THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

Balance Sheet

and

Accounts

31st December 1964

CHELSEA BRIDGE ROAD,
LONDON, S.W.1.
25th May, 1965

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

FINANCIAL REPORT OF THE GOVERNING BODY

1. The Balance Sheet as at 31st December, 1964, shows balances to the credit of the various funds as follows: Capital Fund £873,940, Specific Funds £211,200 and Bequest Funds £25,284. The balance on the Sinking Fund for Freehold Buildings of £148,639 is after transferring £6,982 from Income and Expenditure Account. During the year donations and legacies of £682 have been added to the Re-endowment Fund. The General Fund Investment Reserve has been credited with profits, less losses, on sales of investments amounting to £27,667 and the Sinking Fund Investment Reserve has been debited with a loss on sale of an investment amounting to £4,441.

2. The General Fund Income and Expenditure Account shows the Income for the year as £280,727 compared with £236,110 in 1963. Expenditure amounted to £308,665 against £293,957 last year. The deficit for the year is £27,938 compared with a deficit of £57,847 in 1963.

3. The year's deficit of £27,938 shown by the General Fund Income and Expenditure Account has been deducted from the Capital Fund.

4. Stocks of Sera, Virus Vaccines and Horses on hand at 31st December, 1964, have not been valued in the accounts.

5. Cooper Brothers & Co., the present Auditors, will continue in office pursuant to Section 159 (2) of the Companies Act, 1948.

E. C. DODDS *Chairman of the Governing Body.*

HUGH BEAVER *Hon. Treasurer.*

BALANCE SHEET

(1963) £		£	£	£
	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, 1963	541,411		
	Less deficit 1964	27,938		
541,411			513,473	
901,878				873,940
	Specific Funds:—			
141,657	Sinking fund for freehold buildings	148,639		
33,915	Pension fund	33,589		
28,290	Re-endowment fund	28,972		
			211,200	
	Bequest Funds:—			
10,972	Jenner Memorial studentship fund	17,513		
7,439	Morna Macleod scholarship fund	7,771		
			25,284	
222,273				236,484
	Specific Grants and Legacies Unexpended:—			
772	Cancer research legacies (1937-1950)		272	
4,781	Nuffield Foundation grants (1952-1962)		4,372	
5,398	Guinness Lister research grant (1953-1964)		5,826	
10,951				10,470
	Current Liabilities:—			
59,061	Creditors and accrued charges			46,557
£1,194,163				£1,167,451

E. C. DODDS *Chairman of the 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 properly kept. In our opinion the accounts, amplified by the information given in paragraphs 1 and 4 of the Financial Report, show the deficit of the Institute.

London, 27th May, 1965

t DECEMBER 1964

(1963) £					£	£	£
	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		
	Additions during year			7,073			
	Less depreciation			1,473	5,600		
						8,072	
98,524	*(Note: Additions and replacements between 31st December, 1920, and 31st December, 1963 have been charged to revenue)*						104,124
	General, Specific and Bequest Funds. Investments and Uninvested Cash:—						
		Quoted at cost In Gt. Britain	Quoted at cost Elsewhere	Unquoted at cost	Uninvested cash		
797,263	General	£591,655	£63,365	£42,395	—	697,415	
	Specific—						
	Sinking fund for freehold buildings	147,403	—	—	6,234	153,637	
151,096	Pension fund	34,217	—	—	628 (Cr.)	33,589	
33,915	Re-endowment fund	28,001	—	—	971	28,972	
28,290	Bequest—						
	Jenner Memorial studentship fund	14,550	—	940	2,023	17,513	
10,972	Morna Macleod scholarship fund	6,656	—	—	1,115	7,771	
7,439							
1,028,975		822,482	63,365	43,335	9,715	938,897	
	Less investment reserves—						
81,814	General fund				109,481		
9,439	Sinking fund for freehold buildings				4,998		
						114,479	
937,722							824,418
	Current Assets:—						
111,033	Debtors and payments in advance					83,576	
46,884	Balance at bankers and cash in hand					155,333	
157,917							238,909
	(Notes: (a) Quoted investments at a cost of £885,847 have a market value at 31st December, 1964, of £1,155,080.						
	(b) There is an outstanding capital expenditure commitment of £12,090 in respect of building at Elstree.)						
£1,194,163							£1,167,451

ITORS TO THE MEMBERS

pe kept. We obtained the information and explanations we required.
rt 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 ACCOUN

GENERA

(1963)		Total Expenditure	External Contributions	
£		£	£	£
132,546	Salaries and wages	255,938	97,773	158,165
9,811	Emoluments of two members of the Governing Body in an executive capacity	11,340	—	11,340
6,088	Premiums on federated superannuation policies	12,216	3,824	8,392
1,246	Premiums on group pension policy	2,910	1,073	1,837
5,831	Rent, rates and insurance	6,845	275	6,570
17,159	Gas, water, fuel and electricity	21,145	4,007	17,138
4,658	Office expenses, stationery and printing	5,883	841	5,042
430	Audit fee	420	—	420
1,001	Travelling expenses	2,510	719	1,791
4,186	Biochemistry expenses	4,954	2,241	2,713
2,477	Microbiology, immunology, experimental pathology and electron microscopy expenses	19,789	14,989	4,800
687	Biophysics expenses	569	—	569
200	Virology expenses	937	794	143
35,048	Serum, vaccine and virus vaccine expenses	41,679	1,064	40,615
8,160	Animals	11,171	1,531	9,640
11,448	Animal house expenses and forage	13,055	1,443	11,612
37,581	Buildings, alterations, repairs and renewals (including depreciation £1,473)	10,630	90	10,540
3,071	General apparatus and new installations	1,968	—	1,968
2,020	Library expenses	2,214	—	2,214
1,641	General stores	1,806	—	1,806
2,626	Staff canteen loss	4,874	506	4,368
—	Blood products laboratory expenses	8,243	8,243	—
6,042	Amount transferred to sinking fund for freehold buildings (including £6,557 interest on investments)	6,982	—	6,982
£293,957		£448,078	£139,413	£308,665

...N for the year ended 31st December 1964

...RA FUND

(1963)		£	£
£			
	Interest and dividends on investments:—		
49,637	General fund	48,708	
5,618	Sinking fund	6,557	
			55,265
234	Underwriting commission		292
174,520	Sales of sera, vaccines, virus vaccines, &c.		219,556
6,101	Rent		5,614
	Deficit transferred to Capital Fund after charging to expenditure £12,963		
57,847	(1963 £37,726) for additions to property and equipment		27,938
£293 957			£308,665

PENSION FUND

(1963) £		£	(1963) £		£
2,406	Pensions	2,415	34,008	Balance as at 1st January, 1964 ..	33,915
33,915	Balance carried forward	33,589	2,268	Interest on investments (gross) ..	2,089
			45	Net profit on realisation of investments	—
£36,321		£36,004	£36,321		£36,004

JENNER MEMORIAL STUDENTSHIP FUND

(1963) £		£	(1963) £		£
10,972	Balance carried forward	17,513	10,367	Balance as at 1st January, 1964 ..	10,972
			605	Interest on investments (gross) ..	957
			—	Profit on realisation of investment	10
			—	Legacy	£5,574
£10,972		£17,513	£10,972		£17,513

MORNA MACLEOD SCHOLARSHIP FUND

(1963) £		£	(1963) £		£
7,439	Balance carried forward	7,771	7,121	Balance as at 1st January, 1964 ..	7,439
			311	Interest on investments (gross) ..	332
			7	Profit on realisation of investment	—
£7,439		£7,771	£7,439		£7,771

NUFFIELD FOUNDATION GRANTS

(1963) £		£	(1963) £		£
1,115	Salaries, wages, laboratory expenses and animals	409	5,896	Balance as at 1st January, 1964 ..	4,781
4,781	Balance carried forward	4,372			
£5,896		£4,781	£5,896		£4,781

GUINNESS-LISTER RESEARCH GRANT

(1963) £		£	(1963) £		£
13,415	Salaries and wages	12,405	7,857	Balance as at 1st January, 1964 ..	5,398
4,294	Laboratory expenses	2,167	15,250	Amount received	15,000
5,398	Balance carried forward	5,826			
£23,107		£20,398	£23,107		£20,398

THE LISTER INSTITUTE
OF
PREVENTIVE MEDICINE

REPORT
OF THE
GOVERNING BODY
1965

CHELSEA BRIDGE ROAD, LONDON, S.W.1

THE GOVERNING BODY

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H. P. G. CHANNON, MP
The Rt Hon The EARL OF IVEAGH, KG, CB, CMG, FRS
W. d'A. MAYCOCK, MVO, MBE, MD
Professor A. A. MILES, CBE, MA, MD, FRCP, FRS
Professor J. S. MITCHELL, CBE, MA, MD, FRS
Professor WILSON SMITH, MD, FRCP, FRS

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

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The Rt Hon The EARL OF IVEAGH, KG, CB, CMG, FRS	" "
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Professor J. S. MITCHELL, CBE, MA, MD, FRS	" "
Professor W. T. J. MORGAN, CBE, DSc, PhD, FRIC, FRS	" "
Professor Sir RUDOLPH PETERS, MC, MA, MD, 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
MURIEL ROBERTSON, MA, DSc, LLD, FRS	Members of the Institute
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*)

F. R. Wells, MA, BM, BCh

W. E. Parish, MA, PhD, BVSc, MRCVS

Ruth M. Lemcke, BSc, PhD

Brenda Mason, BSc

GUINNESS-LISTER RESEARCH UNIT

†B. A. D. Stocker, MD, MRCS, LRCP (*Guinness Professor of Microbiology in the University of London*)

*G. G. Meynell, MD, MRCS, LRCP

M. W. McDonough, BSc, PhD

W. T. Drabble, BSc, D.Phil (*Medical Research Council Junior Research Fellow*)

Ursula Pearce, BSc (*Medical Research Council Scholar*)

R. G. Wilkinson, MSc (*Commonwealth Scholar*)

P. Gemski, AB, MS, PhD (*U.S.A.*)

J. G. Michael, BSc, MA, PhD (*U.S.A.*)

VIROLOGY

*L. H. Collier, MD (*also Hon. Director, M.R.C. Trachoma Research Unit*)

M.R.C. Trachoma Research Unit

C. A. Placido de Sousa, MB, ChB
W. A. Blyth, BSc, PhD
Janice Taverne, BA, PhD
P. Reeve, BSc, PhD
Doris M. Graham, MSc
Elizabeth F. Hart, BSc

ELECTRON MICROSCOPY UNIT

A. M. Lawn, BSc, PhD, MRCVS

BIOCHEMISTRY

†W. T. J. Morgan, CBE, DSc, PhD, MD(HC), FRIC, FRS (*Professor of Biochemistry in the University of London*). *Principal Biochemist, Elstree*

*Winifred M. Watkins, DSc, PhD

G. M. A. Gray, BSc, PhD

Sheila M. Lanham, BSc (*Trypanosomiasis Research*)

Barbara J. Dod, BSc.

