Medical Research Council External Scientific Staff).*
M. Garay, B.Sc.

BLOOD PRODUCTS (ELSTREE)

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Shirley M. Evans, B.Sc.
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Ruth Sanger, B.Sc., Ph.D.
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§*A. E. Mourant, M.A., D.Phil., D.M., M.R.C.P.
Dorothy M. Parkin, M.R.C.S., L.R.C.P.
Elizabeth W. Ikin, B.Sc.
Carolyn M. Giles, B.Sc.

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Elstree Secretary and Estate Manager - F. K. Fox

Solicitors:
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52 Bedford Square, W.C.1.

Auditors:
Cooper Brothers & Co.
14 George Street, Mansion House, E.C.4

*Recognised Teacher of the University of London.
§Honorary Member of Institute Staff.

ANNUAL GENERAL MEETING

OF

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

June 17th, 1958

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the work of the Institute for the year 1957/58.

GOVERNING BODY

The Governing Body reports with great regret the death during the year of Lord Waverley, one of the representatives first of Lord Moyne and then of Lord Iveagh. At the time of his death Lord Waverley was the senior member of the Board and had acted as Honorary Treasurer of the Institute since he became a Governor in 1938.

Sir Hugh Beaver, K.B.E., was appointed by Lord Iveagh as a member of the Governing Body and he has accepted the position of Honorary Treasurer in succession to Lord Waverley.

The Council, at a meeting held on 27th June last, re-elected Sir Henry Dale, Sir Charles Dodds and Sir Wilson Jameson as its representatives on the Governing Body until 31st December, 1958.

COUNCIL

At the Annual General Meeting last year the three members of Council retiring were Lord Iveagh and Lord Waverley, representatives of the Members of the Institute and Major L. M. E. Dent, one of the representatives of the Worshipful Company of Grocers. Each of these representatives was re-appointed to the Council.

The three members of the Council due to retire this year in accordance with the Articles of Association, but who are eligible for re-appointment, are Professor F. S. Stewart and the President of the Royal College of Physicians, representing the University of Dublin and the Royal College of Physicians respectively, and Professor A. A. Miles, a representative of the Members of the Institute.

The death of Sir Merrik Burrell, who had represented the Royal Agricultural Society since 1953, is recorded with regret.

MEMBERS

During the year Dr. L. H. Collier and Dr. D. E. Dolby accepted invitations to become Members.

The Governing Body records with regret the death of Sir Ernest Kennaway and Dr. Charles Todd. At the beginning of the century Dr. Todd was for a number of years a member of the scientific staff of the Institute and worked as an assistant bacteriologist in the Antitoxin Department, first at Sudbury, and then at Elstree.

STAFF

The Governing Body takes pleasure in recording that Dr. R. A. Kekwick was awarded the Oliver Memorial Prize for 1957 for his work in connection with blood transfusion.

Miss Brenda Mason was appointed to the Experimental Pathology Department; Miss Janice Edgar, Miss Sylvia Smith and Mr. H. Ozeki to the Guinness-Lister Research Unit; Mrs. Jane Stocker and Miss Lilias MacGregor to the Virology Department; Miss Philomena Glover to the Biochemistry Department; Mm. Sara Derechin to the Biophysics Department; and Mr. A. A. Horner to the Blood Products Laboratory.

The following studentships were also awarded: Mr. J. Joys, Guinness-Lister Research Unit; Mr. I. A. F. Lister Cheese and Mr. H. M. Tyler, Biochemistry Department.

Dr. C. J. Perret, Dr. R. H. Painter, Miss Elizabeth M. Sparrow, Mrs. Dorothy H. Perret (née Card), Dr. W. C. Crimmin, Mr. E. A. Caspary, Miss Patricia M. Gibson and Miss Janet Campbell resigned, and Dr. P. J. Mill, Dr. M. Ruszkiewicz and Miss Valerie Lawton completed the tenure of their studentships during the year.

Professor A. A. Miles was appointed a member of the Medical Research Council and Dr. W. d'A. Maycock a member of the Committee of Experts convened by the Council of Europe to draft specifications for human blood and its derivatives.

At the invitation of the Henry Ford Hospital, Professor Miles took part in a symposium on the "Mechanisms of Hypersensitivity" held at the Henry Ford Hospital, Detroit, U.S.A., in March, 1958.

Professor W. T. J. Morgan, at the invitation of the Medical Faculty, lectured at a Biological Colloquium at the University of Frankfurt in February, 1958.

Dr. D. McClean visited Thailand in January, 1958, as a consultant on behalf of the World Health Organisation to advise on the production of dried smallpox vaccine.

At the invitation of the Protein Foundation and the Brown-Hazen Fund, Dr. R. A. Kekwick spent two weeks in U.S.A. in November, 1957. He presented a paper at the 12th Conference of the Protein Foundation at Albany, N.Y.

Dr. W. J. Whelan lectured by invitation at the University of Freiburg and the Max-Planck Institute, Heidelberg, in February and at the University of Manchester in March, 1958.

Dr. Emmy Klieneberger-Nobel attended by invitation a meeting of the Austrian Society for Microbiology in February, 1958, at which she gave a paper on L-forms of bacteria.

Mr. L. Vallet visited Poland as a consultant on behalf of the World Health Organisation to advise on the equipping of laboratories for the preparation of gamma globulin.

VISITORS

The following visitors worked for short periods in the Institute Laboratories: Dr. G. Curzon, National Hospital for Nervous Diseases, London; Mr. A. Edwards, Department of Genetics, University of Cambridge; Dr. H. Feier, Dr. A. Wander Forschunginstitut, Freiberg; Mr. D. Jones, Genetics Laboratory, University of Oxford; Dr. W. Köhler, Hygiene Institute, Rostock; Miss K. Lebech, State Serum Institute, Copenhagen; Mr. Z. Lorkiewicz, Lublin, Poland; Dr. J. Mohn, Department of Bacteriology and Immunology, University

of Buffalo; Dr. L. Poleff, Centre d'Ophthalmologie et de Trachomatologie Expérimentale, Rabat-Salé, Morocco; Dr. Rozansky, Medical School, Jerusalem; Dr. B. Seydian, Pasteur Institute, Teheran, Iran; Dr. Soemiatno, Pasteur Institute, Bandung, Indonesia.

During the year lectures were given at the Institute by Professor T. Magill, of the State University of New York College, on "Parenteral Digestion and Allergy"; by Dr. John Heller, Director of the New England Institute for Medical Research, Connecticut, on "A new *physical* method for quantitative assay of the *toxicity* of botulinum toxin, of antitoxin and immunologic and therapeutic implications of these techniques"; and by Dr. Anne M. Staub of the Institut Pasteur, Paris, on "Chemical Basis of the Serological Specificity of *Salmonella* Somatic Polysaccharides".

The Blood Group Research Unit and the Blood Group Reference Laboratories are still accommodated at the Institute, and Miss E. M. Hume continues to do editorial work for *Nutrition Abstracts and Reviews* on behalf of the Commonwealth Bureau of Animal Nutrition at the Rowett Research Institute.

The Governing Body notes with satisfaction the successful continuance of the researches under Professor Miles and Professor Morgan, made possible by the benefaction of the Nuffield Foundation.

The Governing Body is also indebted to the Agricultural Research Council for grants in aid of research on protozoa and on the mechanism of starch-carbohydrate action; to the Medical Research Council for a grant in aid of research on acetylphospholipids and related compounds; and to the Department of Scientific and Industrial Research for a special grant for research on the enzymic polymerisation of monosaccharides.

Grateful acknowledgment is made of grants from the Royal Society and Rockefeller Foundation for the purchase of special apparatus and to the Wellcome Trust for a travelling grant to the Director.

RESEARCHES IN 1957

MICROBIOLOGY

PROTOZOOLOGY

Antigenic Structure of Trichomonas. Dr. Robertson's analysis of the antigens of two serological varieties of *Trichomonas fœtus* from the cow has revealed that the various protein antigens demonstrable by the agar-gel diffusion technique are mostly common to the two varieties. The serological distinction between the two revealed by agglutination and precipitin tests appears to depend on differences in polysaccharide components of the two organisms.

No other serological varieties were found among strains of *T. fœtus* isolated during the past 20 years in field outbreaks of cattle trichomoniasis.

Two strains of Trichomas isolated from the cæcum of pigs in Munich had many protein antigens in common with the cow strains. There were some polysaccharides common to the four strains, but others appeared to be characteristic of each one of them. One of the pig strains was found by Dr. W. R. Kerr, of the Ministry of Agriculture of Northern Ireland, to produce a typical vaginal infection of cattle when added to semen used for artificial insemination. It is therefore possible that pig trichomonads could give rise to outbreaks of cattle trichomoniasis; but at present there is no evidence of their doing so.

Cytology and Inheritance in Amœbo-flagellates. Mr. Pittam has continued his study of soil amœbæ, mainly *Nægleria gruberi* and *Tetramitus rostratus*. It has not proved possible to grow them in bacteria-free media, but the food bacteria of the medium may be kept under control by the antibiotic terramycin. The process of nuclear division was studied in *N. gruberi*, *T. rostratus* and *Hartmanella astromyxis* by phase contrast microscopy, and also in terms of changes in amount and disposition of nucleic acids and the carbohydrate, protein and lipid complexes of the cytoplasm, as revealed by histochemical methods.

The reversible change from amœboid to flagellate form which occurs in *Nægleria* and *Tetramitus* species were studied in detail by phase contrast microscopy. The possible dependence on peculiarities of inheritance of the ability of these protozoa to make the change, was tested in single-cell cultures of these organisms. The proportion of amœbæ that became flagellated in cultures of *N. gruberi* proved to be independent of the status of the parent cell; on the other hand, in *T. rostratus* flagellation occurred in 30% of amœboid progeny of a single flagellated cell, whereas only 2% of the progeny of a single amœboid cell did so. The phenomenon is under investigation.

WHOOPING COUGH BACILLUS

Identification of Protective Antigens. Mr. Standfast and Dr. Jean Dolby (Horton) have continued their work on the two protective antigens—"intranasal" and "intracerebral"—of *Bordetella (Hæmophilus) pertussis* and the two antibodies they elicit (Report, 1957). The two antigens can be distinguished only by active or passive protection tests in mice challenged respectively by the intranasal or intracerebral routes.

The action of the two antibodies was examined in lung and brain infections in mice. "Intranasal" antiserum—that is serum prepared against the antigen which protects mice against an intranasal challenge but not an intracerebral challenge—appears to be directly bactericidal, at once decreasing to a sublethal level the dose of *B. pertussis* used to infect the lung. Thereafter the course of infection resembles that following a sublethal infecting dose; the number of viable bacteria in the lung, after a slight initial increase in some cases, decreases slowly over a number of weeks.

The action of antiserum to the "intracerebral" antigen in brain infections is quite different. The number of viable bacteria in the brain of serum-treated mice rises steadily from the initial value for 3 to 4 days and then falls dramatically, even in mice given the serum 4 hours before the challenge, until by the 7th day most of the infected mouse brains are sterile. "Intracerebral" serum appears to have no demonstrable action for the first 3-4 days of the infection.

INHERITANCE IN BACTERIA

Segregation of Lysogenicity and of Transduced Genes after Infection of Salmonella by Phage. Phage transduction of a genetic character entails the infection of a bacterium by a free phage particle carrying some of the genes of the bacterium in which the phage particle has been formed. Dr. Stocker and Madame de Margerie have produced evidence that the association between the transducing phage and the bacterial genes it happens to be carrying is not necessarily maintained in the immediate progeny of the newly infected bacterium.

In pedigree studies of Salmonella exposed to phage, they found that in bacteria each infected with about two phage particles the progeny of a surviving bacterium sorted out into stable lysogenic cells (i.e., cells whose progeny all have a hereditary latent phage infection) and stable non-lysogenic cells only after several generations. In experiments on a non-motile *Salmonella*

strain, cells made motile by transduction, and, as controls, non-motile cells picked at random, were isolated by micromanipulation; the proportions of cells with only lysogenic descendants, only non-lysogenic descendants, or a mixture, were about the same in the cells picked at random and in the cells made motile by transduction. This indicates that if the phage particle which effects a transduction is capable of establishing lysogenicity, then during the first bacterial divisions after it infects a bacterium its gene-set does not necessarily pass to the bacterial descendant which receives the fragment of bacterial genetic material imported by the phage.

Lysogenic Conversion in Salmonella. When a bacterial strain becomes lysogenic, i.e., acquires by infection a hereditary latent virus infection, the character of the bacterial strain is altered in certain respects ("lysogenic conversion"). Dr. Stocker confirmed Japanese reports that lysogenisation of *Salmonella typhimurium* with certain phages invariably causes the appearance of a new bacterial somatic antigen (antigen 1 of the K-W table); he finds that all the phages of the serologically homogeneous A1-A2 group of Boyd have this effect, whereas the generally similar but serologically distinct A3 and A4 phages do not; though, like the A1-A2 phages, they can transduce characters from their last host to a small minority of the bacteria they infect. Of many freshly isolated strains of *S. typhimurium* (supplied by the Central Enteric Reference Laboratory), nearly all the strains with antigen 1, and none of those without it, were found to be latently infected with a phage whose presence induces the appearance of this antigen. The few exceptional strains, possessing antigen 1 but apparently not carrying a "converting" phage, are being examined by Dr. N. Zinder of the Rockefeller Institute, to see if they are, as suspected, carriers of a latent phage with a genetic defect that prevents it from maturing normally. The antigen concerned appears to be present in the cell-wall of the bacterium, and in a form resistant to peptic and tryptic digestion. Antigen 1 was demonstrated, by precipitin and red-cell sensitisation methods, in the complete O antigen extracted by trichloracetic acid from lysogenic cells: and by precipitin tests in the haptenic polysaccharide component obtained by acid hydrolysis of the complete O antigen. Haptenic polysaccharides from lysogenic and non-lysogenic strains, respectively possessing and lacking antigen 1, examined after hydrolysis by chromatography (Dr. Watkins) differed in their monosaccharide composition. It thus appears that the presence of an A1-A2 latent phage in a *S. typhimurium* cell causes it to synthesise a polysaccharide different from that synthesised by uninfected cells. This is of interest in that the other known specific products of active or latent phage infection (including diphtheria toxin) are all proteins or nucleic acids.

Somatic Phase Variation in Salmonella. Some of the components of the somatic antigen complex of *Salmonella*, including factor 1, the phage-determined component in *S. typhimurium*, reported in the preceding paragraph, are subject to phase variation; i.e., when a culture is plated some colonies are strongly and others weakly agglutinable by the corresponding serum. Madame de Margerie and Dr. Stocker are investigating the genetic aspects of phase variation of factor 1. A method for determining the presence or absence of the antigen in single bacteria *in vivo* has been developed. The results establish that a culture of a strain showing somatic phase variation in respect of factor 1, even if grown from a single cell, is heterogeneous, some cells being unaffected by factor 1 serum and others being agglutinable by it; for a few generations the progeny of a cell of either sort resemble their parent. Some cells, however, behave as though intermediate in character, and as a result attempts to estimate rates of change per cell per generation from the non-agglutinable to the agglutinable state, and back, were unsuccessful.

Genetical Observations on Glycerol Utilization by Salmonella typhimurium. Dr. Stocker and Miss Campbell investigated the papillæ (secondary colonies) which appeared after prolonged incubation when certain phage stocks were applied to *S. typhimurium* strain Glasgow O, inoculated so as to produce a continuous "lawn" of growth on a papain-digest nutrient agar; these secondary colonies were found to result from the transduction (genetic transfer by phage), from the previous bacterial host to a few cells of strain Glasgow O, of the ability to utilise some component of the medium unavailable to the remainder of the population. The component in question was identified as glycerol, strain Glasgow O differing from normal strains by an inability to use this substance. Further investigations suggested that this defect resulted from an inability to convert glycerol into αglycerophosphate. Dr. M. Brookes, of Harvard Medical School, examined cell-free preparations of various strains, and found glycerokinase activity in all except Glasgow O, in which this enzyme appeared to be totally absent. Certain stock cultures of strain Glasgow O made glycerol-positive by transduction when re-examined after some weeks were found to contain a proportion of glycerol-negative cells. Such reversion to the ancestral state has not previously been reported in strains whose character has been changed by transduction. Its genetic mechanism is obscure. because transduction is currently believed to consist in the replacement of a piece of bacterial chromosome by a homologous fragment from another bacterium, introduced by a phage particle, the original piece being lost. The phenomenon is therefore being investigated further.

Physiology of the Mating Process in Escherichia coli. Mrs. Bernstein investigated some physiological aspects of the mating process in *E. coli* strain K-12, and others, "infected" with the transmissible F (fertility) agents F12 and F3 (Report, 1956). The fertility of young growing cultures was found generally to be higher than that of non-growing cultures. When growing cells were exposed to streptomycin their viability and ability to mate were both immediately destroyed; but non-growing cells, though rendered unable to form colonies, were still able to mate. These results were obtained regardless of the mating type of the cultures. By the use of streptomycin during cross-mating where one parent was sensitive and the other resistant to the drug, it was found that the parent cell in which the zygote formed depended on whether or not the parent cells were growing at the time of mating. When young growing cells were mated, the transfer of genetic material in crosses of F+ with F− derivatives of strain K-12 was always from F+ to F−, all zygotes forming in cells of the F− strain; in crosses of F+ with F+, and crosses between K-12 and other strains, the transfer could be in either direction and zygotes could form in cells of either parent strain. On the other hand in all crosses between non-growing cells there was transfer in both directions, and zygotes formed in either parent. Thus, the concept of "donor" and "recipient" mating types is not of general application. A study (among F3+, F12+ and F− derivatives of K-12) of the frequency of gene recombination for an unselected nutritional marker showed that this frequency depended not only on the mating types of the parents but also on their phase of growth. There has been controversy as to whether fertilization in *E. coli* K-12 is complete (union of all genetic material of both parents) or partial (union of part of the genetic material of one parent with all that of the other). The present study yields results at different stages of growth supporting either hypothesis. However, neither hypothesis in its present form can account for all the findings. The foregoing investigations suggest that fertility is not a constant state, but both qualitatively and quantitatively variable, depending not only on mating type but also on the physiological state of the cells at the time of mating.

Cell Pairing in Salmonella. Genetic recombination as a result of conjugation like that taking place in *Escherichia coli* (Report, 1957, and the previous paragraph) is not known to occur in *Salmonella;* but the property of colicinogenesis, that is, of producing an antibiotic active on certain coliform bacteria, is sometimes acquired by a non-colicinogenic *Salmonella* strain when it is grown in mixed culture with a colicinogenic organism. Dr. Lorkiewicz used *Salmonella* strains made colicinogenic in this way by Dr. Hamon (Report, 1957, p. 10) to transfer the colicinogenic property to other *Salmonella* strains. In one mixture one hour's contact sufficed to "infect" about 10% of the cells of the recipient strain with the colicinogenic character. To see if the transfer resulted from pairing of cells of the two strains, rather than from transient contact or through the intermediary of some cell-free product, a culture of a motile non-colicinogenic strain was mixed with a culture of a non-motile colicinogenic strain, whose cells contained red formazan granules, as a result of growth in tetrazolium broth. After two hours' incubation of the mixture, motile granule-bearing pairs, formed by the pairing of a motile with a granule-bearing cell, were easily found by microscopy. Such pairs are being isolated by micromanipulation, to see if the pairing is an incomplete kind of conjugation.

BACTERIAL PHYSIOLOGY

Synthesis of Flagella. Dr. Kerridge has continued his studies on the biosynthesis of bacterial flagella, using mutants of *Salmonella typhimurium* requiring various amino-acids. A system suitable for the quantitative study of synthesis of nucleic acid and protein in washed suspensions of organisms was developed and earlier investigations (Report, 1957) were extended to include more mutants.

When the mutants are incubated with a nitrogen and an energy source in the absence of their specific growth requirement, there is little or no net synthesis of ribonucleic acid and protein. However, synthesis of deoxyribonucleic acid continues, and 50% increases of cell DNA occur within two hours. The synthesis of flagella is determined in cultures of "artificially" deflagellated bacilli (Report, 1957). When the culture medium lacks an amino-acid required for multiplication of a particular mutant, the synthesis of flagella continues if the deficient amino-acid is not a constituent of flagellin, the protein constituting bacterial flagella.

The synthesis of adaptive enzymes can also be used as a measure of the ability of bacterial cells to synthesise protein, since increasing enzyme activity reflects increases in the enzyme itself. Studies of adaptive nitratase formation in mutants requiring tryptophan and glutamic-acid showed that adaptation does not occur in the absence of the essential nutrient; for example, the increase in nitratase activity of the glutamic-acid-requiring mutant is only 2% of that in its presence. The increase in nitratase activity is inhibited by concentrations of chloramphenicol which inhibit growth and it appears that the increase in enzyme activity is associated with a synthesis of protein. A similar result was obtained with the tryptophan-requiring mutant. Since flagella resynthesis in the tryptophan-requiring mutant occurs in the absence of tryptophan, it appears that by using suitable mutants of *Salmonella typhimurium* it will be possible to investigate the synthesis of a specific cell protein in the absence of general protein synthesis.

An investigation of the effects of inhibitors on the resynthesis of bacterial flagella has begun. Particular attention is being paid to metabolite analogues known to be incorporated into cellular constituents. Both 8-azaguanine and 2-thiouracil are incorporated into the RNA fraction of bacterial cells; yet in

the presence of these compounds deflagellated suspensions of *Salmonella typhimurium* can resynthesise functional flagella. The amino-acid analogue *p*-fluorophenylalanine appears to have no effect on the resynthesis of flagella but the flagella so produced are non-functional. It is hoped that these studies will throw light on the inter-relations of nucleic acid and protein synthesis.

Bacterial Growth and Division. Dr. Perret has completed a study of the relationship between growth rate, cell division and division of the chromatin bodies (or "nuclei") in *Escherichia coli*, strain K12 (Reports, 1955, 1956, 1957). Samples of *E. coli* grown for many generations at known constant growth-rates and rapidly killed by formalin were examined and photographed under the phase-contrast microscope. In every sample, length, breadth and number of contained chromatin bodies were determined by direct observation of 1000-1500 randomly selected bacteria. Analysis of the data indicate that, on the average, (i) the bacterial diameter remains virtually constant; (ii) the bacteria grow in length at a constant exponential or rectilinear rate throughout their life span; (iii) the mean distance, d, between the centres of adjacent chromatin bodies is independent of growth rate, and has the remarkably constant value of 1.57 $\pm 0.06\mu$; (iv) the time interval, t, between the visible inception of division of chromatin bodies and the subsequent cell division is comparatively independent of growth rate, and is approximately related to the population doubling time, T, by the expression

$$t = 34 + 0.88\ T \text{ min.} \qquad (1)$$

For a population of bacteria dividing asynchronously, with all individuals having the same constant doubling time and lengthening exponentially throughout their life-spans, the following relationships should apply:

$$F = e^{\ln.2\ t/T} \qquad (2)$$

$$L = F.d \qquad (3)$$

where F and L are, respectively, mean number of chromatin bodies per cell, and mean cell length. Substitution of the experimentally determined values of t, T and d in equations (2) and (3) gave strikingly accurate forecasts of F and L for all growth rates tested (which corresponded to doubling times from 22.5 to 240 min.). These results further support the hypothesis (Report, 1955) that, within limits, the times required for cell doubling and for the division of chromatin bodies are independent; and that changes in mean cell size, and in mean numbers of chromatin bodies per cell with growth rate are mainly an inevitable consequence of that independence.

The Relation of Induced Protoplasts and the L-form of Bacteria. Dr. Klieneberger-Nobel has induced the formation of "protoplasts" from *Proteus vulgaris* by Ledeberg's method of growth in a penicillin medium at increased osmotic pressure. Unlike the lysozyme-induced protoplasts of *B. megaterium*, they reverted to the bacterial forms when cultivated on media lacking the inducing agents; but stable L-forms were produced after prolonged sub-cultivation on the penicillin medium.

ABACTERIAL URETHRITIS IN MAN

Dr. Klieneberger-Nobel and Miss Card have continued their investigations on the significance of pleuropneumonia-like organisms (PPLO) in genital infections in man, in particular, in A.U. (abacterial urethritis; "non-specific urethritis"). The investigations were undertaken on behalf of the U.S. Public Health Authority, in collaboration with Dr. G. W. Csonka of St. Mary's Hospital, and Dr. R. D. Catterall of the Whitechapel Clinic.

The Role of Pleuropenumonia-like Organisms (PPLO) in Abacterial Urethritis. Dr. Klieneberger-Nobel's investigation, made to determine the association of abacterial urethritis with PPLO, was completed. The results are consistent with the view that a single type of human genital PPLO is the infecting agent in a substantial proportion of cases of abacterial urethritis in the male, and in a greater proportion of cases of abacterial cervicitis, vaginitis and urethritis in the female; and that it also occurs in a proportion of genital infections with gonococci and *Trichomonas vaginalis.*

Miss Card's investigation of the distribution of complement-fixing antibodies to the human genital PPLO among normal and diseased persons has also terminated. The incidence of PPLO antibodies in the various groups is roughly parallel to that of positive PPLO cultures from the genital tract in similar groups of persons, though the percentage values in the latter are rather higher; and both suggest that PPLO urethritis is a venereal disease. Nevertheless, in individual cases, there is no strong association of PPLO in the genital tract with PPLO antibodies in the blood. A detailed study, both in man and experimental animals, of the relation between antibody level and the course of PPLO infection, is indicated.

The Role of PPLO in Uveitis. Uveitis in the male is frequently accompanied by chronic prostatitis; the aetiology of the syndrome is unknown and the possibility of a PPLO infection is being investigated. PPLO were cultivated from the prostatic fluid and urine of 8 of 77 patients (10.4%) with uveitis and prostatitis; and PPLO antibodies were detected in 20% of the patients. Thus the incidence of PPLO antibodies in this group is higher than in healthy adults and is similar to that in men attending V.D. Clinics (cf. Report, 1957).

The Role of PPLO in Reiter's Disease. No definite association of PPLO with Reiter's disease emerged from cultural and serological tests of affected persons; genital PPLO and PPLO antibodies were only rarely found in these.

Treatment of Abacterial Urethritis. In collaboration with Dr. Csonka, a therapeutic trial of erythromycin is being made on unselected cases of abacterial urethritis treated at random with the antibiotic or a placebo. The results so far are inconclusive, only 8 of the 96 patients admitted to the trial having a genital PPLO infection.

Susceptibility of Human Genital PPLO to Antibiotics. Mr. Blyth has extended his investigation of the susceptibility of PPLO to antibiotics (Report, 1957) to include 48 strains of organisms isolated from the human genital tract. Of the further antibiotics tested, chloramphenical, spiramycin and neomycin inhibited growth of PPLO in concentrations of 4-16 μg./ml. of medium. All strains were highly resistant to sulphonamides, penicillin, erythromycin and oleandomycin. Permanent *in vitro* resistance to streptomycin was acquired very rapidly, whereas repeated subcultivation in the presence of the antibiotic was necessary to increase the tetracycline resistance of the strains.

Animal and Cell Pathogenicity of Human Genital PPLO. In the study of the action of PPLO on living cells *in vitro* Mr. Blyth has shown that a long-standing infection of PPLO can be established in human amnion cells and a strain of human carcinoma cells. Under these conditions, the organisms appear to multiply while attached to the cell surface without, however, significantly damaging the cells.

All his attempts to infect rhesus monkeys with PPLO of human genital origin, by the urethral or other routes, have so far failed. PPLO could not be adapted to monkeys by subcutaneous or intraperitoneal passage in normal or cortisone-treated animals.

Serology of PPLO strains. Miss Card found that all of the 59 human genital PPLO strains so far tested belong to the same serological group. PPLO isolated from the human mouth was distinguishable from the genital strains but the two types had common antigens. PPLO isolated from different animal species also have antigens in common with the human genital PPLO.

Serology of PPLO Infection in Rats. Dr. Klieneberger-Nobel and Miss Card have begun to study the pattern of the antibody response to the PPLO polyarthritic infection of rats. Complement-fixing antibodies to the infecting strain, but not to human, ovine or bovine PPLO, were demonstrable in the infected rats on the 14th day of the disease.

VIROLOGY

TRACHOMA

Dr. Collier assumed charge of the Medical Research Council's Trachoma Unit, and visited the Gambia to organize laboratory and field work there.

Isolation of a Virus. Materials from patients with trachoma were collected for serological study and for attempted virus isolation in embryonate eggs. From nineteen inclusion-positive cases of trachoma, agents were isolated resembling those previously recovered from trachoma patients in China by T'ang and his co-workers. One of the Gambian strains (G1) has now been studied in some detail and the results of electron microscope and serological studies show that strain G1 and the Chinese strains strongly resemble viruses of the psittacosis-lymphogranuloma group. Sera from Frei-negative trachoma patients contain antibodies fixing complement with strain G1 and with the Chinese viruses. After eight passages in eggs, strain G1 was inoculated into the conjunctiva of a human volunteer; it induced lesions resembling those of natural trachoma, and typical cytoplasmic inclusion bodies. The virus was again isolated from this subject by egg incubation and it is concluded that strain G1 is a trachoma virus.

MEASLES

Miss MacGregor has begun a study of measles virus with particular reference to its propagation in chick embryo tissues.

VIRUSES AND ABACTERIAL URETHRITIS

Dr. Collier and Mrs. Stocker are attempting to establish cultures of human urethral cells from material provided by Dr. G. W. Csonka of St. Mary's Hospital. It is hoped that such cultures may be useful for isolating infective agents from cases of abacterial urethritis.

Urethral washings from a small number of patients were inoculated into HeLa and amnion cell cultures, but no evidence of viral activity was obtained. The washings were also examined for the presence of PPLO, and when these organisms were present, even in small numbers, profuse multiplication subsequently occurred in the tissue cultures.

VACCINIA VIRUS

Virus Inactivation. Trials in volunteers of the immunizing activity of vaccine inactivated by ultra-violet irradiation are being made in collaboration with Wing-Commander R. M. Cross, R.A.F. The results obtained so far, suggest that the preparation, though antigenic in man, is not as active as it is in rabbits.

Experiments by Dr. McClean and Dr. Kaplan on the inactivation of vaccinia virus by gamma rays have continued with the collaboration of Mr.

Horne of the Atomic Energy Research Establishment, Wantage. Preparations of dried virus have proved impossible to inactivate with doses of gamma rays as great as 11.6 million rads. There is a residuum of virus which retains infectivity after even the heaviest exposures to ionizing radiation. Up to doses of about 5 million rads, however, the inactivation is directly proportional to the exposure, i.e., it behaves as a first order reaction. Liquid suspensions of virus have been inactivated completely by 1 million rads. This material appears to have retained some protective antigenicity for rabbits.

Dr. Kaplan has re-examined the inactivation of vaccinia virus by ultra-violet irradiation. Under his experimental conditions, which are very different from those in the Habel-Sockrider apparatus used in the preparation of the inactivated vaccine, the inactivation does not behave as a simple, first order reaction. The likeliest explanation of this result is that the virus population is heterogeneous in its sensitivity to ultra-violet light.

Dr. Kaplan also investigated the inactivation of vaccinia virus by heat. The virus is heterogeneous in its response to this agent, too. As far as could be determined, the heterogeneity is not genetically controlled.

Dried Smallpox Vaccine. The use of the dried smallpox vaccine developed by the Institute for routine vaccination has been extended during the last year to several tropical and sub-tropical countries.

Vaccine Production in Tissue Culture. Miss Thomas has continued her work on the optimum conditions necessary for good yields of virus from cultures of chick embryo cells. It appears that the constitution of the medium is important: small additions of lactalbumin hydrolysate, for example, are followed by worthwhile increases in virus titre. Recent results suggest that small scale production of such vaccine some time in 1958 may be possible.

Reproductive Cycle of Vaccinia Virus. From the start of an experimental infection of monolayer cultures of chick embryo cells to the first appearance of new virus, a period of about 9-10 hours elapses, and the production of virus continues for about 10 hours. In the experiments Dr. Kaplan is conducting, the yield of virus is about 100 infectious units per infected cell. Experiments are in progress to determine whether or not the so-called "eclipse phase" is involved in the early stages of the multiplication process of the virus.

Titration of Vaccinia Virus. Dr. Kaplan has titrated repeatedly, by intravenous inoculation of chick embryos, three freeze dried virus preparations. The information is being analysed to determine the reproducibility and precision of the method.

BACTERIOPHAGES

Antiphage Antibiotics. Dr. and Mrs. Asheshov have continued their work on substances produced by actinomycetes active against bacterial viruses. By the collaboration with the Medical Research Council's Antibiotics Research Station at Clevedon large amounts of relatively pure preparations of rutilantin from the actinomycete A220 were prepared for use in more extensive trials against animal viruses. A simpler and more efficient method of processing the active substance was devised and many samples examined chromatographically and biologically. A relatively non-toxic preparation was active against phage in a concentration of $10^{-6.7}$ was found to have a LD50 of 45 mg./kg. for mice. Further purification did not increase the toxicity.

In collaboration with the Station at Clevedon some 250 mould cultures were screened for activity against 60 different bacteriophages. A few were active, one or two of which are worth further investigation.

The active material produced by actinomycete A803 was also studied and a simplified and more effective method of processing devised.

Mrs. Asheshov continued her study of the mode of action of rutilantin on phage, using choleraphage C and Coliphage T2 as test systems. The antibiotic inhibited a stage of intracellular development of the phage directly after adsorption of the phage particles. The results suggest an inhibition of protein synthesis, but it is possible that an early stage in the synthesis of nucleic acid might be involved.

IMMUNOLOGY AND SEROLOGY

DISTRIBUTION OF ANTIBODY IN PLASMA PROTEINS

Dr. Mackay has continued her investigation of the distribution of antibody among the components of animal sera. An attempt was made to show that the association of antibody with beta or gamma globulin might be due to the method of immunization.

In collaboration with Dr. Kekwick, the relation of antibodies to electrophoretic serum components in guinea-pigs immunized with several strains of *Brucella melitensis* and *abortus* was investigated. These animals were part of the experimental material of the late Dr. J. G. Cruickshank and had developed serum agglutinins either to virulent, avirulent or killed cultures of the organism. Rats were immunized by either intravenous or intramuscular injection of a salmonella vaccine and the resultant sera separated by ether precipitation into G2/1, (α + β globulin), G2/2 (α + β + γ globulin) and G3 (γ globulin). The distribution of salmonella agglutinins in the three fractions was not affected by the route of injection. Titration of the agglutinin content of G2/1, G2/2 and G3 fractions pooled serum from the different groups of guinea-pigs revealed that antibody induced by killed or avirulent bacilli was in the G2 fractions and absent or in low titre in G3; whereas that induced by virulent strains was abundant in fraction G3 and sometimes in G2/2.

Immunization with a virulent organism, increased the gamma-globulin. In two serum pools with a high antibody content in the G2/2 fraction, a separate γ_1 globulin was demonstrable electrophoretically.

SEROLOGICAL IDENTIFICATION OF INSECT BLOOD MEALS

Tsetse Flies. The studies by Mr. Weitz and Miss Lee-Jones of the feeding habits of *G. swynnertoni* (Report, 1957) are now complete. The source of the blood meal of large numbers of flies of both sexes collected monthly by Dr. J. P. Glasgow and Dr. P. Isherwood of the Central Tsetse Research Laboratory, E.A.T.R.O. Shinyanga, was identified. Both sexes had a very marked preference for wart-hog blood, the female more than the male flies. This female preference throws some light on the bionomics of *G. swynnertoni;* it is postulated that the activity of females before feeding is much less than that of the males, which make a less discriminate selection of hosts because they leave their resting places earlier, possibly to find a mate; and in so doing perhaps encounter a wider variety of potential hosts than the more static females. Although at Shinyanga *G. swynnertoni* preferred wart-hog to other possible hosts, the preference for wart-hog in some areas was not so evident and in others the fly existed without this host.

Experiments on the feeding habits of *G. longipennis* were made in collaboration with Dr. P. Glover of the Department of Veterinary Services, Veterinary Research Laboratory, Kabete, Kenya. The preferred host of *G. longipennis* on the coast of Kenya is undoubtedly rhinoceros, which provides up to 75% of blood meals. This is in accordance with previous observations from Tan-

ganyika where all the flies that could be found had fed on rhinoceros. A search is being made for areas where *G. longipennis* exists in the absence of this host.

In Uganda, in co-operation with Mr. A. G. Robertson, Director of the Tsetse Control Department, Kampala, various species of fly were collected, mostly in connection with schemes for the eradication of the fly. The habits of the flies concerned *(G. brevipalpis, G. morsitans, G. morsitans sub-morsitans, G. fuscipleuris* and *G. pallidipes)* vary with the district and with the amount of hunting done in the area for the purposes of eradication or control. On the whole, giant forest hog, wart-hog and bush-pig are the preferred hosts of these flies. In areas from which most of the game had been eliminated, *G. brevipalpis* survived on the remaining hippopotami, but disappeared when these animals were removed.

The feeding habits of West African flies (including *G. palpalis, G. longipalpis, G. tabaniformis, G. fusca, G. nigrofusca* and *G. medicorum)* were investigated in collaboration with Dr. T. Nash and his colleagues of the West African Institute for Trypanosomiasis, Kaduna, N. Nigeria. The different species have various feeding habits. But it is noteworthy that in Nigeria the red river hog was a host of importance; a finding consistent with the general preference for pigs observed in East Africa. In the forest, *G. tabaniformis* preferred porcupine blood as its staple diet.

Miss Lee-Jones has begun a study of serological methods for the identification of insect predators of *Glossina*. Antisera prepared in rabbits against the extracts of *G. morsitans* were in precipitin and agar diffusion methods only feebly reactive, although sufficiently so to identify approximately 1/50th of the dry weight of antigenic material of a fly. When specificity of the reaction is established, it will be applied to the stomach contents of various suspected predators of tsetse flies, such as dragon flies.

Mosquitoes. Identification of mosquito blood meals was continued in collaboration with various malaria control schemes. For the first time accurate determinations of the feeding habits of the various culicines have been made, in collaboration with Dr. M. C. Williams and Dr. G. A. H. McClelland of the East Africa Virus Research Institute, Entebbe. Six species of mosquito were tested in significant numbers. The results for *Taeniorynchus (C) pseudoconopas, T. fraseri, T. aurites* and *T. metallicus* were similar. In all four species, 94% had an avian meal. Further tests are required to establish whether this high percentage of avian feeds indicates a host preference, or a large avian population. *T. fuscopennatus* was more variable in its feeding habits, the majority feeding on blood of man or primates and bovids.

In Malaya, in collaboration with Dr. J. A. Reid of the Institute for Medical Research, Kuala Lumpur, the feeding habits of *A. hackeri, A. baezai, A. pujutensis* and *A. barbarostris* were investigated. As was to be expected from Dr. Reid's field work, *A. hackeri* and *A. pujutensis* mainly fed on monkeys *(Macaca irus* and *Presbytis* sp.) or man. *A. baezai* fed chiefly on a bovid which is suspected to be the very small but numerous mouse deer *(Tragulus* sp.). This is being further investigated.

In addition, large numbers of blood meals of various species of mosquito were tested from the Belgian Congo, China, Ethiopia, Formosa, Indonesia, Iran, Iraq, North Borneo, Philippine Islands, Singapore, South Africa, Southern Rhodesia, Saudi Arabia and French West Africa.

TRYPANOSOME ANTIGENS

Mr. Weitz has continued his studies of the antigenic structure of trypanosomes. Relatively large quantities of antigen from *T. brucei* were obtained from infected rats. The antigenic pattern is somewhat obscured by impurities

in the extracts of *T. brucei* which were inevitably contaminated with rat serum proteins, a difficulty it is proposed to avoid by using rat anti-trypanosome sera for the serological tests. The investigations were extended to *T. rhodesiense*.

ANTITOXIN PRODUCTION

Refinement of Therapeutic Antitoxins. Miss Lanham has continued her investigations of methods of fractionation of hyperimmune horse serum, especially by the use of ether at low temperatures. Dr. Dolby is using the albumin and globulins purified by this and other methods, together with purified bovine serum albumin and gamma-globulin, for a study of the kinetics of their break-down by the enzyme pepsin; for this purpose a potentiometric formol titration was devised. In collaboration with Miss Lanham and Mr. Rodican, he is studying by electrophoretic and chromatographic methods the products of the peptic hydrolysis, and also the peptide material found in refined antisera that increases the permeability of small blood vessels.

Dr. Dolby has investigated the electrophoretic mobility of refined antisera produced in the Institute and elsewhere and found, in agreement with most other workers, that the major fraction has the mobility of a gamma-globulin.

Proteolytic Enzymes for Refining Antisera. In continuation of his experiments on the action of pepsin on hæmoglobin at pH 2 and 3.2, Dr. Dolby has found that the activity of the enzyme at pH 3.2 is dependent on the degree of denaturation of the substrate. Further attempts to fractionate the enzyme and separate the activities at these two pH levels have so far proved unsuccessful.

EXPERIMENTAL PATHOLOGY

MECHANISMS OF INFLAMMATION

Permeability Factors in Mammalian Sera. Professor Miles, Dr. Wilhelm, Dr. Mill and Dr. Sparrow completed their survey of the globulin permeability factor and its inhibitor in the serum of man, guinea-pig, rat and rabbit. Among the serum proteins of each species is the inactive precursor of a potent permeability factor activable by dilution in 0.85% saline (except in the rabbit) and by fractionation in aqueous ethyl ether systems. The serum permeability factor in man is associated with the β-globulins, in the guinea-pig and rat with the α-globulins, and in the rabbit with the α- and β-globulins. In each species the permeability factor is susceptible to soya bean trypsin inhibitor, but is not plasmin; and does not appear to owe its permeability-increasing activity to the liberation of histamine. The serum permeability factors of man, guinea-pig and rat increase permeability for 15-20 min., but that of the rabbit has an effect lasting more than $2\frac{1}{2}$ hr.

Among the serum proteins of each species there is also a slowly-acting, low-potency inhibiter of the permeability factor. In man the serum inhibitor is associated with the α- and β-globulins; in the guinea-pig with α-globulins; in the rat with α-globulins and albumins; and in the rabbit with albumins.

The permeability factor-inhibitor system in the sera of the above animals exhibits species differences, but in general is basically similar; it probably is a feature of the sera of all mammalian species, because similar permeability factors are also present in serum fractions containing α- and β-globulins in all other animals investigated—cat, dog, horse, and ox. The mechanism of activation of the system and its possible rôle in physiological and pathological phenomena are under investigation.

Permeability Factor-Inhibitor System in Tissue Fluid and Lymph. Professor Miles and Dr. Wilhelm demonstrated that the pro-permeability factor/inhibitor system in guinea-pig serum is also present in the tissue fluid of the skin and in the lymph of the guinea-pig. As in serum, the permeability factor is activated by dilution, shifts to higher dilutions with age, and is susceptible to fractions containing the serum inhibitor and to soya bean trypsin inhibitor.

The establishment of a general distribution of the permeability factor-inhibitor system in tissue fluid and lymph removes the need to postulate mechanisms whereby the permeability factor present in serum is brought into the tissues at the time of an injury leading to increased permeability of the vessels.

Permeability Factor in Various Tissues. In an attempt to demonstrate that guinea-pig tissues contain a physiological activator of the globulin permeability factor present in serum, tissue fluid and lymph, Dr. Mill investigated the effect of treating normal serum with washed debris from tissue homogenates. Homogenates of brain, lung, heart, skin and liver, but not skeletal muscle, contain a histamine-liberator, but do not activate the globulin permeability factor. The liberator is also present in the soluble portion of the homogenates and can be extracted with saline from acetone-dried homogenate.

Distinction of Hypotensive Factor from Permeability Factor in Serum. The G2 fractions of guinea-pig serum that increase capillary permeability also induce hypotension when injected intravenously into guinea-pigs (Report, 1955). By comparing the hypotensive with the permeability-increasing potencies of a number of G2 fractions from the guinea-pig, rat and rabbit, each tested in all three species, Dr. Sparrow concluded that the substances inducing hypotension are probably distinct from those increasing permeability.

Permeability Factors in Coagulating Gland Fluid of the Guinea-pig. In collaboration with Dr. Jules Freund of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, U.S.A., Professor Miles and Drs. Mill and Wilhelm investigated the toxic principle in the secretion of the guinea-pig's coagulating gland. It proved to be a high-potency permeability factor, more active than histamine in the skin of the guinea-pig and rabbit, and producing a hypotensive shock on intravenous injection into these animals. It appears to be a glycoprotein with a molecular weight of about 70000, and with a high arginine esterase activity. The toxin is quite distinct from the globulin permeability factor which can be isolated from the blood of the guinea-pig.

It was readily separable by starch block electrophoresis from the protein-coagulating principle in the secretion. This second principle is also a moderately toxic and moderately potent permeability factor in the rat and rabbit, acting in the rat by the liberation of histamine. These two substances account for most of the protein in the fluid of the gland. The analogous fluid in the rat also contains two predominant proteins, one of which is the coagulating principle, but neither of which is toxic.

Effect of Various Antihistaminic Drugs on Permeability Factors. Miss Mason and Dr. Wilhelm are investigating the specificity and efficacy of the inhibitory action of various antihistaminic drugs on permeability factors, tested in the skin of laboratory animals.

Permeability Factor present in Antitoxic Sera. Mr. Rodican has completed his studies of the substances responsible for increasing the capillary permeability which occur as impurities in refined therapeutic antitoxins. These are produced during the initial stages of the refining process, a large amount

when the pH of the antiserum is adjusted to 3.2 and a smaller amount during pepsin digestion. The permeability factor is largely removed in subsequent stages of the refining process. The permeability factors in samples taken at different stages of the refining process have very different dialysis rates, indicating that more than one form of active substance is concerned; at least two active substances are indicated by chromatographic separation. The active material is relatively heat-stable and is associated with peptides.

The activity is not inhibited by mepyramine, soya bean trypsin inhibitor or by protein inhibitor present in guinea-pig serum.

MECHANISMS OF INFECTION AND DEFENCE

The Mechanisms of Non-specific Immunity. Professor Miles, in collaboration with Dr. Douglas Smith, has completed a survey of the effect of a short period of hyperthermia during the early stages of infection, on the resistance of the guinea-pig to local infection of the skin by a variety of bacteria. In most of the infections the resistance of the animal is unaltered; in a few it is decreased, and in them the change in resistance is effective only in the first few hours of infection.

The Stimulation of Apparently Non-antibody Immunity by B. pertussis *Vaccines.* Professor Miles investigated the effect of intraperitoneal pertussis and typhoid vaccine on a number of bacterial infections, including infection by *B. pertussis* in the skin of the guinea-pig. Both vaccines, if given in subshocking doses, stimulated an immunity to infection, that appeared within a few hours and lasted a few days. There was no evidence of a specific action of the pertussis vaccine on pertussis infection, as reported in the mouse.

Histamine-release during Bacterial Infection and Intoxication. Dr. Smith and Professor Miles are investigating the effects of intraperitoneal injections of minimally effective doses of bacterial toxins and of various living pathogenic bacteria on the mast cell population of the peritoneum, and on the release of histamine into the peritoneal cavity; with a view to determining the role, if any, that histamine release plays in the inflammatory response to infection.

Mechanisms of Staphylococcal Infection. Dr. Smith is devising methods of separation of the soluble toxins produced by a number of variants of *Staphylococcus aureus,* with a view to analysing the different degrees and manifestations of pathogenicity displayed by these strains.

BIOCHEMISTRY

THE HUMAN BLOOD GROUP SUBSTANCES

Specific Inhibition Studies Relating to the Lewis Blood Group System. One of the most interesting problems in biochemical studies on blood group substances is the elucidation of the chemical structures which are responsible for the complete serological independence of the various blood group characters. Enzyme inhibition experiments with simple sugars suggested that L-fucose plays an important part in Lewis Le^a specificity and Professor Morgan and Dr. Watkins accordingly examined structures containing fucose to see whether they would inhibit the reaction between Le (a+) red cells and anti-Le^a serum. Through the kindness of Professor Kuhn a number of fucose-containing oligosaccharides isolated from human milk were made available. One of these compounds lacto-*N*-fucopentaose II contains an L-fucose unit joined by an α-(1$\rightarrow$4) linkage to *N*-acetylglucosamine, and has considerable capacity to inhibit the specific agglutination reaction, whereas lacto-*N*-tetraose

which is identical in structure with the Pentaose II except that it does not contain fucose, fails to inhibit. This result indicates that an α-L-fucopyranosyl structure is concerned in Le^a specificity. Compounds in which one α-L-fucosyl unit is joined by a (1→2) linkage to galactose or a (1→3) linkage to glucose are inactive, and a compound, lacto-*N*-difucoahexaose, with two fucose units, the second joined to the terminal galactose of Pentaose II, was much less active than Pentaose II. It is suggested that the determinant group in Le^a serological reactions is a branched trisaccharide unit formed by two non-reducing end-units, α-L-fucosyl- and β-D-galactosyl, attached to *N*-acetylglucosamine by (1→4) and (1→3) linkages respectively.

Microbial Enzymes and Blood Group Substances. Dr. Watkins has continued the investigation on the enzymes of the protozoan flagellate *Trichomonas fœtus* which destroy the serological activity of the blood group A, B, H and Le^a substances. She finds in active extracts of the protozoon a number of glycosidases, including α- and β-galactosidases, α- and β-glucosaminidases, α- and β-galactosaminidases and an α-fucosidase, and is determining if any of these glycosidases are responsible for the loss of specificity of the blood group mucopolysaccharides. With Mr. Tyler she is devising methods for the separation and purification of the enzymes, for use in structural studies on the blood group substances.

The Preparation and Properties of Group Substances. Mr. Pusztai and Professor Morgan are examining preparations of specific blood group mucopolysaccharides isolated from (a) the same specimen of ovarian cyst fluid by entirely different methods and, using these methods, from (b) different fluids from persons of the same serological group within the ABO classification. This information is needed before a satisfactory explanation of the influence of genes on the chemical structure, the serological behaviour and the biosynthesis of the group specific substances from a common preformed substrate, can be reached.

Analysis of the Blood Group Substances. The Products of Partial Acid and Alkali Hydrolysis. Mr. Cheese and Professor Morgan are looking for satisfactory methods of forming and isolating small oligosaccharides that still retain some serological reactivity, from the specific group mucopolysaccharides.

Dr. Côté and Professor Morgan have examined further the partial acid hydrolysis products of group A substance and have isolated and identified *O*-α-D-galactosyl (1→3)-*N*-acetylgalactosamine. This *N*-containing disaccharide is the sixth disaccharide obtained from group A substance.

To assist in the identification of fucose-containing disaccharides from blood-group substances and to provide disaccharides of known structure for serological studies, Dr. Coté has isolated from crude fucoidin by acetolysis, three new disaccharides, *O*-α-L-fucosyl (1→2)-fucose and the corresponding (1→3) and (1→4) linked disaccharides. In addition to the above material he also obtained *O*-α-L-fucosyl (1→3)-*N*-acetylglucosamine and the corresponding (1→6) disaccharide by acid reversion from a mixture of L-fucose and *N*-acetylglucosamine.

The earlier work of Dr. Watkins and Professor Morgan indicated that an oligosaccharide which contained non-reducing α-L-fucosyl and β-D-galactosyl units joined to *N*-acetyl-glucosamine would probably prove to be the serologically active structure in Le^a substance. Since α-fucosyl residues in Le^a substance are relatively stable in dilute alkali it was considered that gentle alkaline hydrolysis of the Le^a substance would most probably yield serologically active oligosaccharides. Dr. Thomas and Professor Morgan have now isolated from the products of alkaline hydrolysis (100° pH 8.5) of Le^a substance, by adsorp-

tion on charcoal and partition chromatography on cellulose columns, oligosaccharides which are probably homogeneous, contain the sugars fucose, galactose and *N*-acetylglucosamine and which possess considerable Le^a specificity.

Artificial antigens. Dr. Feier has undertaken the preparation of α-galactosyl disaccharides which are of serological interest. A chemical synthesis was achieved by coupling acetobromogalactose with a glucosamine derivative, employing mucuric cyanide as a condensing agent. This material brings about the synthesis of an α-linkage in contrast to the formation of a β-linkage when silver carbonate is used. An artificial antigen was made by coupling the aminophenyl derivative of lactosamine, galactosyl-(1→4)-*N*-acetylglucosamine, with horse serum protein. The antigen precipitates with pneumococcus type XIV horse anti-serum and the precipitation can be inhibited by lactosamine.

Plant Seed Hæmagglutinins. The presence of hamagglutinins in various plant seeds, some of which are specific for certain human blood group antigens, was studied further by Lt.-Col. Bird. The agglutinins extracted from the seeds of *Dolichos biflorus* are specific for the human blood group antigen A, reacting more strongly with sub-group A_1 than with A_2; and are thus of value in distinguishing A_1 erythrocytes from those of the weaker sub-groups of A. The specificity depends on reaction with a hitherto undescribed component of the human A cell, which is also present in the erythrocytes of sheep, goat, horse, pig and dog, but not in those of chicken, pigeon, rabbit and guinea-pig. By fractional precipitation of crude seed extracts with ethanol at low temperatures, Lt.-Col. Bird and Professor Morgan obtained a purified preparation of the *Dolichos* agglutinin agglutinating A_1 cells to a titre of 16,000. It contained three electrophoretic components; only one of which was highly active. The yield of the active component was insufficient for satisfactory chemical and physical characterization but the agglutinin appears to be a protein or a muco-protein with a molecular weight of about 50,000.

The active substance obtained from *Dolichos biflorus* is also a powerful and specific precipitin which can be used in qualitative and quantitative studies of purified A-substance, and in the identification of A-secretors. The precipitin reaction in agar gels indicate that the *Dolichos* principle is specific for an antigen common to human and hog A-substance.

The seeds of *Crotalania striata* and *Calpurnina aurea* were found to contain agglutinins acting on human erythrocytes of groups A and B but not on those of group O. Absorption and elution experiments indicated that the anti-A and anti-B reactive groups are in each case on the same molecule.

The agglutinins of *Dolichos biflorus* are specifically inhibited by *N*-acetylgalactosamine, and those of *Crotalaria striata* and *Calpurnia aurea* are inhibited by both *N*-acetylgalactosamine and D-galactose; these findings confirm previous observations by Professor Morgan and Dr. Watkins that *N*-acetylgalactosamine and D-galactose are important fundamental structural determinants of A and B specificity respectively.

CARBOHYDRATE STUDIES

Starch-metabolising enzymes. Dr. Whelan and Mr. Parrish examined the effects of reducing and oxidising the reducing-end groups of maltodextrins (α-1:4-linked glucose polymers) on the susceptibility of these sugars to attack by α-amylase, β-amylase, phosphorylase and D-enzyme. In the case of the unmodified sugars, none of the enzymes attacks the disaccharide (maltose), only the D-enzyme acts rapidly on the trisaccharide, but all four rapidly attack the tetra and higher saccharides. When the dextrins are reduced or oxidised the trisaccharide becomes completely resistant to amylase and phosphorylase

action, the tetrasaccharide behaves as did the unmodified trisaccharide, the pentasaccharide behaves as the unmodified tetrasaccharide and so on. This means that although in maltotetraose the reducing-end unit is remote from the site of enzyme action it nevertheless partakes in complex formation with the enzymes. Dr. Walker found that end-group reduction rendered both the tri- and tetra-saccharides resistant to D-enzyme action. This confirms the deductions of the action pattern of D-enzyme outlined in the 1957 Report.

The joint action of phosphorylase and D-enzyme was studied by Dr. Walker and Dr. Whelan. When phosphorylase degrades starch chains it could not shorten the chains to fewer than four glucose units. This means that some 20% of the major starch component, amylopectin, cannot be metabolised by phosphorylase. By adding D-enzyme to the system a further 75% of the resistant residue now becomes accessible to phosphorylase. When conditions favour the synthesis of starch, D-enzyme assists phosphorylase to synthesize the "primer" molecules from which phosphorylase builds the starch macromolecules. D-Enzyme has also been found to incorporate glucose 6-phosphate into starch molecules. This may be the origin of the ester phosphate groups occurring at the C-6 positions in some of the glucose units of native starch.

Mr. Parrish and Dr. Whelan investigated the end products of the α-amylolysis of starch, in particular the limit dextrins containing phosphate groups. Previously only dextrins containing one phosphate group per molecule had been detected but dextrins containing two or more such groups have now been recognised.

Mr. Abdullah and Dr. Whelan found that D-enzyme transfers maltodextrinyl chains to L-sorbose and methyl α-L-sorboside (acceptor molecules) and the products have been isolated and examined. The position of attachment of the maltodextrinyl chains is at C-3 of sorbose. When D-glucose is the acceptor molecule the attachment is at C-4. The steric configurations at and around these respective carbon atoms are so alike that the enzyme cannot distinguish between L-sorbose and D-glucose, except in rate of action. Similarly α-amylase, β-amylase and phosphorylase have been found unable to distinguish between maltotetraose (maltotriosyl-glucose) and synthetic maltotriosyl-sorbose. This is in contrast to the failure of these enzymes rapidly to attack reduced (maltotriosyl-sorbitol) and oxidized (maltotriosyl-gluconic acid) maltotetraose (see above).

Dr. Walker and Dr. Whelan investigated the production of glucose during the salivary α-amylolysis of starch. This is proved to be due to the amylase itself, and not to an enzymic impurity. The rate of attack of maltotriose relative to the higher maltodextrins can be controlled by varying the amount of chloride ion added as an enzyme activator. Under appropriate conditions of chloride and enzyme concentration the action on maltotriose can be eliminated, leaving the hydrolysis of higher maltodextrins to proceed unhindered.

Dr. Feier has examined the chain molecules of α-1:6-linked galactose units which occur in peas, the chains being terminated by a sucrose molecule. This sucrose was removed by chemical methods and sugar polymers containing only galactose were isolated.

Molecular Weights of Polysaccharides. Mr. Parrish continued his study of periodate oxidation as a means of determining the molecular weights of polysaccharides, using maltodextrins and enzymically synthesized amyloses of known molecular size. Neutral periodate gives satisfactory results with small molecules but also induces a random depolymerization which mitigates against its use with large molecules. Variations of the conditions of oxidation were tested and oxidation at pH 2 seems likely to be the basis of a preferred method.

The synthetic amyloses were used to test the permeability of cellophan membranes. Molecules up to 3,200 in weight can pass through.

Enzymic Polymerization of Monosaccharides. Disaccharides based on the naturally occurring monosaccharides are of importance in, for example, studies of the structures of polysaccharides and of serological specificity. Their chemical synthesis is difficult but glucose disaccharides can be formed by direct polymerization of glucose with certain glycosidases. Mr. Haq has found that potato juice will catalyse such a reaction. Dr. Clancy has investigated this and two other enzyme sources, almond emulsin and takadiastase, and finds them to polymerize a wide range of aldohexoses, aldopentoses, and deoxyhexoses. The biologically important disaccharides of L-fucose are in course of preparation.

Structural Studies by Oxidative Degradation. Mr. Hughes is studying the separate actions of sodium periodate and lead tetra-acetate in oxidizing the α-glycol groups of mono- and oligo-saccharides, with the intention of using these reagents to investigate naturally occurring sugar polymers. He has also developed a means of removing electrolyte from its mixture with a monosaccharide, the sugar being adsorbed on charcoal. This is a desirable alternative to the use of ion-exchange resin, which may degrade the sugar.

PHOSPHOLIPIDS OF HEART MUSCLE

Dr. Macfarlane and Mr. Gray have continued work on the separation and constitution of the phospholipids of ox heart muscle, particularly on cardiolipin and the plasmalogens, and have examined the effect of bacterial toxins on several of the components.

Cardiolipin. This complex phosphatidic acid constitutes about 5% of the total phospholipid, and contains only unsaturated fatty acids. Its constitution is not certain, but work reported previously suggested that it has a free hydroxyl group. Evidence for its presence has been sought by identifying the products of oxidation with potassium permanganate. The results though not conclusive, indicate the presence of a hydroxyl group. It is important to establish this as knowledge of the structure may clarify the function and biosynthesis of this compound.

Plasmalogens. (Acetal Phospholipids.) Mr. Gray has completed studies on the structure of choline plasmalogen, which showed conclusively that the fatty aldehyde residue is exclusively linked to the β-carbon atom of the glycerol molecule, thus establishing the natural plasmalogen as an α-acyl-β-aldehyde derivative of glycerophosphorylcholine. The ethanolamine-containing plasmalogen, present in kephalin fractions, was proved to be the α-acyl-β-aldehyde derivative. In both cases the structure was established by identification of the lysophosphatides, formed by removing the aldehyde group from the plasmalogen, as the α-acyl isomers. The work provided material for identification of the fatty acids present in lecithin, choline plasmalogen, kephalin and ethanolamine plasmalogen. There are very interesting differences in the fatty acids of the individual phospholipids; their significance, particularly in the unsaturated essential fatty acids, is at present quite obscure, but must ultimately relate to the biological function.

Action of Bacterial Toxin. The action of the toxins of *Cl.welchii*, *Cl.œdematiens*, *Cl.septicum* and *C.diptheriæ* on the plasmalogens and cardiolipin was examined. None was detected, except with *Cl.welchii* α-toxin (lecithinase) which decomposes choline plasmalogen as readily and in the same manner as it decomposes lecithin.

BACTERIAL PRODUCTS

Carotenoid pigments of Staphylococcus aureus. Miss Lanham has completed work begun elsewhere on the identification of the major carotenoid pigment produced on a milk-agar medium by a single strain of *Staphylococcus aureus*, and on the chemical and physical factors affecting its formation. By its chemical and chromatographic behaviour, the pigment appears to be an ester of the acidic carotenoid, norbixin.

BIOPHYSICS

HUMAN PLASMA PROTEINS

The Antihæmophilic Factor. Although two methods are available for the assay of antihæmophilic factor, neither provide absolute values and with either of them measurements have to be referred to a standard. The selection of a standard which would permit the comparison of measurements made at intervals of some months is a matter of some difficulty, and the lack of such a standard is at present a major obstacle to further advances.

In an attempt to solve this problem Dr. Kekwick and Mr. Caspary stored two samples of fresh platelet-free normal human plasma and two solutions of human antihæmophilic factor concentrate, after distribution in small volumes, at —25°C. The relative activities of these preparations were assayed by the prothrombin conversion ratio method at intervals over an eleven week period. None of the samples remained constant in activity. Moreover it was clear that during a period of a month at —25° platelet free human plasma, collected with the utmost care to avoid the formation of traces of thrombin, may lose 30-40% of its antihæmophilic activity. Plasma stored in this way is therefore obviously unsatisfactory for use clinically as well as for reference purposes.

Plasminogen. It has long been known that in preparations of plasminogen-containing globulin, the plasminogen may be spontaneously activated to plasmin. The presence of organic solvents is known to facilitate this activation but the cause of the variability in behaviour of different batches of plasminogen in this respect has not been found. Dr. and Mrs. Derechin have begun to study this problem.

Pathological Sera. Professor N. H. Martin studied the sera of fourteen patients in which globulins with sedimentation coefficients between 17 and 24S were present in excessive amounts. The immunological characteristics of purified globulins from these sera are being compared with those isolated from the sera of patients suffering from myelomatosis, and with normal gamma globulin.

Blood Group Substances. In collaboration with Prof. Morgan, Mr. Caspary examined the characteristics of blood group H substance preparations obtained by different procedures from the same pseudomucinous cyst fluid. Although the chemical compositions of preparations made by extraction with phenol and by treatment with ficin were indistinguishable, the molecular weights, estimated from determinations of sedimentation coefficient and diffusion coefficient, were found to be 332,000 and 265,000 respectively.

BLOOD PRODUCTS LABORATORY

During the year, staff changes and the demands of a large laboratory with heavy production responsibilities severely restricted research activity.

Hypogammaglobulinæmia. The laboratory continued to carry out the electrophoretic analysis of sera from patients in the field trial organized by the Medical Research Council Working Party on Hypogammaglobulinæmia.

Anti-hæmophilic Factor. Concentrates of human anti-hæmophilic factor were prepared regularly throughout the year. They were used mainly in three hospitals designated by the Medical Research Council Hæmophilia Committee. The preparation has now been given to some thirty hæmophiliacs, some of whom have received repeated infusions of the material at intervals over periods up to a year without any evidence of reaction to suggest that the preparation is antigenic. The potency has been found to vary; this is probably attributable at least in part to the quality of the starting materials; it is sometimes difficult to obtain blood within a few hours of its collection.

Clinically, the preparation controls hæmorrhage effectively.

Cœruloplasmin. With Dr. G. Curzon of the National Hospital for Nervous Diseases, Mr. Vallet is developing a method of preparing cœruloplasmin from the G2 fraction of plasma, which is usually discarded during the routine process of plasma fractionation.

BLOOD GROUP RESEARCH UNIT

The study of blood groups seems still to be in the 'shell-gathering' stage, in which more can be picked up by chance than can be found in searching by design. Most of the time of the Unit is spent in testing samples of blood which, because of some peculiarity, have been selected out of thousands and sent by colleagues in the United States, Great Britain and elsewhere. The serum of people who have had a reaction to transfusion, particularly when the donor belongs to a different race, is now a more hopeful source of new antibodies than is the serum of mothers of children with hæmolytic disease. It seems that mothers have made almost all the varieties of antibody, detectable by present methods, of which they are capable.

The MNSs System. The rare antigens Mi^a and Vw, which are part of the MNSs system, are being investigated in the hope that they will throw some light on the complex genes responsible for the MNSs antigens. During the course of the work, which is being done in collaboration with Dr. J. Mohn of the University of Buffalo and Dr. J. Wallace of Glasgow, a new allele of *M* and *N* has disclosed itself—in a negro family.

Dr. Hackel analysed, by absorption and elution methods the immune antibody sometimes found in the serum of negroes of the genotype S^uS^u (Report, 1956). The work confirmed the previous belief that the serum does not contain separable anti-S plus anti-s. It seems probable that the antibody molecules are all anti-Ss.

The P System. Two years ago Dr. Sanger showed that a rare antibody discovered in the United States belonged to the P system (Report, 1956); so that the P system appeared to have a pattern like that of the A_1 A_2 BO system: P_1 corresponding to A_1, P_2 to A_2 and p to O. Samples of blood from a family in Minneapolis, sent by Dr. G. A. Matson, are adding to the pattern of the P system; the new antigen in this family promises to be analogous with the B of the A_1 A_2 BO system.

The ABO System. A very rare weak form of A was studied in collaboration with Dr. W. Weiner, of the Regional Blood Transfusion Service, Birmingham, and a very weak form of B with Dr. C. Cameron of the East of Scotland

Blood Transfusion Service, Dundee. The latter sample was most curious for though the cells certainly had a weak B antigen the serum contained anti-B.

Family Studies. The analysis of the blood groups of families tested by the Unit has been brought up to date. Such an analysis is made every four years or so. The results serve to consolidate the known manner of inheritance of the groups; a point of particular importance with respect to the more recently discovered systems. Several families were of the rare type needed to confirm the first autosomal linkage to be recognised in Man—that discovered by Mohr, in Copenhagen, between the Lutheran and the Lewis or secretor genes. It is gradually becoming clear that the linkage is, in fact, not between the Lutheran and Lewis genes but between the Lutheran and secretor genes. The appearance of linkage with the Lewis genes was due to the Lewis antigens of the red cells being dependent in part on the secretor genes.

The Unit has continued to collaborate with Dr. Eliot Slater and Mr. James Shields of the Genetics Unit, The Maudsley Hospital, in testing the blood of twins taking part in a psychiatric investigation: blood groups are useful in helping to decide whether the twins are monozygotic. The Unit has also collaborated with Dr. C. N. Armstrong of Durham University and Dr. P. Polani of Guy's Hospital in studies of patients with the sex abnormalities called Klinefelter's and Turner's syndromes: if some chromosomal upheaval were causing these conditions it might be reflected in the blood groups.

For the supply of routine but often very scarce antisera the Unit is indebted to Dr. Mourant and the Blood Group Reference Laboratory. Many thousand blood samples were provided by Dr. R. A. Zeitlin of the South London Blood Transfusion Centre, Sutton. Innumerable samples of blood, without which the work would be impossible, are very kindly given by the staff of the Institute.

BLOOD GROUP REFERENCE LABORATORY

The Laboratory serves as reference centre for blood grouping problems and supply centre for grouping sera for the United Kingdom. It also acts under the auspices of the World Health Organisation as the world reference centre.

Demands for liquid grouping serum for use in the United Kingdom and for dried serum for use overseas have continued to increase. More overseas laboratories were helped to start their own blood grouping services, by the supply of sera and by determining the groups of members of their staffs. Large numbers of specimens received from laboratories in Great Britain and overseas were examined for red cell antigens and serum antibodies, both for clinical purposes and in order to select and identify sera suitable for use as diagnostic reagents.

Dr. Parkin has examined numerous sera from patients with rheumatoid arthritis and other rheumatic diseases for the presence of factors promoting the agglutination of sensitised red cells and which are inhibited by gamma-globulin. She is continuing her work on the effect of reconstituted dried plasma on red cells, in collaboration with the Medical Research Council Burns Unit at Birmingham, but only a small number of suitable cases were available for study during the year.

Dr. Parkin and Miss Giles investigated the serology and genetics of a number of rare blood group antigens.

Miss Ikin has continued the grouping of blood specimens for anthropological purposes and has tested bloods from Arabia, Sarawak (Sea Dyaks), Sardinia, Belgian Congo, Nigeria (numerous tribes), Corsica, Iraq (Assyrians) and from more Burmese resident in England.

In conclusion the Governing body desires to record its great appreciation of the manner in which the scientific, administrative and technical staffs have worked together during the period under review, and to congratulate them on the interest and range of their scientific activities.

H. H. DALE

Chairman.

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THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

Balance Sheet

and

Accounts

31st December 1958

CHELSEA BRIDGE ROAD,
LONDON, S.W.1.
26th May, 1959.

FINANCIAL REPORT OF THE GOVERNING BODY

1. The Balance Sheet as at 31st December, 1958, shows balances to the credit of the various funds as follows: Capital Fund £666,129, Specific Funds £138,487, Bequest Funds £14,820 and Contingency Reserve £109,142. The balance on the Sinking Fund for Freehold Buildings of £98,369 is after adding the profit on sales of Sinking Fund investments amounting to £385 and transferring from income and expenditure account £4,940. The Re-endowment Fund has been increased by donations during the year amounting to £201.

2. The General Fund Income and Expenditure Account shows the income for the year as £213,182 compared with £193,274 in 1957. Expenditure amounted to £177,534 against £168,849 last year. The surplus for the year is £35,648 compared with £24,425 in 1957.

3. The year's surplus of £35,648 shown by the General Fund Income and Expenditure Account has been transferred to the Contingency Reserve.

4. Stocks of Sera, Vaccine Lymph and Horses on hand at December 31st have the nominal value of £17,180, £662 and £5,720 respectively.

5. Messrs. Cooper Brothers & Co., the retiring Auditors, will, subject to the provision of the Companies Act, 1948, be re-appointed.

H. H. DALE, *Chairman of Governing Body.*

HUGH BEAVER, *Hon. Treasurer.*

BALANCE SHEET

(1957) £		£	£	£
	Capital Fund:—			
	Donations, &c., received to date from the following:—			
2,000	Dr. Ludwig Mond (1893)		2,000	
46,380	Berridge Trustees (1893/98)		46,380	
10,000	Worshipful Company of Grocers (1894)		10,000	
250,000	Lord Iveagh (1900)		250,000	
18,904	Lord Lister's Bequest (1913/23)		18,904	
7,114	William Henry Clarke Bequest (1923/6)		7,114	
3,400	Rockefeller Foundation (1935/6)		3,400	
22,669	Other Donations and Legacies (1891-1954)		22,669	
	General Fund Income and Expenditure Account Accumulated Surplus as at 31st December, 1957	285,239		
	Add Profit, less losses on sale of investments	34,326		
	Less amounts written off investments	13,903		
		20,423		
285,239			305,662	
645,706				666,129
	Specific Funds:—			
93,044	Sinking Fund for Freehold Buildings	98,369		
32,964	Pension Fund	33,207		
6,710	Re-endowment Fund	6,911		
			138,487	
	Bequest Funds:—			
8,038	Jenner Memorial Studentship Fund	8,500		
6,241	Morna Macleod Scholarship Fund	6,320		
			14,820	
146,997				153,307
	Specific Grants and Legacies Unexpended:—			
772	Cancer Research Legacies (1937-50)		772	
347	Royal Society Grant (1951)		347	
5,489	Nuffield Foundation Grants (1952-8)		5,665	
9,812	Guinness Lister Research Grant (1953-8)		11,237	
16,420				18,021
	Contingency Reserve:—			
	As at 31st December, 1957		73,494	
	Add Surplus on General Fund Income and Expenditure Account, 1958		35,648	
73,494				109,142
	Current Liabilities:—			
22,810	Creditors and accrued charges			17,730
£905,427				£964,329

H. H. DALE, *Chairman of Governing Body.*

HUGH BEAVER, *Hon. Treasurer.*

REPORT OF THE AUDITORS

We have examined the above Balance Sheet and annexed Income and Expenditure Account which are in all the information and explanations which we considered necessary for our audit. In our opinion these accounts information required by the Companies Act, 1948, and show a true and fair view of the state of the Institute's

London, 28th May, 1959.

31st DECEMBER 1958

(1957) £					£	£	£
	Fixed Assets:—						
	Freehold property at cost:—						
73,548	Land and Buildings, Chelsea				73,548		
20,456	Queensberry Lodge Estate, Elstree				20,455		
2,049	House, Bushey				2,049		
						96,052	
	(Note: Additions and replacements since 1912 at Elstree and 1935 at Chelsea have been charged to Revenue.)						
	Furniture, Fittings, Scientific Apparatus and Books:—						
2,472	At cost *less* depreciation to 31st December, 1920 ...					2,472	
98,525	*(Note: Additions and replacements since 31st December, 1920 have been charged to Revenue)* ...						98,524
	General, Specific and Bequest Funds.						
	Investments and Uninvested Cash:—						
		Quoted at cost, less amounts written off In Gt. Britain	Elsewhere	Unquoted at cost	Cash		
558,343	General	476,451	67,752	51,295	—	595,498	
	Specific—						
93,044	Sinking Fund for Freehold Buildings ...	84,320	7,935	—	6,114	98,369	
32,964	Pension Fund	31,746	—	—	1,461	33,207	
6,710	Re-endowment Fund ...	6,590	—	—	321	6,911	
	Bequest—						
8,038	Jenner Memorial Studentship Fund	5,340	—	1,940	1,220	8,500	
6,241	Morna Macleod Scholarship Fund	5,894	—	—	426	6,320	
705,340		610,341	75,687	53,235	9,542	748,805	748,805
		£686,028					
	(Market Value of Quoted Investments—£837,347)						
	Current Assets:—						
76,966	Debtors and Payments in advance					60,920	
24,596	Balance at Bankers and Cash in hand					56,080	
101,562							117,000
	(Notes: See paragraph 4 Governing Body's Financial Report for nominal values of Sera, Vaccine Lymph and Horses which have not been brought into the accounts. There is a contingent liability of £6,750 in respect of investments not fully called up.)						
£905,427							£964,329

TO THE MEMBERS.

agreement with the books of account. In our opinion proper books of account have been kept. We have obtained amplified by the information given in paragraphs 1 and 4 of the Financial Report of the Governing Body give the affairs at 31st December, 1958, and of the surplus for the year ended on that date.

COOPER BROTHERS & CO.,

Chartered Accountants.

INCOME AND EXPENDITURE ACCOUNT

GENERAL

(1957) £		Total Expenditure £	External Contributions £	£
68,600	Salaries and Wages	129,334	54,611	74,723
7,074	Emoluments of two members of the Governing Body in an Executive Capacity	7,643	—	7,643
2,468	Premiums on Federated Superannuation Policies	4,960	1,537	3,423
3,112	Premium on Group Pension Policy	3,508	328	3,180
3,822	Rent, Rates and Insurance	4,485	179	4,306
11,194	Gas, Water, Fuel and Electricity	14,146	2,205	11,941
2,802	Office Expenses, Stationery and Printing	3,445	234	3,211
326	Auditor's Fee	410	—	410
709	Travelling Expenses	1,257	211	1,046
2,292	Biochemistry Expenses	4,372	1,744	2,628
1,154	Microbiology, Immunology and Experimental Pathology Expenses	3,066	2,117	949
354	Biophysics Expenses	1,657	760	897
428	Virology Expenses	1,470	627	843
14,969	Serum, Vaccine and Vaccine Lymph Expenses	22,653	2,013	20,640
7,645	Animals	9,833	888	8,945
9,138	Animal House Expenses and Forage	11,170	1,330	9,840
22,844	Buildings, Alterations, Repairs and Renewals	14,334	957	13,377
2,432	General Apparatus and New Installations	473	—	473
790	Library Expenses	1,347	—	1,347
722	General Stores	1,397	—	1,397
1,158	Staff Canteen Loss	1,586	211	1,375
—	Blood Products Laboratory Expenses	6,089	6,089	—
4,816	Amount transferred to Sinking Fund for Freehold Buildings (including £4,516 Interest on Investments)	4,940	—	4,940
24,425	Surplus transferred to Contingency Reserve after charging to expenditure £5,905 for additions to property and equipment *(1957 £18,698)*	35,648	—	35,648
£193,274		£289,223	£76,041	£213,182

NUFFIELD FOUNDATION GRANTS

(1957) £		£	(1957) £		£
5,519	Salaries, Wages, Laboratory Expenses and Animals	5,824	5,008	Balance at 1st January, 1958	5,489
5,489	Balance carried forward	5,665	6,000	Amounts received	6,000
£11,008		£11,489	£11,008		£11,489

for the year ended 31st December 1958

FUND

(1957) £		£	£
	Interest and Dividends on Investments:—		
32,435	General Fund	33,782	
4,236	Sinking Fund	4,516	
		———	38,298
	Underwriting Commission:—		
995	General Fund	731	
156	Sinking Fund	—	
		———	731
149,925	Sales of Sera, Vaccines, Vaccine Lymph, &c.		168,348
5,527	Rent		5,805
£193,274			£213,182

GUINNESS-LISTER RESEARCH GRANT

(1957) £		£	(1957) £		£
8,697	Salaries and Wages	9,464	8,307	Balance at 1st January, 1958 ...	9,812
3,798	Laboratory Expenses	3,111	14,000	Amount received	14,000
9,812	Balance carried forward ...	11,237			
£22,307		£23,812	£22,307		£23,812

PENSION FUND

(1957) £		£	(1957) £		£
3,858	Loss on Realisation of investments	—	36,478	Fund as at 1st January, 1958 ...	32,964
1,380	Pensions	1,486	1,663	Interest on Investments (gross)	1,729
32,964	Fund as at 31st Dec., 1958 ...	33,207	61	Underwriting Commission ...	—
£38,202		£34,693	£38,202		£34,693

JENNER MEMORIAL STUDENTSHIP FUND

(1957) £		£	(1957) £		£
354	Stipend of Student	—	7,932	Fund as at 1st January, 1958 ...	8,038
8,038	Fund as at 31st Dec., 1958 ...	8,500	460	Interest on Investments (gross)	462
£8,392		£8,500	£8,392		£8,500

MORNA MACLEOD SCHOLARSHIP FUND

(1957) £		£	(1957) £		£
—	Loss on realisation of investments	218	5,980	Fund as at 1st January, 1958 ...	6,241
6,241	Fund as at 31st Dec., 1958 ...	6,320	261	Interest on Investments (gross)	297
£6,241		£6,538	£6,241		£6,538

INVESTMENTS AS AT 31st DECEMBER 1958

GENERAL FUND

		Balance Sheet Value	Market Value
	QUOTED:—		
Trustee Securities			
£2,900	Australia 4½% Stock 1960/62	£2,666	£2,900
£12,000	Australia 3% Stock 1972/74	12,121	8,880
£20,000	British Transport 3% Gtd. Stock, 1967/72	20,259	14,500
£12,000	3% Savings Bonds 1955/65	12,000	10,980
£66,300	3% Savings Bonds 1960/70	66,417	53,703
£3,000	3% Savings Bonds 1965/75	2,652	2,250
£25,000	New Zealand 3¼% Stock 1962/65	21,989	21,875
Debentures and Loan Stocks			
£4,000	A.P.V. Company Ltd. 5% 1st Mrge. Deb. Stock 1980/85	3,866	3,180
£5,000	Allied Bakeries Ltd. 5% Unsecured Loan Stock 1966/70	4,819	4,625
£5,000	Associated Biscuit Manufacturers Ltd. 6% Deb. Stk. 1978/83	4,995	5,150
£500	Associated Commercial Vehicles Ltd. 5¼% Unsecd. Loan Stk. 1977/82	501	462
£5,000	Associated Electrical Industries Ltd. 6% Deb. Stk. 1978/83	4,988	5,125
£10,000	Australian Estates Co. Ltd. 6½% Conv. Secd. Loan Stk. 1971/76	9,848	9,450
£5,000	Birfield Ltd. 6% Unsecd. Loan Stk. 1976/81	4,977	5,025
£5,000	British Petroleum Co. Ltd. 6% Conv. Deb. Stk. 1976/80	5,179	5,400
£500	Bowater Paper Corpn. Ltd. 5¾% Conv. Unsecd. Loan Stk. 1978/82	531	522
£5,000	George Cohen 600 Group Ltd. 6% Unsecd. Loan Stk. 1975/80	5,000	4,875
£5,000	Debenture Corpn. Ltd. 5¾% Deb. Stock 1979/83	4,806	4,875
£5,000	Edwards High Vacuum Ltd. 6½% Conv. Unsecd. Loan Stk. 1978/83	5,270	6,125
£5,000	Flowers Breweries Ltd. 5½% 1st Mtge. Deb. Stk. 1970/72	4,850	4,875
£4,500	General Electric Co. Ltd. 6% Unsecd. Loan Stk. 1976/81	4,408	4,567
£9,000	Hope & Anchor Breweries Ltd. 5½% Mtge. Deb. Stk. 1980/85	8,842	8,325
£5,000	Kennings Ltd. 5½% Unsecd. Loan Stock 1970/75	4,703	4,125
£10,000	London & Overseas Freighters Ltd. 6% 1st Mtge. Deb. Stk. 1963/82 (45% paid)	4,525	4,150
£9,000	Mitchell Cotts Group Ltd. 6% Unsecd. Loan Stk. 1976/81	8,800	8,775
£10,000	Norvic Shoe Co. Ltd. 5% Unsecd. Loan Stock 1970/75	9,800	9,000
£10,000	Peninsular & Oriental Steam Navigation Co. 5% Deb. Stk. 1975/80	9,542	9,050
£3,000	Port of London 3½% Regd. Stock 1965/75	2,687	2,220
£4,000	Ruston & Hornsby Ltd. 6% Unsecd. Loan Stk. 1975/80	4,000	3,960
£5,000	John Smith's Tadcaster Brewery Co. Ltd. 6% Red. Deb. Stock 1978/83	4,892	5,100
£10,000	Tennant Bros. Ltd. 6% Red. Deb. Stock 1971	10,089	9,900
£10,000	United Gas Industries Ltd. 6% Unsecd. Loan Stk. 1973/75	9,900	9,750
£10,000	Whitbread Investment Co. Ltd. 5¾% Gtd. Deb. Stk. 1980/85	9,950	9,600
United Kingdom Equities			
7,500	Albright & Wilson Ltd. Ordinary 5/- Shares	5,168	8,156
1,500	Associated Electrical Industries Ordinary £1 Stk. Units	4,498	4,312
3,000	Associated Portland Cement Manufacturers Ltd. Ordinary £1 Stock Units	5,522	7,969
1,500	Automatic Telephone & Electric Co. Ltd. Ordinary £1 Stock Units	5,228	6,094
3,000	British Oxygen Co. Ltd. Ordinary Stk. Units £1	6,165	7,687
3,125	British Tabulating Machine Co. Ltd. Ordinary £1 Shares	5,391	10,078
2,500	British Timken Ltd. Ordinary £1 Shares	5,304	7,875
3,500	Cater Brightwen & Co. Ltd. Ordinary £1 Stock Units	10,872	9,537
16,500	Debenture & Capital Investment Trust Ltd. Ordinary 5/- Stock Units	3,071	11,962
5,000	Distillers Co. Ltd. Ordinary 10/- Shares	4,678	6,712
4,000	Dorman Long & Co. Ltd. Ordinary Shares of £1 each	4,981	5,700
300	Hadfields Ltd. Ordinary £1 Shares	393	319
2,500	Alfred Herbert Ltd. Ordinary £1 Shares	4,656	5,156
3,750	Imperial Chemical Industries Ltd. Ordinary £1 Stock Units	5,619	7,125
4,000	Lancashire Steel Corpn. Ltd. Ordinary £1 Shares	5,328	6,300
9,000	London County Freehold & Leasehold Properties Ltd. Ordinary 10/- Stock Units	5,153	7,200
10,000	London & Montrose Investment Trust Ltd. Ordinary 5/- Shares	—	9,250
£2,500	London Scottish American Trust Ltd. Deferred Stock	—	7,125
2,500	Marks & Spencer Ltd. 'A' Ordinary 5/- Shares	5,479	6,937
2,000	Metal Box Co. Ltd. Ordinary £1 Stock Units	5,189	7,312
30,000	Mercantile Investment & General Trust Co. Ltd. Ordinary 5/- Shares	13,401	29,250
550	Prudential Assurance Co. Ltd. 'A' Shares of 4/-	5,268	5,981
15,000	Rio Claro Investment Trust Ltd. Ordinary 5/- Stock Units	991	11,625
10,000	River Plate & General Investment Trust Co. Ltd. Deferred 5/- Stock Units	667	9,500

GENERAL FUND—continued.