Diane Ziderman

G. J. Harrap, BSc (*Grocers' Company Research Student*)

Medical Research Council Grantees:

Zeenat H. Gunja Smith, BSc, PhD

A. S. R. Donald, BSc, PhD

E. P. Adams, BSc

Janet Spencer, BSc

British Empire Cancer Campaign Grantees:

Jennifer J. Wells, BA, PhD

G. B. Hay, BSc

D. R. Body, MSc (*New Zealand National Research Fellow*)

BIOPHYSICS

†R. A. Kekwick, DSc (*Reader in Chemical Biophysics in the University of London*)

*J. M. Creeth, BSc, PhD, FRIC

†Professor N. H. Martin, MA, FRCP, FRIC (*Honorary Research Associate*)

C. G. Knight, MSc (*Medical Research Council Grantee*)

HONORARY MEMBERS OF INSTITUTE STAFF (RET'D)

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
Angela E. R. Taylor, BSc, PhD (*Trypanosomiasis Research*)
N. Mahoney, Dip Tech

BIOCHEMISTRY (ELSTREE)

*D. E. Dolby, BSc, PhD

PREPARATION and STUDY of VIRUS VACCINES (ELSTREE)

C. Kaplan, MB, ChB, MSc, Dip Bact, MCPath
H. G. S. Murray, MD
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*)

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

ADMINISTRATION

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Elstree Secretary and Estate Manager -	G. J. Roderick, BCom
Assistant Secretary - - - -	Barbara A. Prideaux
Assistant Accountant - - - -	E. J. H. Lloyd

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

Auditors:
Cooper Brothers & Co.
Abacus House, 3 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 29th, 1965

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the work of the Institute for the year 1964.

GOVERNING BODY

At a meeting held on 30th June, 1964, the Council reappointed Sir Charles Dodds, Lord Brain and Professor J. S. Mitchell as its representatives on the Governing Body until 31st December, 1965.

COUNCIL

At last year's Annual General Meeting two of the three retiring members of the Council, Sir Howard Florey and Sir Graham Wilson, were reappointed. The third retiring member, Sir Henry Dale, had expressed the wish that he should not be reappointed. Sir Henry's resignation was accepted with regret but the vacancy thus created was not filled.

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 Professor R. E. Glover, the representative of the Royal Agricultural Society, and Dame Harriette Chick and Sir Alan Drury, each a representative of the Members of the Institute.

MEMBERS

Dr. G. G. Meynell and Mr. L. Vallet accepted invitations to become Members.

STAFF AND STUDENTS

The Governing Body has noted with much pleasure that the University of Basel has conferred upon Professor W. T. J. Morgan the degree of Doctor of Medicine (honoris causa) and that Professor Morgan has also received the Conway Evans Award of the Royal College of Physicians for his valuable contributions to medical science.

It is also with much pleasure that the Governing Body records the nomination of Professor B. A. D. Stocker as one of the four " New Generation " Paul Ehrlich Prize-winners for 1964, for scientific work in the field of the recipient of the main prize, Dr. F. Kauffman of Copenhagen.

Dr. A. M. Lawn was appointed to take charge of the new Electron Microscopy Unit; Dr. Angela E. R. Taylor and Mr. N. C. Mahoney to the Serum Department; and Miss Barbara Dod to the Biochemistry Department.

The Governors offer their congratulations to Dr. W. J. Whelan, who resigned on 30th September, 1964, on his appointment as Professor of Biochemistry at the Royal Free Hospital School of Medicine. Dr. M. D. Pittam, Miss G. Sampson and Mr. J. Stone also resigned during the year.

Dr. Marjorie G. Macfarlane has also left the Institute, resigning on 31st March, 1965. A member of the Biochemistry Department since 1931 and a one-time representative of the Scientific Staff on the Governing Body, her loyal and distinguished services to the Institute will be much missed. She has the good wishes of the Governors and Staff.

Professor A. A. Miles lectured by invitation in October, 1964, at the 25th Anniversary Celebrations of the Spanish Higher Council for Scientific Research.

In April, 1964, Professor W. T. J. Morgan lectured in Paris on the occasion of the 59th Anniversary Meeting of the Société de Chimie Biologique, and took part as a Chairman and lecturer in a meeting on Immunochemie held in Mosbach, Germany, by the Gesellschaft für Physiologische Chemie. He lectured by invitation to the Swiss Chemical Society in Basel and acted as Chairman at a discussion on Blood Group Antigens during the Tenth Congress of the International Society of Blood Transfusion in Stockholm, in September.

Professor B. A. D. Stocker, in April, 1964, took part by invitation in a "Workshop" on "Chemical and Genetic Properties of Bacterial and other Cellular Surfaces at Royaumont, France (sponsored by the U.S.A. National Science Foundation). In May, 1964, he contributed by invitation to a Symposium on "Bacterial Structure and Replication" during the 64th Annual Meeting of the American Society for Microbiology, in Washington, D.C., and visited laboratories in New York, Stanford and Eugene, Oregon. In October, he took part by invitation in a Symposium on "Bacteriocins and Bacteriocin-like Substances", at the University of Göttingen, sponsored by the German Societe für Hygiene und Microbiologie.

In May, 1964, Dr. W. d'A. Maycock attended a meeting in Montpelier of the Subcommittee of Specialists in Blood Transfusion of the Public Health Committee of the Council of Europe, and in September took part by invitation in the World Health Organization European Symposium on Viral Hepatitis in Prague. He also attended the Tenth Congress of the International Society of Haematology and Tenth Congress of the International Society of Blood Transfusion in Stockholm.

As a W.H.O. consultant Dr. Kaplan visited India, Burma and Indonesia in February and March, 1964, to advise on smallpox vaccine production.

Dr. Marjorie G. Macfarlane took part by invitation in the Sixth International Congress of Biochemistry in New York in July, 1964, and since October, 1964, has visited, by invitation, the Commonwealth Serum Laboratories in Melbourne and other laboratories in Australia and New Zealand; she also lectured by invitation to the Biochemical Societies in Melbourne and Sydney.

Dr. W. J. Whelan spent June and July, 1964, at Iowa State University, U.S.A., as a visiting professor of biochemistry and took part by invitation in the Gordon Research Conference on Carbohydrates, New Hampshire, U.S.A., and the Carbohydrate Symposium of the Sixth International Congress of Biochemistry, New York. As Chairman of the British Chemical Society's Committee on Carbohydrate Nomenclature he attended an International Union of Pure and Applied Chemistry nomenclature conference in Basel in February, 1964.

Dr. J. M. Creeth attended the 6th International Congress of Biochemistry in New York, July, 1964, and visited other laboratories in the United States.

Dr. Winifred M. Watkins attended by invitation a meeting on "Chemical and Genetic Properties of Bacterial and other Cellular Surfaces", at Royaumont, France, in March, 1964, and gave the opening lecture at a discussion on Blood Group Antigens during the Tenth Congress of the International Society of Blood Transfusion in Stockholm in September.

Mr. L. Vallet attended the Tenth Congress of the International Society of Haematology and Tenth Congress of the International Society of Blood Transfusion in Stockholm in September.

Mr. G. S. Turner attended an International Symposium on measles and rubella vaccines at Lyons, France, in June, 1964.

Dr. M. W. McDonough attended the Sixth International Congress of Biochemistry in New York in August, 1964.

For the academic year 1964/5 there are eighteen postgraduate research workers at the Institute registered for higher degrees of the University. Three Ph.D. degrees were awarded to students during 1964.

DONATIONS AND GRANTS

Of the total grant of £35,000 from the Fleming Memorial Fund for Medical Research, announced in the last Report, £15,000 was received during the year. This has enabled the Institute to purchase and install, in a suite of laboratories specially converted for the purpose, a Philips EM 200 Electron Microscope.

Arthur Guinness, Son & Co., Ltd., continue their generous support of the Guinness-Lister Research Unit. The Governing Body records its appreciation 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 Scientific and Industrial Research for researches on the enzymic polymerization of monosaccharides, on chemically modified polysaccharides and on the carbohydrases of barley and malt; 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 separation and characterization of the antigenic components of *Bordetella pertussis*, on the synthesis of haptens and dextran-antidextran inhibitors, for studies of glycogen-debranching enzyme systems and on the structure of the amino-acid containing moiety in mucopolysaccharides; grants from the Ministry of Overseas Development in aid of research on the blood-meals of insect vectors of disease and on the immunology of trypanosomiasis; 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 Co., 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: Dr. W. R. Bailey, University of Delaware, U.S.A.; Dr. P. C. Basu, National Institute of Communicable Diseases, Delhi, India; Dr. C. Ferreora Crespo, Laboratorio Central de Bakteriologica, Lisbon, Portugal; Dr. Ko Ko Gyi, Burma Pharmaceutical Industry, Rangoon; Dr. Fatma Hassan, Serum and Vaccine Institute, Cairo, U.A.R.; Dr. M. J. Lewis, Public Health Laboratory Service, Colindale, N.W.9; Dr. F. Lopez-Bueno, Escuelo Nacional de Sanidad, Madrid, Spain; Dr. P. H. Makela, University of Helsinki, Finland; Dr. R. Narayanan, Safdarjang Hospital, New Delhi, India; Dr. Neimark, State University of New York, U.S.A.; Dr. J. Novotny, Institute of Sera and Vaccines, Prague, C.S.S.R.; Dr. R. J. Roantree, Stanford University, California, U.S.A.; Dr. J. F. Robyt, Iowa State University, Ames, Iowa, U.S.A.; Dr. El Sarky, Production Laboratories, Agouza, Cairo, U.A.R.; Dr. Sezginman,

Institute Central d'Higiene, Ankara, Turkey; Dr. W. Shepherd, Queen's College, Dundee; Dr. K. N. Slessor, University of British Columbia, Vancouver, Canada; Dr. K. V. Subbarao, Institute of Preventive Medicine, Hyderbad, India; Dr. G. Szabo, National Institute of Traumatology, Budapest, Hungary; Dr. S. M. Zan, Burma Pharmaceutical Industry, Rangoon.

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MICROBIOLOGY

TRYPANOSOMIASIS

Mr. Miller and Dr. Weitz continued the study of the antigens of variant strains of *Trypanosoma brucei*. As revealed by the precipitation and agglutination reactions of rat antisera, the soluble exoantigen of parent strains and of derived variant strains are strain specific. Antigens common to parent and derived strains are present in homogenates of whole organisms. There are indications that homogenates of a number of parent strains have in common antigens that are not present in the respective variant strains derived from them.

The immunity induced in mice by the exoantigens is likewise strain specific; the common antigens in homogenates, on the other hand, induce no corresponding cross-immunity. Homogenates of the one parent strain, cultivated *in vitro* by Dr. Pittam, contained antigens in common with the forms grown *in vivo*, but did not immunize mice against infection by the strain grown *in vivo*.

The significance of these findings in relation to other wild strains and to other species of trypanosomes is being investigated.

Miss Lanham continued the chemical analysis of trypanosomal antigens. The soluble extract of homogenates of *Trypanosoma vivax* fractionated on DEAE Sephadex or Sephadex G200 yielded fractions containing precipitinogens free of nucleic acid and with a characteristic ultra-violet absorption spectrum. Various specific precipitinogenic components were obtained by lowering the pH of the antigenic solution to pH 5.2. The immunogenicity of these components is under investigation.

WHOOPING COUGH BACILLUS

Isolation and Identification of B.pertussis Protective Antigen. Dr. Vincent continued his work on the isolation of the antigens of *Bordetella pertussis*. A fraction from the cell walls was obtained which is chemically similar to the immunogenic macromolecular material isolated from extracts of whole organisms (Report 1964). It was extracted by aqueous buffers from the cell walls of acetone dried organisms. Like the macromolecular material, it is not antigenically pure; besides the protective antigen, it contains agglutinogen, histamine-sensitizing factor and haemagglutinin. So far, further fractionation has decreased the protective activity. An apparatus for the continuous culture of *B.pertussis* was devised, to meet the demand for material for investigation.

The Bactericidal Action of B.pertussis Antisera. Most rabbit antisera to *B.pertussis* are bactericidal in the presence of complement, but only when they are used in low concentrations (Report 1962); at high serum concentrations the antisera are inactive. The inactivity may be due to interference with the attachment of the bactericidal antibody to the corresponding antigen on the cell wall, by another antibody which attaches itself either to the same antigen or to a neighbouring one, thereby blocking access of the " bactericidal " antibody. Drs. Jean and D. E. Dolby fractionated rabbit anti-pertussis serum on columns of the cross-linked dextran, " Sephadex ". The " 7S " fraction, consisting mostly of serum globulins, contained nearly all the inhibitory material, some bactericidal material and most of the other antibodies; but the " 19S " fraction, of high molecular weight proteins, was strongly bactericidal and only slightly inhibitory. This is in agreement with the results obtained by others with other antigen-antibody systems. However, the fraction containing mainly albumin also had considerable bactericidal activity, not associated with the " 19S " globulins. The investigation continues.

TYPHOID BACILLUS

Mr. Banks and Mr. Standfast continued their investigations into the laboratory assay of typhoid vaccines under trial in the field, as part of the collaborative study instituted by the World Health Organization. As there was little typhoid in the areas selected for field trial in Europe, the correlation of field results and laboratory assays has been much slower than was at first expected.

ACTINOMYCOTIC DERMATITIS IN SHEEP

Dr. Roberts concluded his studies (Report 1964) on mechanisms of infection and invasion of skin by *Dermatophilus congolensis*, and on the mechanisms, both humoral and cellular, of resistance of sheep and laboratory animals to infection by this organism.

ESCHERICHIA COLI INFECTION

Dr. Parish is investigating the factors responsible for pathogenicity of *Escherichia coli*, with the intention of developing a simple laboratory test to identify pathogenic strains. Strains of *E.coli* were tested for their ability to cause tissue damage in ligated segments of the small intestine of rabbits and chickens. In rabbits, two strains of *E.coli* isolated from enteritis in rabbits and two from enteritis in infants caused severe changes, but *E.coli* from healthy infants, from urinary infection in man, or enteritis in pigs, calves and chickens caused no significant change.

In young chickens *E.coli* from poultry dying of bacteraemia caused tissue damage at the site of injection and bacteraemia, whereas strains isolated from birds with severe enteritis, if pathogenic, caused tissue damage confined to the injected segment of the intestine. Some strains which were pathogenic in these tests after isolation, became harmless after repeated subculture, without demonstrable change of surface antigens. Only strains isolated from disease in chickens proved to be chicken pathogenic; *E.coli* from other species, even if pathogenic for rabbits, were innocuous.

Attempts are being made to detect enzymes that may be responsible for the damage to the intestinal mucosa and for the species specificity.

INHERITANCE IN BACTERIA

Genetics of Transmissible Drug-Resistance (R) Factors. An increasing proportion of newly isolated strains of *Salmonella* are resistant to an increasing number of antibiotics, because they carry transmissible drug-resistance (R) factors (Reports 1962–1964). Dr. Drabble infected genetically marked sublines of *S.typhimurium* strain LT2 with two new R factors: one (from Dr. G. Lebek, Munich) conferring resistance to chloramphenicol, tetracycline, streptomycin and sulphonamides, and also kanamycin-resistance, an R-factor trait not previously encountered; the other, a representative of the recently encountered R factors which confer penicillin resistance (from Dr. N. Datta, Postgraduate Medical School, Hammersmith, and Dr. E. S. Anderson, Enteric Reference Laboratory, Colindale), conferring resistance to tetracycline, streptomycin, sulphonamides and the penicillin group. The pattern of P22 transduction of resistance traits from the Lebek factor in general resembled that previously observed with other R factors. The newly encountered kanamycin trait was never co-transduced with tetracycline resistance, and usually accompanied the streptomycin, sulphonamide and chloramphenicol traits; but its occasional separate transmission showed it to result from a separate " gene " in the R factor. The transduction pattern of the other new R factor differed; transduction occurred at a higher rate, the tetracycline trait usually accompanied the other traits instead of being separately transduced, and the transduced resistance traits were transmissible by conjugation. The penicillin-resistance factor also conferred resistance to the related cephalosporin antibiotics.

Genetics of Flagellar Characters in Salmonella typhimurium. Mrs. Pearse continued mapping the *fla—H1* region of the chromosome. Transductional crosses extended the fine-structure map (Reports 1959–1961, 1964) to give:—

— *fla*(*58* etc.) — *H1* — *Ah1* — *nml* —

(*fla*, a gene needed for production of flagella; *H1*, gene specifying amino-acid sequence of phase-1 flagellar protein; *Ah1*, " operator " gene controlling activity of adjacent *H1*; *nml*, gene determining methylation of lysine in flagellar protein). Several *mot* loci, determining locomotor function of flagella, and another *fla* locus, *fla-54*, were shown to be linked to the *H1* group. Coarse mapping, using

Hfr or *col*-factor conjugation, indicated the order:—

— *try* — (*fla-54*, *mot*) — (*H1* group) — *his* — etc.

with the orientation of the H1 group uncertain.

Further experiments on the inhibitory effect of anti-flagellar sera on the production of trails through abortive transduction, either of motility (Report 1955, 1964) or of a new flagellar antigen, led to the following conclusions.

(1) The fragments of bacterial chromosome carried by transducing particles are not as previously supposed of determinate composition; some of them that carry *mot*$^+$ (or *fla–54*$^+$) carry also *H1* and some do not. (2) The activity of *H1* is determined by the " state " of *H2* (determinant of phase-2 flagellar antigen) through a non-chromosomal mechanism. The phenomenon of flagellar antigen phase variation can now be interpreted in terms of a repressor locus within the *H2* " operon ", which, so long as this operon is active, produces a cytoplasmic repressor substance which acts on the *H1* operator site, *Ah1*, and thereby prevents the activity of *H1*.

Genetics of Somatic Antigens in Salmonella. Dr. Helen Makela, of Helsinki, continued hybridization of *Salmonella* with different somatic (O) antigens, using her Hfr or F′ lines (highly fertile " males ") to overcome inter-species infertility (Report 1964). When *Salmonella* with O antigens 4,5,12 were crossed with *S.enteritidis* (O antigens 9,12) factors 4 and 9 behaved as alleles of a locus near *his*. Factor 4 reflects the presence of abequose in the S side-chain of somatic polysaccharide, factor 9 that of tyvelose. Several different enzymes, and so several different genes, must be needed for their synthesis and attachment and it is inferred that several genes concerned in the assembly of the S side-chain and in synthesis of some of its sugar precursors are grouped near *his*. The *rouB* gene (Report 1963, 1964) seems to be part of this gene cluster. The factor 5 of some 4,12 species reflects the presence of acetyl in the repeat unit of the S side-chain, determined by a gene near *his* (Report, 1962); none of the recombinants with factor 9 reacted with anti-5 serum, though some probably had the *05*$^+$ gene.

In Dr. Makela's crosses of group B *Salmonella* with group C1 (O antigens 6,7) or C2 (O antigens 6,8) many recombinants selected for possession of the *his*$^+$ gene of the male were intermediate in their behaviour between S and R and resembled a recently recognized class of " semi-R " mutant in strain TL2. They probably have all the *his*-linked cluster of genes for synthesis of the specific sugar components of 4,12 side-chains but lack a gene for attachment of further 4,12 repeat units after the first.