		Balance Sheet Value		Market Value
7,200	Seccombe Marshall & Campion Ltd. Ordinary £1 Shares	10,162	...	13,140
1,000	Shell Transport & Trading Co. Ltd. Ordinary £1 Shares	6,703	...	7,406
2,500	Sphere Investment Trust Ltd. Ordinary £1 Shares	—	...	6,875
12,000	Standard Trust Ltd. Ordinary 5/- Stock Units	3,833	...	12,300
7,500	Sterling Trust Ltd. Ordinary £1 Stock Units	6,824	...	25,500
1,500	Turner & Newall Ltd. Ordinary £1 Stock Units	4,763	...	5,719
11,250	Typhoo Tea Holdings Ltd. Ordinary 5/- Shares	7,505	...	24,750
3,000	Vickers Ltd. Ordinary £1 Stock Units	4,765	...	5,400
12,000	Allen West & Co. Ltd. Ordinary 5/- Stock Units	6,126	...	8,100
3,000	Westinghouse Brake & Signal Co. Ltd. Ordinary £1 Stock Units ...	5,718	...	6,937
£9,450	Witan Investment Co. Ltd. Ordinary Stock	11,745	...	21,498
2,500	F. W. Woolworth & Co. Ltd. Ordinary 5/- Stock Units	5,413	...	6,562
Dollar Equities				
600	American Metal Climax Inc. Common Shares of $1	5,496	...	5,889
350	Central Illinois Public Service Co. Common Shares of $10	4,893	...	5,340
250	Consolidated Edison Co. Common Shares (n.p.v.)	4,980	...	5,800
400	First Bank Stock Corporation Common Shares of $10	4,623	...	7,005
200	First National Bank of New York Common Shares of $20	4,603	...	5,421
700	Firstamerica Corpn. Common Shares of $2	4,967	...	5,278
700	Marine Midland Corpn. Common Shares of $5	4,954	...	5,903
300	Montana Power Co. Common Shares (n.p.v.)	5,421	...	7,201
600	Oklahoma Gas & Electric Co. Common Shares of $5	4,808	...	6,398
400	Southern Natural Gas Co. Common Shares of $7½	5,630	...	5,996
100	Standard Oil Co. New Jersey Common Shares of $7	2,066	...	2,057
480	Tennessee Gas Transmission Co. Common Shares of $5	4,859	...	6,125
450	Tri-Continental Corpn. Common Shares of $1	5,023	...	6,485
400	Washington Power Co. Common Shares (n.p.v.)	5,429	...	6,353
		£544,203	...	£687,899
	UNQUOTED:—			
£9,000	British Titan Products Co. Ltd. 5½% Unsecd. Loan Stock 1970/75	£8,820		
£10,000	Caltex (U.K.) Ltd. 6% Gtd. Loan Stock 1971/76	9,800		
£10,000	Kraft Foods Ltd. 5% Debenture Stock 1965/75	10,000		
£10,000	R. A. Lister & Co. Ltd. 5% Unsecured Loan Stock 1960/65 ...	9,975		
£4,000	R. H. Neal & Co. Ltd. 6½% Unsecured Loan Stock 1966/71... ...	4,000		
£5,000	Powers Samas Accounting Machines Ltd. 6% Unsecured Loan Stk. 1975/78	4,950		
£5,000	Tanker Charter Co. Ltd. 6½% 'B' Secured Loan Stock (75% paid)	3,750		
		£51,295		

SINKING FUND FOR FREEHOLD BUILDINGS

Trustee Securities				
£3,000	British Electricity 3% Gtd. Stock 1968/73	£2,916	...	£2,325
£10,000	3% Savings Bonds 1960/70	9,622	...	8,100
£3,500	3% Savings Bonds 1955/65	3,518	...	3,202
£4,500	3% Funding Stock 1959/69	3,876	...	3,757
Debentures and Loan Stocks				
£5,000	Financial Times Ltd. 5½% Mtge. Deb. Stock 1980/85	4,900	...	4,875
£9,000	Gallaher Ltd. 6% Unsecured Loan Stock 1976/81	8,884	...	9,045
£10,000	New Zealand Loan & Mercantile Agency Co. Ltd. 5½% 2nd Mtge. Deb. Stk. 1970/80	9,525	...	8,750
United Kingdom Equities				
2,000	British Aluminium Co. Ltd. Ordinary £1 Stock Units	5,830	...	8,250
3,000	Ford Motor Co. Ltd. Ordinary £1 Stock Units...	5,550	...	7,687
2,000	English Electric Co. Ltd. Ordinary £1 Stock Units	6,194	...	6,125
2,600	Guest Keen & Nettlefolds Ltd. Ordinary £1 Stock Units	5,373	...	7,540
1,000	A. Reyrolle & Co. Ltd. Ordinary £1 Stock Units	4,463	...	4,875
500	Shell Transport & Trading Co. Ltd. Ordinary £1 Shares	3,352	...	3,703
4,000	South Durham Steel & Iron Co. Ltd. Ordinary £1 Shares	5,329	...	5,600
2,000	Union Discount Co. of London Ltd. Stock Units of £1	4,988	...	6,200
Dollar Equities				
150	Standard Oil Co. New Jersey Common Shares of $7...	3,108	...	3,085
400	Union Tank Car Co. Common Shares (n.p.v.)	4,827	...	5,282
		£92,255	...	£98,401

PENSION FUND

		Balance Sheet Value		Market Value
Trustee Securities				
£12,416	British Electricity 4½% Gtd. Stk. 1967/69	£11,322	...	£11,857
£2,200	3% Savings Bonds 1960/70	2,205	...	1,782
£1,000	3% Savings Bonds 1965/75	1,000	...	750
£5,800	4% Funding Loan 1960/90	3,050	...	5,249
Debentures and Loan Stocks				
£3,000	Lewis Berger & Sons Ltd. 5½% Deb. Stock 1977/82	2,977	...	2,805
£3,000	British Oxygen Co. Ltd. 5¾% Deb. Stock 1981/86	2,860	...	2,985
£3,000	Dowty Group Ltd. 6% Unsecured Loan Stock 1977/82	2,932	...	3,000
£3,000	Ruston & Hornsby Ltd. 6% Unsecured Loan Stock 1975/80... ...	3,000	...	2,970
£2,500	Metal Box Co. Ltd. 5½% Unsecured Loan Stock 1977/80	2,400	...	2,387
		£31,746	...	£33,785

RE-ENDOWMENT FUND

£7,600	3% Savings Bonds 1960/70	£6,590	...	£6,156

JENNER MEMORIAL STUDENTSHIP FUND

	QUOTED:—			
Debentures and Loan Stocks				
£1,000	A.P.V. Company Ltd. 5% 1st Mtge. Debenture Stock 1980/85 ...	£850	...	£795
£1,000	Hope & Anchor Breweries Ltd. 5½% Mtge. Debenture Stock 1980/85	990	...	925
£1,000	Gallaher Ltd. 6% Unsecured Loan Stk. 1976/81	1,000	...	1,005
£1,500	General Electric Co. Ltd. Unsecd. Loan Stock 1976/81	1,500	...	1,522
£1,000	Mitchell Cotts & Co. Ltd. 6% Unsecd. Loan Stock 1976/81 ...	1,000	...	975
		£5,340	...	£5,222
	UNQUOTED:—			
£1,000	British Titan Products Co. Ltd. 5½% Unsecured Loan Stk. 1970/75	£940		
£1,000	R. H. Neal & Co. Ltd. 6½% Unsecured Loan Stock 1966/71 ...	1,000		
		£1,940		

MORNA MACLEOD SCHOLARSHIP FUND

Trustee Securities				
£1,000	Stockton-on-Tees 5½% Redeemable Stock 1966	£992		£1,000
£1,500	Australia 6% Regd. Stock 1974/76	1,496	...	1,552
£1,500	New Zealand 6% Stock 1976/80	1,485	...	1,567
£1,000	Agricultural Mortgage Corpn. Ltd. 5% Debenture Stk. 1979/83 ...	958	...	920
£1,000	South Essex Waterworks Co. 5% Red. Debenture Stock 1986/91...	963	...	845
		£5,894	...	£5,884

THE LISTER INSTITUTE

OF

PREVENTIVE MEDICINE

REPORT

OF THE

GOVERNING BODY

1959

CHELSEA BRIDGE ROAD, LONDON, S.W.1

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Deputy Director: Professor W. T. J. Morgan.

Superintendent of Elstree Laboratories: W. d'A. Maycock.

MICROBIOLOGY, IMMUNOLOGY and EXPERIMENTAL PATHOLOGY

†A. A. Miles, C.B.E., M.A., M.D., F.R.C.P. *(Professor of Experimental Pathology in the University of London).*
Muriel Robertson, M.A., D.Sc., LL.D., F.R.S.
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Emmy Klieneberger-Nobel, Ph.D., D.Sc.
Ruth M. Lemke, B.Sc., Ph.D.
M. D. Pittam, B.A., Ph.D. *(Trypanosomiasis Research)*
Brenda Mason, B.Sc.
J. P. Craig, M.D. *(U.S.A.).*
R. G. Blaker, Ph.D. *(U.S.A.).*

GUINNESS-LISTER RESEARCH UNIT

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Sheila Howarth, B.Sc., Ph.D.
Janice Edgar, M.Sc.
Sylvia Smith, M.Sc.
H. Ozeki, B.Sc.
J. Joys, B.Sc. *(Research Student).*
Helene de Margarie *(Honorary Research Staff)*

VIROLOGY

L. H. Collier, M.D. *(also Hon. Director, M.R.C. Trachoma Research Unit).*
J. Jane Stocker, B.Sc.
Lilias G. Gillies, B.Sc.
A. A. Frohlich, B.Sc., Dip.An.Gen. *(M.R.C. Trachoma Research Unit).*
P. Reeve, B.Sc. *(M.R.C. Trachoma Research Unit).*

BIOCHEMISTRY

†W. T. J. Morgan, C.B.E., D.Sc., Ph.D., F.R.I.C., F.R.S. *(Professor of Biochemistry in the University of London). Principal Biochemist, Elstree.*
*Marjorie G. Macfarlane, D.Sc., Ph.D.
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†R. A. Kekwick, D.Sc. *(Reader in Chemical Biophysics in the University of London).*
Professor N. H. Martin, M.A., B.M., B.Ch., B.Sc *(Honorary Research Associate).*
M. Derechin, M.D. *(British Council Scholar).*
P. W. Walton, B.Sc. *(Medical Research Council Grantee).*

NUTRITION

§Dame Harriette Chick, D.B.E., D.Sc
§E. Margaret Hume, M.A

†Appointed Teacher of the University of London.
*Recognised Teacher of the University of London.
§Honorary Member of Institute Staff.

PREPARATION and STUDY of THERAPEUTIC SERA (ELSTREE)

*B. G. F. Weitz, M.R.C.V.S.
J. Rodican, B.Sc.
Frances M. Lee-Jones, B.Sc. *(Trypanosomiasis Research)*

BIOCHEMISTRY (ELSTREE)

*D. E. Dolby, B.Sc., Ph.D.
Sheila M. Lanham, B.Sc.

PREPARATION and STUDY of SMALLPOX VACCINE (ELSTREE)

*D. McClean, M.B., B.S., M.R.C.S.
C. Kaplan, M.Sc., M.B., Ch.B., Dip.Bact.
L. C. Robinson, B.Sc.

PREPARATION and STUDY of BACTERIAL VACCINES (ELSTREE)

*A. F. B. Standfast, M.A., Dip.Bact.
Jean M. Dolby, M.A., Ph.D. *(Medical Research Council External Scientific Staff).*
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BLOOD PRODUCTS (ELSTREE)

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*Recognised Teacher of the University of London.
§Honorary Member of Institute Staff.

ANNUAL GENERAL MEETING

OF

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

June 23rd, 1959

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the work of the Institute for the year 1958.

GOVERNING BODY

At its last meeting the Council re-appointed Sir Henry Dale, Sir Charles Dodds and Sir Wilson Jameson as its representatives on the Governing Body until 31st December 1959. Professor W. T. J. Morgan was re-appointed as the Scientific Staff's representative.

The Governing Body has noted with pleasure the appointment of Professor Morgan to the rank of Commander of the Order of the British Empire and the election of Mr. Paul Channon as a Member of Parliament.

COUNCIL

At last year's Annual General Meeting the three retiring members of the Council, Professor F. S. Stewart, The President of the Royal College of Physicians and Professor A. A. Miles, were re-appointed. Sir Hugh Beaver was appointed to the Council as a representative of the Members.

The three members of the Council due to retire this year in accordance with the Articles of Association, but who are eligible for re-appointment, are Sir Charles Harington, Sir Paul Fildes and the Rt. Hon. Lord Balfour of Burleigh, each a representative of the Members of the Institute.

MEMBERS

There have been no changes in the Membership of the Institute during the past year.

STAFF

The Governing Body is pleased to record that the Council of the Royal Society appointed Professor W. T. J. Morgan the Croonian Lecturer for 1959.

Mr. B. G. F. Weitz was appointed a member of the Tse-tse Fly and Trypanosomiasis Committee of the Colonial Office.

Dr. M. W. McDonough was appointed to the Guinness-Lister Research Unit; Mr. M. D. Pittam as protozoologist for the trypanosomiasis research programme; Mr. L. C. Robinson to the Smallpox Vaccine Department; and Mr. G. J. Roderick as Elstree Secretary Designate.

Dr. D. Kerridge, Dr. F. W. Parrish, Dr. J. Thomas, Mrs. S. Derechin, Miss P. M. Glover and Miss L. R. Thomas resigned during the year.

Professor A A. Miles spent ten weeks in the early summer of 1958 as a Visiting Professor at the Department of Microbiology and Immunology at the Downstate Medical Centre of New York State University, Brooklyn, N.Y.

At the invitation of the Council for International Organizations of Medical Sciences, Professor Miles and Dr. D. L. Wilhelm presented papers at a symposium on the "Biochemical Response to Injury" held at Semmering, Austria, in September 1958.

Professor Morgan, by invitation, gave a special lecture at the 100th meeting of the Gesellscheft Deutscher Naturforscher und Arzte in Wiesbaden in October 1958. He visited India in January 1959 as the Institute's delegate to the Golden Jubilee Celebrations at the Haffkine Institute in Bombay, and as a delegate of the Royal Society to the Indian Science Congress in Delhi and to the Indian Institute of Science in Bangalore. Whilst in India he visited Colombo University, Ceylon, at the invitation of the British Council. He also represented the University of London at the International Biochemical Congress in Vienna, in September 1958.

On behalf of the World Health Organisation Dr. D. McClean visited Geneva as a consultant to the Study Group on Smallpox Vaccine.

At the invitation of Professor J. E. Jorpes, of the Karolinska Institutet, Stockholm, Dr. R. A. Kekwick visited laboratories in Stockholm, Upsala and Oslo during May 1958.

Dr. B. A. D. Stocker took part by invitation in a symposium on "Genetics in Medical Research" at the University of Wisconsin, Madison, U.S.A., in May 1958, and in a symposium on Recombination Mechanisms in Bacteria at VIIth International Congress for Microbiology, at Stockholm, in September 1958.

Dr. E. Klieneberger-Nobel, by invitation of the New York Academy of Sciences, took part in a meeting on the biology of Pleuropneumonia-like Organisms, in January 1959 and subsequently lectured at eight American universities.

Dr. W. J. Whelan lectured by invitation at the University of Washington, Seattle, the Iowa State College, Ames, the Ohio State University, Columbus and the Höchst-Fabrick, Frankfurt.

The VIIth International Congress for Microbiology held in Stockholm in August 1958 was attended by Professor Miles, Mr. Standfast and Dr. Stocker; the IVth International Congress for Biochemistry held in Vienna in September 1958 by Drs. Kekwick, Macfarlane, Watkins, Wilhelm and Whelan; the VIIth Congress of the International Society of Hematology and the VIIth Congress of the International Society of Blood Transfusion held in Rome in September 1958 by Professor Morgan, Dr. Maycock and Dr. Watkins; the International Scientific Conference on Tse-tse Research, held in Brussels in August 1958 by Mr. Weitz; the General Assembly of the International Organisation Against Trachoma (International Congress of Ophthalmology) held in Brussels in September 1958 by Dr. Collier.

VISITORS

The following visitors, apart from those listed under Staff, worked in the Institute's Laboratories: Miss Stina Bobeck, Forskningslaboratoriet, LKB, Stockholm, Sweden; Miss Birgitte Bohn and Mr. Ole Jørgensen, Department of Technical Biochemistry, Tekniske Højskole, Copenhagen; Dr. G. Curzon Institute of Neurology, Queen's Square, London; Dr. S. Dubiski, Institute of Microbiology, Zabrze-Rokitnica, Poland; Dr. T. Iino, National Institute of Genetics, Japan; Professor T. P. Magill, Downstate Medical Centre, State University, New York, Brooklyn, U.S.A.; Dr. P. Mathews, John Innes Horticultural Institution, Herts.; Dr. A. Umnova, Central Institute of Blood Transfusion, Moscow, U.S.S.R.

During the year a lecture was given at the Institute by Dr. Dexter French, of the Iowa State College, on "Chromatographic Separation of Oligosaccharides".

The Blood Group Research Unit and the Blood Group Reference Laboratory whose researches for 1958 are described on pages 31-34, are still accommodated at the Institute; and Miss E. M. Hume continues to do editorial work for *Nutrition Abstracts and Reviews* on behalf of the Commonwealth Bureau of Animal Nutrition at the Rowett Research Institute.

DONATIONS AND GRANTS

The Governing Body gratefully acknowledges a number of donations to the Institute's Re-endowment Fund from members of the Life Offices' Association of Assurance Companies and a legacy from the late Miss Flora Latter.

The Governing Body records its appreciation of the generosity of many bodies whose benefactions and grants support research work in the Institute.

New funds include a grant by the British Empire Cancer Campaign in aid of research on phospholipids, under the direction of Dr. Marjorie Macfarlane, and another from the Colonial Development and Welfare Fund in aid of researches on the immunology of trypanosomiasis infections under the direction of Mr. B. Weitz. The Nuffield Foundation renewed for a further five years, the grant made to the department of Experimental Pathology for researches on non-specific immunity in the early stages of infection.

The Governing Body is also indebted to the Nuffield Foundation for a grant in aid of Professor Morgan's researches on the immunochemistry of blood group substances; to the Medical Research Council for a grant in aid of research on acetal phospholipids and related compounds under Dr. Macfarlane; to the Agricultural Research Council for a grant in aid of researches on protozoa, and on the mechanism of starch-carbohydrase action; to the Department of Scientic and Industrial Research for special grants for researches under Dr. Whelan on the enzymic polymerisation of monosaccharides and on chemically modified polysaccharides; to the Royal Society and Imperial Chemical Industries Ltd. for grants for the purchase of special apparatus; to the Colonial Welfare Development Fund and to the World Health Organization for grants in aid of researches on the blood-meals of insect vectors of disease; to the U.S. Public Health Authority for grants in aid of researches on abacterial urethritis in man; and finally to the Rockefeller Foundation for a travelling grant to Dr. Whelan.

RESEARCHES IN 1958

SUMMARY

In this summary of the investigations made in 1958, the bracketed numbers refer to the pages of the report where the researches are described in greater detail.

Microbiology. The Guinness-Lister Unit continues its exploration of the genetics of Salmonella bacilli, mainly in terms of the biochemistry and genic control of the synthesis and function of flagella; using the bacteriophages that infect these bacilli to transduce genetic material from one kind of bacillus to another (12). The bacteriophage transduction technique has been extended to staphylococci (13). The other purely microbiological study concerns the cytology of certain free-living, flagellated protozoa (10).

Immunology and pathology of infective diseases. The Institute's studies embrace infections by viruses, pleuropneumonia-like organisms (PPLO), bacteria and protozoa.

The isolation of the *viruses* of trachoma (16) and of inclusion blennorrhoea (17) has opened up a large field of study, both in Gambian laboratories of the Medical Research Council's Trachoma Unit, where the epidemiology of trachoma is under study (17) and in the Council's Unit in the department of Virology. The ready infection of the baboon's conjunctiva (17) with the virus of inclusion blennorrhoea provides an experimental model in which to study the practicability of prophylactic immunization in the related infection by trachoma virus. In the Smallpox Vaccine department, there is continued progress towards making vaccine from vaccinia virus grown in tissue culture, as an alternative to virus harvested from the skin of infected sheep (18).

The investigation of abacterial urethritis in man established a genital type of *PPLO* as a possible cause. In man, however, the incidence of antibodies to PPLO was not correlated with the presence of PPLO in the genitalia, so a detailed study of the relation of PPLO antibody response to PPLO infections is being made, in the first place in experimental infections of the rat (15).

The immunological study of *bacterial* infections includes further progress in identifying the two antigens of the whooping cough bacillus responsible for prophylactic immunization (11) and the exclusion of the histamine-sensitizing antigen as being immunogenic (11); a search for immunizing somatic antigens in the diphtheria bacillus (11); and an analysis of the *iota* toxin of *Clostridium welchii*, a bacillus that may play an aetiological role in infective haemorrhagic fever (12). The study continues of the biologically active substances formed when diphtheria antitoxins are refined by proteolysis, and of the actual enzymic process of refinement (21).

As regards pathogenic *protozoa*, the antigenic analysis of Trichomonas species continues (10); and a new field has been entered in an attack on the immunology of trypanosomiasis. Soluble trypanosome antigens, formed during experimental trypanosomiasis of the rat, are under investigation (10); and the *in vitro* culture of trypanosomes is being attempted, to provide bulk material for antigenic analysis of these protozoa (11).

Epidemiology. The refined serological methods devised to identify the animal source of food for blood-sucking insects continues to provide valuable facts about the feeding habits of Tse-tse flies and mosquitoes in regions where these insects are vectors, or possible vectors, of disease (20).

Pathology. Work on the relation of early tissue reactions to defence against microbial infection continues. The tissue response to various kinds of injury, including infection, was explored to determine the role, if any, of the serum proteases which increase capillary permeability (22). The investigation of early non-specific resistance to bacteria was extended to infections by tubercle bacilli (23).

Biochemistry. The biochemical researches mainly concern three kinds of substances—the blood group substances, the cellular phospholipids, and starches.

During the year the problem of homogeneity of the blood group specific substances isolated from secretions and digests of tissue has received careful attention and new methods of analysis have revealed that in the natural secretions blood group specificity is associated with at least two types of mucopolysaccharide molecule (24). Progress was made in the separation and purification of enzymes which destroy the serological activity of the blood group

substances and the chemical changes associated with loss of activity were investigated (24).

The phospholipid study is at present directed to defining the constitution of tissue phospholipids which, although ill-described, are known to be metabolically active. The structure of one of these, cardiolipin, has been elucidated (28) and work is proceeding on the fatty constituents of the plasmalogens (28) and the polyglycerophosphates (28).

The plant enzymes established as responsible for the synthesis and degradation of starch have been characterized in terms of their individual actions on starch. These enzymes were studied particularly in respect of their combined actions in systems thought likely to reproduce the conditions in which starch is synthesized *in vivo* (25); and of their separate actions on chemically modified substrates, designed to yield information on the specificity of the enzymes (26).

The human plasma proteins. The Institute's work is concerned with the isolation, refinement, characterisation, assay, and in some cases clinical trial of the various biologically active proteins of human plasma.

The difficulties of specifying the potency of preparations of anti-haemophilic globulins for use in haemophiliacs has necessitated a re-examination of the modes of assay (29); clinical studies of the efficacy of the human preparation are in progress (30). The therapeutic value of gamma globulin in the treatment of hypogammaglobulinaemia is the subject of another clinical study (30).

Studies of the isolation of active proteins include that of plasmin, for clinical use (30), and the conditions of its activation from the precursor plasminogen during the fractionation of serum (29); and that of the oxidase caeruloplasmin (30). Pathological studies include investigations of the so-called "macroglobulins" that occur in hyperglobulinaemic sera (29) and of the proteins that appear in the urine of man and animals poisoned by heavy metals (29).

MICROBIOLOGY

PROTOZOOLOGY

Antigenic Structure of Trichomonas. Dr. Robertson's analysis of the antigens of *Trichomonas foetus* from the cow, and *T. suis* from the pig has revealed a close relation between the two protozoa, which suggests that they are varieties of one species. By graded ethanol precipitation of crude extracts of the Belfast strain of *T. fœtus*, it is possible to separate mainly polysaccharide and mainly protein fractions corresponding to the serologically active materials detectable in the whole organism by gel diffusion precipitin tests. Work has begun on the antigens of the ciliate *Tetrahymena pyriformis* and of other *Tetrahymena* species.

Cytology of Flagellates. Dr. Pittam has continued his cytological and cytochemical studies of various protozoa including soil amoebo-flagellates. *Trichomonas foetus* proved to contain two distinct carbohydrates. One of them, present in abundance in the cytoplasmic granules and vacuoles, is a glycogen-like polysaccharide. The other is probably a glyco- or muco-protein, diffusely distributed in the cytoplasm. No lipid globules are present in the cytoplasm. In contrast, *Bodo caudatus* contains many intra-cytoplasmic lipid globules and only a few minute glycogen granules concentrated in the kinetonuclear area. Both by standard staining techniques and by cytochemical tests for phospholipid, mitochondria were demonstrable in *Naegleria gruberi, Tetramitus*

rostratus and *Hartmanella rhysodes*, but not in the *Trichomonas* or *Bodo* species. The presence of mitochondria in *N. gruberi* and their absence in *T. foetus* were confirmed by electromicroscopy.

TRYPANOSOMIASIS

Antigens of T. brucei. Mr. Weitz has continued his studies of the antigens of African trypanosomes. He has observed the liberation of a trypanosome antigen into the blood of rats during infection with *T. brucei*. This "exo-antigen", which is separable from the cells and cell material of the trypanosome, is present at the height of parasitaemia.

Cultivation of Pathogenic Trypanosomes in Bulk. The chief difficulty of studying the antigens and trypanosomes is the production of enough material for analysis. The amount obtainable from infected animals is small, and the material often of low antigenicity. Dr. Pittam has begun a study of the cultivation of pathogenic trypanosomes, with a view to mass-culture of the organisms *in vitro*.

WHOOPING COUGH BACILLUS

Identification of Protective Antigens. Mr. Standfast and Dr. Jean Dolby have continued their work on the two protective antigens of *Bordetella pertussis* and the two antibodies they elicit (Report 1958).

The infective process of *B. pertussis* in mice was investigated by counting the viable bacilli in the lung and brain of animals challenged with lethal and sublethal infecting doses after active and passive immunization. The contention of Danish workers that the events following sublethal doses by the intranasal route corresponds more closely to those following lethal doses by the intracerebral route than to those following lethal doses by the intranasal route, was confirmed. The factors which relate pathogenicity of the inoculum to route of inoculation are under investigation.

The histology of infected brains from normal and immune mice is under investigation in the hope of distinguishing the modes of action of the two antibodies.

The antibody that protects against intranasal infection is bactericidal *in vitro* at 37°.

The Antigen Sensitizing to Histamine. Dr. Dolby has completed her work on the factor in *B. pertussis* that sensitizes mice to histamine. The histamine-sensitizing factor in bacillary extracts is separable by fractionation from the antigen protecting mice against an intracerebral challenge. The addition of ten per cent methanol to a calcium chloride extract of broken cells precipitates a water-soluble fraction containing the histamine sensitizing factor and the protective antigen. On dialysis this fraction yields a precipitate rich in protective antigen and poor in histamine sensitizing factor; the soluble portion has high histamine sensitizing and poor immunizing potency.

The histamine-sensitizing fraction is serologically impure, stimulating antibodies not only to the sensitizing factor, but also to both protective antigens. Sera to other fractions were obtained, however, which protected against either intranasal or intracerebral challenge but contained no antibody to the histamine-sensitizing factor.

DIPHTHERIA BACILLUS

The contribution, if any, of the somatic antigens of the diphtheria bacillus to immunity, is largely undetermined. Dr. Craig is fractionating toxigenic

and non-toxigenic strains of *C. diphtheriae* for immunological analysis, with a view to obtaining an immunogenic fraction that could be used in a survey of the epidemiological importance of the corresponding bacillary antibodies in man.

CLOSTRIDIUM WELCHII

The *iota* toxin of *C. welchii* Type E is tentatively implicated in epidemic haemorrhagic fever in man. Taking advantage of his observation that *iota* toxin increases capillary permeability in the skin of the guinea-pig, Dr. Craig has devised a rapid *in vivo* method of titrating toxin and its corresponding antitoxin. By this technique, he is investigating the nature of the toxin, and has begun a survey of iota antitoxin content of human sera from healthy persons.

INHERITANCE IN BACTERIA

Lysogenic Conversion in Salmonella. As noted in last year's report, the presence in *Salmonella typhimurium* of phage of group A1-A2, either in the active or the latent (lysogenic) state, causes the appearance of a new serological specificity in the polysaccharide component of the somatic antigen of the bacteria, which corresponds to factor 1 of the Kauffman-White scheme. Dr. Stocker has examined acid-hydrolysed polysaccharides from infected and non-infected *S. typhimurium*, i.e., with and without factor 1, by paper chromatography. The infected cells yield a slow-moving fraction absent from the non-infected cells. It appears to be an oligosaccharide of glucose and galactose. A similar oligosaccharide is present in several *Salmonella* species with O antigens made up of factors *1, 3, 19*, in which the factor 1 is not, apparently, determined by phage. The oligosaccharide appears to determine factor 1 specificity. Dr. Stocker is collaborating with Dr. A. M. Staub of the Institut Pasteur, Paris, on the immunology and chemistry of somatic factor 1.

Genetics of Flagellar Characters in Salmonella typhimurium. Mr. Joys has begun a serological and genetical investigation of mutants of *S. typhimurium* differing from the parent strain in the serological character of their phase 1 flagellar antigen, *i*, since it is thought that such mutants may provide peculiarly favourable material for a study both of the chemical basis of the serological specificity of a protein antigen, and of the kind of chemical alteration of a protein which results from a mutation of the chromosomal locus controlling its composition. Eight independently-arising mutants, obtained by selection of variants not immobilized by a limiting concentration of anti-*i* serum, were analyzed serologically. Two are perhaps identical and some of the others have certain similarities. Each differs from the parent type both by loss of one "specificity" or sub-factor, and by the acquisition of another. Each of the mutant antigens has been transduced, *in toto*, into a strain of *S. paratyphi B.* Two of the mutant antigens are peculiar in that their presence on bacteria causes some auto-agglutinability, because of a tendency of the flagella to aggregate: this tendency is detectable in suspensions of the detached flagella, and when the abberant flagellar antigens have been transferred by transduction to another strain.

Dr. Iino made a genetical analysis of 10 non-motile mutants of *S. typhimurium* strain LT2 (7 non-flagellated and 3 'paralysed'). The absence of abortive transduction (Report 1955) indicated that the three paralysed strains were mutated at different sites within the same functional unit (locus) of the chromosome. Two of the non-flagellated mutants did not recombine to give

wild-type (motile), which indicated that they arose by two independent mutations at the same site; no recurrence of this sort has previously been found amongst such mutants. Dr. Iino obtained variants of strain LT2 which, unlike it, produce flagella at 44° as well as at 37°; no linkage of the genes concerned to those controlling other flagellar characters was detected. A strain of *S. typhimurium* with flagella of wavelength half the normal and poorly motile or non-motile displayed these abnormalities only when in phase 1, i.e. when making flagella of antigenic type *i;* when in phase 2, i.e. making flagella of antigenic type *1,2,* the flagella are normal in shape and function. A genetical analysis by transduction showed that the gene responsible for the flagellar abnormality is either part of, or closely linked to, the gene H_i, determining the antigenic character *i.*

Genetic Transduction in Staphylococcus pyogenes. Miss Edgar and Dr. Stocker investigated transduction (gene transfer by phage) in *Staphylococcus pyogenes,* using typing phage 53, since it has been found elsewhere that this phage can transduce antibiotic resistance. Two strains, among more than a hundred tested, were unable to grow on a defined medium containing 12 amino-acids, vitamin B_1 and nicotinic acid, but grew when the medium was supplemented with threonine. The ability to dispense with threonine can readily be transduced to either strain by phage 53 grown on staphylococci not requiring it. Treatment of one of the strains with phage grown on the other gave some, but only a few, non-exacting colonies: this suggests that their chromosomal-lesions are closely linked, but are not at identical sites. Testing of a further 200 strains on a medium containing ten amino-acids and two vitamins has revealed 20 strains exacting for threonine, tryptophan, or not yet identified growth factors. The ability to dispense with the relevant growth-factor has been transduced to several of these strains.

Genetics of nicotinamide-requirement in Salmonella typhimurium. Dr. Stocker and Miss Edgar made a genetical investigation, by transduction, of 15 strains of *Salmonella typhimurium* requiring nicotinamide or nicotinic acid for growth, encountered amongst several hundred recently isolated strains tested for nutritional character. All the 15 strains appeared to have their chromosomal lesions at identical sites, since no pair would recombine to give a non-exacting (wild-type) hybrid; the 15 strains included some from South Africa, Australia and Italy, which makes it unlikely that all are descended from a recent common ancestor exacting for nicotinic acid. A laboratory mutant exacting for nicotinic acid had a mutation at a site closely linked to the common site in the 15 wild strains.

Transfer of colicinogeny and cell pairing in Salmonella. Colicinogeny, i.e. the ability to produce an antibiotic active on certain coliform bacteria, is sometimes transmitted to a high proportion of the cells of a non-colicinogenic strain mixed for a short time, e.g. 60 mins., with cells of a colicinogenic strain; and cell pairs may be seen on microscopy of such mixtures (see Report 1957, 1958). Miss Smith and Dr. Stocker in a further investigation of this phenomenon at first could not reproduce the results obtained last year by Dr. Lorkiewicz (Report 1958), for only a very small proportion of cells ($<1\%$) acquired colicinogeny during one hour's contact, and cell pairs were undetectable. In their earlier experiments, the colicinogenic strains used were ones which had been colicinogenic, in respect of colicine I, for many generations: they later found that cultures which had just acquired colicinogeny in respect of colicine I were much better transmitters of this property to non-colicinogenic strains, so that as many as 30% of the cells of the latter might become colicinogenic

during 30 min. contact: cell pairs and larger clumps were seen in large numbers in such mixtures. Miss Smith and Dr. Stocker investigated the effect of environmental conditions on the transfer of colicinogeny; starved bacteria cannot consummate the transmission, which is also hindered by cyanide or azide. By contrast, streptomycin, even in a concentration which rapidly kills the cells of the colicinogenic partner, has little effect on the transfer of colicinogeny to a streptomycin-resistant strain.

Dr. Ozeki has investigated certain genetic aspects of colicinogeny in *Salmonella typhimurium*, using sub-lines of strain LT2 made colicinogenic by contact with a Shigella strain which produces colicines I and E2. In the *S. typhimurium* strain colicinogeny in respect of either or both of these colicines is a stable character, which under some conditions may be transmitted to other *S. typhimurium* strains either by cell contact (see above, and Report 1957, 1958) or by phage-mediated transduction. A strain which produces colicine I can transmit this character by cell contact; whereas a strain which produces only colicine E2 cannot transmit its character in this way; but a strain which produces both colicines transmits both properties to a proportion of the cells made colicinogenic by contact with it, the remainder acquiring only the ability to produce colicine I. Dr. Ozeki propagated the transducing phage PLT22 on *S. typhimurium* made colicinogenic for various colicines (cf. Stocker & Esterbrook, Report 1957); phage grown on a strain producing colicine E2 can transduce this property to a small proportion (c 10^{-5}) of the cells of a non-colicinogenic strain, or one producing colicine I, whereas in similar experiments no transduction of the ability to produce colicine I has been detected. Dr. Ozeki also investigated the genetics of colicinogeny in respect of several other colicines. In the case of colicines K and E1, as in that of E2, colicinogeny is not transmissible by cell contact, unless colicinogeny for colicine I is being transmitted at the same time; but is, probably, transducible by phage PLT22. By contrast colicinogeny for colicine B resembles that for colicine I in that the character is transmissible, *per se*, by cell contact but is not, apparently, transducible by phage; furthermore, transmission by cell contact of colicinogeny for colicine B makes possible the simultaneous transmission of other colicinogenic properties, not transmissible by themselves.

Heretofore it has only been possible to detect the colicine released by a large number of bacteria, e.g. a colony or culture of a colicinogenic strain. Dr. Ozeki found that when a suitable number of cells of a colicinogenic strain are incorporated in a soft agar layer heavily inoculated with a colicine-sensitive strain, minute clearings may be seen in the confluent growth of the sensitive strain after a few hours incubation; and he showed that each such clearing results from the colicine released by a single bacterium of the colicinogenic strain. Concurrent micro-manipulation experiments, in collaboration with Madame H. de Margerie and Dr. Stocker, also demonstrated the release of colicine by single cells. His results indicate that a bacterium which liberates colicine does not itself survive; in the case of colicine E2 this is true both in ultraviolet-irradiated cultures where half or more of the cells liberate colicine, and in non-irradiated cultures, where only a very small proportion of the cells liberate colicine.

BACTERIAL PHYSIOLOGY

Synthesis of Flagella. Dr. Kerridge continued his experiments on the ability of nutritionally exacting mutants of *S. typhimurium* to regenerate flagella in chemically defined media. He had shown that a mutant requiring a particular amino-acid for growth could regenerate its flagella in the absence

of this amino-acid when the amino-acid concerned was one not present in 'flagellin' (flagellar protein), but not otherwise. He has now found an intermediate situation: several different mutants needing histidine or methionine can regenerate their flagella to a limited extent when starved of the amino-acid needed for growth, even though each of these amino-acids is present, in a small amount, in flagellin. It is probable that some endogenous protein is broken down in the starved bacteria, providing a limited supply of the essential amino-acid for the synthesis of new protein; and that flagellar protein is synthesised to a disproportionate extent because it contains an exceptionally small amount of each of these amino-acids (one residue per molecular weight c. 30,000). Various amino-acid analogues were tested for effect on regeneration of flagella. All inhibited bacterial growth to some extent, but only one, *p*-fluorophenylalanine, affected flagellar synthesis. Flagella formed in the presence of this substance fail to propel the bacteria (Report, 1958), and have a morphological abnormality, viz, a wave-length of half that of normal flagella (1.04μ instead of 2.08μ). Mr. Joys and Dr. Kerridge could not detect any serological difference between normal flagella and non-functional flagella formed in the presence of this analogue.

S. typhimurium grown for several generations at 44° have few or no flagella (Report, 1956), but begin to form them soon after transfer to 37°; the lag period probably represents the time required for the synthesis of some flagellum-secreting apparatus. The metabolic analogues 2-thiouracil and 8-azaguanine, which are known to be incorporated into bacterial ribonucleic acid, do not interfere with the regeneration of flagella by bacteria whose flagella have been torn off. Dr. Kerridge, however, found that they inhibit the formation of flagella by non-flagellated bacteria grown at 44° and transferred to 37°, presumably by affecting the synthesis of the flagellum-secreting apparatus.

ABACTERIAL URETHRITIS IN MAN

Dr. Klieneberger-Nobel has continued to test human sera for the presence of antibodies against pleuropneumonia-like organisms (PPLO), in collaboration with Dr. G. W. Csonka of St. Mary's Hospital and Dr. R. D. Catterall of the Whitechapel Clinic.

The investigation of the role of PPLO in uveitis in the male, made with Dr. R. D. Catterall of the Whitechapel Clinic, is complete.

The organism ("T" strain) isolated from embryonated eggs by Dr. M. C. Shepard of Camp Lejeune, North Carolina after several blind egg passages of material from patients with abacterial urethritis was investigated serologically and culturally in comparison with a number of strains isolated from mammals and fowl. The "T" strain was identical with the "coccobacilliform bodies" identified by Nelson as the cause of fowl coryza in chicken, suggesting that it originated in the eggs used for isolation, and not from the human material originally inoculated.

Serology of PPLO Infection in Rats. Dr. Klieneberger-Nobel has studied the pattern of antibody response to subcutaneous infection of the rat by a PPLO strain of rat origin which produces arthritis and abscesses and by a human genital strain of PPLO which is non-pathogenic to the rat. During the prolonged infection, with invasion of the blood stream by PPLO, induced by the rat strain, PPLO antibody in the blood increased rapidly during the acute phase, and declined very slowly after the regression of the local infection. The local infection with the human strain was mild, did not generalize, and regressed after two weeks; at no time was antibody detectable in the blood.

Therapy of PPLO Infection in Rats. Terramycin hastened the regression of abscesses produced in the rat by PPLO of rat origin, but without destroying all the PPLO in encapsulated abscesses.

VIROLOGY

TRACHOMA

Identification of Trachoma Viruses. Viruses isolated from patients with trachoma can be identified with certainty as the casual agent only by inoculating human volunteers, thereby inducing the characteristic clinical syndrome, and the typical cytoplasmic inclusions in the conjunctival lesions. In collaboration with Sir Stewart Duke-Elder and Mr. Barrie Jones of the Institute of Ophthalmology, Dr. Collier extended the tests of viruses isolated in the Gambia (Report, 1958). The volunteer inoculated with trachoma virus strain G1, has been observed, untreated, for over a year, and has yielded virus on eight occasions. He has developed the conjunctival scarring which is a characteristic feature of the infection in man. Observations could only be made on the conjunctival membrane, because the subject had previously lost both eyes by operation. Since keratitis and pannus are pronounced and characteristic features of natural trachoma, a second test was made with Strain G17, on a blind volunteer with an intact cornea. Strain G17 induced follicular conjunctivitis, and unmistakable keratitis and pannus. Again, typical cytoplasmic inclusions were found, and virus was repeatedly isolated by egg inoculation.

Inoculation of Baboons. Through the courtesy of Dr. J. Newsome of the Medical Research Council's Bilharzia Research Group, Dr. Collier inoculated a number of baboons with trachoma virus. They proved difficult to infect, but typical inclusions were induced in one of them; from the conjunctivae of this animal and of 2 others, virus was isolated some weeks after inoculation, indicating that multiplication had occurred.

Morphological Variation in Trachoma Viruses. When growing in the chick embryo yolk sac, all the viruses isolated in the Gambia appear as free elementary bodies 0.2 – 0.3μ in diameter. Mr. J. Sowa, of the Medical Research Council's Trachoma Group, has isolated two strains, G16 and G17, which in addition give rise to compact aggregates of elementary bodies arranged around a central vacuole. Dr. Collier has shown that the vacuole contains a carbohydrate, probably glycogen. This is interesting because the conjunctival inclusions found in infected man and apes contain a glycogen matrix. Trachoma and the allied virus of inclusion blennorrhoea are the only agents giving rise to inclusions of this kind. It is possibly significant that when inoculated into man, strain G17 induced many more inclusions than did G1. Strain G17 may have a greater capacity than usual for inclusion formation, both in the yolk sac and in the conjunctiva.

Like strain G1, strain G17 contains the complement fixing lymphogranuloma-psittacosis group antigen. In collaboration with Dr. R. Valentine, of the National Institute for Medical Research, its elementary bodies have been shown by electron microscopy to resemble those of strain G1.

Immunological Studies. Dr. Collier and Mr. Reeve are investigating the antigenic composition of trachoma viruses, with the ultimate aim of developing a prophylactic vaccine. In the first instance, they are examining the relationship of trachoma virus to other members of the lymphogranuloma group, and are attempting to distinguish strains of trachoma virus from different sources.

Diagnostic Complement Fixation Tests. Dr. Collier showed that the sera of some, but not all, trachoma patients contain small amounts of antibodies

fixing complement with trachoma and psittacosis antigens. Since, however, sera from some normal Africans also contain these antibodies, the complement fixation test is not likely to be of much diagnostic value.

Dermal Sensitivity. Preliminary results indicate that intracutaneous injection of trachoma virus induces a skin lesion in trachoma patients similar to the Frei reaction in lymphogranuloma. Trachoma patients are however Frei negative. These findings are being further investigated in collaboration with Professor C. F. Barwell, of the London Hospital.

Adaptation to Tissue Culture. Mrs. J. Stocker and Mr. Frohlich are attempting to adapt trachoma virus to grow in a variety of human, animal and avian tissues. So far there is no clear evidence of multiplication in these systems.

Histology of Infected Yolk Sac. Mr. Frohlich has shown that the virus multiplies in the layer of endodermal cells forming the inner lining of the chick embryo yolk sac.

Epidemiological Investigations in West Africa. An augmented Medical Research Council team under Dr. Collier's direction is now investigating trachoma in a village community in the Gambia. It is hoped to determine the usual age of onset, the relation of clinical signs with presence of inclusions and cultivable virus, and the mode of transmission, including the roles of fomites and insect vectors. The influence of secondary bacterial infections is also under examination.

INCLUSION BLENNORRHOEA

Isolation of a Virus. The filterable infective agent causing inclusion blennorrhoea in the newborn, and inclusion conjunctivitis, cervicitis and urethritis in adults, has for long resisted all attempts at laboratory cultivation. Since the cytoplasmic inclusion bodies in these diseases are apparently identical with those of trachoma, it seemed likely that this virus could also be isolated by inoculating infected epithelium into the chick embryo yolk sac. With the clinical collaboration of Mr. Barrie Jones, Dr. Collier in this way isolated viruses from 2 babies with inclusion blennorrhoea, from the cervix of a woman whose newborn baby developed this disease, and from an adult with inclusion conjunctivitis. Two of these isolations were confirmed by Dr. C. H. Smith of the Evans Biological Institute. The agents resemble the virus of trachoma morphologically, and in possessing the psittacosis-lymphogranuloma group antigen.

Inoculation of Baboons. The conjunctivae of 4 baboons (*P. papio*) were inoculated with the 8th egg passage of the virus isolated from the cervix uteri. They developed severe conjunctivitis, and numerous typical inclusion bodies were demonstrable in conjunctival scrapings.

The final proof of the identity of this virus must await inoculation of a human volunteer, but the evidence obtained leaves little doubt that this agent is that causing inclusion blennorrhoea. It is hoped that this work will lead to a long-awaited understanding of the relation between trachoma, inclusion blennorrhoea and conjunctivitis, and the associated infections of the genital tract.

MEASLES

With the ultimate aim of developing a vaccine, Mrs. Gillies (MacGregor) is continuing her studies of the virology of measles, using the Edmonston strain of virus.

Growth of Virus in Tissue Culture. Mrs. Gillies found that the cytopathogenic action of the virus on a stable line of monkey kidney cells (MK2) is more rapid and easily recognizable than on human amnion or primary monkey kidney cultures. Lesions are observable 3 days after inoculation, making MK2 cells a convenient system for infectivity titrations. When a large dose of virus is inoculated into MK2 cells, the titre falls rapidly during the first 8 hours, and then increases, reaching its maximum 3–5 days later. By 6 days, the cultured cells are entirely replaced by abnormal multinucleate cells. Scanty intranuclear inclusions are now present, and become much more numerous by the 9th day. After the 6th day, virus diminishes steadily in amount, and cannot be detected after the 18th day.

Serological Studies. Preliminary results indicate that the neutralizing antibodies developing after measles persist for long periods. Samples of gamma globulin contain about 6 times more neutralizing antibody than sera from subjects with a past history of measles.

Adaptation of Virus to Chick Embryo. Because of the possible value of an embryo attenuated strain in prophylaxis, attempts have been made to grow virus in the chick embryo amniotic and allantoic cavities. Multiplication occurred in the amnion on several occasions, but not consistently.

VACCINIA VIRUS

Virus Inactivation. The work on inactivation of vaccinia virus by gamma rays is complete. Suspensions of virus inactivated by 600 and 800 kilorads conferred protection on rabbits against challenge by scarification with living virus. The concentration of neutralizing antibody in the sera of immunised rabbits is less than was expected from the degree of protection established by the immunization.

Dr. Kaplan is continuing his studies of heat inactivation and of the influence of metal ions on the inactivation of virus adapted to the chick embryo. Sodium and potassium in relatively high concentration (0.1 molar) stabilize infectivity at 50°C. Of the divalent kations tested, Mg^{++} stabilizes the infectivity and Cu^{++} and Co^{++} increase inactivation.

In collaboration with Miss Thomas (Mrs. Micklem) Dr. Kaplan studied the action of thiomersalate on the infectivity of vaccinia virus. He is extending the investigation to certain chemical inactivations which may be related to inactivation by thiomersalate.

Virus Purification. Dr. Kaplan and Dr. R. C. Valentine of the National Institute for Medical Research studied the efficacy of certain methods of purifying suspensions of infective pox viruses. Although treatment with a fluorocarbon compound was useful, the highest degree of purification was obtained by flocculating rabbit-adapted virus with molar NaCl, and then removing the salt by centrifugation. The method was however ineffective with virus grown in chick embryo cells.

Vaccine Production in Tissue Culture. Miss Thomas devised a method of small scale production in monolayer cultures of chick embryo cells which proved successful. About 20 batches of vaccine have been grown so far: although the method is successful, Mr. Robinson is now investigating the possibilities of growth of the virus in cultures of suspended cells with a view to large-scale production by this method.

Field Trials of Tissue Culture Vaccine. Clinical trials of vaccine produced in tissue culture carried out by Wing Commander R. M. Cross, R.A.F. revealed an interesting difference between the virus propagated in tissues cultivated *in vitro* and that propagated in the tissues of the living animal. Vaccine viruses from tissue cultures were all primary subcultures in chick embryo cells of rabbit dermal virus and were of full potency when issued for trial. They consistently produced 100 per cent successful results in primary vaccinations but in re-vaccinations the percentage of positive reactions was consistently lower by 15–20% than the percentage positives in revaccination with ordinary vaccine lymph or dried smallpox vaccine. Neither the rabbit-adapted virus used as seed for the tissue cultures nor virus subcultured once in the chorio-allantois of the embryonated chick had this relative incapacity to 'take' in those previously vaccinated. The cause of this change in the vaccine is being investigated; the evidence so far available suggests that, compared with vaccine lymph, tissue culture virus is relatively unstable when stored at temperatures above 0°C. There are indications that the instability can be allowed for by a slight increase in the initial potency of the virus or overcome by the addition of certain protective substances to the suspending medium.

BACTERIOPHAGES

Antiphage Antibiotics. Dr. Asheshov continued his work on substances produced by actinomycetes active against bacterial viruses, particularly the study of the actinomycetes A220 and A855, producing rutilantin and actinomycete A803, producing a similar but not identical substance.

In collaboration with the Medical Research Council's Antibiotics Research Station at Clevedon crude picrates of rutilantin and fractions derived from them were studied extensively, mainly with regard to activity, and structure, and to the relationship of various fractions and their behaviour in different solvents, as determined by paper chromatography.

In an attempt to elucidate the chemical and physical behaviour of different rutilantin compounds, several new bases and salts were prepared from picrates. A method was devised for the chromatography not only of the bases, but also of the salts of rutilantin, making it possible to determine the behaviour of different salts and bases in different solvents, which disclosed peculiar characteristics of the rutilantin compounds.

Active product from actinomycete A803 was studied on the same lines and a method for its paper chromatography devised.

All three cultures—A220, A855, and A803 — were submitted to numerous reisolations with the object of obtaining better antibiotic-producing strains.

Cultures of all the actinomycete previously reported to produce antiphage agents were revived and prepared for storage as type cultures.

A Bacteriophage which Attacks only Motile Bacteria. Dr. Stocker is collaborating with Dr. E. Meynell, of the London School of Hygiene, on a phage which attacks many motile *Salmonella* strains, but no non-motile ones. In sensitive species only bacteria with flagella of an antigenic type other than g . . . (and related forms) are susceptible to the phage. When the bacteria are non-flagellated, through mutation or as a result of mechanical or chemical removal of their flagella, they do not absorb the phage; and when the original flagellar antigen of a sensitive strain is changed to g . . . by transduction, the strain becomes resistent. All "paralysed" strains so far tested are resistent to the phage, which suggests that it attacks only bacteria with functioning flagella.

SEROLOGICAL IDENTIFICATION OF INSECT BLOOD MEALS

Tsetse Flies. The collaborative study of the feeding habits of tsetse flies of East and West Africa was continued by Mr. Weitz and Miss Lee-Jones. The identification of blood meals of *Glossina longipennis* collected monthly from the dry country on the Nairobi-Mombasa road by Dr. P. Glover and his colleagues of the Department of Veterinary Research Laboratory, Kabete, Kenya, revealed a striking association of the fly with the larger mammals. Rhinoceros was the main host; it was fed on by about 70 per cent of the flies during most months of the year, in spite of the presence of a very wide variety of possible hosts. This preference, however, was easily diverted to elephants when they appeared in the region, and in February one per cent of all flies had fed on these animals. For the first time since the beginning of the survey elephant blood was identified. The percentage rose to 50-60 per cent in March and April, and diminished in May and June to 24 per cent; no elephant feeds were found in August. The increase in the rate of feeding on elephant was at the expense of feeding on rhinoceros, although rhinoceros was constantly present. A similar, but less spectacular, diversion of feeding towards buffalo occurred during sporadic visits of these animals in the region. Although *G. longipennis* is probably of very little importance in the transmission of trypanosomiasis, it has proved to be a most useful test fly for an ecological study of the feeding habits of *Glossina*.

Results like these indicate the need for caution in attempts to eliminate flies by selective game destruction. The fly may become habituated to new hosts before the initially preferred sources of food are destroyed.

G. swynnertoni, on the other hand, failed to change in its habits throughout the year, its main host being warthog; whereas in the same region *G. pallidipes* had no discrimination at all, feeding with almost equal frequency on all the large bovids present, as well as on man, rhinoceros and elephant.

Another example of the characteristic feeding habits of individual species of *Glossina* is shown by the regular, although infrequent, feeding of *G. morsitans submorsitans* on hartebeest in Northern Uganda. Until now, no flies of any kind were known to feed on this bovid and it is noteworthy that this subspecies of *G. morsitans* behaves so differently in this respect from any other species of *Glossina*. The adaptation of tsetse fly to its environment is illustrated in the West African forest by the preference of most of the resident species of *Glossina (tabaniformis, fusca, medicorum)* for the Red River Hog; there is no warthog in that region. The feeding habits of *G. morsitans* in West Africa are identical with those of the same species in East Africa; and both *G. longipalpis* and the corresponding East African species *G. pallidipes* feed primarily on bushbuck. It is hoped to study the adaptability of *Glossina* in the face of selective game destruction, but an opportunity for such a study in a suitable region has not yet arisen.

In collaboration with Mr. A. G. Robertson, the Director of Tsetse Control, Uganda, Mr. H. M. Lloyd, the Director of Tsetse Control, Tanganyika and with members of the staff of East African Trypanosomiasis Research Organisation and of West African Institute of Trypanosomiasis Research, the importance of domestic cattle in maintaining fly populations is under investigation in Uganda and Tanganyika. As a result of chemotherapy, cattle are now present in regions where the density of fly is still high, and an attempt is being made to determine whether the presence of cattle will interfere with the fly eradication schemes in operation.

Mosquitoes. In collaboration with the World Health Organisation, the identification tests of mosquito smears collected from all over the world continues. Approximately 30,000 mosquitoes, representing a very wide variety of species were tested during the year. The examination of the results is incomplete, but it will probably provide new information about the feeding habits of numerous malarial mosquitoes, particularly about the feeding habits of mosquitoes found fully-fed in houses. The problems of mosquitoes which feed on man in houses and then rest outdoors, or conversely feed outdoors on animals and then come in to rest in houses, is being explored for a large number of species of anophelines from many different countries.

ANTITOXIN PRODUCTION

Refinement of Therapeutic Antitoxins. In continuation of his comparison of antitoxins from various parts of the world refined by treating hyperimmune horse plasma with pepsin, Dr. Dolby confirmed that the refined antitoxin has the mobility of a gamma-globulin, but in all cases found 5–10% of a component with the properties of a beta-globulin. He is separating the two proteins for further study.

Further work on the protein breakdown products in refined antitoxins showed that by treatment with fullers' earth at two stages in the refining process it is possible to decrease the breakdown products in the final material to less than three per cent. Miss Lanham has fractionated these products, which are only in part dialysable through cellophan, by chromatography on charcoal and ion-exchange resins, by fractional precipitation and by paper chromatography and paper electrophoresis; and is investigating the chemistry of the isolated materials and their biological activity on vascular permeability. The products of the action of pepsin on individual fractions of horse sera are also under investigation.

Proteolytic Enzymes for Refining Antisera. Dr. Dolby has shown that when the breakdown of serum proteins by pepsin is studied by conventional methods the enzyme has optimum activity only at pH 2, differing in this respect from its action on haemoglobin, which is maximal at pH 2 and 3.2. If, however, the splitting of peptide bonds is measured, serum proteins are also found to be broken down maximally at these two pHs. Albumin is attacked about three times as rapidly as gamma globulin.

Permeability Factors Present in Refined Antitoxic Sera. Mr. Rodican used electrophoretic methods to isolate, purify and characterise the vascular permeability factors present in refined therapeutic antitoxins. For various reasons supporting media of starch, celite and powdered glass proved unsatisfactory for this purpose. With a block constructed of strips of filter paper, two major fractions and several minor fractions with permeability-increasing activity were isolated from antitoxins that had been refined by peptic digestion. All the active substances pass a cellophane membrane and the two major fractions are separable into a number of polypeptides.

EXPERIMENTAL PATHOLOGY

MECHANISMS OF INFLAMMATION

Enzymic Nature of Serum Globulin Permeability Factor. In collaboration with Dr. E. L. Becker, Walter Reed Army Institute of Research, Washington, U.S.A., Professor Miles and Dr. Wilhelm studied the susceptibility of the globulin permeability factor in mammalian serum to di-*iso*propyl phospho-

fluoridate (DFP). *In vitro* treatment with DFP, 10^{-3}M to 10^{-5}M, irreversibly inhibits the serum factor, inhibition being progressive with the duration of treatment. Furthermore, the inactivation is prevented when DFP itself is first mixed with the methyl esters of p-toluene sulphonyl L-arginine and of benzoyl L-arginine. The results establish more firmly that the serum factor is a protease which probably hydrolyses ester linkages.

Other esterase inhibitors like diethyl p-nitrophenyl phosphate, tetraethyl pyrophosphate, bis-mono*iso*propyl aminofluorophosphine oxide have no substantial effect on the serum factor.

Rat serum factor is much more susceptible than the guinea-pig and rabbit factors to lima bean trypsin inhibitor; and the guinea-pig factor is strongly antagonized by potato trypsin inhibitor.

The Activation of Permeability Profactor in Serum. The activation of permeability factor by dilution of guinea-pig serum in glass tubes appears to be identical with its activation when undiluted serum is exposed to large surfaces of glass, silica, cellulose, starch and agar. Professor Miles is investigating the relation of this activation to the similar activation of serum anaphylatoxins and of factors concerned in coagulation. The permeability factor does not appear to be anaphylatoxin, or the Hagemann factor, but it may be concerned in other phenomena consequent on the activation of Hagemann factor.

Permeability Factor-Inhibitor System in Tissue Fluid and Lymph. The general distribution of the permeability profactor and of the inhibitor of activated factor in tissue and lymph is established for the guinea-pig (Report 1958). As an extension of this work Professor Miles is investigating the permeability factors of rabbit lymph.

Vascular Permeability Changes in Injury. The role of known endogenous permeability factors, including the serum factor, as mediators of the permeability changes in representative kinds of injury, is under investigation.

Dr. Wilhelm observed that, in guinea-pig skin, a mild heat injury induces a biphasic increase of permeability — an immediate and minor response mediated by histamine; and a delayed and major response, of which the mediator has not been identified. Inhibition by antihistamine of the immediate response in no way affects the development of the delayed response. In the rat, mild thermal injury induces similar permeability changes, though the immediate response is minimal. Miss Mason found that *in vitro* heating at 65° for 5 minutes liberates nearly all the histamine in guinea-pig, rat and rabbit skin. However, despite the comparative ease of its *in vitro* liberation, histamine does not seem to mediate the major delayed permeability response to *in vivo* heating.

In the guinea-pig, injury by ultraviolet light, like that by heat, induces a biphasic permeability response of which the immediate response is mediated by histamine. Chemical injury by organic solvents (e.g. xylol or chloroform) induces a single phase of increased permeability; in which, histamine again appears to be only an early and minor mediator.

Dr. Craig has observed that the increase of permeability in guinea-pigs during locally induced, active and passive anaphylaxis, varies widely between animals, and is only slightly susceptible to intravenous antihistamine.

Professor Miles and Dr. Wilhelm are studying the pattern of permeability response to bacterial infection in the rat.

In general, the permeability response in experimental injury appears to be biphasic, and the major, second response to be mediated by a hitherto undescribed factor.

Effect of Antihistaminic Drugs on Permeability Factors. The histamine antagonists commonly used to study the *in vivo* effects of histamine liberated in injury all have non-antihistaminic side-actions. To select those most suitable, Miss Mason and Dr. Wilhelm are investigating the effects of 9 antihistamines on permeability factors in guinea-pigs, rats and rabbits.

In intravenous doses small enough to minimize their non-specific side-actions, the selected antihistamines decreased the permeability effects of histamine in the guinea-pig and rabbit in the following descending order of potency: triprolidine, chlorprophenpyridamine, mepyramine, promethazine, tripelennamine, chlorcyclizine, diphenhydramine, antazoline and thenalidine. The order is similar in tests in the rat, except that promethazine has the highest inhibitor potency. The permeability effects of a histamine liberator are considerably less susceptible to triprolidine and mepyramine in the guinea-pig, and not substantially affected in the rat.

Tested intracutaneously in the guinea-pig and rat, the same antihistamines have less inhibitor potency against histamine; and many of the drugs seem to have non-specific local actions, probably consequent on the larger doses required to induce substantial inhibition.

Of the various histamine liberators that increase permeability in the guinea-pig and rat, Miss Mason found that none has substantial effects in the rabbit, although the content of liberable histamine in the skin is of the same order in all three animals.

MECHANISMS OF INFECTION AND DEFENCE

The Mechanisms of Non-specific Immunity. Professor Miles, in collaboration with Dr. Blaker, has extended his investigation of stimulators and depressors of local tissue immunity in the early stages of infection, to include acid-fast pathogens. The antimicrobial action of the tissues observed with fast-growing pathogens does not appear to be effective in B.C.G. or vole bacillus infections.

Histamine-release during Bacterial Infection and Intoxication. Dr. Smith and Professor Miles completed their examination of the effects of minimal bacterial infections on the mast-cell population of the rat peritoneum. Typical inflammatory responses could be induced without materially affecting the mast cells and without significant liberation of histamine. The epiphenomenal status of histamine in such infections was confirmed by the failure of antihistamine to alter the inflammatory response.

The Role of Hormones in the Response to Mycobacterial Infections. Dr. Blaker has observed that in local infection of the guinea-pig skin, the state and character of invasion by B.C.G., a vole bacillus, is partly determined by the amount of circulating thyroid hormone. As already described for the rabbit, excessive hormone increases the inflammatory response and the destruction of the bacilli, and deprivation of the hormone has the opposite effects.

BIOCHEMISTRY

THE HUMAN BLOOD GROUP SUBSTANCES

The Preparation and Properties of Group Substances. One of the most difficult problems encountered in the study of the blood group substances, and related materials, is that of establishing the homogeneity of the isolated product. Before any conclusions can be reached concerning macromolecular structure or any significance given to components isolated in small amounts,

however, it is essential to have a homogeneous starting material. Extraction with cold 90% phenol is the method most generally used for the isolation and purification of blood group substances from freeze dried tissue fluids or secretions. This process eliminates the major part of the accompanying non-specific protein and other impurities and leaves a phenol-insoluble residue which has most of the specific activity of the original secretion. Mr. Pusztai and Professor Morgan on further examination of the blood group mucopolysaccharides prepared from ovarian cyst fluids by phenol extraction, found that they can be fractionated into two parts by treatment with saturated ammonium sulphate at 70°. The two fractions differ significantly in their serological chemical and physico-chemical behaviour. The material insoluble in ammonium sulphate is serologically more active in both the haemagglutination inhibition and quantitative precipitation tests, has a higher viscosity, a different optical rotation and probably a larger molecular size than the material soluble in ammonium sulphate. These results indicate that in the natural secretions blood group specificity is associated with at least two different types of molecule.

Enzymes and Blood Group Substances. Proteolytic enzymes have been used to remove adventitious protein during the isolation and purification of the group substances from materials of human and animal origin, and it has been generally accepted that the specific mucopolysaccharides are not changed by such treatment. Professor Morgan and Mr. Pusztai found however that after treatment of crude ovarian cyst fluids with ficin, the proteolytic enzyme from fig latex, the isolated blood group substances had a lower capacity to neutralise the corresponding antibody in haemagglutination tests than substances recovered from the same source by simple phenol extraction. This reduced activity is not always obvious when cyst fluids are given a single short treatment at pH 7.0 especially when they contain a large excess of unspecific protein. Repeated treatment of highly purified blood group substances with crystalline ficin or papain, however, brings about extensive but limited changes in their serological activity without releasing simple sugars or diffusible oligosaccharides. The fragments which remain after repeated treatment are resistant to further proteolytic action and the fraction which is most active serologically has only 3-6% of the activity of the original mucopolysaccharide. The fragments are very similar in chemical composition to the original substance, but loss in serological activity is associated with a decrease in viscosity, a small but consistent decrease in the nitrogen value and a change in optical rotation. The action of ficin and papain on the blood group substances is inhibited by proteolytic enzyme inhibitors such as p-chloro-mercuribenzoate. The changes induced by these enzymes can be explained on the assumption that ficin and papain degrade the blood group specific carbohydrate-amino acid complexes by the rupture of a limited number of peptide bonds and thus release relatively large units similar in composition to the original substance.

The chemical changes in the blood group substances brought about by ficin and papain are very different from those induced by enzymes in extracts of *Trichomonas foetus*, which cause an extensive release of reducing sugars together with complete destruction of serological activity. Mr. Tyler and Dr. Watkins have continued their investigations of methods for the separation and purification of the enzymes in extracts of *T. foetus*. The crude extracts contain a number of glycosidases in addition to the enzymes which destroy the serological activity of A, B, H and Le^a substances. By fractionation on calcium phosphate gel the B-destroying and H-destroying enzymes in the crude extract can be separated almost completely from the enzymes destroying A and Le^a substances and from the accompanying β-galactosidase and β-*N*-acetylglucos-

aminidase. Alcohol fractionation yields the H-destroying enzyme free from those attacking A, B or Le^a substances, but the preparation still contains β-galactosidase and β-*N*-acetylglucosaminidase. The progressive action of the H-destroying enzyme preparation on H substance has been studied in an attempt to correlate the release of monosaccharides with the specificity changes that occur.

Dr. Watkins continued the investigation of the capacity of the enzymes in extracts of *T. foetus* to synthesize oligosaccharides by transgalactosylation. In addition to the disaccharide (Report 1957) two trisaccharides were isolated from the enzyme digest obtained after incubation of melibiose and *N*-acetylglucosamine with the *T. foetus* preparation. One is the known compound manninotriose and the other a new trisaccharide which probably has the structure O-α-D-galactosyl-(1→6)-O-α-D-galactosyl-(1→6)-*N*-acetylglucosamine.

Structural Studies on Blood Group Substances. Professor Morgan and Mr. Lister Cheese continued their study of the oligosaccharide fragments liberated by mild acid hydrolysis of the group specific mucopolysaccharides and turned their attention to the 'Forssman' or heterophile component of A substance. The activity of this component, measured by the inhibition of haemolysis of sheep cells, is accepted as a different, although closely related serological property of the A substance from that measured by the isoagglutination inhibition technique. Materials which are diffusible through a cellophane membrane and which retain some capacity to inhibit sheep cell haemolysis were obtained after partial acid hydrolysis. Fractionation of the complex mixture of diffusible products is being attempted in order to isolate the serologically active fragments and to determine their chemical structure.

CARBOHYDRATE STUDIES

Enzymic Synthesis of Starch. Most starches contain two components, amylose and amylopectin. Enzymes which synthesize these polysaccharides separately have been known for a long time but when these are allowed to act together only one product, amylopectin, is formed; the amylose is lost by conversion into amylopectin. A scheme was devized to explain how both polysaccharides could be synthesized from a single oligosaccharide substrate. The hypothesis requires the presence in the plant of an amylose-synthesizing system in addition to the already known synthesis by phosphorylase, and requires also that only one of the amyloses synthesized by the two systems is converted into amylopectin. Such a system has now been demonstrated by Dr. Walker and makes use of D-enzyme, which reversibly transfers glucose units between maltodextrin molecules. Acting alone on maltotetraose D-enzyme produces glucose and maltodextrins containing up to about 10 glucose units. This reaction has been modified by disturbing the equilibrium. When hexokinase and adenosine triphosphate are added, the glucose is converted into the 6-phosphate and this disturbance of the equilibrium resulting in the formation of more glucose, also results in the conversion of the oligosaccharides into the polysaccharide amylose. In the proposed synthesis of amylose and amylopectin, the glucose 6-phosphate is converted into glucose 1-phosphate and used by phosphorylase to synthesize amylose. Here, also, it has been found necessary to disturb an enzymic equilibrium in order to prove the point. In this case the equilibrium is between 6-phosphate, 1-phosphate and amylose, when only 16% of the 6-phosphate is normally converted into amylose. By including magnesium and ammonium salts in the digest, the inorganic phosphate released on the conversion of glucose 1-phosphate into amylose is precipitated and the conversion of glucose 6-phosphate then approaches completion.

Mechanism of Carbohydrase Action. Dr. Walker has re-examined the structure of the dextrin which remains after exhaustive phosphorolysis of glycogen by crystalline rabbit muscle phosphorylase; and has proved that the structure assigned to the dextrin by Cori and Larner is incorrect. In consequence the mechanism of action of an enzyme acting on the glycogen dextrin, amylo-1:6-glucosidase, needs re-interpretation in terms of the new structure for the dextrin.

Dr. Walker tested the actions of potato phosphorylase and D-enzyme on the branched polysaccharides amylopectin and glycogen. The enzymes readily attack the first but not the second. Glycogen seems insusceptible to attack because its structure is much more compact than that of amylopectin. When the glycogen is slightly degraded by α-amylase, to render the molecule less compact, the two enzymes are able to attack it.

Dr. Walker and Mr. Abdullah synthesized glucose derivatives in which some of the hydroxyl groups of glucose were replaced by hydrogen (deoxyglucoses). D-enzyme reacts with three of these sugars almost as rapidly as with unmodified glucose and each of these three sugars is better in this respect than any other glucose substitute previously known. The three sugars are 1-deoxy-, 2-deoxy- and 6-deoxy-glucose. In contrast, 1:2-dideoxyglucose is inactive.

Mr. Hughes and Dr. Whelan are identifying the products of action of three crystalline α-amylases on amylopectin. The products are glucose, maltose and unfermentable oligosaccharides (α-limit dextins) containing the α-1:6-branch linkages of the original polysaccharide. Semi-micro techniques of structural analysis, using periodate oxidation, lead tetra-acetate oxidation and enzymic breakdown were devised for this study (see below).

α-1:6-Glucose Transferase in Potato. Mr. Abdullah and Dr. Whelan have discovered a new enzyme in the potato capable of transferring α-1:6-linked glucose units from one sugar molecule to another. The α-1:6-glucose bond is the main linkage in the polysaccharide dextran.

Chemical Synthesis of Modified Starches. The aim of this work is to synthesize starches modified at specific points in the molecule to discover whether or not they are substrates for the starch-metabolizing enzymes and so to learn new facts about the specificity of these enzymes.

Dr. Bines examined amylose methylated by a method said to lead to specific substitution at the C-2 hydroxyl group. In fact the substitution is highly unselective and all three available hydroxyl groups of amylose at C-2, C-3 and C-6, are methylated. Nevertheless, the methylated amylose was degraded by α-amylase to yield methyl-substituted maltose.

Dr. Allen synthesized a starch mainly esterified with acetyl groups at C-6. These groups are found completely to inhibit the actions of phosphorylase, β-amylase and D-enzyme, but not α-amylase, which can still degrade the polysaccharide to acetylated maltodextrins. Acetylation of glucose at C-6 renders the glucose inert towards D-enzyme (cf. 6-deoxyglucose, above).

Molecular Weights and Structural Analysis of Polysaccharides. Mr. Parrish and Dr. Whelan have designed conditions of periodate oxidation suitable for the accurate determination of molecular size of polysaccharides containing up to about 100 sugar units. The method was tested on synthetic amyloses of known size and gave accurate results with only 2 mg. of material. Although the method is unsuitable for the examination of very large molecules the viscometric method of measurement is suitable. Amyloses standardized by the periodate

method were used to calibrate the viscometric method and the accurate determination of molecular size of large amylose molecules is now possible. The periodate method can also be used with small oligosaccharide molecules both to measure the molecular weight and to locate the position in the molecule of sugar units substituted at C-6 (cf. α-limit dextrins, above). In this way the molecular size and the position of the 6-phosphate group in phosphomaltodextrins were determined. The phosphodextrins are found in the α-amylase degradation products of tuber starches, e.g. potato starch; in contrast to cereal starches, they contain phosphate groups in the amylopectin component. A further application of the periodate method arises with polysaccharides terminated by sugar alcohols, e.g. the seaweed polysaccharide laminarin, which contains mannitol. The molecular size and mannitol content of laminarin were measured.

Mr. Hughes and Dr. Whelan devized conditions suitable for the complete and quantitative periodate oxidation of amino sugars, as the hydrochlorides or as the acetylamino sugars. The pH dependance of the periodate oxidation of glucose was studied. Lead tetra-acetate can be used selectively to oxidize the chain-ends of maltodextrins. This property is being used in the structural examination of the α-limit dextrins of amylopectin (see above). The measurement of optical rotation of sugars in alkaline sodium borate proved to be of value in their identification.

Enzymic Polymerization of Monosaccharides. In the search for enzyme systems capable of directly polymerizing monosaccharides, Dr. Clancy, investigated whole yeast. When brewer's yeast fermented galactose a series of di- and tri-saccharides was formed, in substantial yield. These appear to be α-linked galactose oligosaccharides and include hitherto unknown sugars. Polmerization also takes place when fermentation is suppressed by toluene, and the composition of the mixture is different from that in the absence of toluene. The mixtures of oligosaccharides were separated. In preparation for the structural examination of these substances the action of sodium periodate on disaccharides of known constitution was examined, in order to develop semi-micro techniques of structural analysis. Sodium periodate normally oxidizes all the α-glycol groups of a sugar but in very dilute solution only the reducing-end sugar unit of a disaccharide was oxidized. This greatly simplifies the interpretation of the results and makes possible a ready distinction between 1:3- and 1:4-linked sugars by the periodate technique.

Fractionation of Potato Enzymes. Miss Bohn, Mr. Jørgensen, and Dr. Whelan examined the ion-exchange resin *N*, *N*-diethylaminoethyl cellulose as a means of purifying the starch-metabolizing enzymes of potato. Although the resin does not separate the enzymes very well, it effects a very considerable purification with respect to the removal of other protein.

PHOSPHOLIPIDS OF HEART MUSCLE

Studies on Phospholipids. The importance of phospholipids in the economy of all kinds of cells, particularly in relation to the function of the cell membrane, is universally admitted, but little is known in fact of their exact function. This is partly due to the difficulty of separating and identifying different kinds of phospholipid. The object of the studies reported below is to elucidate the structure and function of certain tissue phospholipids, the polyglycerophosphatides, which though present in only small amounts, are known to be metabolically active, and the plasmalogens.

Polyglycerophosphatides. Cardiolipin. Dr. Macfarlane and Dr. Gray suggested previously that this complex phosphatidic acid, found in heart muscle, contained two phosphoric acid, three glycerol and four fatty acid residues, a simpler structure than that proposed by Pangborn. Dr. Macfarlane obtained evidence of this structure by oxidative degradation of the deacylated polyglycerophosphate; on oxidation of the terminal glycerol groups with periodate, the formaldehyde produced was in equimolar proportion to the phosphorus present, indicating that the polyglycerophosphate skeleton of cardiolipin consists of three glycerol residues linked by two phosphoric acid residues. The position of the fatty acids in the intact phospholipid has been ascertained by Dr. Macfarlane and Dr. Wheeldon, by identification of the fragments obtained in conditions in which the splitting of the phosphoric diester bonds occurs without rupture of the fatty acid ester bonds. On heating cardiolipin in 90% acetic acid, the main products were glyceroldiphosphate and diglycerides, and only traces of free fatty acids and free glycerol were found.

These results are incompatible with the structure for cardiolipin proposed by Pangborn. They afford substantial evidence that the simpler structure suggested by Dr. Macfarlane and Dr. Gray is correct, the four fatty acids being linked to the two terminal glycerol residues, leaving a free hydroxyl group on the middle glycerol residue.

The polyglycerophosphatide present in liver is being prepared by chromatographic methods for similar structural studies.

Plasmalogens. Two of the main classes of phospholipids hitherto recognised in tissues, kephalin and lecithin, are frequently mixtures of the classical esters, containing two fatty acids, with closely related substances, the kephalin and choline plasmalogens, in which a long chain fatty aldehyde, linked as an α-β unsaturated ether, replaces one of the fatty acids, the remaining fatty acid being generally highly unsaturated. Chemical evidence indicating that in ox heart plasmalogen the fatty acid was in the α position was obtained previously by Dr. Gray. In view of the suggestion of Marinetti & Erbland that the structure of plasmalogens in ox and pig heart was different, Dr. Gray and Dr. Macfarlane prepared choline plasmalogen fractions from both tissues and found that they behaved identically in three tests. Both were decomposed by snake venom (which is known to attack the α-acyl linkage only) and both gave on treatment with acetic acid an unsaturated lysolecithin identified as the α-acyl isomer; these tests indicate that the original plasmalogen was the α-acyl compound. On catalytic reduction of the plasmalogens, followed by removal of the fatty acid and phosphoric acid moieties by hydrolysis, the alkoxyglyceryl ether derivative obtained from both ox and pig preparations was found by periodate oxidation to be predominantly the α-glyceryl ether: this test indicates that the original plasmalogen was predominantly the β-acyl derivative. This last finding is quite discordant with the enzymic and earlier chemical evidence, and further work is required to resolve the discrepancy and establish the structure of the compound.

The proportion of plasmalogens differs markedly in different tissues, but nothing is known as yet of the functional significance of this variation. Dr. Gray is investigating the pattern of phospholipids, particularly of plasmalogens, in selected tissues by quantitative separation of individual phospholipids and identification of their fatty components by gas chromatography, with a view to correlating the pattern with the biological function. To further this work he has devised various systems for partition chromatography, particularly for the fractionation of the acidic phospholipids.

HUMAN PLASMA PROTEINS

Components of the Clotting Mechanism. In all procedures used for the assay of antihaemophilic factor, Factor V is an essential constituent of the clotting reaction mixtures The difficulty of preparing human Factor V has led to the use of such things as adsorbed human haemophilic plasma or rabbit serum as crude sources of Factor V, for adding to the reaction mixtures In a laboratory divorced from immediate access to patients, the human haemophilic plasma is virtually unobtainable and the rabbit serum has proved to be unreliable. In the hope of overcoming the difficulty, Dr. Horner is attacking the problem of preparing human Factor V free from other components of the clotting mechanism.

Owing to staff changes, experimental work on human antihaemophilic factor has been in abeyance for almost the whole year, but has been started again by Dr. Kekwick and Mr. Walton.

The Globulins of Fraction G.2. In the separation of human plasma proteins with ether the fraction G.2 is obtained. In addition to most of the alpha and beta lipoproteins, it contains caeruloplasmin, and the high molecular weight globulins with a sedimentation coefficient of approximately 20S. Mrs. S. Derechin has examined the suitability of various buffers for the differential extraction of the high molecular components from the G.2 precipitate at 0°C.

Plasminogen. In certain circumstances human plasminogen is activated to plasmin by treatment with some organic solvents, and this may occur when human plasma proteins are fractionated in systems containing such solvents. As a preliminary to defining the conditions for activation by solvents, Dr. M. Derechin has examined procedures for purifying plasminogen, using plasma from outdated human blood. The extraction with citrate buffers of a crude plasminogen fraction, obtained by precipitation with ether, followed by precipitation from the extract, has led to a forty fold increase in plasminogen content, with a recovery of about 50% of the total plasminogen.