Genetics of Phage-Resistance and Roughness in Salmonella typhimurium. Mr. Wilkinson isolated from *S.typhimurium* strain LT2 several " semi-R " mutants which resembled R (rough) mutants of classes *rouA* and *rouB* (Reports 1962–1964) by their resistance to the S-specific phage, P22; but with cultural and serological properties intermediate between S and typical R strains. Their pattern of susceptibility to various anti-R phages divided them into two classes, *rouC* and *rouD*. The *rouC* locus was found to map between *gal* and *try*. The semi-R character of some of Dr. Makela's group B × group C hybrids (above) probably results from their lack of the *rouC*$^+$ gene of group B. Dr. Gemski mapped the *rouD* locus near *xyl*, so that it is perhaps adjacent to *rouA*. Mutants lacking the enzyme phospho-mannose-isomerase are R because they cannot make the mannose component of the S side-chain of somatic lipopolysaccharide. Mr. Wilkinson and Professor Stocker found the gene affected in such mutants to lie between *gal* and *try*. Similarly, mutants lacking the enzyme UDP galactose-epimerase are R (Report 1962) since they cannot make the galactose component of lipopolysaccharide. Mr. Wilkinson found them to have a characteristic

pattern of susceptibility to anti-R phages; this pattern was encountered also in a new class of phage-resistant mutant, able to ferment galactose and therefore presumably not deficient in the epimerase. The site of mutation in several such mutants, which are presumed to lack a transferase involved in synthesis of lipopolysaccharide, was mapped between *xyl* and *metA*.

Genetics of Serum-Sensitivity in S.typhimurium. Dr. R. J. Roantree (Stanford Medical School) made a genetic investigation of a strain of *S.typhimurium* which though S (smooth) resembled R (rough) mutants by its sensitivity to killing by normal serum (Report 1964). In crosses of nutritionally exacting mutants of this strain to one of Dr. Makela's *S.abony* Hfr (highly fertile " male ") strains (Report 1964), serum sensitivity was inherited as though determined by a gene in the *HI —his* region of the chromosome—i.e. near the complex locus determining the structure of the specific side-chain of the S antigen.

Properties of Colicine Factors. Dr. Lewis and Professor Stocker investigated colicines (antibiotic proteins produced by bacteria) of group E (Report 1959, 1960). Amongst some thousand *Salmonella typhimurium* strains tested seven produced colicines identified as E1, which, in transmissibility and ability to interfere with the "epidemic spread" of a *col I* factor, fell into two groups, both distinguishable from the " type " E1 factor previously studied. E2 colicines were produced by five *S.typhimurium* strains and all were alike; though in neutralization by E2 antisera and by a cross-immunity test they differed both from the previously studied colicine E2 (Report 1959) and from two further, distinguishable, E2 factors carried by " standard " E2-colicinogenic strains of *Escherichia coli*.

Professor Stocker and Dr. W. R. Bailey (of the University of Delaware) investigated colicines produced by *Shigella sonnei* strains of various " colicine types " and confirmed the unpublished observation of Professor P. Fredericq, of Liege, that both type 2 and type 4 strains produce colicine I. There were two sorts of colicine I factor, distinguished by the immunity they confer on sensitive strains. The I factor previously studied (Reports 1959–1961) is of the sort characteristic of type 4 *S.sonnei*. Some type 2 and type 4 strains produce an additional colicine, previously undetected, which is probably the same as the unclassified colicine produced by type 7 strains.

Properties of Salmonella typhimurium Strains from Natural Sources. Dr. M. J. Lewis and Professor Stocker tested various characters of c. 1500 strains of *S.typhimurium*, previously phage-typed by Dr. E. S. Anderson and tested by Dr. Naomi Datta for R factors. About 6% of the strains were colicinogenic, nearly all of these producing one or other of the newly recognized sorts of colicine I. About 8% were nutritionally exacting strains, most of them requiring nicotinic acid (Report 1959) and of phage-type 1a or 1a variety 2. The growth of some strains on a defined medium was inhibited by meso-tartrate. The very prevalent phage type 1a was divided into four groups by nicotinic requirement, meso-tartrate sensitivity and inositol fermentation—a subdivision perhaps of epidemiological value. For example, during simultaneous type 1a outbreaks in Manchester, Leeds, Bradford and Wakefield all the Manchester strains were of one sub-group, and all the rest of another, proving that at least two sources of infection were involved, not only one as suspected.

BACTERIAL PHYSIOLOGY

Somatic Polysaccharide of Semi-R Salmonella. The somatic lipopolysaccharides (LPS) of semi-R *Salmonella* (*rouC* and *rouD* mutants, and semi-R recombinants from group B × group C crosses) were investigated in collaboration with Dr. H.

Nikaido and his colleagues at Massachusetts General Hospital, Boston. All three sorts of semi-R LPS, unlike that of *rouA* and *rouB* mutants (Report 1964), contained the S-specific sugars mannose, rhamnose, and abequose, but only in very small amounts. Drs. Makela and Nikaido showed that the semi-R bacteria and their LPS possessed antigen factor 04, characteristic of S *Salmonella* of group B. The LPS of *rouC* strains perhaps has a normal number of side-chains, in each of which the R stub is covered by only a single S (smooth) repeat unit (probably comprising one unit each of glucose, galactose, mannose, rhamnose and abequose) in consequence of the absence, through mutation or recombination, of a " transferase " enzyme needed for elongation of the 4,12 S side-chain. The defect in *rouD* LPS is probably different from the *rouC* defect—despite the similarity of the quantitative sugar composition of *rouC* and *rouD* LPS.

Environmental Control of Phage-sensitivity and LPS Composition. Mr. Wilkinson investigated the phage susceptibility pattern of mutants lacking UDP galactose-epimerase and, in collaboration with Dr. Mary Jane Osborn of Albert Einstein College of Medicine, New York, of mutants lacking phosphomannose-isomerase. In each class growth of a mutant on a medium containing the relevant sugar resulted in loss both of resistance to the S-specific phage P22, and of sensitivity to various anti-R phages; as is to be expected if the mutants, possessing all the necessary enzymes for assembly of the side-chain of somatic lipopolysaccharide, produce a normal lipopolysaccharide when the block in synthesis of its galactose or mannose component is circumvented by provision of the sugar in the medium. The new class of phage-resistant mutant resembling galactose-epimerase mutants in phage-resistance pattern (above) but differing from them by possession of this enzyme (as inferred from their ability to ferment galactose) differed also in that their phage pattern was unaffected by provision of galactose. The inference that the abnormal lipopolysaccharide of these mutants results from lack of a sugar-transferring enzyme, not from block in synthesis of any sugar " building block ", was confirmed by enzymic assay in Dr. Osborn's laboratory.

Properties of Salmonella Flagellar Protein. Dr. McDonough prepared flagellar protein of antigenic type *1,2* for examination in the ultracentrifuge by Dr. Creeth. Specimens dialysed against buffer after exposure to 8M urea appeared homogeneous in the ultra-centrifuge. The Archibald approach-to-equilibrium method gave molecular weight values of 39,000 and 39,500, and calculation from measurements of sedimentation and diffusion coefficients a value of 41,500. The mean of these estimates, c. 40,000, is slightly higher than the minimum molecular weight previously calculated from the amino-acid composition (Report 1964); it accounts satisfactorily for the number of peptide spots in tryptic digest maps.

Dr. McDonough completed his series of amino-acid analyses of allelic forms of *Salmonella* flagellar proteins (Report 1964). To investigate the amino-acids at the carboxy-terminal end of flagellar protein of antigenic type *i*, denatured protein was incubated with carboxy-peptidase A, which removes amino-acids one by one from this end. The result suggests that the sequence at this end is:— (serine, leucine) — (alanine, valine, serine) — (leucine, threonine) — isoleucine—.

The re-aggregation of isolated *Salmonella* flagellar protein was investigated by Dr. McDonough. Neutralization, ammonium sulphate precipitation and dialysis of flagellar protein of antigenic type *1,2* in solution as a monomer at pH 2.0 gave an opalescent solution which on centrifugation at 105,000 G yielded a gelatinous-looking pellet. On electron-microscopy, by Dr. J. Lowy (Medical Research Council Biophysics Research Unit, King's College, London), the material proved to consist of filaments of the same diameter and fine structure as intact flagella. The few which were several microns long had the typical undulations of

intact flagella. Similar results have been reported with other species. It seems that the helical filamentous form of bacterial flagella is implicit in the structure of the molecules of the protein of which they are composed.

Capsule Formation by Bacillus anthracis. Dr. G. G. Meynell, with Dr. Elinor Meynell (Medical Research Council Microbial Genetics Research Unit, Hammersmith Hospital), continued the study of the biosynthesis of the capsule of the anthrax bacillus. Virulent strains form capsules only when provided with a minimum concentration of bicarbonate (corresponding at pH 7.4 to 5% CO_2 in the atmosphere), but this minimum concentration is raised by the presence of fatty acids (Report 1964). They confirmed the old observation that virulent strains produce mutants which form capsules even on incubation in air. The capsular material made by the mutants seems to be chemically like that of the parent, i.e., the mutants differ only in not requiring extra bicarbonate for its production. They are perhaps "constitutive" mutants but as they resemble the parent strain by failing to form toxin unless provided with CO_2 during growth, it is unlikely that their "constitutive capsulated" character results from an acquired ability to utilize and respond to some other component of the medium instead of bicarbonate. When "constitutive capsulated" mutants were incubated on bicarbonate charcoal agar with added CO_2 many failed to grow, i.e., they are CO_2-sensitive. Fatty acid prevented this growth-inhibitory effect, as well as the capsule-inducing effect, of CO_2. Two sorts of CO_2-resistant mutant were isolated from CO_2-sensitive mutants: one is apparently a revertant to the parental form; the other has lost the ability to form capsules in any environment tested. Some, but not all, non-capsulated mutants isolated from CO_2-sensitive strains had also become CO_2-resistant.

Bacterial Multiplication in Infected Animals. Dr. Meynell continued his studies (Reports 1961–1964) on model microbial infections. Certain discrepancies between observation and theoretical predictions have now been resolved by considering a microbial infection as a "birth-death" process—that is, a process whose outcome is determined by successive chance events which, in microbial infections, are the chances per organism of dividing or of being killed in each interval of time after inoculation. The mathematical development of this model is due to Dr. Trevor Williams, now at Duke University, North Carolina, who is collaborating with Dr. Meynell and Professor Peter Armitage, of the London School of Hygiene and Tropical Medicine, in an analysis of the data from experimental infections and in the examination of epidemiological reports. The latter provide useful data, because many quantitative characteristics of human infections, e.g. microbial growth rates in man, can on this model be deduced from the distribution of individual incubation periods in epidemics.