The estimation of total plasminogen depends on its conversion to plasmin with streptokinase, and the measurement of the proteolytic activity of the plasmin produced. Improvements in the precision of this assay have been made. Some observations were made of the effect of ethyl-ether on the spontaneous activation of plasminogen to plasmin.

Pathological Sera. Professor Martin continued his detailed study of hyperglobulinæmic sera with special reference to those containing an abnormally high proportion of globulins with sedimentation coefficients between 17S and 24S, the so-called "macroglobulins".

At the request of pathologists from several hospitals Dr. Kekwick examined the sera from a number of patients in the ultracentrifuge, in order to establish for diagnostic purposes whether they display a macroglobulinæmia.

Miscellaneous. In collaboration with Dr. Kench and Dr. Smith of the Nuffield Department of Occupational Health, Manchester University, Dr. Kekwick examined physico-chemically the urinary proteins from a series of patients and experimental animals suffering from cadmium and mercury poisoning. In man the urinary protein of mercury poisoning appears to be closely similar to serum albumin, but the cadmium urinary proteins have a low sedimentation coefficient of the order of 2.0S and are electrochemically and immunologically very heterogenous.

With Dr. Morgan and Mr. Pusztai he is making an ultracentrifugal study of the products of enzymic degradation of blood group H-substance.

BLOOD PRODUCTS LABORATORY

Hypogammaglobulinæmia. The laboratory continues to participate in the investigation of this disease organized by the Medical Research Council Working Party on Hypogammaglobulinæmia, and the field trial of its treatment with gamma globulin. Several batches of gamma globulin were prepared for use in the trial, and a quantity of highly purified gamma globulin was made to serve as a reference standard in the immunological measurement of gamma globulin used by two of the collaborating laboratories.

Anti-hæmophilic Factor. The efficacy of human anti-hæmophilic factor in controlling hæmorrhage in hæmophilia was confirmed although the problems caused by variation in batch potency have not yet been solved. Altogether some 75 hæmophiliacs have now been treated, most of them at Lewisham Hospital, the Radcliffe Infirmary and Hammersmith Hospital.

Cæruloplasmin. Cæruloplasmin is a cuproprotein with oxidase activity, the plasma content of which is decreased in certain clinical conditions, particularly hepato-lenticular degeneration (Wilson's disease), a disease in which copper metabolism is profoundly disturbed. Mr. Vallet, with Dr. G. Curzon of the Institute of Neurology, Queen's Square, London, has devised a method of separating from normal plasma cæruloplasmin and concentrating it. A saline suspension of fraction G2 of Kekwick and Mackay is extracted with ether. The extract is then precipitated at pH 5.35 and 4.8. The pH 4.8 precipitate is subjected to fractional precipitation at low ionic strengths, and finally chromotographic separation on a diethylaminoethylcellulose column. This procedure yields a material that is about 90% cæruloplasmin.

During the freeze-drying of a cæruloplasmin preparation by the conventional technique, the product lost oxidase activity. By freezing the material very rapidly and subsequently drying below —22.5°C, little loss of activity occurred. This observation is of interest because it may shed light upon the reported failures to freeze-dry cuproproteins without loss of activity.

Plasmin. Dr. Mackay has undertaken a study of human fibrinolytic enzymes with the particular object of finding a method of preparing an active enzyme suitable for clinical use. It is generally recognized that the system in human blood consists of an activator, which combines with a tissue or bacterial kinase to convert the circulating proenzyme, plasminogen, to plasmin. Plasmin lyses fibrin clots and causes hydrolysis of casein or fibrinogen; and the activity of a preparation may be measured by the rate at which this hydrolysis occurs.

In the system of plasma fractionation which makes use of ether as a precipitant in aqueous systems, plasminogen is precipitated with other blood clotting factors in fraction P; and recovery in this fraction is complete if the ionic strength is reduced to 0.037. A further concentration is achieved by dissolving fraction P in phosphate-acetate buffer at pH 4.6, and re-precipitating at pH 5.0-5.2, ionic strength 0.01. This fraction, fraction CP, represents about 50% of the initial protein in fraction P and, when made from plasma not more than 24 hours old, is 6-10 times as active as fraction P. Fraction CP is at present being used as a crude material for further experiments in purification.

Christmas Factor. Facilities were given to Dr. P. W. Wolf of the Lewisham Group Laboratory to prepare concentrates of Christmas Factor, which were used for the treatment of patients with Christmas Disease.

Freeze-drying. By using an automatic balance, it has been possible to make extensive observations of the moisture content of dried plasma and plasma fractions. This information has made it possible to plan modifications of the primary desiccators in the plasma-drying plant, which will allow a more uniform degree of dryness to be attained.

BLOOD GROUP RESEARCH UNIT

The genetic aspect of human blood groups continues to be the main interest of the Unit. Each new system to be recognized represents one more identifiable point on the 23 pairs of chromosomes and each subdivision of a system makes the identification easier. More than 60 red cell antigens can now be detected, and most of them fall into nine well established independent systems; the independence of three more systems is very nearly established.

The search for new antigens continues to be through the testing of sera mostly from patients who have had a reaction to blood transfusion or who have presented some cross-matching difficulty before transfusion. Such sera are the pick of thousands and are kindly sent to us by colleagues in the United States, Great Britain and elsewhere.

ABO System: An acquired B-like Antigen. In the last Report it was noted that the blood of a patient, sent by Dr. C. Cameron of Dundee, was peculiar in that the red cells appeared to belong to a weak kind of group B though the serum contained anti-B. In collaboration with Dr. Cameron, Dr. I. Dunsford of Sheffield and Dr. A. Cahan of New York, seven such patients have now been studied. Tests on the blood of relatives of these seven patients show that the B-like antigen is not an inherited character. All the patients were group A_1: the absence of group O from the seven is statistically highly significant. The acquisition of the B-like antigen may be associated with old age or disease or both, for the average age of the patients was 72 and five of the seven had cancer. Young and middle-aged healthy people certainly do not acquire the antigen, otherwise it would have been recognized long ago in blood donors. These observations clearly call for investigation of the ABO groups of old people, ill and well, and this has already started in Dundee.

The MNSs System. In collaboration with Dr. J. J. van Loghem an antigen called Vr, discovered in Amsterdam, was investigated. Family studies showed Vr to be part of the MNSs system: in the three families so far tested the gene responsible for Vr is travelling on an *Ms* chromosome. *Vr* is not an allele of *M* or *N* or of *S* or *s* and must represent a separate site where Mendelian substitution can go on. Whether *Vr* is allelic to the other unplaced genes of the system, *Hu, He, Mi*^{a} and *Vw* (Report, 1957), will probably remain unknown for a long time owing to the extreme rarity, at any rate in Caucasians, of families having any two of the corresponding antigens.

The P System. Earlier work of the Unit (Report, 1956) showed that this system which before seemed simple was, in fact, complex and apparently constructed on the A_1A_2O pattern. An antigen, now called P^k, found in a Finnish patient in Minneapolis at first looked like the analogue of group B in the system (Report, 1958) but samples from many relatives of the patient, sent to us by Dr. G. A. Matson, showed that the manner of inheritance of P^k was quite unlike that of B. Either P^k is a straightforward recessive character or it is one that can be expressed only when the gene P^k is present and the genes P_1 and P_2 are absent—that is to say, in the genotypes P^kP^k or P^kp. But, whatever the genetic mechanism, P^k is inherited in a way previously unknown in blood groups.

Further examples of P^k were found amongst samples of blood sent to us by Professor E. Mustakallio from another Finnish family, this time living in Finland. No relationship to the Minneapolis family has yet been traced. The antigen was inherited in the same surprising manner.

The antigen P^k must be extremely rare though, curiously, the corresponding antibody is relatively very common, being found in most strong anti-P_1 sera. P^k is of unusual interest because of the principle of its inheritance and for the further glimpse it gives of the complexity of the P system.

The Kidd System. In collaboration with Dr. F. J. Pinkerton and Dr. L. E. Mermod of Honolulu and with Dr. A. Cahan of New York the Unit investigated the blood of a Filipina who has some Chinese ancestry. The red cells react neither with anti-Jk^a nor with anti-Jk^b: this is the first time that the phenotype Jk(a-b-) has been observed. The serum of the patient contains an antibody which reacts with the cells of all people so far tested; one component of the antibody is clearly anti-Jk^b but most of it appears to be inseparable anti-Jk^aJk^b. If the phenotype Jk(a-b-) exists in Whites and Negroes it must be rare: it can most hopefully be looked for in Asiatics.

When there is no evidence of activity at a locus there are several possible explanations. In the present case, for example, the patient may be homozygous for an unknown Kidd allele, say Jk^c; but this could only be established by the finding of the corresponding antibody, anti-Jk^c. On the other hand inhibitor genes may be at work.

Towards the Establishment of Three New Blood Group Systems. When a "new" blood group antibody has been found the antigen thereby defined may belong to one of the systems known at the time or it may represent a new system. The only certain way of proving that a new antigen does not belong to an old system is to find a family in which the respective genes are being inherited independently. This is rather laborious for only a minority of families are of informative types.

The rare antigen Wr^a was discovered in 1953 by Holman who was able to show from the original family that Wr^a did not belong to the ABO, MNSs, P, Rh or Kidd systems. In collaboration with Dr. Dunsford of Sheffield, the Unit studied families with Wr^a and was able to show that it is independent also of the Kell and Duffy systems.

The antigen Di^a was first found in Venezuela. Amongst Carib Indians it may reach a frequency of 40 per cent. Di^a is evidently an Asiatic character for it is found amongst Chinese and Japanese but not in Whites or Negroes. Previous work had shown that the antigen was independent of the ABO, MNSs, P, Rh and Kidd systems. In collaboration with Dr. M. Layrisse of Caracas, nine Venezuelan families of mixed Negro-Indian people who had the antigen have been studied; as a result Kell and Duffy were added to the systems excluded.

An antigen Js^a, which seems practically to be confined to Negroes was discovered in 1958 by Giblett, who was able to show that it was independent of the ABO, MNSs and Rh systems. The Venezuelan families were tested with anti-Js^a serum, kindly given by Dr. E. Giblett of Seattle, and Duffy and Kidd were added to the systems excluded. One of the families showed that Js^a does not belong to the same system as Di^a.

Red Cell Antigens Possessed by Most People. During the course of the year several antibodies have been investigated which reacted with all cells tested save their owner's. The corresponding antigens doubtless reflect the presence of genes of almost universal distribution, though the proof that the

antigens are genetically determined depends on finding a sib whose red cells are also negative: one family of this kind was investigated in collaboration with Dr. J. J. Griffitts, of Miami. The demonstration that such antigens are not part of any of the established systems will be particularly difficult.

In collaboration with Dr. W. J. Jenkins of Brentwood and Dr. A. Cahan of New York, the blood of two most unusual donors was studied. The red cells of both donors lack an antigen called I and the serum of both donors contains anti-I. People whose red cells lack I are a great rarity: no example was found by the North East London Blood Transfusion Centre though 17,000 donors have been tested. The problem is proving difficult though it is becoming clear that I is connected in some way with the ABO groups and perhaps with the P groups as well.

The blood grouping of twins, mostly sent by Dr. E. Slater and Mr. J. Shields of the Genetics Unit, the Maudsley Hospital, and of patients with sex abnormalities, mostly sent by Dr. P. Polani of Guy's Hospital, continues. (Report, 1958).

For routine antisera the Unit is indebted to many colleagues, particularly Dr. A. E. Mourant and Miss E. W. Ikin of the Medical Research Council Blood Group Reference Laboratory, Dr. I. Dunsford of the Sheffield Blood Transfusion Service and Dr. R. A. Zeitlin and Dr. T. E. Cleghorn of the South London Blood Transfusion Centre. Dr. Zeitlin has also provided on many occasions large numbers of random blood samples. Again we are grateful to the Staff of the Institute for innumerable samples of their blood which, having been tested for almost all the known antigens, is invaluable in the identification of antibodies.

BLOOD GROUP REFERENCE LABORATORY

The Laboratory serves as reference centre for blood grouping problems and supply centre for grouping sera for the United Kingdom. It also acts under the auspices of the World Health Organisation as the world reference centre.

Demands for liquid grouping sera for use in the United Kingdom have continued at a high level, and in the case of anti-human-globulin serum have increased considerably, as have those for dried sera of nearly all kinds for overseas use. Once again a number of overseas laboratories have been supplied with an initial stock of serum and members of their staff fully blood-grouped in order to help them to start their own grouping services. Numerous specimens received from laboratories in Great Britain and elsewhere have been examined for blood-group antigens and antibodies, to guide the management of clinical cases, to select and identify suitable diagnostic sera and for research purposes.

A detailed investigation, by Dr. Parkin, Miss Giles and Miss Ikin, of the D^u antigens of the Rh blood group system, and of the best means for detecting them, is in progress.

Dr. Parkin has continued her work on sera from patients with rheumatic diseases, for the presence of factors which bring about the agglutination of red cells sensitised with certain non-agglutinating antibodies, and the effects of which are inhibited by gamma globulin.

Dr. Parkin and Miss Giles have investigated serologically and genetically a considerable number of cases with unusual antigens and antibodies, first detected in the course of examination for clinical reasons.

Miss Ikin has continued the grouping of blood specimens for anthropological purposes and has carried out tests on specimens from Saudi Arabia and from Nigeria (Kanouri and Bade), and she and Dr. Parkin have jointly tested numerous specimens from Ferrara, Italy.

Miss Ikin has also carried out extensive statistical analyses of the results of tests carried out in recent years on specimens from the Near East and elsewhere.

In conclusion the Governing body desires to record its great appreciation of the manner in which the scientific, administrative and technical staffs have worked together during the period under review, and to congratulate them on the interest and range of their scientific activities.

H. H. DALE

Chairman.

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THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

Balance Sheet

and

Accounts

31st December 1959

CHELSEA BRIDGE ROAD,
LONDON, S.W.1.
24th May, 1960.

FINANCIAL REPORT OF THE GOVERNING BODY

1. The Balance Sheet as at 31st December, 1959, shows balances to the credit of the various funds as follows: Capital Fund £859,460, Specific Funds £153,533 and Bequest Funds £15,509. The balance on the Capital Fund is after adding the Contingency Reserve of £109,142, and the balance on the Sinking Fund for Freehold Buildings of £112,556 is after adding the profit, less losses, on sales of Sinking Fund investments amounting to £8,539 and transferring from income and expenditure account £5,648. The Re-endowment Fund has been increased by donations during the year amounting to £787.

2. The General Fund Income and Expenditure Account shows the income for the year as £252,665 compared with £213,182 in 1958. Expenditure amounted to £201,866 against £177,534 last year. The surplus for the year is £50,799 compared with £35,648 in 1958.

3. The year's surplus of £50,799 shown by the General Fund Income and Expenditure Account has been transferred to the Capital Fund.

4. Stocks of Sera, Vaccine Lymph and Horses on hand at 31st December, 1959, have the nominal value of £11,802, £990 and £5,915 respectively.

5. Messrs. Cooper Brothers & Co., the retiring Auditors, will, subject to the provision of the Companies Act, 1948, be re-appointed.

HENRY H. DALE, *Chairman of Governing Body.*

HUGH BEAVER. *Hon. Treasurer.*

(1958) £		£	£	£
	Capital Fund:—			
	Donations, &c., received to date from the following:—			
2,000	Dr. Ludwig Mond (1893)		2,000	
46,380	Berridge Trustees (1893/98)		46,380	
10,000	Worshipful Company of Grocers (1894)		10,000	
250,000	Lord Iveagh (1900)		250,000	
18,904	Lord Lister's Bequest (1913/23)		18,904	
7,114	William Henry Clarke Bequest (1923/6)		7,114	
3,400	Rockefeller Foundation (1935/6)		3,400	
22,669	Other Donations and Legacies (1891-1954)		22,669	
	General Fund Income and Expenditure Account Accumulated Surplus, as at 31st December, 1958	305,662		
	Add Surplus, 1959	50,799		
	Transfer from Contingency Reserve	109,142		
	Profit, less losses on sale of investments	46,950		
	Less amounts written off investments	13,560		
		33,390		
305,662			498,993	
666,129				859,460
	Specific Funds:—			
98,369	Sinking Fund for Freehold Buildings	112,556		
33,207	Pension Fund	33,279		
6,911	Re-endowment Fund	7,698		
			153,533	
	Bequest Funds:—			
8,500	Jenner Memorial Studentship Fund	8,857		
6,320	Morna Macleod Scholarship Fund	6,652		
			15,509	
153,307				169,042
	Specific Grants and Legacies Unexpended:—			
772	Cancer Research Legacies (1937-50)		772	
347	Royal Society Grant (1951)		347	
5,665	Nuffield Foundation Grants (1952-9)		6,301	
11,237	Guinness Lister Research Grant (1953-9)		11,341	
18,021				18,761
	Contingency Reserve:—			
	As at 31st December, 1958		109,142	
	Less Transfer to Capital Fund (General Fund Income and Expenditure Account Accumulated Surplus)		109,142	
109,142				—
	Current Liabilities:—			
17,730	Creditors and accrued charges			21,120
£964,329				£1,068,383

HENRY H. DALE. *Chairman of Governing Body.*
HUGH BEAVER. *Hon. Treasurer.*

REPORT OF THE AUDITORS

The accounts set out on pages 4 to 8 are in agreement with the books which, in our opinion, have been
In our opinion the accounts, amplified by the information given in paragraphs 1 and 4 of the Financial
state of affairs and the surplus of the Institute.

London, 25th May, 1960.

31st DECEMBER 1959

(1958) £		£	£	£
	Fixed Assets:—			
	Freehold property at cost:—			
73,548	Land and Buildings, Chelsea	73,548		
20,455	Queensberry Lodge Estate, Elstree	20,455		
2,049	House, Bushey	2,049		
			96,052	
	(Note: Additions and replacements since 1912 at Elstree and 1935 at Chelsea have been charged to Revenue.)			
	Furniture, Fittings, Scientific Apparatus and Books:—			
2,472	At cost *less* depreciation to 31st December, 1920 ...		2,472	
98,524	*(Note: Additions and replacements since 31st December, 1920, have been charged to Revenue)* ...			98,524

General, Specific and Bequest Funds.

Investments and Uninvested Cash:—

		Quoted at cost, less amounts written off In Gt. Britain	Quoted at cost, less amounts written off Elsewhere	Unquoted at cost	Cash		
595,498	General	545,277	82,868	47,595	—	675,740	
	Specific—						
98,369	Sinking Fund for Freehold Buildings ...	97,904	13,032	—	1,620	112,556	
33,207	Pension Fund	31,746	—	—	1,533	33,279	
6,911	Re-endowment Fund ...	6,590	—	—	1,108	7,698	
	Bequest—						
8,500	Jenner Memorial Studentship Fund	5,481	—	1,940	1,436	8,857	
6,320	Morna Macleod Scholarship Fund	5,894	—	—	758	6,652	
748,805		692,892	95,900	49,535	6,455	844,782	844,782
		£788,792					

(Market Value of Quoted Investments—£1,125,922)

	Current Assets:—		
60,920	Debtors and Payments in advance	87,316	
56,080	Balance at Bankers and Cash in hand	37,761	
117,000			125,077

(Notes: See paragraph 4 Governing Body's Financial Report for nominal values of Sera, Vaccine Lymph and Horses which have not been brought into the accounts.
There is a contingent liability of £3,000 in respect of investments not fully called up.)

£964,329	£1,068,383

TO THE MEMBERS

properly kept. We obtained the information and explanations we required.
Report of the Governing Body, comply with the Companies Act, 1948, and give a true and fair view of the

COOPER BROTHERS & CO.,

Chartered Accountants.

INCOME AND EXPENDITURE ACCOUNT

GENERAL

(1958)		*Total Expenditure*	*External Contributions*	
£		£	£	£
74,723	Salaries and Wages	140,539	57,101	83,438
7,643	Emoluments of two members of the Governing Body in an Executive Capacity	7,028	—	7,028
3,423	Premiums on Federated Superannuation Policies	4,985	2,295	2,690
3,180	Premium on Group Pension Policy	3,260	396	2,864
4,306	Rent, Rates and Insurance	4,540	135	4,405
11,941	Gas, Water, Fuel and Electricity	14,808	2,437	12,371
3,211	Office Expenses, Stationery and Printing	3,891	460	3,431
410	Auditor's Fee	409	—	409
1,046	Travelling Expenses	1,193	265	928
2,628	Biochemistry Expenses	5,022	1,841	3,181
949	Microbiology, Immunology and Experimental Pathology expenses	2,917	1,657	1,260
897	Biophysics Expenses	568	100	468
843	Virology Expenses	1,909	1,458	451
20,640	Serum, Vaccine and Vaccine Lymph Expenses	19,965	1,350	18,615
8,945	Animals	9,998	954	9,044
9,840	Animal House Expenses and Forage	10,558	1,493	9,065
13,377	Buildings, Alterations, Repairs and Renewals	32,043	303	31,740
473	General Apparatus and New Installations	461	—	461
1,347	Library Expenses	1,009	—	1,009
1,397	General Stores	1,689	—	1,689
1,375	Staff Canteen Loss	1,912	241	1,671
—	Blood Products Laboratory Expenses	6,497	6,497	—
4,940	Amount transferred to Sinking Fund for Freehold Buildings (including £4,949 Interest on Investments)	5,648	—	5,648
35,648	Surplus transferred to Capital Fund after charging to expenditure £28,830 for additions to property and equipment *(1958 £5,905)*	50,799	—	50,799
£213,182		£331,648	£78,983	£252,665

NUFFIELD FOUNDATION GRANTS

(1958)			(1958)		
£		£	£		£
5,824	Salaries, Wages, Laboratory Expenses and Animals ...	5,364	5,489	Balance at 1st January, 1959 ...	5,665
5,665	Balance carried forward ...	6,301	6,000	Amounts received	6,000
£11,489		£11,665	£11,489		£11,665

FUND

(1958) £		£	£
	Interest and Dividends on Investments:—		
33,782	General Fund	35,835	
4,516	Sinking Fund	4,949	
			40,784
	Underwriting Commission:—		
731	General Fund	740	
—	Sinking Fund	275	
			1,015
168,348	Sales of Sera, Vaccines, Vaccine Lymph, &c.		204,622
5,805	Rent		6,244
£213,182			£252,665

GUINNESS-LISTER RESEARCH GRANT

(1958) £		£	(1958) £		£
9,464	Salaries and Wages	10,837	9,812	Balance at 1st January, 1959 ...	11,237
3,111	Laboratory Expenses	3,059	14,000	Amount received	14,000
11,237	Balance carried forward ...	11,341			
£23,812		£25,237	£23,812		£25,237

PENSION FUND

(1958) £		£	(1958) £		£
1,486	Pensions	1,656	32,964	Fund as at 1st January, 1959 ...	33,207
33,207	Fund as at 31st Dec., 1959 ...	33,279	1,729	Interest on Investments (gross)	1,728
£34,693		£34,935	£34,693		£34,935

JENNER MEMORIAL STUDENTSHIP FUND

(1958) £		£	(1958) £		£
—	Loss on Realisation of Investment	46	8,038	Fund as at 1st January, 1959 ...	8,500
8,500	Fund as at 31st Dec., 1959 ...	8,857	462	Interest on Investments (gross)	403
£8,500		£8,903	£8,500		£8,903

MORNA MACLEOD SCHOLARSHIP FUND

(1958) £		£	(1958) £		£
218	Loss on realisation of investments	—	6,241	Fund as at 1st January, 1959 ...	6,320
6,320	Fund as at 31st Dec., 1959 ...	6,652	297	Interest on Investments (gross)	332
£6,538		£6,652	£6,538		£6,652

INVESTMENTS AS AT 31st DECEMBER 1959

GENERAL FUND

		Balance Sheet Value	Market Value
	QUOTED:—		
Trustee Securities			
£12,000	Australia 3% Stock 1972/74	£12,121	£9,180
£20,000	British Transport 3% Gtd. Stock, 1967/72	20,259	15,100
£66,300	3% Savings Bonds 1960/70	66,417	55,029
£3,000	3% Savings Bonds 1965/75	2,652	2,325
£5,000	Borough of Luton Loan	5,000	5,000
Debentures and Loan Stocks			
£5,000	Allied Bakeries Ltd. 5% Unsecured Loan Stock 1966/70	4,819	4,725
£5,000	Associated Biscuit Manufacturers Ltd. 6% Deb. Stk. 1978/83	4,995	5,200
£500	Associated Commercial Vehicles Ltd. 5¾% Unsecd. Loan Stk. 1977/82	501	462
£5,000	Associated Electrical Industries Ltd. 6% Deb. Stk. 1978/83	4,988	5,175
£10,000	Australian Estates Co. Ltd. 6½% Conv. Secd. Loan Stk. 1971/76	9,848	9,450
£5,000	Birfield Ltd. 6% Unsecd. Loan Stk. 1976/81	4,976	4,925
£5,000	British Petroleum Co. Ltd. 6% Conv. Deb. Stk. 1976/80	5,179	5,250
£5,000	Cheapside Land Development Co. Ltd. 5¾% Gtd. 1st Mtge Deb. Stock 1978/83	4,988	4,875
£5,000	George Cohen 600 Group Ltd. 6% Unsecd. Loan Stk. 1975/80	5,000	5,025
£5,000	Debenture Corpn. Ltd. 5¾% Deb. Stock 1979/83	4,806	4,925
£5,000	Edwards High Vacuum Ltd. 6½% Conv. Unsecd. Loan Stk. 1978/83	5,270	8,000
£10,000	English Electric Co. Ltd. 5½% Debenture Stock 1979/84	9,912	9,950
£5,000	Flowers Breweries Ltd. 5½% 1st Mtge. Deb. Stk. 1970/72	4,850	4,975
£4,500	General Electric Co. Ltd. 6% Unsecd. Loan Stk. 1976/81	4,408	4,657
£9,000	Hope & Anchor Breweries Ltd. 5½% Mtge. Deb. Stk. 1980/85	8,843	8,775
£9,000	Hunting (Eden) Tankers Ltd. 6½% Debenture Stock 1979	8,921	9,000
£5,000	Kennings Ltd. 5½% Unsecd. Loan Stock 1970/75	4,703	4,550
£5,000	International Computers & Tabulators Ltd. 6% Debenture Stock 1975/80	4,950	5,125
£5,000	International Computers & Tabulators Ltd. 5½% Debenture Stock 1979/84	4,883	5,125
£10,000	London & Overseas Freighters Ltd. 6% 1st Mtge. Deb. Stk. 1963/82 (70% paid)	7,025	6,250
£9,000	Mitchell Cotts Group Ltd. 6% Unsecd. Loan Stk. 1976/81	8,800	8,775
£10,000	Norvic Shoe Co. Ltd. 5% Unsecd. Loan Stock 1970/75	9,800	9,400
£10,000	Peninsular & Oriental Steam Navigation Co. 5% Deb. Stk. 1975/80	9,542	9,250
£3,000	Port of London 3½% Regd. Stock 1965/75	2,687	2,280
£4,000	Ruston & Hornsby Ltd. 6% Unsecd. Loan Stk. 1975/80	4,000	4,000
£5,000	John Smith's Tadcaster Brewery Co. Ltd. 6% Red. Deb. Stock 1978/83	4,892	5,100
£10,000	Tennant Bros. Ltd. 6% Red. Deb. Stock 1971	10,089	10,200
£10,000	United Gas Industries Ltd. 6% Unsecd. Loan Stk. 1973/75	9,900	9,600
£10,000	Whitbread Investment Co. Ltd. 5¾% Gtd. Deb. Stk. 1980/85	9,950	9,850
£10,000	Witan Investment Co. Ltd. 4½% Conv. Deb. Stock 1991/96	10,000	10,250
United Kingdom Equities			
9,375	Albright & Wilson Ltd. Ordinary 5/- Shares	5,168	13,945
1,500	Associated Electrical Industries Ltd. Ordinary £1 Stock Units	4,498	4,781
3,750	Associated Portland Cement Manufacturers Ltd. Ordinary £1 Stock Units	5,522	13,828
6,156	Automatic Telephone & Electric Co. Ltd. Ordinary 5/- Stock Units (6,000 Old, 156 New)	5,227	6,618
5,000	Beecham Group Ltd. Ordinary 5/- Shares	8,301	12,062
3,000	British Oxygen Co. Ltd. Ordinary £1 Stock Units	6,165	12,656
6,000	Boots Pure Drug Co. Ltd. Ordinary 5/- Shares	6,694	9,300
4,200	Cater Brightwen & Co. Ltd. Ordinary £1 Stock Units	10,872	12,390
16,500	Debenture & Capital Investment Trust Ltd. Ord. 5/- Stock Units	3,071	18,150
5,000	Distillers Co. Ltd. Ordinary 10/- Shares	4,678	9,750
2,000	English China Clays Ltd. Ordinary £1 Shares	6,363	8,125
3,000	Grattan Warehouses Ltd. Ordinary 5/- Stock Units	8,017	9,937
3,850	Great Universal Stores Ltd. "A" Ordinary 5/- Stock Units	7,704	10,010
5,000	Alfred Herbert Ltd. Ordinary £1 Shares	10,307	15,000
1,600	General Accident Fire & Life Assurance Corpn. Ltd. Ordinary 5/- Stock Units	5,757	6,160
3,750	Imperial Chemical Industries Ltd. Ordinary £1 Stock Units	5,619	11,438
2,750	International Computers & Tabulators Ltd. Ordinary £1 Shares	3,194	10,484
3,500	Land Securities Investment Trust Ltd. Ordinary 10/- Shares	5,336	5,469

GENERAL FUND—continued

		Balance Sheet Value	Market Value
10,000	London & Montrose Investment Trust Ltd. Ordinary 5/- Shares ...	—	14,000
£2,500	London Scottish American Trust Ltd. Deferred Stock	—	11,625
1,250	J. Lyons & Co. Ltd. "A" Ordinary £1 Stock Units	4,767	5,273
3,000	Marks & Spencer Ltd. "A" Ordinary 5/- Shares	5,479	11,400
2,250	Metal Box Co. Ltd. Ordinary £1 Stock Units	3,493	8,508
30,000	Mercantile Investment & General Trust Co. Ltd. Ord. 5/- Shares	13,401	48,000
10,000	Monsanto Chemicals Ltd. Ordinary 5/- Shares	8,163	13,500
550	Prudential Assurance Co. Ltd. "A" Shares of 4/-	5,268	9,694
15,000	Rio Claro Investment Trust Ltd. Ordinary 5/- Stock Units ...	991	17,063
10,000	River Plate & General Investment Trust Co. Ltd. Deferred 5/- Stock Units	667	14,250
9,600	Seccombe Marshall & Campion Ltd. Ordinary £1 Shares	10,162	19,440
2,500	Sphere Investment Trust Ltd. Ordinary £1 Shares	—	10,469
12,000	Standard Trust Ltd. Ordinary 5/- Stock Units	3,832	18,600
7,500	Sterling Trust Ltd. Ordinary Stock Units of £1	6,824	35,156
3,500	John Summers & Sons Ltd. Ordinary £1 Shares	12,497	13,037
1,500	Turner & Newall Ltd. Ordinary £1 Stock Units	4,763	8,297
5,000	Transparent Paper Ltd. Ordinary 5/- Shares	5,224	5,156
8,000	Typhoo Tea Holdings Ltd. Ordinary 5/- Shares	—	18,800
3,500	United Steel Companies Ltd. Ordinary £1 Shares	13,512	14,437
3,000	Vickers Ltd. Ordinary £1 Stock Units	4,765	5,925
12,000	Allen West & Co. Ltd. Ordinary 5/- Stock Units	6,126	9,300
3,000	Westinghouse Brake & Signal Co. Ltd. Ordinary £1 Stock Units ...	5,718	8,812
9,450	Witan Investment Co. Ltd. Ordinary £1 Shares	11,745	34,256
2,500	F. W. Woolworth & Co. Ltd. Ordinary 5/- Stock Units	5,413	8,562
Dollar Equities			
600	American Metal Climax Inc. Common Shares of $1	5,496	5,313
350	Central Illinois Public Service Co. Common Shares of $10	4,893	5,560
250	Consolidated Edison Co. Common Shares (n.p.v.)	4,925	5,352
682	Consolidated Foods Corpn. Common Shares of $1⅓	5,796	7,347
65	E. I. du Pont de Nemours & Co. Inc. Common Shares of $5 ...	4,921	6,266
250	Federated Department Stores Inc. Common Shares of $2½	4,984	6,436
400	First Bank Stock Corpn. Common Shares of $10	4,623	7,468
200	First National City Bank of New York Common Shares of $20 ...	4,603	6,760
700	Firstamerica Corpn. Common Shares of $2	4,968	7,733
200	Jones & Laughlin Steel Co. Common Shares of $10	4,625	6,153
735	Marine Midland Corpn. Common Shares of $5	5,227	7,448
900	Montana Power Co. Common Shares (n.p.v.)	5,421	7,724
600	Oklahoma Gas & Electric Co. Common Shares of $5	4,808	6,874
400	Southern Natural Gas Co. Common Shares of $7½	5,630	5,204
100	Standard Oil Co. New Jersey Common Shares of $7	2,066	1,812
480	Tennessee Gas Transmission Co. Common Shares of $5	4,859	6,223
450	Tri-Continental Corpn. Common Shares of $1	5,023	6,450
		£628,145 ...	£941,544
	UNQUOTED:—		
£9,000	British Titan Products Co. Ltd. 5½% Unsecd. Loan Stock 1970/75	£8,820	
£10,000	Caltex (U.K.) Ltd. 6% Gtd. Loan Stock 1971/76	9,800	
£10,000	Kraft Foods Ltd. 5% Debenture Stock 1965/75	10,000	
£10,000	R. A. Lister & Co. Ltd. 5% Unsecured Loan Stock 1960/65 ...	9,975	
£4,000	R. H. Neal & Co. Ltd. 6½% Unsecured Loan Stock 1966/71... ...	4,000	
£5,000	Tanker Charter Co. Ltd. 6½% 'B' Secured Loan Stock 1970/82 ...	5,000	
		£47,595	

SINKING FUND FOR FREEHOLD BUILDINGS

Trustee Securities			
£3,000	British Electricity 3% Gtd. Stock 1968/73	£2,916 ...	£2,385
£10,000	3% Savings Bonds 1960/70	9,622 ...	8,300
£4,500	3% Funding Loan 1959/69	3,876 ...	3,825
Debenture and Loan Stocks			
£5,000	Debenhams Ltd. 5½% 2nd Debenture Stock 1979/83	4,907 ...	5,000
£5,000	Financial Times Ltd. 5½% Mtge. Deb. Stock 1980/85	4,900 ...	4,850
£9,000	Gallaher Ltd. 6% Unsecured Loan Stock 1976/81	8,884 ...	9,135
£10,000	New Zealand Loan & Mercantile Agency Co. Ltd. 5½% 2nd Mtge. Deb. Stk. 1970/80	9,525 ...	9,200
£5,000	New Zealand Loan & Mercantile Agency Co. Ltd. 6% 2nd Mtge. Deb. Stock 1977/80	4,963 ...	4,875

		Balance Sheet Value	Market Value
United Kingdom Equities			
1,500	Eagle Star Insurance Co. Ltd. Ordinary 10/- Shares	5,322	5,325
3,000	Ford Motor Co. Ltd. Ordinary £1 Stock Units	5,550	16,500
5,000	English Electric Co. Ltd. Ordinary £1 Stock Units	10,386	13,125
2,600	Guest Keen & Nettlefolds Ltd. Ordinary £1 Stock Units	5,373	11,862
1,000	A. Reyrolle & Co. Ltd. Ordinary £1 Stock Units	4,463	4,875
5,000	Steel Company of Wales Ltd. Ordinary £1 Shares	12,229	12,750
2,400	Union Discount Co. of London Ltd. Stock Units of £1	4,988	7,440
Dollar Equities			
250	Commercial Credit Corpn. Common Shares of $10	5,098	5,295
150	Standard Oil Co. New Jersey Common Shares of $7	3,107	2,718
400	Union Tank Car Co. Common Shares (n.p.v.)	4,827	4,400
		£110,936	£131,860

PENSION FUND

Trustee Securities			
£12,416	British Electricity 4½% Gtd. Stk. 1967/69	£11,322	£12,106
£2,200	3% Savings Bonds 1960/70	2,205	1,826
£1,000	3% Savings Bonds 1965/75	1,000	775
£5,800	4% Funding Loan 1960/90	3,050	5,423
Debentures and Loan Stocks			
£3,000	Lewis Berger & Sons Ltd. 5¼% Deb. Stock 1977/82	2,977	2,910
£3,000	British Oxygen Co. Ltd. 5¼% Deb. Stock 1981/86	2,860	3,075
£3,000	Dowty Group Ltd. 6% Unsecured Loan Stock 1977/82	2,932	3,075
£3,000	Ruston & Hornsby Ltd. 6% Unsecured Loan Stock 1975/80	3,000	3,000
£2,500	Metal Box Co. Ltd. 5½% Unsecured Loan Stock 1977/80	2,400	2,462
		£31,746	£34,652

RE-ENDOWMENT FUND

£7,600	3% Savings Bonds 1960/70	£6,590	£6,308

JENNER MEMORIAL STUDENTSHIP FUND

QUOTED:—

Debentures and Loan Stocks			
£1,000	Gallaher Ltd. 6% Unsecured Loan Stk. 1976/81	£1,000	£1,015
£1,500	General Electric Co. Ltd. 6% Unsecd. Loan Stock 1976/81	1,500	1,553
£1,000	Hope & Anchor Breweries Ltd. 5½% Mtge. Debenture Stock 1980/85	990	975
£1,000	Hunting (Eden) Tankers Ltd. 6½% Debenture Stock 1979	991	1,000
£1,000	Mitchell Cotts Group Ltd. 6% Unsecd. Loan Stock 1976/81	1,000	975
		£5,481	£5,518

UNQUOTED:—

£1,000	British Titan Products Co. Ltd. 5½% Unsecured Loan Stk. 1970/75	£940
£1,000	R. H. Neal & Co. Ltd. 6½% Unsecured Loan Stock 1966/71	1,000
		£1,940

MORNA MACLEOD SCHOLARSHIP FUND

Trustee Securities			
£1,000	Stockton-on-Tees 5½% Redeemable Stock 1966	£992	£1,000
£1,500	Australia 6% Regd. Stock 1974/76	1,496	1,590
£1,500	New Zealand 6% Stock 1976/80	1,485	1,605
£1,000	Agricultural Mortgage Corpn. Ltd. 5% Debenture Stk. 1979/83	958	960
£1,000	South Essex Waterworks Co. 5% Red. Debenture Stock 1986/91	963	885
		£5,894	£6,040

THE LISTER INSTITUTE

OF

PREVENTIVE MEDICINE

REPORT

OF THE

GOVERNING BODY

1960

CHELSEA BRIDGE ROAD, LONDON, S.W.1

THE GOVERNING BODY

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The Rt. Hon. The EARL OF IVEAGH, K.G., C.B., C.M.G.

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Clerk to the Governors ... ... S. A. WHITE, A.A.C.C.A.

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Sir CHARLES HARINGTON, M.A., Ph.D., F.R.S.	Members of the Institute.
D. W. W. HENDERSON, C.B., D.Sc., Ph.D., F.R.S.	" "
The Rt. Hon. The EARL OF IVEAGH, K.G., C.B., C.M.G.	" "
Sir WILLIAM WILSON JAMESON, G.B.E., K.C.B., M.A., M.D., F.R.C.P., LL.D. ...	" "
Professor H. B. MAITLAND, M.D., M.R.C.S., L.R.C.P.	University of Manchester.
Professor A. A. MILES, C.B.E., M.A., M.D., F.R.C.P.	Members of the Institute.
Professor W. T. J. MORGAN, C.B.E., D.Sc., Ph.D., F.R.I.C., F.R.S.	" "
Professor Sir RUDOLPH PETERS, M.C., M.A., M.D., F.R.S.	" "
The President of the ROYAL COLLEGE OF PHYSICIANS	Royal College of Physicians, London
The President of the ROYAL COLLEGE OF SURGEONS	Royal College of Surgeons of England
The President of the ROYAL COLLEGE OF VETERINARY SURGEONS	Royal College of Veterinary Surgeons
MURIEL ROBERTSON, M.A., D.Sc., LL.D., F.R.S.	Members of the Institute.
Professor WILSON SMITH, M.D., F.R.S. ...	Royal Society.
Professor F. S. STEWART, M.D., B.Ch. B.A.O.	University of Dublin.
WILLIAM J. THOMPSON	Worshipful Company of Grocers
Professor E. B. VERNEY, M.A., M.B., B.Ch., F.R.C.P., F.R.S.	University of Cambridge.
Professor D. WHITTERIDGE, D.M., B.Sc., F.R.S.	University of Edinburgh.
G. S. WILSON, M.D., B.Sc., F.R.C.P. ...	University of London.

THE STAFF

Director: Professor A. A. Miles.

Deputy Director: Professor W. T. J. Morgan.

Superintendent of Elstree Laboratories: W. d'A. Maycock.

MICROBIOLOGY, IMMUNOLOGY and EXPERIMENTAL PATHOLOGY

†A. A. Miles, C.B.E., M.A., M.D., F.R.C.P. *(Professor of Experimental Pathology in the University of London).*
Muriel Robertson, M.A., D.Sc., LL.D., F.R.S.
Emmy Klieneberger-Nobel, Ph.D., D.Sc.
Ruth M. Lemke, B.Sc., Ph.D.
M. D. Pittam, B.A., Ph.D. *(Trypanosomiasis Research)*
Brenda Mason, B.Sc.
Ann M. Brimacombe, B.A.
J. A .Mills, B.A., M.D. *(Canada).*

GUINNESS-LISTER RESEARCH UNIT

*B. A. D. Stocker, M.D., M.R.C.S., L.R.C.P
G. G. Meynell, M.D., M.R.C.S., L.R.C.P.
M. W. McDonough, B.Sc., Ph.D.
Sheila Howarth, B.Sc., Ph.D.
Sylvia Smith, M.Sc.
H. Ozeki, B.Sc.
T. M. Joys, B.Sc. *(Research Student).*

VIROLOGY

L. H. Collier, M.D. *(also Hon. Director, M.R.C. Trachoma Research Unit).*
I. J. Payne, Ph.D.
G. Furness, B.Sc., Ph.D., Dip.Bact. *(M.R.C. Trachoma Research Unit).*
Doris M. Graham, M.Sc. *(M.R.C. Trachoma Research Unit).*
P. Reeve, B.Sc. *(M.R.C. Trachoma Research Unit).*
Elizabeth F. Fraser, B.Sc. *(M.R.C. Trachoma Research Unit).*

BIOCHEMISTRY

†W. T. J. Morgan, C.B.E., D.Sc., Ph.D., F.R.I.C., F.R.S. *(Professor of Biochemistry in the University of London). Principal Biochemist, Elstree.*
*Marjorie G. Macfarlane, D.Sc., Ph.D.
*W. J. Whelan, D.Sc., Ph.D., F.R.I.C.
Winifred M. Watkins, B.Sc., Ph.D.
D. Doyle, B.Sc., Ph.D.
Joan Allen, B.Sc.
I. A. F. Lister Cheese, B.Sc. *(Grocers' Company Research Student).*
H. M. Tyler, B.Sc. *(Research Student).*
Pamela M. Taylor, B.Sc. *(Research Student).*
G. M. A. Gray, B.Sc., Ph.D. *(Beit Memorial Research Fellow).*
M. J. Clancy, M.Sc., Ph.D., A.R.I.C. *(Dept. Scientific and Industrial Research Grantee).*
B. J. Bines, B.Sc., Ph.D., A.R.I.C. *(Dept. Scientific and Industrial Research Grantee).*
Regina Pietruszko, M.Sc. *(British Empire Cancer Campaign Grantee).*
A. J. Pusztai *(Ford Foundation Grantee).*
Zeenat H. Gunja, Ph.D. *(Agricultural Research Council Grantee).*
M. Abdullah, M.Sc. *(Agricultural Research Council Grantee).*
T. J. Painter, M.A., B.Sc., Ph.D. *(Medical Research Council Grantee).*
L. G. Egyud, B.Sc. *(Mental Health Research Fund Grantee).*
E. E. Smith, B.Sc. *(Dept. Scientific and Industrial Research Student).*
P. Z. Allen, A.B., Ph.D. *(U.S.A.).*
I. J. Goldstein, Ph.D. *(U.S.A.).*
G. Uhlenbruck, Dr.Med. *(Germany).*

BIOPHYSICS

†R. A. Kekwick, D.Sc. *(Reader in Chemical Biophysics in the University of London).*
†Professor N. H. Martin, M.A., F.R.C.P., F.R.I.C. *(Honorary Research Associate).*
P. W. Walton, B.Sc. *(Medical Research Council Grantee).*
M. Derechin, M.D. *(Argentina).*
D. S. Chandrasekhar, B.Sc., M.B., B.S. *(India).*

NUTRITION

§Dame Harriette Chick, D.B.E., D.Sc.
§E. Margaret Hume, M.A.

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†Appointed Teacher of the University of London.
*Recognised Teacher of the University of London.
§Honorary Member of Institute Staff.

PREPARATION and STUDY of THERAPEUTIC SERA (ELSTREE)

*B. G. F. Weitz, M.R.C.V.S.
J. Rodican, B.Sc.
Sheila M. Lanham, B.Sc. *(Trypanosomiasis Research).*
Frances M. Lee-Jones, B.Sc. *(Trypanosomiasis Research)*

BIOCHEMISTRY (ELSTREE)

*D. E. Dolby, B.Sc., Ph.D.

PREPARATION and STUDY of SMALLPOX VACCINE (ELSTREE)

*D. McClean, M.B., B.S., M.R.C.S.
C. Kaplan, M.Sc., M.B., Ch.B., Dip.Bact.
L. C. Robinson, B.Sc.

PREPARATION and STUDY of BACTERIAL VACCINES (ELSTREE)

*A. F. B. Standfast, M.A., Dip.Bact.
Jean M. Dolby, M.A., Ph.D. *(Medical Research Council External Scientific Staff).*
M. Garay, B.Sc.

BLOOD PRODUCTS (ELSTREE)

*W. d'A. Maycock, M.B.E., M.D.
L. Vallet, M.A.
§Margaret E. Mackay, M.Sc., Ph.D. *(Medical Research Council External Scientific Staff).*
Constance Shaw, M.Sc., Dip.Bact.
Shirley M. Evans, B.Sc.

MEDICAL RESEARCH COUNCIL UNITS HOUSED AT THE INSTITUTE

BLOOD GROUP RESEARCH UNIT.

§R. R. Race, Ph.D., F.R.C.P., F.R.S.
Ruth Sanger, B.Sc., Ph.D.
Jean Noades, B.Sc.
Patricia Tippett, B.Sc.
Florence J. Hamper, B.Sc.

BLOOD GROUP REFERENCE LABORATORY.

§*A. E. Mourant, M.A., D.Phil., D.M., M.R.C.P.
K. L. G. Goldsmith, Ph.D., M.B., B.S.
Elizabeth W. Ikin, B.Sc.
Carolyn M. Giles, B.Sc.

ADMINISTRATION

Secretary and Accountant - - - S. A. White, A.A.C.C.A.
Elstree Secretary and Estate Manager - G. J. Roderick, B.Comm.

Solicitors:
Field, Roscoe & Co.
52 Bedford Square, W.C.1.

Auditors:
Cooper Brothers & Co.,
Abacus House, 33 Gutter Lane, E.C.2.

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*Recognised Teacher of the University of London.
§Honorary Member of Institute Staff.

ANNUAL GENERAL MEETING

OF

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

June 21st, 1960

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the work of the Institute for the year 1959.

GOVERNING BODY

At its last meeting the Council re-appointed Sir Henry Dale, Sir Charles Dodds and Sir Wilson Jameson as its representatives on the Governing Body until 31st December 1960.

In accordance with the Articles of Association, Professor Morgan retired from the Governing Body and was succeeded by Dr. Marjorie G. Macfarlane as the Scientific Staff's representative.

COUNCIL

At last year's Annual General Meeting the three retiring members of the Council, Sir Charles Harington, Sir Paul Fildes and the Rt. Hon. Lord Balfour of Burleigh, were re-appointed. Dr. D. W. W. Henderson was appointed to the Council as a representative of the Members.

The three members of the Council due to retire this year in accordance with the Articles of Association, but who are eligible for re-appointment, are Professor Wilson Smith and Professor Edward J. Conway, the representatives of the Royal Society and the Royal Irish Academy respectively, and Professor W. T. J. Morgan, a representative of the Members of the Institute.

MEMBERS

The Governing Body records with regret the death during the year of Dr. Korenchevsky. Dr. Korenchevsky retired in 1944, after working at the Institute for twenty-four years.

STAFF

The Governing Body is pleased to record that Dr. Collier was awarded the Medaille d'Or for 1959 by the Legue contre le Trachome.

Dr. W. J. Whelan was appointed an Honorary Secretary of the Biochemical Society.

Dr. G. G. Meynell was appointed to the Guinness-Lister Research Unit; Dr. I. J. Payne to the Virology Department; Dr. D. Doyle to the Biochemistry Department; Miss A. M. Brimacombe to the Experimental Pathology Department; and Miss E. A. Bell to the Blood Products Laboratory.

Dr. D. L. Wilhelm resigned from the Department of Experimental Pathology on his appointment to the Foundation Chair of Pathology, University of

New South Wales. Mrs. L. G. Gillies, Mrs. J. Stocker, Miss Edgar, Miss E. A. Bell and Dr. A. A. Horner also resigned during the year.

Mr. F. K. Fox, Elstree Secretary and Estate Manager, retired on 31st December 1959 after thirty-eight years' service and is succeeded by Mr. G. J. Roderick. The Institute has also lost by retirement the services of Mr. C. D. Bevis and Mr. S. Foskett. Mr. Bevis, the most senior laboratory technician at Chelsea, entered the Institute's service in 1908. Mr. S. Foskett, an animal attendant, had been employed at Elstree since 1921. The Governing Body has pleasure in recording its appreciation of the very many years of loyal service they have given to the Institute.

In April 1959 Professor Miles delivered the Office of Naval Research Lecture at the Annual Meeting of the Society of American Bacteriologists in St. Louis, Mis., U.S.A.

Professor Morgan and Dr. Watkins took part, by invitation, in a Symposium on "The Biochemistry of Human Genetics", arranged by the Ciba Foundation in Naples in May 1959; and, at the invitation of the Polish Academy of Science, atended a conference on "The Biologically Active Mucoids" in Warsaw in October 1959.

Dr. Maycock visited Oslo at the invitation of the Norwegian Medical Society to lecture on the Blood Transfusion Service.

During September 1959, Dr. R. A. Kekwick attended a symposium in Amsterdam on the occasion of the opening of the new Central Laboratory of the Netherlands Red Cross Blood Transfusion Service.

On behalf of the World Health Organisation, Dr. L. H. Collier visited Paris and Geneva as a member of the Study Group on Trachoma.

In March 1959 Mr. Weitz visited the Pasteur Institute, Bandung, Java, as a World Health Organisation Consultant on Serum Production; and in January 1960, as Representative of the Colonial Secretary, attended the East African Trypanosomiasis Research Committee in Dar-es-Salaam and the East Africa Agriculture and Fisheries Research Council in Nairobi.

Dr. Stocker by invitation visited the Institut Pasteur, Paris.

Dr. Pittam, on a Colonial Office Grant, during September-December 1959 toured U.S. Laboratories concerned with protozoology and tropical medicine.

VISITORS

The following visitors, in addition to those listed under Staff, worked in the Institute's Laboratories: Dr. L. P. Andral, Institut Pasteur, Addis Ababa, Ethiopia; Dr. H. Bad el Din, Serum and Vaccine Institute, Agouza, Cairo; Mr. Chang Hong Min, National Institute of Health, Seoul, S. Korea; Mr. H. Chojnowski, Serum and Vaccine Production Laboratory, Lublin, Poland; Mr. J. H. Darbyshire, Central Veterinary Laboratory, New Haw, Weybridge, Surrey; Dr. J. P. Duguid, Department of Bacteriology, University of Edinburgh; Dr. J. Fonseca da Cunha, Oswaldo Cruz Institute, Rio de Janeiro, Brazil; Mlle. M. M. Gex, Centre Transfusion Sanguine, Strasbourg; Dr. Sutas Guptarak and Dr. Prakorb Tuchinda, Department of Medical Sciences, Bangkok, Thailand; Dr. M. A. Haseeb, Stack Medical Research Laboratories, Khartoum, Sudan; Dr. J. Moor-Jankowski, Institute for the Study of Human Variation, Columbia University, New York; Dr. G. Modiano, Institute of Genetics, University of Pavia; Dr. Nasution, Institut Pasteur, Bandung, Java; Miss W. A. F. Webber, East African Trypanosomiasis Research Organisation, Tororo, Uganda; Dr. P. Wolf, Southern Group Laboratory, Lewisham Hospital, London, S.E.

The Blood Group Research Unit and the Blood Group Reference Laboratory, whose researches for 1959 are described on pages 31-33 are still accommodated at the Institute; and Miss E. M. Hume continues to do editorial work for *Nutrition Abstracts and Reviews* on behalf of the Commonwealth Bureau of Animal Nutrition.

DONATIONS AND GRANTS

The Governing Body records its appreciation of the generosity of many bodies whose benefactions and grants support research work in the Institute. These include a grant from the Agricultural Research Council for research on the mechanism of starch-carbohydrase action; a grant from the British Empire Cancer Campaign for research on phospholipids; grants from the Colonial Development and Welfare Fund and the World Health Organisation in aid of research on the blood-meals of insect vectors of disease; a further grant from the Colonial Development and Welfare Fund in aid of researches on the immunology of trypanosomiasis; grants from the Department of Scientific and Industrial Research for researches on the enzymic polymerization of monosaccharides and on chemically modified polysaccharides; a grant from Imperial Chemical Industries Ltd. for the purchase of special apparatus; a grant from the Medical Research Council in aid of research on the isolation and purification of proteins involved in the clotting mechanism in human plasma; grants from the Nuffield Foundation for researches on the immunochemistry of blood group substances and on non-specific immunity in the early stages of infection; and a grant from the U.S. Public Health Authority in aid of researches on abacterial urethritis in man.

The Governing Body also gratefully acknowledges donations to the Institute's Re-endowment Fund from the following Assurance Companies: The General Life Assurance Company, the Norwich Union Insurance Societies and The Prudential Assurance Company Ltd.

RESEARCHES IN 1959

SUMMARY

In this summary of the investigations made in 1959, the bracketed numbers refer to the pages of the report where the researches are described in greater detail.

Microbiology. The Guinness-Lister Unit continues its exploration of bacterial genetics, mainly of the Salmonella bacilli, and to some extent (13) of staphylococci. Among the genetically-determined characters investigated are the chemical structure of the somatic antigens (12), the biosynthesis and chemical structure of flagella (13, 15), flagellar antigens (12), fimbriae (15) and the production of colicines (14). The other purely microbiological study concerns the cytology of certain free-living flagellated protozoa (10).

Immunology and pathology of infective diseases. The Institute's studies embrace infections by viruses, pleuropneumonia-like organisms (PPLO), bacteria and protozoa.

The Trachoma Research Group exploited its isolation of the viruses of trachoma and inclusion blenorrhoea. The field study of trachoma in terms of virus and bacilli of the Haemophilus group continued in the Gambia (17),

and extensive bench studies of the trachoma and blenorrhoea virus were made at the Institute (16). Measles virus was also studied. In the Smallpox Vaccine department, there is continued progress towards making vaccine from vaccinia virus grown in tissue culture, as an alternative to virus harvested from the skin of infected sheep (18).

The investigation of abacterial urethritis in man established a genital type of PPLO as a possible cause. In man, however, the incidence of antibodies to PPLO was not correlated with the presence of PPLO in the genitalia, so a detailed study of the relation of PPLO antibody response to PPLO infections is being made, both in experimental infections of the rat and in natural infection in man (16).

The immunological study of *bacterial* infections includes a detailed analysis of the immunology of infection in the mouse by the whooping cough bacillus, in terms of the two protective antigens (11), and an analysis of the *iota* toxin of *Clostridium welchii* (12). The study continues of the biologically active substances formed when diphtheria antitoxins are refined by protolysis, and of the enzymic process of refinement (20).

As regards pathogenic *protozoa*, the antigenic analysis of Trichomonas species (10) and the investigation of the immunology of trypanosomiasis continues. Soluble trypanosome antigens, formed during experimental trypanosomiasis of the rat, are under investigation (10); and trypanosomes cultivated *in vitro* to provide bulk material for antigenic analysis of these protozoa (11).

Epidemiology. The refined serological methods devised to identify the animal source of food for blood-sucking insects continues to provide valuable facts about the feeding habits of Tse-tse flies and mosquitos in regions where these insects are vectors or possible vectors of disease (19).

Pathology. Work on the relation of early tissue reactions to defence against microbial infection continues. The tissue response to various kinds of injury, including infection, was explored to determine the role, if any, of the serum proteases which increase capillary permeability (21). The investigation of early non-specific resistance to bacteria was extended to infections by Leishmania (22).

Biochemistry. The biochemical researches again concern three kinds of substances—the blood group substances, the cellular phospholipids, and starches.

The occurrence of more than one blood-group-specific chemical structure on a single mucopolysaccharide macromolecule and the genetical implications of this finding have been considered (23); and possible pathways for the later stages of the synthesis of the group mucopolysaccharides were embodied into a scheme which proved to be in very close agreement with the genetical ideas elaborated on the basis of serological results with family material (24).

The polyglycerophosphatide of liver was identified as cardiolipin, and the distribution and fatty acid composition of the phospholipids in the sub-cellular particles of liver was examined (27). The nature of the plasmalogens in heart muscle, liver, semen and spleen was investigated (28).

The enzymes of plants and animals synthesizing and degrading starch and glycogen were studied with regard to their specific effects on the natural polysaccharides and on modified polysaccharides produced by chemical methods (25). A new enzyme acting on starch-like substances was discovered in the potato (27). New disaccharides were synthesized by the action of brewer's yeast on galactose and N-acetylglucosamine.

Human and animal plasma proteins. The Institute's work is concerned with the isolation, refinement, characterization, assay and in some cases clinical trial of the various biologically active proteins of human plasma.

The difficulties of specifying the potency of preparations of anti-haemophilic globulins for use in haemophiliacs necessitated a re-examination of its mode of assay (29); clinical studies of the efficacy of the human preparation are in progress (30). The therapeutic value of gamma globulin in the treatment of hypogammaglobulinaemia is the subject of another clinical study (30).

Studies of the isolation and characterization of active proteins include that of plasmin (31), plasminogen (29, 31) and fibrinogen (31) for clinical use; that of Human Factor V (28), Christmas Factor (31), and of antibodies to red blood cells (29). Pathological studies include investigation of the so-called macro-globulins (30).

Physiological studies include that of the absorption of proteins from the gut of young mice (30).

MICROBIOLOGY

PROTOZOOLOGY

Antigenic Structure of Trichomonas. Dr. Robertson concluded her analysis of the antigens of the two serologically differing strains of *Trichomonas foetus* isolated from the uterus of infected cows and of two strains isolated from the caecum of pigs in Germany. The four strains are all serologically related to one another. The major protein antigens are common to them all, but the major polysaccharide antigens are only partially shared. The two cow strains, though slightly related, are readily distinguished from each other. The two pig strains are closely related to one another but are not identical. One cow strain is more closely related than the other to the pig strains. The serological distinctions do not justify the separation of the cow and the pig strains into two species.

Work on the antigens of the ciliate *Tetrahymena pyriformis* and other *Tetrahymena* species is in progress.

Cytology of Flagellates. Dr. Pittam continued his cytological and cytochemical studies (Report 1958) on *Trichomonas foetus.* Special attention was paid to the cytoplasmic granules in the hope of determining whether they contained mitochondria. Granules isolated by differential centrifugation were examined by manometric techniques to identify mitochondrial enzymes; no such enzymes were detected.

TRYPANOSOMIASIS

Antigens of T. brucei. Mr. Weitz continued his work on the antigens of *Trypanosoma brucei.* The serum of infected rats contains measurable amounts of trypanosomal antigen which is liberated in the circulation at the height of parasitaemia. This antigen, called "exo-antigen" in view of its solubility, is by itself harmless on injection into normal mice or rats. Added to suspensions of living trypanosomes, it greatly increases the infectivity of the suspension for mice, which die sooner than those receiving the organism alone; it appears to act by preserving the viability and infectivity of living *T. brucei in vitro.* Exo-antigen is also present on the surface of T. brucei, since antibody to it agglutinates whole organisms, and exo-antigen removes agglutinins from antisera to whole organisms. In addition, mice hyperimmunized with exo-antigen are either wholly or partly resistant to infection, according to the severity of challenge with living trypanosomes.

These observations indicate that the exo-antigen of *T. brucei* occurs on the surface of the organisms and is liberated in its surrounding medium, plays

an essential part in the infection of rats and mice and is a means by which laboratory animals can be immunized against the *T. brucei* infection following syringe inoculation. The immunogenic efficiency against infection transmitted cyclically by tsetse flies is being investigated.

In addition to the soluble exo-antigen, two somewhat labile antigens were extracted from mechanically disintegrated trypanosomes. Their serological relation to the exo-antigen and their biological and immunogenic properties are being studied.

Cultivation of Pathogenic Trypanosomes in Bulk. Dr. Pittam continued with his work on the establishment of mass culture of trypanosomes *in vitro*. Small-scale cultures of *Trypanosoma rhodesiense* and *T. gambiense* were established, and some of the problems connected with their mass-culture examined. Successful trial cultures were grown in Roux bottles and in large flasks, but some of the difficulties of harvesting the cultures are not yet overcome. A liquid medium was devised for the maintenance of trypanosomes which gave promising results.

WHOOPING COUGH BACILLUS

Identification of Protective Antigens. Mr. Standfast and Dr. Jean Dolby continued their work on the protective antigens of *Bordetella (Haemophilus) pertussis* and the corresponding antibodies.

Infections via lung and brain, and their modification by antibody. The course of infection by the two commonly used routes of challenge and the effect of IN and IC antibody was studied in detail.

(i) *Intranasal Infections. Bordetella pertussis* instilled by the intranasal route into the lungs of mice multiplies without difficulty even from small inocula, although it is not a natural pathogen for mice. There is a critical number of viable organisms in the lung, usually between 10^8 and 10^9, which is lethal for the mouse. When the viable count fails to reach this number the mouse recovers, though the lung is not freed from the infecting organism for several weeks or even months.

In lethal infections, deaths occur from the fifth day onwards. In non-lethal infections, on the other hand, the bacterial numbers begin to decline at 8—10 days, about the time when an active antibody response to the infecting bacteria is to be expected. Death occurs in the mouse therefore when the parasite grows fast enough to reach critical numbers before the host begins to build up a specific antibody-immunity. Protective IN antiserum given systemically in intranasal infection immediately reduces the number of bacteria in the lung from a lethal to a non-lethal level. The reduction is complete in a few hours, after which the mice harbour non-lethal amounts of bacteria in the lung for some weeks. IC antiserum has no effect in lethal intranasal infection.

(ii) *Intracerebral Infections.* In some respects, the course of intracerebral infection differs substantially from that of intranasal infection. There is an immediate decrease in numbers of viable bacilli, so that after 5 minutes not more than 10% of the initial dose can be recovered from the brain. From this low figure the count rises steadily for 5—6 days, to the critical figure of about 10^8, when the mouse dies. Sub-lethal brain infections with virulent *B. pertussis* cannot be established experimentally, because whenever the survivors of the inoculum succeed in mutliplying, the mouse inevitably dies. The effect of protective IC antiserum is quite different from that of IN antiserum in intranasal infections. The viable count rises as in untreated mice for four days, and then starts to decline, the brain eventually becoming

sterile. The four days' delay in the antibody effect appears to be due to the inaccessibility of the brain lesions to the antibody, because at about the fourth day the blood-brain barrier, hitherto impermeable, becomes leaky to indicator substances like pontamine blue or human γ-globulin introduced into the circulation, suggesting that only at this time can circulating antibody reach infected tissues in effectively bactericidal quantities. This suggestion is consistent with the fact that IC antiserum is equally effective, whether given one day before or three days after infections. IN antiserum, on the other hand, must be given at the time of intranasal inoculation if it is to modify the subsequent lung infection.

Properties of the Two Protective Antibodies. As regards the mechanism of action of these two antibodies, IN antibody but not IC antibody is bactericidal *in vitro* in the presence of complement. The action of IC antibody is under investigation.

CLOSTRIDIUM WELCHII

Dr. Craig completed his study of the *iota* toxin of *Cl. welchii*. Its lethal activity in mice parallels its capacity to increase vascular permeability or to induce necrotic lesions in the skin of the guinea-pig. The rapid detection and assay by permeability tests facilitated the determination of the toxins production to culture, activation from prototoxin, and neutralization by *Cl. welchii* antisera.

INHERITANCE IN BACTERIA

Lysogenic Conversion in Salmonella. Dr. Stocker, and Dr. A. M. Staub and her colleagues at the Institut Pasteur, Paris, continued the investigation of the chemical basis of the salmonella O factor *1*; they examined polysaccharide from *S. typhimurium*, in which the presence of factor *1* results from phage infection (see Report 1958, 1959), and from a salmonella with O antigen made up of factors *1*, *3* and *19*, in which factor *1* is not so determined. Oligosaccharides obtained by partial hydrolysis were tested for their inhibitory effect on the precipitation of an anti-factor-*1* serum by a factor-*1* polysaccharide. The results obtained suggest that in each of the two salmonella polysaccharides factor *1* specificity results from the presence of a side chain of which the terminal three sugars are:—Glucose-α-(1-6 or 1-3)-Galactose-Mannose-, the fourth sugar in from the free end of the side chain differing in the two polysaccharides. This chemical difference is reflected by a serological difference, because the antiserum to the phage-determined antigen of *S. typhimurium*, "factor *1*", though it reacts with factor *1* of salmonellae that have factors *1*, *3*, *19*, also contains an antibody which does *not* combine with these salmonellae. The acid-resistant oligosaccharide detected by Stocker in his preliminary examination of the factor-1-containing polysaccharides (Report 1959) has proved to be an α-glucoside of galactose.

Genetics of Flagellar Characters in Salmonella typhimurium. Mr. Joys has continued his genetical and serological investigation of mutants of *S. typhimurium* differing from the parent strain in respect of their phase *1* flagellar antigen *i*. Two of the original eight mutants, obtained by independent mutations, were serologically indistinguishable, which indicates that they probably arose by a recurrence of the same mutation. Full serological study of the original and mutant forms of antigen *i* gave complex results whose meaning is not clear. Interpreted in the classical way they indicate that the serum of a rabbit immunized with any form of antigen *i* contains many different

kinds of antibodies, each specific for a different "subfactor" of *i*. Mr. Joys also attempted to obtain recombination between various pairs of mutants, using phage-mediated transduction as the method of hybridization. For technical reasons he found it necessary to use non-flagellated variants as gene recipients in these experiments: variants of this sort resulting from mutation in a gene locus linked to the antigen-determining locus were obtained by selection with a phage which attacks only motile bacteria (see Report 1959). When such a non-motile variant of a serological mutant is treated with phage grown on some other serological mutant, motile forms are produced by transduction of the gene for production of flagella. They may show either the mutant form of antigen *i* characteristic of the recipient strain; or the form of the donor strain, if the antigen-determining gene has been transduced along with the gene for flagella production. By the use of appropriate sera Joys detected, in some pairs, a third class of motile bacteria, with a flagellar antigen different from either parental form, and shown to be indistinguishable from the original, unmutated, antigen *i*. These are interpreted as the result of recombination between the two mutated forms of the antigen gene, leading to reconstitution of the ancestral form of the gene.

It has recently been discovered at Cambridge that the flagellar proteins of some Salmonella strains contain N-methyl-lysine, an amino-acid not previously known to occur naturally. Dr. Stocker is collaborating with Mr. R. Ambler, at Cambridge, in an investigation of the genetic aspects of this phenomenon. The majority of Salmonella strains so far tested yield flagellar protein containing N-methyl-lysine. Of three strains of *S. derby* tested, one yielded flagellar protein lacking N-methyl-lysine. Several flagellated recombinants were obtained by the simultaneous transduction of the linked genes for flagellation and for flagellar antigen from strains producing N-methyl-lysine to non-flagellated strains which (when flagellated) do not produce N-methyl-lysine; all were found to produce flagella containing N-methyl-lysine. This is interpreted as evidence that the methylation, or lack of it, of a proportion of the lysine of the flagellar protein is regulated by a gene which is closely linked to that which determines the amino-acid composition and sequence (and hence the antigenic character) of the flagellar protein.

Genetic Transduction in Staphylococcus pyogenes. Miss Edgar and Dr. Stocker investigated the physiology and genetics of several strains of *Staphylococcus pyogenes* unable to grow on a complex defined medium unless provided with the amino-acid threonine. Two strains, previously shown to result from mutation at non-identical but closely linked sites (Report 1959), grew in the absence of threonine if provided instead with homoserine, a known precursor of threonine and of methionine; these strains did not grow, even in the presence of threonine, when methionine was omitted from the basal medium. By contrast three other strains requiring threonine did not respond to homoserine; and grew in the absence of methionine if provided with threonine. When cells of the latter type were treated with phage 53 grown on a homoserine-responding strain and plated on the basal medium supplemented with homoserine, about one bacterium in a million formed a colony as a result of transduction; about half these colonies consisted of bacteria which required either homoserine or threonine + methionine for growth; i.e., they resembled the strain on which the phage had been grown. These results indicate the existence in *S. pyogenes* of a pair of genes regulating the successive biosynthetic steps needed for the making of homoserine, and for its conversion to threonine; and that these genes are closely linked in the bacterial "chromosome".

Transfer of Colicinogeny and Cell Pairing in Salmonella. Miss Smith and Dr. Stocker have continued their investigation (Report 1959) of a system in which the ability to produce an antibiotic substance, colicine I, is rapidly transmitted from one strain of *Salmonella typhimurium* to another. They find that in cultures which have newly acquired the colicinogenic property about half the bacteria are able to transmit this character to non-colicinogenic ones. This highly "infectious" state persists for at least four generations after the acquisition of colicinogeny; but after eleven generations the proportion of "infective" bacteria has fallen from one in two to about one in ten thousand. The transfer of colicinogeny evidently occurs as a result of a kind of conjugation: pairing occurs very rapidly, so that 3 mins. after mixing a non-colicinogenic culture with a culture newly made colicinogenic, about 10% of the cells of the former strain have paired with cells of the latter strain. The transmission of the colicinogenic character is, however, almost entirely prevented when the cell-pairs are broken up, 3 mins. after mixing, by violent stirring. Experiments in which blendor treatment was given at various times indicate that transmission of the colicinogenic character is generally not completed until about 20 mins. after pairing.

Cells of strains producing only colicines E1, E2, or both do not transmit their colicinogenic properties to other strains (see Report 1959). Dr. Ozeki and Miss Smith found that when such cells have just acquired the ability to produce colicine I they readily transmit not only their new property, but also their original colicinogenic properties. In the case of a strain producing colicine E1 and E2, most pairings result in transmission of all three characters. However, when the culture was treated in the blendor 1 or 2 mins. after mixing, the small proportion of cells which acquired colicinogeny included cells with all possible combinations of the three colicinogenic characters. These results are thought to indicate (i) that cells which have newly acquired the transmissible agent conferring ability to synthesize colicine I have an altered cell surface, which results in their sticking to other cells with which they collide, and (ii) that such pairing permits the transmission to the other cell of one or more particles of any of the colicine agents which may be present in the cell, probably in the cytoplasm.

Genetic Recombination in Salmonella typhimurium *occurring during Transmission of Colicinogeny.* Dr. Ozeki and Dr. Howarth looked for the occurrence of recombination of chromosomal genes in systems in which colicinogeny is being transmitted as described above. Their results indicate that, in about one pairing in a million, genes believed to be located in the bacterial chromosome (e.g. genes regulating various biosynthetic steps and a gene for resistance to streptomycin) undergo recombination. Recombinants can be detected in small numbers whenever colicinogeny -I is being transmitted at high frequency, and in larger numbers when colicinogeny-E1 is also being transmitted. Many pairs of genes cannot be simultaneously transduced by a single phage particle, probably because they are not close together in the bacterial chromosome, only a very small fraction of which can be enclosed within a single phage particle. A number of crosses involving such pairs of apparently unlinked genes were made by the newly discovered method of hybridization, and in every instance a fraction of the recombinants were found to have received both of the pairs of genes concerned from the strain which was transmitting colicinogeny. This suggests that in this new kind of hybridization either the whole or at least a large fraction of the chromosome of the transmitting strain is enabled to recombine with the chromosome of the acceptor strain, in contrast to the situation in phage-mediated transduction.

It therefore seems likely that the new method of hybridization may prove of great value in determining the arrangement of the genes along the whole length of the Salmonella chromosome, a problem which could not be investigated with the previously available method of hybridization by phage-mediated transduction.

Bacterial Mutation induced by Treatment with an Ethylating Agent. Dr. Howarth collaborated with Dr. A. Loveless, of the Chester Beatty Research Institute, in an investigation of the production of mutations in bacteria exposed to the ethylating agent, ethyl methyl sulphonate. Treatment of a cystine-exacting strain of *Salmonella typhimurium* resulted in the production of large numbers of mutants able to grow without cystine; and after treatment of a non-exacting strain five different nutritionally-deficient mutants were detected amongst 1,090 colonies tested. Unlike most other agents which induce mutations, this substance was effective even in concentrations which killed only half or less of the treated bacteria.

Genetics of Fimbriation. Dr. J. P. Duguid investigated some genetic aspects of the character *fimbriation; i.e.*, the production by bacteria of numerous filamentous appendages (fimbriae) smaller than flagella and of unknown function. Nearly all of a large number of strains of *Salmonella typhimurium* were shown to possess the ability to manufacture fimbriae; fimbriate cells were generally in a small minority, so that fimbriation could only be detected after several successive sub-cultures in unaerated broth, a medium in which fimbriated bacteria outgrow non-fimbriated ones. Duguid attempted to transfer fimbriation to one of the uncommon permanently non-fimbriate strains of *S. typhimurium;* treatment with phage grown on a fimbriate strain in several experiments resulted in the appearance of fimbriation in the phage-treated culture, which probably means that the permanent absence of fimbriation in the strain concerned resulted from mutation of a gene capable of being transduced from one strain to another by phage. Fimbriation was not acquired by a non-fimbriate strain when it acquired colicinogeny, by "conjugation" (see p. 14).

BACTERIAL PHYSIOLOGY

Chemistry of Flagellar Protein. Dr. McDonough began an investigation of the protein *flagellin*, constituting the flagella of salmonella bacteria. Flagella detached from bacteria by blendor treatment were concentrated and purified by high-speed centrifugation; treatment of the purified flagella with acid or concentrated urea results in their lysis, to give a solution of flagellin. On electrophoresis the urea-lysed material gives two major bands; subsequent acid treatment reduces these to a single major band, perhaps through depolymerization of a dimer or polymer. Flagellins from flagella of antigenic types *i, i M5* (a mutant form of *i*, see above) and *1,2,3* were compared by electrophoresis. At pH 8.4, *1,2,3* flagellin moved most rapidly towards the anode, folowed by *i* M5, and then by *i*. The difference in electrophoretic mobilities of *i* and *i* M5 flagellins was also observed in flagellins obtained by urea treatment of flagella. Dr. McDonough also began an examination of tryptic digests of flagellins, by combined paper chromatography and electrophoresis ("fingerprint" technique). Differences between digests of *i* flagellin and the serologically unrelated *1,2,3* flagellin were observed; but digests of *i* and its mutant form *i* M6 could not be distinguished.

ABACTERIAL URETHRITIS IN MAN AND RELATED DISEASES

Dr. Klieneberger-Nobel and Dr. Lemcke tested the association of pleuropneumonia-like organisms (PPLO) and of antibodies against them in patients with the following conditions: salpingitis, Bartholin's abscesses, pelvic abscesses, non-specific vaginitis, an abdominal wound infection and post-partum pyrexia (women); Reiter's disease, epididimytis and non-gonococcal urethritis (men). From some of the patients, material for examination was obtained several times during the course of the disease. Rising titres were observed in three cases which were also culturally positive. This supports the view that the human genital PPLO were responsible for the pathological conditions concerned. The sera of 6 out of 12 patients with salpingitis contained PPLO antibodies, suggesting that the infection was due to PPLO in at least some of these cases.

Although previous work failed to reveal any human PPLO other than the buccal and the genital types, other workers have isolated yet other, possibly saprophytic, varieties. A search for new human strains from non-pathological material was started; so far no new types have been found.

The survival and multiplication of human genital PPLO in tissue cultures was demonstrated by their recovery, after several passages, from chick embryo cell cultures set up by Dr. G. Furness (Wright Fleming Institute) to detect virus inclusion bodies. The tissue cultures were originally seeded with material from patients with abacterial urethritis known to be harbouring PPLO. The organisms isolated from the chick embryos were typical of human genital PPLO; and could not therefore have been derived from natural infection of the chick embryos with latent avian PPLO.

Serology of PPLO Infections in Rats and Mice. The cultural and serological study of rat PPLO arthritis (Report 1959) was extended to bronchiectasis in rats. Rats of various ages from the foetal stage up to one year and more were examined. Young rats naturally become infected with PPLO soon after birth, the organism being first carried in the nasopharynx and later infecting the lungs. This disease serves as a model for human genital PPLO infection in that it also is usually localized and induces only a feeble or moderate specific antibody response. The immunology of infection with another rat strain, distinct from the lung and arthritis strains, is still under investigation.

Five strains of PPLO were isolated from both lungs and brains of mice, and their relationship and pathogenicity studied.

Serological Typing of PPLO Strains. A battery of hyperimmune sera prepared against PPLO from various sources was used in complement fixation tests, to identify strains from rats, mice, man and various tissue cultures.

The serology of a human genital PPLO and a corynebacterium reported to have been derived from it was also studied; the two organisms proved to be unrelated.

VIROLOGY

TRACHOMA

Adaptation to Cell Cultures. During the year much work was devoted to the adaptation of trachoma virus to growth in cell cultures; for accurate quantitative studies this technique offers considerable advantages over propagation in the chick embryo yolk sac. The Chinese strain of trachoma virus was propagated in a variety of cell cultures by Mrs. Stocker, Miss Graham, Mr. Reeve and Mr. Frohlich; Miss Fraser was responsible for maintaining the cell lines. The virus induces the formation of large inclusion bodies in human

carcinoma cells (strain HeLa), embryo rabbit kidney (strain ERK), and monkey kidney (strain MK2). Dr. Furness, Miss Graham and Mr. Reeve are now exploiting these findings for the development of more accurate methods of assaying the virus, and for detailed observations of its mode of replication. Because of its comparative freedom from extraneous material, virus propagated in cell culture may prove useful for serological studies.

Adaptation to Mice. In collaboration with Dr. Weston Hurst of Imperial Chemical Industries, Mr. Reeve has investigated the properties of trachoma virus adapted to the mouse brain. Only the Chinese strain of trachoma can be shown to multiply in mice; when inoculated intracerebrally, adapted virus induces a syndrome similar to that caused by lymphogranuloma virus. Adult mice are more susceptible than sucklings. Several attempts to adapt two African strains of trachoma were unsuccessful.

After repeated brain passages, the virus reaches an infective titre similar to that attained in the chick embryo yolk sac, and can be isolated from mouse brain by inoculation of chick embryos or cell cultures. Complement fixation tests reveal the characteristic psittacosis-lymphogranuloma-trachoma group antigen in infected mouse brains.

Adaptation to chick embryo allontoic sac. Miss Graham attempted to adapt several strains of virus to grow in the allantoic and amniotic sacs of chick embryos. The Chinese strain of trachoma was recovered after 5 consecutive allantoic passages, but no growth was detected in the amnion.

Dermal Reactions in Laboratory Animals. Mr. Reeve and Miss Graham showed that intracutaneous inoculation of partially purified trachoma and inclusion blennorrhoea virus induces skin lesions in rabbits and guinea-pigs. They are investigating the possibility of using this technique to demonstrate antigenic differences between various strains.

Serological Studies. Mr. Reeve and Miss Graham are studying the serological responses of rabbits and guinea-pigs to trachoma and inclusion blennorrhoea viruses, with particular reference to the formation of neutralizing antibodies.

Trachoma Vaccine. Dr. Collier is conducting immunization experiments in monkeys and baboons with the object of developing a vaccine effective against trachoma.

Influence of Therapy on Experimental Trachoma. In collaboration with Sir Stewart Duke-Elder and Mr. Barrie Jones of the Institute of Ophthalmology, Dr. Collier continued his observations of experimental trachoma in a human volunteer (Report 1959). The influence of large parenteral doses of penicillin on the clinical course and on the morphology of the conjunctival inclusions was observed. Although penicillin induced changes in the appearance of the inclusions, it did not eradicate the infection which, however, was rapidly cured by oral administration of sulphadiazine.

Investigation of Trachoma in West Africa. Under Dr. Collier's direction, a Medical Research Council team is continuing its investigation of trachoma in a Gambian village community. They have made repeated clinical, virological and bacteriological examinations of the eyes of the entire population (about 400). The results are still incomplete, but show that there is a very high incidence of trachoma, particularly in young children; 91% of children aged

5-9 years are affected. By inoculation of chick embryos, trachoma virus was isolated from a high proportion of subjects in the earlier stages of the disease; thus of 36 patients with Trachoma Stage II, 25 (70%) yielded virus. This figure will undoubtedly be higher when the results of later examinations are available. Material from those patients who consistently failed to yield virus by chick embryo inoculation is now being inoculated into mice and cell cultures in an attempt to detect other agents that might give rise to the trachoma syndrome.

A number of bacterial species are often isolated from the eyes both of healthy and trachomatous subjects; streptococci, micrococci and neisseriae are fairly evenly distributed among the different age groups, but organisms of the haemophilus group are found more frequently in younger subjects. It is not yet clear whether this increased prevalence is related to the greater incidence of trachoma in young children, or whether organisms of the haemophilus group play any part in the pathogenesis of trachoma. It is hoped to gain further information on these points.

Mr. Standfast and Dr. Jean Dolby examined over 100 strains of haemophilus isolated in this survey and found that they fall into three main categories: *Haemophilus aegyptius, H. influenzae* and *H. parainfluenzae*. Other strains resemble one or other of these species more or less closely; a few strains cannot be classified.

INCLUSION BLENNORRHOEA

Isolation and Identification of Viruses. With the clinical collaboration of Mr. Barrie Jones, more viruses were isolated from patients with inclusion blennorrhoea. One agent (LB1) isolated from the *cervix uteri* (Report 1959) has undergone numerous chick embryo passages, and will shortly be inoculated into the eye of a human volunteer to find whether it induces the syndrome characteristic of adult inclusion conjunctivitis.