MYCOPLASMA INFECTIONS IN MAN

Dr. Lemcke continued her investigations on the antigens of *Mycoplasma hominis* type 1, which is frequently associated with human genital infections. A mixture of soluble antigens not sedimenting at 100,000G and giving the full complement of precipitation lines on Ouchterlony plates with homologous antiserum (Report 1964) was obtained for chromatographic analysis. Serological tests after heat and periodate treatment of the soluble antigens suggested that they were mainly protein.

The survey of mycoplasma in the eyes of neonates and the genital tracts of their parents, undertaken as part of an investigation by Professor Barrie Jones (Institute of Ophthalmology) of the aetiology of ophthalmia neonatorum (Report 1964), was concluded. In five of ten families, mycoplasmas were isolated from the genital tract of one or both parents, but not from the eyes of the infants. In

contrast, four infants were found to be infected with TRIC (trachoma/inclusion conjunctivitis) agent.

Mycoplasmas are reported to have been isolated from tissue cultures inoculated with material from patients with leukaemia and other neoplastic diseases. The necessity of identifying such mycoplasmas, in view of the unknown aetiology of these conditions and the frequency with which tissue cultures are contaminated with mycoplasmas, is obvious. A mycoplasma isolated by Dr. L. Hayflick (Philadelphia) from tissue cultures inoculated with human hemangioma tissue was identified by Dr. Lemcke as belonging to the species *M.hominis* type 1, an organism recognized as a frequent contaminant of tissue cultures. A mycoplasma isolated from human embryo kidney cells in which bone marrow from patients with leukaemia had been passaged by Dr. G. Negroni (Imperial Cancer Research Fund) proved to be distinct from sixteen serological types or species of mycoplasma, including all the known types of human origin. However, it had some antigenic relationship to *M.pulmonis*, which frequently occurs in the respiratory tract of rats and mice. In view of these results, the significance of these mycoplasmas in human neoplastic disease seems doubtful, but final conclusions must await attempts to isolate mycoplasmas directly from neoplastic tissues.

With the increasing interest in mycoplasmas and in the absence of a reference laboratory for these organisms, assistance was given during the year to a number of other laboratories in the isolation and identification of mycoplasmas from man and animals.

VIROLOGY

TRACHOMA AND INCLUSION BLENNORRHOEA (CONJUNCTIVITIS)

Growth Characteristics. After repeated passage in chick embryos, some strains of trachoma/inclusion conjunctivitis (TRIC) agents acquire the property of killing the embryos faster with a given dose than the parent strain (Report 1964). Apparent differences in the growth rates of slow and fast killing strains were observed in the period between inoculation and death of the embryo; but since many cycles of intracellular replication take place within this period, the differences might not reflect differences in multiplication rate, but variations in the rate of absorption before each cycle begins, or in the rate of release after each is complete. To investigate this problem, Dr. Reeve and Dr. Taverne obtained growth curves in terms of egg lethal doses for various strains multiplying in the yolk sac. With three fast killing strains, new virus appeared 24 hours after infection and continued exponentially until about 60 hours, when diminution of the amount of infective virus indicated that one growth cycle was complete. During this first cycle the virus multiplied 100-fold. With three slow killing strains new virus was first detected 26 hours after infection; the subsequent rate of increase was similar to that of the fast killing strains, but the highest yield of infective virus was only 5 times greater than the inoculum. It thus appears that both varieties of virus multiply at the same rate, but that over a period of several cycles the fast killing strains yield the critical amount necessary to kill within a comparatively shorter time.

Whereas the fast killing strains multiply readily in cell cultures, slow killing strains do not; American workers have, however, shown that at least one cycle of growth can be induced by centrifuging the virus into the cells. Dr. Reeve and Dr. Taverne are using a specially devised centrifuge to study the factors governing growth of various strains in cell cultures. To determine whether increased virulence for chick embryos is a general property of strains that grow readily in cell culture, they are studying a number of such strains obtained from other laboratories.

Preservation of TRIC Agents. Mrs. Hart continued attempts to preserve the infectivity of TRIC agents grown in cell cultures, which in this form are highly labile. This work is important both for maintaining stock cultures and for preserving live vaccines for field use. She showed that the concentration of dimethyl sulphoxide previously used to preserve infected cell suspensions in the frozen state (Report 1964) can be decreased from 10% to 2% (v/v); and that of many other substances tested for preservative action, sodium glutamate (5% w/v) gave similar results when added to infected cells before slowly freezing them to −60° C. Unlike dimethyl sulphoxide, sodium glutamate can also be added before freeze-drying. By slowly freezing in the presence of this additive before drying from the frozen state, it is possible to preserve about 3% of the original infectivity.

Immunological Studies. Miss Graham continued her researches on the relationship between various TRIC agents in terms of neutralization tests (Report 1964). Cross tests in HeLa cells with unabsorbed sera from immunized rabbits revealed minor differences between several strains, but they were too small to serve as a basis for serological classification.

Absorption of rabbit antiserum with group antigen, prepared by desoxycholate treatment of elementary bodies, removed all complement-fixing group antibody without affecting the neutralizing capacity of the serum. This result implies that the antigen responsible for eliciting neutralizing antibody is not the same as the complement-fixing group antigen. Further experiments with adsorbed sera are in progress.

Miss Graham showed that large doses of TRIC agent given intranasally to mice cause pneumonitis and death within 3 days; with sublethal doses there is 100-fold multiplication of virus by the 4th or 5th day. The finding that the lung lesions can be prevented by prior vaccination is potentially important in relation to studies of immunity. Preliminary work with this system suggests that the mutation of strains from the slow to the fast killing variety (see above) may be accompanied by an antigenic change.

Dr. Blyth is studying the mechanism of immunity to TRIC agents, using the guinea pig as host; should this animal prove suitable it will serve as a useful alternative to the baboon, which is much more expensive and difficult to handle. The first step is to induce an easily recognizable and reproducible infection; subconjunctival injection of the 4 strains so far tested causes hyperaemia and oedema of the lid, associated with a pronounced polymorphonuclear response in the conjunctiva, and with the occasional presence of intracellular bodies resembling inclusions. These signs persist for about 3 days; during this time the infecting agent can readily be isolated from the conjunctiva but is rarely detectable by the 7th day.

As part of this research, Dr. Blyth is investigating the reaction of guinea pigs to intracutaneous injection of TRIC agent (Report 1963). It can be isolated from skin lesions for up to 3 weeks after injecting only 100 viable organisms, implying that multiplication has taken place. The addition of hyaluronidase to the inoculum increases the areas of erythema, but TRIC agent cannot then be isolated later than 1 week after injection.

Trachoma Vaccine. Dr. Collier and Dr. Blyth extended their researches on the immunogenicity of experimental vaccines. It was previously shown (Report 1964) that the LB4 strain grown in cell culture protected baboons against conjunctival infection with the same strain. In this experiment the animals were challenged 1 month after the last dose of vaccine. Eleven months later, however, the same animals failed to resist a second challenge.

It is important to know the extent to which various strains of TRIC agent confer immunity against each other. Cross-protection tests with stock strains are limited, because repeated passage in chick embryos may impair pathogenicity for the conjunctiva. Nevertheless, one test was made with a strain of trachoma (G187) and one of inclusion conjunctivitis (LB4). The respective vaccines contained the same amount of live virus, and the challenge inocula of the 2 strains were similarly matched. Neither strain protected baboons against conjunctival infection with the other; and although, as in previous experiments, strain LB4 protected against itself, the slight protection afforded by the G187 vaccine against homologous challenge was statistically insignificant. This confirms a previous finding that strain G187 appears to be a relatively inefficient immunogen.

Investigations Overseas. Under Dr. Collier's direction the Medical Research Council's Trachoma Research Unit has virtually completed its investigations of trachoma and allied infections in a Gambian village; the results are shortly to be published in the Council's Special Report Series. In a field trial of trachoma vaccine (Report 1964) a live antigen given with a mineral oil adjuvant failed to influence the trachoma attack rate in young children. In collaboration with the Institute of Ophthalmology, London, the University of Teheran, the Lister Institute and two British pharmaceutical firms, further field trials are soon to be started in Iran; the antigens to be tested will be more highly concentrated and purified than that used in the Gambia.

SHOPE FIBROMA

In collaboration with the Chester Beatty Research Institute, Dr. Placido de Sousa continued to examine the influence of carcinogens on Shope fibroma virus in rabbits and in rabbit kidney cell cultures. In a study of the influence of chemical agents on the benign growths normally induced by this virus he found that treatment of infected rabbits with dimethyl dibenzanthracene or methylcholanthrene resulted in malignant tumours (sarcomata), but the first of these carcinogens alone induced tumours in normal animals. The role of virus in this response is under investigation.

Dr. Placido de Sousa also showed that another carcinogen, *N*-hydroxyurethane, seems to depress to some extent the growth of fibroma virus in rabbit skin, but has a much more pronounced inhibitory action on this agent growing in cell cultures. Inhibition is almost complete when the carcinogen at 0.01 M concentration is added to the cells at periods up to 24 hours after infection, and is still demonstrable up to the 30th hour. Preliminary treatment of either cells or virus with this compound does not result in viral inhibition, which seems to take place only during actual multiplication. Of a number of other urethane derivatives tested in this way, only hydroxyurea, dihydroxyurea and hydroxymethylcarbamate behaved similarly. Thiosemicarbazones, which are active against other pox viruses, failed to inhibit fibroma virus in the test system used.

VACCINIA AND VARIOLA VIRUSES

Mr. Turner and Dr. Kaplan continued their investigation of the inactivation of vaccinia virus by the photodynamic action of methylene blue. They confirmed, quantitatively, many of the findings of older workers. The mechanism of virus inactivation is oxygen dependent, but enzyme studies failed to demonstrate the participation of peroxides in the reaction. Water appeared to be necessary for the photodynamic activity, since even intense illumination of dried vaccinia virus with adsorbed methylene blue did not reduce the infectivity. As regards the site of the photodynamic inactivation of vaccinia virus, significant damage appears to be done to the nucleic acid, but virus protein may also be

involved. Experiments on genetical reactivation of inactivated vaccinia virus strongly suggested the participation of protein; photoinactivation therefore may affect both sites in the virus particle. Inactivated vaccine of proved immunogenicity in rabbits was tested by Dr. K. Cantell of the Finnish State Serum Laboratory in 133 young men without adverse reactions; the antibody content of their sera is now being assayed.