Adaptation to hosts other than chick embryos. In collaboration with Dr. Weston Hurst, the LB1 strain has been adapted to mouse brain. Mr. Reeve and Miss Graham have also propagated this virus in HeLa cells, in which it forms inclusion bodies similar to those of trachoma.

MEASLES

Dr. Payne began a study of the properties of measles virus grown in cell culture, with particular reference to the development of a vaccine. In the first instance, he is examining methods of purification, including treatment by fluorocarbon and ultracentrifugation. Dr. Payne also hopes to investigate purified virus preparations by electron microscopy.

VACCINIA VIRUS

Virus inactivation. Dr. Kaplan continued his studies of heat inactivation and of the influence of metal ions on the stability of virus grown in chick embryos. The work is being extended to vaccinia virus from mammalian hosts.

A significant proportion of the infectivity of vaccinia virus abolished by exposure to mercuric chloride may be restored by treatment of the virus with cysteine or other -SH donors. Dr. Kaplan concluded that the relative ease with which infectivity was restored pointed to the -SH groups attacked by the mercury being superficially placed in the virus and to the possibility of their being involved in the mechanism of attachment of virus to host-cell.

Tissue culture and virus infection. Dr. Kaplan is attempting to isolate and propagate in series a strain of chick embryo muscle cells suitable for experimental work on interactions of virus and host-cell; and perhaps for titrations of virus and antivaccinial antibody.

Tissue culture vaccine. Smallpox vaccine produced in tissue culture is at 0°—5° less stable than conventional vaccine (Report 1959). In a direct application of his experimental work on the influence of metal ions on the heat inactivation of vaccinia virus, Dr. Kaplan was able satisfactorily to stabilize the potency of vaccine in bulk storage by increasing to 0.1 M the concentration of Na^+ in the suspending fluid. Further trials of tissue culture vaccine are being made in two civilian vaccination clinics, in collaboration with Dr. W. C. Cockburn of the Public Health Laboratory Service and the two medical officers concerned.

International Reference Smallpox Vaccine. Dr. McClean and Dr. Kaplan, in association with Group Captain R. M. Cross, R.A.F., took part in the international collaborative study organized by the International Laboratory for Biological Standards in the State Serum Institute, Copenhagen, of the smallpox vaccine, proposed for establishment of the International Reference Preparation of Smallpox Vaccine. The preparation of vaccine tested for this purpose was presented to the World Health Organisation by the Institute. Its properties were explored in terms of rabbit scarification, pock counting in chick embryo membranes and re-vaccination of young adult volunteers, the last being undertaken by Group Captain R. M. Cross.

BACTERIOPHAGES

A Bacteriophage which Attacks only Motile Bacteria. Dr. Stocker continued to collaborate with Dr. E. Meynell, of the London School of Hygiene, on a phage which attacks only motile salmonellae (see Report 1959). Electron microscopy of sensitive bacteria which have been exposed to phage shows numerous phage particles attached to the bacterial flagella; non-flagellated bacteria, flagellated but non-motile bacteria and motile bacteria with flagella of antigenic type *g*, . . . , all of which are resistant to the phage (see Report 1959), seem to owe their immunity to lack of absorption of the phage, for no phage particles are visible in electron micrographs of washed cells which have been exposed to phage.

IMMUNOLOGY AND SEROLOGY

SEROLOGICAL IDENTIFICATION OF INSECT BLOOD MEALS

Tsetse Flies. The continued study of the feeding habits of tsetse flies by Mr. Weitz and Miss Lee-Jones has confirmed and extended earlier findings. In collaboration with the Veterinary Department of Kenya under Dr. Glover, investigations were made to establish the food preferences of *Glossina swynnertoni* and *G. longipennis*. The availability of game to the flies calculated on the basis of spoor records was compared with the blood meals identified in flies caught in a given area. Both species of tsetse fly have strong preferences in their selection of host. *G. swynnertoni* fed repeatedly on warthog in spite of the presence of large numbers of other animals in the area, and *G. longipennis* preferred rhinoceros to other available hosts.

In Uganda, in an area under reclamation where the game has been largely eliminated, it was not possible to establish that the cattle introduced into the reclaimed area contributed to the survival of *G. morsitans*, though the identification of blood meals clearly showed that the fly was using the cattle as a source of food. The problem is under investigation.

Mosquitoes. The survey of the feeding habits of Anopheline mosquitoes is proceeding. Some 25,000 were tested during the year, making a total of over 70,000 tested on a world-wide basis in collaboration with the World Health Organization. More than 75% of the blood meals of the following species were from man:—*A. minimus, A. gambiae, A. leucosphyrus, A. moucheti, A. nili, A. sundiacus* and *A. wellcomei.* The behaviour of anophelines after feeding varies greatly. Some remain in the house after feeding, i.e. "endophilous" species. These include *A. barbirostris, A. fluviatilis, A. funestus, A. gambiae, A. nili, A. pharaensis* and *A. sundiacus;* over 70% of these mosquitoes collected from human habitations had fed on man. Others go outdoors after feeding on man or into animal shelters; when mosquitoes of these species are caught on outside shelters, over 70% are found to have fed on man. Such "exophilous" species include *A. flavicosta, A. leucosphyrus, A. moucheti, A. nili* and *A. wellcomei.* It is clearly essential to identify the blood meals of mosquitoes caught in all types of habitat in order to establish the anthropophilic tendencies of any species.

ANTITOXIN PRODUCTION

Proteolytic Enzymes for Refining Antisera. The results of various physical treatments of pepsin, such as its inactivation by heat, give no indication of its being heterogenous. Dr. Dolby confirmed the work of others that pepsin nevertheless may be split into fractions with different properties by chromotography on ion-exchange resins, and began a study on the part the fractions play in the refinement of antitoxic sera.

Refinement of Therapeutic Antitoxins. Antitoxins refined by the treatment of hyperimmune horse plasma with enzymes still contain a high proportion of inactive protein and peptide. Dr. Dolby previously showed that much of the peptide was removed by treatment with fullers' earth, and now finds that the addition of a cationic detergent in high dilution to the plasma during its digestion by pepsin leads to the removal of a considerable amount of the inactive protein without appreciably affecting the yield of antitoxin. The application of this process on a larger scale is under study.

Miss Lanham continued her investigation of the peptide breakdown products in refined antitoxins and, in collaboration with Mr. Rodican, tested the fractions obtained for vascular permeability effect. Paper electrophoresis revealed a complex mixture of peptides, in a pattern repeatable from batch to batch of antitoxin. Some of the peptides have a fairly high molecular weight and are precipitable by saturated sodium sulphate, 80% acetone or 80% ethanol; they are not permeability factors.

The smaller peptides are nearly all precipitable by 95% acetone or by phosphotungstic acid, and some of these are permeability factors.

Permeability Factors in Refined Antitoxic Sera. Mr. Rodican has devised a new apparatus and method for the electrophoretic separation of heterogeneous biological material into individual components, by which the separated

components may be removed directly from the apparatus in solutions. A supporting medium, and the need for subsequent elution of the components is thereby obviated. The apparatus can be used for large or small scale work.

In continuing the work on the isolation and purification of vascular permeability factors in refined antitoxin, horse serum was fractionated by this technique, and the isolation of the peptides from refined therapeutic antitoxins was greatly facilitated. Several active and several non-active components were isolated; all appeared to be polypeptides.

EXPERIMENTAL PATHOLOGY

MECHANISMS OF INFLAMMATION

Vascular Permeability Changes in Injury. The biphasic permeability response induced by mild thermal injury in guinea-pig skin (see 1959 Report) was further investigated by Dr. Wilhelm and Miss Mason in guinea-pigs, rats and rabbits.

In all three species the general pattern of permeability response is biphasic In guinea-pigs, heating at 54° induces immediate and delayed responses. The immediate response is maximal in 5 minutes, and subsides in 10 minutes. It is followed by a prolonged delayed response that appears in ½-1 hour, is maximal in 3-4 hours, and declines in 6-8 hours. The delayed response occurs progressively more quickly as the temperature of heating is increased to 58°.

In rats, heating at 54° induces immediate and delayed responses like those in guinea-pigs, except that the immediate is very small or absent. In rabbits both responses to heating at 54° are feeble. The delayed response to heating at a higher temperature (56°), as in the guinea-pig, is more rapid in onset and increased in magnitude.

In all three species the *immediate* response is mediated by histamine—entirely so in guinea-pigs and rabbits, and largely so in rats.

The *delayed* response does not appear to be induced by histamine. Furthermore, it is unaffected by the local administration of various trypsin inhibitors, which are strong antagonists of the globulin permeability factor *in vitro*. However, these negative results do not satisfactorily exclude the globulin factor as the mediator of the delayed response because of the pharmacological limitations of the inhibitors *in vivo*.

In guinea-pigs and rats the response is insusceptible to local treatment with ovomucoid trypsin inhibitor, or with the metal-chelating agent, disodium ethylene diamine tetra-acetate; and to systemic sodium α-naphthyl acetate.

Effect of Antihistamine Drugs on Permeability Factors. Miss Mason and Dr. Wilhelm completed their survey of antihistamine drugs to select those most suitable for further studies of permeability responses in injury (see 1959 Report). Triprolidine and mepyramine in small doses intravenously are the most reliable drugs for suppressing permeability responses in guinea-pigs, rats and rabbits; and are considerably more effective intravenously than intracutaneously.

In all three species, treated either systemically or locally, the antihistamines most effective against histamine only moderately suppress the permeability response to established histamine-liberators; and do not affect that to non-liberators like globulin permeability factor.

Distinction Between Globulin Permeability Factor and Kallikrein. In collaboration with Dr. Marion E. Webster, National Institutes of Health, Bethesda,

U.S.A., Dr. Wilhelm found that the permeability responses to human globulin factor and kallikrein have a similar susceptibility in guinea-pigs to the trypsin inhibitors in soya bean and potato, as well as to the plasma inhibitor of the globulin factor; and that each are insusceptible to the trypsin inhibitors in lima bean and ovomucoid, and to an anti-histamine drug. However, preliminary comparisons of the "kallikrein" and permeability-increasing potency of the two kinds of preparation suggest that the active principles are distinct.

Identity of Hypotensive Factor and Permeability Factor in Serum. Miss Mason has extended the study begun by Miss Sparrow (1958 Report). By developing a more sensitive assay of the hypotensive potency of test substances, she established that various preparations of globulin permeability factor in guinea-pig serum have corresponding hypotensive and permeability-increasing potencies when tested in guinea-pigs and rats. Furthermore, in guinea-pigs, the hypotensive and permeability-increasing responses were similarly susceptible to soya bean trypsin inhibitor. The investigation has also to be made in rabbits before a definite conclusion is justified; but, to date, the results suggest that preparations of guinea-pig globulin factor owe their hypotensive activity to the substance which increases vascular permeability.

The Activation of Permeability Factor in Serum. Prof. Miles continued to explore the relation of permeability factor to other serum factors. The parallel between pain-producing substance, smooth muscle contractor substance and the permeability factor, based on the similarity of activation rates, appears on several grounds to be superficial. Glass surfaces activate only a small fraction of the permeability factor in native serum and the activated factor rapidly combines with its inhibitors; but continuing exposure to glass inhibits this inactivation, and eventually destroys the complex of factor and inhibitor, setting free active permeability factor once more. Like the contact factor of coagulation in human serum, the permeability factor is selectively absorbed from guinea-pig serum by silica and highly active material is eluted from the silica by 8.5% saline.

Dr. Mills surveyed the permeability factors present in foci of various bacterial infections of the skin of rabbits and guinea-pigs, sampled by intercellular perfusion of excised skin. He confirmed that in the guinea-pig, at the height of the delayed permeability response to infection at 4 hours, the extravascular globulin permeability factor and profactor is increased, and found no small-molecular permeability factors. In the rabbit, in which the delayed permeability phase proved to be maximum within 2 hours, the skin lesions yielded a mixture of factors, some resembling the globulin factor and some having the characters of histamine-releasers and of polypeptides.

MECHANISMS OF INFECTION AND DEFENCE

The mechanisms of Non-specific immunity. Dr. Blaker, in collaboration with Prof. Miles, studied local infection of guinea-pig skin with *Leishmania henrietti*. The infectivity of this protozoon is strikingly enhanced in tissue treated with adrenaline and, as with bacterial pathogens, the local antimicrobial responses of the tissues that are suppressed by the adrenaline, are effective only in the first few hours of infection. These defences, unlike those against bacteria, are quite insusceptible to liquoid, a substance destroying the microbicidal power of the blood.

Miss Brimacombe, in collaboration with Prof. Miles, studied the potentiation of local adrenaline toxicity by systemically administered bacterial endo-

toxins, extending the American observations to the reaction of the guinea-pig. Potentiation occurs in this animal, though it is not as pronounced as in the rabbit. The endotoxins of Gram-negative bacteria are not exclusive in this respect. Relatively pure diphtheria and tetanus toxoid are also active in small doses.

The relation of adrenaline potentiation by free "gram-negative" endotoxin to the terminal vascular events in infection by endotoxin-producing bacteria is obscure. The anti-adrenaline drug phenoxybenzamine decreases infectivity of gram-positive and gram-negative pathogens alike; and whereas it antagonizes the enhancement of such infections by zymosan (a preparation of yeast cell walls) it has little effect on enhancement by gram-negative endotoxin.

Miss Brimacombe began a study of the bactericidal power of cultures of tissue cells other than macrophages and microphages; in the hope of elucidating the rapid kill of bacteria during the first hour or so of infection, when neither bactericidal fluids nor phagocytes are present in detectable amounts in the infected tissue.

In an attempt to elucidate the role of auto-antigenation in diseases of joints, Dr. Mills studied the induction in guinea-pigs of allergy to guinea-pig synovial tissue.

Use of Bacteriophages for Determining Bacterial Multiplication in Infected Animals. Dr. Meynell has investigated the A phages of *Salmonella typhimurium*, and has found a phage-bacterium system suitable for investigations of the rate of multiplication of bacteria in inoculated mice, by a method he has used with *Escherichia coli*. He intends to use this system in an investigation to determine why mice survive inoculation with large numbers of *Salmonella paratyphi* B, whereas they succumb to very small numbers of the very similar species *Salmonella typhimurium*.

BIOCHEMISTRY

THE HUMAN BLOOD GROUP SUBSTANCES

Molecular nature of the water-soluble blood group mucopolysaccharides. Prof. Morgan and Dr. Watkins continued their investigation on the presence of more than one blood group-specific structure on a single macromolecule (Report 1957), and considered the genetical implications of finding that the associated specificities are determined by allelic genes, such as *A* and *B*, or by genes at independent loci, such as *A* and Le^a. Possible pathways for the later stages in the synthesis of the blood group specific mucopolysaccharides were proposed, in which the molecules are built up by a series of synthetic steps, each of which is controlled by a different gene and which can take place only in a given sequence. Thus, the first structure with blood group specificity to be built on to a precursor mucopolysaccharide is that associated with Le^a character. Subsequent steps convert this mucopolysaccharide to an H active substance and, finally, if the appropriate genes are present, to A and B substances.

It is essential for this scheme to establish that the multiple specificities arise from the interaction of the blood group genes on a common precursor substance, and not from the secondary polymerization or aggregation of molecules formed separately under the influence of their respective genes. Attempts were accordingly made to dissociate the A and B specificities in the secretions

of AB individuals. Exposure of the substances to ultrasonic vibrations failed to bring about demonstrable dissociation of the A and B activities, but it was observed that relatively short treatment decreased the power to inhibit A and B iso-agglutination. This unexpected finding suggested an investigation of the action of ultrasonic vibrations on the serological specificity and physical and chemical properties of the blood group substances. The specificity of H substance, measured by inhibition of agglutination, was slightly more resistant, and that of Le^a substance considerably more resistant to ultrasonic vibration than were A and B specificities. The capacity of A substance to precipitate antibody from a rabbit anti-A serum, however, decreased to about 85% of its original value, whereas the capacity to inhibit iso-agglutination almost disappeared. Viscosity measurements, the levels at which the substance could be precipitated from aqueous solution with ethanol and its behaviour in the ultracentrifuge (kindly examined by Dr. Kekwick), indicated that the molecules had been reduced in size by the ultrasonic treatment, although less than 1% of the material was small enough to diffuse through a cellophan membrane. Loss of specificity during exposure to ultrasonic vibration appears, therefore, to arise from changes in the secondary structure of the macromolecules and not from the liberation or destruction of the carbohydrate structures which play a dominant role in specificity.

The capacity of a number of AB substances to precipitate antibody nitrogen from a rabbit anti-A serum was examined by Mrs. Allen who found that the AB substances precipitate almost as much antibody nitrogen as A substance alone. The curves obtained can be readily distinguished from those given by artificial mixtures of A and B substances. Mrs. Allen is also investigating a method, earlier proposed by Kabat, which determines the purity of blood group mucopolysaccharides by estimating the amount of fucose in the group substance carried down in the antigen-antibody precipitate.

Microbial enzymes and Blood Group Substances. Dr. Watkins and Mr. Tyler are continuing their studies on the enzymes in extracts of *Trichomonas foetus* which destroy the serological activity of A, B, H and Le^a substances. Mr. Tyler examined additional methods for the separation of these enzymes. Chromatography of the crude extract on a diethylaminoethyl cellulose column, using a gradient elution technique, facilitated separation of the enzymes which destroy A, B and H specificity. The A and B enzymes, however, are of low potency and conditions for reproducible results are not yet known. The powerful β-galactosidase and β-glucosaminidase in the crude *T. foetus* extract are eluted from the resin column along with the enzyme destroying H specificity. A comparison is being made of the action on H substance of this preparation and of an H enzyme preparation almost free from β-galactosidase and β-glucosaminidase activity to determine whether these two glycosidases play any part in the breakdown of H substances.

The separation of *T. foetus* enzymes by zone electrophoresis on starch columns (Report 1956) was re-examined by Miss Watkins, and the weakly active enzyme destroying Le^a specificity—not previously detected after electrophoresis—was shown to migrate in the same position as the enzyme destroying B specificity. H enzyme can be obtained free from Le^a enzyme activity and, by treatment of H substances with these H enzyme preparations, it was shown that loss of H serological activity is accompanied by a considerable increase in Le^a activity. This finding agrees well with the suggestion that Le^a substance is the substrate for the conversion step controlled by the H gene.

A sialomucopolysaccharide. Prof. Morgan and Mr. Pusztai isolated from human ovarian cyst fluid a new type of mucopolysaccharide, provisionally called sialomucopolysaccharide, which contains sialic acid as a major component as well as the four sugars, L-fucose, D-galactose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine and the eleven or twelve amino acids which are usually found in blood group specific mucopolysaccharides. The substance behaves electrophoretically and in the ultracentrifuge as a single molecular species which is somewhat polydisperse and has a molecular weight of about 2.3×10^5, calculated from sedimentation and viscosity data. The substance has powerful Le^a specificity and is, at the same time, a potent inhibitor of the haemagglutination of the indicator forms of type A (ASH) and type B (ROB) influenza virus, but not of an Asian strain. The sialic acid is quantitatively released by the action of Receptor-Destroying Enzyme (R.D.E.) obtained from *Vibrio cholerae* filtrates or by treatment with weak acid, and was identified as *N*-acetyl-neuraminic acid.

After removal of sialic acid the substance does not inhibit viral haemagglutination, whereas its Le^a activity remains unchanged. It seems that although sialic acid is a component of certain mucopolysaccharides it has no influence upon their blood group character; it determines another property, that of specifically inhibiting viral haemagglutination.

Structural Studies of Blood Group Substances. From blood group A mucopolysaccharide partially hydrolysed by mild acid, Mr. Cheese isolated several disaccharide and oligosaccharide fragments that inhibit iso-haemagglutination. The most active disaccharide, *O*-α-*N*-acetylgalactosaminoyl-(1→3)-galactose, is also the strongest inhibitor of the haemolysis of sheep cells by rabbit anti-A serum and complement. Trisaccharides, not yet completely identified structurally but nevertheless known to contain the *O*-α-*N*-acetylgalactosaminoyl end-group, are even stronger inhibitors of sheep cell haemolysins. These, and the oligosaccharides, are being separated for examination in detail.

The Analysis and Oxidation of Mucopolysaccharides. Dr. Doyle developed a quantitative micro-method for the identification and assay of the different sugar components of mucopolysaccharides, and applied it to the determination of the unoxidized sugar residues which remain after oxidation of the group substances with periodic acid, reduction with lithium borohydride and hydrolysis with mild acid. By the sequential use of these techniques, a step-wise oxidation of the group substances can be carried out, and some insight gained into the sugar sequence, linkage, etc., in the different group specific mucopolysaccharides.

The oxidation of the group substances with lead tetra-acetate was studied by Mr. Hughes who, in the light of his experience using the reagent on the α-limit dextrins of amylopectin (Report 1959), is determining by this additional approach the sugar sequence in the group specific mucopolysaccharides.

Mr. Pusztai and Professor Morgan continued the work (Report 1959) on the purification of the specific blood group mucopolysaccharides by treatment with saturated ammonium sulphate at 60°, and examined in greater detail the breakdown of these materials with ficin and papain.

CARBOHYDRATE STUDIES

Chemical Synthesis of Modified Starches. The aim of this work is to modify starch chemically at specified points in the molecule and then to use the resulting substances as substrates for the starch-metabolizing enzymes, in

order to define the structures determining susceptibility to these enzymes.

Dr. Allen studied the action of α-amylase on amylose (the linear component of starch) modified by acetyl or triphenylmethyl groups. Both modified polysaccharides are degraded to oligosaccharides and in general the substituent group affects the enzyme in the same way as does a point of branching in the branched component of starch, amylopectin. A synthetic acetate of maltotriose was also attacked by α-amylase. Dr. Allen also tested the action of α-amylase on the polysaccharide mannan from the tubers of the salep orchid, which is reported as being attacked by the enzyme. By using crude and crystalline specimens of four different α-amylases the degradation was traced to enzymic impurities in the amylase preparations.

Dr. Bines developed methods for introducing *p*-toluenesulphonyl groups at specified points in amylose. These polymers lead to the formation of amyloses containing units of 6-deoxyglucose, 3:6-anhydroglucose and altrose. Each type of polymer was obtained for test with starch-splitting enzymes.

Dr. Goldstein is investigating the use of the benzyl group as a means of selectively substituting the secondary hydroxyl groups of starch, so as to allow the preparation of derivatives selectively modified at the primary hydroxyl groups.

Dr. Gunja is carrying out the structural analysis of the synthetic polymer of glucose and altrose. This is produced by the action of alkali on amylose selectively substituted at the primary hydroxyl groups by triphenylmethyl and at the secondary hydroxyl groups by *p*-toluenesulphonyl. Selective substitution of one of the two secondary hydroxyls by the *p*-toluenesulphonyl group takes place, making possible the synthesis of new types of modified amylose.

Enzymic Synthesis of Modified Starches. An alternative way of preparing modified starches may be their enzymic synthesis by phosphorylase from modified glucose 1-phosphates. Mr. Egyud and Dr. Whelan are preparing the 6-phosphates of glucose analogues by chemical and enzymic methods, first to study the specificity of phosphoglucomutase, which interconverts glucose 6- and 1-phosphates, and then to use the 1-phosphates as substrates for phosphorylase.

Structures of Limit Dextrins. The limit dextrin remaining after the action of rabbit-muscle phosphorylase on glycogen and amylopectin was said by Cori and Larner to contain side chains only one glucose unit long. Dr. Whelan has shown that these side chains are four units long. The enzyme preparation described by Cori and Larner as detaching these side chains from the limit dextrin in fact depends for its action on three enzymes, each of which is present in the Cori-Larner preparation.

Mr. Hughes and Dr. Whelan analysed the structures of the α-limit dextrins resulting from the actions of three crystalline α-amylases on amylopectin. Those formed by pig-pancreatic and *Aspergillus oryzae* α-amylases are identical with those formed by salivary α-amylase. The dextrins formed by *Bacillus subtilis* α-amylase differ structurally; confirmation of this unusual behaviour of the enzyme is being sought by Mr. Smith.

Molecular Weights and Structural Analysis of Polysaccharides. In the last Report (1959) a semi-micro method was described for determining the molecular weights of polysaccharides containing up to 100 glucose units, based on the periodate oxidative release of formaldehyde from the polymer. Mr. Hughes and Dr. Whelan extended the range of the method to polymers containing up to 1,000 glucose units, and with a series of amyloses obtained good agreement with estimates derived from measurement of osmotic pressure. The

viscosities of these amyloses were also measured; contrary to statements in the literature, there is no simple relation by which viscosity can be used to determine the molecular size of amylose.

Dr. Goldstein is determining the structures of dextrans by fragmentation analysis and of the polymer from Iceland moss, isolichenin, by fragmentation of the periodate-oxidized polysaccharide.

α-1:6-Glucose Transferase in Potato. Mr. Abdullah and Dr. Whelan further characterized the action of a new enzyme (T-enzyme) in the potato. The enzyme transfers α-1:4-linked glucose units from oligosaccharides and rejoins them to other molecules by α-1:6-bonds. The splitting of the α-1:4-bond is irreversible but the formation of the α-1:6-bond is reversible. Several transformations of oligosaccharides were performed to demonstrate this behaviour. The potato is already known to contain Q-enzyme, which performs the same reaction, but with polysaccharides instead of oligosaccharides. It was proved that T-enzyme is not Q-enzyme.

Enzymic Polymerization of Monosaccharides. Last year Dr. Clancy (Report 1959) synthesized a mixture of galactose disaccharides by the action of brewer's yeast on galactose in presence of toluene. The disaccharides were separated and characterized as the α-1:6-, α-1:5-, α-1:4- and α-1:3-disaccharides, partly by the use of the periodate oxidation method.

It was found that brewer's yeast also couples galactose with *N*-acetylglucosamine; the resulting mixture of oligosaccharides is under examination. The reaction occurs much more rapidly when phenyl α-galactoside is substituted for galactose and an improved preparation of the galactoside was devised.

Dr. Clancy isolated the rare β-1:2-glucobiose from *Sophora japonica* by an improved method and prepared new derivatives.

TISSUE PHOSPHOLIPIDS

The object of this work is to elucidate the structure of phospholipids present in different tissues and cellular components, since this may help to clarify their function, of which little is known. In particular the pattern of the constituent fatty acids and fatty aldehydes were examined, using gas chromatography for their identification.

Dr. Macfarlane and Dr. Wheeldon previously obtained evidence that cardiolipin, the polyglycerophosphatide present in heart muscle, which is used as a component of the Wasserman antigen, has the structure bis (diacylglycerophosphoryl) glycerol. Dr. Macfarlane found that the polyglycerophosphatide present in ox liver has the same general structure as cardiolipin and likewise contains only unsaturated acids, predominantly linoleic acid, together with oleic and linolenic acid. The phosphoinositide of liver was isolated and found to be similar in fatty acid composition to that of heart muscle, with about 50% of saturated acids, almost entirely stearic acid, and 25-30% of C_{20} and C_{22} polyenoic acids. The kephalin and lecithin fractions were also examined.

Dr. Macfarlane, Dr. Gray and Dr. Wheeldon examined the nature of the neutral lipid and phospholipids bound in the subcellular particles of rat liver. Significantly more cardiolipin, about 9% of the total phospolipid, was present in mitochondria than in the microsomes, but the proportion of phosphoinositide, kephalin and lecithin were similar in the two kinds of particle. The fatty acid composition of the component phospholipids and of the neutral lipid

was virtually the same in the mitochondria and microsomes, suggesting either a common origin or a similar balance of synthesizing enzymes. The neutral lipid contained mainly oleic (45%) palmitoleic (10%) and palmitic (24%) acids. The kephalin and lecithin contained high proportions of highly unsaturated C_{20} and C_{22} fatty acids. The lecithin, like that of ox liver, is unusually rich in these "essential" fatty acids compared with lecithin from other sources such as heart muscle or egg; this is of interest in relation to the effects of irradiation or peroxidation on the metabolic activity of mitochondria, since it is known from previous work that the integrity of the lecithin in the particles is essential for succinoxidase activity.

Dr. Gray studied the relationship between the classical ester phosphatides, kephalin and lecithin, and the aldehydogenic compounds, ethanolamine and choline plasmalogen, in ox spleen, ox heart, pig heart, ram semen and ox liver. With the exception of liver, the kephalins differ from the lecithins in containing stearic acid (C_{18}) as the main saturated acid, together with large proportion of the highly unsaturated C_{20} acid, arachidonic acid; the lecithins contained small amounts of C_{20} acids and had palmitic acid (C_{16}) as the main saturated acid. There seems to be a general relationship between the chain length of the major saturated acid in the kephalin and lecithin and that of the major aldehyde in the corresponding plasmalogen, which was stearaldehyde (C_{18}) in ethanolamine plasmalogen and palmitaldehyde (C_{16}) in choline plasmalogen. In ox liver, however, which contains little plasmalogen, the aldehydes were mainly branched compounds with 13, 15 or 17 carbon atoms, which occurred only in traces in the other tissues.

Dr. Wheeldon examined the phospholipids of cabbage leaf, which were fractionated by chromatography on silicic acid. The presence of a phosphatidyl glycerol in plant lipids was confirmed and another non-nitrogenous phospholipid, not yet identified, was detected but no cardiolipin was present. The fatty acid composition of the phosphatidic acid, kephalin and lecithin fractions was qualitatively very similar, with large proportions of palmitic, linoleic and linolenic acids, but no C_{20} or C_{22} unsaturated acids.

Dr. Macfarlane and Mr. Shaw are examining the phospholipids in different cellular components of muscle, particularly with regard to the distribution of plasmalogens.

BIOPHYSICS

HUMAN PLASMA PROTEINS

Components of the Clotting Mechanism

(i) *Human Factor V.* It became clear early in Dr. Horner's work on Factor V that the one-stage assay as described by Wolf was unsatisfactory. The assay depends on the shortening of the clotting time of human plasma depleted of Factor V when suitable samples are added to it, the clotting being induced by added brain thromboplastin and calcium. A study of the method showed that the clotting time was very sensitive to slight variations in calcium ion concentration, necessitating the use of diluting fluids which would ensure that a constant citrate concentration was maintained in the test system; constancy of ionic strength and pH also proved to be important. The optimum concentration of calcium had to be determined for each preparation of brain thromboplastin used in the test.

Attempts were made to devise a two-stage test system in which either a defibrinated plasma or a purified prothrombin-containing eluate was used as a source of prothrombin, and the rate of conversion to thrombin was measured by the addition of samples to aliquots of fibrinogen solution. These systems proved to be rather unstable and troublesome to handle.

There is evidence that in removing prothrombin from human citrated plasma with aluminium hydroxide, an essential step in the preparation of Factor V, up to 50% of the Factor V might also be absorbed, depending on the batch and amount of absorbent used.

(ii) *Human Antihaemophilic Factor.* A new procedure for the assay of antihaemophilic factor, depending on the measurement of intrinsic thromboplastin generation, was published early in the year by Pool and Robinson. The reagents used are a bovine factor V concentrate, normal human serum and crude brain phospholipid. The assay was examined by Mr. Walton and modified to improve accuracy. A more highly purified bovine factor V concentrate than that recommended by the authors was advantageous.

The modified assay measures the antihaemophilic factor activity of separated plasma protein fractions with precision, and the results were consistent with those of parallel assays carried out under the direction of Dr. Maycock at Elstree, in a system in which haemophilic plasma is the main constituent of the assay mixture. Fresh, platelet-free normal human plasma gives anomalously high values with the Pool and Robinson assay; the discrepancy appears to be determined by the amount of the "activated product" formed in the plasma under test as a result of contact with glass surfaces.

The antihaemophilic factor, in the standard concentrate issued for clininal use by the Blood Products Laboratory over the past three years, was examined for stability with respect to pH; it is most stable at pH 7.1. The pH of the salt solution in which the concentrate is dissolved before freeze-drying has accordingly been modified by buffering to this pH value. Work on the further purification of human antihaemophilic factor is in progress.

Plasminogen. Dr. Derechin continued his work on the purification of plasminogen from plasma derived from outdated blood. An assay depending on the proteolysis of commercial casein purified by precipitation with perchloric acid, a widely used substrate, was found unsatisfactory; the estimates of activity of streptokinase-activated plasminogen varied considerably with the period of incubation, an effect due to an initial non-linear period of proteolysis. Pure α-casein was prepared by the fractionation of aqueous casein solutions with urea. In the proteolysis of the pure α-casein and of mixtures containing varying proportions of α and β caseins, the liberation of non-protein nitrogen depended to a considerable extent on the composition of the substrate. Work on the improvement of the plasminogen assay continues.

Antibodies to Human Erythrocytes. In collaboration with Dr. P. L. Mollison (M.R.C. Blood Transfusion Research Unit), Dr. Kekwick examined the characteristics of various human antibodies to human red cell antigens with special reference to anti-D and anti-Le^a activity. Some of these antibodies are associated with the "19S" globulin and others with the "7S" globulin component. These may be separated from one another by the ether fractionation procedure, the "19S" component being quantitatively segregated into the G2/2 fraction whereas the "7S" globulin is predominantly in the G3 fraction. The "19S" globulin can be obtained free from other contaminants by differential

ultracentrifugal treatment of the G2/2 fraction. The behaviour of the separated antibody fractions when they are treated with sulphydryl compounds is also being studied.

Pathological Sera. Prof. N. H. Martin has continued in investigations of hyperglobulinaemic sera containing excessive amounts of globulins with sedimentation co-efficients between 17S and 24S, and of the "macroglobulins" separated from these sera.

Dr. Kekwick continued to examine in the ultracentrifuge sera submitted by hospital pathologists to establish, for diagnostic purposes, whether they display a macroglobulinaemia. This helps to distinguish a benign condition from multiple myelomatosis.

ANIMAL PLASMA PROTEINS

The gut of the young rat up to the age of about 21 days is capable of transmitting undegraded protein to the circulation, although it is very selective in this function. In much of the experimental work establishing this selectivity, the transmission of antibody has been studied and some of the results suggested strongly that the species of origin of the antibody is a controlling factor. However, in some animals antibody activity can be associated either with the beta or gamma globulins and the distribution of total antibody between them may vary with the stage of immunization.

In order to elucidate some observations made by Dr. R. Halliday (A.R.C. Unit of Embryology, University College, Bangor), that certain antibodies were absorbed from the gut with unexpected difficulty when the animal was fed with whole serum, Dr. Kekwick fractionated several antibacterial sera by the ether method and by moving boundary electrophoresis in the flow-through U-tube. Antisera to *Brucella abortus* and *Salmonella pullorum* induced in the rat, guinea-pig and rabbit were so treated and the antibody distribution examined. The results are consistent with the suggestion that the gut transmits gamma globulin far more readily than beta globulin and that the species of origin of the protein may be of less importance than was formerly believed.

BLOOD PRODUCTS LABORATORY

Hypogammaglobulinaemia. In the field trial by the Medical Research Council Working Party on Hypogammaglobulinaemia, the gamma globulin concentrations in the sera of patients were determined by three different methods: moving boundary electrophoresis performed by Dr. Kekwick and Mr. L. Vallet; immunological tests (made in two laboratories) namely, measurement of inhibition by gamma globulin of the agglutination by anti-D serum of Rh (D) positive red cells, and gel-diffusion precipitin reaction of patients sera with antisera to gamma globulin. The results are being analysed by the Working Party.

Anti-haemophilic Factor and Fibrinogen: The preparation of human anti-haemophilic factor on a limited scale has continued. A Working Party has been formed by the Medical Research Council to conduct a clinical trial, in the hope of devising a scheme of dosage of human anti-haemophilic factor related to the clinical severity of haemophilia and the scope of the surgical procedure envisaged, and to investigate the reactions which occur after giving certain preparations of the plasma fraction containing the factor. None of the available methods of assaying anti-haemophilic factor is entirely satisfactory; most of them require

haemophilic plasma, which is difficult to obtain, and the 'standard', against which the anti-haemophilic factor is assayed, is normal human plasma, the AHF content of which varies from person to person and possibly in the same person from time to time. During the year, Mr. Vallet began to investigate the preparation of batches of human anti-haemophilic factor which are being examined by Dr. Kekwick to determine whether they can be used as 'standards' by the laboratories taking part in the clinical trial.

Dr. Mackay, with the assistance of Miss Bell, investigated the pharmacologically active substances present in fibrinogen prepared from fresh and from time-expired plasma (i.e. older than 21 days) and in the fraction rich in anti-haemophilic factor, some 60% of which is fibrinogen. Reactions are seldom, if ever, associated with transfusion of the first two fractions, but occur in some patients after transfusion of the fraction rich in anti-haemophilic factor. Substances contracting isolated smooth muscle strips are present in each type of fraction, but in each the activity is affected differently by dialysis, by exposure to plasmin and by certain other procedures. Isolation and identification of the contractor substances are under way.

Christmas Factor. Mr. Vallet collaborated with Dr. Wolf in the preparation of a concentrate of Christmas factor (Factor IX). In the preparation of plasmin by fractionation with ethyl ether, a solution of precipitate P is treated with barium sulphate, which absorbs an elutable substance with Christmas factor activity *in vitro*. Christmas factor suitable for clinical use may prove to be obtainable from this source without diminishing the yields of other plasma fractions. It would be of value in the treatment of bleeding caused by deficiency of Christmas factor, an hereditary condition broadly resembling haemophilia.

Plasminogen. Dr. Mackay continued her work on proteolytic enzymes of human plasma. Batches of plasminogen concentrate, prepared from large pools of human plasma by the method described previously (Report 1959), have a similar content of caseinolytic and fibrinolytic material. Plasminogen in this fraction may be treated with streptokinase and the activated plasmin further concentrated by precipitation. Both the plasmin and the plasminogen preparations are soluble in phosphate buffer at pH 7.0, and may be Seitz filtered and dried from the frozen state without loss of proteolytic activity. During several months of storage at room temperature, the dried plasminogen was stable but the dried plasmin slowly lost activity.

The lipid content of this fraction is undesirably high and attempts were made to bring the enzyme into solution without the lipoprotein. Owing to the nature of the starting material, fraction P, the extraction with dilute mineral acids described by Kline, could not be used; but extraction with glycine buffers was successful, and is under investigation.

BLOOD GROUP RESEARCH UNIT

As in past years the course of the Unit's work has been set mainly by problems submitted by colleagues in the United States, Great Britain and elsewhere. Each year fewer samples come from people who have suffered adverse reaction to transfusion: troublesome antigens and antibodies nowadays are more often found at the cross-matching stage, before the transfusion. This is one index of the increasingly high skill of the services concerned.

The Rh System. The greater part of the year's work was on Rh. Several of the problems remained unsolved, but did at least serve to show how complex the system is. On the other hand, one long investigation ended with a clear answer. The serum of a patient in San Antonio, Texas, sent by Dr. A. Cahan of the Knickerbocker Foundation, New York, reacted with cells containing either of two predominantly negro antigens—V (Report 1956) and R^s The exact place of these antigens in the Rh system was previously unknown, and a relationship between them unsuspected. The new antibody showed that both contained an antigen which is now called e^s, the antigen V corresponds to ce^s and e^s, and R^s to Ce^s and e^s: anti-V is anti-ce^s and the new anti-body anti-e^s.

In other Rh work the Unit collaborated with Dr. Cahan, Dr. R. E. Rosenfield, of New York, Dr. F. H. Allen, Jnr., of Boston, Dr. I. Dunsford, of Sheffield, Dr. M. N. Metaxas, of Zurich, and many others.

The MNSs System. In collaboration with Dr. Cahan, a previously unsuspected component of certain human anti-M sera was recognized. It was called anti-M_1 because of resemblances to anti-A_1 of the ABO system. The corresponding antigen, M_1, is at least four times more frequent in New York negroes than it is in whites. Some human anti-M sera are almost wholly anti-M_1 in specificity: such sera had been sent to the Unit in the past, but had not been recognized.

The ABO System. Several more examples of the B-like antigen, which is an acquired character (Report 1959), were referred to the Unit. Again all the samples were from A_1 people. Over three-quarters of these people are old and suffering from carcinoma of the colon. Since Springer found that *E.coli* O_{86} contains a substance closely related to the human B antigen, opinion has leaned towards a bacterial cause of the acquired B. This aspect of the problem is under investigation elsewhere.

Towards the Establishment of New Blood Group Systems. During the course of the year three of the samples of serum sent as containing a "new" antibody were found to have the same specificity. The name anti-Ge was given to the antibody. In collaboration with Dr. Rosenfield and Dr. Cahan of New York and Dr. F. Kissmeyer-Nielsen of Arhus, it was shown, by family tests, that the corresponding "new" antigen, Ge, is a dominant character which does not belong to the ABO, MNSs, P, Rh, Kell or Kidd systems. Ge is an extremely common antigen: no person lacking it was found in tests on 11,000 random New York blood samples; it is estimated that at least 1,500 of these must have been from negroes and at least 100 from Asians.

I and anti-I. The work on the I antigen and antibody (Report 1959) continued, in collaboration with Dr. W. J. Jenkins and Mr. W. L. Marsh of Brentwood, Dr. C. A. Holman of Lewisham, Dr. Cahan and Miss L. Sausais of Baltimore. The I antigen is extremely common: the lack of I is not quite such a rarity in negroes as it is in whites. Though the lack of the antigen is so rare, the antibody has turned out to be relatively common: the auto-agglutinin in the serum of people with acquired haemolytic anaemia of the cold antibody type is auto-anti-I, and so is the antibody which used to be called non-specific cold auto-agglutinin. The antigen I is but feebly developed at birth. Samples from a negro family in Baltimore provided the first evidence that I is, as expected, under genetic control. Since the last Report it has become clear that I is not connected with the P system though some association with the ABO system is still suspected.

The blood-grouping of twins, as an aid to establishing their zygosity, continues. In this the Unit collaborated with colleagues at the Canadian Red Cross Hospital, Taplow, and the Postgraduate Medical School of London (rheumatic diseases), the Maudsley Hospital (psychiatric conditions) and the Royal Eye Hospital (eye abnormalities). The blood groups of patients at Guy's Hospital with various sex abnormalities continue to be disappointingly normal.

For routine antisera the Unit is indebted to many Colleagues, particularly Dr. A. E. Mourant and Miss E. W. Ikin of the Medical Research Council Blood Group Reference Laboratory, Dr. I. Dunsford of the Sheffield Blood Transfusion Service and Dr. R. A. Zeitlin and Dr. T. E. Cleghorn of the South London Blood Transfusion Centre. Dr. Zeitlin has also provided on many occasions large numbers of random blood samples. Again the Unit is grateful to the Staff of the Institute for the numerous samples of their blood which, having been tested for almost all the known antigens, are invaluable in the identification of antibodies.

BLOOD GROUP REFERENCE LABORATORY

The Laboratory serves as reference centre for blood grouping problems and supply centre for grouping sera for the United Kingdom. It also acts under the auspices of the World Health Organization as the world reference centre.

Liquid blood grouping sera and anti-human-globulin serum are supplied for use in the United Kingdom and dried sera for use overseas. Demands for these have been maintained, and have in some cases increased considerably. Help to overseas laboratories generally, and those in the British Commonwealth in particular, continues to be an important activity. Several laboratories starting or expanding blood grouping activities were supplied with initial stocks of serum, and the red-cell antigens of members of their staffs were identified in readiness for the investigation of sera. Numerous blood specimens from Great Britain and abroad were investigated for blood group antigens and antibodies to assist in clinical investigations, for research purposes, and for routine blood grouping purposes, especially in connection with the control and supply of grouping sera.

Miss Ikin and Miss Giles investigated the use of ficinized red cells in the detection of weak haemagglutinins. Miss Giles has continued to work on the D^u antigens of the Rh blood group system and the means for their detection.

Dr. Parkin and Miss Giles carried out serological and genetical investigations of numerous examples of unusual antigens and antibodies, found in the course of tests carried out for clinical purposes.

Miss Ikin continued her work on the distribution of blood groups in a number of populations in Nigeria, Pakistan, Nepal and Ethiopia, and her statistical studies of the results of these and previous tests.

In conclusion the Governing Body desires to record its great appreciation of the manner in which the scientific, administrative and technical staffs have worked together during the period under review, and to congratulate them on the interest and range of their scientific activities and achievements.

H. H. DALE,
Chairman.

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BLOOD GROUP RESEARCH UNIT

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BLOOD GROUP REFERENCE LABORATORY

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THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

Balance Sheet

and

Accounts

31st December 1960

CHELSEA BRIDGE ROAD,
LONDON, S.W.1.
30th May, 1961.

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

FINANCIAL REPORT OF THE GOVERNING BODY

1. The Balance Sheet as at 31st December, 1960, shows balances to the credit of the various funds as follows: Capital Fund £882,204, Specific Funds £167,622 and Bequest Funds £16,298. The balance on the Sinking Fund for Freehold Buildings of £118,476 is after transferring from income and expenditure account £5,922. The Re-endowment Fund has been increased by donations and a legacy during the year amounting to £8,492.

2. The General Fund Income and Expenditure Account shows the income for the year as £236,179 compared with £252,665 in 1959. Expenditure amounted to £217,484 against £201,866 last year. The surplus for the year is £18,695 compared with £50,799 in 1959.

3. The year's surplus of £18,695 shown by the General Fund Income and Expenditure Account has been transferred to the Capital Fund.

4. Stocks of Sera, Smallpox Vaccine and Horses on hand at 31st December, 1960, have the nominal value of £10,261, £2,005 and £7,575 respectively.

5. Messrs. Cooper Brothers & Co. the retiring Auditors, will, subject to the provisions of the Companies' Act, 1948, be re-appointed.

HENRY H. DALE, *Chairman of the Governing Body.*

HUGH BEAVER, *Hon. Treasurer.*

BALANCE SHEET

(1959) £		£	£	£
	Capital Fund:—			
	Donations, &c., received to date from the following:—			
2,000	Dr. Ludwig Mond (1893)		2,000	
46,380	Berridge Trustees (1893-1898)		46,380	
10,000	Worshipful Company of Grocers (1894)		10,000	
250,000	Lord Iveagh (1900)		250,000	
18,904	Lord Lister's Bequest (1913-1923)		18,904	
7,114	William Henry Clarke Bequest (1923-1926)		7,114	
3,400	Rockefeller Foundation (1935-1936)		3,400	
22,669	Other donations and legacies (1891-1954)		22,669	
	General fund income and expenditure account accumulated surplus, as at 31st December, 1959	498,993		
	Add surplus, 1960	18,695		
	Profit, less losses, on sale of investments £5,300			
	Less amounts written off investments 1,251			
		4,049		
498,993			521,737	
859,460				882,204
	Specific Funds:—			
112,556	Sinking fund for freehold buildings	118,476		
33,279	Pension fund	32,956		
7,698	Re-endowment fund	16,190		
			167,622	
	Bequest Funds:—			
8,857	Jenner Memorial studentship fund	9,307		
6,652	Morna Macleod scholarship fund	6,991		
			16,298	
169,042				183,920
	Specific Grants and Legacies Unexpended:—			
772	Cancer research legacies (1937-1950)		772	
347	Royal Society grant (1951)		347	
6,301	Nuffield Foundation grants (1952-1960)		5,385	
11,341	Guinness Lister research grant (1953-1960)		8,518	
18,761				15,022
	Current Liabilities:—			
21,120	Creditors and accrued charges			28,886

HENRY H. DALE, *Chairman of the Governing Body.*

HUGH BEAVER, *Hon. Treasurer.*

£1,068,383	£1,110,032

REPORT OF THE AUDITO

The accounts set out on pages 4 to 8 are in agreement with the books which, in our opinion, have be
In our opinion the accounts, amplified by the information given in paragraphs 1 and 4 of the Financ
state of affairs and the surplus of the Institute.

London, 6th June, 1961

(1959) £					£	£	£
	Fixed Assets:—						
	Freehold property at cost:—						
73,548	Land and buildings, Chelsea				73,548		
20,455	Queensberry Lodge estate, Elstree				20,455		
2,049	House, Bushey				2,049		
						96,052	
	(Note: Additions and replacements since 1912 at Elstree and 1935 at Chelsea have been charged to revenue.)						
	Furniture, fittings, scientific apparatus and books:—						
2,472	At cost, less depreciation to 31st December, 1920					2,472	
98,524	*Note: Additions and replacements since 31st December, 1920, have been charged to Revenue)*						98,524

General, Specific and Bequest Funds.

Investments and Uninvested Cash:—

		Quoted at cost, less amounts written off in Gt. Britain	Elsewhere	Unquoted at cost	Uninvested cash		
675,740	General	£593,065	£78,238	£47,595	—	718,898	
	Specific—						
112,556	Sinking fund for freehold buildings	98,489	13,032	—	6,955	118,476	
33,279	Pension fund	31,746	—	—	1,210	32,956	
7,698	Re-endowment fund	6,590	—	—	9,600	16,190	
	Bequest—						
8,857	Jenner Memorial studentship fund	6,479	—	1,940	888	9,307	
6,652	Morna Macleod scholarship fund	6,295	—	—	696	6,991	
844,782		742,664	91,270	49,535	19,349	902,818	902,818
		£833,934					

(Market value of quoted investments—£1,113,667)

	Current Assets:—		
87,316	Debtors and payments in advance	56,279	
37,761	Balance at bankers and cash in hand	52,411	
125,077			108,690

(Notes: See paragraph 4 Governing Body's Financial Report for nominal values of sera, smallpox vaccine and horses which have not been brought into the accounts.
There is a contingent liability of £1,425 in respect of investments not fully called up.)

£1,068,383	£1,110,032

THE MEMBERS

...erly kept. We obtained the information and explanations we required.
...rt of the Governing Body, comply with the Companies Act, 1948, and give a true and fair view of the

COOPER BROTHERS & CO.,

Chartered Accountants.

INCOME AND EXPENDITURE ACCOUNT

GENERAL

(1959)		Total Expenditure	External Contributions	
£		£	£	£
83,438	Salaries and wages	151,530	58,089	93,441
7,028	Emoluments of two members of the Governing Body in an executive capacity	7,225	—	7,225
2,690	Premiums on federated superannuation policies	7,268	2,940	4,328
2,864	Premiums on group pension policy	2,880	628	2,252
4,405	Rent, rates and insurance	5,628	432	5,196
12,371	Gas, water, fuel and electricity	17,091	2,887	14,204
3,431	Office expenses, stationery and printing	4,592	531	4,061
409	Auditor's fee	384	—	384
928	Travelling expenses	1,361	448	913
3,181	Biochemistry expenses	8,046	3,256	4,790
1,260	Microbiology, immunology and experimental pathology expenses	3,308	2,188	1,120
468	Biophysics expenses	1,277	500	777
451	Virology expenses	1,992	1,784	208
18,615	Serum, vaccine and smallpox vaccine expenses	25,307	1,649	23,658
9,044	Animals	10,594	1,284	9,310
9,065	Animal house expenses and forage	10,602	2,285	8,317
31,740	Buildings, alterations, repairs and renewals	25,311	609	24,702
461	General apparatus and new installations	1,741	—	1,741
1,009	Library expenses	1,379	—	1,379
1,689	General stores	1,725	—	1,725
1,671	Staff canteen loss	2,075	244	1,831
—	Blood products laboratory expenses	7,620	7,620	—
5,648	Amount transferred to sinking fund for freehold buildings (including £5,498 interest on investments)	5,922	—	5,922
50,799	Surplus transferred to Capital Fund after charging to expenditure £17,787 for additions to property and equipment (1959 £28,830)	18,695	—	18,695
£252,665		£323,553	£87,374	£236,179

OF PREVENTIVE MEDICINE

For the year ended 31st December 1960

FUND

(1959) £		£	£
	Interest and dividends on investments:—		
35,835	General fund	40,030	
4,949	Sinking fund	5,498	
			45,528
	Underwriting commission:—		
740	General fund	587	
275	Sinking fund	—	
			587
204,622	Sales of sera, vaccines, smallpox vaccine, &c.		183,449
6,244	Rent		6,615
£252,665			£236,179

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

PENSION FUND

(1959) £		£	(1959) £		£
1,656	Pensions	2,051	33,207	Balance as at 1st January, 1960 ..	33,279
33,279	Balance carried forward	32,956	1,728	Interest on investments (gross) ..	1,728
£34,935		£35,007	£34,935		£35,007

JENNER MEMORIAL STUDENTSHIP FUND

(1959) £		£	(1959) £		£
46	Loss on realisation of investment	—	8,500	Balance as at 1st January, 1960 ..	8,857
8,857	Balance carried forward	9,307	403	Interest on investments (gross) ..	450
£8,903		£9,307	£8,903		£9,307

MORNA MACLEOD SCHOLARSHIP FUND

(1959) £		£	(1959) £		£
6,652	Balance carried forward	6,991	6,320	Balance as at 1st January, 1960 ..	6,652
			332	Interest on investments (gross) ..	339
£6,652		£6,991	£6,652		£6,991

NUFFIELD FOUNDATION GRANTS

(1959) £		£	(1959) £		£
5,364	Salaries, wages, laboratory expenses and animals	6,916	5,665	Balance at 1st January, 1960 ..	6,301
6,301	Balance carried forward	5,385	6,000	Amounts received	6,000
£11,665		£12,301	£11,665		£12,301

GUINNESS-LISTER RESEARCH GRANT

(1959) £		£	(1959) £		£
10,837	Salaries and wages	13,924	11,237	Balance at 1st January, 1960 ..	11,341
3,059	Laboratory expenses	2,899	14,000	Amount received	14,000
11,341	Balance carried forward	8,518			
£25,237		£25,341	£25,237		£25,341

THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE

REPORT
OF THE
GOVERNING BODY
1961

CHELSEA BRIDGE ROAD, LONDON, S.W.1

THE GOVERNING BODY

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Deputy Director: Professor W. T. J. Morgan.

Superintendent of Elstree Laboratories: W. d'A. Maycock.

MICROBIOLOGY, IMMUNOLOGY and EXPERIMENTAL PATHOLOGY

†A. A. Miles, C.B.E., M.A., M.D., F.R.C.P., F.R.S. (*Professor of Experimental Pathology in the University of London*).
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Doris M. Graham, M.Sc.
P. Reeve, B.Sc.
Elizabeth F. Fraser, B.Sc.
} *M.R.C. Trachoma Research Unit*

BIOCHEMISTRY

†W. T. J. Morgan, C.B.E., D.Sc., Ph.D., F.R.I.C., F.R.S. (*Professor of Biochemistry in the University of London). Principal Biochemist, Elstree.*
*Marjorie G. Macfarlane, D.Sc., Ph.D.
*W. J. Whelan, D.Sc., Ph.D., F.R.I.C.
Winifred M. Watkins, B.Sc., Ph.D.
G. M. A. Gray, B.Sc., Ph.D.
Joan Allen, B.Sc.
G. G. Birch, B.Sc.
B. J. Bines, B.Sc., Ph.D., A.R.I.C. (*Dept. Scientific and Industrial Research Grantee*).
T. J. Painter, M.A., B.Sc., Ph.D. (*Medical Research Council Grantee*).
A. J. Pusztai, Ph.D. (*Medical Research Council Grantee*).
M. Abdullah, M.Sc. (*Agricultural Research Council Grantee*).
Regina Pietruszko, M.Sc. (*British Empire Cancer Campaign Grantee*).
Pamela M. Taylor, B.Sc. (*Medical Research Council Grantee*).
L. G. Egyud, B.Sc. (*Mental Health Research Fund Grantee*).
E. E. Smith, B.Sc. (*Dept. Scientific and Industrial Research Student*).
G. Uhlenbruck, Dr.Med. (*Germany*).
E. J. Koscielak, Dr. Med. (*Poland*).

BIOPHYSICS

†R. A. Kekwick, D.Sc. (*Reader in Chemical Biophysics in the University of London*).
J. M. Creeth, B.Sc., Ph.D.
†Professor N. H. Martin, M.A., F.R.C.P., F.R.I.C. (*Honorary Research Associate*).
P. W. Walton, B.Sc. (*Medical Research Council Grantee*).

NUTRITION

§Dame Harriette Chick, D.B.E., D.Sc.
§E. Margaret Hume, M.A.

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†Appointed Teacher of the University of London.
*Recognised Teacher of the University of London.
§Honorary Member of Institute Staff.

PREPARATION and STUDY of THERAPEUTIC SERA (ELSTREE)

*B. G. F. Weitz, M.R.C.V.S.
Sheila M. Lanham, B.Sc. (*Trypanosomiasis Research*).
Frances M. Lee-Jones, B.Sc. (*Trypanosomiasis Research*).
G. Stone, B.Sc.

BIOCHEMISTRY (ELSTREE)

*D. E. Dolby, B.Sc., Ph.D.

PREPARATION and STUDY of SMALLPOX VACCINE (ELSTREE)

*D. McClean, M.B., B.S., M.R.C.S.
C. Kaplan, M.Sc., M.B., Ch.B., Dip. Bact.
H. G. S. Murray, M.B., B.Ch.
L. C. Robinson, B.Sc.

PREPARATION and STUDY of BACTERIAL VACCINES (ELSTREE)

*A. F. B. Standfast, M.A., Dip.Bact.
Jean M. Dolby, M.A., Ph.D. (*Medical Research Council External Scientific Staff*).
M. P. Banks, B.Sc.
W. A. Vincent, B.Sc., Ph.D. (*Medical Research Council Grantee*).
Georgina Sampson, B.Sc. (*M.R.C. Trachoma Research Unit*).

BLOOD PRODUCTS (ELSTREE)

*W. d'A. Maycock, M.B.E., M.D.
L. Vallet, M.A.
§Margaret E. Mackay, M.Sc., Ph.D. (*Medical Research Council External Scientific Staff*).
Constance Shaw, M.Sc., Dip.Bact.
Shirley M. Evans, B.Sc.

MEDICAL RESEARCH COUNCIL UNITS HOUSED AT THE INSTITUTE

BLOOD GROUP RESEARCH UNIT.

§R. R. Race, Ph.D., F.R.C.P., F.R.S.
Ruth Sanger, B.Sc., Ph.D.
Patricia Tippett, B.Sc.
Florence J. Hamper, B.Sc.
June Gavin, B.Sc.

BLOOD GROUP REFERENCE LABORATORY.

§*A. E. Mourant, M.A., D. Phil., D.M., M.R.C.P.
K. L. G. Goldsmith, Ph.D., M.B., B.S.
Elizabeth W. Ikin, B.Sc.
Carolyn M. Giles, B.Sc.

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Elstree Secretary and Estate Manager -	G. J. Roderick, B.Comm.
Assistant Secretary - - - -	Barbara A. Prideaux

Solicitors:
Field, Roscoe & Co.
52 Bedford Square, W.C.1.

Auditors:
Cooper Brothers & Co.,
Abacus House, 33 Gutter Lane, E.C.2.

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*Recognised Teacher of the University of London.
§Honorary Member of Institute Staff.

ANNUAL GENERAL MEETING

OF

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

June 27th, 1961

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the work of the Institute for the year 1960.

GOVERNING BODY

At its last meeting the Council re-appointed Sir Henry Dale and Sir Charles Dodds as its representatives on the Governing Body until 31st December 1961. The Council accepted with much regret the decision of Sir Wilson Jameson, who had been a representative of the Council since 1950, not to offer himself for re-appointment. The Governing Body takes this opportunity of expressing its gratitude to Sir Wilson Jameson for his warm interest and wise counsel in the affairs of the Institute. The Governors welcome Sir Russell Brain as the new representative of the Council.

COUNCIL

Last year the three retiring members of the Council were Professor E. J. Conway, Professor W. T. J. Morgan and Professor Wilson Smith. The Royal Irish Academy appointed Dr. V. C. Barry as its representative in place of Professor E. J. Conway, and at the Annual General Meeting Professor W. T. J. Morgan and Professor Wilson Smith were re-appointed. Sir Russell Brain was also appointed to the Council as one of the representatives of the Members.

The three members of the Council due to retire this year in accordance with the Articles of Association, but who are eligible for re-appointment, are The President of the Royal College of Veterinary Surgeons, Professor E. B. Verney and Professor D. Whitteridge, the representatives of the Royal College of Veterinary Surgeons, the University of Cambridge and the University of Edinburgh respectively.

MEMBERS

The Governing Body records with regret the death during the year of Professor H. R. Dean, a Member since 1922 and a former member of the Governing Body from 1938 to 1950.

STAFF

The Governing Body takes much pleasure in recording the election of Professor A. A. Miles to the Fellowship of the Royal Society.

Dr. L. H. Collier was appointed a member of the World Health Organisation Expert Advisory Panel on Virus Diseases.

Dr. J. M. Creeth was appointed to the Biophysics Department; Dr. H. G. Murray to the Smallpox Vaccine Department; Dr. G. M. Gray, Dr. A. Pusztai and

Mrs. Pamela Taylor to the Biochemistry Department; Mr. M. P. Banks to the Bacterial Vaccines Department; and Mr. G. Stone to the Serum Department. Dr. D. Doyle, Dr. Sheila Howarth, Dr. J. I. Payne, Mr. J. Rodican and Mr. M. Garay resigned during the year, and Mr. I. A. F. Lister-Cheese, Mr. H. M. Tyler and Mr. T. M. Joys completed the tenure of their Studentships.

In December, 1960, Professor A. A. Miles took part, by invitation, in a Conference on " Recent Progress and Present Problems in the Field of Shock " organised by the National Academy of Sciences, and held at the Walter Reed Army Institute of Research in Washington, D.C.

In November 1960, Dr. W. d'A. Maycock attended a meeting of the Council of Europe Working Party held in Amsterdam to examine the reports of holders of Council of Europe Travelling Fellowships in Blood Transfusion and to prepare recommendations on certain aspects of the Practice of Blood Transfusion for the Council.

Professor Morgan took part in the Chemical Society Symposium on Polysaccharides in Edinburgh and, together with Dr. Watkins and Dr. Whelan, contributed to a Colloquium on the Biochemistry of Glucosides at Gif-sur-Yvette, France, in July 1960.

Dr. L. H. Collier took part in the Annual Assembly of the Ligue Contre le Trachome, held in Paris in May 1960.

In January 1961, Dr. B. A. D. Stocker took up a six-month Visiting Professorship in the Department of Genetics, Stanford University, California.

Mr. B. G. F. Weitz visited the West African Institute for Trypanosomiasis Research at Von, Nigeria, in April 1960, at the invitation of the Director; and in January 1961, attended a meeting of the East African Trypanosomiasis Research Committee in Nairobi.

Dr. W. J. Whelan lectured at the Stärke-Tagung in Detmold, Germany, in April 1960; at the British Nylon Spinners Ltd., Pontypool, in June 1960, and at the Chemical Society Symposium on Polysaccharides in Edinburgh.

Mr. L. Vallet visited Warsaw in January 1961, as a World Health Organisation Consultant, to advise the Polish Government on the preparation of human plasma fractions and problems relating to freeze-drying.

Dr. Winifred M. Watkins was awarded a Wellcome Trust Travelling Fellowship in October 1960, to enable her to spend one year at the University of California, Berkeley, U.S.A.

DONATIONS AND GRANTS

The Governing Body records its appreciation of the generosity of many bodies whose benefactions and grants support research work in the Institute. These include a grant from the Agricultural Research Council for research on the mechanism of starch-carbohydrase action; a grant from the British Empire Cancer Campaign for research on phospholipids; grants from the Colonial Development and Welfare Fund in aid of researches on the blood-meals of insect vectors of disease and on the immunology of trypanosomiasis; grants from the Department of Scientific and Industrial Research for researches on the enzymic polymerization of monosaccharides, on chemically modified polysaccharides and on the characterisation of biological macro-molecules; a grant from Imperial Chemical Industries Ltd. for the purchase of special apparatus; grants from the Medical Research Council in aid of researches on the isolation and purification of proteins involved in the clotting mechanism in human plasma and on the

chemical basis of blood group specificity in man; grants from the Nuffield Foundation for researches on the immunochemistry of blood group substances and on non-specific immunity in the early stages of infection; a grant from the U.S. Public Health Authority in aid of researches on abacterial urethritis in man; and grants from the World Health Organisation for serological identification tests on material submitted by the World Health Organisation.

The Governing Body also gratefully acknowledges donations to the Institute's Re-endowment Fund from the following Assurance Companies: The General Life Assurance Company, The Prudential Assurance Company Ltd. and the Royal London Mutual Insurance Society Ltd.

VISITORS

The following visitors, in addition to those listed under Staff, worked in the Institute's Laboratories: Mr. A. R. Gray, West African Institute for Trypanosomiasis Research, Kaduna, Northern Nigeria; Dr. A. E. Kortekangas, Blood Transfusion Service, University Hospital, Turku, Finland; Dr. K. V. Kulkarni and Dr. Y. S. Nimbkar, Haffkine Institute, Bombay 12, India; Miss J. Mas, Laboratorios Grifols, Barcelona; Dr. P. D. Meers, Royal Army Medical Corps., Millbank; Dr. G. Modiano, Institute of Genetics, University of Pavia; Dr. J. Triginer, Laboratory Grifols, Barcelona; Dr. A. M. Vilches, Pan American Sanitary Bureau, Washington, D.C.; Dr. R. G. Wittler, Walter Reed Army Institute for Medical Research, Washington, D.C.

The Blood Group Research Unit and the Blood Group Reference Laboratory, whose researches for 1960 are described on pages 27-28 are still accommodated at the Institute; and Miss E. M. Hume continues to do editorial work for Nutrition Abstracts and Reviews on behalf of the Commonwealth Bureau of Animal Nutrition.

RESEARCHES IN 1960

SUMMARY

In this summary of the investigations made in 1960, the bracketed numbers refer to the pages of the report where the researches are described in greater detail.

Microbiology. The Guinness-Lister Unit continues its exploration of bacterial genetics, mainly of the Salmonella bacilli. The problems studied include conjugation and gene transfer initiated by the colicine type of fertility factor (12) and by a mutant of the classical fertility factor, F of Hayes (13); the effect of a colicine factor on resistance to ultraviolet irradiation (13); the genetics of resistance of *E.coli* to colicines (13); the genic control of flagellation in a number of Salmonella mutants (13) and of the production of different varieties of flagellar protein (14); and the chemistry of flagellar proteins and of the cell-wall polysaccharides (14-15).

The rates of bacterial multiplication in the infected mouse were studied with pathogenic Salmonella marked either with a non-replicating prophage, or with an abortively-transduced gene from another strain of Salmonella (15).

The other purely microbiological study concerns the antigenic analysis of certain free-living protozoa (10).

Immunology and pathology of infective diseases. The Institute's studies embrace infections by viruses, pleuropneumonia-like organisms, bacteria and protozoa.

The Trachoma Research Unit continued its epidemiological field study in the Gambia of trachoma in terms of virus and of bacilli of the Haemophilus group (17). The laboratory studies at the Institute include growth of trachoma and inclusion conjunctivitis virus in tissue culture (16), serological classification of the viruses (16), the vaccination of baboons against inclusion conjunctivitis (17) and the serology of Gambian *Haemophilus* strains (12). In the Smallpox Vaccine department, there is continued progress towards making vaccine from virus grown in tissue culture (18). The properties of measles virus were also studied (17).

As an indirect means of determining the significance of antibody to PPLO in human subjects with abacterial urethritis and other kinds of genital infection, the study continues of the relation of PPLO antibody to the infecting agent and stage of infection in rats and mice (16).

The immunological study of bacterial infections includes an analysis of the action of two types of protective antibody in mice infected with the whooping cough bacillus (11); the biological activity of the C polysaccharide of *Strep. pyogenes* (12); and the process of refinement of antitoxin by proteolysis (18).

As regards pathogenic protozoa, the investigation of the immunology of experimental trypanosomiasis continues, in terms of soluble and bound immunizing antigen (10); and of methods of bulk growth of the trypanosomes for antigenic analysis (11).

Epidemiology. The refined serological methods devised to identify the animal source of food for blood-sucking insects continues to provide valuable facts about the feeding habits of Tse-tse flies in regions where these insects are vectors or possible vectors of disease (18).

Pathology. Work on the relation of early tissue reactions to defence against microbial infection continues. The non-specific resistance to various bacteria was investigated during the course of streptococcal infection in the guinea-pig.

Biochemistry. The biochemical researches again concern three kinds of substances—the blood group substances, the cellular phospholipids, and starches.

The investigations of the substances of the ABO blood group system include analysis by improved methods of partial hydrolysis (20), determination of the amino-acid moiety of the substances and its relation to serological specificity (21), transformation of blood group specificity by the action of selected enzymes (21) and measurement of the molecular properties of the substances (21). The analysis of M and N blood group substances was begun (21).

Work on the structure of phospholipids, their distribution in tissues, cells and cellular particles, and their possible function in the cell includes analysis of the phospholipids of *Micrococcus lysodeikticus* (23) of pig kidney, lung and spleen (23), and of mammalian plasmalogens (24).

The enzymes of plants, animals and bacteria synthesizing and degrading starch and glycogen were studied with regard to their specific effects on natural carbohydrates (22) and on modified carbohydrates produced by chemical methods including starches (22) and various glucose phosphates (22). A disaccharide other than isomaltose was obtained from a dextran, and a new trisaccharide (22). Carbohydrates were synthesized for use as haptens in the immunochemical

analysis of dextrans (22); and the products of the enzymic polymerization of certain monosaccharides identified (23).

Human and animal plasma proteins. The Institute's work is concerned with the isolation, refinement, characterization, assay and in some cases clinical trial of the various biologically active proteins of human plasma.

A reliable assay of human antihaemophilic globulins was devised (24), and improvements made in separation of stable preparations of the globulins from plasma (25).

Studies of the isolation and characterization of biologically active proteins include those of plasmin (26), plasminogen (25, 26), albumin (26), caeruloplasmin (26) and Christmas factor (27). Investigations of hyperglobulinaemic sera were made (25).

MICROBIOLOGY

PROTOZOOLOGY

Dr. Robertson concluded the work on *Trichomonas foetus*, but continued to grow the organisms in bulk for the production of the various enzymes attacking human blood group substances (see page 21). A serological study of certain strains of Tetrahymena reveals that most of them, considered to be *Tetrahymena pyriformis* by the usual methods of classification, nevertheless differ antigenically.

By agglutination tests with live organisms, there is an absence of cross-reaction between certain strains; but considerable cross-reaction by precipitin tests with saline extracts of homogenised cells.

The temporary insensitiveness to homologous antisera, previously studied (1939), of Tetrahymena organisms that survive the first exposure to antibody is being reinvestigated.

TRYPANOSOMIASIS

Mr. Weitz continued his studies of the antigens of *Trypanosoma brucei* and *T.vivax*. Two kinds of antigens occur: (1) soluble antigens, called "exo-antigens", which are released in the serum of infected animals and (2) " bound " antigens obtained by the mechanical disintegration of trypanosome suspensions. The exo-antigens appear to be species-specific. Mice immunised with the exo-antigen of one species are protected against infection with the homologous species only, and antisera from animals thus immunised react specifically *in vitro* with the homologous exo-antigen only. However, the " bound " antigens are not species-specific, since extracts of disintegrated trypanosomes react *in vitro* with antisera derived from animals immunised with whole extracts from either species of trypanosomes.

Animals given repeated sublethal doses of *T.vivax* produce antibodies reacting with the homogenate of both species but with the exo-antigen of *T.vivax* only. Such animals are, however, immune to infection with either *T.brucei* or *T.vivax* because of the presence of antibodies to the interspecific " bound " antigens presumably liberated as the living trypanosomes break up *in vivo*.

Miss Lee-Jones developed a technique for the qualitative and quantitative determination of trypanosome antigens *in vitro*. Tanned red blood cells, sensitized with the antigen, are agglutinated by the corresponding antibodies. The antigen content of solutions is determined and measured by the amount required to inhibit specifically the agglutination of sensitized cells. The inhibition of antiserum in a given dilution is measured in terms of a standard antigen solution. Significant differences of 5-10 per cent are measurable.

Cultivation of African Trypanosomes in vitro. Dr. Pittam continued his investigations of methods for large-scale culture of trypanosomes *in vitro*.

A strain of *Trypanosoma rhodesiense*, recently taken from a man, was brought into *in vitro* culture by Weinman's method. Attempts were made to grow this strain in large vessels (1 litre flasks, Roux bottles) using a medium consisting of a blood-agar slope with an overlay of Locke's saline, but were discontinued because of the poor yield.

The search for an entirely liquid medium continues. Many media support reasonably good growth of trypanosomes, but only when they are used in small volumes (5-20 ml.) and receive a massive inoculum. Some success was achieved with a chemically-defined medium to which yeast extract and whole blood is added. This medium yields good growth of trypanosomes when used in volumes of 250-500 ml.

Miss Lanham is investigating the biochemical aspects of trypanosomal antigens. Preliminary results suggest that the main exo-antigen of the Shinyanga " O " strain of *T.brucei* is a polypeptide-polysaccharide complex.

WHOOPING COUGH BACILLUS

Properties of the Two Protective Antibodies. Mr. Standfast and Dr. Jean Dolby continued their search for an *in vitro* test to distinguish the two protective antibodies of *Bordetella pertussis* (the intranasal protective antibody " IN " and the intracerebral protective antibody, " IC ") so far distinguishable only by *in vivo* tests (see Report 1960). Both antibodies are bactericidal *in vivo*; and *in vitro* in the presence of fresh plasma or serum, including that of the guinea-pig, or peritoneal exudate.

Experiments on Fractionation, salt sensitivity, rate of deterioration and on the effect of ethylene diamine tetra-acetic acid, strongly suggest that the factor rendering both antibodies bactericidal *in vitro* is complement. The first assumption (1960 Report) that the " IC " antibody required a factor other than complement was based on the inactivity of undiluted preserved guinea pig serum, in which the complement proves to have been reversibly inhibited by reason of the hypertonicity of the preparation.

The two bactericidal antibodies are distinguishable *in vitro* by their different requirements for complement, the " IC " antibody requiring more than that required for an equipotent amount of the " IN " antibody. Further, whereas the bactericidal effect of " IN " antibody can be detected only on media containing blood, that of " IC " antibody is detectable on media without it. The reason for this is not yet known.

The differential test is difficult to apply because both antibodies may be inhibited by other constituents. The inhibition of the antiserum which takes place only *in vitro*, is not due to agglutinins, anti-haemagglutins, antitoxin or antihistamine sensitizing factor, nor to any anti-complementary activity of the antisera. It may be due to blocking of the " IC " antibody by traces of the "IN" antibody, and *vice versa*.

Mr. Standfast and Miss Sampson continued the examination of haemophilus strains isolated from the eyes of trachomatous patients in the Gambia. In other surveys of this kind only two *Haemophilus* spp., *H.influenzae* and *H.aegyptius*, with an occasional intermediate form, were found. The proportion of types among 157 isolations from the Gambian patients were *H.influenzae*, 26%; *H.aegyptius*, 33%; Intermediate 1, 10%; and Intermediate 2, 31%.

Recently 450 isolates from 57 Gambian patients were examined; 62% of the patients carried one type of haemophilus, 29% carried two and 9% carried three types.

A method for rapid serological sorting of these strains by a simple gel-diffusion technique is being developed.

STREPTOCOCCUS PYOGENES

Dr. Schwab studied the chronic remittent lesions of dermal connective tissue produced by extracts of sonic disrupted group A streptococci. The results obtained with streptococcal protoplasts and L-forms, with strains of streptococci varying in cell wall components, and the detoxifying action of an enzyme lysing the cell wall, are consistent with the cell-wall polysaccharides being the toxic component. The binding of the toxin to skin tissue is rapid, and although reversed *in vivo* by specific antibody and an enzyme that destroys the serological activity of the polysaccharide, it is not reversible by the cell-wall lytic enzyme, which *in vitro* leaves polysaccharide fragments with serological activity intact.

INHERITANCE IN BACTERIA

Conjugation in Salmonella. Bacterial conjugation and gene transfer initiated by two different kinds of fertility factors were studied. The first type are the colicine factors, governing formation of colicines, antibiotics produced by many enterobacteria (Report 1959, 1960). The second kind of factor, F′, is a mutant form of the "classical" fertility factor, F, of *Escherichia coli*.

Conjugation Initiated by Colicine Factors. The last Report (1960) contained an account of this process, whereby lengths of chromosome longer than those transferred in phage-mediated transduction can be transferred from colicinogenic donor bacteria to non-colicinogenic recipient bacteria.

Miss Smith found, in crosses between sublines of *Salmonella typhimurium* strain LT 2, that genes which were found linked by phage-mediated transduction appeared to be linked in this analysis, e.g. H_1 determining the phase 1 flagellar antigen, and an *fla* gene controlling flagellar production. Moreover, linkage was demonstrated between genes too widely separated to behave as linked in transduction. This finding facilitated a tentative determination of the order of fifteen different genes in the salmonella chromosome which, like that of *Escherichia coli*, can be mapped as a loop able to open at any point during mating to form a linear chromosome.

Similarities of the chromosomes of *E.coli* and *S.typhimurium* were also studied by Dr. Howarth and Dr. Ozeki who found that the genes *str*, *isol*, *ara*, and *leu* have the same order in both species, *ara* and *leu* being closely linked.

The discovery of techniques for inducing gene transfer in *Salmonella* may clearly be of medical importance in allowing identification of unknown virulence factors by study of hybrids of species of differing virulence. Unfortunately, *S.typhimurium*, strain LT 2, on which most of the genetic analysis of *Salmonella* has been performed, is almost avirulent for mice. Dr. Meynell therefore isolated a number of auxotrophic mutants of a highly mouse-virulent strain of *S.enteriditis*, using the potent mutagen, ethyl methane sulphonate, described by Loveless and Howarth (Report 1960). Nutritionally exacting mutants of *S.typhi*, strain Ty 22, were kindly provided by Dr. T. W. Burrows (Microbiological Research Station, Porton). During the transfer of colicinogeny by cell contact with *S.enteriditis* as donor and *S.typhi* as recipient, conjugation readily occurred with more than 50% of recipient cells; but transfer of other genes was not observed.

Conjugation Determined by F′. The classical fertility factor F, of *E.coli* is usually transmitted alone from F+ to F− strains. Occasionally, the factor mutates and becomes linked to other markers such as *lac* (F'_{13}) or *gal* (F'_{8}) so that it can be recognised in *lac-* (lactose non-fermenting) or *gal-* (galactose non-fermenting) strains. These factors were studied by Dr. Hirota, and he and Dr. Meynell isolated strains of *S.typhimurium* and *S.enteriditis* which had acquired these factors after growth in mixed culture with F′+ strains of *E.coli*. In *E.coli*, the presence of F′ leads to transmission of genes unlinked to F′ at a relatively high frequency but apparently this does not occur in crosses either between sub-lines of the same *Salmonella* species or between different salmonella species such as *S.enteriditis* (F'_{13}) and *S.typhi*. The frequency of transfer of F'_{13} itself varies greatly in different crosses.

Genetics of Resistance to Colicine. Mr. Gratia studied one type of mutant of *E.Coli*, strain K12, resistant to colicine B, which is also tryptophane-requiring and resistant to colicines V and I, and to phage T_1. When the donor strain F+ *tryp*+ $(T_1—VBI)^r$ conjugates with the recipient F− strain *tryp-* $(T_1—VBI)^s$, all *tryp* + recombinants are $(T_1—VBI)^r$, indicating close linkage between these five markers. Their separation therefore requires methods with greater resolving power than that obtained by normal conjugation induced by the F agent. This is being attempted by transduction mediated by phage P1; and by analysis of the progeny of recombinant clones formed by conjugation, many of which appear to contain chromosomal fragments of both parents (i.e. they are unstable heterozygotes). In such heterozygotes the parental chromosomes have repeated opportunities of recombining over a long period so that uncommon recombinant types, such as are formed by cross-overs between closely linked markers, are more likely to be detected than in the usual form of conjugation where cross-overs must occur in a relatively short time.

Effect of Colicine Factor I on Resistance to Irradiation. Dr. Howarth found that colicine factor I substantially increased resistance to killing by ultraviolet irradiation. Noncolicinogenic strains were killed exponentially but *col* I+ strains gave a multi-hit dose-survival curve, the ultimate slopes of both curves being the same. The only other difference found between these strains was that after irradiation, the colicinogenic strains had a longer lag period before entering logarithmic growth than the non-colicinogenic. Factor I also increased the resistance to ultraviolet irradiation of *E.coli*, strain K12, lysogenised by phage λ, at least part of this effect being due to an inhibition of the induction by irradiation of vegetative phage growth.

Resistance to the mutagens, di-2-chloroethyl methylamine (HN2) and ethyl methane sulphonate, was only slightly increased by factor I. Factor I did not affect the resistance of *S.typhimurium cys-36* to X-rays in the dose-range, 0-9,600 r. (the latter killing *c.* 90% cells).

Genetic Control of Flagellation in S.typhimurium. Mutants of *S.typhimurium* possessing varieties of the normal phase I antigen, *i*, were further studied by Mr. Joys. The mutants isolated had undergone mutation at 8 different sites in the H_1 locus (Report 1960). The order of these sites in relation to the neighbouring *fla* loci was determined by crosses between pairs of different mutants mediated by phage transduction.

The *fla* locus is genetically complex (Report 1959). In the sixteen non-motile (*fla-*) strains used in his work on the *i* variants, Mr. Joys found that the mutations fell into five functionally different groups (cistrons), one of which was divisible into three sub-groups. The order of five *fla-* mutants was determined in relation to the H_1 locus.

N-Methyl-lysine in Salmonella Flagellar Protein: Genetics and Serology. Dr. Stocker and Dr. McDonough, in collaboration with Dr. R. P. Ambler (Department of Biochemistry, University of Cambridge), continued their work (Report 1960) on the genetic control of the presence or absence of ε-N-methyl-lysine (NML) in salmonella flagellar protein. Chemical analysis of flagella produced by bacterial hybrids (obtained by phage-mediated transduction) led them to conclude that the presence or absence of the uncommon amino-acid in *both* of the flagellar proteins made by a diphasic salmonella strain is determined by a gene which is closely linked to, but distinct from, the H_1 gene determining the antigenic character of the phase 1 flagellar protein; and which is unlinked to the H_2 gene determining the phase 2 flagellar antigen. It is generally believed that the whole composition (i.e. the complete amino-acid sequence) of a protein made up of a single polypeptide chain is determined by a single gene. The present work shows that the composition of salmonella flagellar protein, however, is determined by two genes. One gene, it seems, specifies the sequence of common amino-acids used in building up the polypeptide chain; and the other gene determines whether NML, the uncommon amino-acid, shall be present. It is believed that this is the first instance in which the genetic control of the presence or absence in a protein of an amino-acid other than one of the twenty common ones has been demonstrated. The results are consistent with the hypothesis that only twenty different amino-acid specifying "signals" exist in the genetically active nucleic acids, in which, in all organisms, the specification of the protein is embodied.