Dr. Murray demonstrated changes in the antibody formed in rabbits during immunization with vaccinia virus. Antibody appearing early after infection was less avid than later antibody and had some of the characteristics of 19S γ globulin. He also began to investigate the growth of vaccinia virus in cultures of calf kidney cells. Cultures infected with more than 1 infectious unit of virus per cell underwent substantial cytopathic change within 24 hr.; but little infectious virus could be recovered from the cultures.

Mr. Robinson continued his studies on the purification of vaccinia virus by phase separation. The counter-current distribution of vaccinia virus in a 2-phase system of higher polymers provided small amounts of highly purified virus for research purposes. He also built a stable-flow electrophoresis apparatus for the continuous separation and concentration of vaccinia virus from large volumes of relatively crude suspensions. A single electrophoretic treatment of crude suspensions from sheep pulp concentrated infectivity about 10-fold and reduced protein from 2–5 mg. to 15–50 µg./ml. A combination of phase separation and stable-flow electrophoresis may be expected to provide virus pure enough for a detailed biochemical examination.

The production of dried smallpox vaccine was considerably mechanized during the year, thus greatly increasing the potential output. A new formulation of glycerolated (liquid) smallpox vaccine was prepared and tested. The new product is more economical to produce, more uniform and of greater purity than the traditional vaccine lymph. The first few batches issued on behalf of the Ministry of Health gave very satisfactory results in the field. Laboratory and field tests continue.

IMMUNOLOGY AND EXPERIMENTAL PATHOLOGY

SEROLOGICAL IDENTIFICATION OF BLOOD MEALS

The serological identification of the blood meals of mosquitoes and tsetse flies has continued. The results of world wide studies in conjunction with the World Health Organization, on the feeding habits of *Anopheles*, related to the control of malaria, are being correlated. A study of the feeding habits of *Culicines* in relation to filariasis was started in Ceylon for the World Health Organization, under Dr. M. H. M. Abdulcader. In Trinidad the survey of feeding habits of bats possibly concerned with rabies transmission continues.

Special studies in progress with the Department of Tsetse Control in Southern Rhodesia are concerned with the elimination of the favourite hosts of *Glossina morsitans*. The results indicate that these tsetse flies are probably slow to adapt themselves to new hosts. In Nigeria, Dr. D. A. T. Bawldry, of the West African Institute for Trypanosomiasis Research, has collected large numbers of blood meals of *G.tachinoides*; these flies prove to have a marked preference for domestic pigs in certain regions, a fact of possible importance in the epidemiology of sleeping sickness in these areas.

ANTITOXIN PRODUCTION

Dr. D. E. Dolby has continued his studies on the digestion of β_2 and γ globulin antitoxins by pepsin and papain and the comparison of the breakdown products by immunological methods (Report 1964), and has applied the same

techniques to the " heavy " and " light " polypeptide chains obtained from these globulins by reduction of the sulphydryl groups and fractionation of the acidified product on Sephadex. As has been found previously, the " light " chains of the β_2 and γ globulins appear to be identical, but the " heavy " ones differ; the effect of enzyme treatment of the latter is being investigated.

Dr. Dolby has also tried to apply to fresh and freeze-dried rabbit sera his method for fractionation of the antitoxic globulins of horse sera (Report 1962). Although pure γ globulin is easily obtained he has not yet succeeded in preparing any other globulin free from contaminating proteins.

ANAPHYLAXIS

An Eosinophil-stimulating Factor in Anaphylaxis. Dr. Parish continued his attempt to isolate the factor causing the sudden rise in the number of eosinophils in the peripheral blood and some organs in anaphylaxis (Report1964). Identification of this factor may be of importance in the examination of the post-mortem body to detect anaphylaxis as a possible cause of death. It was confirmed that the factor can be extracted from anaphylactic lung. The eosinophilia was not due to any histamine in the extract.

Antigen-antibody complexes, made with antibody from rabbit, guinea-pig or man and the appropriate antigen, when given intraperitoneally in normal guinea-pigs induced a great increase in the number of circulating eosinophils. Antiserum alone, or with unrelated antigens, were ineffective.

Since the anaphylactic lungs implanted to elicit eosinophilia might also contain inhaled environmental antigens for which antibodies were by chance present in the lungs, and so induce eosinophilia by means of the resultant antigen-antibody complexes, the experiments were repeated with diaphragm from anaphylactic guinea-pigs. The number of eosinophils in the recipients remained constant or slightly increased, but no eosinophils remained following implantation of non-anaphylactic diaphragm.

Pieces of lung taken post mortem from infants were implanted in guinea-pigs. Eosinophils were present in the majority of animals with implants from infants believed to have died of anaphylaxis. None was present after implants from infants dying of infections or congenital abnormalities.

The lung implantation technique in guinea-pigs thus provides some evidence of recent anaphylaxis in human tissues.

CYTOPHILIC ANTIBODY AND OPSONINS

In order to compare the efficiency of cytophilic antibody and opsonin to potentiate phagocytosis and limit bacterial infection, Dr. Parish fractionated mouse antisera to bacterial polysaccharide and to bovine γ globulin on DEAE cellulose columns. Two main fractions were obtained, identified as γ_1 and γ_2 globulins. The γ_1 fraction contained the antibody capable of sensitizing skin to passive cutaneous anaphylaxis. The γ_2 fraction contained the greater concentration of agglutinating antibody. Subfractions of γ_2 globulin prepared by electrophoresis in agar gel were tested on monolayers of mouse macrophages obtained from the peritoneal cavity. The cytophilic antibody and opsonin in γ_2 fractions were completely separable.

Antigen particles first adhered to, and sometimes were ingested by, macrophages treated with cytophilic antibody. Adherence still occurred after killing the macrophages so treated by drying. Prior exposure of antigen to cytophilic antibody did not enhance phagocytosis. Both macrophages and antigen can adsorb the antibody from serum fractions.

In contrast, opsonin did not sensitize the macrophages to ingest the unsensitized antigen particles, but was highly effective in sensitizing the antigen to ingestion by unsensitized macrophages. Opsonin caused no adherence of particles on the surface of killed macrophages. It was readily absorbed from serum fractions by antigen, but not by macrophages.

It was possible to elute the opsonin from the antigen, but insufficient cytophilic antibody could be eluted from macrophages for further tests. This may be due to the low concentration of cytophilic antibody as compared with opsonin.

The relative ability of these two antibodies to limit bacterial infection is under test.

MECHANISMS OF INFLAMMATION

Vascular Changes in Injury. Dr. Wells continued his studies of vascular changes induced by various forms of injury. The single, early initial phase of venular change in rat tissue after mild thermal injury, described last year (Report 1964) proved to be double, the immediate initial peak response being followed within 20 minutes by a second smaller peak response. The delayed capillary response could be made to start during the venular response by increasing the degree of injury.

The studies on the pattern of injury induced by *Cl.welchii* toxins was completed, and those on the pattern in *Staph. aureus* infection extended. In this infection there are two venular phases, maximum at about $\frac{1}{4}$ and 5 hours respectively, followed, at about the twelfth hour, by a long-lasting capillary phase, apparently associated with the onset of massive tissue leucocytosis, in which the circulating colloidal carbon used as an indicator of vascular damage was not only deposited in the vessel walls, but also extravasated.

The biphasic nature of the response of hypersensitive animals to tuberculin was confirmed; attempts to demonstrate vascular damage by the carbon-deposition method, however, were negative in the rat.

The genetically "hairless" mouse was explored for skin tests of vascular injury. The results with thermal injury largely confirm those in the rat. As regards injected permeability factors, in comparison with the rat cremaster, the mouse dorsal skin is much more susceptible to histamine and much less so to serotonin. Vascular reactions to injury in the mouse ear also have the same general pattern.

Attempts were made by Miss Mason to confirm the isolation—described by Dr. H. Hayashi (Kumamoto Medical School)—from mature Arthus lesions in the rabbit of a cysteine-dependent protease capable of inducing a delayed permeability response.

Except for the euglobulin fractions, all protein fractions from inflamed and normal skin contained similar permeability-increasing activity. The euglobulins derived from pathological skin differed from those in normal skin in containing activity susceptible to protease inhibitors. Unlike Hayashi's protease this factor was heat stable, had no caseinolytic activity and was not cysteine-dependent. None of the protein fractions induced a delayed phase reaction in the guinea-pig.

Permeability Proteins and Kininogenase Activators. The PF globulin of human plasma was distinguished from plasma kininogenase and identified as an activator of the kinin-releasing system. As such, it bears a resemblance to Hageman factor and investigations were made to distinguish this clotting factor from the globulin PF. The evidence so far is inconclusive.

ELECTRON MICROSCOPY UNIT

The electron microscope (Philips EM 200) and the ancillary equipment required for specimen preparation was installed and a number of investigations were commenced in collaboration with various other departments. These include:—

With Professor Stocker: some aspects of flagella structure.

With Dr. Gemski and Professor Stocker: the mechanism of conjugation in bacteria.

With Dr. Meynell: electron microscope examination of the capsule of *Bacillus anthracis*.

With Dr. Blyth and Dr. Taverne: particle counts of preparations of the agent of Trachoma.

BIOCHEMISTRY

THE HUMAN BLOOD GROUP SUBSTANCES

As a result of their earlier investigations Mrs. Rege, Dr. Painter, Dr. Watkins and Professor Morgan established from an examination of the partial acid hydrolysis products the probable sequence of the sugar residues in the main chains of the serological determinant structures of the A, B, H and Le^a blood-group specific substances (Reports 1962, 1963). It was pointed out, however, that one important sugar residue, L-fucose, was readily liberated as free sugar because of the sensitivity of its glycosidic bond and that, even under the mild acid conditions employed, the di- and oligosaccharides obtained were largely devoid of L-fucose. Since it was believed, on the basis of the results of inhibition studies, that L-fucose was involved in both H and Le^a specificity, it was not surprising to find that the sugar fragments obtained from H and Le^a substances were serologically inactive. In order to establish the position and the nature of the linkage of the L-fucose residues in the carbohydrate chains it was therefore necessary to carry out similar studies on fragments obtained as a result of alkaline degradation with methanolic triethylamine. Under these conditions the L-fucose units are retained to a considerable extent, especially those attached near the non-reducing ends of the specific determinant structures, and the fucose-containing fragments were serologically active.

It was possible to isolate from A substance, 2-*O*-α-L-fucopyranosyl-3-*O*-(*N*-acetyl-α-D-galactosaminopyranosyl)-D-galactose, and the tetrasaccharide

O-α-L-fucosyl
|
↓(1→2) (1)

O-α-*N*-acetyl-D-galactosaminyl-(1→3)-β-D-galactosyl-(1→4)-*N*-acetyl-D-glucosamine

From B substance the trisaccharide 2-*O*-α-L-fucopyranosyl-3-*O*-α-D-galactopyranosyl-D-galactose, and the tetrasaccharide

O-α-L-fucosyl
|
↓(1→2) (2)

O-α-D-galactosyl-(1→3)-*O*-β-D-galactosyl-(1→4)-*N*-acetyl-D-glucosamine

were obtained. Similar structures in which the second glycosidic linkage in each specific substance is (1→3) in place of (1→4) are also believed to be present.

Similarly, from the alkaline hydrolysis products of H substance, 2-*O*-α-L-fucosyl-D-galactose, and the trisaccharide

O-α-L-fucosyl
|
↓(1→2) (3)
O-β-D-galactosyl-(1→4)-*N*-acetyl-D-glucosamine

were recovered and identified. A corresponding structure in which the first glycosidic linkage in the main chain was not (1→4) but (1→3) was also identified in small amounts in the mild acid hydrolysis products of H substance.

It was established through the isolation and examination of the trisaccharide

O-β-D-galactosyl-(1→3)-*N*-acetyl-D-glucosamine
|
↓(1→4) (4)
O-α-L-fucosyl

that this structure is responsible for the serological specificity of Le^a substance and the structure proposed earlier (Report 1958) is therefore completely confirmed.

The results taken together offer an explanation, in terms of chemical structure, of inherited differences in the blood-group specific glycoproteins and thus make a useful contribution to the important and rapidly growing subject of biochemical genetics.

Enzymic Degradation of Blood-group Substances. Mr. Harrap and Dr. Watkins continued their studies on the characterization of enzymes from *Trichomonas foetus*. The tentative identification of the enzyme that destroys the serological activity of blood-group A substance as an α-*N*-acetylgalactosaminidase (Report 1964) is supported by the results of experiments on a number of different highly purified preparations of human A substance. The main product of enzyme action in each instance is *N*-acetylgalactosamine; small amounts of fucose are also released. Attempts to remove the last traces of fucosidases have so far proved unsuccessful, but the ratio of fucose to *N*-acetylgalactosamine increases on successive treatments of the substrate. This result is consistent with the interpretation that the fucosidase acts, at least in part, only after the release of *N*-acetylgalactosamine. Loss of A activity is accompanied by the development of H activity, and it is evident from the A specific structure (1) given above that removal of the terminal non-reducing *N*-acetylgalactosamine residue would result in the exposure of an H specific grouping (3).

Biosynthesis of Blood-group Substances. In attempts to characterize the enzymes formed under the influence of the blood-group genes, Dr. Gompertz and Dr. Watkins continued their examination of tissue homogenates for glycosyl transferases that will transfer sugar units from nucleotide sugars to low molecular weight acceptors of known structure (Report 1964). B specificity is associated with a terminal non-reducing α-galactosyl unit and therefore tissue homogenates were tested for the presence of specific α-galactosyl transferases. Rabbit stomach mucosa, which has blood-group specificity related to human group B substance, was used in the absence of suitable human material. Evidence for two different transferases was obtained; one transferred galactose in α-linkage from uridine diphosphate galactose to the disaccharide β-galactosyl-(1→4)-*N*-acetylglucosamine, and the second transferred galactose in β-linkage from the same donor substrate to *N*-acetylglucosamine, or to the disaccharide β-*N*-acetylglucosaminyl-(1→4)-*N*-acetylglucosamine. These experiments demonstrate that within a single tissue the same nucleotide-bound sugar can function as a donor of sugars to be transferred in either α- or β-linkage and that the specificity therefore resides in the enzyme and the nature of the acceptor

sugar. Miss Ziderman is extending these experiments in an attempt to isolate the trisaccharides formed, and to compare the compound containing the α-linked galactosyl residue with the serologically active fragments isolated from human B substance.

CARBOHYDRATE STUDIES

Chemical Synthesis of Modified Carbohydrates. The search continued for analogues of natural carbohydrates susceptible to the enzymes engaged in the synthesis of starch and glycogen. Encouraged by the successful substitution of methylated, halogeno and deoxy derivatives of β-glucose 1-phosphate for β-glucose 1-phosphate itself, in a reaction with maltose phosphorylase (Report 1964), Dr. Beattie synthesized nine analogues of α-glucose 1-phosphate. She and Dr. Slessor tested them with starch, sucrose and laminaribiose phosphorylases, with negative results, indicating very high degrees of specificity of these enzymes for the natural metabolite.

Glycogen and Starch Metabolism. Mr. Qureshi continued his attempt to verify a report that synthesis of glycogen from α-glucose 1-phosphate by phosphorylase can take place in absence of a primer (Report 1964); he found additional evidence that such *de novo* synthesis does not take place.

Dr. Taylor studied the carbohydrases of sweet corn, and detected α-glucosidase, β-glucosyl transferase, α- and β-amylases, and a new enzymic activity, manifested by the ability of the enzyme(s) to detach α-maltose joined in 1→6-combination to a glucose polymer, and to re-attach the maltose through the same link to another glucose polymer. The enzyme also appears in potatoes and malted barley and appears to take part in the construction of the branched (1→6 linked) starch component amylopectin.

The specificity of pullulanase, an enzyme hydrolysing the 1→6-bonds of amylopectin, was tested by Dr. Robyt and Mr. Catley. The smallest molecule that it will attack rapidly is a tetrasaccharide in which α-maltose is joined through a 1→6-bond to another maltose. Pullulan, the high polymeric α-glucan substrate for pullulanase, is more complex than its discoverers had supposed and is not simply a 1↓6-linked polymer of α-maltotriose. Maltotetraose is also present in the molecule.

Mr. Lee and Dr. Whelan studied the debranching (hydrolysis of 1→6-linkages) of amylopectin and glycogen by pullulanase, using β-amylase to hydrolyse the products set free; this latter enzyme enables the chain lengths of the split products to be determined. A hitherto unsuspected α-glucosidase contaminant of the crystalline β-amylase was detected. Specific inhibition was accomplished as a result of a discovery by Dr. Kelemen of a new family of inhibitors of α-glucosidases. A charged aminopolyhydroxy compound (tris), already known to inhibit α-glucosidases and thought to do so through its amino group, was shown to do so mainly, if not entirely, through its hydroxyls. The outcome is the finding that neutral and inert compounds such as glycerol and erythritol are excellent inhibitors of α-glucosidases, and can be used under conditions where the corresponding ionic amino compound tris would cause undesirable side effects.

PHOSPHOLIPIDS AND GLYCOLIPIDS

The boundaries of mammalian cells and cell organelles mostly consist of lipids and proteins which are intimately associated in such a way that they form a physical lipoprotein membrane. Other workers have shown that membrane components are responsible for many of the important functions of the cell and

its organelles and in this respect a knowledge of the composition and structure of a membrane is important if its functions are to be understood at a molecular level. The work carried out in the last few years on the nature and distribution of lipids in different mammalian tissues has provided knowledge of the lipid composition of both normal and abnormal whole cells. Most of the phospholipids and glycolipids are known to be components of the various membranes of the cell and its organelles and work is aimed at making clean preparations of mammalian cell membranes on which studies of lipid composition, lipid-protein interactions and membrane (lipoprotein) structure can be made.

The main lines of work during the year were the isolation and characterization of the glycolipids from ascites tumour cells grown in mice; the comparison of the glycolipids found in different strains of ascites tumour cells; the analysis of the lipids isolated from an antigenic lipoprotein carrying H2 histocompatibility specificity; and the isolation of a surface-active lipoprotein from the fluid secreted by the linings of mammalian lung.

Lipids of Ascites Tumour Cells. Dr. Wells (née Gallai-Hatchard) continued the investigation of the glycolipids of CL2/Balb/c ascites tumour cells. The glycolipids, which accounted for about 0.5% of the total lipid extract of the cells, were separated by silicic acid chromatography into five components. The major component (47% of the total glycolipids) was identified as ceramide glucoside (glucose cerebroside). A ceramide dihexoside (17%) characterised as ceramide galactosylglucoside and an aminoglycolipid (27%) characterised as a ceramide *N*-acetylgalactosaminylgalactosylglucoside were the other main components. The latter compound, which was not found in the BP8/C3H cells (Report 1964) appeared to replace two other glycolipids, the ceramide trihexoside and the ceramide *N*-acetylhexosaminyltrihexoside, which were found in the BP8/C3H cells and not in the CL2/Balb/c cells.

Dr. Gray continued the investigation of the glycolipids of various strains of ascites tumour cells and compared those isolated from CL2/Balb/c (Leukaemia), EL4/C57BL (Leukaemia), SA1/A (Sarcoma), MC1M/C3H (Sarcoma) and TA3/A (Carcinoma) ascites tumour cells. The glycolipids were minor components of the cells and represented only 0.05% (SA1/A) to 0.5% (EL4/C57BL) by weight of the total lipids. The glycolipid type defined as a ceramide *N*-acetylhexosaminyldihexoside was found only in the CL2 cells: all of the other strains contained ceramide monohexoside,-dihexoside, trihexoside and -*N*-acetylhexosaminyltrihexoside compounds but there was considerable variation in the relative proportions. The CL2, MC1M and TA3 cells also contained significant amounts of two glycolipids with carbohydrate residues which appeared to be larger than a tetrasaccharide. A possible relationship between the glycolipid composition of the cells and mouse specificity was not supported by the large differences in the glycolipid compositions of TA3 and SA1 cells which were both grown in A strain mice. Tumour and/or mouse specificity may be reflected in more subtle differences such as variations in the fine structure of glycolipids of a similar type. Variations of this kind were found in the ceramide dihexosides isolated from BP8 cells (Report 1964) and CL2 cells. Both dihexosides contained glucose and galactose in equal proportions and the glycolipid fractions, which included the dihexosides, from the two strains of cells were tested for cytolipin H (ceramide lactoside) activity by Dr. M. M. Rapport (Albert Einstein College of Medicine, New York) who used a very sensitive immunochemical technique. The presence of cytolipin H was confirmed in the BP8 cells but not in the CL2 cells, and it was concluded that the CL2 cells contained a dihexoside in which the linkage between galactose and glucose was different from the β 1→4 linkage (lactose) in cytolipin H.