One hypothetical function of the NML gene is that it determines the production of an enzyme which transfers a methyl group from some methyl donor, probably activated methionine, to the ε-amino group of lysine already incorporated in a polypeptide chain. Dr. McDonough, however, could not detect the known methyl donor, S-adenosyl-methionine, in extracts of NML-positive salmonella.

Dr. Stocker and Mr. Joys are comparing the serological characters of flagella differing only in the presence or absence of NML in the flagellar protein. The presence of NML in flagella of several antigenic types introduced a new specificity into the corresponding H antisera. Conversely, NML-negative flagella carrying H antigen *1, 2* (but not those with antigen *f,g*) have a specificity lacking in their NML-positive form.

BACTERIAL PHYSIOLOGY

Chemistry of Flagellar Protein. Dr. McDonough continued his examination of enzymic digests of flagellar proteins (flagellins) by chromatography and high voltage electrophoresis, and obtained reproducible peptide maps with respect to strongly staining spots.

Definite differences between flagellins possessing antigens *i* and *1, 2, 3* were found in the basic peptides in tryptic and chymotryptic digests.

Peptide maps of digests of flagellins carrying antigen *i* and certain of its varieties differ little but there is scope for better resolution. Any chemical differences between these proteins are probably slight—perhaps involving only one amino-acid residue—and unless they are large, will be difficult to detect. By one-dimensional electrophoresis, enzymic digests of *b*, and *e, n, x* flagellins are similar to *i* and *1, 2* flagellins. The latter differ substantially from the *g* series and from the flagellins of *B.subtilis*.

Cell-wall Polysaccharides of Salmonella. Dr. Stocker confirmed Japanese reports that *S.typhimurium* mutants unable to interconvert galactose and glucose (UDP-gal-epimerase mutants), make a cell wall polysaccharide containing only glucose instead of the polysaccharide containing glucose, galactose, rhamnose, mannose and abequose produced by normal bacteria of the species. It is thought that this glucose polymer may be present as a "back-bone" in the normal polysaccharide, with side-chains containing galactose and other sugars acting as determinants of the antigenic character of the polysaccharide and so of the somatic antigen of the bacteria (see Report, 1960 for the immunochemical and genetic studies of somatic antigen 1). Dr. Stocker and Mr. Abdullah are therefore studying the polysaccharide produced by mutant bacteria of this sort. Polysaccharide obtained by acid hydrolysis of a Boivin type extract contains only glucose, with a preponderance of β-linkages.

Measurement of Bacterial Division Rates in Infected Animals. The apparent rate of bacterial division as obtained by viable counts on material from infected animals may be less than the true rate *in vivo* where the bacteria are affected by bactericidal mechanisms of their host. The true rate can be measured by two methods studied by Dr. Meynell.

The first entails superinfection of lysogenic bacteria by a mutant of their prophage. Many phage-bacterium combinations were found unsuitable, for the bacteria die after superinfection, but *S.java*, strain SW 546, lysogenised by phage A2c and superinfected by phage A2e, appears satisfactory.

The second method uses histidine-requiring bacteria, some of which have been made transitorily histidine-independent following abortive transduction (Report 1955, 1956). The number of generations occuring in a given time is obtained by comparing the proportion of abortively transduced cells in the bacterial population with the initial proportion. This method was previously considered impractical, principally because abortive transductants usually occur very rarely. However, their frequency in histidine-requiring cultures is often 0.5% which is adequate for the experiment.

ABACTERIAL URETHRITIS IN MAN AND RELATED DISEASES

Dr. Klieneberger-Nobel and Dr. Lemcke, in collaboration with Dr. G. W. Csonka of St. Mary's Hospital, extended the investigation on the association of pleuropneumonia-like organisms (PPLO) with salpingitis. Sera from 35 patients with salpingitis were examined for PPLO antibodies; two thirds were positive. As a positive serum reaction is rare in healthy controls, these figures suggest that PPLO are often the cause of this condition.

A search for various types of PPLO on the external genitalia of healthy human beings was concluded. Although a few other workers have reported the isolation of saprophytic strains from these sites, only one isolate distinct from the common human genital type was found during this investigation.

The origin of PPLO contaminants found in tissue cultures is a subject of current controversy. Dr. Lemcke isolated PPLO from nine tissue cultures from seven different laboratories, including cultures of embryo rabbit kidney, monkey kidney and HeLa cell lines. All these PPLO isolates belong serologically to the common human genital type. It seems likely that HeLa cells, originally derived from a cancer of the uterus, were infected with PPLO from the source and that other tissue cultures have become infected by cross-contamination. The result is inconsistent with the widespread idea that PPLO found in tissue cultures are derived from bacterial contaminants which, under the influence of antibiotics, have been transformed first to L-forms and then to PPLO.

The studies of PPLO infection in rats and mice were concluded. Of the two PPLO diseases investigated in rats, the rat lung disease (bronchopneumonia) is a localised infection, and the other, the polyarthritis, is more generalised. Polyarthritic infections are associated with high specific serum titres, whereas the localised lung disease, even when severe, produces a limited although distinct antibody response. A low to moderate PPLO antibody level is often detectable in the serum of human patients with genital tract infections. In this respect the rat lung disease resembles the human genital infection. Although it was not possible to study the antibody response at different stages of human infection in man this was done in the rat lung disease. Little or no antibody was present in the serum of very young rats when PPLO were confined to the nasopharynx, but titres rose when the lungs became infected, although never to the high levels found in polyarthritic infections. Specific antibody to two distinct PPLO were demonstrated in naturally and experimentally infected mice. As in rats the antibody titres are proportional to the extent and severity of the PPLO infection. These animal experiments suggest that PPLO antibody in the human patients also indicates PPLO infection.

During the year PPLO sent by a number of laboratories were typed by the complement-fixation test.

VIROLOGY

TRACHOMA AND INCLUSION BLENNORRHOEA

These two viruses have proved to be so similar in their serology and growth characters that they are considered under the same heading.

Growth in Cell Cultures. More strains of virus were adapted to grow in a variety of cells with formation of inclusions. Dr. Furness, Miss Graham and Mr. Reeve showed that the distribution of inclusions is random in HeLa cell monolayers infected with suitably diluted virus suspensions, and devised a titration method in which the infected cells in a given area of monolayer are counted by low power microscopy. This method has a standard error of $\pm 10\%$, and is much more accurate than assays with chick embryos. It was used by Dr. Furness and Miss Fraser to investigate the replication of inclusion blennorrhoea virus in HeLa cells. Since free virus is rapidly inactivated at 37°, adsorption to cells is best done at 30°. During subsequent incubation at 37°, less than 1% of the infectivity of the original inoculum can be recovered from disrupted cells during the first 24 hours. Thereafter, the amount of intracellular infective virus rapidly increases; at least thirty infective units are eventually formed by each infected cell. These experiments, which are continuing, also yielded valuable information about the stability of the virus at different temperatures.

Serological Studies. Mr. Reeve and Miss Graham showed that sera from immunized rabbits neutralized the growth of virus in cell cultures. The antisera have low neutralization titres; attempts are being made to obtain more potent antisera and to investigate antigenic differences among virus strains. Purified virus injected intracutaneously into guinea-pigs induces lesions whose diameter is proportional to virus concentration; since, however, the lesions are not affected by antiserum, the technique has been abandoned as unsuitable for demonstrating antigenic differences.

Dr. Taverne and Mr. Reeve are studying the complement-fixing antigens of virus grown in cell cultures. They treated trachoma and inclusion blennorrhoea viruses with heat, periodate and lecithinase in an attempt to distinguish

these agents from other members of the psittacosis-lymphogranuloma group. Although a specific antigen could not be detected, their results suggest that the group antigen is a lipo-polysaccharide-protein complex, and confirm Benedict and O'Brien's work with detergent-extracted psittacosis virus. In collaboration with Dr. Collier, they are investigating the serological reactions of baboons immunized with experimental trachoma vaccines.

Trachoma Vaccine. To test the efficacy of trachoma vaccines, a primate host is inoculated and the response to subsequent infection of the eye with a pathogenic strain of virus observed. Since simians are liable to so-called "spontaneous" folliculosis which can be confused with specific infection, it is essential to test their immunity with a strain of virus capable of inducing an easily recognizable clinical syndrome, and formation of typical inclusion bodies in the conjunctival epithelium. Dr. Collier tested the response of several species of monkeys and baboons to conjunctival inoculation with different strains of virus. An African strain of trachoma was insufficiently pathogenic, even when freshly isolated from a human volunteer (Report, 1960). A strain of inclusion blennorrhoea in its eighth chick embryo passage induced a severe conjunctival infection in baboons, but lost this property during subsequent transfers through chick embryos. Another inclusion blennorrhoea virus (strain LB4) in its fourth chick embryo passage satisfied the above requirements, and a stock of this material was prepared for use in subsequent vaccination experiments.

Dr. Collier showed that a live antigen made from chick embryos infected with LB4 virus, given as two subcutaneous and one intravenous dose spaced at weekly intervals, conferred virtually complete protection against conjunctival inoculation with a highly infectious preparation of the same strain. Three subcutaneous doses conferred partial immunity. These experiments demonstrated the feasibility of parenteral immunization against viruses of the trachoma-inclusion blennorrhoea group. With Dr. Blyth, Dr. Collier is investigating the use of live and killed antigens prepared from various strains of virus, with the object of producing a vaccine suitable for field trial in the Gambia. Dr. Blyth, Miss Graham, Mr. Reeve and Dr. Taverne are seeking methods of purifying antigens for use in these researches, to which the serological and cell culture studies are also contributing.

Investigation of Trachoma in West Africa. The Medical Research Council Trachoma Unit, directed by Dr. Collier, completed the first stage of its investigation of trachoma in a Gambian village. The results, now being analysed in detail, provide epidemiological, clinical and microbiological data essential for staging a field trial of trachoma vaccine. The Unit is now preparing for this trial, which is expected to start this year. As part of this study, children below the age of 15 years diagnosed as trachomatous during 1959-60 are under observation to determine the natural course of the untreated disease in its active stages.

A clinical and virological investigation of infected babies and their parents is in progress, to determine whether their disease should be properly described as inclusion blennorrhoea or trachoma; and if trachoma, whether it exists as a genital infection of adults transmissible to infants at birth.

MEASLES

The viruses of measles, rinderpest and canine distemper are antigenically related, and their cytopathic effects on cultured cells are similar. Further evidence of close relationship was found in a joint investigation in which Dr. Payne collaborated with Dr. W. Plowright (East African Veterinary Research Organization) and Dr. S. E. Piercy (Wellcome Research Laboratories). These

workers demonstrated respectively that the viruses of measles, rinderpest and canine distemper are inactivated by exposure to 20% ethyl ether for 24 hours at 4°, and can thus be placed together in the ether-sensitive group of viruses.

Dr. Payne also investigated haemagglutination with measles virus, but found that this agent failed to agglutinate red cells from man, baboon, calf, sheep, rabbit, rat, mouse or fowl at 37° or 4°.

VACCINIA VIRUS

Tissue Culture Vaccine. The potency of smallpox vaccine prepared in cultures of chick embryo cells was stabilized in bulk storage by increasing the concentration of Na^+ in the suspending fluid. When dispensed in single dose capillary tubes, however, the potency of the vaccine diminished rapidly. Because of this, the clinical trials being made in two civilian vaccination clinics were terminated. This loss of potency has now been obviated by the simple expedient of incorporating 1% gelatine in the suspending fluid, and clinical trials are expected to recommence soon.

Mr. Robinson has defined the conditions for cultivation of chick embryo cells in constantly stirred suspensions, and successfully maintained cultures for periods of up to 10 days. Preliminary experiments in the propagation of vaccinia virus to high titre in such cultures have given promising results.

Anti-vaccinial Antibody. Dr. Kaplan hyperimmunized a series of sheep against vaccinia virus and Dr. Mackay fractionated their sera. It is intended to offer a dried gamma globulin to the World Health Organization as an international reference preparation for the assay of anti-vaccinial antibody for use in the treatment of smallpox.

Dr. Murray has begun to determine whether there is any qualitative difference between antibody produced early and late in vaccinial infection.

Titration of Vaccinia Virus. Dr. Murray, with Mr. Robinson, is investigating the suitability for titration of vaccinia virus of the so-called haemadsorption phenomenon in cultures of chick embryo cells. The method, if sensitive enough, will be more convenient than the pock count titrations now used, and may lend itself to the titration of anti-vaccinial antibodies.

IMMUNOLOGY AND SEROLOGY

SEROLOGICAL IDENTIFICATION OF INSECT BLOOD MEALS

Feeding Habits of Blood-sucking Insects. The study of the natural feeding habits of tsetse flies was completed this year after the identification of over 25,000 blood meals from twelve different species of *Glossina* captured in various areas in all parts of East and West Africa over the last five or six years. The results are too complex to be detailed but it is evident that each species of fly, from whatever area, has specific feeding habits. Different preferences of various species of flies were found for animals in the same area, while some animals (e.g. zebra) were never fed on by any fly. These findings suggest that the feeding preferences of *Glossina* are genetically determined and are not mainly the result of opportunity.

ANTITOXIN PRODUCTION

Proteolytic Enzymes for Refining Antisera. Dr. Dolby fractionated pepsin on ion-exchange resins (see 1960 Report) and tested the separated components on

antitoxic horse sera. Parapepsin I is without action on serum proteins; parapepsin II hydrolyses them but is present in so small a proportion in the purified pepsin used in refining antitoxic sera that its total effect is negligible. The activity of pepsin in the refining process must therefore be attributed to pepsin itself and not to either of these other enzymes.

Refinement of Therapeutic Antitoxins. The use of modified cellulose ion-exchangers makes it possible to isolate fractions from protein mixtures in fairly large quantities. Dr. Dolby used this method to obtain from horse sera pure γ-globulin, and a β-globulin fraction which has not yet been freed from α-globulins. Antitoxic activity is present in both these fractions from hyperimmune horses.

Degradation experiments with mixtures of bovine albumin and γ-globulin showed that, despite the speed with which the albumin is degraded by pepsin, 10—15% of it remains in the material after refining; 40% of the γ-globulin remains intact. The effect of pepsin on the antitoxin content and physical and chemical properties of fractions from horse sera is now under study.

EXPERIMENTAL PATHOLOGY

MECHANISMS OF INFLAMMATION

Vascular Permeability Changes in Injury. Dr. Mill determined the time course of increased permeability and increased endothelial stickiness during the first eight hours of a number of bacterial infections in the skin of rabbits. As in the guinea-pig, the main response was delayed, but there was evidence, in some infections, of two or more phases of increased permeability in this response. There was also evidence of slightly increased amounts of globulin permeability factor in the intercellular spaces during the delayed response. All attempts, made in collaboration with Miss Mason, failed to demonstrate kininogenases or kinin in intercellular perfusates of infected lesions. Of the various infecting agents studied only a strain of *E.coli* contained detectable amounts of easily released substances increasing vascular permeability.

Identity of Globulin Permeability Factor and Plasma Kininogenases. Miss Mason completed an assay of fractions containing permeability factors from human, rat, rabbit and guinea-pig serum, including preparations of human kallikrein from Dr. Marion Webster of the Walter Reed Army Institute of Research, Washington, U.S.A., for hypotensive effect in guinea-pig, rat and rabbit; and concluded that within the rather wide limits of error of the method, hypotensive activity in general was proportional to permeability factor content. The human permeability factor was also a kininogenase but no kininogenase activity could be detected in fractions of guinea-pig serum until a very heavy contamination with a powerful bradykininase was removed. For identification of the globulin factor with serum kallikrein, it remains to devise a reliable assay of kininogenase to test the proportionality, in preparations of globulin factor and of kallikrein, of permeability-increasing, hypotensive and kininogenic potency.

Globulin Factors in Shock. With Professor Miles, Miss Mason investigated the plasma of guinea-pigs in severe, reversible shock induced by intraperitoneal hypotonic glucose. No gross change, either in activable permeability factor, hypotensive factors, or inhibitor of the permeability factor was detected.

The Activation of Permeability Factor in Serum. Professor Miles studied the permeability factor in human and guinea-pig sera activable by dilution, by contact with foreign surfaces, including antigen-antibody complexes. The same type of factor appears to be activated in all cases. The time course of activation by

dilution and by contact with glass appears to be determined not only by the content of profactor and inhibitor of the factor, but to some extent by the presence of other inhibitors, including perhaps bradykininases.

An association of permeability factor and anti-haemophilic factor in fractions of human plasma prepared for the treatment of haemophilia was found to be accidental, the two activities being independent. Moreover, as judged by the activability by dilution, the plasma of haemophiliac patients is normal with respect to permeability factor and its inhibitor.

Dr. Schwab investigated the anti-bradykinin action of blood exudate leucocytes of the guinea-pig and of blood leucocytes in man. Sonic disintegration of leucocytes in both species, and especially of guinea-pig macrophages, releases moderate amounts of a substance with properties similar to the plasma bradykininases.

MECHANISMS OF INFECTION AND DEFENCE

Non-specific Immunity. Miss Brimacombe, with Professor Miles, studied the resistance to intracutaneous infection by wholly unrelated organisms, during the course of a streptococcal septicaemia in the guinea-pig. Non-specific immunity rises within hours of intravenous inoculation of the infecting streptococci, and with fluctuations dependent on the resistance to the septicaemia, persists during active infection, declining just before death, or with recovery. No single factor has been detected as the cause of the increase.

Miss Brimacombe continued her attempts to demonstrate microbicidal activity of tissue cells other than microphages and cells of the lymphoid-macrophage system.

BIOCHEMISTRY

THE HUMAN BLOOD-GROUP SUBSTANCES

The substances which are responsible for blood-group specificity are complex mucopolysaccharides of high molecular weight. About 80% of these macromolecules is carbohydrate and the remaining part is built up of amino-acids. Previous work has shown that the serological specificity is determined primarily by the chemical structure of the carbohydrate portion. During the year, Dr. Painter and Professor Morgan examined procedures which will allow the carbohydrate structures in the group specific substances to be fragmented into suitably sized units, the detailed structure of which can then be determined. The ordinary approach through partial acid hydrolysis with mineral acid is unsatisfactory because the yields of suitable fragments are low and the scission of the glycosidic linkages is accompanied by N-deacetylation of the amino sugar residues. After studying the kinetics of degradation of various model compounds by polystyrene sulphonic acid, the changes induced by this reagent proved to be quite different from those obtained with mineral acid. The specific blood group substances yielded much larger amounts of oligo-saccharides, and N-deacetylation of the amino sugars was considerably decreased. The polystyrene sulphonic acid is water-soluble and is a macromolecule which does not diffuse; so that the small molecular weight products of degradation of the group substances are readily recovered free from the hydrolytic agent. An apparatus was designed to bring about the rapid and continuous removal of the diffusible products which were then no longer exposed to further degradation. The results with inulin as a model substance confirmed the superiority of the method over those usually

employed to fragment polysaccharide, and the procedure is now being used to study the partial hydrolysis products of the specific blood-group substances.

Dr. Pusztai and Professor Morgan continued their work on the amino-acid-containing moiety of the specific blood-group substances and made a number of quantitative amino-acid analyses of materials of high purity and of the fragments obtained after they were degraded by the enzymes papain and ficin. The fragments were closely similar in chemical composition to the original substance, but inexplicably lost much of their serological activity, as measured by haemagglutination inhibition tests. The serological properties appear to be determined by the nature, linkage and sequence of certain of the carbohydrate units and these determinants were intact after incubation with ficin and papain. These results give some support to the idea that the biological properties of the specific mucopolysaccharides are strongly influenced by the overall conformation of the macromolecule and that the maintenance of a regular relationship between those carbohydrate structures which specifically combine with antibody and other parts of the macromolecule depends on the structural integrity of the amino acid-containing moiety.

Dr. Watkins extended her studies on the isolation and purification of the enzymes from *Trichomonas foetus* and succeeded in obtaining preparations of blood-group-destroying enzymes which brought about a selective and stepwise modification of A or B substance to an H specific material almost devoid of A or B specificity. This material could then be converted to an Le^a active substance and finally to a material which was not serologically active for any of the normal group specific characters, but which reacted strongly with Pneumococcus Type XIV anti-serum. These findings give strong experimental support for the scheme for the biosynthesis of the human blood-group substances suggested earlier by Dr. Watkins and Professor Morgan.

In an attempt to determine the nature of any reducing sugar end-units in the blood-group specific mucopolysaccharides, Dr. Doyle reduced the substances with borohydride, hydrolysed the products with weak acid and fractionated the sugar fragments. No sugar alcohols were detected, and it is concluded that if reducing-sugar end-groups exist in the group specific substances they account for less than 1% of the mucopolysaccharide molecule.

Dr. Uhlenbruck studied methods for the recovery of substances responsible for the M and N blood-group serological specificity of the human erythrocyte. M or N-active stromata preparations in saline were extracted at 60° with 45% phenol. The mixture on cooling separated into two layers, the upper layer or water-phase of which contained a material which was strongly M or N specific. The substances contained about 7% nitrogen, 6% amino sugar, 14% sialic acid and 20% total carbohydrate. The M substance has a weakly active N specificity. Specific precipitation of this material with a mono-specific anti-M serum and recovery of the M substance gave an M active substance which retained its original N character. It seems probable, therefore, that the N reactive structure is part of the molecule responsible for M specificity. Mumps virus, which did not destroy the M N specific structures on the intact erythrocyte surface, nevertheless released 30% of the sialic acid of the M substance. Crystalline neuraminidase liberates the whole of the sialic acid. The destruction of the M or N specificity of erythrocytes by neuraminidase renders the cells pan-agglutinable and the new serologically reactive structure which is formed is the T agglutinogen.

CARBOHYDRATE STUDIES

Chemical Synthesis of Modified Starches. Three chemically modified amyloses were prepared. These are 6-deoxyamylose, 3,6-anhydro-amylose, prepared

by Dr. Bines, and a polymer of glucose and altrose, prepared by Dr. Bines and Dr. Gunja. The degrees of conversion of the glucose units into their various modifications are 90%, 60% and 50%, respectively, and the modified sugar units were isolated and characterized. α-Amylase readily attacks 6-deoxyamylose but not 3,6-anhydro-amylose. Neither potato phosphorylase nor β-amylase attacks the two polysaccharides. The extreme insolubility and chemical inertness of the altrose polymer have so far precluded a study of its behaviour with enzymes. Dr. Gunja also prepared an amylose specifically substituted at the C-3 hydroxyl by a methyl group.

A further modification of amylose, made by Dr. Bines, is the formation of deoxy groupings at C-3 and C-6. Preliminary observations indicate that amylose can be converted into a polymer of 3,6-dideoxyglucose. This sugar is important as a determinant of immunological specificity in certain *Salmonella* polysaccharides.

Chemical and Enzymic Synthesis of Glucose Phosphates. This work by Dr. Whelan and his colleagues is designed to yield information on the specificity of carbohydrases. Mr. Egyud is synthesizing the 6-phosphates of modified glucoses to study the actions of enzymes normally acting on glucose 6-phosphate itself. Some of the compounds are prepared by chemical synthesis, e.g. 2- and 3-*O*-methyl glucose phosphates; others such as the 6-phosphates of allose, altrose, 2- and 3-deoxyglucose are prepared by enzymic procedures. New analogues of glucose were prepared in which some of the oxygen atoms have been replaced by sulphur.

Chemical Synthesis of Haptens and Serological Inhibitors. Dr. Allen and Mrs. Taylor prepared two types of substance for studying the mechanism of the dextran-antidextran precipitation reaction. First, sugars were combined severally to polyhydric alcohols so as to form the "multivalent" molecules thought to be necessary for precipitation with dextran antibody. Second, the isomalto-dextrins, potent inhibitors of the precipitation reaction, were chemically modified at their reducing ends. The effect of the modifications are under test by Dr. E. A. Kabat at the Columbia-Presbyterian Medical Center, New York. Dr. Allen oxidised some of the primary hydroxyl groups in the glucose units of dextran, producing glucuronic acid. As was expected, the dextran thereby acquired cross-reactivity with antiserum of Type II pneumococcus polysaccharide. Dr. Goldstein synthesized 6-*O*-α-glucosylgalactose, which proved to be identical in chemical and serological inhibition tests with a disaccharide obtained from a *Salmonella* polysaccharide by Dr. A. M. Staub of the Institut Pasteur, Paris.

Structures of Polyglucoses. Dr. Goldstein isolated and characterized the first disaccharide other than isomaltose to be obtained from a dextran. This is nigerose, 3-*O*-α-glucosylglucose, obtained in very high yield by subjecting dextran to partial acetolysis instead of the usual partial acid hydrolysis. A new trisaccharide was also obtained, having the structure 3-*O*-α-isomaltosylglucose and Dr. Goldstein and Mr. Abdullah confirmed its structure by enzymic synthesis from nigerose, using potato T-enzyme, newly discovered by Mr. Abdullah (Report, 1959). Dr. Goldstein also found evidence of a regular repeating unit within the Iceland moss polysaccharide isolichenin, and he and Mr. Abdullah showed that the blue iodine stain of isolichenin, long thought to be characteristic of the polysaccharide, is due to contamination by starch.

Starch-Metabolizing Enzymes. Mr. Abdullah made further studies of the specificity of potato T-enzyme and found it to use phenyl α-glucoside as a substrate from which glucose units can be transferred. This is a far more convenient and accessible substrate than any previously discovered.

Mr. Smith is examining the structure of a branched pentasaccharide liberated from amylopectin by *Bacillus subtilis* α-amylase. The structure appears to be unique within the group of branched saccharides formed by different α-amylases from amylopectin. It was also proved that highly purified and crystalline *B.subtilis* α-amylase exerts a synthetic as well as hydrolytic action when acting on starch-type substrates. The cause of the synthetic action, the amylase itself or an impurity, is being investigated.

Enzymic Polymerization of Monosaccharides. Dr. Clancy found that acetone-dried yeast couples galactose to *N*-acetylglucosamine (Report, 1959). The products have been identified as 6-*O*-α-galactosyl-*N*-acetylglucosamine and the 3-*O*-isomer.

TISSUE PHOSPHOLIPIDS

The object of this work is to examine the structure and distribution of phospholipids in different tissues and cells and in sub-cellular components, and to attempt to relate the findings to the function of the phospholipids.

It appears probable that the family of phosphatidylglycerols, to which cardiolipin (diphosphatidylglycerol) belongs, is intimately linked with the respiratory activity of osmotically-active sub-cellular particles. It was noted previously that in rat liver the diphosphatidylglycerol is concentrated in the mitochondria, which carry the cytochrome oxidase activity. Dr. Macfarlane has now found that about 70% of the phospholipid in the protoplast membrane of *Micrococcus lysodeikticus* is diphosphatidylglycerol; the protoplast membrane carries the main oxidising enzymes of the bacterial cell. In contrast to the cardiolipin of animal cells, which contains only unsaturated fatty acids, mainly the "essential" linoleic acid, the phospholipid of *M.lysodeikticus* contains branched chain saturated acids, mainly the *ante iso* and *iso acids*, 12-methyl and 13-methyl tetradecanoic acids, so the biological activity of the phosphatidylglycerols is apparently not dependent on the presence of unsaturated acids. The protoplast membrane also contained a mannose-containing phospholipid and a diglyceride with similar branched chain acids, and a small proportion of phosphatidylinositol.

Dr. Gray has studied the phospholipids in pig kidney, lung and spleen. The major component in all these tissues is phosphatidylcholine (lecithin), but the major aldehyde-containing phospholipid is ethanolamine plasmalogen (8—15%), choline plasmalogen and serine plasmalogen occurring only in small amounts. The fatty constituents fell in the pattern previously noted in ox spleen, ox heart, pig heart and ram semen, the saturated acids having a chain length of sixteen carbon atoms in the lecithins and eighteen carbon atoms in the kephalins. The aldehydes from the choline plasmalogen of ox spleen differed from those of pig spleen in containing a considerable proportion of saturated branched chain compounds, of twelve to seventeen carbon atom chain lengths, of the *ante iso* or *iso* series, predominantly 12-methyltetradecanal; this difference may be a species specificity or possibly due to a difference in diet. The structure of these unusual aldehydes was determined by identification of the acids formed on oxidation, using the gas chromatographic technique.

The composition of a phospholipid mixture isolated from a tissue is frequently deduced by identification and estimation of the products obtained by treatment with mild alkali, followed by mild acid. Various workers have noticed discrepancies with mixtures containing plasmalogens. Dr. Pietruszko and Dr. Gray have therefore studied the hydrolysis of choline- and ethanolamine-plasmalogen, using a standard method of estimation of the aldehyde component

which involves the liberation of the free aldehyde in acetic acid, followed by condensation with the Feulgen reagent. Results with the method indicated that the product of alkaline hydrolysis was a mixture of the expected lyso-plasmalogen —an αβ-unsaturated ether structure labile in acetic acid—and a saturated cyclic form relatively stable in this acid. However, when the product of alkaline hydrolysis was hydrogenated, the aldehyde test became negative, i.e. all the aldehydogenic material in the alkaline hydrolysis product was present as the αβ unsaturated lysoplasmalogen. Part of this is converted during the subsequent treatment with acetic acid to the more stable cyclic form. The presence of the two forms must be taken into account in the usual technique for the analysis of phospholipid mixtures.

In the different mammalian tissues so far examined, the content of choline plasmalogen varies greatly and is notably high in muscle. Dr. Macfarlane and Mr. Shaw found that the myofibrils of rabbit skeletal muscle contained the same proportion of choline plasmalogen (22% of the total phospholipid) as the whole muscle. Pigeon breast and heart muscle contained 13%. However, the presence of choline plasmalogen is apparently not directly associated with contractility, for trout muscle contained virtually none.

The accumulated analysis of phospholipids from a variety of normal tissues and cells will serve as a basis for the detection of abnormalities in pathological tissues, particularly in a study of malignant tissues now being undertaken.

BIOPHYSICS

A Spinco analytical ultracentrifuge is now being installed. The interferometric optical system available with this instrument and other ancillary equipment will widen the scope and precision of sedimentation measurements made. The Svedberg oil turbine ultracentrifuge installed in 1936 will be used for more routine measurements.

The diffusion equipment has been extensively modified by Dr. Creeth and fitted with a Rayleigh interferometric optical system, to enhance the utility and accuracy of the apparatus, particularly for studying the dependence on concentration of the diffusion coefficient of macromolecules.

A modified toolmaker's projecting microscope, capable of measuring directly to 2 microns in two directions at right-angles, was installed for the interpretation of Rayleigh interferometric patterns obtained from sedimentation, diffusion and electrophoresis experiments.

HUMAN PLASMA PROTEINS

Antihaemophilic Factor. The difficulties (Report 1960) of assaying antihaemophilic factor in whole human plasma by the method devised by Mr. Walton and Dr. Kekwick have been overcome. Whole plasma and separated plasma fractions can be directly compared, by dissolving the fractions initially in a small amount of plasma from a severe haemophiliac, before making the dilutions appropriate to the titration. Direct comparison of this kind is important because freeze-dried concentrates of antihaemophilic factor are far more stable than plasma under conditions of storage.

The assay was adopted by the M.R.C. Working Party on Human Antihaemophilic Globulin, as the definitive method for use at the centres carrying out clinical trials of the human antihaemophilic factor concentrates produced at the Blood Products Laboratory, Elstree.

In human plasma the antihaemophilic factor has a sharply optimum stability at pH 7.2. In concentrates it has an optimum stability at pH 7.1. The result suggests, contrary to published information, that the lability of antihaemophilic factor is not due to its destruction by plasmin.

In attempts to obtain more potent preparations of human antihaemophilic factor suitable for intravenous injection, exploration has been restricted to procedures operable under aseptic conditions. Many were tried; a 10% increase in recovery was attained by adjusting the plasma to pH 7.1 from 7.5-7.6 for the initial precipitation of the fibrinogen—AHF fraction. By extracting at low ionic strength at pH 7.1, later in the purification, the potency was doubled, with a 90% recovery in activity, providing material about 50 times as potent as plasma on a dry weight basis. By this procedure about half the fibrinogen of the initial fraction is also recovered separately with a purity of 85% in terms of protein clottable with thrombin.

Attempts to repeat the procedure of M. Blombäck, by which concentrates one hundred times as potent as plasma are said to be produced by using glycine-citrate buffers in the presence of ethanol, were unsuccessful.

Methods of purification that could not easily be applied aseptically are now under examination, to provide purer material for a sharper definition of the nature of antihaemophilic factor, as a guide to other possibilities for aseptic fractionation.

Plasminogen. Dr. Derechin extended his studies of the proteolysis of purified alpha$_1$, alpha$_2$ and beta casein by streptokinase-activated human plasminogen. The hydrolysis curves of the fractions suggested that with α_1-casein large fragments are formed initially which are subsequently broken down into smaller acid-soluble products, whereas during the hydrolysis of α_2-casein small acid soluble products are liberated immediately hydrolysis begins.

The purification of human plasminogen from the plasma of out-dated blood, was carried further using adsorption methods with concentrates obtained by solvent-fractionation. By the use of cellulose ion-exchange resins, and including lysine in the eluting buffers, a 400-fold purification was obtained with a 70% yield of plasminogen. The product, unlike those produced by variations of the Kline procedure, which includes extraction at extreme pH values, 3 and 11, is freely soluble at pH 7.0, and preliminary measurements indicate that it is homogenous in the ultracentrifuge.

Pathological sera. In a collaboration with Dr. F. V. Flynn of University College Hospital to establish for diagnostic purposes whether certain pathological sera contain excess amounts of macroglobulin components, recently examined sera fell into two categories. In one a small proportion of a still heavier component is present in addition to the main macroglobulin constituent, which itself may account for as much as 40% of the total serum protein. In the second there is only a single macroglobulin representing about 10-15% of the total serum protein.

Professor Martin continued his ultracentrifugal and electrophoretic examinations of hyperglobulinaemic sera.

BLOOD GROUP SUBSTANCES

Dr. Creeth began a detailed physico-chemical investigation of blood-group H substance. By differential preparative centrifugations a fraction was obtained displaying essentially one component on ultracentrifugation and electrophoresis.

This fraction is being examined to specify any heterogeneity quantitatively and to define the concentration-dependence parameters relating to sedimentation coefficient and apparent molecular weight. The first is essential for the application of hydrodynamic theories, and the second gives information about the thermodynamic properties of the solution: both sets of data can be interpreted, in principle, in terms of molecular shape and configuration.

BLOOD PRODUCTS LABORATORY

Pharmacologically Active Substances in Plasma. Dr. Mackay and Dr. Maycock continued to investigate these substances. Dried anti-haemophilic factor, as prepared for clinical use, when dissolved in a hypertonic buffered citrate saline solution and incubated, produces a smooth muscle contracting substance activity. The effect is delayed by soya bean trypsin inhibitor. There appears to be a kininogen in these preparations which is slowly activated to form a plasma kinin. There is at present no direct evidence of the mode of activation, but the presence in this fraction of plasminogen and the increase of smooth muscle contracting activity and of acid soluble tyrosine when the fraction is incubated with streptokinase, suggest that kinin production may be mediated by plasmin, although there is no direct correlation between the time courses of the two reactions.

Combination of Citrate with Albumin. Professor C. Rimington observed that albumin fraction AP_2, used for transfusion, contains an appreciable quantity of citrate. Since this might be of clinical significance the citrate content of human plasma fractions prepared for clinical use by precipitation with ether was investigated. Samples of typical plasma pool, from which these fractions are separated, contained 100-160 mg. citrate per gm. protein; gammaglobulin separated from it contains less than 1 mg., albumin AP_2 16-19 mg. and albumin AP_3 about 25 mg. per gram. The bulk of the original citrate in the starting plasma remains on the residues discarded during fractionation, but 10% is contained in the albumin fractions, the ratio of citrate to protein rising during the purification of albumin. The reason for the inclusion of this large amount of citrate in the albumin remains to be investigated.

Plasminogen. Continuing the investigation of proteolytic enzymes in human plasma (Annual Reports 1959, 1960), Dr. Mackay examined their heat stability. Proteolysis by streptokinase-activated plasminogen was estimated from the rate of hydrolysis of casein and fibrinogen and of fibrin clot lysis. The heat stability of the enzyme was determined in 1% concentration of protein in glycine and phosphate buffers over a pH range 3.8-7.0 at temperatures from 40°—60° for periods of 15 to 240 minutes. At pH 7.0 and temperatures above 45° there is a progressive slow destruction of enzyme; at 60°, 80% of the protein is precipitated. At pH 4.0 at 60° for 4 hours, there is no detectable denaturation or loss of caseinolytic activity, but fibrinogenolytic activity and clot lysis are progressively depressed. When crude plasminogen is precipitated with 25% ethanol at pH 5.3 a fraction is obtained, soluble in 0.1 M glycine, which can be heated at 60° for 4 hours without loss of caseinolytic or fibrinogenolytic activity.

Caeruloplasmin. Mr. Vallet simplified the method of preparing this fraction (Report 1959). For preparing approximately 1.0 gm. quantities G_2 precipitate is resuspended and extracted with ether. Crude caeruloplasmin is precipitated at pH 4.8 and redissolved in pH 5.7 acetate buffer at low ionic strength, from which it is adsorbed on diethyl amino ethyl cellulose. It is eluted with pH 5.2 acetate buffer in 0.25 M sodium chloride. A high yield of caeruloplasmin is obtained with a ratio $(E_{1cm,\ 610\ m\mu})/(E_{1cm,\ 280\ m\mu})$ between 0.020 and 0.024. For

preparing smaller amounts caeruloplasmin can be adsorbed on DEAE cellulose directly from fraction G_2 without preliminary extraction with ether.

The laboratory collaborated with the M.R.C. Blood Coagulation Research Unit on the separation of Christmas Factor from plasma and with the M.R.C. Working Parties on Hypogammaglobulinaemia and Human Antihaemophilic Globulin.

BLOOD GROUP RESEARCH UNIT

Once again most of the problems studied during the year began as cross-matching difficulties during preparation for transfusion. Again the majority of the samples were sent by colleagues in the United States. Such problems nowadays often involve very rare groups, which would not in themselves be particularly interesting did they not, quite frequently, give a glimpse of a larger pattern behind the common forms of the system to which they belong.

The ABO system. A very rare group known as "Bombay" or Oh, was investigated (Report 1952) in collaboration with Dr. Y. M. Bhende and his colleagues in Bombay and Professor Morgan and Dr. Watkins. Oh red cells have neither A, B nor H antigens and the serum contains anti-A, anti-B and anti-H. In 1955 a particularly informative family studied by Dr. P. Levine in New Jersey showed the condition to be due to the homozygous absence of a gene, named *X*, necessary for the appearance in red cells and saliva of the antigens B and H. Two families investigated in collaboration with Dr. H. Fudenberg of San Francisco and Dr. A. Cahan of New York have given the long awaited information that *X* is necessary also for the expression of the antigens A_1 and A_2.

The MNSs System. All Caucasians so far tested have the antigen S or s or both. One per cent, or less, of Negroes lack both S and s (Report, 1956); such S-s- people may become immunized by pregnancy or transfusion and make anti-Ss. Certain rare S-s- negro red cell samples are being investigated which, surprisingly, react with anti-Ss; this hints that suppressor genes, rather than a third allele of *S* and *s*, may be responsible for the S-s- condition.

The P System. A third family with the rare antigen P^k (Report, 1959) was investigated during the visit of Dr. A. E. Kortekangas, of Turku. Samples from over 100 members of the family were taken in Finland and sent to the Institute.

From the beginning P^k promised, rare though it is, to be of importance in the understanding of the P system as a whole. The previous two families had shown that P^k, unlike practically all blood group antigens, is not a straightforward dominant character. The latest family rules out the two possible genetical backgrounds suggested in the 1959 Report; and it raises the possibility that P^k may represent the product of a gene not belonging to the P locus but acting on a P precursor substance. If this proved true the P system would seem to be following the ABO type of genetical pathway illuminated by the work of Morgan and Watkins.

The Rh System. Ten years ago a very rare but informative Rh group was recognized in the Unit (Report, 1951): the red cells had only that part of the Rh complex called D, they lacked any representation of the parts called C and E. Further examples have since been found in other Caucasians, in Japanese and in North American Indians. Such people are brought to notice because of the complexity of the Rh antibodies they make when they become immunized by transfusion or pregnancy.

Early in the year samples were sent from a Louisiana family of French extraction which disclosed the existence of an Rh group having D and c but no

representation of E. This work was done in collaboration with Miss Hermine Tate of Lafayette and Dr. A. Cahan.

Later in the year a more surprising sample arrived, sent by Dr. R. L. Kirk and Mr. and Mrs. G. H. Vos of Perth; it was from an Australian aboriginal woman caught in an anthropological survey while prospecting for gold in the Western Desert. She has no Rh antigens at all: nor, unfortunately, has she any relatives.

On inspecting the records of the people who lack part of the Rh groups it was noticed that in all of ten families in which the question had been asked the parents of the propositus were cousins. The consanguinity rate of parents of people with homozygous conditions such as these is expected to be higher than that of the general population, but 100 per cent is significantly too high. The traditional explanation of such a high rate is that more than one pair of genes is involved, that is to say, genes outside the Rh locus may be influencing the groups.

Further hints that modifying genes can influence the Rh groups are being gathered from families whose Rh antigens appear to be depressed: an English family was investigated in collaboration with Dr. I. Dunsford, of Sheffield, and several negro families in collaboration with Dr. R. E. Rosenfield of New York and Dr. J. Grundorfer of New Jersey. The work on negro samples was aided by the antibody anti-e^s (Report 1960), several further examples of which were identified during the year.

The Lutheran and the Kidd Systems. For several years Lutheran has been the only blood group system which could be called simple: there were two antigens, Lu^a and Lu^b, and people had one or the other or both, according to their genetic constitution. A family was recently found in which several members have neither Lu^a nor Lu^b, and the peculiarity is clearly inherited as a dominant character. Such negative groups are known in other blood group systems but they are all recessive characters. The present interpretation is that the family has normal Lutheran genes and a separate and very rare modifying gene, dominant in effect, which prevents the gene Lu^b expressing itself as an antigen. The Kidd groups also are unconventional in this family and the segregation of the two abnormalities is such that they could have a common cause, one gene affecting the action of two independent genes. The work is being done in collaboration with Dr. T. J. Greenwalt of Milwaukee and Dr. Mary N. Crawford of Philadelphia.

The blood-grouping of twins and of patients with various sex abnormalities continues (Report 1960).

For routine antisera the Unit is indebted to many colleagues, particularly Dr. Mourant and Miss Ikin of the Medical Research Council Blood Group Reference Laboratory, Dr. I. Dunsford of the Sheffield Blood Transfusion Service, Dr. R. A. Zeitlin and Dr. T. E. Cleghorn of the South London Blood Transfusion Centre and Dr. W. J. Jenkins and Mr. W. L. Marsh of the North East London Blood Transfusion Centre. Again the Unit is grateful to the Staff of the Institute for the numerous samples of their blood which, having been tested for almost all the known antigens, are invaluable in the identification of antibodies.

BLOOD GROUP REFERENCE LABORATORY

The Unit acts as the central reference laboratory for the investigation of blood group problems, and the supply centre for grouping sera, for the United Kingdom. It also acts under the auspices of the World Health Organisation as the world reference centre.

Liquid blood-grouping sera and anti-human-globulin serum are supplied for use in the United Kingdom, and dried serum for use overseas. The quantities issued of nearly every kind of serum have increased substantially since the previous year. Help to overseas laboratories generally, and especially to those in the British Commonwealth, continues to be an important activity. Several laboratories beginning or expanding blood grouping work have been supplied with initial stocks of serum, and members of the staff of these and other laboratories have been fully blood-grouped so that their red cells may be used for control purposes. Numerous blood specimens from Great Britain and overseas have been investigated for blood-group antigens and antibodies, to assist in clinical investigations, for research purposes, and for routine blood-grouping purposes, especially in connection with the control and supply of grouping sera.

The methods of testing anti-human-globulin sera before issue have been revised and extended. All sera are now titrated against cells sensitised with gamma-globulin antibodies, and also against cells sensitised with complement-binding antibodies. Every anti-globulin serum is also tested by immuno-electrophoresis against whole human serum. It is thus possible to give precise advice on the use of routine sera for special purposes, or to issue selected sera for such purposes.

Dr. Goldsmith initiated investigations into the presence of antibodies against leucocytes and against platelets in sera from cases of such conditions as leucopenia and thrombocytopenic purpura.

Miss Ikin continued her work on the varying distribution of the blood groups in different populations.

Miss Giles carried out serological and genetical investigations of unusual antigens and antibodies, especially the E^u antigen, found in the course of tests carried out for clinical purposes.

In conclusion, the Governing Body desires to record its great appreciation of the manner in which the scientific, administrative and technical staffs have worked together during the period under review, and to congratulate them on the interest and range of their scientific activities and achievements.

H. H. DALE,

Chairman.

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THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

Balance Sheet

and

Accounts

31st December 1961

CHELSEA BRIDGE ROAD,
LONDON, S.W.1.
29th May, 1962

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

FINANCIAL REPORT OF THE GOVERNING BODY

1. The Balance Sheet as at 31st December, 1961, shows balances to the credit of the various funds as follows: Capital Fund £934,932, Specific Funds £196,186, and Bequest Funds £16,683. The balance on the Sinking Fund for Freehold Buildings of £140,070 is after adding a net profit on the realisation of investments of £15,002 and transferring £6,592 from income and expenditure account. During the year donations and legacies of £8,382 have been added to the Re-endowment Fund and there was a loss of £1,120 on the realisation of investments.

2. The General Fund Income and Expenditure Account shows the income for the year as £271,993, compared with £236,179 in 1960. Expenditure amounted to £218,717 against £217,484 last year. The surplus for the year is £53,276 compared with £18,695 in 1960.

3. The year's surplus of £53, 276 shown by the General Fund Income and Expenditure Account has been transferred to the Capital Fund.

4. Stocks of Sera, Smallpox Vaccine and Horses on hand at 31st December, 1961 have the nominal value of £9,678, £3,016 and £7,800 respectively.

5. Messrs. Cooper Brothers & Co., the retiring Auditors, will, subject to the provisions of the Companies Act, 1948, be re-appointed.

E. C. DODDS, *Chairman of the Governing Body.*

HUGH BEAVER, *Hon. Treasurer.*

BALANCE SHEET

(1960) £		£	£	£
	Capital Fund:—			
	Donations, &c., received to date from the following:—			
2,000	Dr. Ludwig Mond (1893)		2,000	
46,380	Berridge Trustees (1893-1898)		46,380	
10,000	Worshipful Company of Grocers (1894)		10,000	
250,000	Lord Iveagh (1900)		250,000	
18,904	Lord Lister's Bequest (1913-1923)		18,904	
7,114	William Henry Clarke Bequest (1923-1926)		7,114	
3,400	Rockefeller Foundation (1935-1936)		3,400	
22,669	Other donations and legacies (1891-1954)		22,669	
	General fund income and expenditure account accumulated surplus, as at 31st December, 1960	521,737		
	Add surplus, 1961	53,276		
	profit, less losses, on sale of investments	5,026		
		580,039		
	Less amounts written off investments	5,574		
521,737			574,465	
882,204				934,932
	Specific Funds:—			
118,476	Sinking fund for freehold buildings	140,070		
32,956	Pension fund	32,664		
16,190	Re-endowment fund	23,452		
			196,186	
	Bequest Funds:—			
9,307	Jenner Memorial studentship fund	9,822		
6,991	Morna Macleod scholarship fund	6,861		
			16,683	
183,920				212,869
	Specific Grants and Legacies Unexpended:—			
772	Cancer research legacies (1937-1950)		772	
347	Royal Society grant (1951)		40	
5,385	Nuffield Foundation grants (1952-1961)		6,453	
8,518	Guinness Lister research grant (1953-1961)		8,812	
15,022				16,077
	Current Liabilities:—			
28,886	Creditors and accrued charges			35,172

E. C. DODDS, *Chairman of the Governing Body.*

HUGH BEAVER, *Hon. Treasurer.*

£1,110,032		£1,197,050

REPORT OF THE AU

The accounts set out on pages 4 to 8 are in agreement with the books which, in our opinion, have been ke
In our opinion the accounts, amplified by the information given in paragraphs 1 and 4 of the Financial Re th
the surplus of the Institute.

London, 30th May, 1962

ETt DECEMBER 1961

(1960) £						£	£	£
	Fixed Assets:—							
	Freehold property at cost:—							
73,548	Land and building, Chelsea					73,548		
20,455	Queensberry Lodge estate, Elstree					20,455		
2,049	House, Bushey					2,049		
							96,052	
	(Note: *Additions and replacements since 1912 at Elstree and 1935 at Chelsea have been charged to revenue.*)							
	Furniture, fittings, scientific apparatus and books:—							
2,472	At cost, less depreciation to 31st December, 1920						2,472	
98,524	*Note: Additions and replacements since 31st December, 1920, have been charged to revenue)*							98,524

General, Specific and Bequest Funds.
Investments and Uninvested Cash:—

		Quoted at cost, less amounts written off in Gt. Britain	Quoted at cost, less amounts written off Elsewhere	Unquoted at cost	Uninvested cash		
718,898	General	£602,726	£52,094	£115,665	—	770,485	
	Specific—						
118,476	Sinking fund for freehold buildings	112,830	4,827	11,500	10,913	140,070	
32,956	Pension fund	31,746	—	—	918	32,664	
16,190	Re-endowment fund	15,052	—	—	8,400	23,452	
	Bequest—						
9,307	Jenner Memorial studentship fund	6,479	—	2,940	403	9,822	
6,991	Morna Macleod scholarship fund	5,773	—	—	1,088	6,861	
902,818		774,606	56,921	130,105	21,722	983,354	983,354
			£831,527				

(*Market value of quoted investments*—£1,220,145)

	Current Assets:—		
56,279	Debtors and payments in advance	84,428	
52,411	Balance at bankers and cash in hand	32,744	
108,690			117,172

(*Note: See paragraph 4 Governing Body's Financial Report for nominal values of sera, smallpox vaccine and horses which have not been brought into the accounts.*)

£1,110,032	£1,199,050

AS TO THE MEMBERS

kept. We obtained the information and explanations we required.
the Governing Body, comply with the Companies Act, 1948, and give a true and fair view of the state of affairs and

COOPER BROTHERS & CO.,
Chartered Accountants.

INCOME AND EXPENDITURE ACCOUNT

GENERAL

(1960)		Total Expenditure	External Contributions	
£		£	£	£
93,441	Salaries and wages	172,341	72,990	99,351
7,225	Emoluments of two members of the Governing Body in an executive capacity	7,321	—	7,321
4,328	Premiums on federated superannuation policies	8,353	3,354	4,999
2,252	Premiums on group pension policy	2,791	845	1,946
5,196	Rent, rates and insurance	5,409	259	5,150
14,204	Gas, water, fuel and electricity	17,178	3,217	13,961
4,061	Office expenses, stationery and printing	4,737	819	3,918
384	Auditor's fee	368	—	368
913	Travelling expenses	1,829	433	1,396
4,790	Biochemistry expenses	4,868	2,227	2,641
1,120	Microbiology, immunology and experimental pathology expenses	2,611	1,304	1,307
777	Biophysics expenses	9,163	8,557	606
208	Virology expenses	1,707	1,345	362
23,658	Serum, vaccine and smallpox vaccine expenses	29,622	1,865	27,757
9,310	Animals	9,343	1,132	8,211
8,317	Animal house expenses and forage	12,415	1,702	10,713
24,702	Buildings, alterations, repairs and renewals	15,958	357	15,601
1,741	General apparatus and new installations	789	—	789
1,379	Library expenses	1,593	—	1,593
1,725	General stores	2,046	—	2,046
1,831	Staff canteen loss	2,332	243	2,089
—	Blood products laboratory expenses	7,122	7,122	—
5,922	Amount transferred to sinking fund for freehold buildings (including £6,168 interest on investments)	6,592	—	6,592
18,695	Surplus transferred to Capital Fund after charging to expenditure £7,057 (1960 £17,787) for additions to property and equipment	53,276	—	53,276
£236,179		£379,764	£107,771	£271,993

Nor the year ended 31st December 1961

ERGND

960)

£		£	£
	Interest and dividends on investments:—		
,030	General fund	43,648	
,498	Sinking fund	6,168	
			49,816
587	Underwriting commission		461
,449	Sales of sera, vaccine, smallpox vaccine, &c.		214,900
615	Rent		6,816
£236,179			£271,993

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

PENSION FUND

(1960)			(1960)		
£		£	£		£
2,051	Pensions	2,019	33,279	Balance as at 1st January, 1961 ..	32,956
32,956	Balance carried forward	32,664	1,728	Interest on investments (gross) ..	1,727
£35,007		£34,683	£35,007		£34,683

JENNER MEMORIAL STUDENTSHIP FUND

(1960)			(1960)		
£		£	£		£
			8,857	Balance as at 1st January, 1961 ..	9,307
9,307	Balance carried forward ..	9,822	450	Interest on investments (gross) ..	515
£9,307		£9,822	£9,307		£9,822

MORNA MACLEOD SCHOLARSHIP FUND

(1960)			(1960)		
£		£	£		£
—	Loss on realisation of investments	486	6,652	Balance as at 1st January, 1961 ..	6,991
6,991	Balance carried forward	6,861	339	Interest on investments (gross) ..	356
£6,991		£7,347	£6,991		£7,347

NUFFIELD FOUNDATION GRANTS

(1960)			(1960)		
£		£	£		£
	Salaries, wages, laboratory ex-		6,301	Balance as at 1st January, 1961 ..	5,385
6,916	penses and animals	4,932	6,000	Amounts received	6,000
5,385	Balance carried forward	6,453			
£12,301		£11,385	£12,301		£11,385

GUINNESS-LISTER RESEARCH GRANT

(1960)			(1960)		
£		£	£		£
13,924	Salaries and wages	11,367	11,341	Balance as at 1st January, 1961 ..	8,518
2,899	Laboratory expenses	2,339	14,000	Amount received	14,000
8,518	Balance carried forward	8,812			
£25,341		£22,518	£25,341		£22,518

THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE

REPORT
OF THE
GOVERNING BODY
1962

CHELSEA BRIDGE ROAD, LONDON, S.W.1

THE GOVERNING BODY

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Professor A. A. MILES, CBE, MA, MD, FRCP, FRS
MARJORIE G. MACFARLANE, DSc, PhD
Professor WILSON SMITH, MD, FRCP, FRS

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The President of the ROYAL COLLEGE OF SURGEONS	Royal College of Surgeons of England.
The President of the ROYAL COLLEGE OF VETERINARY SURGEONS	Royal College of Veterinary Surgeons
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Professor WILSON SMITH, MD, FRCP, FRS	Royal Society
Professor F. S. STEWART, MD, BCh, BAO	University of Dublin
WILLIAM J. THOMPSON	Worshipful Company of Grocers
Sir GRAHAM S. WILSON, MD, BSc, FRCP	University of London

THE STAFF

Director: Professor A. A. Miles

Deputy Director: Professor W. T. J. Morgan

Superintendent of Elstree Laboratories: W. d'A. Maycock

MICROBIOLOGY, IMMUNOLOGY and EXPERIMENTAL PATHOLOGY

†A. A. Miles, CBE, MA, MD, FRCP, FRS (*Professor of Experimental Pathology in the University of London*)

Emmy Klienberger-Nobel, PhD, DSc

Ruth M. Lemcke, BSc, PhD

M. D. Pittam, BA, PhD (*Trypanosomiasis Research*)

Brenda Mason, BSc

Anne M. Brimacombe, BA

O. D. Ratnoff, AB, MD, (U.S.A)

GUINNESS-LISTER RESEARCH UNIT

*B. A. D. Stocker, MD, MRCS, LRCP

G. G. Meynell, MD, MRCS, LRCP

M. W. McDonough, BSc, PhD

Sylvia Smith, MSc

Eugene Dubnau, MA (U.S.A.)

Maija I. Vitolins, BSc

VIROLOGY

L. H. Collier, MD (*also Hon. Director, M.R.C. Trachoma Research Unit*)

C. A. Placido de Sousa, MB, ChB

G. Furness, BSc, PhD, Dip Bact

W. A. Blyth, B.Sc., Ph.D

Janice Taverne, BA, PhD

Doris M. Graham, MSc.

P. Reeve, BSc, PhD

Elizabeth F. Fraser, BSc

M.R.C. Trachoma Research Unit

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†W. T. J. Morgan, CBE, DSc, PhD, FRIC, FRS (*Professor of Biochemistry in the University of London*). *Principal Biochemist, Elstree*

*Marjorie G. Macfarlane, DSc, PhD

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Zeenat H. Gunja Smith, BSc, PhD (*Dept. Scientific and Industrial Research Grantee*)

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†R. A. Kekwick, DSc (*Reader in Chemical Biophysics in the University of London*)

J. M. Creeth, BSc, PhD

†Professor N. H. Martin, MA, FRCP, FRIC (*Honorary Research Associate*)

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Dame Harriette Chick, DBE, DSc

E. Margaret Hume, MA

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†Appointed Teacher of the University of London.

*Recognised Teacher of the University of London.

PREPARATION and STUDY of THERAPEUTIC SERA (ELSTREE)

*B. G. F. Weitz, DSc, MRCVS
Sheila M. Lanham, BSc (*Trypanosomiasis Research*)
J. K. Miller, BVetSc, MRCVS (*Trypanosomiasis Research*)
G. Stone, BSc

BIOCHEMISTRY (ELSTREE)

*D. E. Dolby, BSc, PhD

PREPARATION and STUDY of SMALLPOX VACCINE (ELSTREE)

C. Kaplan, MSc, MB, ChB, Dip Bact
H. G. S. Murray, MB, BCh
G. S. Turner, BSc
L. C. Robinson, BSc

PREPARATION and STUDY of BACTERIAL VACCINES (ELSTREE)

*A. F. B. Standfast, MA, Dip Bact
Jean M. Dolby, MA, PhD (*Medical Research Council External Scientific Staff*)
M. P. Banks, BSc
W. A. Vincent, BSc, PhD (*Medical Research Council Grantee*)
Georgina Sampson, BSc (*M.R.C. Trachoma Research Unit*)

BLOOD PRODUCTS (ELSTREE)

*W. d'A. Maycock, MVO, MBE, MD
L. Vallet, MA
§Margaret E. Mackay, MSc, PhD (*Medical Research Council External Scientific Staff*)
Constance Shaw, MSc, Dip Bact
Shirley M. Evans, BSc

MEDICAL RESEARCH COUNCIL UNITS HOUSED AT THE INSTITUTE

BLOOD GROUP RESEARCH UNIT

§R. R. Race, PhD, FRCP, FRS
Ruth Sanger, BSc, PhD
Patricia Tippett, BSc
F. Jean Hamper, BSc
E. June Gavin, BSc

BLOOD GROUP REFERENCE LABORATORY

§*A. E. Mourant, MA, DPhil, DM, MRCP
K. L. G. Goldsmith, PhD, MB, BS
Elizabeth W. Ikin, BSc
Carolyn M. Giles, BSc

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Elstree Secretary and Estate Manager -	G. J. Roderick, B.Comm
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52 Bedford Square, W.C.1

Auditors:
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*Recognised Teacher of the University of London
§Honorary Member of Institute Staff

ANNUAL GENERAL MEETING

OF

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

June 26th, 1962

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the work of the Institute for the year 1961.

GOVERNING BODY

The Council, at a meeting held on 27th June 1961, reappointed Sir Henry Dale, Sir Russell Brain and Sir Charles Dodds as its representatives on the Governing Body until 31st December 1962. At a meeting held on 12th December 1961 the Governors accepted with much regret the decision of Sir Henry Dale to resign from the Governing Body. The Governing Body takes this opportunity of paying warm tribute to Sir Henry's interest in the affairs of the Institute and to his patience and help in dealing with the many problems which arose during the twenty years of his distinguished Chairmanship.

On the proposal of Sir Henry Dale, seconded by Lord Iveagh, Sir Charles Dodds was unanimously elected the new Chairman of the Governing Body.

The Governing Body has noted with pleasure the conferment of a barony on Sir Russell Brain and the election of Sir Charles Dodds to the Presidency of the Royal College of Physicians.

COUNCIL

Last year the three retiring members of the Council were The President of the Royal College of Veterinary Surgeons, Professor E. B. Verney and Professor D. Whitteridge. As their new representatives the University of Cambridge appointed Dr. R. I. N. Greaves in place of Professor Verney and the University of Edinburgh appointed Professor Robert Cruickshank in place of Professor Whitteridge. The President of the Royal College of Veterinary Surgeons being *ex officio* a member of the Council, retained his appointment.

The three members of the Council due to retire this year in accordance with the Articles of Association, but who are eligible for re-appointment, are the President of the Royal College of Surgeons, Mr. A. Lawrence Abel, the representative of the Royal College of Surgeons of England and the British Medical Association respectively, and Dr. Muriel Robertson, a representative of the Members of the Institute.

MEMBERS

The Governing Body records with regret the death during the year of Professor T. R. Elliott. Professor Elliott had been a member since 1931.

STAFF

The Governing Body takes much pleasure in recording the appointment

of Dr. W. d'A. Maycock to the rank of member (4th Class) of the Royal Victorian Order and the appointment of Professor W. T. J. Morgan as a Vice President of the Royal Society.

Dr. C. A. Placido de Sousa was appointed to the Virology Department; Dr. Sheila Gompertz to the Biochemistry Department; Mr. G. S. Turner to the Smallpox Vaccine Department; Mr. J. K. Miller to the Trypanosomiasis Unit; Miss M. I. Vitolins temporarily to the Guinness-Lister Research Unit; and Mr. E. J. H. Lloyd to the administrative staff as Assistant Accountant. Miss F. M. Lee-Jones resigned during the year.

Dr. Muriel Robertson has completed her work at the Institute after a total of fifty-one years research as a member of the Institute Staff.

Dr. D. McClean, Bacteriologist-in-charge of the Smallpox Vaccine Department since 1936, retired on 31st May 1961, after 32 years service with the Institute and has been succeeded by Dr. C. Kaplan. The Institute has also lost by retirement the services of Mr. J. Crawley and Mr. W. C. Ling. Mr. Crawley, Head Stableman at Elstree for the past twenty-two years, entered the Institute's service in 1920. Mr. Ling, a senior technician, had been employed at Elstree since 1924. The Governing Body takes this opportunity of recording its appreciation of the very many years of loyal service they have all given to the Institute.

In May 1961 Professor A. A. Miles gave the Warner-Lambert Lectures in Medical Microbiology at Rutgers State University, New Brunswick, New Jersey, U.S.A. and the first Rosenau Memorial Lecture at Harvard University School of Public Health, Boston. In January 1962 he served as Chairman of the World Health Organisation Scientific Group on Immunoprophylaxis and Immunotherapy, held in Geneva.

Professor W. T. J. Morgan, in May 1961, lectured by invitation at the Rockefeller Institute and other research institutes in the U.S.A., and in August attended the International Congress of Biochemistry in Moscow as a Royal Society representative of the British National Committee for Biochemistry.

In May 1961, Dr. W. d'A. Maycock served as Chairman of a meeting convened in Geneva by the World Health Organisation to consider the preparation of a manual on blood transfusion, and in September attended a Council of Europe Working Party on Blood Transfusion in Amsterdam.

In December, with Dr. R. A. Kekwick, he attended a symposium on human antihaemophilic globulin and Christmas factor arranged by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, under the auspices of the University of Amsterdam.

Dr. Kekwick also visited various laboratories in the United States in connection with plasma fractionation and blood coagulation, in September.

In May 1961 Dr. L. H. Collier took part by invitation in the New York Academy of Sciences Symposium on "The Biology of the Trachoma Agent" and in July, in the Oxford Ophthalmological Congress. In August he served as Chairman of the second meeting of the World Health Organisation Scientific Group on Trachoma Research, held in Geneva.

Dr. C. Kaplan visited the Ministry of Public Health Vaccine Laboratory, Bangkok, Thailand in February and March as a World Health Organisation Consultant in dried smallpox vaccine manufacture.

Dr. G. G. Meynell spent three months as a Visiting Associate Professor in the Department of Microbiology, Washington University School of Medicine, St. Louis, U.S.A. at the end of 1961.

Dr. W. J. Whelan took part by invitation in the 1st Gordon Conference on Carbohydrates at Tilton, U.S.A. in July, and the Conference on Bacterial Polysaccharides at the Massachusetts Institute of Technology, Boston, and the Starch Round Table at Woodruff, U.S.A., in September. He also lectured at Yale, Seattle, Ames, Chicago, Decatur, Ottawa, New York and Washington.

In July Mr. L. Vallet spent a week at the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service.

During the tenure of her Wellcome Research Fellowship in Berkeley, California, Dr. Winifred Watkins lectured by invitation in Philadelphia, Stanford and Salt Lake City.

Mr. R. Grundon, Senior Technician in the Smallpox Vaccine Department, visited the Pasteur Institute, Bandung, Indonesia in November, as a World Health Organisation Consultant, to supervise the assembly and commissioning of a freeze-drying plant for the production of dried smallpox vaccine by methods developed at this Institute.

DONATIONS AND GRANTS

The Governing Body again records its appreciation of the generosity of many bodies whose benefactions and grants support research work in the Institute. These include a grant from the Agricultural Research Council for research on the enzymic synthesis and degradation of glycogen in rabbit muscle; a grant from the British Empire Cancer Campaign for research on phospholipids; grants from the Colonial Development and Welfare Fund in aid of research on the blood-meals of insect vectors of disease and on the immunology of trypanosomiasis; grants from the Department of Scientific and Industrial Research for researches on the enzymic polymerization of monosaccharides and on chemically-modified polysaccharides; grants from Imperial Chemical Industries Ltd. for the purchase of special apparatus; grants from the Medical Research Council in aid of researches on the isolation and purification of proteins involved in the clotting mechanism of human plasma, on the chemical basis of blood group specificity in man, on the identification of *Bordetella pertussis* antigens, on the action of certain rabbit muscle enzymes and the synthesis of haptens and inhibitors in the dextran-antidrextan system, and on the structure of the amino-acid containing moiety in mucopolysaccharides; grants from the Nuffield Foundation for researches on the immunochemistry of blood group substances and on non-specific immunity in the early stages of infection; a grant from the U.S. Public Health Authority in aid of researches on abacterial urethritis in man; and grants from the World Health Organisation for researches on the relationship between circulating antibody and immunity to challenge with vaccinia virus on sheep vaccinia hyperimmune gamma globulin, and for serological identification tests on material submitted by the World Health Organisation.

The Governing Body also gratefully acknowledges donations to the Institute's Re-endowment Fund from the following Assurance Companies : The General Life Assurance Company, The Prudential Assurance Company Ltd., and the Royal London Mutual Insurance Society Ltd.

VISITING WORKERS

The following visitors, in addition to those listed under Staff, worked in the Institute's Laboratories: Dr. Laura Ayres, Instituto Superior de Higiene Dr. Ricardo Jorge, Lisbon, Portugal; Dr. Nicolle Grasset, Institut Pasteur, Paris; Mr. A. R. Gray, West African Institute for Trypanosomiasis Research,

Kaduna, Northern Nigeria; Mr. H. Hamer, University of Toronto Medical School; Dr. N. B. Kuppurajan, King Institute, Madras; Dr. P. Lhoas, Facultes N. D. Namur, Belgium; Dr. P. B. McCay, Oklahoma Medical Research Institute, Oklahoma; Dr. G. Modiano, Institute of Genetics, University of Pavia, Italy; Dr. A. Natarajan, King Institute, Madras; Mr. P. Plieger, Potato Research Institute, Gröningen, Netherlands; Dr. L. Roden, Institute of Medical Chemistry, Upsala, Sweden; Mr. T. V. Subbaiah, Tuberculosis Chemotherapy Centre, Madras; Dr. P. K. Topa, State Vaccine Institute, Patwadangar, U.P.; Dr. Marian V. Waller, Medical College of Virginia, Richmond, U.S.A.

The Medical Research Council's Blood Group Research Unit and Blood Groud Reference Laboratory, whose researches for 1961 are described on pages 27-29, and its Trachoma Research Unit, are accommodated in the Institute.

RESEARCHES IN 1961

SUMMARY

In this summary of the investigations made in 1961, the bracketed numbers refer to the pages of the report where the researches are described in greater detail.

Microbiology. The Guinness-Lister Unit continues its exploration of bacterial genetics, mainly of the Salmonella bacilli. The problems studied include conjugation and hybridization of *S. typhimurium* (12); the genic control of production of flagella and fimbriae (13), and of virulence (13); interspecies hybridization (13); and the structural chemistry of flagella and of the cell-wall polysaccharides (14).

The study of bacterial multiplication in the infected mouse by means of marked salmonellae (14) was continued.

The other purely microbiological study concerns the antigenic analysis of certain free-living protozoa (10).

Immunology and pathology of infective diseases. The Institute's studies embrace infections by viruses, pleuro-pneumonia-like organisms, bacteria and protozoa.

The Trachoma Research Unit continued its epidemiological field study in the Gambia of trachoma in terms of virus and of bacilli of the *Haemophilus* group (17) and completed a field trial of a therapeutic trachoma vaccine (17). The laboratory studies at the Institute include the growth characteristics of trachoma and inclusion conjunctivitis virus in tissue culture (16) and the possible identity of the two viruses (16), serological classification of the viruses (16), and the serology of Gambian *Haemophilus* strains (17). In the Smallpox Vaccine department, there is continued progress towards making vaccine from virus grown in tissue culture (18), and the development of an irradiated vaccine (17); and a study of antibody immunity in experimental vaccinia (17).

As an indirect means of determining the significance of antibody to PPLO in human subjects with abacterial urethritis and other kinds of genital infection, the study continues of the relation of PPLO antibody to the infecting agents and stage of infection in rats and mice (15); and of the distinction between PPLO and L-forms (15).

The immunological study of bacterial infections includes an analysis of the

action of two types of protective antibody in mice infected with the whooping cough bacillus (11); the laboratory assay of typhoid vaccines (12); and the process of refinement of antitoxin by proteolysis (18).

As regards pathogenic protozoa, the investigation of the immunology of experimental trypanosomiasis continues, in terms of soluble and bound immunizing antigen (11); and of methods of bulk growth of the trypanosomes for immunochemical analysis (11).

Epidemiology. The refined serological methods devized to identify the animal source of food for blood-sucking insects continues to provide valuable facts about the feeding habits of tsetse flies in regions where these insects are vectors or possible vectors of disease (18).

Pathology. Work continues on the relation of early tissue reactions to defence against microbial infection (19); and on the relation of serum permeability factors to plasma kininogenases and the components of the clotting system (19). The non-specific resistance to various bacteria was investigated during the course of streptococcal infection in the guinea-pig (19).

Biochemistry. The biochemical researches again concern three kinds of substances—the blood group substances, the cellular phospholipids, and starches.

The investigations of the substances of the ABO and other blood group systems include definition of the terminal sugar groups responsible for serological specificity (20); the rôle of nucleotides in biosynthesis of the substances (21); analysis by improved methods of partial hydrolysis (21), determination of the amino-acid moiety of the substances and its relation to serological specificity (21), transformation of blood group specificity by the action of selected enzymes (21) and measurement of the molecular properties of the substances (25).

Work on the structure of phospholipids, their distribution in tissues, cells and cellular particles, and their possible function in the cell includes analysis of the phospholipids of various bacteria (23) of bone marrow and of normal and neoplastic mammalian cells (24); and studies of their effect on cellular metabolism (24).

Carbohydrate studies mainly concern the relation of structure of both natural and synthetic polysaccharides to the specificity of enzyme and antibody reactions (22). Other studies include mammalian enzymes concerned in glycogen metabolism (23); and the structure of polyglucose (23).

Human and animal plasma proteins. The Institute's work is concerned with the isolation, refinement, characterization, assay, and in some cases clinical trial, of the various biologically active proteins of human plasma.

Some progress was made in the isolation and identification of contaminating enzymes that destroy antihaemophilic factor in therapeutic preparations of the factor (25); and reference standard of the factor made (25).

Studies of the isolation and characterization of other biologically active proteins include those of plasma globulins associated with lymphoreticular disease (26); of urinary proteins in Fanconi's syndrome (26); iodine-modified ovalbumin (26); plasmin and plasma kininogenases (26); and plasminogen (26).

MICROBIOLOGY

PROTOZOOLOGY

Dr. Robertson continued her serological study of strains of *Tetrahymena* spp. By agglutination tests of living organisms two strains of *T.pyriformis* proved to be closely related, and different from a third; all three differed from a strain of *T.vorax*. The distinctions were also evident in gel-diffusion tests of extracts,

though all four strains had certain antigens in common. Strong *pyriformis* antisera induced *T.vorax* to shed its periplast, with subsequent flocculation of the shed material only.

TRYPANOSOMIASIS

With Dr. Weitz, a visiting worker, Mr. A. R. Gray from the West African Institute for Trypanosomiasis Research, studied the antigenic variation of *T.brucei* in chronic infections of laboratory animals. The antigenic pattern of the variants isolated during the course of infection was studied in terms of the specificity of the antibodies produced in animals infected with these variants. During infection trypanosomes undergo a number of antigenic variations so that the strain in the final stages of the disease bears little or no antigenic resemblance to the original infecting strain. These changes were shown to be a direct response to antibody formed in the animals. Predetermined variants were obtained in rabbits passively immunized with antibodies to given variants, and then infected with the parent strain.

Miss Lee-Jones, in a survey of the cross-reactions of the various antibodies during infection, showed that the antigenic relationships between different species and strains of the *brucei* group of trypanosomes varied continuously during the course of infection. It appears impracticable to classify serologically strains of trypanosomes which undergo such continual changes. Work in progress is designed to determine the nature of these changes in relation to the soluble ' exoantigen ' (Report 1961) and the bound antigens of these trypanosomes.

Dr. Weitz began a study of the distribution of antigens in the individual trypanosome by the fluorescent antibody technique.

Cultivation of African Trypanosomes in vitro. Dr. Pittam evolved a method for the bulk growth of *Trypanosoma rhodesiense* yielding about 20-30 million organisms per c.mm. The organisms were grown for 4 days in 1 litre amounts of a liquid medium. Deep cultivation, with continuous stirring and aeration of the medium, increased the yield. So far similar methods have not succeeded with *T.congolense* and *T.vivax*.

Immunochemistry of Trypanosomal Antigens. Miss Lanham continued her studies of the properties of exoantigen of *Trypanosoma brucei*. Since the antigen is obtained from the serum of infected rats, attempts were made to separate it from the serum proteins, by protein precipitants, heat coagulation and ion exchange separation. In the more purified fractions of exoantigen, electrophoresis analysis indicated that it had similar properties to slow moving components of serum proteins and may be, in fact, conjugated to the more stable serum γ-globulin.

Exoantigen is a protein, stable when heated briefly at 100°C, non-diffusable through cellophane membranes and is destroyed by pepsin. Similar investigations are in progress to identify the nature of the " bound antigens " released from the disintegrated cell.

WHOOPING COUGH BACILLUS

Identification of Protective Antigens. Mr. Standfast, Dr. Jean Dolby and Dr. Vincent continued their work on the two protective antigens of *Bordetella pertussis* (the IN, inducing protection against intranasal and the IC, inducing protection against intracerebral challenge) and the antibodies they elicit.

Antisera to various fractions of *B.pertussis* were tested for specific *in vitro* bactericidal activity, an effect (Report 1961) requiring components of fresh guinea-pig serum which may be similar to but not identical with those required

for immune haemolysis. Antisera were divisible into (i) those bactericidal at low concentrations of guinea-pig serum and relatively high concentrations of antiserum; and (ii) those bactericidal only at very low antiserum concentrations but requiring relatively more guinea-pig serum, and which inhibit the activity of antisera in the first group.

Antisera protecting mice only against an intranasal challenge usually fall into group (i) and those protective against an intracerebral challenge, with or without intranasal protection, in its group (ii). Many non-protective sera, however, were strongly bactericidal *in vitro*. It is probable that the *in vitro* test is more sensitive than the mouse test as a measure of protective antibodies and not that the bactericidal activity is due to an unrecognized antibody. The bactericidal power of an antiserum was not correlated with its content of any of the known antibodies other than the protective. Antisera to fractions of *B.pertussis* made by Dr. Vincent, were studied in the same way; and the fractions were also tested as specific inhibitors of the bactericidal system.

Dr. Vincent began the chemical isolation and identification of the IN and IC antigens, from the methanol precipitable material in an aqueous extract of crushed pertussis cells. This fraction, rich in IC and IN antigens, had in Dr. Dolby's hands already yielded a relatively pure preparation of the IC antigen.

The fraction was resolved into three proteins with which the protective activity was associated. It is not yet clear whether the proteins are degradation products of macromolecules originally present. Some immunogenic activity was lost during fractionation; nevertheless, the IC and IN activities appear to be associated respectively with different molecular species. Samples of the different molecular species, carrying the different antigens, will be prepared in quantities large enough for thorough *in vivo* testing.

TYPHOID BACILLUS

Laboratory Assay of Typhoid Vaccines. As part of a collaborative study initiated by the World Health Organisation in conjunction with current field trials of typhoid vaccines in British Guiana, Yugoslavia and Poland, Mr. Banks continued his investigation of laboratory assays of the vaccines under trial (Report 1957), with particular attention to the test devised by Mr. Standfast using a Vi-negative strain of *S.typhi* for challenge. The vaccines differed in mouse-protective potency; the significance of the differences awaits the results of the field trials in man.

INHERITANCE IN BACTERIA

Conjugation in Salmonella. The Guinness-Lister Unit has continued its investigation of inheritance in bacteria of the *Salmonella* (food-poisoning and enteric fever) groups, using two different kinds of fertility factors to induce conjugation (i) The colicine factors, which determine the production of antibiotics called colicines (see Reports 1959-1961) and (ii) the F factor and some variants of it, transferred from *Escherichia coli* (Report 1961).

Conjugation Determined by Colicine Factors. Miss Smith continued her work (Report 1961) on mapping the chromosome of *Salmonella typhimurium*, obtaining conjugation (and hybridization) by use of colicine factors *col* I and *col E* 1. Additional hereditary characters were introduced into the strains used, by the isolation of mutants unable to synthesize particular aminoacids, to ferment particular sugars or to produce the fraction of the polysaccharide component of the O antigen called factor 5. Many of these mutants were obtained by treating

bacteria with the mutagen ethyl methane sulphonate (Report 1960). The genes regulating all the characters concerned were mapped in the single, closed-loop, linkage map already tentatively established (Report 1961). Dr. Stocker, Mr. Subbaiah and Mrs. Dubnau, using the same system, investigated the segregation of some additional characters, resulting from mutation in the laboratory or pre-existing in strains isolated from natural sources. The linkage map so far obtained is represented:—*ara—azi—proA—gal—fla*$_{29}$*—tryB—(H1, fla*$_1$*, nml)—his—O5—adeC—H2—(cysC, cysD)—str—(isol, gl-kin, rha)—metA—ara—etc.*

From analogy with the situation in mating of *Escherichia coli* resulting from activity of the fertility factor F it was suspected that in conjugation of *Salmonella* induced by colicine factors the partner carrying the colicine factor acts as a " male " and injects a part, or perhaps the whole, of its chromosome into the cytoplasm of the other, " female " partner. Some data on frequency of various recombinant classes cast doubt on this idea, and suggested that perhaps the partner lacking the colicine factors sometimes acted as " male ". However, Miss Smith, in a further investigation of crosses in which a reversal of sexual roles was suspected, has now shown that if in such a cross the partner carrying the colicine factors is resistant to streptomycin and the other partner sensitive, exposure to streptomycin prevents the appearance of nearly all the streptomycin-resistant recombinants which develop in the absence of the antibiotic. This is the result expected if only the colicinogenic partner can inject its chromosome into its partner; for sensitive " female " bacteria which had received a chromosome fragment from the streptomycin-resistant colicinogenic partner would be killed by the antibiotic, even when the injected chromosome fragment carried the gene for resistance to streptomycin, because of the streptomycin-sensitivity of their own cytoplasm.

Mr. Subbaiah and Dr. Stocker established that two genes, regulating, respectively, ability to ferment rhamnose and ability to produce the filamentous bacterial appendages called fimbriae (Duguid, see Report 1960) are well separated in the chromosome and functionally independent, despite the almost complete correlation of the two characters in " wild " strains of *Salmonella typhimurium*.

Now that the chromosome of *Salmonella typhimurium* is reasonably well mapped, the genetics of virulence in this species can more fruitfully be investigated. As a start Mr. Subbaiah has isolated a series of *rough* mutants in strain LT2, as the *rough* character entails loss of virulence. The *rough* character of one mutant segregates in crosses just like any other character; the gene concerned seems to lie close to *try* (ability to synthesise tryptophan) and to *fla*$_1$*—H1* (a cluster of genes concerned with synthesis and chemical composition of flagella).

Dr. Meynell completed his study (Report 1961) on intra-species and inter-species crosses in *Salmonella*, mediated by colicine factors. In all crosses cell pairing was very frequent (c. 50% of acceptor population acquiring a colicine factor); the frequency of chromosomal recombination was about one in ten million in crosses between members of the same species (*S. typhimurium* or *S. enteritidis*), 1 in a thousand million in a cross of *S.enteritidis* with *S.typhimurium*; and apparently zero in a cross of *S.enteritidis* with *S.typhi*. The results suggest that the low recombination rates in inter-species crosses result from failure of integration of transferred chromosome fragments, rather than from failure of pairing or of chromosome transfer.

Conjugation Determined by F'. Miss Smith transferred the variant fertility factor F'$_{13}$ (Report 1961) to genetically marked stocks of *S.typhimurium* strain LT2, and found that its presence resulted not only in conjugation and transfer

of the agent itself, but also in production of chromosomal recombinants. The rate of recombination was about 1 in a million, i.e. rather higher than in crosses using the *col* factors. The classes of recombinants obtained were generally similar to those from *col* factor crosses. Recombinants produced progeny many of which did not show the ability to ferment lactose conferred by the presence of the F'_{13} factor; it seems that this factor, unlike the colicine factors, often fails to establish itself in recombinants.

Mr. Hamer and Dr. Meynell transferred F'_{13} between various *Salmonella* strains. The F'_{13}-infected strains produce a small minority of descendants which have apparently lost the lactose-fermenting ability which the factor confers. However, they encountered a new kind of lactose-negative variant in one strain given F'_{13}; this variant on sub-culture produced some lactose-positive descendants. This suggests that a reversible suppression of the activity of the agent is involved, rather than its total loss.

BACTERIAL PHYSIOLOGY

Chemistry of Flagellar Protein. Dr. McDonough continued his examination, by paper chromatography and electrophoresis, of enzymic digests of flagellar proteins (Report 1960, 1961). In tryptic digests of *i* flagellin some 18 basic, 7 neutral and 8 acidic peptide spots are now recognizable. Three of seven mutant forms of flagellin *i* yield recognizably different tryptic digest " maps ". In all three the slowest moving basic peptide of digests of normal *i* is affected; in *i M7* this peptide is more basic than in normal *i*, and in *iM10* and *iM11* it moves faster on chromatography. Tryptic digests of *iM9* apparently do not differ from those of normal *i*; but in peptic digests of *iM9* an acidic tyrosine-containing band is missing. The serologically unrelated flagellin *1,2* yields a tryptic digest map very different from that of *i*; but 10 peptide spots correspond in position to those of *i* peptides.

The peptide changes so far detected accord well with the theoretical expectation that the changes in serological character (Report 1960, 1961) resulted from mutational alterations in amino-acid composition of the polypeptide chain of flagellin, only one very small region, perhaps a single amino-acid, being changed in any one mutant.

Cell-Wall Polysaccharide of Salmonella. Dr. McDonough made boiling-water extracts of cells of an *S.typhimurium* mutant unable to inter-convert glucose and galactose (Report 1961). After fractionation and hydrolysis glucose, mannose, ribose, rhamnose and abequose were detected. This confirms the surmise that the absence of the normal components mannose, rhamnose and abequose as well as of galactose from the cell-wall polysaccharide of this strain (Report 1961) does not mean that these sugars are normally synthesized *via* galactose, which the mutant cannot make, but results from some other cause; perhaps absence of galactose prevents the attachment of side-chains containing the three other sugars to a polyglucose backbone. Mr. Abdullah, of the Department of Biochemistry, is continuing his examination (Report 1961) of this polyglucose; on partial hydrolysis it yields mainly or entirely β-1,6- and β-1,2-glucose disaccharides.

Measurement of Bacterial Division Rates in Infected Animals. Dr. Meynell and Mr. Subbaiah continued experiments (Report 1961) to determine the true rate of multiplication of bacteria in infected animals. The use of the abortive transduction method with histidine-exacting *S.typhimurium* was greatly facilitated by development of a medium on which abortive transductant bacteria form colonies 1-2 mm. in diameter, instead of scarcely visible ones.

ABACTERIAL URETHRITIS IN MAN AND RELATED DISEASES

Dr. Lemcke, in collaboration with Dr. G. W. Csonka (St. Mary's Hospital), concluded an investigation of the association of pleuropneumonia-like organisms (PPLO) with salpingitis. Antibody against human genital PPLO was found in 55% of the patients compared with 4% in healthy female blood donors. The results suggest that PPLO, either alone or in association with other agents such as the gonococcus, can play an active part in the disease.

Serological typing of PPLO from various sources was carried out by Dr. Lemcke using a complement fixation test. The relationships of some 80 PPLO strains were investigated. Representative strains of the so-called " human genital type 2 " PPLO, isolated from human genitals in America but not in the U.K., proved to be serologically indistinguishable from rat polyarthritis PPLO. This observation supports the view, suggested by previous investigations in this laboratory into PPLO urogenital infections, that there is only one type of human genital PPLO.

The investigation of PPLO isolated from tissue cultures continued. All strains previously typed in this laboratory were human genital PPLO, but four strains sent from one laboratory in America belong to a distinct serological type not identifiable with any known strain of human, animal or saprophytic PPLO. Dr. Klieneberger-Nobel studied the morphology of these new strains by light and electron microscopy and found it to be typical of PPLO, and not of L-forms of bacteria. The results indicate that the widespread contamination of tissue cultures is due to PPLO and not to L-forms derived from bacterial contaminants under the influence of antibiotics.

A strain of PPLO said to have been isolated from the skin lesions of a human patient was studied. It proved to be serologically related to a saprophytic avian PPLO and was probably derived from the embryonated eggs in which the human pathological material was passaged. It is already known that pathogenic chicken coryza strains can be isolated from embryonated eggs in which human pathological material has been passaged. These observations show the necessity of identifying unknown PPLO before ascribing to them a rôle in the aetiology of human disease, especially when animal or egg passages have been used in their isolation.

Dr. Klieneberger-Nobel made a microscopical comparison of PPLO and L-forms of bacteria. The hypothesis was tested that bacteria can, *via* the L-form, transform into PPLO; as, e.g., that an apathogenic diphtheroid organism can become a pathogenic PPLO. By colour photomicrography of stained material, and electron microscopy carried out in collaboration with Dr. R. C. Valentine (National Institute for Medical Research), PPLO proved to be well organized microbes of great slenderness, whose filamentous forms measure as little as 70-140 mμ in diameter. L-forms are morphologically quite distinct; in particular there is always a considerable amount of disintegration and re-organisation, a feature consistent with the observation that PPLO are much more resistant than L-forms to variations in osmotic pressure. Stable L-forms appear to be a special type of protoplast, whereas PPLO are a class of organism, both pathogenic and apathogenic, widely distributed in nature.

VIROLOGY

TRACHOMA AND INCLUSION BLENNORRHOEA

Inoculation of Man with Inclusion Blennorrhoea Virus. In collaboration with Mr. Barrie Jones (Institute of Ophthalmology) Dr. Collier inoculated the eye

of a blind volunteer with a virus (LB4) isolated from an English baby with inclusion blennorrhoea of the newborn. It induced not only severe conjunctivitis with characteristic inclusion bodies, but also the keratitis and corneal vascularization typical of trachoma. This finding strongly supports Jones' contention that there is in fact no clear demarcation between the syndromes caused by trachoma and inclusion blennorrhoea viruses.

Growth in Cell Cultures. Dr. Furness and Miss Fraser continued their investigation of the replication of inclusion blennorrhoea virus (LB1) in HeLa cells. With Dr. G. W. Csonka (St. Mary's Hospital) and Dr. W. G. Henderson (Paddington Green Hospital), they used fluorescence microscopy to study the formation of viral nucleic acids within the host cell. When related to infectivity measurements (Report 1961), their findings suggest that the infective unit is a single elementary body rich in deoxyribonucleic acid (DNA). About 3 hours after it enters the cell, a larger body appears near the nucleus; this gives the red fluorescence characteristic of ribonucleic acid, is non-infective, contains a core of DNA and is presumably derived from the original infecting particle. This form corresponds to the basophilic " initial body " seen in Giemsa-stained preparations. Following a latent period of about 20 hours during which this body increases in volume, new DNA elementary bodies appear. They attain their maximum number after a further 20 hours and are then released from the cell; some, but not all, are capable of infecting other cells. Lymphogranuloma venereum virus (strain JH provided by Professor C. F. Barwell of the London Hospital) behaves somewhat differently, the initial body itself undergoing a series of divisions before the appearance of the DNA elementary bodies.

Dr. Furness is also studying the influence on viral growth of various constituents of cell culture media.

Dr. Blyth adapted a Chinese (TE55) strain of trachoma and the LB1 strain of inclusion blennorrhoea to growth in cultures of trypsinized chick embryo cells, and is investigating the conditions for high yields of infective virus.

Serological Studies. Dr. Reeve and Miss Graham devized an accurate method of measuring neutralizing antibody, based on inhibition of inclusion formation in HeLa cells. With Dr. Blyth and Dr. Taverne, they studied the use of adjuvants for preparing neutralizing antisera in rabbits. Antigens adsorbed on calcium phosphate or precipitated with alum are of little use, but those suspended in water-in-oil emulsion are effective. It is noteworthy that sera with large amounts of complement-fixing group antibody do not necessarily neutralize virus. Using water-in-oil emulsion adjuvants, antisera to several strains are now being prepared in order to study their serological relationships.

Dr. Reeve and Dr. Taverne continued their work on the complement-fixing antigens of these agents. Specific antigens are difficult to demonstrate; to obtain large amounts of virus for fractionation, they are investigating ways of increasing the yield from HeLa cells; infection and maintenance of cells in suspension, rather than as monolayers on glass, has given promising results. These studies also demand a high degree of purification; electron micrographs made by Dr. K. Pedler (Institute of Ophthalmology) confirm that trypsin is useful for this purpose. Unlike fluorocarbon treatment, it is innocuous to the virus.

Trachoma Vaccine. To obtain a standard challenge inoculum for baboon experiments, Dr. Collier and Dr. Blyth measured the infectivity of LB4 virus for the conjunctiva, and estimated that from 1 to 10 egg infective doses can induce infection. In an attempt to find a strain of trachoma suitable as challenge in immunization experiments, 3 Gambian strains were tested for pathogenicity

in baboons. All induced mild infections; these experiments are continuing. Further vaccination studies (Report 1961) suggested that immunogenicity of a given antigen depends more on its content of live virus than on the total amount of group antigen; and that the level of complement-fixing antibody in the sera of immunized animals does not reflect their state of immunity.

Investigations in West Africa. Under Dr. Collier's direction the Medical Research Council Trachoma Unit completed a small field trial to test the immunogenicity of a live trachoma antigen by assessing its power to modify the natural course of the disease. Twenty children with virus-positive active trachoma each received 3 subcutaneous doses of vaccine at fortnightly intervals; their subsequent progress was compared with that of a similar group that was given a dummy vaccine. The results of repeated clinical and virological examinations, analyzed statistically by Dr. I. A. Sutherland (M.R.C. Tuberculosis Research Unit) showed a significant benefit from vaccination at the 8th week thereafter; however, this effect was of short duration. There was evidence that the vaccine benefited only a proportion—about one third—of those who received it. This experiment was designed not to test the value of vaccine therapy in trachoma, but as a means of detecting an immunological response to the vaccine, before embarking on a much larger prophylactic trial. The demonstration of even a limited effect on a well-established, chronic and localized disease is encouraging, and it is hoped to proceed to more extensive trials in 1962/3.

Researches on the clinical aspects and virology of trachoma were also pursued, with emphasis on its onset and course in infants; it was established that in young children the diagnostic criteria laid down by the World Health Organization are not always applicable. In a study of genital tract infections caused by trachoma-like agents, virus was isolated from the uterine cervices of three women whose babies developed inclusion-positive eye infections shortly after birth.

Conjunctival Strains of Haemophilus spp. from Trachoma Patients. Miss Sampson investigated 244 strains of Haemophilus bacilli isolated from the conjunctivae of 58 trachoma patients in the Gambia. Her results confirmed the results of the 1960 series of tests in that patients may carry more than one type of Haemophilus: 72% carried one type (62% in 1960), 26% carried two types (29% in 1960) and one patient carried three types (9% in 1960). The 244-cultures were typed culturally and biochemically, and sorted serologically by a simple gel-diffusion test.

VACCINIA VIRUS

Dr. Kaplan continued his studies on the inactivation of vaccinia virus infectivity; and, with Mr. Vallet, started to prepare a batch of ultraviolet-irradiated vaccine of high immunogenic potency for a clinical trial early in 1962. He began a study for the World Health Organization of the virus content of vaccinal material from water-buffaloes. The results so far indicate that the water buffalo is not as good a vaccinifer as the sheep. Aided by a grant from the World Health Organization, he is investigating the effect on vaccinia virus infection in rabbits and mice of antivaccinial antibody prepared in an unrelated species.

Anti-vaccinial Antibody. Dr. Murray continued his studies on immunity to vaccinia virus. He has some evidence that antibody produced in the early stages of convalescence may be less avid than that produced later. He also continued to develop a rapid diagnostic method for variola virus by immuno-fluoresence

microscopy. Together with Dr. Kaplan he is studying the relationship between circulating antibody and skin immunity to vaccinia virus infection. This work is also assisted by a grant from the World Health Organization.

Mr. Robinson continued his experiments on the culture of chick embryo cells in stirred suspensions, and began a study of mammalian kidney cells in monolayer and suspension cultures for the propagation of vaccinia virus.

Tissue Culture Vaccine. With the assistance of the Epidemiological Research Laboratory of the Central Public Health Laboratory, several trials are being conducted in civilian clinics of smallpox vaccine prepared in chick embryo cell cultures. The vaccine has already been successfully used in the Royal Air Force.

IMMUNOLOGY AND SEROLOGY

SEROLOGICAL IDENTIFICATION OF BLOOD MEALS

Feeding Habits of Bloodsucking Insects. The information obtained by Dr. Weitz and Miss Lee-Jones on the natural feeding habits of tsetse flies was extended to studies of the epidemiology of trypanosomiasis in the field. For example, Mr. H. A. W. Southon (East African Trypanosomisasis Research Organization, Tororo, Uganda,) is carrying out large scale surveys of infection of *Glossina palpalis, G.pallidipes* and *G.brevipalpis* in S.E. Uganda and the identification of the blood meals of these tsetse flies should throw some light on the mode of transmission, the reservoir hosts and the persistence of infection of *Trypanosoma rhodesiense* in endemic areas. Similarly, Miss B. M. Leggate (Department of Veterinary Services, Tsetse and Trypanosomiasis Branch, Salisbury, Southern Rhodesia) is studying the rate of infection of *G.morsitans* with *T.brucei, T.vivax* and *T.congolense* and the correlation of her findings with the feeding habits of the fly in some areas in Southern Rhodesia indicates that some game animals, mainly bovids, are largely responsible for the infection of the flies. It is hoped to continue these studies over several years to determine whether these correlations, which show seasonal variations, are consistently observed.

Work on the relative importance of different mosquitoes in the epidemiology of malaria in many parts of the world is continuing in collaboration with the World Health Organization.

SCORPION VENOMS

Mr. Stone is investigating the immunological and pharmacological properties of scorpion venoms of *Buthus occitanus, Androctonus australis* and *Parabuthus transvaalicus.* Antisera were prepared by immunizing rabbits with venom obtained by electrical stimulation of live scorpions and with extracts of telsons. The telson extracts appear to contain toxic factors not present in the venom ejected after electrical stimulation. The additional toxic factors in telsons and their separation from the venom toxins is under investigation.

ANTITOXIN PRODUCTION

Refinement of Therapeutic Antitoxins. The use of modified cellulose ion-exchangers to fractionate antitoxic sera (see Report 1961) was continued by Dr. Dolby, and β-globulin fractions of fairly high purity were obtained from horse antisera. It is clear, however, that both β- and γ-globulins are altered during the chromatographic fractionation; although about 95% of the protein can be recovered in the fractions, the yield of antitoxic activity is usually only about

50%, and sometimes considerably less, a feature also of antisera fractionated with ether or alcohol. Attempts to improve the yield by such methods as the inclusion of a reducing agent in the solvent used for chromatography, have so far proved unsuccessful, and the study of other possible causes is now under way.

Further evidence that these fractions contain modified protein came from a study of the action of pepsin on them. When antitoxic horse sera are purified by pepsin treatment the product contains 15-20% of the initial protein and 50-60% of the initial antitoxic activity; the same process carried out on β- and γ-globulin fractions isolated by chromatography yields a product with 30-50% of the protein and 35-45% of the antitoxic activity of the original fraction. The structure of the fractionated globulins has apparently been altered so that the pepsin is no longer able to split the globulin molecule into an inactive portion and a portion retaining the bulk of the antitoxic activity.

EXPERIMENTAL PATHOLOGY

MECHANISMS OF INFLAMMATION

Vascular Permeability Changes in Injury. Professor Miles began a comparative study of the various types of substances said to be "antiphlogistic", testing their effect on permeability changes in guinea-pigs induced by various permeability factors, chemical burns, bacterial toxins and bacterial infections. None of the agents so far tested, including salicylates and iproniazid, had much effect, and positive effects were not readily correlated with any of the postulated specific actions of the drugs. He also investigated the possibility that the apparent exudation of circulating dye into the skin under the influence of permeability factors was, not an index of increased permeability, but only the expression of an increased affinity of treated tissue for the dye; with negative results.

Miss Mason and Dr. Mackay used chromatographic methods to fractionate preparations of guinea-pig permeability factors, and to separate contaminating peptidases and plasmins.

Identity of Globulin Permeability Factor and Kininogenase. The association of hypotensive and permeability-increasing potency in preparations of mammalian permeability globulins and of human plasma kallikrein suggests that both are kininogenases and both act on small vessels by catalysing the production of a kinin. Miss Mason showed that preparations of guinea-pig permeability globulin, when treated to destroy both a heat-labile kininogenase that is present and the peptidases that interfere with the detection of kinins, do not produce kinins from the accepted pseudo-globulin substrates. They produce kinins from dilute guinea-pig plasma, reacting with a system different from the presumed substrate of kallikrein, and apparently doing so, not as a kininogenase, but as an activator of a kininogenase in the plasma. The preparations retained both their hypotensive and their permeability-increasing potency.

Miss Mason also established that potent preparations of the permeability-factor in guinea-pig coagulating gland (Report 1958) contained a potent kininogenase, insusceptible to soya bean trypsin inhibitor.

The Activation of Permeability Factor in Plasma. Dr. Ratnoff is studying the rôle of Hageman factor in the activation of permeability factor in human and guinea-pig plasma, and the relation of the activating process to blood-coagulation.

MECHANISMS OF INFECTION AND DEFENCE

Non-specific Immunity. Miss Brimacombe, with Professor Miles, continued her study of changes in resistance to intracutaneous infection during the course

of acute systemic streptococcal infection of the guinea-pig; testing the skin with the infecting streptococcus and two unrelated pathogens. The course of skin-immunity was similar during the first week of infections by two strains of Group C streptococcus, but differed in the later weeks according to the infecting strain; one of which induced a short acute infection, the other a late chronic infection as well. The rise in non-specific resistance in the earlier stages resembles that induced by endotoxins, including a concomitant biphasic change—hypersusceptibility succeeded by hyposusceptibility—to local adrenaline. But results with other stimuli, not obviously related to endotoxin, suggest that the response is common to many types of injury. In it, the skin tissues appear to be stimulated much in the way reported for the reticulo-endothelial system. The interpretation of local resistance to the systemically infecting streptococcus was in some instances complicated by the development, within 3-4 days of infection, of substantial, delayed bacterial hypersensitivity.

The adrenaline hypersusceptibility was not obviously associated with decreased resistance; in some circumstances administration of the anti-adrenaline drug, phenoxybenzamine, enhanced, and in other circumstances, depressed resistance to cutaneous infections.

BIOCHEMISTRY

THE HUMAN BLOOD GROUP SUBSTANCES

Dr. Watkins and Professor Morgan continued to investigate, by the indirect method of haemagglutination and precipitation inhibition with simple substances of known structure, the chemical nature of the serological determinant units in the water-soluble A, B, H and Le^a and Le^b blood-group substances. The results allow the following inferences to be drawn (1) that β-*N*-acetyl-D-glucosaminoyl groupings are present as non-reducing terminal units in H specific substances, in addition to the α-L-fucosyl units previously shown to play an important part in H specificity, (2) that the terminal α-D-galactosyl-units in B substance are joined by 1 : 3 glycosidic linkage to the next sugar and (3) that a terminal 4-*O*-β-D-galactosyl-*N*-acetylglucosamine disaccharide unit, which is believed to be the structure responsible for the cross-reactivity of blood-group substances with horse anti-Type XIV pneumococcus serum, is probably joined by a 1 : 4 glycosidic linkage to a third galactose unit. Further support was obtained for the earlier suggestion (Report 1958) that a branched trisaccharide unit is an important part of the Le^a determinant structure, and that two α-L-fucosyl units joined as branches to each of two adjacent sugars play a part in Le^b specific structure.

The P blood-group system was also investigated by the inhibition method, and of seventy small-molecular compounds examined, only 3-*O*-α-D-galactosyl-galactose inhibited the agglutination of P_1 cells by a human anti-P_1 serum. An α-D-galactosyl grouping therefore seems to be involved in P_1 specificity. Macromolecular substances with P_1 specificity do not occur in a water-soluble form in the secretions or tissue fluids in man, and the P_1 antigen has not yet been isolated from red-cells. The possible association of P_1 specificity with a carbohydrate structure prompted an examination of sheep hydatid cyst fluid known to be a powerful inhibitor of P_1 agglutination. Treatment of dried cyst fluid with 95% phenol gave a phenol-insoluble water-soluble residue with a 200-fold greater serological activity than the crude cyst substance. The material does not diffuse through a Visking membrane and on acid hydrolysis yields 39% reducing sugars (as glucose) and 12% amino sugar (as glucosamine), and

amino acids; indicating that the P_t inhibitor in hydatid cyst is mucopolysaccharide in nature.

Dr. Koscielak studied methods for extraction of blood-group specific A substance from human erythrocytes by organic solvents, and its purification by chromatography on silicic acid columns with chloroform-methanol-water systems. The most active material contained sphingosine, a long-chain fatty acid, *N*-acetylgalactosamine, and galactose and could be regarded as a glycolipid.

Dr. Watkins, Dr. Koscielak and Professor Morgan compared the serologically active (determinant) groupings in the water-soluble A mucopolysaccharide with those in the A active ganglioside-like material obtained by Dr. Koscielak from human group A red-cells. Precipitation of both these materials by the plant seed anti-A reagent from *Dolichos biflorus* is inhibited by *N*-acetylgalactosamine and not by any other sugar in the A substance; precipitation of both materials by a rabbit anti-A serum is inhibited by the A active disaccharide 3(?4)-*O*-α-D-*N*-acetylgalactosaminoyl-D-galactose, isolated earlier from the water-soluble A substance by Dr. Côte and Professor Morgan (Report 1957). The A activity of both preparations is destroyed by an enzyme in extracts of *Trichomonas foetus*, and this destruction can be specifically inhibited by *N*-acetyl-galactosamine. In gel-diffusion tests with anti-A serum, the substance from group A cells was identical with the water-soluble A substance. These results indicate that although the blood-group substance obtained from the erythrocyte surface differs in its gross chemical composition and properties from the water-soluble A active mucopolysaccharide, the groupings which determine serological specificity are chemically similar and contain a terminal non-reducing *O*-α-D-*N*-acetylgalactosaminoyl unit.

Dr. Joyeux and Dr. Watkins are investigating the presence in single macromolecules of more than one blood group specificity. Specificities determined by allelic genes A and B are present on single molecules (Report 1957), and it is now proposed to examine specificities determined by genes at independent loci, such as A and H, and A and Le^a, to find the extent to which these characterize a single molecule.

Recent work on the biosynthesis of carbohydrates and mucopolysaccharides has shown that the most common pathway for synthesis is via sugar nucleotide intermediates. The specific polysaccharide from *Trichomonas foetus* contains some of the sugar components present in the water-soluble A, B, H and Le^a blood-group mucopolysaccharides, and Dr. Gompertz and Dr. Watkins are therefore examining this organism for sugar nucleotides that might be used for biosynthetic studies on the blood-group substances.

Several methods have been studied for the separation of the enzymes in extracts of *T.foetus* which destroy the serological activity of the blood-group substances and hydrolyse low molecular weight glycosides (Report 1956, 1959, 1960). Some success was achieved but, owing to the lability of the enzymes, considerable losses in activity occur during purification. Dr. Romanowska and Dr. Watkins therefore investigated the separation of the enzymes by gel filtration on the cross-linked dextran Sephadex-75. Partial separation of the enzyme destroying H serological activity from those destroying A and B activities was accomplished and the neuraminidase activity in the crude extract separated into two fractions. The two neuraminidase-containing fractions are under examination to determine differences in specificity and properties other than molecular size.

Dr. Pusztai and Professor Morgan continued their work on the amino-acid composition of the specific blood-group substances. Contrary to expectation,

the high content of carbohydrate in the substances protected certain of the more acid-labile amino acids from destruction during the hydrolysis of the amino-acid-containing moiety. Six specimens of A substance, three of B substance and one specimen of each of H and Le^a substances had closely similar amino-acid composition. The sulphur-containing amino-acids were absent or present in very small amounts; the hydroxy amino-acids threonine and serine were present in greatest amount, and together with proline, made up about half the total amino-acid-containing component. Irrespective of the group specificity the amino-acid composition of each of the water-soluble blood-group substances is closely similar.

Dr. Painter and Professor Morgan examined the behaviour of blood-group A and B mucopolysaccharides when these acid-labile materials were hydrolyzed with a water-soluble polystyrene sulphonic acid under the conditions described in last year's Report. The separation and identification of the di- and oligo-saccharides is as yet incomplete, but nevertheless has already confirmed that the unit responsible for A serological activity is 3(?4)-*O*-α-D-*N*-acetylgalacto-saminoyl-galactose and for B activity is 3-*O*-α-D-galactosyl-galactose.

CARBOHYDRATE STUDIES

Chemical Synthesis and Uses of Modified Carbohydrates. Most of the carbo-hydrate studies are directed to the examination of enzyme specificity and the eelated subject of specificity of antibodies. In each such investigation the normal rinzyme substrate is being subjected to chemical modification at specific points in the molecule. The product is then tested with the appropriate enzymes to discover whether or not they " recognize " the modification by failing to attack the carbohydrate. In this way the parts of the substrate molecules important to the enzymes can be distinguished. These researches, which open up the prospect of the synthesis of 3,6-dideoxyhexoses from polysaccharides by a far more convenient method than the customary synthesis from monosaccharides, have implications in other biological fields.

Dr. Bines studied the action of α-amylase on 6-deoxyamylose, a polymer produced by chemical modification of potato amylose. Hydrolysis took place, but at so low a rate as to indicate that the primary hydroxyl groups in amylose, absent in the modified polymer, are important to the enzyme in its action. 3,6-Dideoxyamylose was synthesized and the sugar paratose (3,6-dideoxyglucose) isolated from it. Paratose is a determinant of immunological specificity in *Salmonella paratyphi* and the dideoxyamylose is to be tested for possible anti-genicity. Dr. Himmelspach is applying the same modifications to a mannan and a galactan to provide further polymers of naturally occurring dideoxy sugars. Dr. Bines also synthesized deoxysucroses for use as substrates for sucrose-metabolizing enzymes.

Mr. Egyud has obtained a series of sugar phosphates by enzymic and chemical synthesis. These are either isomers or close structural analogues of glucose 6-phosphate and are being tested against the enzymes normally acting on glucose 6-phosphate.

Dr. K. Morgan examined the specificity of maltose phosphorylase, an enzyme from *Neisseria meningiditis* which synthesizes maltose from β-glucose 1-phosphate and glucose. It was found that glucose can be replaced by 2-deoxy-, 6-deoxy- and 2-methyl-glucose, resulting in the synthesis of several new disaccharides. Methylation of glucose at C-3 or C-6, or deoxygenation at C-1, destroyed the capacity to substitute for glucose. A series of sugar phosphates,

analogues of β-glucose 1-phosphate, is in course of preparation to examine the specificity further.

Mrs. Taylor continued the synthesis of chemically modified isomaltodextrins for use as substrates for dextran-splitting enzymes and as inhibitors of the dextran-antidextran system. The compounds so far synthesized are the methyl β-glycosides, benzyl β-glycosides, 6-deoxy derivatives and 6-carboxy derivatives. Clinical dextran was oxidized selectively at the primary hydroxyl groups to induce cross-reactivity against type 11 pneumococcus polysaccharide. An outcome of the work was a modification of the charcoal chromatographic method for separating oligosaccharides. By eluting the sugars with an acid solvent much sharper separations were obtained.

Glycogen and Starch Metabolism. Dr. Abdullah discovered a new enzyme in rabbit muscle that dismembers the branched structure of glycogen. The enzyme is the same as is used by the potato to attack the plant analogue of glycogen, amylopectin. Mr. Smith examined the synthetic activity of the crystalline α-amylase of *Bacillus subtilis* (Report 1961), and the indications are that this is not the property of the α-amylase but of an impurity.

Structures of Polyglucoses. Dr. Abdullah continued the application of the acetolysis method of breakdown of dextrans (Report 1961) and for the first time maltose and the related trisaccharide panose were obtained from a dextran. Similarly the dextran used clinically was shown to contain α-1, 3-bonds, by virtue of its breakdown to the disaccharide nigerose. A polysaccharide from *Salmonella typhimurium*, provided by Dr. Stocker, was shown to consist of β-glucose joined through 1,2- and 1,6-bonds and as such is unique.

PHOSPHOLIPIDS

The object of this work is to examine the structure and distribution of phospholipids and other lipids in different tissues and cells and to attempt to relate the findings to their function.

Dr. Macfarlane found previously that in the protoplast membrane of *Micrococcus lysodeikticus* the main phospholipid was diphosphatidylglycerol (cardiolipin) which occurs also in the mitochondria of animal cells. In extracts of whole cells, however, the phospholipid occurred mainly as monophosphatidylglycerol, which was isolated for the first time; a glycolipid, identified as a mannosyldiglyceride, and a polymannose were also isolated from these cells. Comparative studies were made on the lipids of *Staphylococccus aureus*, strain Duncan, and *Salmonella typhimurium*, strain LT_1. The staphylococcal lipids were similar to those of *Micrococcus lysodeikticus* in that the main phospholipid was phosphatidylglycerol and the fatty acids contained a high proportion of C_{16} branched acids; instead of a mannosylglyceride, however, a glucosylglyceride was found, and no phosphatidylinositol was detected. The *Salmonella* lipids also contained phosphatidylglycerols, but in small proportion, the main component being phosphatidylethanolamine, which was absent from *Micrococcus* and *Staphylococcus*; the fatty acids included palmitic, palmitoleic and oleic acids, and two other acids identified as cis-9,10-methylene hexadecanoic acid and the homologous lactobacillic acid, which have been previously found in *Escherichia coli*.

None of the three species of bacteria contained lecithin and only one contained phosphatidylethanolamine. These phospholipids appear to be less ubiquitous than previously supposed and can no longer be regarded as a necessary constituent of the living cell. On the other hand, phosphatidylglycerols have

been found in every kind of cell (plant, animal and bacteria) in which they have been looked for.

Dr. Gray studied the total lipid composition of Landschutz ascites carcinoma cells with particular emphasis on the distribution of the phospholipids, the composition of their fatty acids and aldehydes and the possible presence of unusual minor components. Previous study of the phospholipid composition of a variety of normal tissues formed a basis for comparison. A preliminary fractionation of the lipid extract from the cells gave three fractions: the phospholipids (6.6 g./100g. dry wt. of cells), other lipids (7.0 g./100 g. dry wt. of cells) and a lipoprotein (1.6 g./100 g. dry wt. of cells, 65% protein). Cardiolipin, phosphatidic acid, phosphatidylethanolamine, phosphatidylserine, ethanolamine plasmalogen, serine plasmalogen, choline plasmalogen, phosphatidylcholine, sphingomyelin and lysophosphatidylcholine were quantitatively determined. The phospholipid distribution did not vary significantly from those in normal tissues. The phospholipids from the lipoprotein fraction had the same composition as those of the main extract.

The distribution of fatty acids in the various phospholipids varied far less from class to class than those present in the normal tissues examined, especially with respect to the proportions of stearic, palmitic, oleic and linoleic acids. Stearic acid was the predominating saturated acid in all the phospholipids, thus differing from normal tissues in which the choline-containing phospholipids have palmitic acid as the major saturated acid.

The lack of variation in the general chemical composition of tumour cells and normal cells gives greater significance to the presence of unusual minor components. A minor component isolated from the phospholipid extract of the tumour cell was identified as a cerebroside containing only glucose. This was unusual, because cerebrosides from tissues normally contain galactose, if not exclusively, at least as the major carbohydrate component. A different strain of tumour cells is being studied to determine whether the minor components, if present, differ in type or quantity from those of the Landschutz ascites cell.

Dr. Pietruszko examined the lipid composition of red bone marrow from pigs, which presented several features of interest. The phospholipids, which were only 0.1% of the total fat, contained lecithin as the main component, and an unusually high proportion (16%) of a glycerylether derivative of phosphorylcholine. The second main component was a nitrogen-containing acidic phospholipid, which was isolated, but the nitrogenous base, which appears to be an unusual type of hydroxyamino acid, has not yet been identified. The fatty acid composition of all the phospholipid fractions and the glycerides was very similar, which is also unusual, and polyenoic acids were present in very small amounts.

Dr. McCay has examined the inhibiting effect of liver phospholipids on the synthesis *in vitro* of ascorbic acid from L-gulonolactone by an enzyme from liver microsomes. The effect appears to be correlated with the presence of highly unsaturated fatty acids, possibly as a result of their peroxidation.

BIOPHYSICS

The installation of the Spinco analytical ultracentrifuge was completed. Its wide variety of rotors and cells proved very useful in extending the range of experimental measurement.

BLOOD GROUP SUBSTANCES

The physico-chemical investigation of blood-group specific mucopolysaccharides was continued by Dr. Creeth. About twenty preparations and sub-fractions were examined in the ultracentrifuge and two samples, both B-active, were found sufficiently homogeneous to warrant detailed study. The purpose of this work is to determine the properties of the mucopolysaccharides in transport and equilibrium, to interpret their properties in terms of molecular parameters, and to correlate, if possible, changes in molecular parameters with changes in biological activity. One of the B-active substances is currently being examined to determine the effect of temperature on the concentration-dependence of the sedimentation coefficient.

In conjunction with Dr. Koscielak, the properties of two A-active fractions isolated from red cells were investigated. The serological activity of these materials, when present together in aqueous solution, was markedly different from their activity when separated. From their behaviour in the ultracentrifuge it was shown that some degree of complex formation occurred; the combination, however, was not in simple ratio nor was it in reversible equilibrium.

HUMAN PLASMA PROTEINS

Purification of Antihaemophilic Factor. The lability of antihaemophilic factor (AHF) in both plasma and concentrates is a major hazard during purification. It was established (Report 1961) that in concentrates AHF has an optimum stability at pH 7.1, suggesting that the lability is not predominantly due to the destruction of AHF by plasmin. In the concentrates obtained 70% of the protein is fibrinogen, and the presence also of small amounts of active plasmin can be detected by the eventual loss of the capacity of this fibrinogen to form a clot, when thrombin is added to the preparation. The length of time required for the fibrinogen to lose the capacity to clot, the " fibrinogen life ", is inversely related to the concentration of plasmin present. From measurements made by Mr. Walton and Dr. Kekwick on a series of antihaemophilic factor preparations, it is clear that there is no direct correlation between the instability of the antihaemophilic activity and the amount of plasmin present. The addition of ε-aminocaproic acid to such preparations, in concentrations known to be highly inhibitory to the action of plasmin, although considerably prolonging the " fibrinogen life ", only slightly increased the stability of the AHF. However, treatment of the AHF preparations with di-isopropyl-phospho-fluoridate (DFP) substantially increased stability, suggesting that a DFP-sensitive enzyme other than plasmin may be a major factor contributing to the instability of AHF in solution. A fraction was separated from concentrates of antihaemophilic factor containing little AHF but much of the labilising factor.

Reference Standard of Antihaemophilic Factor. The wide range of AHF activity in normal human plasma, 50 to 200% of the mean, and the instability of the factor in drawn plasma, make the standardization of assays a difficult problem. To overcome this a series of intercomparisons of the activities of several batches of freeze-dried concentrate of antihaemophilic factor has been made during long term storage at temperatures from +20° to —25°. Losses in activity occur during the storage of freeze-dried preparations at room temperature (20°), of the order of 15% in 9 months, whereas no loss in activity was detected during similar peroids of storage at +4° or —25°.

The results indicate that freeze-dried reference preparations should be stored at no higher a temperature than 4° and it is also obviously desirable to store material intended for clinical use at this temperature, a more practicable possibility in most circumstances than —25°.

Plasma Globulins. Professor Martin has investigated the sedimentation characteristics of the globulins associated with lymphoreticular hypo- and hyperplasia.

OTHER PROTEINS

Human Urinary Proteins In collaboration with Dr. F. V. Flynn (University College Hospital), a series of urinary protein preparations are being examined in the ultracentrifuge by Dr. Kekwick. Some years ago (Report 1954) when the urine proteins from cases of cadmium poisoning were studied, they were found to have a sedimentation coefficient of about 2.0 S. It was thought that such proteins might be pathognomonic of the condition; but proteins with similar characteristics were found in the urine of patients with Fanconi's syndrome. In addition to their sedimentation coefficients the molecular weights of the urinary proteins are being estimated by the Archibald procedure in the Spinco analytical ultracentrifuge.

Iodine-modified Ovalbumin. Comparisons of the properties of native and chemically modified proteins may provide valuable information about structure and configuration, particularly when the modification is both specific and limited in extent. The reaction of iodine with ovalbumin was studied electrochemically by Dr. Creeth, and conditions were found under which only two sulphhydryl residues of the ovalbumin reacted. It was shown from ultracentrifuge measurements that no dimer was produced in the reaction, and from this and other evidence it was deduced that the modified ovalbumin differs from the native protein only by the introduction of one sulphenyl iodide and one sulphenic acid group.

BLOOD PRODUCTS LABORATORY

Pharmacologically Active Substances in Plasma. Dr. Mackay and Dr. Maycock continue to investigate these substances, in particular the kininogenetic system of the fraction rich in antihaemophilic globulin (AHG) and chemically related fractions.

Activation of a kininogenase by streptokinase and by a fraction separated from intact plasma (i.e. collected without glass contact) was studied, the latter activator being obtained by elution from Celite used to adsorb the intact plasma. Kinin production was demonstrable in AHG from fresh citrated blood (18-24 hours old) but not in a similar fraction prepared from blood stored at 4° for three or more weeks. Activation of plasminogen and hydrolysis of fibrinogen occurred with both preparations, so kininogen is neither plasminogen or fibrinogen. Kininogenic activity disappeared from AHG on storage in solution at 4° at about the same rate as antihaemophilic factor, but was not destroyed by conditions which destroyed antihaemophilic factor.

Chromatographic separation of the components of AHG on DEAE Sephadex suggests that the kininogenase is plasmin, but so far has given no information about the nature of the kininogen.

The plasma kinin may be separated from inactive protein by chromatography on Sephadex G75 and by extraction with boiling 75% ethanol. These observations and dialysis experiments suggest that the kinin is a small molecule like bradykinin.

Plasminogen. During the year the large scale technique developed at the Michigan State Laboratories was modified to produce plasminogen from a

fraction from fresh human plasma precipitated by ether during the routine plasma fractionation carried on in the laboratory.

BLOOD GROUP RESEARCH UNIT

Once again most of the year's work has arisen from cross-matching problems in preparation for transfusion; and again most of the samples have been sent by friends abroad.

The Xg System. All the inherited blood group antigens of man and animals when adequately studied have been found to be controlled by genes carried on the autosomes. The realization that a 'new' antigen was controlled by genes on the X-chromosome gave, therefore, a lot of pleasure and a prospect of exciting work to follow.

Serum from a much transfused patient containing an antibody which defined an apparently 'new' antigen was sent by Dr. J. D. Mann, of the Butterworth Hospital, Grand Rapids, to Dr. Amos Cahan, of the Knickerbocker Foundation, New York. Dr. Cahan confirmed the presence of a new antibody and sent the serum on to this Unit, where the sex-linked nature of the corresponding antigen was recognized.

About 64% of men have the antigen and about 88% of women. Family tests are also startling: for example from the mating father Xg (a+) by mother Xg (a—) all the sons are Xg (a—) and all the daughters Xg (a+), and from the mating Xg (a+) by Xg (a+) any Xg (a—) children must be sons. Hitherto, blood groups have ignored sex differences.

The antigen should make a good contribution to fixing the relative position of genes on the X chromosome. To this end the Unit is testing families with other X-borne conditions such as: glucose-6-phosphate dehydrogenase deficiency (G6PD) (with Mr. A. Adam and Dr. C. Sheba of Tel-Hashomer, Israel, Dr. M. Siniscalco of Sardinia and Dr. V. McKusick, of the Johns Hopkins Hospital); haemophilia and Christmas disease (with Dr. J. R. O'Brien of Portsmouth, Dr. C. A. Holman of Lewisham, Dr. R. M. Hardisty, of the Hospital for Sick Children, and Dr. A. C. Stevenson of Oxford, and Dr. J. B. Graham of the University of North Carolina, Dr. Goldsmith of the Blood Group Reference Laboratory and Dr. T. Gwynfor Jones of the Westminster Hospital; Duchenne's type of muscular dystrophy (with Dr. Helen Blyth of Leeds, Dr. Stevenson, Dr. J. N. Walton of Newcastle and Dr. J. van den Bosch of University College, London); familial hypophosphatemia (with Dr. Graham); sex-linked hypogammaglobulinaemia (with the Medical Research Council); red-green colour-blindness (with Mr. Adam and Dr. Sheba, Professor P. E. Polani, of Guy's Hospital, and Mr. J. S. S. Stewart and Dr. C. Cameron of St. Andrew's) and various other more exotic conditions such as familial ichthiosis and partial albinism.

This work is only just beginning, but already it is becoming clear that the locus for Xg is well away from the loci for G6PD and red-green colour-blindness which are close to each other. This is a good thing: it would have been a pity indeed if the three best markers on the X chromosome were all marking more or less the same spot.

Several women are known who lack part of one of their X chromosomes: they and their families are being grouped in the hope that the position of the Xg locus may thereby be assigned to the long or to the short arm of the X, that is, below or above the centromere. This work is being done with Professor

Polani, with Dr. W. M. Court Brown of Edinburgh, Dr. M. Fraccaro of Oxford, and Dr. J. Lindsten of Stockholm.

People with the wrong number of sex chromosomes are being grouped, notably XO females (Turner's syndrome) and XXY males (Klinefelter's syndrome) in the hope that something may be learnt about the origin of these conditions —whether the causal sticking together of two Xs or of an X and a Y at cell division, has happened at oogenesis, spermatogenesis or at a post-zygotic stage of development. This work is being done in collaboration with many physicians and cytogeneticists.

The Auberger System. Through the kindness of Dr. C. Salmon of the Centre Départemental de Transfusion Sanguine, Paris, the Unit shared the enjoyment of establishing the existence of a ' new ' blood group system called Auberger. The antigen, Au^a, is present in the red cells of about 82% of people tested in Paris and London. The patient who made the antibody is dead so the continuing usefulness of the system will depend on further examples of the antibody being found: this should happen sooner or later, and recognition should be easy by means of the red cells of people already grouped, amongst whom are most of the Institute Staff.

The P System. The work on the rare Finnish antigen P^k (Report 1961) adumbrated the existence of genes not at the P locus but acting on hypothetical P precursor substance. A serum sent by Dr. W. T. Snoddy, of Oklahoma City, contains an antibody which identifies a very common antigen which also appears to be controlled by genes associated with P rather than allelic to P.

The Rh System. There seems no end to the complexity of this system. More than twenty samples of blood from apparently D positive white and negro women who have disobeyed the rules and made anti-D during pregnancy were tested in the Unit. That D positive people can, on rare occasion, make anti-D is not an original observation, but it is hoped that the pattern which is emerging as a result of these tests will make some sense. A similar pattern is disclosing itself in e positive Negroes who have made anti-e. It seems that people with D and with e can lack parts of these antigens and can make antibody to the missing part.

Some more examples of cells with ' depressed ' Rh antigens (Report 1961) have been tested without further light being thrown on the genetic background.

Duffy and Kidd Systems. There are two antigens, Fy^a and Fy^b, in the Duffy systems and all Europeans so far tested have one or the other or both: the majority of Negroes, on the other hand, lack both and it is assumed that they are homozygous for a third allele, *Fy*, which either makes an antigen for which no antibody has yet been found or makes no antigen at all (Report 1956).

In the Kidd system also there are two antigens, Jk^a and Jk^b, and all Europeans tested have one or the other or both: but in Hawaii several people are known who lack both (Report 1959) and again a third and ' silent ' allele, *Jk*, is presumed to be responsible.

Analysis of tests on random English people and on families made by Dr. T. E. Cleghorn (South London Blood Transfusion Centre, Sutton), and by the Unit shows that heterozygotes for both *Fy* and *Jk* (or for genes indistinguishable in effect) must on occasion also be present in our population. This observation will somewhat limit the future use of the Duffy and Kidd groups in medico-legal work.

Some of the G6PD families tested for Xg (see above) were Yemenite Jewish: several members lack both Fy^a and Fy^b which suggests that *Fy* must be very common in these people.

For routine antisera the Unit is indebted to many colleagues, notably those in the Medical Research Council Blood Group Reference Laboratory and the South London, North East London, Birmingham and Sheffield Blood Transfusion Centres. Dr. Cleghorn has provided samples of blood from many normal families which were invaluable in the Xg investigations. Again the Unit is grateful to the Staff of the Institute, and sometimes to their families also, for samples of blood which have played a major part in many of the investigations.

BLOOD GROUP REFERENCE LABORATORY

The Unit acts as the central Reference Laboratory for the investigation of blood group problems, and as the supply centre for grouping serum, for the United Kingdom. It also acts under the auspices of the World Health Organisation as the world reference centre.

Liquid blood grouping sera and anti-human-globulin serum are supplied for use in the United Kingdom, and dried sera for use overseas. Demands for serum of nearly every kind have continued to increase and have in nearly all cases been met. Help to overseas laboratories has continued to be an important activity. Through the initiative of the World Health Organisation, national blood group reference laboratories have been designated during the year in a large number of countries which did not possess them before and these have been helped in various ways, but especially by the full blood grouping of members of their staffs, to create panels of donors of red cells for use in the identification of unknown haemagglutinins. Large numbers of blood and serum specimens from Great Britain and overseas have been examined for blood group antigens and antibodies, as part of clinical investigations, for research purposes, and for routine blood grouping purposes, especially in connection with the control and supply of grouping sera.

The methods of production and standardisation of anti-human-globulin serum have been under review by Dr. Goldsmith and Miss Ikin, in order to meet both the rapidly growing demands for the serum and advances in knowledge of its properties.

Dr. Goldsmith continued his investigations into antibodies against leucocytes, and extended his work on antibodies against platelets in sera from cases of such conditions as thrombocytopenic purpura. Investigations into agglutinins of vegetable origin, with a view to their possible use as routine reagents, were begun.

Miss Ikin continued work on the varying distribution of the blood groups in different populations. A special study is being made of the former inhabitants of Tristan da Cunha, in collaboration with other Medical Research Council Units.

Miss Giles carried out serological and genetical investigations of unusual antigens and antibodies, especially variants of the A and B antigens, found in the course of tests performed for clinical purposes.

Finally, the Governing Body would like to praise the scientific, administrative and technical staff for their enthusiastic devotion to the work of the Institute. Without this the successful results which are recorded in this Report would not have been achieved.

E. C. DODDS,
Chairman.

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BLOOD GROUP REFERENCE LABORATORY

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THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

Balance Sheet

and

Accounts

31st December 1962

CHELSEA BRIDGE ROAD,
LONDON, S.W.1.
22nd May, 1963

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

FINANCIAL REPORT OF THE GOVERNING BODY

1. The Balance Sheet as at 31st December, 1962, shows balances to the credit of the various funds as follows: Capital Fund £974,850, Specific Funds £206,629, and Bequest Funds £17,488. The balance on the Sinking Fund for Freehold Buildings of £145,028 is after deducting a loss on the realisation of investments of £1,011 and transferring £5,969 from Income and Expenditure Account. During the year donations and legacies of £4,141 have been added to the Re-endowment Fund.

2. The General Fund Income and Expenditure Account shows the income for the year as £322,664 compared with £271,993 in 1961. Expenditure amounted to £294,454 against £218,717 last year. The surplus for the year is £28,210 compared with £53,276 in 1961.

3. The year's surplus of £28,210 shown by the General Fund Income and Expenditure Account has been transferred to the Capital Fund.

4. Stocks of Sera, Smallpox Vaccine and Horses on hand at 31st December, 1962 have the nominal value of £8,630, £6,396 and £7,650 respectively.

5. Messrs. Cooper Brothers & Co., the retiring Auditors, will, subject to the provisions of the Companies Act, 1948, be re-appointed.

E. C. DODDS, *Chairman of the Governing Body.*

HUGH BEAVER, *Hon. Treasurer.*

BALANCE SHEET

(1961) £		£	£	£
	Capital Fund:—			
	Donations, &c., received to date from the following:—			
2,000	Dr. Ludwig Mond (1893)		2,000	
46,380	Berridge Trustees (1893-1898)		46,380	
10,000	Worshipful Company of Grocers (1894)		10,000	
250,000	Lord Iveagh (1900)		250,000	
18,904	Lord Lister's Bequest (1913-1923)		18,904	
7,114	William Henry Clarke Bequest (1923-1926)		7,114	
3,400	Rockefeller Foundation (1935-1936)		3,400	
22,669	Other donations and legacies (1891-1954)		22,669	
	General fund income and expenditure account accumulated surplus, as at 31st December, 1961	574,465		
	Add surplus, 1962	28,210		
	profits, less losses, on sales of investments	28,921		
		631,596		
	Less amounts written off investments	17,213		
574,465			614,383	
934,932				974,850
	Specific Funds:—			
140,070	Sinking fund for freehold buildings	145,028		
32,664	Pension fund	34,008		
23,452	Re-endowment fund	27,593		
			206,629	
	Bequest Funds:—			
9,822	Jenner Memorial studentship fund	10,367		
6,861	Morna Macleod scholarship fund	7,121		
			17,488	
212,869				224,117
	Specific Grants and Legacies Unexpended:—			
772	Cancer research legacies (1937-1950)		772	
40	Royal Society grant (1951)		40	
6,453	Nuffield Foundation grants (1952-1962)		5,896	
8,812	Guinness Lister research grant (1953-1962)		7,857	
16,077				14,565
	Current Liabilities:—			
35,172	Creditors and accrued charges			43,528

E. C. DODDS, *Chairman of the Governing Body.*

HUGH BEAVER, *Hon. Treasurer.*

£1,199,050		£1,257,060

REPORT OF THE AUDITORS

The accounts set out on pages 4 to 8 are in agreement with the books, which, in our opinion, have been properly prepared.
In our opinion the accounts, amplified by the information given in paragraphs 1 and 4 of the Financial Report, show correctly the surplus of the Institute.

London, 24th May, 1963

(1961) £					£	£	£
	Fixed Assets:—						
	Freehold property at cost:—						
73,548	Land and building, Chelsea				73,548		
20,455	Queensberry Lodge estate, Elstree				20,455		
2,049	House, Bushey				2,049		
						96,052	
	(Note: Additions and replacements since 1912 at Elstree and 1935 at Chelsea have been charged to revenue.)						
	Furniture, fittings, scientific apparatus and books:—						
2,472	At cost, less depreciation to 31st December, 1920					2,472	
98,524	*(Note: Additions and replacements since 31st December, 1920, have been charged to revenue)*						98,524
	General, Specific and Bequest Funds. Investments and Uninvested Cash:—						
		Quoted at cost, less amounts written off in Gt. Britain	Elsewhere	Unquoted at cost	Uninvested cash		
770,485	General	£6,699,971	£38,220	£106,995	—	845,186	
	Specific—						
140,070	Sinking fund for freehold buildings	136,018	—	—	9,010	145,028	
32,664	Pension fund	34,172	—	—	164 (Cr.)	34,008	
23,452	Re-endowment fund	25,939	—	—	1,654	27,593	
	Bequest—						
9,822	Jenner Memorial studentship fund	7,081	—	2,940	346	10,367	
6,861	Morna Macleod scholarship fund	6,662	—	—	459	7,121	
983,354		909,843	38,220	109,935	11,305	1,069,303	1,069,303
		£948,063					
	(Market value of quoted investments—£1,309,350)						
	Current Assets:—						
84,428	Debtors and payments in advance					72,458	
32,744	Balance at bankers and cash in hand					16,775	
117,172							89,233
	(Notes: See paragraph 4 Governing Body's Financial Report for nominal values of sera, smallpox vaccine and horses which have not been brought into the accounts.						
	There is an outstanding capital expenditure commitment of £23,870 in respect of building at Elstree).						
£1,199,050							£1,257,060

TO THE MEMBERS

...rately kept. We obtained the information and explanations we required.
...pect of the Governing Body, comply with the Companies Act, 1948, and give a true and fair view of the state of affairs and

COOPER BROTHERS & CO.
Chartered Accountants.

INCOME AND EXPENDITURE ACCOUNT

GENERAL FU

(1961)		Total Expenditure	External Contributions	
£		£	£	£
99,351	Salaries and wages	182,788	69,928	112,860
7,321	Emoluments of two members of the Governing Body in an executive capacity	8,098	—	8,098
4,999	Premiums on federated superannuation policies	8,793	3,633	5,160
1,946	Premiums on group pension policy	2,642	899	1,743
5,150	Rent, rates and insurance	5,606	365	5,241
13,961	Gas, water, fuel and electricity	20,464	3,767	16,697
3,918	Office expenses, stationery and printing	5,266	845	4,421
368	Audit fee	410	—	410
1,396	Travelling expenses	2,613	1,045	1,568
2,641	Biochemistry expenses	7,571	3,845	3,726
1,307	Microbiology, immunology and experimental pathology expenses	7,101	5,837	1,264
606	Biophysics expenses	771	—	771
362	Virology expenses	1,507	1,304	203
27,757	Serum, vaccine and virus vaccine expenses	41,932	1,370	40,562
8,211	Animals	11,696	1,995	9,701
10,713	Animal house expenses and forage	11,497	1,199	10,298
15,601	Buildings, alterations, repairs and renewals	59,708	678	59,030
789	General apparatus and new installations	960	—	960
1,593	Library expenses	1,995	—	1,995
2,046	General stores	1,607	—	1,607
2,089	Staff canteen loss	2,413	243	2,170
—	Blood products laboratory expenses	7,031	7,031	—
6,592	Amount transferred to sinking fund for freehold buildings (including £5,545 interest on Investments)	5,969	—	5,969
53,276	Surplus transferred to Capital Fund after charging to expenditure £56,608 (1961 £7,057) for additions to property and equipment	28,210	—	28,210
£271,993		£426,648	£103,984	£322,664

TEC PREVENTIVE MEDICINE

IT For the year ended 31st December 1962

AL FUND

(1961)				
£		£	£	£
	Interest and dividends on investments:—			
43,648	General fund		50,768	
6,168	Sinking fund		5,545	
				56,313
461	Underwriting commission..			522
214,900	Sales of sera, vaccine, smallpox vaccine, &c.			259,134
6,816	Rent			6,695
£271,993				£322,664

PENSION FUND

(1961) £		£	(1961) £		£
2,019	Pensions	2,069	32,956	Balance as at 1st January, 1962 ..	32,664
32,664	Balance carried forward	34,008	1,727	Interest on investments (gross) ..	1,781
			—	Net profit on realisation of investments	1,632
£34,683		£36,077	£34,683		£36,077

JENNER MEMORIAL STUDENTSHIP FUND

(1961) £		£	(1961) £		£
9,822	Balance carried forward	10,367	9,307	Balance as at 1st January, 1962 ..	9,822
			515	Interest on investments (gross) ..	545
£9,822		£10,367	£9,822		£10,367

MORNA MACLEOD SCHOLARSHIP FUND

(1961) £		£	(1961) £		£
486	Loss on realisation of investments	6	6,991	Balance as at 1st January, 1962 ..	6,861
6,861	Balance carried forward	7,121	356	Interest on investments (gross) ..	266
£7,347		£7,127	£7,347		£7,127

NUFFIELD FOUNDATION GRANTS

(1961) £		£	(1961) £		£
4,932	Salaries, wages, laboratory expenses and animals	3,557	5,385	Balance as at 1st January, 1962 ..	6,453
6,453	Balance carried forward	5,896	6,000	Amount received	3,000
£11,385		£9,453	£11,385		£9,453

GUINNESS-LISTER RESEARCH GRANT

(1961) £		£	(1961) £		£
11,367	Salaries and wages	13,159	8,518	Balance as at 1st January, 1962 ..	8,812
2,339	Laboratory expenses	6,996	14,000	Amount received	19,200
8,812	Balance carried forward	7,857			
£22,518		£28,012	£22,518		£28,012

THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE

REPORT
OF THE
GOVERNING BODY
1963

CHELSEA BRIDGE ROAD, LONDON, S.W.1

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Deputy Director: Professor W. T. J. Morgan

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---

†Appointed Teacher of the University of London.

*Recognised Teacher of the University of London.

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---

*Recognised Teacher of the University of London
§Honorary Member of Institute Staff

ANNUAL GENERAL MEETING
OF
THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

June 25th, 1963

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the work of the Institute for the year 1962.

GOVERNING BODY

At its last meeting the Council re-appointed Sir Charles Dodds and Lord Brain as two of its representatives on the Governing Body until 31st December 1963. At this meeting Professor J. S. Mitchell was also appointed to the Governing Body as the Council's other representative.

In accordance with the Articles of Association, Dr. Marjorie Macfarlane retired from the Governing Body and was succeeded by Dr. W. d'A. Maycock as the Scientific Staff's representative.

COUNCIL

At last year's Annual General Meeting the three retiring members of the Council, Dr. Muriel Robertson, Mr. Lawrence Abel and the President of the Royal College of Surgeons, were re-appointed.

Professor H. B. Maitland, who had been the representative of the University of Manchester for thirty years, was succeeded by Professor P. Collard in July last.

The three members of the Council due to retire this year in accordance with the Articles of Association, but who are eligible for re-appointment, are Professor P. Collard, the representative of the University of Manchester, and Professor Sir Charles Dodds and Professor Sir Rudolph Peters, each a representative of the Members of the Institute.

MEMBERS

The following persons accepted the invitation of the Governing Body to become Members: Professor G. Belyavin, Professor A. C. Cunliffe, Professor A. Haddow, Dr. C. Kaplan, Dr. Margaret Mackay, Dr. P. Medawar, Dr. A. E. Mourant, Dr. Emmy Klieneberger-Nobel, Dr. R. R. Race, Dr. Winifred M. Watkins and Mr. S. A. White.

The Governing Body records with regret the deaths during the year of Sir Wilson Jameson, a member since 1931 and a former member of the Governing Body from 1950 to 1960, and of Dr. A. B. Rosher, a member since 1946.

STAFF

The Governing Body has noted with pleasure that the Société de Médecine de Paris awarded Dr. Collier the Luys Prize for 1962.

The Governors are grateful to Dame Harriette Chick for representing the Institute at the dedication ceremony of the Clemens von Pirquet Memorial, in Vienna in October.

Dr. F. R. Wells was appointed to the Experimental Pathology Department, Miss G. Sampson to the Bacterial Vaccines Department, Miss J. Ritchie and Miss M. L. Rissik to the Biochemistry Department. Studentships in the Biochemistry Department were awarded to Mr. G. Harrap (Grocers' Company Student) and Mr. E. Y. C. Lee.

Dr. Emmy Klieneberger-Nobel has completed her work at the Institute after a total of twenty-eight years as a Jenner Memorial Student, research fellow and member of the staff of the Institute.

Miss A. Brimacombe, Dr. B. J. Bines and Dr. K. Morgan resigned during the year.

Professor A. A. Miles took part by invitation in three Symposia organised by Miles Laboratories Ltd. on " Injury, Inflammation and Immunity " in May 1962 at Stoke Poges, Bucks, Elkhart, Indiana, U.S.A., and Mexico City.

Professor W. T. J. Morgan took part, by invitation, in the New York Academy of Sciences Symposium on " Mucus Secretions " in May 1962.

Professor Morgan and Dr. Winifred M. Watkins lectured, by invitation, at the Genetics Institutes of the Universities of Pavia and Turin in March and in August attended a symposium on the "Chemistry and Genetics of Cellular Surface Structures ", arranged by the Massachusetts Institute of Technology at Stowe, Vermont, U.S.A. They also attended the International Blood Transfusion Congress in Mexico City in September and lectured in the School of Medicine, Louisiana State University, New Orleans.

In April 1962 Dr. W. d'A. Maycock attended a meeting in Rome of the Sub-committee of Specialists in Blood Transfusion of the Public Health Committee of the Council of Europe and in September he attended the IXth International Congress of Blood Transfusion in Mexico City.

Dr. B. A. D. Stocker attended, by invitation, a meeting on the " Chemistry and Genetics of Cellular Surface Structures ", at Stowe, Vermont, in August 1962.

In February 1962, Dr. L. H. Collier lectured by invitation to the Société de Médecine de Paris. In November he lectured to the World Health Organization Trachoma Training Course at Aligarh, India; he served as a Temporary Adviser to the W.H.O. Inter-Regional Conference on Trachoma, in New Delhi; attended the Annual Meeting of the International Organization against Trachoma, and took part in a Symposium on Trachoma at the All India Institute of Medical Research.

On behalf of the World Health Organization Mr. A. F. B. Standfast visited the National Institutes of Health and the Walter Reed Army Medical Centre in Washington and the Harvard School of Public Health, Boston, U.S.A. In November he went to Geneva as a consultant on Whooping Cough and participated in the meetings on Laboratory and Field Studies of Cholera Vaccines, and on the Laboratory Evaluation of Typhoid Vaccines.

Mr. Standfast and Dr. Jean Dolby took part in the Round Table Conference on Pertussis Immunization, at Prague, in June 1962.

Dr. C. Kaplan attended the VIIth International Congress of Microbiological Standardization at Berne, Switzerland, in June. He acted as W.H.O. consultant on the production of dried smallpox vaccine, in India, during November and December.

In October 1962, Dr. R. A. Kekwick gave a lecture to the Manchester Medical Society entitled " Human antihaemophilic factor; some biochemical problems ".

Dr. Marjorie Macfarlane spent three months in the autumn as a Visiting Research Associate Professor at the Lipid Research Center, College of Medicine, Baylor University, Houston, U.S.A., and also lectured by invitation at Harvard Medical School, Johns Hopkins Medical School, Institute of Enzyme Studies at Wisconsin, University of California, Los Angeles, and other research centres.

Dr. W. J. Whelan presented papers, by invitation, at the Journées de l'Amidon, Institut Pasteur, in March 1962, at the Stärke-Tagung, Detmold, Germany, in May, at the Carbohydrate Symposium, Birmingham, in July, at the Starch Round Table, Chestertown, U.S.A., and the American Chemical Society Meeting, Atlantic City, U.S.A., in September. He also lectured at a number of University Centres in Eire, Germany, the U.S.A. and Canada.

Dr. J. M. Creeth took part in a conference on " The theory and practice of sedimentation analysis " sponsored by the National Academy of Sciences and held in New York in June 1962.

Dr. R. M. Lemcke attended, by invitation, a conference on the " Molecular Biology of Pleuropneumonia-like Organisms " sponsored by the University of Connecticut, Yale University and the National Science Foundation at Stoors, Connecticut, U.S.A., in June 1962, and gave a paper on " The serology of PPLO ". She also lectured at Rahway, New Jersey, and acted as a consultant at an informal conference on Pleuropneumonia-like Organisms at the National Institutes of Health, Bethesda, U.S.A.

Dr. G. M. Gray was invited as a speaker to the Gordon Conference on Lipids in New Hampshire, U.S.A., in June 1962, and also lectured by invitation at Tulane University Medical School and the Oklahoma Medical Research Institute.

Dr. H. G. S. Murray took part in an International Symposium on Smallpox Control held at the Merieux Institute, Lyon, France, in December.

In August 1962, Professor Miles, Mr. Standfast, Dr. Emmy Nobel, Miss Sylvia Smith, Dr. W. Blyth and Dr. P. Reeve participated in the VIIIth International Congress for Microbiology, in Montreal, Canada.

DONATIONS AND GRANTS

The Governing Body is most grateful to Messrs. Arthur Guinness Son & Co. Ltd. for its continued support of research in the Institute. The Company has, for the past ten years, provided funds for the work of the Guinness-Lister Research Unit and it has now very generously agreed to increase its endowment to £15,000 per annum for a further ten years.

The Governing Body also records its appreciation of the generosity of the many other bodies whose benefactions and grants support research work in the Institute. These include a grant from the British Empire Cancer Campaign

for research on lipids; grants from the Department of Technical Co-operation in aid of research on the blood-meals of insect vectors of disease and on the immunology of trypanosomiasis; grants from the Department of Scientific and Industrial Research for researches on the enzymic polymerization of monosaccharides, on chemically modified polysaccharides and on biological matter in macromolecules; a grant from Imperial Chemical Industries Ltd. for the purchase of special apparatus; grants from the Medical Research Council in aid of researches on the isolation and purification of proteins involved in the clotting mechanism of human plasma, on physico-chemical studies of blood group substances, on the chemical basis of blood group specificity in man, on the enzymic decomposition of blood group specific substances, on the identification of *Bordetella Pertussis* antigens, on the synthesis of haptens and dextran-antidextran inhibitors, for studies of glycogen-debranching enzyme systems, on the structure of the amino-acid containing moiety in mucopolysaccharides and on the genetics of virulence in Salmonella; a grant from the Nuffield Foundation for research on non-specific immunity in the early stages of infection; a grant from the U.S. Public Health Authority in aid of researches on abacterial urethritis in man.

The Governing Body also gratefully acknowledges donations to the Institute's Re-endowment Fund from the following Assurance Companies: The General Life Assurance Company, The Prudential Assurance Company Ltd., and the Royal London Mutual Insurance Society Ltd.

VISITING WORKERS

The following visitors, in addition to those listed under Staff, worked for short periods in the Institute's Laboratories: Mr. P. T. L. Cook, Department of Genetics, Cambridge; Dr. S. Danish, Serum and Vaccine Institute, Agouza, Cairo; Dr. A. H. Esterabady, Institut d'etat des Serums et Vaccins Razi, Teheran; Professor Dexter French, Iowa State University, Ames, U.S.A.; Dr. A. E. Kulkarni, Haffkine Institute, Bombay; Mr. P. E. Lemoine, Virology Laboratory, Institute of Hygiene, Brussels; Dr. S. S. Marennikova, Smallpox Prophylaxis Laboratory, Moscow Institute for Viral Prophylactics, U.S.S.R.; Dr. Paul B. McCay, Oklahoma Medical Research Institute, Oklahoma City, U.S.A.; Dr. J. K. Moor-Jankowski, National Institutes of Health, Bethesda, U.S.A.; Dr. Elhan Özlüarda, Reyfik Saydam Central Institute of Hygiene, Ankara, Turkey; Dr. R. H. Pain, Wright-Fleming Institute of Microbiology, London; Dr. P. Poszwinski, Institute of Haematology, Warsaw, Poland; Dr. P. Plackett, Animal Health Research Laboratories, C.S.I.R.O., Australia; Dr. M. R. Radovanovic, World Health Organization Regional Office, New Delhi, India; Dr. Arnaldo Sampaio, Instituto Superior di Higiene " Dr. Ricardo Jorge ", Lisbon, Portugal; Dr. Marion Waller, Medical College of Virginia, Richmond, U.S.A.; Nurse E. Zachari, Indonesian Red Cross, Jakarta, Indonesia.

The Medical Research Council's Blood Group Research Unit and Trachoma Research Unit are accommodated in the Institute. The Council's Blood Group Reference Laboratory has now moved to its new laboratories, built on a site adjoining the Institute's main building.

RESEARCHES IN 1962

SUMMARY

In this summary of the investigations made in 1962, the bracketed numbers refer to the pages of the report where the researches are described in greater detail.

Microbiology. The Guinness-Lister Unit continues its exploration of bacterial genetics, mainly of the Salmonella bacilli. The problems studied include conjugation and hybridization of *S.typhi-murium* (13); exploitation of the colicine and the fertility factors in the mapping of the bacterial chromosome (13–15); the genic control of the synthesis of S and R cell-wall polysaccharides, and its relation to S→R variation and to virulence (17); the genic control of the synthesis of flagella and the structural chemistry of cell-wall polysaccharides and flagella protein (16–17).

Marked salmonella bacteria were used to investigate the factors in the normal mouse gut, destroyed by streptomycin, that contribute to resistance to oral infection (17).

Immunology and Pathology of Infective Diseases. The Institute's studies embrace infections by viruses, pleuropneumonia-like organisms, bacteria and protozoa.

The Trachoma Research Unit continued its epidemiological field study in the Gambia of trachoma in terms of virus and of bacilli of the *Haemophilus* group and is preparing to test the prophylactic value of a virus vaccine in the Gambia (20–21). The laboratory studies at the Institute include the growth characteristics of trachoma and inclusion conjunctivitis viruses in tissue culture and chick embryo (18), serological classification of the viruses (20) and the bacteriology of trachomatous and non-trachomatous conjunctivitis in Gambian subjects (21).

Other virus studies include the replication of shope fibroma virus in hamster cell-lines and attempts to cultivate the virus of *molluscum contagiosum* (21). In the Virus Vaccine department, there is continued progress towards making smallpox vaccine from vaccinia virus grown in tissue culture and the development of an irradiated vaccine ; in the study of antibody immunity in experimental vaccine ; and the laboratory diagnosis of variola by immunofluorescent methods (22).

The possible aetiological rôle in abacterial urethritis of a recently described agent, thought to be an aberrant form of PPLO, was investigated with negative results. The work on the electronmicroscopic structure of PPLO, and on the antigenic analysis of human and animal strains of PPLO was continued (18–19).

The immunological study of bacterial infections includes an analysis of the action of two types of protective antibody in mice infected with the whooping cough bacillus (12); the laboratory assay of typhoid vaccines and the nature of the protective antigen in typhoid vaccines for human use (13); the immunopathology of actinomycotic dermatitis of sheep (13); and the process of refinement of antitoxin by proteolysis (22).

As regards pathogenic protozoa, the investigation of the immunology of experimental trypanosomiasis continues, in terms of soluble and bound immunizing antigen (11); and of methods of bulk growth of the trypanosomes for immunochemical analysis (12).

Epidemiology. The refined serological methods devised to identify the animal source of food for blood-sucking insects continues to provide valuable facts about feeding habits of the possible vectors of trypanosomiasis, malaria and yellow fever (21).

Pathology. Work continues on the relation of serum permeability factors to plasma kininogenases, and their respective rôles in vascular reactions to injury. Researches are in progress on the gross and microscopic location of the site of the response of both blood and lymphatic vessels to various forms of inflammatory injury (23).

With a view to the improvement of therapeutic antitoxins for scorpion bites, the antigenic components of scorpion venoms are under investigation (22).

Biochemistry. The biochemical researches again concern three kinds of substances—the blood group substances, the cellular phospholipids, and starches.

The investigations of the substances of the ABO and other blood group systems include definition of the chemical heterogeneity of preparations of mucopolysaccharides (24); definition of the terminal sugar groups determining serological specificity (24); analysis by improved methods of partial hydrolysis; transformation of blood group specificity, and biosynthesis of determinant groups, by selected enzymes (24–25); measurement of the molecular properties of blood group substance B (29); and the relation of the P_1 blood substance to an antigenically similar substance in sheep hydatid fluid (25).

Work on the structure of phospholipids, their distribution in tissues, cells and cellular particles, and their possible function in the cell includes the identification of lipoaminoacids in bacteria as aminoacid esters of phosphatidylglycerol; of cyclopropane ring fatty acids in salmonella bacteria; and the isolation of new classes of glycolipid from ascites tumour cells (26–28).

Carbohydrate studies mainly concern the relation of structure of both natural and synthetic polysaccharides to the specificity of enzyme and antibody reactions. Other studies include mammalian enzymes concerned in glycogen metabolism ; and the structure of polyglucoses (24–25).

Human and Animal Plasma Proteins. The Institute's work is concerned with the isolation, refinement, characterization, assay, and in some cases clinical trial, of the various biologically active proteins of human plasma.

Further progress was made in the isolation and identification of contaminating enzyme-like substances that destroy antihaemophilic factor in therapeutic preparations of the factor (28).

Studies of the isolation and characterization of other proteins include those of the macroglobulins of normal human plasma (29); of urinary proteins in renal tubular disorders (29); plasmin and plasma kininogenases ; and plasminogen (30).

MICROBIOLOGY

TRYPANOSOMIASIS

Dr. Weitz continued his study of the exoantigen and bound antigens of *Trypanosoma brucei* and *T.vivax.* The species specificity of exoantigens of each species, previously demonstrated by immunodiffusion and active protection tests in mice, was confirmed by immunofluorescent methods. Conjugated antibodies to the exoantigens were specific for the homologous species only, and those to the bound antigens reacted equally with both species. Immunofluorescent methods are also being used to explore the antigenic variation of the parasites that occurs in the infected host as the result of antibody formation. It is hoped to apply these methods to the current field studies on the identity of the hosts of tsetse flies, by demonstrating in the blood meals the immune state of the host concerned, so as to identify the wild animals that are carriers of trypanosomes.

Mr. Miller studied the antigenic variations of *T.brucei* in infected rabbits. Each variant isolated at intervals during infection was inoculated into rats; the soluble antigen of each was obtained from the serum of the infected rat, and the bound antigen from homogenates of the washed trypanosomes in the blood. Antisera to these exoantigens were each specific only for the homologous variant, and when used to immunize mice, the exoantigens protected only against infection by the homologous variant.

The rabbits infected with *T.brucei* produced a succession of antibodies specific for each variant as it appeared. Thus, at any time during the infection, antibodies to the current variant and to each previous variant were present, but none to subsequent variants. These antibodies were absorbed specifically by the exoantigens of the respective variants. These studies confirm that antigenic variations of trypanosomes are related to changes in the exoantigens.

Mr. Miller is also investigating the lethal effect of normal sera on trypanosomes *in vitro*. In normal rat sera, trypanosomes that have been stored outside the body disintegrate or are immobilized, according to the concentration of serum. The sera of other species of animals contain a similar lethal factor, some in large, some in smaller amounts. The factor is separable from the serum globulins and albumin, and is associated with the ceruloplasmin and " post-albumin " chromatographic fraction.

Dr. Pittam has tried to establish *T.congolense* and *T.vivax* in culture. In a medium containing α and β globulins from human serum, and adenosine diphosphate, *in vitro* cultures from infected rat blood were established which lasted through three subcultures over a period of eight days. With Mr. Miller, he succeeded in maintaining cultures of *T.congolense*, from the gut of tsetse flies (*Glossina morsitans*) fed on infected rats, through five subcultures over a period of twenty-one days in a modified Tobie medium.

Miss Lanham continued her investigation of the antigens of a rat-adapted strain of *T.vivax*. The exoantigen of *T.vivax*, purified by fractionation of infected rat serum on ion-exchange celluloses, has properties very similar to those of the exoantigen of *T.brucei*. The " bound antigen " released from washed, disintegrated trypanosomes proved to contain at least six antigens, two of them diffusible through cellophan. The diffusible antigens are similar to those observed by Brown & Williamson (*Nature, Lond.* (1962), **194**, 1253) in strains of *T.brucei* and *T.rhodesiense*. Fractionation of the non-diffusible cellular material yielded three fractions, one of which contained the other four antigens. All six antigens were distinguishable by immuno-electrophoretic analysis.

WHOOPING COUGH BACILLUS

Dr. Vincent continued the fractionation of the antigens of *Bordetella pertussis* with the purpose of isolating and characterizing the protective components of whooping cough vaccines. Cruder fractions containing the protective antigen appeared to be protein; but further purification revealed that the protective activity is associated with a phosphorus-containing carbohydrate present in very small amounts.

The comparison of rabbit antisera to the purified antigens with those containing known *B.pertussis* antibodies, carried out by Dr. Jean Dolby, shows that the separation of the various antigens of the bacillus is gradually being achieved. Chemically, agglutinogen, haemagglutinin and the two protective antigens are very closely related.

In Vitro Tests with B. pertussis *Antisera*. The bactericidal test (Report 1962) is being applied by Dr. Jean Dolby to all Dr. Vincent's antisera, some of which contain only two antibodies. The analysis of results with over 300 sera may make it possible to determine whether all sera protecting mice against an intracerebral challenge, and hence presumably containing the child protective antibody, have a characteristic bactericidal reaction. If so, the bactericidal test would be a useful alternative to the mouse test for detecting protective antibody. The association of bactericidal and protective activity may prove to be significant for our understanding the mechanism of protection.

Bordetella pertussis *Infection and Immunity in Mice.* Mr. Standfast and Dr. Jean Dolby have continued their work on the effects of the IC protective antibody on brain infections in mice (Report 1961) by investigating the part played by local immunity, both specific and non-specific, in the brain. For understanding of the processes in the infected brain it is important to determine the extent to which the infecting dose used to test immunity itself induces a local or a general antibody response. Estimates were made of the degree of non-specific local immunity, of specific local immunity and of general immunity. There is some local non-specific immunity against *B.pertussis* organisms introduced into the brain. The distinction between local and general specific immunity is under investigation. It was possible to detect small variations in the degree of the different types of immunity in immunized mice by establishing the time-course of changes in the number of viable organisms in the brain of infected mice.

TYPHOID BACILLUS

Laboratory Assay of Typhoid Vaccines. Mr. Banks continued his investigation of methods of laboratory assay of the typhoid vaccines used in the field trials in British Guiana, Yugoslavia, Poland and the U.S.S.R., as part of the collaborative study initiated by the World Health Organization. The collaborative work carried out so far has shown that it is not possible to select any one method of assay as the method of choice. Mr. Banks is also investigating the antigen in the typhoid bacillus responsible for inducing immunity in man.

ACTINOMYCOTIC DERMATITIS IN SHEEP

Mr. Roberts investigated the *in vitro* capacity of *Dermatophilus dermatonomus*, the causative organism of ovine actinomycotic dermatitis, to grow through agar gels, as an experimental model for its penetration of the animal epidermis during infection. The failure of hyphae to penetrate far into the epidermis does not appear to be due to lack of nutrients, since *in vitro* hyphae can grow into non-nutrient regions; nor is it due to the absence of the relatively high concentrations of CO_2 needed for growth, since these obtain in the epidermis. Penetration fails presumably because of antimicrobial factors, physical or biological, in the dermis.

Mr. Roberts is also investigating dermal infection of mice, guinea-pigs and rabbits by *D.dermatonomus*, and the nature of the immunity and specific hypersensitivity induced in these animals as a result of infection. Immunization to infection is associated with the appearance of agglutinins for the flagella and bodies of the zoospores of the organisms. The relation of the immune response to the antigenic components of *Dermatophilus* species is also under investigation.

INHERITANCE IN BACTERIA

Recombination in Salmonella. The investigations (Report 1961, 1962) of inheritance in bacteria of the *Salmonella* (food-poisoning and enteric fever) group continue. Conjugation of the bacteria is brought about either by use of the *col* factors, which determine the production of antibiotics called colicines, or of the F or fertility factor, transferred from *Escherichia coli*. The transmission from strain to strain both of the *col* and F agents, previously studied, and of another kind of transmissible agent, the R factor, have also been investigated.

Conjugation Determined by Colicine Factors. Miss Smith, Mr. Subbaiah and Dr. Stocker continued to map the chromosome of *Salmonella typhi-murium*, in crosses in which conjugation was induced by the colicine factors *col* I and *col* E 1. They tested additional nutritional, fermentative, serological and other characters, displayed by the laboratory mutants or already present in strains isolated from natural sources. The genes regulating all these characters were found to map

within the single closed-loop linkage group already established. Mr. Subbaiah examined 18 more R mutants in *S.typhi-murium* strain LT2; six of them resulted from mutation near the *try* region (tryptophan requirement), all the rest from mutation near *ile* (isoleucine requirement). Such R mutants are of special interest because R mutants of S virulent strains are non-virulent. Unfortunately *S.typhi-murium* strain LT2, the classical strain used in most of the work on the genetics of *S.typhi-murium*, is, for unknown reasons, of very low virulence; Mr. Subbaiah and Dr. Stocker prepared another strain which is highly virulent to mice, hybridizes well with strain LT2, and has several good marker characters on its chromosome. This strain is now being used for experiments on the genetic control of virulence.

The bacterial virus P22, which can carry or transduce bacterial genes from the bacterium in which it is formed to its new host, can set up a stable (lysogenic) relationship with its bacterial host, such that the bacterium multiplies normally but carries, as a latent, heritable parasite, the gene set or prophage form of the virus (Report 1958). Miss Smith made crosses in which one, both or neither parent carried P22 in its latent form: her results indicate that the latent virus is attached to the chromosome near the *proA* (proline requirement) locus, in agreement with the conclusions of Zinder, of the Rockefeller Institute, using other methods; and that when this part of the chromosome with the attached prophage is transferred into a female bacterium not already carrying the latent virus and is integrated into its chromosome, the bacterium usually dies, perhaps as a result of activation (induction) of the latent virus.

The lipoprotein-polysaccharide somatic antigen complex of bacteria—like the mucoprotein of animal cells—is a complicated structure of great importance; and though a good deal is known about its chemical structure, little has yet been discovered about its biosynthesis and genetic control. Mapping experiments on *S.typhi-murium* have now shown that several different genes regulate different features of the synthesis of the lipoprotein-polysaccharide constituting the somatic antigen of this bacterium. Thus bacteria blocked in the inter-conversion of glucose and galactose through mutation in the *gal* region cannot make the galactose component of the normal polysaccharide and in consequence produce a grossly abnormal somatic complex (Report 1961). A new kind of mutant discovered by Miss Smith is unable to make glucose at 37° and when grown on mannitol makes an abnormal somatic polysaccharide (see below); the gene concerned maps near *metA* (methionine requirement). The locus for the somatic antigen factor 5, known to represent the presence of acetyl groups on a terminal sugar of the polysaccharide, maps near *adeC* (adenine requirement) (Report 1962). Somatic antigen factor 1, resulting from the presence of an α1–6–linked terminal glucose on a side chain of the polysaccharide (Report 1960), reflects the presence of the gene set of an A1–A2 phage such as P22, now shown by Miss Smith to be attached to the chromosome near *proA* (proline requirement). Mr. Subbaiah's results show that inability to form the normal, smooth O antigen may result from mutation either near the *try* or near the *ile* loci. No one of these six loci is immediately adjacent to any other.

A bacterium—or a human cell—possesses many genes conferring the ability to synthesize particular proteins, which are only brought into action, or full action, when circumstances necessitate the production of the protein(s) concerned; the way in which such genes come into operation, or cease to operate, is not yet well understood. The order of eight adjacent genes concerned with histidine biosynthesis in *S.typhi-murium* is known from the work of Hartman, in Philadelphia, who used phage transduction to obtain recombination; he finds that a *his* " operator " gene simultaneously adjusts the level of activity of all the

other genes in the *his* group, to match the rate at which histidine must be synthesized, and that this operator gene lies at one end of the *his* region. Dr. Stocker examined the orientation of the *his* region with respect to the rest of the chromosome; his results strongly suggest that the *his* operator gene lies at the " early " end (using the conventional clockface map of the chromosome) of the *his* region. What is probably another operator gene, controlling several genes concerned in galactose metabolism, has now been identified; Miss Smith is investigating its position relative to the other galactose genes, which it controls. Knowledge of whether all operator genes lie at the " early " end of the group of genes which they control may throw light on the way in which they act.

Conjugation Induced by the F Factor. Though all the genes controlling ordinary biochemical, morphological and the like characters so far tested have been found to map in a single linkage map, the *col* factors, determining production of the colicine antibiotics and used to obtain conjugation, themselves behave in a way which shows that they can multiply and be transferred from one bacterium to another independently of the chromosome. In this respect they typify the class of genetic determinants in bacteria called plasmids. It is known that some plasmids, though at times they multiply autonomously, at other times become attached to and integrated with the bacterial chromosome. Miss Smith's demonstration that the bacterial virus P22 of *S.typhi-murium* in its latent (prophage) form is closely linked to *proA* provides a new example of such behaviour. No chromosomal location for the *col* factors can be inferred from the study of conjugation induced by the *col* factors themselves, for nearly all female exconjugants become infected with all the *col* factors of the male, regardless of which part of the male chromosome, if any, they acquire (Report 1960). Mrs. Dubnau has therefore studied the inheritance of the *col* factors in a cross of an LT2 Hfr (high frequency of recombination) strain, received from Dr. N. Zinder, of the Rockefeller Institute, which has an F (fertility) factor originally derived from *Escherichia coli* attached to its chromosome at one point, and which on conjugation with ordinary LT2 bacteria injects a part of its chromosome in a characteristic order. Mrs. Dubnau found that the relative frequency of different recombinant classes, and the time of entry of different genes as judged from the effect of breaking up mating pairs at various times after the beginning of conjugation, indicate that these Hfr bacteria inject their chromosomes in the order:

ile—rha—mal—inl—(azi, ara)—proA—gal—

which confirms and extends Zinder's unpublished map. Between 10 and 80% of the recombinants which obtained the leading *ile* gene of the donor also acquired one, two or all three of the *col* factors of the Hfr donor when this carried *col* I, *col E* 1 and *col E* 2, whereas much less than 0.1% of the female population at large acquired any of the *col* factors under the same conditions. A recombinant which obtained one *col* factor commonly also acquired one or both the other *col* factors. Recombinants in which a long piece of chromosome from the male had been incorporated were more likely than others to be infected with one or more of the *col* factors; but Mrs. Dubnau did not detect any definite linkage of any one of the *col* factors to any one of the chromosomal genes transferred—nor did the time of entry of the *col* factors, inferred from interrupted mating experiments, indicate a special location for any of the three *col* factors. Mrs. Dubnau also studied the behaviour in this cross of a representative of another class of plasmid, recently discovered in Japan, the R (resistance) factors, which are transmissible from one bacterium to another by contact, presumably through conjugation, and are recognized by the resistance to various antibacterial agents which they confer on bacteria harbouring them. A transmissible R factor conferring resistance to tetracycline,

streptomycin and sulphonamides, originally isolated from a wild strain during an outbreak of *S.typhi-murium* infection in a London hospital, was transferred to the Hfr strain of LT2, also carrying *col E* 1, *col E* 2 and *col* I. A few recombinants which acquired a chromosomal gene from this Hfr male also acquired the R factor, and these recombinants generally also acquired all three *col* factors. This result suggests that the R factor concerned is in some way linked to one or more of the *col* factors in the Hfr strain.

Transduction of the R factor and Other Plasmids. Mrs. Dubnau has also transferred the R factor, or parts of it, by transduction, using phage P22. In confirmation of Japanese work, some transductants acquired resistance to sulphonamides and streptomycin but remained sensitive to tetracycline; others acquired tetracycline resistance only. Some at least of these resistant forms which have acquired resistance by transduction differ from the parent strain carrying the complete R factor in that they cannot transmit their resistance by cell contact. Some of the transductants which had acquired either tetracycline-resistance or resistance to sulphonamides and streptomycin had acquired also *col E* 1 or *col E* 2. This seems strong evidence that at least in some bacteria the R plasmid becomes attached to a *col* plasmid, so that both it and the *col* factor can be enclosed within the coat of a single phage particle, which then carries both factors to a new host.

BACTERIAL PHYSIOLOGY

Chemistry of Flagellar Protein. Dr. McDonough has continued his study of the two-dimensional patterns obtained by paper chromatography and electrophoresis of tryptic digests of *Salmonella* flagellar proteins. Spraying with specific colour reagents has shown the presence of histidine, arginine, tyrosine and methionine in some of the c. 30 peptides present in digests of *i* flagellins; but peptide T1, the slowest moving basic peptide, whose position in the pattern is changed in some mutant forms of *i* (Report 1962), does not react with any of these reagents. The reagents have revealed no new differences between the wild-type *i* and the mutant patterns. Acid hydrolysates of peptide T1, eluted from heavily loaded preparations, suggest that the T1 peptides of wild-type *i*, *iM10*, *iM11* and *iM7* all contain asparagine, glutamine, threonine, glycine and/or serine and probably lysine and/or N-methyl lysine, and that the *i*, *iM10* and *iM11*, but not the *iM7*, peptides contain leucine or isoleucine. As the amounts of peptide which can be recovered from paper are insufficient for structural studies Dr. McDonough devised column chromatographic methods, using Dowex 50–X2 cation exchange resin. Elution with a volatile pyridine-acetic-acid-water buffer gave good separation of most of the peptides.

The EEL automatic amino-acid analyser has been standardized with an artificial mixture of amino-acids. The first satisfactory analyses, of *I* and *iM5* flagellins, confirm the characteristic amino-acid composition of flagellin (absence of cysteine, very little histidine and methionine, little proline, tyrosine and phenylalanine, presence of N-methyl lysine, etc.) but reveal no difference between wild-type *i* and *iM5*.

Flagella of antigenic type *1,2* containing N-methyl lysine have an antigenic specificity or sub-factor absent from flagella identical except for the absence of N-methyl lysine (Report 1961). Mrs. Pearce used serum specific for this subfactor to select mutants lacking this subfactor in their flagellar antigen; Dr. McDonough has confirmed chemically that the mutants do indeed produce flagella lacking N-methyl lysine, presumably because of a block in some methyl-transferring enzyme system.

Somatic Polysaccharide of Salmonella. Mr. Subbaiah and Dr. Stocker are collaborating with Drs. I. Beckmann and O. Westphal, of Freiberg, in a chemical and serological investigation of the somatic lipopolysaccharide and polysaccharides of Mr. Subbaiah's R mutants of *S.typhi-murium* strain LT2. Phenol extraction of dried crops of two R mutants yielded, as expected, the characteristic R lipopolysaccharide, with glucose and galactose as the main components of its polysaccharide and without the mannose, rhamnose and abequose found in the corresponding S lipopolysaccharide of the parent strain. Surprisingly, however, Dr. Beckmann found that her phenol extracts of these two mutants, after centrifugal removal of the macromolecular R lipopolysaccharide, contained in solution material which on hydrolysis yielded glucose, galactose, mannose, rhamnose and abequose, i.e. the characteristic constituent sugars of the normal S polysaccharide; and material which reacted, in precipitation and haemagglutination tests, with antiserum for the normal S somatic antigen. Mr. Subbaiah, using a different fractionation procedure, confirmed the presence in phenol extracts of material which reacts with serum specific for the major factor 4 of the S somatic (O) antigen. Dr. Beckmann is investigating the material in these extracts, which appears to be heterogeneous. The presence in extracts of these apparently typical R strains of material containing the characteristic sugars and with some at least of the serological specificity of the normal S somatic antigen shows that their R character does not result from inability to synthesize mannose, rhamnose or abequose; it suggests rather that they are unable either to polymerize a relatively small S polysaccharide or to combine it with the other components, to form the normal S lipopolysaccharide-protein O antigen complex. Both the mutants so far examined are of the class which map near *ile*. Bulk crops of further R mutants are now being grown at the Microbiological Research Establishment, Porton, for chemical examination.

The glucose-negative mutant mentioned above is also of interest in respect of its somatic polysaccharide. Miss Smith found that at 37° it does not ferment or utilize glucose, galactose or some related compounds which it attacks normally at 30°, but that it ferments and utilizes fructose, mannitol and some related compounds both at 30° and at 37°; her conclusion that the mutant at 37° is unable to effect the normal interconversion of glucose-6-phosphate and fructose-6-phosphate has been confirmed by enzyme studies by Dr. A. E. Fraenkel, of New York University Medical School. When grown at 37° on medium lacking glucose the mutant has the R character; when grown at 30°, or at 37° on medium supplemented with glucose, it is S. This presumably means that at 37° the mutant, not supplied with glucose and unable to synthesize this sugar, a component of the S polysaccharide and precursor of some of its other sugar components, manufactures an abnormal polysaccharide; the chemical composition of this polysaccharide is being studied by Dr. Fraenkel in New York.

Bacterial Multiplication in Infected Animals. Dr. Meynell and Mr. Subbaiah perfected their abortive transduction method (Report 1962) for the determination of the true rate of bacterial division in the infected animal, where bacterial multiplication may be obscured by loss of bacteria through destruction by the host or other causes. They found that when the histidine-exacting mutant of *S.typhi-murium* strain LT2, *hisBc-482*, was treated with phage grown on another histidine-exacting mutant, *hisBb-206*, the small abortive transductant colonies obtained on an enriched histidine-free medium, which are attributed to the activity of a non-integrated and therefore non-replicated gene, were, unexpectedly, of two sizes. The larger class, but not the smaller, contained not one but several or many bacteria able to grow to a limited extent without histidine.

These bacteria probably result, not from any multiplication of the supranumerary gene, but from the spontaneous production by the recipient bacteria of very rare phenotypic variants which have a limited ability to grow without histidine (*cf.* the rare phenotypic motile variants produced by some non-motile strains (Report 1956, 1957)).

Dr. Meynell and Mr. Subbaiah used the abortive transduction method of measuring bacterial division rates to study the fate of *S.typhi-murium* given to mice by the oral route; although this bacterium produces a natural disease in mice many millions of bacteria must be given by mouth to induce infection. The experiments showed that in normal mice the bacteria do not divide more than once in the first six hours after ingestion (in broth they would divide ten or eleven times in this period); most of them neither multiply nor die but pass through the gut unchanged and are voided in the faeces. The reason why these bacteria do not multiply in the normal mouse intestine was not known; for the caecal contents of such mice support an abundant natural population of other bacteria and caecal extracts proved to contain amino-acids sufficient to support the multiplication of the salmonella. Elimination of the normal bacteria of the mouse intestine by oral streptomycin greatly increases the susceptibility of mice to *S.typhi-murium* given by mouth, so that now even a very few bacteria (of a streptomycin-resistant strain) will multiply and cause infection. Dr. Meynell and Dr. Gray found that the caecal contents of normal mice have a low oxidation-reduction potential (Eh about –0.2v) and contain about 0.2M fatty acids, mostly acetic with some butyric and propionic; broth containing this concentration of fatty acids and with a similar Eh did not support the multiplication of *S.typhi-murium*. By contrast in the caeca of streptomycin-treated mice the concentration of fatty acids was ten-fold lower, and conditions were less reducing (Eh about +0.2v); this combination of factors did not inhibit multiplication of *S.typhi murium* in broth. The high fatty acid concentration and the reducing conditions in the caeca of normal mice sufficed to explain the failure of ingested *S.typhi-murium* to multiply in the normal mouse gut; both factors presumably result from the metabolic activity of the normal bacteria of the gut, which are eliminated when streptomycin is given by mouth. Dr. Meynell is investigating the relevance of these observations to the severe staphylococcal enterocolitis which sometimes occurs in patients being treated with antibiotics.

ABACTERIAL URETHRITIS IN MAN AND RELATED DISEASES

In order to test the hypothesis that pathogenic PPLO are derived from apathogenic bacteria via L-forms (Report 1962), Dr. Klieneberger-Nobel continued her collaboration with Dr. R. C. Valentine of the National Institute for Medical Research on the comparative morphology of PPLO and L-forms as determined by electron microscopy. A method of fixation was devised that preserved the filamentous and complex asteroid forms of PPLO. In this way PPLO structures of extreme delicacy which previously had not been demonstrated were revealed. In view of these findings it is possible that the disruptive effects of standard fixation procedures are responsible for the disorganized appearances characteristic of many electromicrographs of L-forms. Dr. Lemcke, with Dr. Valentine, is continuing this work on the L-forms of various bacteria.

Dr. Lemcke investigated the " tiny " colonies (" T " strains) isolated by Dr. D. K. Ford of Vancouver, B.C., from patients with abacterial urethritis. Subcultures of Dr. Ford's strains did not appear to be PPLO; their failure to propagate in serial subculture made any determination of their exact nature impossible. Although it was not possible consistently to grow a bacterial form from them, these colonies may be aberrant growth forms of some bacterium.

Specimens from 15 patients with abacterial urethritis obtained by Dr. G. W. Csonka (St. Mary's Hospital), when inoculated on to suitable media, failed to produce the characteristic colonies described by Dr. Ford and by Dr. M. C. Shepard of Camp Lejeune, N. Carolina. As Drs. Shepard and Ford obtain these colonies from 70–80% of patients with abacterial urethritis, it was to be expected that they would be found in some of the 15 patients examined, had they any aetiological significance in the disease in this country. Authentic PPLO were isolated from 5 of the 15 patients.

Most PPLO isolated from the urogenital tract belong to one broad serological group, which, however, is not antigenically homogenous. To determine whether strains isolated from different disease conditions differ in their antigenic structure and whether strains isolated from patients with infections differ from those from people without clinical symptoms, an investigation of human genital PPLO by immunodiffusion techniques was begun.

A serological comparison was begun of several different PPLO isolated from tissue and embryonated egg cultures of Eaton's Agent of primary atypical pneumonia.

During the year strains of PPLO from various laboratories in this country and abroad were identified serologically.

VIROLOGY

TRACHOMA AND INCLUSION BLENNORRHOEA

Following common practice, the closely related micro-organisms causing trachoma and inclusion conjunctivitis (blennorrhoea) are sometimes jointly referred to as " TRIC agents " in this Report.

Particle Counts. Dr. Reeve and Dr. Taverne devised a new technique for counting the numbers of elementary bodies in suspensions of TRIC agents; it depends on the finding that Giemsa-stained elementary bodies appear as bright yellow-green particles when viewed by dark field microscopy, and can readily be distinguished from background material. Apart from simplicity, an advantage over electron microscopy is that counts can be made on comparatively crude suspensions. The method was used to show that the amount of complement-fixing antigen in a given suspension is directly proportional to the number of elementary bodies; about 10^7 particles are required to fix one of complement in the presence of excess antibody. This technique is also being used for purification studies, and for researches on the relationships between total particle count, infectivity for chick embryos and HeLa cells, and toxicity.

Growth in HeLa Cells. Dr. Furness and Miss Fraser completed their study of the replication of inclusion blennorrhoea virus strain LB1 in HeLa cells (Report, 1962) with observations by fluorescent microscopy on the earliest stages of infection. With Dr. W. G. Henderson (Paddington Green Hospital) and Dr. G. W. Csonka (St. Mary's Hospital), they showed that after adsorption, infective virus particles stained with acridine orange disappear rapidly, whereas inactivated particles remain visible for at least six hours. By infecting cells with varying numbers of virus particles, it was shown that the infective unit is almost certainly a single elementary body, rich in deoxyribonucleic acid.

Although there is now considerable information about the mode of replication of TRIC agents, the mechanism of entry into the cell is not well understood; Miss Fraser has begun a study of this process in HeLa cells.

Growth in Chick Embryo Cell Cultures. Dr. Blyth continued to study the factors influencing the yield of TRIC agents from whole chick embryo cultures

For such experiments it is preferable to use as inoculum virus propagated in eggs, since it withstands storage much better than that grown in cell cultures. Since crude yolk sac suspensions are deleterious to chick embryo monolayers, a method of purifying the inocula with trypsin and centrifugation was devised. Virus purified in this way was rapidly inactivated at 37° C. during adsorption, but the loss was reduced to acceptable limits by adding serum to a concentration of 50%. Even under optimal conditions, adsorption to chick embryo cells is relatively inefficient; nevertheless, virus that enters cells multiplies up to 100-fold.

Changes in Growth Characteristics. Dr. Reeve and Dr. Taverne noticed that although all the TRIC agents they examined multiplied at the same rate in the chick embryo yolk sac, some killed embryos more rapidly at a given dose than did others. The dark field counting method was used to show that relatively fewer elementary bodies of such strains are needed to kill the embryos. Furthermore, only the fast-killing strains induced formation of cytoplasmic inclusions in cell cultures and could be propagated in mouse brain. Retrospective examination of the records of 13 strains then revealed that 1 strain of trachoma and 2 of inclusion blennorrhoea had apparently acquired these properties during the frequent chick embryo passages by which they had for some years been maintained. These findings—which may possibly be explained by the emergence of mutants—are under investigation; in this connection Miss Graham is investigating the pathogenicity of various strains for mouse lung.

Serological Studies. Miss Graham continued to study the serological relationships of TRIC and allied agents, using the neutralization test in cell cultures (Report, 1962). Since the number of strains inducing inclusion bodies in cultured cells is limited, complete cross-neutralization tests cannot always be done. Furthermore, some strains apparently fail to elicit neutralizing antibody in rabbits, although inducing the formation of complement-fixing antibody. Nevertheless, Miss Graham demonstrated that antisera to both trachoma strain T'ang and inclusion blennorrhoea strain LB1 neutralize over 90% of the infectivity of either virus. Both these antisera, and one prepared against a Gambian trachoma agent (G221), neutralize lymphogranuloma venereum virus; by contrast, T'ang antiserum fails to neutralize Saudi Arabian (SA2) and American (Bour) strains of trachoma.

Toxicity Studies. Most, if not all, TRIC agents are toxic to mice when injected intravenously, but such tests are difficult and tedious. In an attempt to devise a simpler method, Dr. Blyth, Dr. Reeve and Miss Graham showed that these agents induce lesions in guinea pigs when injected intracutaneously; it is thought that these lesions are more likely to be due to toxin than to viral multiplication. Dr. Blyth found that approximately 5×10^4 elementary bodies of strain SA2 are needed to induce a 5 × 5 mm. lesion; he and Miss Graham are comparing the results of these tests with infectivity measurements in chick embryos and HeLa cells.

Trachoma Vaccine. Dr. Collier has now tested eight Gambian and one Saudi Arabian trachoma virus for pathogenicity in the baboon conjunctiva (Report 1962); none of them induced such severe infections as the inclusion blennorrhoea agents previously examined. A Gambian virus (G221) was titrated in parallel in chick embryos and baboons; it proved about 10 times less infective for baboons than the inclusion blennorrhoea strain LB4.

Baboons that were successfully immunized against LB4 virus during 1960 were again challenged 15 months later; they still showed pronounced resistance to infection. In another experiment, antigen prepared from yolk sac material and suspended in a mineral oil adjuvant failed to induce immunity. These tests,

including some with antigens prepared in cell cultures, are being continued.

Mr. Turner undertook at Elstree the production of trachoma vaccine for field trials by the Trachoma Research Unit.

Investigations in West Africa. Sera from children receiving trachoma vaccine (Report 1962) were tested for complement-fixing group antibody; it was detected in only about half the vaccinated group, and then in low titre. Dr. I. A. McGregor suggested that the poor response might be due to intercurrent malaria, which affected all these children; to test this possibility 8 vaccinated children were rendered free of malarial parasites with Daraprim, and 8 others were left untreated. One month after beginning treatment, all received a booster dose of trachoma vaccine; again the serological response was poor, and did not differ in treated and control children.

Preparations are now almost complete for a prophylactic trial of trachoma vaccine in villages on the north bank of the River Gambia.

Conjunctival Strains of Haemophilus from Trachoma Patients. Miss Sampson found that culturally and biochemically the Haemophilus strains isolated from the eyes of trachoma patients fall into 4 "groups", (1) *Haemophilus influenzae*, (2) *Haemophilus aegyptius*, (3) Intermediate 1—similar to *H.influenzae* but haemagglutination-positive and (4) Intermediate 2—similar to *H.aegyptius* but haemagglutination-negative. There is reason to believe that strains of the two "Intermediate" groups are variants of *H.influenzae* and *H.aegyptius*. The investigation shows (a) that multiple infections with Haemophilus strains occurred in the same patient; (b) that *H.aegyptius* and/or Intermediate 2 were more commonly associated with trachoma than *H.influenzae* or Intermediate 1, and they did not predominate in the non-trachomatous patients and (c) that Haemophilus strains were not particularly associated with any stage in trachoma or with any type of secondary infection, except that there was an indication of an association of *H.aegyptius* with mucoid or mucopurulent discharges.

SHOPE FIBROMA

Dr. Placido de Sousa began a study of the mechanism of virus-induced cell proliferation, using as a model Shope virus which causes fibroblastic tumours in rabbits. He showed that this agent is pathogenic for a continuous line of baby hamster kidney (BHK) cells, kindly provided by Professor M. G. P. Stoker (Institute of Virology, Glasgow); this is the first report of its cytopathogenic effect in a cell line other than of rabbit origin. Dr. de Sousa also devised a titration method using primary cultures of young rabbit kidney, which gave results that are directly related to those of titrations in the intact rabbit skin. He is using this method in a study of the role of interferon in the Shope virus/rabbit cell system.

MOLLUSCUM CONTAGIOSUM

This infective skin disease is characterized by the presence of epithelial cell inclusions containing elementary bodies that are probably viral in nature. Although the disease is of minor importance, its causal agent is interesting because, like Shope virus, it induces cell proliferation; so far it has not been isolated and grown in the laboratory. Through the generosity of several dermatologists, Dr. de Sousa obtained material from 34 patients with molluscum lesions. Attempts to isolate virus from these specimens gave negative or equivocal results; the method most commonly used was inoculation of the chick embryo chorioallantoic membrane, with variations in the age of embryo, and length and temperature of incubation. Lesions were often observed on membranes inoculated directly with molluscum material, but not on control mem-

branes; however, they could not be transmitted to other embryos, and did not contain inclusion bodies. Inoculations of various cell cultures, and into the skin and conjunctivae of baboons all gave negative results.

VACCINIA VIRUS

The research work in the Department was somewhat disturbed in the first quarter of the year by the smallpox emergency. As a result of the emergency certain technical changes in production were made which should enable the Department to meet very large demands for smallpox vaccine with a minimum of disturbance.

Virus Inactivation. Dr. Kaplan showed that a vaccine prepared from vaccinia virus inactivated by ultraviolet irradiation stimulated a secondary immune response in previously vaccinated subjects. The work is being continued in non-immune volunteers. Mr. Turner extended the range of the studies on inactivation of vaccinia virus which have been made in the Department for several years.

Immunity to Variola and Vaccinia Viruses. The studies by Dr. Kaplan and Dr. Murray on immunity to smallpox and vaccinia viruses, supported by the World Health Organization, are continuing. The devising by Dr. Murray and Mr. Robinson of a method for the precise assay of virus and of neutralizing antibodies in monolayers of cultivated cells has greatly facilitated this work. Dr. Murray was able to identify, by immunofluorescence microscopy, smallpox virus in preparations made from patients' lesions. Similar preparations from chickenpox patients were negative.

Tissue Culture Vaccine. Mr. Robinson began the purification of vaccinia virus from several sources for a chemical study of the virus. He investigated the suitability of cultures of lamb kidney and baby rabbit kidney cells for the production of vaccinia virus for smallpox vaccine. This was made necessary by the decision to withdraw the vaccine prepared in cultures of chick embryo cells in view of the possibility of transmission to man of latent fowl viruses.

IMMUNOLOGY AND SEROLOGY

SEROLOGICAL IDENTIFICATION OF BLOOD MEALS

The serological identification of blood meals from insect vectors of trypanosomiasis, malaria and yellow fever continued in collaboration with overseas workers.

SCORPION VENOMS

Mr. Stone continued his work on the immunology of scorpion venoms, particularly those from North African species *Buthus occitanus*, *Androctonus australis* and *Leirurus quinquestriatus*. Rabbits produced only small amounts of neutralizing antibodies when immunized with scorpion venoms unless adjuvants were added; particularly Freund's adjuvant or calcium phosphate.

Several antigenic components were revealed by gel diffusion precipitin tests, but only a few were related to the lethal antigens; some of them were antigens of the scorpion tissues. The antigens lethal to mice are dialysable and thermostable; the non-lethal antigens are not dialysable and are thermolabile. Immunological identity of the lethal antigens of the venoms of *Buthus occitanus* and *Leirurus quinquestriatus* was established.

ANTITOXIN PRODUCTION

Refinement of Therapeutic Antitoxins. Further experiments by Dr Dolby on the

isolation of β- and γ-globulins from horse antisera on cellulose ion-exchange columns (Report 1962) confirmed and modified previous conclusions. The flocculation of some of the separated fractions with toxin is inhibited by the phosphate buffer used for dilution in the measurement of antitoxic activity. When this buffer is replaced by isotonic borate-saline of the same pH, 86–100% of the antitoxic activity is recovered in the isolated fractions, the bulk of it in the β-globulin fraction.

Pepsin treatment of γ-globulin fractions under the conditions normally used in refining antisera results in the loss of 60% of both protein and antitoxic activity, so that no purification results; with β-globulin fractions, 50–55% of the protein and 36–40% of the antitoxic activity are lost, a purification of about 1.3-fold. 90% of a fraction consisting largely of albumin with no antitoxic activity is degraded in this way, as happens (Report 1961) with bovine albumins.

When all the fractions are combined in the proportions in which they were isolated from the serum, and then treated with pepsin, the yield of antitoxin and the purification factor are of the same order as those with the original serum treated with pepsin. Thus the purification attained in the refining of antitoxic sera by pepsin is due primarily to the breakdown of inactive protein and only to a lesser extent to the splitting of antitoxic globulin into active and inactive fragments.

EXPERIMENTAL PATHOLOGY

MECHANISMS OF INFLAMMATION

Vascular Changes in Injury. Dr. Wells investigated the changes in the blood and lymphatic vessels of the mouse ear induced by traumatic injury, using increased permeability to circulating dye as the index of blood vascular injury; and as indices of lymphatic vascular injury the diffusion of dyes from the lumen of the vessels into the surrounding tissues and the increased adhesion to the vessel walls of carbon particles injected into the lumen. Mechanical injury sufficient to alter blood vascular permeability induces lymphatic vascular changes of at least the same duration, some 90–180 minutes; but lymphatic changes can persist after the blood vessels have recovered. None of the various endogeneous factors increasing blood vascular permeability—histamine, bradykinin, etc.—even in high doses, affects the lymphatic vessel wall.

Increased permeability of small venules and of the blood capillaries can be distinguished by the rat cremaster muscle technique devised by Dr. G. Majno (Harvard School of Pathology). Dr. Wells and Professor Miles are exploring the phases of increased permeability that occur in various forms of injury, including that induced by bacterial toxins and bacterial infection, in terms of the anatomical site of injury in the vascular bed. It is already evident that in thermal injury the immediate phase of increased permeability is a venular event, and the late delayed phase a capillary event.

The Relation of Permeability Globulins to other Plasma Proteins. Miss Mason completed her demonstration that activation of the γ globulin permeability factor of guinea-pig plasma is a precursor of the activation of kininogenase; and that the two substances are distinct. The same relation is proving to hold with human plasma, the kininogenase (serum kallikrein) being distinct from the main permeability globulin. The hypotensive activity of the permeability factor in both species appears to be due to its acting as an activator of kininogenases in the test animal; that of the kininogenases to a direct action on the kininogens in the test animal.

Dr. O. Ratnoff completed his study of the relation of Hageman factor to the activation of permeability factor in human plasma. It was confirmed that the activation of Hageman factor is a necessary precursor of the activation of permeability factor; but this step alone of the known sequence of events leading to clotting was common to the two processes.

BIOCHEMISTRY

THE HUMAN BLOOD GROUP SUBSTANCES

Further studies on the heterogeneity of preparations of blood-group specific mucopolysaccharides obtained from individual specimens of human ovarian cyst fluids were made by Dr. Pusztai and Professor Morgan. In this instance, the mucopolysaccharide macromolecules in a preparation of the specific substance were examined for their behaviour on columns of diethylaminoethylether (DEAE) cellulose eluted with increasing concentrations of sodium chloride. The overall charge of those specific substances which were closely similar to each other in amino acid composition (Report 1962) is due to the presence of sialic acid, and it was found that whereas some preparations of the specific mucopolysaccharide contained molecules, all of which had almost identical amounts of sialic acid, the molecules in other preparations differed considerably in their content of sialic acid. Because of the different amounts of sialic acid in the mucopolysaccharide molecules, they could be separated from each other on DEAE cellulose.

Dr. Painter and Professor Morgan continued their investigations on the isolation of serologically active fragments from human blood-group specific mucopolysaccharides (Report 1961, 1962). Kinetic studies of the acid hydrolysis of model substances indicated that a higher yield of suitable fragments could be obtained by using polystyrene sulphonic acid as the hydrolytic agent and the results are consistent with predictions based upon the theory of random depolymerization. Preliminary work indicated that good yields of fucose-containing oligosaccharides could be obtained by alkaline degradation of the mucopolysaccharides.

Mrs. Rege and Dr. Painter subjected human H and Le^a substances to partial acid hydrolysis and isolated four disaccharides from each hydrolysate. Preliminary examination of these disaccharides indicated that they are probably identical with the four disaccharides common to the hydrolysates of A and B substances, i.e. 3-*O*-β-D-galactosyl-*N*-acetylgalactosamine, 3-*O*-β-D-galactosyl-*N*-acetylglucosamine, 4-*O*-β-D-galactosyl-*N*-acetylglucosamine and 3-*O*-β-*N*-acetylglucosaminoyl-galactose.

The finding that, with the exception of the A-active disaccharide, 3-*O*-α-*N*-acetylgalactosaminoyl-D-galactose, isolated only from A-substance (Report 1957, 1962) and the B-active disaccharide, 3-*O*-α-D-galalactosyl-galactose isolated only from B substance (Report 1962), the same four disaccharides can be isolated from A, B, H and Le^a substances is consistent with the view that the specific blood-group characters arise through the addition, by genetically determined enzymes, of single terminal sugar units to a common precursor mucopolysaccharide. On the assumption that the steps controlled by the A and B blood-group genes involve the transfer to the precursor substance of the sugar residue from nucleotide-bound *N*-acetylgalactosamine or galactose, respectively, Dr. Gompertz and Dr. Watkins examined extracts of *T. foetus* for enzymes which would synthesize uridine diphosphate (UDP) galactose and UDP-*N*-acetylgalactosamine from simpler substrates. Synthesis of these compounds would give a source of nucleotide sugars which could be used in biosynthesis experi-

ments on the blood-group substances. Since the *T.foetus* extract proved to contain hexokinase, phosphoglucomutase, UDP-glucose-pyrophosphorylase and UDP-galactose-4-epimerase activities, it may be possible to use it for the synthesis of UDP-galactose, using glucose as a starting material. A UDP-*N*-acetylglucosamine-4-epimerase that converts UDP-N-acetylglucosamine to UDP-*N*-acetylgalactosamine was also demonstrated.

Dr. Romanovska completed her studies on the neuraminidases in *T.foetus* (Report 1962). Separation of the enzymes on columns of DEAE-Sephadex A-50 gave four different zones of neuraminidase activity. Each of the neuraminidases released sialic acid from a variety of substrates, but differences were observed in the pH optima of the different fractions and in the relative rates at which liberation of sialic acid occurred. The *T.foetus* extracts therefore appear to contain a family of neuraminidases which differ, not only in molecular size and/or ionic properties, but also in enzymic behaviour.

Studies on the water-soluble blood-group substances showed that, by the sequential use of enzymes from *T.foetus*, A and B substances can be broken down to give first H, and then Le^a, active substances (Report 1956, 1960). Miss Rissik is examining the action of *T.foetus* enzymes on the intact red cell, and on red cell stromata, to determine whether the same series of changes can be induced in the A and B antigens on the red cell surface.

The enzyme in extracts of *T.foetus* which destroys the serological activity of blood group A substance is thought from indirect evidence to be an α-*N*-acetylgalactosaminidase. Mr. Harrap is attempting to isolate and purify the A-decomposing enzyme and to determine its specificity by following the chemical changes induced in A substance by the purified enzyme preparation.

Dr. Watkins and Professor Morgan continued their investigations on the substances with blood-group P_1 specificity found in sheep hydatid cyst fluid. The cyst material insoluble in 95% phenol is highly active as an inhibitor of human anti-P_1 serum (Report 1962). Further purification was achieved by fractionation with ethanol of an aqueous solution of the phenol-insoluble residue. The most active fraction contained 3.4% N, 23% hexosamine and 56% reducing sugar; galactose, glucosamine and several amino acids were detected chromatographically. This P_1 preparation, when coupled with the conjugated protein of *Shigella shigae* gave rise to powerful anti-P_1 agglutinins and precipitins in rabbits. Examination in the ultracentrifuge at pH 4, and by double diffusion in agar with the homologous rabbit antiserum, demonstrated the presence of two components in the P_1 preparation, indicating the need for further purification. Specimens of human hydatid cyst fluid are being examined for their content of P_1 active material, and a comparison made with the material isolated from the hydatid cysts of sheep origin.

CARBOHYDRATE STUDIES

Chemical Synthesis of Modified Carbohydrates. These researches are concerned with the specific chemical alteration of derivatives of glucose, usually glucose polymers. The modified molecules are tested as substrates for enzymes that attack the unmodified molecules, to gain a closer insight into the specificity of the enzymes. Some of the modified molecules are also intended for immunological studies. Thus, Kabat had predicted that after oxidation dextran would cross-react with antiserum to type II pneumococcus polysaccharide. This has now been verified.

Amylose, a linear polyglucose, was previously the main starting material for the chemical modifications. Attention has now turned to much more con-

venient substances, a family of crystalline cyclic polyglucoses (Schardinger dextrins) synthesized from starch with an enzyme from *Bacillus macerans*. Dr. Whelan and Professor Dexter French developed the method for a large-scale synthesis of these compounds, and their chemical modifications are in progress. Mrs. Taylor completed the modifications of the isomaltodextrins (Report 1962) by oxidizing their terminal non-reducing end groups. Dr. Együd and Dr. Morgan have synthesized modified sugar phosphates to test the specificities of phosphorylases.

Studies of Enzyme Specificity. Dr. Gunja Smith found that α- and β-amylase attack a synthetic 3-methylamylose, liberating methylated oligosaccharides. Maltose phosphorylase, from *Neisseria meningiditis*, synthesizes maltose from β-glucose 1-phosphate and glucose. Dr. Morgan was able to replace both the sugar phosphate (by 3-methylglucose 1-phosphate) and the glucose by analogues, synthesizing many new modifications of maltose. This is the first example, in a long record of failure by many workers, of a phosphorylase acting on a " foreign " sugar phosphate. Dr. Együd found wide variations in the specificities of glucose 6-phosphate dehydrogenases from different sources and discovered, *inter alia*, that the rabbit-brain enzyme also rapidly oxidizes mannose 6-phosphate, a naturally occurring compound. Phosphoglucose isomerase proved to be completely specific, since it failed to act on any of nine analogues of glucose 6-phosphate; but phosphoglucomutase, converting glucose 6-phosphate into 1-phosphate, was unspecific, acting on all nine phosphates. Preliminary results suggest that some of these 1-phosphates are polymerized to analogues of starch and glycogen, and Dr. Együd, Mr. Lee, Dr. Beattie and Dr. Smith are chemically synthesizing several 1-phosphates for this purpose. Mrs. Taylor found a glucosidase in rabbit muscle that attacks the isomaltodextrins in endwise fashion, liberating the monosaccharide glucose. When used with chemically modified dextrins in which the non-reducing end was converted into 6-deoxyglucose, the enzyme failed to act.

Glycogen and Starch Metabolism. The enzyme system, found by Dr. Abdullah in rabbit muscle (Report 1962), that causes the direct splitting of the branch linkages of glycogen, has now been resolved into two interdependent enzymes. It constitutes a new pathway of glycogen breakdown, operating without the assistance of phosphorylase. Mrs. Taylor and Dr. Abdullah examined the range of glucosidases in muscle and found two distinct enzymes, both of which hydrolyse the polymeric 1,4-bonds of glycogen. These are again new pathways of non-phosphorolytic glycogenolysis. Dr. Smith completed the structural study of a pentasaccharide formed by degradation of amylopectin with *B.subtilis* α-amylase, and synthesized the sugar by an enzymic method.

PHOSPHOLIPIDS AND GLYCOLIPIDS

The object of this work is to examine the structure and distribution of phospholipids and other lipids in different tissues and cells and to relate it to their function. The main lines of work were the characterization of a new class of phospholipids found in bacteria as *O*-amino acid esters of phosphatidylglycerol, and the isolation from ascites tumour cells of sugar-containing lipids which may be of antigenic significance.

Lipoamino Acids and Phosphatidylglycerols. The presence of phosphatidylglycerols in all kinds of cells, and particularly in the protoplast membrane of certain bacteria, suggested that they have some ubiquitous function, possibly in membrane function (Report 1962). Dr. Macfarlane found that the phospholipid extracted from *Clostridium welchii* cells, which had been harvested while still metabolizing, contained bound amino acids. On fractionation on silicic

acid, a small amount of glycosylglyceride was isolated, and most of the phospholipid was recovered in two fractions, both containing amino acids and with a molar ratio amino N to phosphorus of 1 : 1, indicating that the amino group of the amino acids was free. After brief treatment in weak aqueous alkali, the phosphorus from both fractions was recovered in the ether-soluble fraction as phosphatidylglycerol and the amino acids became water-soluble; on mild saponification in methanolic soda, the phosphorus was recovered as the water-soluble glycerylphosphorylglycerol, together with amino acids. These properties indicated that the phospholipid comprised a series of *O*-amino acid esters of phosphatidylglycerol; the constitution was confirmed by the preparation of dinitrophenyl derivatives and with infra-red spectra. The amino acids present included alanine and lysine as major components. Similar amino acid esters were found in phospholipid from *Staphylococcus aureus*.

The chemical and chromatographic properties of these amino acid esters are consistent with those of the lipoamino acids detected by other workers, by use of radioactive tracers, in metabolizing mammalian tissues, tumour cells, etc., but not previously characterized. The identification of lipoamino acids as defined compounds of a metabolically active phospholipid and the previous recognition of glycosylglycerides in protoplast membranes offer valuable clues to the function of phospholipids in membranes, and the mechanism of transport of water-soluble substances across membranes.

Bacterial Fatty Acids. Dr. Gray found the fatty acids in *Clostridium welchii* phospholipids were mainly even-numbered *n*-saturated acids from C_{10} to C_{20} chain-length, predominantly C_{12} (24%) and C_{20} (30%). The cyclopropane ring fatty acids obtained previously from *Salmonella typhi-murium* were further examined. The C_{19} component was found to be a mixture of approximately 80% of cis-11, 12-methylene octadecanoic acid (lactobacillic acid), and 20% of cis-9, 10-methylene octadecanoic acid (dihydrosterculic acid).

Lipids of Ascites Tumour Cells. Dr. Gray continued his investigation of the lipids of various strains of ascites tumour cells, generously provided by Dr. A. L. Davies (Experimental Station, Porton, Wilts.). The composition of the phospholipids, including the nature and distribution of fatty acid and fatty aldehyde components, of PB8/C3H ascites sarcoma cells was very similar to that previously found for Landschutz ascites carcinoma cells (Report 1962). Preliminary studies with Dr. Plackett on CL_2 ascites leukaemia cells indicated that these cells were also similar in lipid composition to the Landschutz cells. No differences between the tumour and normal cells likely to be pathologically significant were observed.

The isolation of glucose cerebroside from Landschutz cells, reported by Dr. Gray, prompted an exhaustive examination of the PB8/C3H cells for similar components, which may well be of antigenic significance. The isolation presented considerable technical difficulty, since the amount of glycolipid present is very small, and perhaps varies with the type of tumour. The quantities present were about 0.1% and 0.4% by weight of the crude total phospholipid in Landschutz and PB8/C3H cells respectively. A satisfactory recovery of the glycolipid was obtained by preferential alkaline hydrolysis of the phospholipids and repeated chromatography of the alkali-stable lipids on silicic acid. Further fractionation revealed three distinct classes of glycolipid, which were similar in the lipid moiety, consisting of a long chain fatty acid joined to sphingosine by an amide linkage, but different in the carbohydrate moiety.

Glycolipids of the simplest class contained a monosaccharide, either glucose or galactose, linked to the terminal hydroxyl of the sphingosine in the usual cerebroside structure. The second class of glycolipid contained a disaccharide unit, and a mixture of two substances of this class in about equal proportions was obtained. One substance contained lactose, linked to sphingosine through the

glucose residue, and is probably identical with cytolipin H, a lipid hapten isolated by Rapport from epidermoid tumour cells; the other substance contained β 1-6 galactosyl-galactose. The third class of glycolipid isolated was a mixture of two substances containing trisaccharide units. The major component (72%) was tentatively identified as a glucosyl-galactosyl-*N*-acetylgalactosamine derivative, linked to the sphingosine of the glycolipid through the glucose residue, and the minor component as a β 1-6 galactosyl-galactosylgalactose. The trisaccharide-containing glycolipids are similar in structure to glycolipids with blood group specificity isolated by Dr. Koscielak from human erythrocytes (Report 1962), but were found by Dr. Watkins to have no serological activity against human anti-A, human anti-B and rabbit anti-H sera. The serological activity of the three types of glycolipid isolated from ascites tumour cells is being examined by Dr. Davies.

TISSUE PHOSPHOLIPIDS

Dr. Wajda has examined the nature of the alkali-stable phospholipids, other than sphingomyelin, reported to be present in brain tissue. The results indicate that the major component of this fraction in extracts from fresh tissue is an ethanolamine-containing cyclic acetal, probably formed from ethanolamine plasmalogen during the fractionation procedure; small amounts of ethanolamine glyceryl ether derivatives are also present. The chromatographic behaviour of lipids isolated from brain tissue fixed in formalin differs from those extracted by the same technique from fresh tissue.

X-Irradiation and Peroxide Content of Phospholipids. In collaboration with Dr. P. B. McCay (Oklahoma Medical Research Institute) and Professor E. Boyland (Chester Beatty Research Institute), Dr. Macfarlane examined the peroxide content of liver phospholipids from rats exposed *in vivo* to X-irradiation. The peroxide content of unwashed butanol extracts from irradiated rats was higher than the controls, but the difference disappeared in washed extracts; the nature of this peroxide-like material needs further investigation. No change in the nature of the phospholipids was observed.

BIOPHYSICS

Characterization of Macromolecular Heterogeneity. Many biological materials are polydisperse when isolated, a fact which frequently complicates the interpretation of physical measurements. Existing methods for characterizing polydispersity are laborious, and often inaccurate. Dr. Creeth attempted to develop a new method, based on the well-known phenomenon of boundary sharpening in the ultracentrifuge. The principle of this method is that substances with which there is high concentration-dependence of sedimentation coefficient may be made to attain a steady state in a gravitational field, in which the diffusion is balanced by the sedimentation sharpening effect. Mathematical analysis of the steady state condition showed that an apparent diffusion coefficient may be obtained, which, for monodisperse substances, will be the same as that found in free-diffusion experiments, but will differ from the static value when polydispersity is present. The method holds promise of great sensitivity and further analysis is in progress.

HUMAN PLASMA AND OTHER PROTEINS

Antihaemophilic Factor (AHF). The stabilizing effect of treatment with di-isopropyl phosphofluoridate (DFP) on the activity of AHF in concentrates made from normal human plasma was examined in more detail. A maximum improvement in stability was obtained with 2×10^{-3} M DFP, whereas 2×10^{-4} M was

almost ineffective. The pH stability range of AHF is extended on the acid side by treatment with DFP, and thus may facilitate purification by providing a wider range of fractionating conditions.

It is possible that the circulating anticoagulant associated with the gamma globulin of some severe haemophiliacs, which specifically inactivates AHF in a manner indicative of an enzymic reaction, may be related to the DFP-sensitive destructive agent of normal plasma.

A great many experiments were made to discover conditions for removing the fibrinogen, which is the major constituent of AHF concentrates, in attempts to obtain purer preparations of AHF. These included precipitation with tannic acid, with anionic polymers such as polyglutamic acid, polystyrene sulphonic acid and polymethacrylic acid; but these were uniformly unsuccessful. So far adsorption procedures with cellulose ion exchange resins have also proved unfruitful.

Macroglobulins of Normal Human Plasma. In fractionating normal human plasma with ether, the macroglobulins with a sedimentation coefficient of about 19S, which occur among both the alpha and gamma globulins, are almost quantitatively segregated into the G2/2 fraction of the plasma globulins obtained by standard procedures, though mixed with other lower molecular weight components. The 19S gamma globulin is associated with blood group isoagglutinin activity, but the function of the " 19S alpha globulin " is not known.

Work on the separation of these two macroglobulins has started, the primary intention being to obtain 19S gamma globulin in a state of purity sufficient for immunological purposes. Antisera against 19S gamma globulin are required for some aspects of blood grouping and on this aspect of the work Dr. Kekwick is collaborating with Dr. Goldsmith.

Human Urinary Proteins. The examination of human urinary proteins with special reference to the proteinuria of renal tubular disorders, by Dr. Kekwick and Dr. Creeth, in collaboration with Dr. F. V. Flynn (University College Hospital) is complete. The proteinuria accompanying renal tubular dysfunction was shown to differ from that associated with glomerular dysfunction by sedimentation velocity analysis in the ultracentrifuge, in that a single peak only resolves in the first condition and more than one in the second.

The sedimentation coefficients of the single ultracentrifuge component in the urine from nine patients with renal tubular dysfunction fell within the range 2.2 to 2.8S. Measurements of molecular weight, made with the Archibald procedure, showed that though sedimenting as a single peak, these proteins are very inhomogeneous with respect to molecular weight. The average molecular weights obtained in two instances were 46,200 and 41,800, rather higher than was expected from the corresponding sedimentation coefficients.

BLOOD GROUP SUBSTANCES

The physico-chemical investigation of these substances was continued by Dr. Creeth and Mr. Knight along the lines described in the previous report. The effect of temperature on the sedimentation coefficient of a preparation with group B activity was determined over a wide range of concentration; the results indicate a small but well-defined change of corrected frictional coefficient, suggesting flexibility in molecular conformation. The data from an investigation of the intrinsic viscosity and partial specific volume at different temperatures yielded results confirming the conclusions from the sedimentation investigation, which indicate that the molecule has a configuration approaching the random coil. Thus, unlike biologically specific proteins, the molecule probably has no tertiary structure, indicating, in agreement with immunochemical findings, that specificity must be due to relatively small areas of the molecule.

BLOOD PRODUCTS LABORATORY

Pharmacologically Active Substances in Plasma. Dr. Mackay and Dr. Maycock continued to investigate these substances with particular reference to the endogenous plasma proteolytic enzyme system.

Activation of kininogenase and fibrinogenolysin by a plasma fraction (C.E.), obtained by elution from celite used to adsorb intact plasma, was further investigated. Kininogenesis by C.E. could be demonstrated by incubating with the fraction rich in antihaemophilic globulin, when the contained fibrinogen was first clotted and then lysed. Kininogenetic potency of C.E. could be related to the rate of clot lysis, and the amount of fibrinogen hydrolysed. When C.E. was added to purified plasminogen, activation to plasmin could be demonstrated by the hydrolysis of purified fibrinogen and of casein.

A concentrated preparation of C.E. in veronal buffer pH 8.0, examined by electrophoresis on acetyl cellulose, was shown to be 12% α_1, 78% α_2 and 10% β globulin. Differences in the decay rate of the properties of C.E. indicated that its clotting properties were more stable than those related to fibrinogenolysis or kinino-genesis.

Freeze-drying. The refitting of the plasma drying plant with large capacity single stage vacuum pumps and refrigerator compressors, which give lower condenser temperatures, made it possible to shorten the period needed to dry the plasma. To achieve this in routine drying requires a new method of controlling heat input in the later stages of drying; and a modification, by which both temperature and duration of heating are regulated, is under trial.

BLOOD GROUP RESEARCH UNIT

In previous years the greater part of the time of the Unit has been spent in looking for " new " blood group antibodies and investigating the inheritance of the antigens thereby defined: this year was unusual in that most of it was spent in the application of a blood group to human genetics.

The Xg System. This sex-linked blood group system, rather breathlessly described in the 1962 Report, lives up to expectation and contributes steadily to the cartography of the X-chromosome and to the investigation of abnormalities of that chromosome.

During the year nearly five thousand samples of blood were sent to the Unit for Xg testing; the samples were from families in which X-linked conditions, like haemophilia, are segregating, or from families with a member who has some abnormality of number or of structure of the X chromosome. The samples came from many countries. In previous Reports a point was made of naming collaborators, but this is not possible in the Xg work, for more than a hundred people are involved. Generous gifts of the precious anti-Xg^a serum from Dr. A. Cahan of Knickerbocker Biologics, New York, and Dr. J. D. Mann of Butterworth Hospital, Grand Rapids, alone made the work possible. The majority of blood samples were sent by Dr. J. B. Graham of the University of North Carolina, Dr. V. A. McKusick of Johns Hopkins, Dr. A. Motulsky and Dr. G. Fraser of the University of Washington, Professor M. Siniscalco of Naples, Mr. J. Lindsten of Stockholm, Mr. A. Adam of Tel-Hashomer, Israel, Professor P. E. Polani of Guy's Hospital, Dr. J. Harrison of the General Hospital, Birmingham, Dr. G. I. C. Ingram of St. Thomas' Hospital, Dr. T. E. Cleghorn of the South London Blood Transfusion Centre, Sutton, and Dr. J. R. O'Brien of Portsmouth.

The X is one of the longer human chromosomes—about 150 units (centimorgans) long. It is divided by the centromere into a short arm of about 50 units and a long arm of about 100 units. If two genes are more than 50 units apart linkage cannot be directly detected between them. A series of Israeli

families showed that the locus for the genes controlling the presence or absence of the enzyme glucose-6-phosphate dehydrogenase (G6PD) is within measurable distance of the Xg locus—about 27 units away. G6PD was known to be about 5 units from deutan red green colour vision, and haemophilia A was known to be about 12 units from deutan. The Xg grouping of deutan and haemophilia A families has shown the genes for these two conditions to be further away from Xg than is G6PD, thus establishing the following order on the X.

Xg . . . G6PD . . . deutan . . . haemophilia A

The Xg:G6PD linkage was, as expected, shown also by a series of Greek families sent to the Unit by Dr. Fraser. On the other hand, a series of Sardinian families from Professor Siniscalco did not show measurable linkage; Professor Siniscalco has an explanation for this finding, the possibility of which he had anticipated.

Enough families were tested to show that certain genes are not close to Xg: haemophilia B (Christmas disease) is more than 33 units away; hypophosphataemia more than 12 units away; hypogammaglobulinaemia probably more than 16 units away; Duchenne's muscular dystrophy more than 26 units away. More families are needed to decide whether the genes for any of these conditions be within measurable distance (50 units) of Xg.

Families with renal diabetes insipidus, ectodermal dysplasia, keratosis follicularis, pyridoxine responsive anaemia, deaf mutism, or with ichthyosis so far tested serve only to show that the genes responsible are not very close to Xg.

In one family sent from the University of North Carolina three X-linked genes were segregating: haemophilia B, protan colour vision and Xg. The family provides the first illustration in man of a double cross-over.

There is reason to think that the Xg locus is sited on the short arm of the X: two girls who break the rigid rules of X-linked inheritance by lacking their father's Xg^a antigen also lack the short arm of one of their X chromosomes. The simple interpretation that the gene for the antigen has vanished with the short arm would be entirely convincing but for present doubt whether the remaining long arm of such defective X chromosomes is genetically active. If the long arm is indeed inactive then the missing antigen of the two girls gives no information about the position, on the short or the long arm, of the Xg locus.

Samples of blood from 71 females with Turner's syndrome and the caryotype XO, instead of the usual XX, were tested. The parents of the majority of these patients were also tested. Having only one X, the Xg distribution was expected to be that of males and so it proved to be. In two families the Xg groups gave the first proof that the sole X of XO females can be of paternal origin: belief was growing that the missing sex chromosome was always the fault of the sperm.

Samples were also tested from 40 males with Klinefelter's syndrome and caryotype XXY, XXXY and XXXXY instead of the usual XY: the frequency of the Xg groups is, for what the small numbers are worth, supporting a current theory of the origin of these conditions.

The frequency of Xg(a+) and Xg(a–) amongst unrelated Caucasians (mostly British) fluctuated very little as the numbers grew: 66 per cent of males and 88 per cent of females were Xg(a+) in the latest count of 1550. In a small series of 138 unrelated Negroes 55 per cent of males and 80 per cent of females were Xg(a+): this lower frequency of Xg(a+) in Negroes compared with Caucasians verges on statistical significance.

In families without visible abnormalities of the sex chromosomes the antigen Xg^a, as expected, continues to obey the rules of X-linked inheritance.

The P System. The pressure of work on Xg unfortunately crowded out attention to the nice problem presented by the serum sent by Dr. Snoddy of Oklahoma (Report 1962).

The Rh System. Samples of blood from D positive persons who have made anti-D (Report 1962) continue to come in. They usually mirror previous samples and it now looks as if there is perhaps a limit to the complexity of the D antigen (compare Report 1962).

A kind of basic Rh antigen adumbrated 11 years ago by Dr. J. Murray of Queen Charlotte's Hospital has recently been studied by Dr. Philip Levine of New Jersey. The antigen, not surprisingly, was absent from the blood of the Australian aborigine with no detecable Rh groups (Report 1961) but, surprisingly, was absent from the blood of two persons of pedestrian Rh groups tested in the Unit. The meaning of this will, without doubt, be illuminating.

For routine antisera the Unit is indebted to many colleagues, notably those in the Medical Research Council Blood Group Reference Laboratory and the South London, North East London, Birmingham and Sheffield Blood Transfusion Centres. Dr. T. E. Cleghorn has continued to provide samples of blood from many normal families which were invaluable in the Xg investigations. Again the Unit is grateful to the Staff of the Institute for samples of blood which have played a major part in many of the investigations.

BLOOD GROUP REFERENCE LABORATORY

The Unit acts as the central Reference Laboratory for the investigation of blood group problems, and is the supply centre for grouping serum, for the United Kingdom.

Liquid blood grouping sera and anti-human-globulin serum are supplied for use in the United Kingdom, and dried sera for use overseas. Help to overseas laboratories has continued to be an important activity. Such laboratories have included those designated as national reference laboratories through the World Health Organization. It is thought that the most valuable part of such help is the full blood-grouping of members of staff, in order to create panels of donors of red cells for use in the identification of unknown haemagglutinins. Large numbers of blood and serum specimens from Great Britain and overseas have been examined for blood group antigens and antibodies, as part of clinical investigations, for research purposes, and for routine blood grouping purposes, especially in connection with the control and supply of grouping sera.

Dr. Goldsmith continued his investigations into antibodies against leucocytes and platelets, including a special study of cases of neonatal purpura. Numerous cases of thrombocytopenic purpura and of leucopenia are now being submitted by hospitals for routine investigation.

In cases arising in several parts of the country where tissue grafting is planned blood specimens from recipients and intended donors are being examined by Miss Ikin and Miss Giles for all possible red-cell antigens and, in selected cases in the London area, by Dr. Goldsmith for leucocyte antigens with some twenty antisera. At present these sera are chosen on an empirical basis and with increasing knowledge they may be found to be neither completely specific nor mutually exclusive, but in a field where the life of a patient may depend upon tissue compatibility every clue to this must be followed.

Miss Ikin, in addition to having charge of serum production, continued work on the varying distribution of blood groups in different populations. She, together with Dr. Mourant and a senior technician, visited the American University of Beirut to help in setting up a laboratory for population blood group studies, and investigated the blood group distribution of the ancient and unique

Samaritan community at Nablus, Jordan.

Miss Giles carried out serological investigations of unusual antigens and antibodies found in the course of tests performed for clinical purposes. These included anomalies involving the ABO, Rh and Lutheran systems.

The demand for anti-human-globulin serum for clinical use has continued to increase. At the same time it has been widely realized that several distinct antibodies, each of adequate titre, are required for the proper performance of the anti-human-globulin test both in research and in clinical work. The Unit is collaborating with a sub-committee of the Medical Research Council Blood Transfusion Committee in planning and carrying out work designed to improve the quality and quantity of serum produced, and to standardize it more precisely. Dr. Goldsmith is also collaborating with Dr. Kekwick in work on the production of a specific reagent for the recognition of sensitization with antibodies of the 19S γ globulin type.

During the year new premises for the Unit, planned many years ago, have been under construction on the " Tennis Court " side, north of the Biophysics Department. The Unit moved into these on February 25th. It is anticipated that increased staff and space will enable a greater range of research work to be done, and will allow larger quantities of testing sera, especially anti-human-globulin serum, to be produced.

Finally, the Governing Body would like to praise the scientific, administrative and technical staff for their enthusiastic devotion to the work of the Institute. Without this the successful results which are recorded in this Report would not have been achieved.

E. C. DODDS,
Chairman.

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BLOOD GROUP RESEARCH UNIT

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BLOOD GROUP REFERENCE LABORATORY

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THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

Balance Sheet

and

Accounts

31st December 1963

CHELSEA BRIDGE ROAD,
LONDON, S.W.1.
26th May, 1964

FINANCIAL REPORT OF THE GOVERNING BODY

1. The Balance Sheet as at 31st December, 1963, shows balances to the credit of the various funds as follows: Capital Fund £901,878, Specific Funds £203,862 and Bequest Funds £18,411. The balance on the Sinking Fund for Freehold Buildings of £141,657 is after transferring £6,042 from Income and Expenditure Account and £9,413 to Investment Reserve. During the year donations and legacies of £697 have been added to the Re-endowment Fund.

2. The General Fund Income and Expenditure Account shows the income for the year as £236,110 compared with £322,664 in 1962. Expenditure amounted to £293,957 against £294,454 last year. The deficit for the year is £57,847 compared with a surplus of £28,210 in 1962.

3. The year's deficit of £57,847 shown by the General Fund Income and Expenditure Account has been deducted from the Capital Fund.

4. Investments are now shown in the balance sheet at original cost. Realised profits, less losses, on sales of investments which had in previous years either been applied against the investments or credited to the respective funds, have been transferred during the year to Investment Reserves as follows:—

General Fund		
Previous years:—		
Profits applied against investments	£45,258	
Profits, less losses, credited to fund	15,125	
	60,383	
Current year:—		
Profits, less losses, on sales of investments	21,431	
		81,814
Sinking Fund for Freehold Buildings		
Previous years:—		
Profits applied against investments	26	
Profits, less losses, credited to fund	9,413	
		9,439
		£91,253

5. Stocks of Sera, Smallpox Vaccine and Horses on hand at 31st December, 1963, have not been valued in the accounts.

6. Cooper Brothers & Co., the retiring Auditors, will, subject to the provisions of the Companies Act, 1948, be re-appointed.

E. C. DODDS, *Chairman of the Governing Body.*

HUGH BEAVER, *Hon. Treasurer.*

BALANCE SHEET

(1962) £		£	£	£
	Capital Fund:—			
	Donations, &c., received to date from the following:—			
2,000	Dr. Ludwig Mond (1893)		2,000	
46,380	Berridge Trustees (1893-1898)		46,380	
10,000	Worshipful Company of Grocers (1894)		10,000	
250,000	Lord Iveagh (1900)		250,000	
18,904	Lord Lister's Bequest (1913-1923)		18,904	
7,114	William Henry Clarke Bequest (1923-1926)		7,114	
3,400	Rockefeller Foundation (1935-1936)		3,400	
22,669	Other donations and legacies (1891-1954)		22,669	
	General fund income and expenditure account accumulated surplus, as at 31st December, 1962	614,383		
	Less deficit 1963 57,847			
	transfer to investment reserve 15,125			
		72,972		
614,383			541,411	
974,850				901,878
	Specific Funds:—			
145,028	Sinking fund for freehold buildings	141,657		
34,008	Pension fund	33,915		
27,593	Re-endowment fund	28,290		
			203,862	
	Bequest Funds:—			
10,367	Jenner Memorial studentship fund	10,972		
7,121	Morna Macleod scholarship fund	7,439		
			18,411	
224,117				222,273
	Specific Grants and Legacies Unexpended:—			
772	Cancer research legacies (1937-1950)		772	
40	Royal Society grant (1951)		—	
5,896	Nuffield Foundation grants (1952-1962)		4,781	
7,857	Guinness Lister research grant (1953-1963)		5,398	
14,565				10,951
	Current Liabilities:—			
43,528	Creditors and accrued charges			59,061

E. C. DODDS, *Chairman of the Governing Body.*

HUGH BEAVER, *Hon. Treasurer.*

£1,257,060	£1,194,163

REPORT OF THE AUDITORS

The accounts set out on pages 4 to 8 are in agreement with the books, which, in our opinion, have been properly kept. In our opinion the accounts, amplified by the information given in paragraphs 1, 4 and 5 of the Financial Report, show the deficit of the Institute.

London, 27th May, 1964

(1962) £		£	£	£
	Fixed Assets:—			
	Freehold property at cost:—			
73,548	Land and building, Chelsea	73,548		
20,455	Queensberry Lodge estate, Elstree	20,455		
2,049	House, Bushey	2,049		
			96,052	
	(Note: Additions and replacements since 1912 at Elstree and 1935 at Chelsea have been charged to revenue.)			
	Furniture, fittings, scientific apparatus and books:—			
2,472	At cost, less depreciation to 31st December, 1920		2,472	
98,524	*(Note: Additions and replacements since 31st December, 1920, have been charged to revenue)*			98,524

General, Specific and Bequest Funds.
Investments and Uninvested Cash:—

		Quoted at cost In Gt. Britain	Quoted at cost Elsewhere	Unquoted at cost	Uninvested cash		
845,186	General	£708,092	£42,476	£46,695	—	797,263	
	Specific—						
145,028	Sinking fund for freehold buildings	143,310	—	—	7,786	151,096	
34,008	Pension fund	34,217	—	—	302 (*Cr.*)	33,915	
27,593	Re-endowment fund	25,926	—	—	2,364	28,290	
	Bequest—						
10,367	Jenner Memorial studentship fund	8,099	—	1,940	933	10,972	
7,121	Morna Macleod scholarship fund	6,666	—	—	773	7,439	
1,069,303		926,310	42,476	48,635	11,554	1,028,975	
	Less investment reserves—						
	General fund				81,814		
	Sinking fund for freehold buildings				9,439		
						91,253	
							937,722
	Current Assets:—						
72,458	Debtors and payments in advance					111,033	
16,775	Balance at bankers and cash in hand					46,884	
89,233							157,917

(Note: Quoted investments at a cost of £968,786 have a market value at 31st December, 1963, of £1,389,699.)

£1,257,060	£1,194,163

AUDITORS TO THE MEMBERS

In our opinion proper books of account have been kept. We obtained the information and explanations we required.
In our opinion the Balance Sheet and Income and Expenditure Account, which are in agreement with the books of account, are properly drawn up so as to exhibit a true and correct view of the state of affairs of the Governing Body, comply with the Companies Act, 1948, and give a true and fair view of the state of affairs and of the surplus for the year ended 31st December, 1963.

COOPER BROTHERS & CO.
Chartered Accountants.

INCOME AND EXPENDITURE ACCOUNT

GENERAL

(1962)		Total Expenditure	External Contributions	
£		£	£	£
112,860	Salaries and wages	224,071	91,525	132,546
8,098	Emoluments of two members of the Governing Body in an executive capacity	9,811	—	9,811
5,160	Premiums on federated superannuation policies	11,191	5,103	6,088
1,743	Premiums on group pension policy	2,403	1,157	1,246
5,241	Rent, rates and insurance	6,186	355	5,831
16,697	Gas, water, fuel and electricity	20,901	3,742	17,159
4,421	Office expenses, stationery and printing	5,491	833	4,658
410	Audit fee	430	—	430
1,568	Travelling expenses	1,516	515	1,001
3,726	Biochemistry expenses	12,228	8,042	4,186
1,264	Microbiology, immunology and experimental pathology expenses	7,583	5,106	2,477
771	Biophysics expenses	4,461	3,774	687
203	Virology expenses	1,153	953	200
40,562	Serum, vaccine and virus vaccine expenses	36,669	1,621	35,048
9,701	Animals	9,806	1,646	8,160
10,298	Animal house expenses and forage	12,824	1,376	11,448
59,030	Buildings, alterations, repairs and renewals	38,438	857	37,581
960	General apparatus and new installations	3,071	—	3,071
1,995	Library expenses	2,020	—	2,020
1,607	General stores	1,641	—	1,641
2,170	Staff canteen loss	3,079	453	2,626
—	Blood products laboratory expenses	7,118	7,118	—
5,969	Amount transferred to sinking fund for freehold buildings (including £5,618 interest on investments)	6,042	—	6,042
28,210	*Surplus transferred to Capital Fund*	—	—	—
£322,664		£428,133	£134,176	£293,957

(1962) £		£	£
	Interest and dividends on investments:—		
50,768	General fund	49,637	
5,545	Sinking fund	5,618	
			55,255
522	Underwriting commission		234
259,134	Sales of sera, vaccine, smallpox vaccine, &c.		174,520
6,695	Rent		6,101
	Deficit transferred to Capital Fund after charging to expenditure £37,726		
—	(1962 £56,608) for additions to property and equipment		57,847
£322,664			£293,957

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

PENSION FUND

(1962) £		£	(1962) £		£
2,069	Pensions	2,406	32,664	Balance as at 1st January, 1963 ..	34,008
34,008	Balance carried forward	33,915	1,781	Interest on investments (gross) ..	2,268
			1,632	Net profit on realisation of investments	45
£36,077		£36,321	£36,077		£36,321

JENNER MEMORIAL STUDENTSHIP FUND

(1962) £		£	(1962) £		£
10,367	Balance carried forward	10,972	9,822	Balance as at 1st January, 1963 ..	10,367
			545	Interest on investments (gross) ..	605
£10,367		£10,972	£10,367		£10,972

MORNA MACLEOD SCHOLARSHIP FUND

(1962) £		£	(1962) £		£
6	Loss on realisation of investments	—	6,861	Balance as at 1st January, 1963 ..	7,121
7,121	Balance carried forward	7,439	266	Interest on investments (gross) ..	311
			—	Profit on realisation of investment	7
£7,127		£7,439	£7,127		£7,439

NUFFIELD FOUNDATION GRANTS

(1962) £		£	(1962) £		£
3,557	Salaries, wages, laboratory expenses and animals	1,115	6,453	Balance as at 1st January, 1963 ..	5,896
5,896	Balance carried forward	4,781	3,000	Amount received	—
£9,453		£5,896	£9,453		£5,896

GUINNESS-LISTER RESEARCH GRANT

(1962) £		£	(1962) £		£
13,159	Salaries and wages	13,415	8,812	Balance as at 1st January, 1963 ..	7,857
6,996	Laboratory expenses	4,294	19,200	Amount received	15,250
7,857	Balance carried forward	5,398			
£28,012		£23,107	£28,012		£23,107

THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE

REPORT
OF THE
GOVERNING BODY
1964

CHELSEA BRIDGE ROAD, LONDON, S.W.1

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G. S. Turner, BSc
L. C. Robinson, BSc

PREPARATION and STUDY of BACTERIAL VACCINES (ELSTREE)

*A. F. B. Standfast, MA, Dip Bact
§Jean M. Dolby, MA, PhD (*Medical Research Council External Scientific Staff*)
M. P. Banks, BSc
Georgina Sampson, BSc
W. A. Vincent, BSc, PhD (*Medical Research Council Grantee*)

BLOOD PRODUCTS (ELSTREE)

*W. d'A. Maycock, MVO, MBE, MD
L. Vallet, MA
§Margaret E. Mackay, MSc, PhD (*Medical Research Council External Scientific Staff*)
Constance Shaw, MSc, Dip Bact
Shirley M. Evans, BSc
Elsie Silk, MSc
E. D. Wesley, B.Pharm

MEDICAL RESEARCH COUNCIL UNITS HOUSED AT THE INSTITUTE

BLOOD GROUP RESEARCH UNIT

§R. R. Race, PhD, FRCP, FRS
Ruth Sanger, BSc, PhD
Patricia Tippett, BSc, PhD
E. June Gavin, BSc
Jean E. Noades, BSc

BLOOD GROUP REFERENCE LABORATORY

§*A. E. Mourant, MA, DPhil, DM, FRCP, FCPath
K. L. G. Goldsmith, PhD, MB, BS, MCPath
Elizabeth W. Ikin, BSc, PhD
Carolyn M. Giles, BSc
Hilary D. Nunn, BSc
R. Narayanan, BA, MB, BS (*Colombo Plan Fellow*)

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*Recognised Teacher of the University of London
§Honorary Member of Institute Staff

ANNUAL GENERAL MEETING

OF

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

June 30th, 1964

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the work of the Institute for the year 1963.

GOVERNING BODY

The Council, at a meeting held on 25th June, 1963, reappointed Sir Charles Dodds, Lord Brain and Professor J. S. Mitchell as its representatives on the Governing Body until 31st December, 1964.

The Governing Body has noted with pleasure the conferment of a baronetcy on Sir Charles Dodds and the election of Lord Iveagh and Lord Brain to the Fellowship of the Royal Society.

COUNCIL

At last year's Annual General Meeting the three retiring members of the Council, Professor P. Collard, Sir Charles Dodds and Sir Rudolph Peters were reappointed.

The three members of the Council due to retire this year in accordance with the Articles of Association, but who are eligible for reappointment, are Sir Henry Dale, a representative of the Members of the Institute, Sir Howard Florey the representative of the University of Oxford and Sir Graham Wilson, the representative of the University of London.

MEMBERS

The Governing Body records with regret the death during the year of Dr. R. St. John Brooks, a member since 1936. Dr. St. John Brooks was for over twenty years Curator of the National Collection of Type Cultures, during which time the Collection was housed at the Institute.

STAFF AND STUDENTS

The Governing Body has noted with much pleasure the election of Professor A. A. Miles as Biological Secretary of the Royal Society and his appointment as a Vice President of the Society. The Governors are also pleased to record the reappointment of Professor W. T. J. Morgan for a third year of office as a Vice President of the Royal Society.

During the year the University of London approved the establishment of the Guinness Chair of Microbiology at the Institute. The Governing Body notes with pleasure the appointment of Dr. B. A. D. Stocker as the first holder of the Chair.

Dr. W. E. Parish was appointed to the Experimental Pathology Department; and Mrs. E. Silk and Mr. E. D. Wesley to the Blood Products Laboratory. Mrs. J. Allen, Miss M. L. Rissik and Miss S. M. Smith resigned during the year.

Professor A. A. Miles took part by invitation in a Conference on "The Acute Inflammatory Response", organised by the New York Academy of Sciences held in New York in November 1963.

Professor W. T. J. Morgan, in March 1963, took part in the opening ceremony of the Max-Planck-Institut für Immunbiologie, in Freiburg, Germany, at the invitation of Professor A. Butenandt, the President of the Max-Planck Society.

In March 1963, Dr. W. d.'A Maycock attended a meeting in West Berlin of the Subcommittee of Specialists in Blood Transfusion of the Public Health Committee of the Council of Europe.

Professor B. A. D. Stocker, sponsored by the British Council, lectured in Copenhagen and Aarhus; he also lectured at the Max-Planck-Institut für Immunbiologie, Freiburg; and attended a meeting on O antigens and colicines at the Institut Pasteur, Paris.

In December 1963, Dr. L. H. Collier participated in the Third Meeting of the World Health Organization Scientific Group on Trachoma Research in Geneva.

Dr. R. A. Kekwick attended by invitation the conference at Gleneagles, Scotland, in July 1963, of the International Committee for the Nomenclature of Blood Clotting Factors.

In May 1963, Dr. C. Kaplan spent two weeks at the Moscow Institute of Viral Prophylactics exchanging information about the preparation and assay of smallpox vaccine. He made the trip as a grantee of the World Health Organization.

Dr. B. G. Weitz took part by invitation in a conference on "Some Biological and Immunological Aspects of Host-Parasite Relationships" organized by the New York Academy of Sciences in New York in April 1963. In September he represented the Department of Technical Co-operation at a meeting in Lagos of experts on Trypanosomiasis organized by the Commission for Technical Co-operation in Africa South of the Sahara; and visited the West African Institute for Trypanosomiasis Research, Vom and Kadune, Northern Nigeria; the East African Trypanosomiasis Research Organization, Tororo, Uganda; the Veterinary Research Laboratory, Kabete, Kenya; and the Department of Veterinary Services, Tsetse and Trypanosomiasis Control Branch at Salisbury, Southern Rhodesia. In December he attended a meeting at the World Health Organization, Geneva to discuss an information service on trypanosomiasis.

Dr. W. J. Whelan lectured by invitation at Makerere University College, Uganda and at University Centres in Bristol and London.

Dr. Marjorie G. Macfarlane and Dr. G. M. Gray were invited as speakers at a NATO Advanced Study Institute on "The Metabolism and Physiological Significance of Lipids", held in Cambridge in September 1963.

At the invitation of Professor A. L. Lehninger, and with the aid of a travel grant from the Wellcome Trust, Dr. Gray spent three months in the spring as Visiting Research Fellow in the Department of Physiological Chemistry, Johns Hopkins Medical School, Baltimore.

During November and December 1963, Mr. L. Vallet attended the annual meeting of the Protein Foundation, Boston, the International Symposium on Haemophilia, Washington, D.C. and visited various laboratories in Canada and U.S.A. concerned with the preparation of plasma fractions for clinical use.

Miss S. M. Evans worked in the laboratories of the Central Transfusion Laboratory of the Netherlands Red Cross Society during May and June 1963,

Dr. G. G. Meynell, Mr. T. V. Subbaiah, Miss S. Smith, Mrs. E. Dubnau and Mrs. U. Pearce attended a meeting on bacterial genetics and bacteriophage in Brussels.

For the academic year 1963/4 there are fourteen postgraduate research workers at the Institute registered for higher degrees of the University.

One D.Sc. and six Ph.D. degrees were awarded to students and other research workers during 1963.

DONATIONS AND GRANTS

The Trustees of the Fleming Memorial Fund for Medical Research made a grant of over £30,000 to the Institute. The Governing Body is most grateful for this very generous gift which will enable the Institute to establish its own electron microscope unit. Arthur Guinness, Son & Co. Ltd. continue their generous support of the Guinness-Lister Research Unit.

The Governing Body also records its appreciation of the generosity of the many other bodies whose benefactions and grants support research work in the Institute. These include a grant from the British Empire Cancer Campaign for research on lipids; grants from the Department of Technical Co-operation in aid of research on the blood-meals of insect vectors of disease and on the immunology of trypanosomiasis; grants from the Department of Scientific and Industrial Research for researches on the enzymic polymerization of monosaccharides, on chemically modified polysaccharides, on the carbohydrases of barley and malt and on intermediary metabolism of lipids, carbohydrates and proteins; grants from Glaxo Ltd. and Imperial Chemical Industries Ltd. for the purchase of special apparatus; grants from the Medical Research Council in aid of researches on physico-chemical studies of blood group substances, on the chemical basis of blood group specificity in man, on the enzymic decomposition of blood group specific substances, on the identification of *Bordetella pertussis* antigens, on the synthesis of haptens and dextran-antidextran inhibitors, for studies of glycogen-debranching enzyme systems, on the structure of the amino-acid containing moiety in mucopolysaccharides and on the genetics of virulence in Salmonella; a grant from the Royal Society for the purchase of special apparatus; a grant from the U.S. Public Health Authority in aid of researches on abacterial urethritis in man; and a grant from the U.S. Department of Agriculture for fundamental studies of the nature and specificity of starch- and glycogen-debranching enzymes.

The Governing Body also gratefully acknowledges donations to the Institute's Re-endowment Fund from the following Assurance Companies: The General Life Assurance Company, the Prudential Assurance Company Ltd., and the Royal London Mutual Insurance Society Ltd.

VISITING WORKERS

The following visitors, in addition to those listed under Staff, worked for short periods in the Institute's laboratories: Miss Catherine P. Bingham, The Moore Clinic, Johns Hopkins Hospital, Baltimore, Maryland, U.S.A.; Dr. T. Camera, Pasteur Institute, Kindia, Guinea; Mr. A. Clarke, Anthropological Blood Grouping Laboratory, American University of Beirut, Lebanon; Professor E. H. Fischer, University of Washington, Seattle, U.S.A.; Dr. A. R. Gray, West African Institute for Trypanosomiasis Research, Vom, Northern Nigeria; Dr. P. E. Hartman, Department of Biology, Johns Hopkins University, Baltimore, Md. U.S.A.; Dr. P. H. Makela, University of Helsinki, Finland; Dr. B. M. Mizen, Turkish Red Crescent Society, National Headquarters, Ankara, Turkey; Dr.

B. M. Mukherji, Indian Statistical Institute, Calcutta, India; Mr. M. Naidoff, Medical School, University of Pennsylvania, U.S.A.; Dr. D. P. Rimal, Blood Bank, Khatmandu, Nepal; Dr. Arnaldo Sampaio, Instituto Superior de Higiene " Dr. Ricardo Jorge ", Lisbon, Portugal; Dr. Halina Seyfried, Institute of Haematology, Warsaw, Poland; Dr. Tuchinda Soodsakorn, Department of Paediatrics, Siriraj Hospital, Bangkok, Thailand.

The Medical Research Council's Blood Group Research Unit, Blood Group Reference Laboratory and Trachoma Research Unit are accommodated at the Institute.

The new Virus Laboratory at Elstree was completed early in the year. It was formally opened on 22nd May by Sir Howard Florey, P.R.S. and was occupied in full working order by the staff of the Virus Vaccine Department by the end of the year.

The Library at Chelsea was extended by the addition of another room, which has been set aside as a writing room. The extra shelf space made available will accommodate the growth of the library for another five years.

RESEARCHES IN 1963

SUMMARY

In this summary of the investigation made in 1963, the bracketed numbers refer to the pages of the report where the researches are described in greater detail.

Microbiology. The Guinness-Lister Unit continues its exploration of bacterial genetics, mainly those of the Salmonella bacilli. The problems studied include the transmission of drug-resistance factors (14); the inter-relations of genes controlling the production of flagella (15); the mapping of the genes determining " smoothness " (S) and " roughness " (R), and the correlation of two types of roughness with defects in the synthesis of the polysaccharide side chains characterizing the S forms (15-16); and differences in the structure of the flagellar proteins from various antigenic types of Salmonella (17).

The *in vitro* conditions for the formation of capsules by the anthrax bacillus were investigated (17), and the study of the factors in the normal mouse gut that are destroyed by streptomycin and that contribute to resistance to oral salmonellae (Report 1963) was extended to staphylococci (18).

Immunology and Pathology of Infective Diseases. The Institute's studies embrace infections by viruses, pleuro-pneumonia-like organisms, bacteria and protozoa.

The Trachoma Research Unit continued its epidemiological field study in the Gambia of trachoma in terms of virus and of bacilli of the *Haemophilus* group and is testing the prophylactic value of a virus vaccine in the Gambia (21). The laboratory studies at the Institute include the maintenance of trachoma virus in cell cultures (20); the differentiation of strains of virus rapidly lethal for the chick embryo from slowly lethal strains (19); the serological relation of strains of the viruses of both trachoma and inclusion conjunctivitis (20); and the perfection of prophylactic vaccines (20).

Other virus studies include the effect of interferons and carcinogens on the replication of Shope fibroma virus in cell culture (21). In the Virus Vaccine department (22), there is continued progress towards making a non-infectious smallpox vaccine; in the purification of vaccinia virus; and in the study of antibody immunity in vaccinia and variola infections.

The antigenic analysis of human and animal strains of *Mycoplasma* (pleuropneumonia-like organisms) continues, as a contribution to the classification of these organisms and as a means of providing specific antigens for serological diagnosis in human infections (18-19).

The immunological study of bacterial infections includes an analysis of the contribution of local and general specific immunity to the outcome of infection in the mouse by the whooping cough bacillus, an attempt to measure protective antibody by an *in vitro* bactericidal test, and the successful isolation of a protective antigen from the bacillus (12); the laboratory assay of typhoid vaccines (13); the immunopathology of actinomycotic dermatitis of sheep with respect both to antibody formation and delayed hypersensitivity (13); and the process of refinement of antitoxin by proteolysis with especial regard to the antigenic structure of the β-globulins (23).

As regards pathogenic protozoa, the investigation continues of the immunology of experimental trypanosomiasis with especial reference to the protective trypanosomal exoantigens characterizing the successive variants of the organisms that appear in the animal during the course of infection (11); and of the immunochemical analysis of trypanosomes harvested from the infected animal and from cultures *in vitro* (11).

Epidemiology. In addition to the epidemiological studies of trachoma in the Gambia, the large-scale identification tests of insect blood meals was continued, as part of the epidemiological field work on transmission by insects of trypanosomiasis and of malaria (22).

Pathology. Work continues on the relation of serum permeability factors to plasma kininogenases, and on their possible roles in vascular reactions to injury (25). The search for substances mediating increased vascular permeability was extended to inflammatory lesions of the Arthus reaction (25). Researches are in progress on the immediate and delayed phases of the response of small blood vessels to inflammatory injury, with particular reference to the association of venular damage within the immediate, and capillary damage with the delayed phase (24).

Immunopathological studies are in progress on a factor in anaphylactic tissues that induces eosinophilia in the blood (23); on the local production in the human cervix uteri of antibodies lethal to spermatozoa, as a possible cause of infertility (23); and on the nature of reagins.

With a view to the improvement of therapeutic antitoxins for scorpion bites, the antigenic analysis of components of venoms from four different species continues; some antigens proved to be common to all the species (23).

Biochemistry. The biochemical researches again concern three kinds of substances—the blood group substances, the cellular phospholipids, and glycogen

The investigations of the substances of the ABO and other blood group systems include definition of the chemical heterogeneity of preparations of mucopolysaccharides and definition of the terminal sugar groups determining serological specificity (25-26); analysis by improved methods of partial hydrolysis; transformation of blood group specificity, and biosynthesis of determinant groups, by selected enzymes and enzyme systems (26); measurement of the molecular properties, including heterogeneity, of preparations of blood group substance B (31); and the relation of the P_1 blood substance to an antigenically similar substance in sheep hydatid fluid (26).

Work on the structure of phospholipids, their distribution in tissues, cells and cellular particles, and their possible function in the cell includes the nature and functions of the lipoamino acids discovered in bacteria (29); the identification

of various glycolipids from different strains of ascites tumour cells (29); examination of a mouse lipoprotein carrying a histo-compatibility specificity; and comparison of the phospholipids of normal and leukaemic human bone marrow (30).

Carbohydrate studies mainly concern the relation of structure of both natural and synthetic polysaccharides to the specificity of enzyme and antibody reactions (27-28). Other studies include mammalian enzymes concerned in glycogen metabolism; a bacterial α-amylase, and a barley α-glucosidase (28).

Human and Animal Plasma Proteins. The Institute's work is concerned with the isolation, refinement, characterization, including refined interpretation of sedimentation and viscosity data (30), assay, and in some cases clinical trial, of the various biologically active proteins of human plasma.

An anticoagulant solution was devised to ensure the stability of antihaemophilic factor in blood required for isolation of the factor and another for the preservation of red blood corpuscles (31).

Studies of the isolation and characterization of other proteins include those of the 7S γ-globulins and the macroglobulins of normal human plasma (32), of gamma globulin from pooled plasma by the ethanol method (32) and of albumin purified after heating to destroy hepatitis virus (32); of plasmin, plasma kininogenases and plasminogen (32-33).

MICROBIOLOGY

TRYPANOSOMIASIS

Dr. Weitz continued the study of the specificity of the soluble and other antigens of *Trypanosoma brucei* and *T.vivax* and confirmed the specificity of the exoantigen by fluorescent antibody techniques. This technique was used to demonstrate specific differences between the exoantigen of trypanosome variants, and is being developed to establish the nature of the antigens of the trypanosomes in the insect vector.

Mr. Miller continued his studies of the antigenic variation of *Trypanosoma brucei* in infected rabbits. A number of variants was isolated; the soluble exoantigen obtained from each variant proved by precipitin tests to be specific for each variant. Antigenic differences between successive variants occurring *in vivo* were demonstrable by absorption of the homologous agglutinins in the serum of rabbits infected with the different variants, indicating that the agglutinins in infected serum were induced specifically by exoantigens. Mice were specifically protected against each variant by immunization with the corresponding exoantigens.

Dr. Pittam continued experiments to establish *Trypanosoma congolense* and *T.vivax* in culture. A strain of *T.congolense* was maintained in serial culture for approximately 20 subcultures over a period of 6 months using a modified Tobie medium. Attempts were made to establish these two trypanosomes in chick embryos. The trypanosomes survived, and even multiplied, for the first 3–4 days in the embryonic bloodstream, but did not survive passage. Allantoic and yolk sac inoculations were unsuccessful.

Trypanosoma brucei, which has not previously been cultured for any long period, was established successfully in a diphasic medium and transferred subsequently to a liquid medium in 500 ml. Thompson bottles, a method suitable for the supply of material for immunological and biochemical investigations.

Miss Lanham continued her investigations on the antigens of *Trypanosoma vivax*. Fractions of infected rat serum (IRS) containing the trypanosomal antigens and trypanosomal homogenate (TH) extracts were prepared by " molecular sieving " on Sephadex columns, and characterised by gel-diffusion techniques.

The three antigens found in IRS were partly separated on Sephadex G200, but in each fraction they were associated with serum proteins, suggesting that they had molecular weights of the same order as those of the serum globulins. On Sephadex G200, the non-diffusible components of the TH extracts were partially separable in four antigens. The first two antigens resembled two of the IRS antigens, the third was an antigen common to many trypanosomes, and the fourth was distinct from any of the IRS antigens. Fractionation on Sephadex G100 of the two diffusible antigens in crude TH diffusate did not separate them, but served to purify them from the other diffusate constituents. A non-diffusible antigen and the serum albumin present in infected rat urine were separated from the non-protein urine constituents on Sephadex G50.

WHOOPING COUGH BACILLUS

In vitro Tests with Bordetella pertussis Antisera. Dr. Jean Dolby is still not able to conclude with certainty that the *in vitro* bactericidal test (Report 1962) with antisera to a number of Dr. Vincent's fractions measures the antibody capable of protecting mice against an intracerebral challenge; in about 3% of the antisera tested there was no association of the two activities. The antibody protecting mice against intranasal infection and the anti-histamine sensitizing factor were not correlated in any way with bactericidal activity.

The bactericidal activity of antisera was influenced not only by complement (Report 1962) but also by the concentration of lysozyme or similar enzymes present and the strain of *B.pertussis* used. Three highly mouse virulent strains and one strain of lesser virulence (about two thousand times less) were killed by suitable antisera, i.e. they were serum-sensitive; ten avirulent strains were serum-insensitive. This is contrary to the finding in bactericidal systems with other Gram-negative organisms. Coating *B.pertussis* with its own somatic lipopolysaccharide renders serum-sensitive strains insensitive.

Bordetella pertussis Infection in the Brains of Mice under Different Degrees of Active and Passive Immunity. Mr. Standfast and Dr. Jean Dolby continued to investigate the cerebral infection along the lines noted in the previous Report (1963). Factors influencing the course of infection were the general immunity, the local immunity, the general immunity boosted by the challenge dose and the local immunity either initiated or boosted by the challenge dose. The outcome of infection depended on the efficacy of a combination of these factors. These findings throw some light on the unexpected behaviour in the brain of organisms sensitized with minute amounts of antiserum.

Isolation and Chemical Identification of B.pertussis Protective Antigen. Dr. Vincent continued his work on the isolation and chemical identification of pertussis antigens. Although purification on ion exchange cellulose of cell extracts of *B.pertussis* destroyed most of the protective activity in the extract, it gave much indirect evidence of the chemical nature of the protective antigen. It is apparently a complex macromolecule containing a protein—which has been isolated and analysed—and other material, including carbohydrate and phosphorus. The whole is known to be part of the bacterial cell wall, but when pure preparations of cell walls were dissociated in order to study the constituent antigens, the protective activity was lost in all cases studied.

The fractionation of a solution of *B.pertussis* in detergent gave a soluble, protective product with a low toxicity and high stability. Ultracentrifugation of this preparation revealed a rapidly-sedimenting species of macromolecule in the active fraction. The investigation of its chemical nature continues.

TYPHOID BACILLUS

Laboratory Assay of Typhoid Vaccines. Mr. Banks has continued his investiga-

tion into the laboratory assay of typhoid vaccines under trial in the field, as part of the collaborative study instituted by the World Health Organization. The preliminary results of the field trials are now coming in, and it should soon be possible to correlate field results and laboratory assays and to select for further work those assays which arrange vaccines in the same order of potency as the field trials.

If one of the laboratory methods of immunization and challenge proves to give an index of immunizing potency of vaccines in man, it is intended to study the mechanisms of immunity in mice artificially infected in this way, in the hope of throwing some light on the mechanisms of immunity in human typhoid fever.

ACTINOMYCOTIC DERMATITIS IN SHEEP

Mechanisms of Infection. Mr. Roberts continued his investigation of this disease. In further studies of the invasion of agar gels by *Dermatophilus congolensis*, the hyphae were found to penetrate most rapidly when the gel was in equilibrium with an atmosphere containing about 15% CO_2. When CO_2 gradients were established, with CO_2 diffusing from the surface of the gel as it does from the skin, there was no observable chemotropic effect on penetrating hyphae. This is in contrast with the zoospores, whose chemotactic response to CO_2 is thought to accelerate their movement to susceptible sites on the skin surface.

That penetration of the skin by hyphae is partly mechanical was suggested by the ready penetration of hyphae into tough, 3% agar gels. On the skin, anchorage for penetration is perhaps provided by the capsule of the budding zoospore, which was found to have hydrophobic properties associated with the property of firm adherence to glass and other surfaces.

D.congolensis releases small amounts of a variety of substances that might determine its pathogenicity and invasive powers. They include a protease, a lipase, a lecithinase A, and two substances acting together to lyse sheep red blood cells. The nature of the two substances is unknown, except that one of them precipitates lipid from serum. This effect is accentuated in the presence of lecithinase A but not lecithinase C.

The inflammatory action of the organism seems to result almost entirely from the intensive epidermal perforation. No endotoxin- or exotoxin-like substances are detectable. The hydrolytic enzymes noted above are produced in amounts too small to have a general toxic effect. The possession of a capsule, which is a highly coherent and resilient polysaccharide gel, was without effect on the irritant properties of organisms injected intradermally. Although it is relatively hydrophobic, the capsule does not resemble similar lipid-rich envelopes associated with the pathogenic properties of *Nocardia* spp. and of tubercle bacilli.

Mechanisms of Resistance. A dense accumulation of granulocytes always occurs immediately beneath regions of the epidermis where invaded by the hyphae of *D.congolensis*. The failure of hyphae to penetrate into the dermis suggests that the accumulations of granulocytes are solely responsible for the confinement of hyphae to the epidermis. The rapid and intensive invasion of the dermis of rabbits made agranulocytic by nitrogen mustard is consistent with this view. The effect was not due to other actions of nitrogen mustard, since the drug had no effect on granulocytes or on resistance to the hyphae when given to rabbits with part of the bone-marrow protected during administration of the nitrogen mustard.

In contrast to dermatophytic fungi, whose failure to invade deeply is attributed to labile inhibitory factors present in fresh serum, the fresh serum of sheep, rabbit or guinea-pig neither kills zoospores nor inhibits their growth.

Infection induces in the animal delayed hypersensitivity, which is manifested as an accelerated inflammatory response to re-infection and quicker healing. The invasion of the hair follicle sheaths is diminished and accumulation of granulocytes around them intensified. The greater accumulation may be due to the greater hyperaemia in the affected region, and the restricted invasion of the follicle sheaths to inhibitory facters diffusing from the accumulated granulocytes. Compared with that in normal animals, the resistance of sheep and guinea-pigs inoculated with formalin-killed organisms, as judged by the dose of zoospores inducing a given intensity of infection, was increased 20-fold. The increased resistance is associated with increased phagocytosis of the infecting zoospores. It was observed in animals with moderate amounts of antibody to the surface antigens of the zoospore and no detectable antibody to the soluble antigens of the interior. That the protective antibody acts by opsonization is indicated by the low resistance of immunized animals made agranulocytic by nitrogen mustard and the absence of any antimicrobial action *in vitro* of fresh high-titre antiserum.

Serology of Dermatophilus species. Comparison of eight strains of *Dermatophilus* from four countries by agglutination and gel-precipitation techniques revealed only very minor inter-strain differences and justified the classification of all the strains as *D.congolensis*.

INHERITANCE IN BACTERIA

Genetics of a Transmissable Drug-Resistance (R) Factor. The R Factors (transmissable agents conferring resistance to one or more antibacterial drugs on otherwise sensitive bacteria which harbour them) have recently been detected in bacteria of the *Salmonella* group in this country, and probably they will, as in Japan, become prevalent and accentuate the difficulties already caused by the frequency of antibiotic-resistant strains of disease-producing bacteria. Mrs. Dubnau continued her studies of the genetics of R3ND, an agent conferring resistance to tetracycline, streptomycin and sulphonamides (Report 1963). The agent was transferred by conjugation to *Proteus mirabilis*, a bacterium whose genetic deoxyribonucleic acid (DNA) differs in specific gravity from that of *S.typhimurium*. Dr. S. Falkow, of Washington, D.C., found that the DNA of *Proteus* given R3ND contained a new component with the specific gravity of *S.typhimurium* DNA; this material, the DNA constituting the R3ND agent, made up some 5% of the total bacterial DNA. In continuation of her studies on the transduction of resistance traits, Mrs. Dubnau used the *col*-factor system (Reports 1960–1963) to hybridize drug-sensitive LT2 stocks with stocks given R3ND resistance traits by transduction. The resistant transductants chosen for use as females were inferred to carry a defective form of the gene-set of the transducing phage (i.e., to be defectively lysogenic). In each cross, drug-resistance and defective lysogeny segregated together, closely linked to *pro*, a gene whose site on the chromosome is at or near the point of attachment of prophage P22 in lysogenic bacteria (Report 1963). Thus in at least some resistance-trait transductants DNA derived from the R agent has become associated, perhaps by exchange, with the DNA of the transducing phage, and in consequence fixed to the chromosome at the prophage attachment site; this would account for the failure of such transductants to transmit their resistance traits by conjugation (Report 1963). Cell-free filtrates of cultures of some of the defectively lysogenic resistance-trait transductants transmitted drug resistance at a low rate, but only to bacteria able to absorb phage P22; probably because such transductants liberate defective phage particles, not able to establish normal phage infection but able to confer drug-resistance and defective lysogeny.

Genetics of Flagellar Characters. In *S.typhimurium* several other genes affecting flagellar characters are closely linked to *H1*, the gene which determines the antigenic character of phase 1 flagella (Reports 1959–63). Mrs. Pearce made transductional crosses of strains carrying: a "curly" *H1* gene, determining production of short-wave length, functionally deficient, phase 1 flagella; *Ah1*$^-$ preventing the production of phase 1 flagella; and *nml*$^-$, which prevents the appearance of N–methyl–lysine in flagellar protein. Her results establish the order *nml–Ah1–H1*. Crosses involving non-flagellated mutants (*fla*$^-$) have not permitted unambiguous mapping of the *fla* genes (Report 1960, 1961) in relation to *H1*. Mrs. Pearce used the inhibitory effect of antisera on the production of "trails" (by abortive transduction of motility Report 1955) to infer the flagellar antigens of rare bacteria carrying two different alleles of *H1*. Such bacteria if they carry *Ah1*$^-$ do not manifest the antigen determined by the *H1* gene immediately adjacent to the *Ah1*$^-$ gene; only the *H1* gene adjacent to the *Ah1*$^+$ gene on the other chromosome fragment is expressed. This observation supports the hypothesis that *Ah1* is an "operator" gene, which switches on or off the protein-specifying activity of its contiguous *H1* locus, probably in response to a cytoplasmic repressor substance.

Mapping of a Repressor Locus. It is supposed that in bacteria specific "repressor" genes determine the production of cytoplasmic repressor substances, each of which after reaction with a relevant metabolite acts on an "operator" gene, causing it to switch on or switch off the activity of its adjacent genes in a manner appropriate to the prevailing metabolic conditions. Dr. P. Hartman, of Johns Hopkins University, who has mapped the *his* region (determining the synthesis of the enzymes needed for making histidine, Report 1963) during his visit to the Unit used the *col* and F'13-*lac* systems of hybridization to map a *his* repressor locus. The mutants used make large amounts of the *his* enzymes even when histidine is supplied *ab externo*. Recombinants with this character could be recognised because of the characteristic appearance of their colonies on certain media. The *his* repressor locus was found to map between *ile* and *rha*, that is remote from the *his* genes controlled by its product; this control being effected by a cytoplasmic product, there is presumably no necessity for the repressor gene to be adjacent to the repressible group of genes.

Genetics of Mouse Virulence in Salmonella typhimurium. Strain LT2, much used in genetic work on *S.typhimurium*, differs from most S ("smooth") strains of this species by its very low virulence for mice. Mr. Subbaiah and Professor Stocker investigated the genetic determination of this character by crossing strain LT2 with a strain of high mouse virulence. A cross of an LT2 line as donor and a virulent line as recipient yielded four recombinants in which, taken together, the whole length of the LT2 chromosome was represented. Three of them had the virulence of the virulent parent; but the fourth one, in which the chromosome region *cys-xyl* was derived from LT2, was no more virulent than strain LT2. This suggests that the low virulence of strain LT2 is determined by a gene, or genes, only in the *cys-xyl* region.

Genetics of Serum-Sensitivity in S.typhimurium. Most S ("smooth") strains of *S.typhimurium* are mouse-virulent and resistant to the killing effect of the antibody plus complement present in, for instance, normal human serum; some serum-sensitive mutants are also resistant to penicillin. Dr. R. J. Roantree, of Stanford, has established that such mutants have a lesser virulence for the mouse. During his stay in the Unit, Dr. Roantree is investigating the genetics of these correlated changes. One class of such mutants proved to be R ("rough"); in a more interesting class, the mutants are S. Dr. Roantree prepared nutritionally exacting mutants in a mouse-virulent, serum-resistant strain, and obtained serum-sensitive, but still S, mutants in some of them. He

is now trying to map the (presumed) locus for serum-sensitivity by appropriate crosses.

Genetics of Somatic Antigens in Salmonella. The O or somatic antigenic constitution of S *Salmonella* is determined by the chemical composition of sugar-containing side-chains on the somatic lipopolysaccharide. Investigation of the genetic control of these various O antigens has in the past been thwarted by the infertility of the necessary crosses between *Salmonella* of different species (e.g. Report 1961, 1962). Dr. Helen Makela, of Helsinki, had developed Hfr and F' forms (" males ") of *S.abony* and *S.montevideo* which permit inter-species hybridization. *S.abony*, like *S.typhimurium*, has O antigens 4, 5, 12 and mannose, rhamnose, and abequose as S-specific sugars; *S.montevideo* has 0 antigens 6, 7 and mannose as its only S-specific sugar. During her stay in the Unit Dr. Makela is investigating the inheritance of the 0 antigens in crosses between these species. The gene(s) determining 0 antigen 4 (reflecting abequose at the end of a side-chain) and the gene(s) for 0 antigen 7 (chemical basis unknown) map in the same part of the chromosome, near *aro* and *his*. These crosses have yielded some S recombinants of 0 antigenic constitution different from those of either parent; and also R (rough) and " semi-R " recombinants.

BACTERIAL PHYSIOLOGY

Somatic Polysaccharides of S and R Salmonella. Mr. Subbaiah and Professor Stocker continued their investigations, in collaboration with Drs. I. Beckmann and O.Westphal at Freiburg, of the chemical and serological properties of the somatic lipopolysaccharides and polysaccharides of genetically investigated R (rough) mutants of *S. typhimurium* strain LT2 (Report 1962, 1963). Dried bacterial crops of further R mutants (many of them grown at the Microbiological Research Establishment, Porton, through the kind co-operation of Dr. D. W. Henderson) were examined at Freiburg and in the Unit. R mutants of both the *rouA* class (resulting from mutation near the *ile* locus on the bacterial chromosome) and of the *rouB* class (due to mutation in the *try—his* region) on phenol extraction yielded typical R macromolecular lipopolysaccharides, i.e. containing glucose, galactose, heptose and glucosamine but lacking the mannose, rhamnose and abequose found in S lipopolysaccharide. Serological analysis by techniques developed by Dr. Beckmann showed that the R lipopolysaccharides of *rouA* mutants had antigen RI, those of *rouB* mutants antigen RII, these being antigens previously recognised by Dr. Beckmann in the lipopolysaccharides of genetically unanalysed R variants of *Salmonella* of many different O groups. The phenol extracts of six *rouA* mutants also yielded material of relatively low molecular weight containing the S-specific sugars mannose, rhamnose and abequose (and also glucose, galactose and glucosamine, but not heptose), and reacting with anti-S sera in precipitation and passive haemagglutination tests—that is similar to the " S-specific " non-sedimentable fractions found in extracts of the first two *rouA* mutants examined (Report 1962); by contrast none of six *rouB* mutants yielded such material. R mutants were tested by Drs. H. and K. Nikaido, in Boston, for the enzymes concerned in most of the reactions involved in the synthesis of the " activated " forms of glucose, galactose, mannose, rhamnose and abequose, since these sugar nucleotides are believed to be the sources of the sugars in the synthesis of somatic polysaccharide. Of six *rouA* and six *rouB* mutants examined, all had normal enzymic activities in all the reactions, except one *rouB* mutant, found to be blocked in the conversion of thymidine diphospho-4-keto-6-deoxyglucose to thymidine diphosphorhamnose. An hypothesis to account for all these observations proposes that all *rouB* mutants are unable to synthesize the rhamnose-containing S side-chain through inability to transfer rhamnose—either because they cannot make activated rhamnose (the proven defect in one *rouB*

mutant) or through lack of a presumed enzyme for the transfer of rhamnose from activated rhamnose; and that their failure to build up the S side-chain on its normal point of attachment on the " core " lipopolysaccharide leaves exposed a terminal unit X which determines antigen RII. The *rouA* defect is inferred to be in the attachment to the core of unit X, to which the S side-chain is normally attached: resulting in exposure of some underlying unit determining antigen RI; and in failure to attach normally synthesized S side-chain material to the core, with consequent accumulation of low-molecular-weight S-specific material. This hypothesis predicted that a strain with both *rouA* and *rouB* lesions would not accumulate non-sedimentable S-specific material and would produce RI lipopolysaccharide. A putative *rouA rouB* recombinant had this predicted unique combination of characters.

Chemistry of Flagellar Protein. Dr. McDonough continued his studies of the chemical composition of *Salmonella* flagellar proteins, and extended them to the flagella of several different, naturally-occurring, antigenic types. An unexpected, and unwelcome, finding was the presence in many purified flagellins of up to 10% of carbohydrate; unexpected, because previously examined flagellins from strain LT2 contain only about 0.3% of carbohydrate. It is suspected that this carbohydrate is somatic polysaccharide (or lipopolysaccharide) tightly bound to flagellin. Its presence did not affect the proportional amino-acid composition of a flagellin. The amino-acid composition of the hydrolysed flagellins was determined by use of the automatic amino-acid analyser (Report 1962). All the flagellins tested had the general characters of flagellar protein previously noted (Report 1962); three antigenically related flagellins of the *g* series (*g,m; g,p;* and *g,s,t*) lacked the small amount of histidine found in all the other flagellins tested, and were otherwise very similar to each other. Flagellins of type *i* from three unrelated strains gave identical or nearly identical analyses; serologically unrelated flagellins differed in abundance of several amino-acids. In the flagellins of bacteria lacking the *nmI*$^+$ gene determining the presence of N-methyl-lysine in flagellar protein (Report 1961, 1962) N-methyl-lysine was quantitatively replaced by lysine; this accords with the hypothesis that the N-methyl-lysine in flagellin results from the action of a methylating enzyme on lysine units during or immediately after their incorporation into the polypeptide chain (Report 1962). The ratio of lysine to N-methyl-lysine was constant for a given antigen; but varied in serologically distinct flagellins. Of six lysine-containing peptides isolated from tryptic digests of *i* flagellin, three contained both lysine and N-methyl-lysine, in approximately equal amounts; three others contained lysine but lacked N-methyl-lysine. It thus appears that some lysine radicals in the polypeptide chain are not susceptible to methylation. The content of some of the minor amino-acid components in flagellin indicate a (minimum) molecular weight of 36,000—37,000.

The constancy of the amino-acid composition of flagellin of a given antigenic type, even when the gene determining this antigen has been transferred into bacteria of a different species, confirms the hypothesis that the flagellar antigen-determining genes specify the whole primary structure (amino-acid sequence) of the polypeptide chain of flagellar proteins.

Capsule Formation by Bacillus anthracis. The capsule of virulent anthrax bacilli consists entirely of polymerized D-glutamic acid. It is invariably formed in the animal body but on nutrient agar is formed only if this contains either bicarbonate or serum and is incubated in concentrations of CO_2 higher than those present *in vivo*. Dr. Meynell, working with Dr. Elinor Meynell of the Wright-Fleming Institute, found that capsulation requires a critical concentration of bicarbonate ion in the medium. This concentration is about 0.003 M in serum agar and is produced by about 5% atmospheric CO_2, provided steps are taken

to prevent the pH falling below 7.2 (since the lower the pH, the more CO_2 is required to produce a given bicarbonate ion concentration in the medium). Capsulation can thus be produced in the laboratory by physiological concentrations of CO_2. Serum apparently acts by binding an inhibitor of capsule formation, since activated charcoal has the same effect. In bicarbonate agar, the critical bicarbonate ion concentration is about 20 times greater than in serum agar, and capsulation is thought to occur because the organisms themselves bind the postulated inhibitor, as dead organisms added to nutrient agar encourage capsule formation. This hypothesis was also supported by microscopical observation, which showed that, whereas organisms growing on serum or charcoal agar became capsulated after 1—2 hr. incubation, those on bicarbonate agar remained uncapsulated for many hours until the bacteria had become very dense. The inhibitor occurring naturally in nutrient agar may be a fatty acid since both lipid material extracted from agar and pure fatty acids inhibited capsulation.

Bacterial Multiplication in Infected Animals. Intestinal infections by *Salmonella* can be produced in mice which have been given streptomycin by mouth, probably because the streptomycin kills the gut flora, whose metabolism normally renders the gut unsuitable for the multiplication of *Salmonella* (Report 1963). This undesirable effect of antibiotics also occurs in human patients, who sometimes suffer from severe intestinal infections by *Staphylococcus aureus*. Dr. Meynell therefore tried to reproduce this infection by giving streptomycin-resistant staphylococci to streptomycin-treated mice as well as to untreated mice. They did not proliferate in untreated mice; *in vitro* their growth was found to be inhibited by the oxidation-reduction potential and the concentration of fatty acid present in the normal mouse caecum. Nor did the staphylococci proliferate as much as salmonellae in streptomycin-treated mice; and, in many mice, only Gram-negative species like *Proteus* were found, even though staphylococci were not detectably inhibited *in vitro* by the oxidation-reduction potential and fatty acid concentrations obtaining in the caecum after streptomycin treatment. Presumably staphylococci failed to proliferate in such mice either because they divided less rapidly than they were excreted in the faeces, or because they were displaced by *Proteus* and other bacteria derived from the environment.

Dr. Meynell continued his work on the kinetics of microbial infection by studying the times required for a response to occur in animals each inoculated with the same dose of a disease-producing organism. The distributions of response times in various systems have sometimes been described as discontinuous, as if the response time of some animals was longer than that of the rest, perhaps because they acquired immunity before they had time to respond. Dr. Meynell showed that most of the reported instances were artefacts, derived from a misleading calculation; but some were certainly valid and therefore yielded valuable information about the time at which acquired immunity became manifest, e.g., with mice, discontinuity occurred 40 hr. after injection of a dose of 100 pneumococci.

ABACTERIAL URETHRITIS IN MAN AND RELATED DISEASES

Dr. Lemcke continued her serological investigation of the *Mycoplasmataceae* (pleuropneumonia-like organisms—PPLO). A gel-diffusion precipitin method provided an alternative to the complement fixation test (CFT) for comparing and identifying different species. A previous observation that strains of the so-called "human genital type 2" were identical with rat polyarthritis strains (Report 1962) was confirmed. Also, a serologically homogeneous group of tissue-culture contaminants unrelated to any known species of *Mycoplasma* (Report 1962) was found both by the CFT and gel-diffusion to belong to a newly-recognized type occurring in the human mouth and throat. This suggests the

possibility of tissue cultures being contaminated by workers during transfer and maintenance.

As a preliminary to an investigation of the antigenic structure of certain species, the gel-diffusion method was used to resolve the constituents of the complex antigenic mixture contained in disrupted suspensions of organisms. For example, all the strains examined of the common human genital organism *M.hominis type I* gave the same six precipitin lines, a finding contrary to the view that this species is antigenically heterogeneous. Other types or species of human origin viz. *M.fermentans*, two serologically distinct oral types and a type from umbilical dermatitis also reacted with *M.hominis type I* antisera, but the precipitin lines were not the same as those given by the homologous antigen. It appears that different species of *Mycoplasma* possess similar but not identical antigens.

With a view to the separation and purification of antigens for use in the serological diagnosis of *Mycoplasma* infections in man, work on the separation of the cell contents from the cell membrane of *M.hominis type I* was begun. Ultrasonically treated suspensions of the organism on centrifugation yield a serologically active supernatant and a serologically inert sediment of cell debris.

As part of an investigation by Professor Barrie Jones (Institute of Ophthalmology) of the aetiology of neonatal ophthalmia, material from the eyes of neonates and from the genital tracts of their parents was cultured for *Mycoplasma*. None was isolated from the eyes. This survey continues.

VIROLOGY

TRACHOMA AND INCLUSION BLENNORRHOEA (CONJUNCTIVITIS)

Growth Characteristics and Toxicity. The trachoma/inclusion conjunctivitis (TRIC) agents contain a toxin that kills mice when injected intravenously; American workers report that the lethal effect can be prevented by immunization, and that two immunological groups can be recognized by cross-protection tests. The finding by Dr. Reeve, Dr. Taverne and Dr. Blyth that this method gives variable results suggested investigation of toxin production; it was also desirable to ascertain whether strains that kill chick embryos comparatively quickly (Report, 1963) contain more toxin than do slow-killing strains. Two fast-killing and two slow-killing strains were grown in chick embryos, with daily assays of infectivity (in HeLa cells), total particle count, and toxin (in guinea pig skin and in mice); the maximum toxin/particle ratio attained was the same for all 4 strains, suggesting that this factor does not govern the ability to kill chick embryos quickly. Although dose response curves seemed to imply that all strains of TRIC agent multiply at the same rate in the yolk sac (Report, 1963), direct measurement of growth rates by total particle counts suggested that the faster-killing strains in fact multiply more rapidly, and perhaps for this reason kill the embryo sooner. This might also explain the emergence of the mutant strains previously described, since in a mixed population particles multiplying faster would have a selective advantage.

In terms of lethal dose, fast-killing strains reach the same titre in eggs incubated at 35° C. or 37° C., whereas slow-killing strains do not multiply at the higher temperature; this finding is not due to gross differences in the rate of inactivation at 37° C. The relationship between growth rate in chick embryos, ability to multiply at 37° C., and ability to form inclusions in cell cultures is now under study.

Cell Culture Experiments. As well as maintaining the Department's supply of cell cultures, Mrs. Hart (née Fraser) investigated adsorption of TRIC agent to HeLa cells, and methods for storing virus grown in them. Suspended cells adsorb virus more effectively than monolayers, and for a given virus/cell ratio,

efficiency of adsorption is increased by decreasing the amount of suspending fluid.

TRIC agent grown in HeLa cells is highly labile; Mrs. Hart examined the influence on viability of slow and rapid freezing in the presence of various additives. It was best preserved by adding dimethyl sulphoxide (10% v/v) to infected cell suspensions, cooling them slowly to –40° C. and then rapidly to –70° C. Preliminary results suggest that infectivity is well maintained on subsequent storage at –70° C.

Mrs. Day found that TRIC agents attain the same titre in primary monkey kidney cells as in HeLa cultures, provided that the cells are healthy and undergoing division.

Serological Studies. Miss Graham extended her researches on the serological relationship of TRIC agents (Report, 1963). A strain of trachoma (SA2) that previously failed to elicit neutralizing antibody in rabbits was induced to do so by intravenous injection of a high concentration of elementary bodies. Cross-neutralization tests in HeLa cells with this and other antisera demonstrated close relationship between trachoma strains TE55 and SA2, and inclusion blennorrhoea strains LB1 and LB4. Neutralizing antibody to lymphogranuloma venereum agent (LGV) has still not been induced in rabbits, but this virus is neutralized by antisera to several TRIC agents. By contrast, 3 TRIC agents are neutralized by serum from a patient with lymphogranuloma to a significantly lesser degree than the JH strain of LGV.

Neutralization tests were also made in mice by Bernkopf's method. Consolidation of the lungs is induced by intranasal instillation of all 5 TRIC agents so far tested, and is inhibited by preliminary treatment of the inoculum with antiserum. Although this method is less sensitive than the cell-culture technique, it permits neutralization tests on strains that do not readily form inclusions in HeLa cells; but it too failed to reveal serological differences among the strains so far tested. During these experiments, it was noted that serial passage of TRIC agents in mouse lung did not enhance the pulmonary infection, by contrast with what has been reported of mouse pneumonitis agent, a related micro-organism.

Trachoma Vaccine. Dr. Collier and Dr. Blyth continued to test the immunogenicity of experimental vaccines in baboons. Hitherto, immunity to conjunctival infection had been induced by TRIC agent grown in the chick embryo yolk sac; it has now been shown that the LB4 strain retains its immunizing property after 11 consecutive passages in cell culture, a finding of potential importance in relation to vaccine production.

Although use of a mineral oil adjuvant seems to prolong the complement-fixing antibody response, further experiments confirmed doubts about the efficacy of such vaccines in preventing conjunctival infection in baboons (Report, 1963).

The minimum dose of cell-grown antigen needed for maximum protection of baboons was determined; and it was found that inadequately immunized animals tend to react more severely to conjunctival challenge than do normal controls.

In collaboration with Messrs. Pfizer Ltd., and with the advice of Mr. Vallet, Dr. Collier showed that TRIC agent irradiated with ultraviolet light is inactivated about 7 times more rapidly than a suspension of vaccinia virus of similar turbidity.

Mrs. Day compared the keeping properties of live antigens suspended respectively in aqueous solution and in mineral oil adjuvant, an important consideration in the conduct of field trials. Both maintained full viability for 4 days at 4° C. but thereafter lost titre; the rate of loss was much faster in the mineral oil suspension.

Investigations in West Africa. Under Dr. Collier's direction, the Medical Research Council's Trachoma Research Unit mounted a field trial of a prophylactic trachoma vaccine, using a live antigen prepared by Mr. Turner in the Institute's laboratories at Elstree.

The Unit also continued its study of TRIC agent infection in young infants (Report, 1962). Seventy-nine babies were examined monthly from birth for periods up to 2 years; in 5, two of whom were twins, TRIC agent infection of the eyes was diagnosed in the neonatal period, and the virus was isolated from the genital tracts of 3 of the 4 mothers. In 3 of these babies, the course of infection resembled trachoma more than inclusion conjunctivitis. In 11 other infants, TRIC agent of the eyes was first confirmed at times varying from 3 to 24 months after birth; in them, the disease often resembled inclusion conjunctivitis more than trachoma. These findings support the view that there is no clear demarcation between these syndromes, and that some TRIC agents existing in the genital tract cause lesions characteristic of trachoma when transmitted to the infant eye at birth.

Conjunctival Strains of the Genus Haemophilus from Trachoma Patients. Miss Sampson completed the main part of the investigation of the 500 cultures isolated from trachoma patients at Marakissa in the Gambia. Three were *Haemophilus parainfluenzae*—confirming the finding of American workers that this organism is occasionally found in the eye. All other strains resembled *H.influenzae* or *H.aegyptius* (Koch-Weeks bacillus). It has been assumed that indole-positive strains were *H.influenzae* and haemagglutinin-positive strains *H.aegyptius*. However, the haemagglutinin proved to be a much less stable character than indole production. The indole test is a more reliable method of distinguishing *H.influenzae* (positive) from *H.aegyptius* (negative).

According to cultural characteristics (though these were not as clear as in the American reports) and the distribution of the ten serological types recognised, haemagglutinin-positive, indole-positive strains more closely resembled type strains of *H.influenzae*, and the haemagglutinin-negative indole-negative strains, type strains of *H.aegyptius*. About equal numbers of influenzae-like and aegyptius-like strains were found in the non-trachomatous patients, whereas the aegyptius-like strains predominated significantly in the trachomatous patients; it is not known, however, whether the presence of these organisms predisposes to trachoma or influences the cause of the disease. The serological sorting of the strains by the gel-diffusion technique was rapid and easy, and gave consistent results. The serological types did not correspond exactly with the biochemical types, nor was any serological type closely associated with any particular clinical stage of the disease.

SHOPE FIBROMA

Dr. Placido de Sousa continued his study of Shope fibroma virus in rabbits, with reference to the influence of interferon and carcinogens on tumour formation. Interferon, a viral inhibitor produced by virus-infected cells, was prepared in rabbit kidney cultures infected with influenza virus. Treatment of cell cultures with high concentrations of interferon prevents the cytopathic action of fibroma virus added subsequently; this system can also be used to assay the interferon itself. Similarly, injection of interferon into rabbit skin diminishes the size of tumours formed by Shope virus injected 24 hours later.

It has been reported that crude tar preparations enhance the tumours induced by fibroma virus; on the other hand, such tumours can be completely suppressed by prior immunization. Dr. de Sousa found that treatment with methylcholanthrene and dimethylbenzanthracene does not result in tumour formation in immune rabbits subsequently challenged with Shope virus, although

It depresses the amount of neutralizing antibody induced by immunization. In non-immune animals, carcinogen treatment enhances the size of tumour and prolongs the regression period, but does not affect the small antibody response to the challenge virus. Dr. de Sousa is now examining the possibility that carcinogens enhance tumour formation by inhibiting interferon production.

VACCINIA AND VARIOLA VIRUSES

Virus Inactivation. In collaboration with Dr. P. F. Benson of the Paediatric Research Unit, Guy's Hospital Medical School, Dr. Kaplan continued his study of a non-infectious smallpox vaccine. About 20 one-year-old children each received 2 doses of the vaccine. All had measurable circulating antibody 3 weeks after the second injection. The significance of this finding in relation to protection against complications of live virus vaccination and immunity to smallpox is still unclear. Further studies are planned.

Mr. Turner studied the mechanism of inactivation of vaccinia virus by ascorbic acid; and obtained some evidence that the immunogenicity of such inactivated virus was not completely destroyed. With Dr. Kaplan, he is investigating the inactivation of viruses by the photodynamic action of dyes. The results so far obtained are not consistent with the generally accepted theory that the site of inactivation is in the virus nucleic acid.

Immunity to Vaccinia and Variola Viruses. Dr. Murray's studies have shown differences in reactivity between sera of early and late bleedings from animals infected with vaccinia virus. He is continuing the investigation of these differences by studying the kinetics of inactivation in whole sera, as well as various chromatographically separated serum fractions.

At the request of the Division of Biological Standards of the National Institute for Medical Research, Dr. Kaplan assayed for neutralizing antibody several sera and pools of sera from patients convalescent after smallpox. The material so tested is intended to serve as an international reference preparation of antibody against variola virus, to be established in due course by an international collaborative assay.

Mr. Robinson continued his study of methods of purifying large amounts of vaccinia virus. The method promising the best yields of purified virus from large volumes of infected fluids depends on the partition of the components of a fluid into the different phases of a mixture of two high polymer solutions of different viscosity and specific gravity. The most successful system is a mixture of polyethylene glycol and dextran sulphate. The virus concentrates in the dextran sulphate phase, in a considerably purified state in terms of infectivity per mg. nitrogen. It is difficult, however, to separate the virus from the dextran sulphate without recourse to centrifugation.

Mr. Turner and Mr. Robinson investigated the purification of suspensions of vaccinia virus by organic solvents. Diethyl ether is the solvent least damaging to virus infectivity. This is not unexpected, since vaccinia virus has for long been regarded as "ether resistant." This resistance, however, is a function of the pH, ionic strength and initial protein content of the suspending fluid.

IMMUNOLOGY AND EXPERIMENTAL PATHOLOGY

SEROLOGICAL IDENTIFICATION OF BLOOD MEALS

The serological identification of blood meals of tsetse flies has continued. In collaboration with workers at the Department of Veterinary Services, Salisbury, Southern Rhodesia, the effect on the bionomics of the tsetse fly of the elimination of preferred hosts is being studied in a large experimental area. Investigations of the anthropophilic characters of different *Anopheles* were

continued in collaboration with the World Health Organization, Malaria Eradication Division.

SCORPION VENOMS

Mr. Stone continued his investigations on the antigens of scorpion venoms. At least two common antigens, as shown by gel-diffusion precipitin tests and absorption tests, are present in the venoms of four species of scorpions. These antigens have similar electrophoretic mobilities in agar. The lethal effect in mice of the venom of *Androctonus australis* and *Androctonus crassicauda* is neutralized by both homologous and heterologous antisera. The characters and nature of these common antigens are being investigated.

ANTITOXIN PRODUCTION

Refinement of Therapeutic Antitoxins. When antitoxins are treated with pepsin at pH 3.2 there is a fall in their antitoxic activity. Using purified antitoxic β-globulin isolated from horse serum (Report, 1963), Dr. Dolby found a very rapid fall to about 90% of the original activity, followed by a slower fall to 70–75%, and a concomitant decrease in the flocculation time of the antitoxin with toxin. He is now correlating these changes with alterations in the electrophoretic and chromatographic properties of the globulins.

Dr. Dolby also used pepsin and papain, in combination with a reducing agent for sulphydryl groups, to split the purified β-globulin, and isolated fragments by chromatography, in order to compare their properties with those of γ-globulin split in the same way. Preliminary tests revealed a close similarity in chromatographic properties between some of the fragments of the β- and γ-globulins, and a comparison by immunological methods is in progress.

Highly purified antisera for experimental purposes have been produced for a number of years by the pepsin treatment of precipitates from diphtheria toxin-antitoxin flocculation. An attempt to purify antisera to tetanus toxin in the same way gave very low yields, and a study of the conditions required to improve yields was started.

ANAPHYLAXIS

An Eosinophil-stimulating Factor in Anaphylaxis. Dr. Parish is attempting to isolate the factor causing the sudden rise in the number of eosinophils in the peripheral blood and some organs in anaphylaxis. This might provide information about the processes of anaphylaxis and its mediators: and might have a forensic application in the post-mortem body in detecting anaphylaxis as a possible cause of death.

It was established that lung of a guinea-pig dying of anaphylaxis implanted in the peritoneal cavity of a normal guinea-pig in 24 hours usually resulted in an increase in the number of eosinophils in the blood; normal lung was ineffective.

Slow freezing to –25° C. or –70° C., with subsequent thawing, largely destroyed the stimulating factor in fresh anaphylactic lung.

The possibility that part of the rise in the eosinophil count in the recipient's blood was due to eosinophils in the donor's lung, was tested by extracting pieces of lung in tissue culture medium and separating cells and soluble extract by centrifugation. Preliminary tests suggest that the stimulating factor is present in the soluble extract and that the cells cause little or no rise in eosinophils of the recipient.

HUMAN INFERTILITY

Local Antibody Formation in the Uterus and Antibody Cytotoxic to Spermatozoa in Cervical Mucus. In collaboration with the staff of Addenbrooke's Hospital

Cambridge, and the Blood Group Research Unit, Dr. Parish investigated the presence and specificity of antibodies in cervical mucus to test the possibility that antibody could be formed locally in the human uterus in the absence of systemic stimulation, and that infertility could result from the local formation of antibodies cytotoxic for spermatozoa.

Saline extracts of cervical mucus at any phase of the menstrual cycle contained γ-globulin, identified by immunoelectrophoretic and gel-diffusion precipitin tests. Natural anti-A or anti-B blood group antibodies were found in some samples. Immune type anti-A or anti-B antibody was also found, and in two group O women an immune type anti-A was present in the cervical mucus and absent in the serum. Antibody to the H25 antigen of *Escherichia coli* occurred in the cervical mucus but not in the serum, of three women.

Cytotoxic antibody to spermatozoa was found in the cervical mucus but not in the serum, of three women. It proved to be a complement dependent 7S γ globulin lethal to spermatozoa of any ABO blood group and toxic to neither macrophages nor granulocytes. Serum samples containing immune type anti-A or anti-B antibody proved to be harmless to spermatozoa of the corresponding blood groups, so the cytotoxic effect was independent of the corresponding blood group antigens.

MECHANISMS OF INFLAMMATION

Vascular Changes in Injury. Dr. Wells continued his investigation of the changes in blood and lymphatic vessels of the mouse ear induced by traumatic injury. Of several anaesthetics tested, methoxyfluorane proved to be without the detectable depressant action that masks the reaction in animals otherwise anaesthetized. Stroke injury sufficient to induce an immediate blanching of the vascular bed lasting 20–50 seconds was adopted as the standard injury. The blanching is followed by increased permeability of the small blood vessels to dyed plasma proteins and increased deposition of carbon particles on the lymphatic vessel wall after intralymphatic injection of a carbon suspension. These changes occur over an area of 1.5 mm. on each side of the stroke, and persist for 40 minutes, leaving an area of damage immediately under the line of the stroking that persists for up to 6 hours. The 40-minute phase appears to be due to the liberation of histamine and serotonin, since it is partly inhibited by the antihistaminic triprolidine, and by the serotonin antagonist Bol 148, given both locally and systemically.

Dr. Wells and Professor Miles continued their exploration of the anatomical site of vascular injury in the biphasic reactions—immediate and delayed—that characterize local injury by heat, bacterial exotoxins and bacterial infection; using permeability to circulating dye and trapping of intravenously injected carbon particles in the vessel wall as indices of damage. They established, both in the rat cremaster muscle and in the skin of mice, that the immediate phase in *mild* thermal injury was associated solely with change in the small venules, and the delayed phase largely with damage to the fine capillaries. In *moderate* thermal injury, an attempt was made to associate the duration of the delayed phase with the subsequent development, 6–24 hours after injury, of vascular stasis and tissue necrosis; there was no obvious association.

In the rat cremaster, Dr. Wells found that the pattern of injury after *Cl.welchii* alpha toxin closely resembled that after thermal injury. With *Cl.welchii* iota toxin, however, both fine capillaries and the larger venules were affected in the delayed phase, the venular injury persisting for up to 24 hours. *Staph.aureus* infection induced a prolonged venular phase (3–5 hours) followed by a largely capillary phase lasting 18–24 hours.

Since all substances of endogenous origin that increase vascular permeability

so far tested—histamine, serotonin, bradykinin, serum kininogenase, globulin permeability protease, and the recently described lymph-node permeability factor of Willoughby and his colleagues—induce only venular damage, the participation of any of them in the major, delayed permeability phase of inflammation is in doubt. Attempts to induce capillary changes in thermally injured tissue after the subsidence of the venular phase and before the onset of the capillary phase, by the injection of these substances, were uniformly negative.

A specimen of a cysteine-dependent protease kindly supplied by Dr. H. Hayashi of the Kumamoto University Medical School was found by Miss Mason to induce only an immediate phase reaction in the guinea-pig. This protease occurs in increased amounts at the site of Arthus reactions in the rabbit at the height of the delayed phase, and was found by Dr. Hayashi to induce a delayed phase of increased permeability in the rabbit. Miss Mason is repeating his work with Arthus reactions of the guinea-pig.

The Relation of Permeability Globulins to other Plasma Proteins. Miss Mason examined the relation of PF globulin to kininogenase in human plasma. As with guinea-pig plasma (Report 1963) the globulin PF appears to be activated first when human plasma comes into contact with glass, and the PF in turn activates kininogenase (kallikrein), which then releases kinins from the serum kininogens. In human plasma, the activation of the PF is mediated by activated Hageman factor. It is not yet clear whether PF preparations owe their capacity to activate kininogenases to traces of Hageman factor; though quantitative considerations suggest that they do not. The investigation continues.

In a search for models of substances that might activate the kinin system *in vivo*, Miss Mason examined a series of polysaccharides and polysaccharide esters both for capacity to activate permeability globulin in dilute guinea-pig serum, and for permeability effect in guinea-pig skin. Chondroitin sulphate was inert. Levan, dextran and sodium alginate were relatively inactive as permeability factors but had some potency as activators of serum. Low molecular weight dextran sulphates were active both as permeability factors and as activators.

BIOCHEMISTRY

THE HUMAN BLOOD GROUP SUBSTANCES

Mrs. Rege, Dr. Painter, Dr. Watkins and Professor Morgan continued their work on the chemical and serological characterization of the oligosaccharides formed from the A, B, H and Le^a blood-group specific substances by partial hydrolysis by polystyrene sulphonic acid. Ten di- or tri-saccharide units from both A and B substances, and seven from each of the H and Le^a substances have now been identified (see Reports 1961, 1962). As a result, tentative conclusions about the sequence of the sugar residues in the main carbohydrate structures in each group substance are possible. It is highly probable that a specific substance from an A or B individual contains at least two different serologically active carbohydrate chains, as follows:

A-substance

(1) α-GalNAc-(1→3)-β-Gal-(1→3)-β-GNAc-(1→3)-β-Gal-(1→3)-GalNAc
(2) α-GalNAc-(1→3)-β-Gal-(1→4)-β-GNAc-(1→3)-β-Gal-(1→3)-GalNAc.

B-substance

(1) α-Gal-(1→3)-β-Gal-(1→3)-β-GNAc-(1→3)-β-Gal-(1→3)-GalNAc
(2) α-Gal-(1→3)-β-Gal-(1→4)-β-GNAc-(1→3)-β-Gal-(1→3)-GalNAc.

The following chains are probably present both in H and Le^a substances:

(1) β-Gal-(1→3)-β-GNAc-(1→3)-β-Gal-(1→3)-GalNAc
(2) β-Gal-(1→4)-β-GNAc-(1→3)-β-Gal-(1→3)-GalNAc.

These have no structural differences associated with H and Le^a specificities but, as L-fucose, which is believed to play an important part in H and Le^a specificity, is not a component of the oligosaccharides isolated, owing to its ready removal during mild acid hydrolysis, it is not surprising that they have no H and Le^a specificity. The structures suggested for the chains in A, B, H and Le^a substances are, owing to the absence of L-fucose and sialic acid units, incomplete compared with their counterpart in the intact or native blood-group substance. The L-fucose and sialic acid units are believed to be attached as acid-labile, branching units to the sugars of the main carbohydrate chain. Means to recover these oligosaccharides with their L-fucose and sialic acid units intact are being devised.

Dr. Gompertz and Dr. Watkins continued their experiments on the biosynthesis and interconversion of nucleotide sugars in order to prepare possible donor substrates for the biosynthesis of blood-group substances. The UDP-galactose-4-epimerase in extracts of *Trichomonas foetus* (Report 1963) was more thoroughly characterized and conditions determined for its use in the assay of UDP-galactose. Methods for the enzymic synthesis of UDP-galactose were investigated and finally radioactive labelled UDP-galactose-C^{14} was prepared by a two-stage process starting from D-galactose-C^{14}.

The presence of blood-group active substances in stomach mucosa suggests that these mucopolysaccharides are synthesized by the cells of the mucosal lining. The stomachs of man and of baboons, all from donors of known ABO blood groups, were homogenized and the homogenates were examined for enzymes transferring galactose-C^{14} from UDP-galactose-C^{14} to simple sugars and possible precursor mucopolysaccharides. There was evidence in both A and B stomach preparations of galactosyl transferases which add D-galactose-C^{14} to *N*-acetyl-D-glucosamine or β-*N*-acetyl-D-glucosaminoyl-(1→4)-*N*-acetyl-D-glucosamine to give labelled compounds with chromatographic mobilities corresponding to di- and trisaccharides, respectively. No evidence was obtained for the addition of D-galactose-C^{14} to unlabelled galactose or to L-fucose. When transfer of D-galactose-C^{14} occurred, the degree of incorporation of radioactivity was small, but the results are encouraging since units containing galactose joined to *N*-acetylglucosamine are known to occur in the carbohydrate chains of the blood-group substances.

Mr. Harrap continued his investigation on the purification and characterization of the enzyme in extracts of *T.foetus* which destroys the serological activity of blood-group A substance (Report 1963). Separation of the enzymes on columns of Sephadex G200 yielded A-enzyme free from H-enzyme activity. The loss of A activity of a water-soluble human blood-group A substance brought about by this A-enzyme preparation, was accompanied by a substantial increase in H activity. Preliminary tests indicate that *N*-acetylgalactosamine is the main sugar liberated by the enzyme. Confirmation of these results will provide the first direct evidence that the enzyme decomposing A activity is an *N*-acetylgalactosaminidase.

Attempts to determine the chemical nature of the human blood-group P_1 antigen led Professor Morgan and Dr. Watkins to investigate sheep hydatid cyst fluids, which contain a material serologically related to the human P_1 substance (Reports 1962, 1963). The fractionated hydatid cyst material was examined for substances related to human blood-group I antigen, as the crude hydatid cyst fluid is reported to inhibit certain anti-I sera. Two such sera kindly supplied by Dr. Race were tested for inhibition with four different hydatid cyst fractions. The fractions most active in the I system did not correspond to the most active P_1 fraction. The P_1 and I inhibitory activities thus appear to be associated with different molecules in the cyst fluid. The I-active fraction contained, after

hydrolysis, a number of sugars and amino acids. Treatment of this fraction in various ways revealed considerable differences in the two anti-I sera. The inhibition of the first serum was destroyed by treatment of the fraction with periodate, or with enzymes in *T. foetus* extracts or with ficin, but not by treatment with trypsin or pepsin; this suggests that the antigen is a mucopolysaccharide. Inhibition of the second serum, however, was scarcely changed by any of these treatments. Further studies are required to elucidate the chemical nature of the I antigenic complex.

Dr. F. W. Michel studied methods for the isolation and identification of an A-active glycolipid substance that occurs on the surface of group A erythrocytes, and confirmed the earlier work of Dr. Koscielak (Report 1962). He was again able to demonstrate that the weak A-activity of a glycolipid component was considerably enhanced by combination with a serologically inactive material, somewhat similar in composition, that also occurs on the human erythrocyte surface. Dr. Michel also determined the changes in the concentration of blood-group active material in the plasma and serum of stored A blood. There was a rise in the A-inhibiting activity in both serum and plasma; blood-group active material is clearly released from the red cell into the serum or plasma with the ageing of blood *in vitro*.

Dr. J. Dunstone and Professor Morgan investigated means whereby preparations of ovarian cyst mucopolysaccharide that are sparingly soluble, or insoluble, in water can be completely solubilized by reducing agents, such as thioglycollate, cystine and sulphite, without inducing significant chemical change. These mucopolysaccharides were found to contain a higher proportion of acidic amino acids and cysteine than did those isolated from the water-soluble part of ovarian cyst fluids. The results suggest that the sparingly soluble forms are molecular aggregates held together by intermolecular disulphide bonds; the possibility is under investigation.

CARBOHYDRATE STUDIES

Chemical Synthesis of Modified Carbohydrates. These studies involve the chemical modification of glucose and its derivatives, to provide new substrates with which to test the specificity of enzymes that synthesize and degrade glycogen and starch. Two types of modification were employed, the phosphorylation of modified glucoses to provide α– and β–1-phosphates, and the chemical modifications of the cyclic α–1,4-bonded polyglucoses (Schardinger dextrins). The dextrins themselves are not substrates for the glycogen-metabolizing enzymes but become so on conversion into acyclic dextrins. These two types of material were used in the following investigations of enzyme specificity.

Studies of Enzyme Specificity. Dr. Gunja Smith synthesized the 6–*O*–methyl– and 2,3–di–*O*–methyl derivatives of α–Schardinger dextrin and found that, like the unmodified dextrin, the first inhibited β-amylase whereas the second did not; indicating that the C-6-hydroxyl groups of glycogen play no part in complex formation with the enzyme, which requires the C-2- and/or C-3-hydroxyls for this purpose.

Dr. Beattie and Dr. Együd prepared and tested several analogues of α-glucose 1-phosphate with enzymes that metabolize the unmodified sugar, i.e., sucrose phosphorylase, starch phosphorylase and phosphatase. Only the last enzyme acted on the analogues and there is therefore still no known alternative substrate for polymerization by these phosphorylases. Maltose phosphorylase, by contrast, accepts the unnatural substrate 3-*O*-methyl-β-glucose 1-phosphate and the product of its polymerization has been characterized (Report 1963). A further six modified β-glucose phosphates were synthesized by Dr. E. E. Smith and Mr. Lee, and two more unnatural substrates were found by Mr. Lee, 6-deoxy-6-fluoro-

and 6-deoxy-β-glucose 1-phosphates. Furthermore 3-O-methyl-β-glucose 1-phosphate is a substrate for β-phosphoglucomutase, and all the unnatural phosphates are split by β-glucose phosphatase.

Glycogen and Starch Metabolism. Dr. Taylor investigated the complex of glycogen-hydrolysing enzymes in rabbit muscle. Other workers have indicated hydrolysis as an important alternative *in vivo* to phosphorolysis during glycogenolysis. Five distinct hydrolases have been recognised; two are α-amylases and three are *exo* α-glucosidases, and the action patterns of the glucosidases have been examined with a variety of oligo- and poly-saccharides as substrates. Dr. Abdullah has re-investigated the structure of glycogen phosphorylase limit dextrin with a bacterial enzyme, pullulanase, specific for the hydrolysis of 1,6-links in glycogen. The structure first put forward on indirect evidence by Dr. Walker (Report 1960) has been confirmed directly. With Professor E. H. Fischer and Dr. Whelan, Dr. Abdullah investigated the contention by Cori and his colleagues that phosphorylase can synthesize glycogen from α-glucose 1-phosphate without the aid of a primer. It was proved that a substantial part, if not all, of the synthesis observed was due to contamination of the sugar phosphate by primer. A procedure was also devised for freeing crystalline muscle phosphorylase of the last traces of α-amylase. Dr. Abdullah also devised a milligram-scale method for the enzymic determination of the average unit-chain length of glycogen, likely to be of value in examining the small amounts of material from biopsy specimens.

Dr. Robyt has examined a reported change in the action pattern of *Bacillus subtilis* α-amylase caused by addition of calcium ions. The change has been found to be an artifact of the method used in determining the reducing properties of the products of the enzyme action.

Dr. Kelemen studied the α-glucosidase activity in barley and malted barley. A powerful α-glucosidase, or mixture of glucosidases, is present in the malted barley, and its capacity to degrade starch is a major part of the total amylolytic activity present. The enzyme has a pH optimum so much lower than that of the malt amylases normally used for such degradation in the brewing process that its action under brewery conditions would be slight. It may, however, prove advantageous to lower the pH of the " mashing " (starch-degrading) process to take advantage of this glucose-producing activity.

PHOSPHOLIPIDS AND GLYCOLIPIDS

Some important functions of cells, such as selective permeability to food stuffs and drugs, certain enzyme activities and specific antigens, are known to reside in the membranes that form the boundary of the cell or of cell organelles and are loosely characterized as lipoprotein. The work carried out in the last few years on the nature and distribution of phospholipids and other lipids in different mammalian tissues and in micro-organisms has been fruitful, not only in the isolation and identification of lipid substances previously unknown, but in giving an approach to the correlation of particular lipids with particular cellular functions. Eventually this knowledge may facilitate the preparation of artificial lipoprotein structures resembling those of the cell, with which precise studies may be carried out.

The main lines of work during the year were further investigations on the nature and function of lipoamino acids discovered in bacteria; the separation and identification of glycolipids from different strains of ascites tumour cells; the examination of an antigenic lipoprotein carrying H_2 histocompatibility specificity; and a comparison of phospholipids present in bone marrow from normal and leukaemic persons.

Amino Acid Esters of Phosphatidylglycerol. Dr. Macfarlane isolated lipoamino acids of this type from several species of Gram-positive bacteria, including *Staphylococcus*, *Lactobacillus*, *Sarcina* and *Clostridium* species, and identified the amino acids present. The results do not support the idea, current for some years, that lipoamino acids play some part in the synthesis of protein or transport of amino acids, for the full range of amino acids required for protein synthesis was not found. In any one species usually only one amino acid was present in this form of ester, the most common being lysine; alanine was found in *Cl. welchii*. Some Gram-positive bacteria contain virtually no amino acid esters during the period of active protein synthesis.

In *Staphylococcus albus*, *Sarcina lutea* and *Streptococcus faecalis*, the main phospholipid was identified as phosphatidylglycerol. The proportion of this lipid bound to lysine as a lipoamino acid varied in different phases of the growth of the bacteria; it was at a minimum in the exponential phase and increased rapidly as the stationary phase was reached. The conversion of the acidic phosphatidylglycerol to a more basic compound by linkage to lysine may, in some way, stabilize the cytoplasmic membrane of the mature protoplast. It appears probable that in rapidly dividing cells the new cytoplasmic membrane synthesized in each cell is different from the older membrane of that cell, and hence may differ in permeability and other properties, such as susceptibility to drugs.

Dr. Gray examined the phospholipids of rat liver mitochondria and was unable to detect any lipoamino acids, though a small amount of phosphatidylglycerol was isolated. Dr. Marfarlane also failed to find these lipoamino acids in liver or pancreas.

Lipids of Ascites Tumour Cells. Dr. Gray continued the investigation of the glycolipids of various strains of ascites tumour cells, which it is thought may be of antigenic significance. A glycolipid fraction isolated from BP8/C3H cells, and previously identified (Report 1963) as a mixture of ceramide-trihexoside and an amino glycolipid, was separated into two components by rechromatography on silicic acid. Each component was homogeneous in several different chromatographic systems. The structure of one component as a ceramide-trihexoside was confirmed, and the analyses indicated that the trisaccharide unit, which was linked through glucose to the sphingosine of the ceramide moiety, was a glucosylgalactosylgalactose. The absolute amounts of glucose and galactose in the fraction suggested that a ceramide-trihexoside containing galactosylgalactosylgalactose was present as a minor component. The second component was an amino glycolipid containing a tetra-saccharide unit (not a trisaccharide as thought previously); this was identified as a glucosylgalactosylgalactosyl-*N*-acetylgalactosamine, linked through the glucose to the sphingosine of the ceramide moiety. The structural similarity of the four classes of glycolipid isolated from BP8/C3H cells suggests that the ceramide-trihexoside, –dihexoside and –monohexoside may be successively formed by enzymic degradation of the amino glycolipid.

Dr. Gallai-Hatchard began a parallel investigation of the glycolipids of CL2/BA1 ascites tumour cells. Preliminary results showed that these differed significantly from those of the BP8/C3H cells.

The differences in the amounts and types of glycolipid found in the Landschutz (carcinoma), the BP8/C3H (lympho-sarcoma) and CL2/BA1 (leukaemia) strains of ascites tumour cells are very interesting; it may be that the glycolipid composition is a characteristic of the tumour specificity, or alternatively, a characteristic of the mouse strain in which the tumour was grown.

Lipids of Mouse H_2 Antigen. A mouse antigen, characterized as a lipoprotein, which carries H_2 histo-compatibility specificity has been isolated from BP8/C3H ascites tumours by Dr. D. A. L. Davies, (M.R.E., Porton) who generously provided a quantity for investigation. Dr. Gray compared the lipid composition of this

antigen with that of the whole tumour cells. The total lipid amounted to 23% of the dry weight, and consisted of phospholipid (59%), neutral lipids (37%) and glycolipids (4%). The phospholipid closely resembled that of the whole cell and no significant differences were apparent. The neutral lipid was mainly cholesterol (95%) with small amounts of triglycerides and fatty acids, whereas that of the whole cell was mainly triglycerides with 20% only of cholesterol. The relative glycolipid content of the antigen was at least ten times that of the whole cell; the major component was a ceramide-dihexoside, with small amounts of ceramide mono- and tri-hexosides. No amino glycolipid was found in the antigen.

Phospholipids of Human Bone Marrow. Dr. Wajda found that the phospholipid of normal human bone marrow comprised 1–3% of the total fat; it contained lecithin as the major component, together with phosphatidylethanolamine and minor amounts of plasmalogens, phosphatidylserine and sphingomyelin. A nitrogenous phospholipid similar to sphingomyelin in chromatographic properties was found; on acid hydrolysis this compound gave a phosphoric ester not yet identified, and an amino compound. Glyceryl ether derivatives of phosphorylcholine and phosphorylethanolamine were present in unusually high proportions in the bone marrow lipids, totalling 11–13%, compared with other mammalian tissues. Small amounts of glycolipids were also found.

In bone marrow from leukaemic patients there was a striking decrease in the proportion of phosphatidylethanolamine; this was balanced by an increase in the proportion of lecithin, the proportions of the other phospholipids being unchanged.

BIOPHYSICS

SEDIMENTATION AND VISCOSITY CHARACTERISTICS OF MACROMOLECULES

The work on the molecular characterization of blood-group substances described in previous reports led Dr. Creeth and Mr. Knight to extend and modify existing theories for interpreting sedimentation and viscosity data. The intrinsic viscosity $[\eta]$ of a macromolecular substance is greater than the limiting value required by the Einstein equation if the molecular configuration is expanded as in random-coil polymers, or is rigid but asymmetric as, for example, in the tobacco mosaic virus nucleoprotein. Given that a molecule has one of these two types of configuration the appropriate theoretical expressions can be applied to define the characteristic dimensions of a macromolecule.

It was shown that the combination of Burgers' theory for the concentration dependence of sedimentation coefficients, S, with Einstein's viscosity equation gives a limiting value for the ratio $K_s/[\eta]$ which is characteristic of spherical particles, where K_s is the coefficient of the concentration-dependence of S. This value is the same for compact spherical particles and for expanded random-coil configurations, so that the experimental determination of the quantities K_s and $[\eta]$ allows a macromolecule to be classified only in respect to symmetry. However, this is sufficient for the selection of the appropriate model; consequently the intrinsic viscosity alone can be interpreted quantitatively with greater confidence. A survey of existing data for a wide variety of well characterised substances showed the need for modifying Burgers' constant, but the procedure retains its usefulness on a partly empirical basis.

Applied to blood-group substances, the procedure revealed that whereas some preparations have a random-coil configuration with a very high degree of expansion, the hydrodynamic volume being up to sixty times the partial specific volume, others have a more rigid and asymmetric configuration. Some correlation with solubility was observed.

CHARACTERIZATION OF MACROMOLECULAR HETEROGENEITY

The principles of a new method for estimating the degree of polydispersity of biological preparations, based on the ultracentrifugal " steady-state " were discussed in the previous report (1963). The method was further developed by Dr. Creeth and subjected to a variety of experimental tests. The simple theory, valid for two component systems, proved to be adequate for the interpretation of the behaviour of a purified protein. In agreement with expectation, the method proved sensitive to the presence of impurities occurring in too low a concentration to be evident by other tests.

When applied to blood-group substance preparations, the method gave useful estimates of relative homogeneity, but it became evident that an adequate theory, valid for multicomponent systems, will be necessary before the full advantages of the method can be realised. Some progress has been made in this direction and the work continues.

HUMAN PLASMA PROTEINS

Antihaemophilic Factor (AHF). The stability of human AHF is very dependent on the pH of its environment; in plasma there is a sharp stability optimum at pH 7.1 to 7.2, and below pH 6.5 and above pH 7.5 the rate of loss of activity rises steeply. When blood is taken into trisodium citrate anticoagulant the resulting plasma has a pH about 7.5 to 7.6 and tends to become slowly more alkaline because of loss of carbon dioxide. When blood is taken into acid -citrate-dextrose anticoagulant the initial mixtures have a pH as low as 5.0 and as more blood is added the pH rises gradually to 6.8.

Both of these anticoagulant mixtures therefore have obvious defects for obtaining plasma with as high as possible a content of AHF, and maintaining it during transport of the blood over moderate distances to a central fractionating laboratory. Blood constituents other than AHF may also be deleteriously affected.

A phosphate-buffered citrate anticoagulant solution was devised such that the pH of initial mixtures is little lower than 6.5 and the final pH stabilises at about 7.1–7.2, with little tendency to drift to more alkaline reactions. The stability of erythrocytes during storage at 4° of blood taken into such mixtures was estimated in terms of red cell fragility tests, and by determinations of the concentration of potassium in the plasma, for periods up to three weeks. Preliminary results obtained by Dr. Kekwick in collaboration with Dr. K. L. G. Goldsmith (Blood Group Reference Laboratory) are encouraging.

Macroglobulins of Normal Human Plasma. By a combination of solvent fractionation and preparative ultracentrifuging, a concentrate was obtained, from the G2/2 fraction produced from normal human plasma by precipitation with ether, in which components having a sedimentation coefficient of about 19S account for 93% of the total protein. The overall recovery of these macroglobulins at this stage is about 70%. The concentrate, which is a mixture of 19S alpha and 19S gamma globulins is being separated into its constituents by chromatographic methods. So far, 19S alpha globulin appears to be rather homogeneous in its chromatographic behaviour, but the 19S gamma globulin can be resolved into subfractions with differing elution characteristics, in this respect resembling 7S gamma globulin.

The identification of the numous chromatographic fractions obtained in these experiments depends largely on analysis by immunoelectrophoresis. In collaboration with Dr. K. L. G. Goldsmith, a new device was made for micro-immuno-electrophoresis; the results of simultaneous electrophoresis of six samples in agar gel can subsequently be analysed and compared on the same agar plate, using a variety of antisera. The device is also very convenient for

the identification of components producing individual arcs of precipitation by the Osserman procedure.

Normal Human 7S Gamma Globulin. In conjunction with a Medical Research Council sub-committee concerned with the provision of highly specific anti-globulin reagents, Dr. Kekwick undertook the preparation of specially purified batches of normal human 7S gamma globulin from the plasma of Le^a positive donors.

An opportunity is being taken to examine the variation of the sedimentation coefficient with pH and concentration of some of this material, to determine whether there is any correlation with the variations known to occur in the optical dispersion of gamma globulin when the pH is varied.

Equipment. A second Spinco Model L preparative ultracentrifuge, bought with the aid of a grant from the Department of Scientific and Industrial Research, was installed. This model has a maximum speed of 50,000 r.p.m. and has accessories for density gradient separation experiments.

BLOOD PRODUCTS LABORATORY

Production. The laboratory continued to prepare dried plasma and plasma fractions for the National Health Service and operated at the limits of its capacity.

Mr. Vallet carried out a series of small-scale fractionations of plasma, using ethanol, in order to obtain data or develop procedures in readiness for the large scale preparation of ethanol fractions for clinical use. Gamma globulin fractions, containing high titre antibody against tetanus toxin, chicken pox, vaccinia virus and the Rh (D) antigen, were made from plasma or serum pools from selected donors. The anti-tetanus gamma globulin was prepared in collaboration with Professor D. G. Evans and Dr. J. W. G. Smith (London School of Hygiene and Tropical Medicine) for use in a treatment trial of tetanus. Gamma globulin containing anti-D was prepared in collaboration with the Department of Medicine, Liverpool University, for the clinical investigation of its value in preventing sensitization of Rh-negative women bearing Rh-positive children. To obtain further experience in using ethanol on a large scale, an experimental cold laboratory, equipped to fractionate 60 litre pools of plasma, was designed. It was brought into use by Mr. Vallet and Mr. Wesley in September, 1963.

Albumin. It was found that the albumin (fraction AP2) prepared for clinical use, which is heated at 60° for 10 hours to inactivate the hepatitis virus, was unsuitable for labelling with radio-active iodine for metabolic studies. Changes occur during heating which are associated with a rapid initial excretion of iodine after injection of the labelled protein. Ideally, unheated albumin, prepared from the plasma of individuals known not to be carriers of hepatitis, should be used. To meet the need for a preparation of albumin suitable for metabolic studies, Mr. Vallet devised a method of purifying the heated fraction AP2. Denatured albumin of decreased solubility and globulin contaminants are precipitated at pH 5.4 in 40% ethanol and discarded. Albumin of normal solubility is then precipitated by adjusting the pH to 4.8. In metabolic studies this albumin was shown to behave like unheated albumin.

Pharmacologically Active Substances in Human Plasma. Dr. Mackay, Dr. Maycock and Mrs. Silk continued their investigations of the kininogenetic systems in plasma. The kininogen from fractions of fibrinogen rich in antihaemophilic globulin (AHF), mentioned in previous reports, was partly separated from fibrinogen and plasminogen by precipitation at pH 4.5. Incubation with streptokinase showed that the fibrinogen and plasminogen were precipitated, and incubation with plasmin that the greater part of the kininogen remained in the supernatant. For example, the precipitate from one preparation of AHF containing 5 ng kinin/mg. protein, contained 1.7 ng kinin/mg. protein and the

supernatant, 3.7 ng kinin/mg. protein. An attempt was made to identify the different components of the proteolytic enzyme system in fractions prepared from fresh plasma by the method of Kekwick and Mackay. An $\alpha + \beta$ lipoprotein globulin fraction (G2/1R), contained, in terms of the original plasma, 33 times as much plasminogen, 30 times as much kininogenase and 50 times as much esterase (p-tosyl-l-arginine methyl ester). The esterase was also concentrated 14 times in the fibrinogen fraction (F_1W) and 7 times in a globulin fraction (G2/2). Kininases were found in the globulin fraction G2/1 and G2/2 and their activity was increased by removal of lipid. Fraction G2/1R will be further fractionated.

Plasminogen. Dr. Mackay and Mrs. Silk have compared, by several different methods of assay, preparations of plasminogen separated from ether-precipitated fraction P and from ethanol precipitated fraction III. Differences were observed in their esterolytic properties, lysine ethyl esterase being relatively more active in plasminogen from fraction P and p-tosyl-l-arginine methyl esterase in plasminogen from fraction III.

The staff of the laboratory continued to take an active part in the trials being conducted by the Medical Research Council's Working Parties of Hypogammaglobulinaemia and Human Antihaemophilic Globulin.

BLOOD GROUP RESEARCH UNIT

The main themes of 1962, the Xg system and the Rh system, continued to take up most of the time of the Unit throughout 1963.

The Xg System. This X-linked blood group system is so far being applied chiefly in two lines of investigations: attempt are being made to map the relative position of some of the 50 or so genes known to be carried on the X chromosome, and families of people with sex abnormalities are being tested in the hope of tracing the source of the accidents at cell division responsible for their condition.

The mapping may seem rather academic since many of the abnormal genes dealt with are very rare, but some justification for this pleasurable work may be found in remembering that these rare genes mark the sites of their more common alleles responsible for the normal function of the body. For example, the gene for angiokeratoma, a very rare X-linked condition, looks, from the family samples sent to us by Dr. J. M. Opitz, to be fairly close to Xg. This in itself may not seem very important, but it may be useful thus to have fixed the position of the ' not-angiokeratoma ' allele which is necessary if this sombre sounding disability is to be avoided.

On the whole, the mapping work has been rather disappointing. The X chromosome is probably longer than it was previously thought to be. After the early finding that Xg was in measurable distance of the gene for g-6-pd, and so of the genes for deutan colour-blindness and haemophilia (Report, 1963), all that we and our collaborators, whose labour has been far greater than ours, seem to have achieved is the knowledge that a number of genes known to be X-linked are not close to Xg. The evidence is becoming stronger that the genes for the following conditions are well away from Xg: Duchenne's muscular dystrophy, Christmas disease, hypogammaglobulinaemia and hypophosphataemia. Less weighty evidence continues to show that the genes for the following X-linked conditions are at least not close to Xg: renal diabetes insipidus, ectodermal dysplasia, keratosis follicularis, Hurler's syndrome (gargoylism), chorio-retinal dystrophy, pyridoxine responsive anaemia, deaf mutism, spastic paraplegia, total colour-blindness, Aland eye-syndrome, pseudoglioma and ichthyosis.

More satisfying, this year, were the tests on people with various sex abnormalities.

Ninety-three females with Turner's syndrome and of the caryotype XO have now been tested and the distribution of the Xg groups is that expected of

males. In about 30% of the families the Xg groups show whether the single X is of maternal or paternal origin.

Eighty-four males with Klinefelter's syndrome and of the caryotype XXY have now been tested. The distribution of the Xg groups is halfway between that expected of males and that expected of females. In two families an XXY Klinefelter son was Xg(a+) and his mother was Xg(a–), thus showing that he had received an X and a Y from his father. That XXY could arise in this way had been demonstrated only in one mouse: this was the first proof in man. Furthermore the time of the causative accident of non-separation of the X and Y is fixed as being not later than the first meiotic division of spermatogenesis.

Families with abnormalities of the sex chromosomes frequently break the rules of X-linked inheritance but in nearly 1,200 families, apparently normal as far as the sex chromosomes were concerned, two were found whose Xg groups did not conform with the usual manner of inheritance: two Xg(a–) women with Xg(a+) husbands had respectively three and two Xg(a+) sons. The explanation is not known: an attractive possibility is that in the ancestry of the fathers a small piece of the X was translocated on to the Y.

The incidence of the groups in unrelated white people (mostly British) fluctuates hardly at all now that the numbers are large: from the latest count, of 2,000 tests, it can be calculated that two out of three X chromosomes carry the gene Xg^a which results in the presence of the antigen.

A sufficient number of Negroes has now been tested to show that in these people the gene Xg^a is significantly less frequent than in Whites: about 55% of their X chromosome carry Xg^a.

The antigen Xg^a could not be detected in the cells of those animals so far tested: baboons (6), marmoset (1), dogs (36), mice (20). Arrangements are being made to test chimpanzee, orangutan and gorilla samples from the States (in collaboration with Dr. J. K. Moor-Jankowski).

Without generous gifts of the precious anti-Xg^a plasma from Dr. Amos Cahan of Knickerbocker Biologics, New York, and Dr. J. D. Mann of Butterworth Hospital, Grand Rapids the work would not have been possible.

Some of our collaborators who send the family samples were acknowledged in the last Report (1963). In addition we have recently had a great deal of help from Dr. J. M. Opitz of the University of Wisconsin, Dr. Jessica Lewis of the University of Pittsburgh, Dr. J. H. Edwards of the University of Birmingham, Dr. C. Kerr of the M.R.C. Population Genetics Research Unit, Dr. C. N. Went of the University of Leiden, Dr. V. Dubowitz of the University of Sheffield, Dr. A. W. Eriksson and Dr. A. de la Chapelle of the Institute for Genetics, Helsinki, Dr. Mette Warburg of the University of Arhus, Dr. A. Frøland of the University of Copenhagen and Dr. M. A. Ferguson-Smith of the University of Glasgow.

The Rh System. Work with Dr. P. Levine of Raritan, Dr. J. Wallace of Glasgow and Dr. A. Matson and Mrs. Jane Swanson of Minneapolis showed that the antigen which is detected by the original rabbit anti-rhesus serum of Lansteiner and Wiener is not controlled by what we call the CDE complex locus but by a gene which is independent of this locus. It is now clear that the name *rhesus* should not have been applied to the system that is responsible for transfusion reactions and for haemolytic disease of the newborn; but it is too late to give up the name Rh by which the system has been known for 24 years. Instead, it is proposed to change the name of the clinically unimportant antigen recognized by the rabbit anti-rhesus serum to LW, in honour of Lansteiner and Wiener.

Dr. Patricia Tippett is studying the blood of the rare people who have the antigen D on their red cells yet have anti-D in their plasma: such antibodies do not react with their owner's cells. By cross-testing cells and sera of these

people six different categories of rare D antigens can be distinguished. (Reports 1962, 1963). For more than a year the cell samples have fallen into already defined categories so there seems to be a limit to diversity at this level. It looks as if the D antigen has a certain number of parts any one of which may, as a great rarity, be missing or altered so that the owner is at liberty to make antibody to the missing part. Tests on the families show that the differences defining the six categories are strictly inherited characters.

The P Systems. The work on the antigen P^k, so far found only in Finns, continues with Dr. A. E. Kortekangas of Turku (Reports 1961, 1962). The manner of inheritance of this rare antigen is proving a very difficult problem but if a solution could be reached it should illuminate the P system as a whole.

Tests were resumed with the serum sent by Dr. W. T. Snoddy of Oklahoma City (Report 1962). The reactions of this serum are now seen to be influenced by the A_1A_2BO groups as well as by the P groups of the cells being tested. The agglutinating agent in the serum is extraordinary in that it is not absorbed by exposure to positive cells.

The Phenotype Lu(a–b–) and the Auberger System. Most people have the 'allelic' antigens Lu^a or Lu^b, or both, but rare people exist of the phenotype Lu(a–b–) who have neither (Report 1961). The Auberger systems of groups was first found in France (Report 1962). Two families were tested in the Unit and in them ten members are Lu(a–b–) and all ten are Au(a–), the rest being (Au(a+). The association of Lu(a–b–) and Au(a–) cannot possibly be due to chance.

The Lu(a–b–) phenotype is a dominant character unlike other 'minus-minus' phenotypes, which are recessive. It remains to be found out whether the Auberger groups belong to the Lutheran system or whether they are independent but under the influence of some mutual modifying gene. The problem is particularly tantalizing because Madame Auberger, the sole source of anti-Au^a, is dead and the supply of her serum is almost exhausted.

The Antigen Rd. This is a very rare antigen studied with Dr. T. E. Cleghorn of Sutton and with a number of collaborators in the States. The antibody, four examples of which have now been found, can be the cause of haemolytic diseases of the newborn. Tests on the families of Rd(+) people have excluded the antigen from being part of the ABO, MNSs, Rh, Lutheran, Kell or Kidd systems and it is not X- or Y-linked.

For routine antisera the Unit is indebted to many colleagues, notably those in the Blood Group Reference Laboratory and the Blood Transfusion Centres at Sheffield, Sutton, Brentwood, Birmingham, Bristol and Glasgow.

Again the Unit is grateful to the Staff of the Institute for samples of their blood which have played a major part in many of the investigations.

BLOOD GROUP REFERENCE LABORATORY

The Unit has been working for the greater part of the year in its new building north of the Biophysics Department. It moved in on February 25th, and the building was opened officially on May 7th, by Lord Newton, Joint Parliamentary Secretary, Ministry of Health. The increased space and staff have enabled new activities to be undertaken, and supplies of animal sera to be increased.

The Unit acts as the central Reference Laboratory for the investigation of blood group problems and is the supply centre for grouping serum, for the United Kingdom.

Liquid blood-grouping sera and anti-human-globulin serum are supplied for use in the United Kingdom, and dried sera for use overseas. Help to overseas laboratories has continued to be an important activity. Such laboratories have included those designed as National Reference Laboratories through

the World Health Organization. One of the most valued parts of such help is the full blood grouping of members of Staff, in order to create panels of donors of red cells for use in the identification of unknown haemagglutinins. Large numbers of blood and serum specimens from Great Britain and overseas have been examined for blood-group antigens and antibodies, as part of clinical investigations, for research purposes, and as part of routine blood-grouping especially in connection with the control and supply of grouping sera.

Dr. Goldsmith continued his investigations into antibodies against leucocytes and platelets. This is a field in which methods are far from being standardized, and in which the results obtained by different laboratories have differed considerably from one another. In the case of platelet antibody investigations, in the course of three years some hundreds of sera have been investigated independently by Dr. Goldsmith and by Dr. W. J. Jenkins of the North-East Metropolitan Blood Transfusion Centre, with an encouraging and steadily improving degree of agreement between them in results.

More and more cases requiring the grafting of tissues and organs, especially kidneys, are being referred for investigation. Blood from patients and potential donors is being investigated for all possible red-cell antigens by Dr. Ikin and Miss Giles, and for leucocyte antigens by Dr. Goldsmith.

These tests, though they must for the present be continued as giving at least some chance of assessing compatibility, are far from ideal, as they depend upon reactions between humoral antibodies and cells which may differ antigenically from the tissues to be grafted. Other means of assessing the reactions of host lymphocytes to donor tissues are therefore being explored, and some promising results have been obtained by Dr. Goldsmith in applying to human volunteers the method of injecting living lymphocytes, developed in animals by Brent and Medawar.

Dr. Ikin, who is in charge of serum production, is investigating modified methods of rabbit antiserum production, and has also considerably increased the quantities of serum produced. With the collaboration of the Ministry of Agriculture, Fisheries and Food, seeds containing haemagglutinins, or " lectins " are now beginning to be produced in sufficient quantities for routine use.

Dr. Ikin resumed work on the Standardization of Rh antisera, with a view to the making of Standard Preparations for the World Health Organisation. She is also investigating the not uncommon weak variants of the A antigen that are sent as such for investigation, or are picked up in specimens sent for other purposes. She continued her investigations, in collaboration with Dr. H. Lehmann, then at St. Bartholomew's Hospital, and others, on the stability or otherwise of the antigens of red cells frozen in liquid nitrogen.

Miss Giles, assisted by Mrs. Nunn, made highly detailed examinations of large numbers of sera sent in for testing for a variety of clinical reasons, including many from overseas. In the course of this work, a considerable number of new or very rare antibodies and antigens were discovered and made the subject of full serological and genetical studies, some of which are still continuing. The blood group systems in which these were found were the ABO, MNSs, Rh, Yt and Au systems. Particular attention was paid to weakly reacting variants in the Rh system. Work on the comparative immunology of primates is being carried out by Dr. Goldsmith and Miss Giles, in collaboration with Dr. L. H. Collier.

Work on the production of animal antisera to the components of human globulin was continued by Dr. Goldsmith, in collaboration both with Dr. R. A. Kekwick, and with a sub-committee of the Medical Research Council Blood Transfusion Committee. The production of anti-human-globulin sera, both experimentally and for routine purposes, in both goats and rabbits, is being

carried out on behalf of the Blood Group Reference Laboratory at the Medical Research Council's Laboratory Animals Centre at Carshalton, through the kindness of Dr. W. Lane-Petter and in collaboration with Mr. J. Bleby.

Dr. Narayanan began an investigation of the various anti-gamma-globulin (anti-Gm) antibodies present in the sera of healthy blood donors, and of the distribution in normal sera of the corresponding antigens.

An apparatus for fluorescence microscopy has been installed primarily to facilitate the production of fluorescent-labelled antibodies, but it can be made available for other purposes.

In conclusion the Governing Body desires to record its great appreciation of the manner in which the scientific, administrative and technical staffs have worked together during the period under review, and to congratulate them on the interest and range of their scientific activities and achievements.

E. C. DODDS,
Chairman.

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