It is very difficult to obtain a sufficient amount of a particular glycolipid from the ascites tumour cells for complete chemical characterization. Dr. Wells has started to isolate a series of similar glycolipids from more readily available sources, such as normal pig lung tissue, to provide a source of material for studies to improve present methods of characterization. Mr Hay began an investigation on possible methods of synthesis of glycolipids. The availability of synthetic compounds of known structures would make the immunochemical technique of Rapport an excellent means of characterizing glycolipids in very small amounts of tissue.

Lipids of Mouse H2 Antigen. Dr. Gray continued the investigation of the lipids of mouse antigens carrying H2 histocompatibility specificity with a study of the antigen of CL2/Balb/c ascites tumour. The antigen was isolated from ascites tumours and characterized as a lipoprotein by Dr. D. A. L. Davies (Queen Victoria Hospital, East Grinstead), who generously provided a quantity of the substance for investigation.

The total lipid (23% by weight of the lipoprotein) consisted of phospholipid (21%), neutral lipid (78.6%) and glycolipid (0.4%). The phospholipid composition was very similar to that of the whole cell and to that of the H2 antigen from the BP8/C3H ascites tumours (Report 1964). The major components of the neutral lipid fraction were cholesterol ester (32%) and cholesterol (16%), free fatty acids (14%) and di- and mono-glycerides (10%). The major component of the neutral lipids of the whole tumour cells was tri-glyceride. The composition of the antigen neutral lipids and the high ratio of neutral lipids to phospholipids were significantly different from those of the H2 antigen from the BP8/C3H ascites tumours. These results suggest the possibility that the lipids indirectly influence the antigenic specificities since the stability and stereochemical structure of a lipoprotein (the antigen) are likely to be closely related to the composition of the lipid moiety.

Surface-active Lipoprotein in Mammalian Lung. Hyaline membrane disease is an organic disease of the newborn which involves acute respiratory distress. Evidence accumulated from the studies of this disease by other workers has shown that the lungs of affected infants lack a component of the fluid lining which possesses surface-active properties and its presence in the lungs of normal infants lowers surface tension and prevents the lungs from collapsing. The active component is a lipoprotein. Dr. Gray with Mr. Body has carried out some preliminary experiments on the isolation of this material from normal rabbit and pig lungs by perfusion of the lungs with physiological saline. A very stable white foam was gradually washed from the lungs and collected. It was washed thoroughly with distilled water and the lipoprotein was obtained by freeze-drying the foam. The lipoprotein is very stable and its chemical and physical structure will be studied in detail. It may be possible to use the lipoprotein as a model compound for studies on the more labile and complex lipoproteins which constitute the mammalian cell membranes.

Mammalian Cell Membranes. The isolation of the outer membrane of a mammalian cell free from contamination by the cell contents is technically very difficult and it has been achieved only recently by other workers with rat liver cells. Dr. Wells and Miss Dod studied methods for rupturing the cells of both rat and rabbit liver, and conditions were found which gave a good proportion of " membrane fragments " which were then isolated by differential centrifugation. Studies were also carried out on certain enzyme systems in the cells whose presence or absence were a possible indication of the purity of the membranes. Miss Dod synthesized a sample of 4-acetamido-4'-isothiocyanostilbene 2, 2'

disulphonic acid which has been reported to be an excellent fluorescent label for cell membranes. This label will provide a simple means of membrane identification at all stages of the isolation procedure and suitable methods for using it are being devised.

BIOPHYSICS

SEDIMENTATION AND VISCOSITY CHARACTERISTICS OF MACROMOLECULES

Further refinements were made by Dr. Creeth in the theory of the relationship between the intrinsic viscosity [η] and the concentration-dependence factor (K_s) of the sedimentation coefficient. A general expression was derived which relates the ratio $K_s/[\eta]$ directly to the molecular asymmetry, and is thought to be valid for all types of particle. Because any easily measurable property of a macromolecular system which is sensitive to changes in molecular conformation is likely to be valuable in interpreting the behaviour of the system, this work is being tested experimentally, with human serum albumin at various pH values, as a model system.

The characterization of a blood-group specific substance believed to be typical of those low in sialic acid was completed by Dr. Creeth and Mr. Knight. Values for the diffusion coefficient were obtained and these results, together with sedimentation coefficient and partial specific volume data obtained previously, permitted the determination of the molecular weight and the second virial coefficient of the substance. The latter quantity is a measure of the thermodynamic non-ideality of solutions of the blood-group substance, and so provides an independent means of estimating molecular parameters. When interpreted as an excluded volume, the value found (36 ml./g.) is in very close agreement with that determined from viscosity measurements. This result provides satisfactory confirmation that the substance has the random-coil configuration in solution previously ascribed to it on other grounds. The configuration remains unchanged over the pH range 3–10 and is unaffected by variations of ionic strength, even though 10% of the constituent amino-acids are known to carry ionizable side-chains.

CHARACTERIZATION OF MACROMOLECULAR HETEROGENEITY

The " ultracentrifugal steady state " method mentioned in previous reports was further studied by Dr. Creeth, with a view mainly to improving the theory for multi-component systems. The main problem is that of differential dilution in the plateau region, and a method of allowing for its effect was devised. The refined theory provides a satisfactory interpretation of the behaviour of very polydisperse systems, but at the price of greater complexity in application. Thus the provision of a general theory that matches the experimental simplicity of the method remains to be achieved; the method is probably most reliable in its earlier semi-quantitative form.

NORMAL HUMAN γ G GLOBULIN

During the course of some tests made by Dr. Kekwick at the request of the Division of Biological Standards, National Institute of Medical Research, it was established that certain aqueous preparations of normal human γ G globulin of foreign commercial origin were partially degraded. The breakdown product of the γ G globulin had a sedimentation coefficient of about 4S in comparison with 7S for the native molecule. During storage for 1 month at 37° the 4S component increased from 15 to 47%, with a corresponding proportional decrease of 7S component. Even at 4°, the 4S component increased by 10% in seven months. The breakdown was probably due to plasmin activated from plasminogen during the preparative fractionation of the material.

A number of batches of human γ G globulin (Fraction G3) prepared for clinical use at the Blood Products Laboratory, Elstree, including one that had been stored in solution for 4 years at 4°, contained no 4S component and none was formed after one month at 37°. The 37° incubation test is being incorporated into the specification for γ G globulin for clinical use.

MACROGLOBULINS OF NORMAL HUMAN PLASMA

Dr. Kekwick continued work on the separation and purification of the α M (19S α globulin) and γ M (19S γ globulin) globulins from a concentrate obtained from normal human plasma by a combination of solvent fractionation and preparative ultracentrifuging (Report 1964). Sub-fractionation procedures involving ion exchange chromatography on DEAE-cellulose and molecular sieve chromatography on Sephadex are being devised.

Although the separation of purified γ M globulin on columns of DEAE-cellulose is regarded by some as a simple matter, it is clear that the molecule polymerizes considerably during the process. Subsequent depolymerization, yielding almost entirely a 19S component, occurs on the addition of suitable concentrations of sulphydryl compounds; but it was not possible to prevent polymerization on the columns by adding sulphydryl compounds to the eluting buffers, and carrying out the chromatography under an atmosphere of nitrogen.

Dr. Goldsmith of the Blood Group Reference Laboratory showed that rabbit antisera to such partially polymerized γ M globulin preparations, after absorption with γ G globulin, specifically agglutinate human red cells sensitized with incomplete human γ M blood group specific antibodies. The cross-reaction with γ G globulin is of course due to an antigenic grouping common to γ G and γ M globulin; the γ M preparation used as antigen contains no demonstrable γ G globulin.

Preparations of α M globulin obtained by fractionating the initial concentrate on DEAE-cellulose columns have proved by sedimentation analysis to be 95% pure. The remaining proteins, with sedimentation coefficients of 7S or less, are removable by molecular sieve chromatography on Sephadex G-200. Although purified α M globulin is stable at 4° when dissolved in phosphate-NaCl buffers at pH 7.0, it is deleteriously affected when such solutions are frozen to –25° and thawed; aggregates form as a result of this treatment. The conditions controlling the stability of this protein are being examined.

BLOOD PRODUCTS LABORATORY

Mr. Vallet and Mr. Wesley made full use of the experimental cold laboratory (Report 1964) to gain experience of moderately large scale plasma fractionation using ethanol. Fibrinogen, γ globulin and plasma protein fraction (PPF) were prepared for clinical trial. The latter fraction contains 85 to 90% albumin together with heat-stable α and β globulins and can be prepared more easily and in greater yield than albumin. By virtue of the absence of heat labile components PPF can be sterilized by heating at 60° C. for 10 hours and thus be freed of the risk of transmitting serum hepatitis, which, though small, is inseparable from the transfusion of dried 10-donor pool plasma. PPF, unlike whole human plasma, is stable in the fluid state for at least 2 years. Similar preparations of PPF have been shown in other countries to be as effective as whole plasma for restoring blood volume. During the year two methods of preparing PPF were investigated and a clinical trial of the fraction prepared by one of these methods begun.

The kinds of globulins present in PPF were studied immunoelectrophoretically by Mr. Vallet. They vary with the method of preparation of PPF but are mainly siderophilin and other glycoproteins. Some lose their identity (as

disclosed by reaction with antisera against whole human plasma) after PPF is heated at 60° C. for 10 hours. Work on the characterization of these globulins is continuing.

Further amounts of γ globulin containing anti-D were prepared in collaboration with the Department of Medicine, Liverpool University (Report 1964). A small batch of convalescent chicken pox γ globulin was prepared for the Public Health Laboratory Service from the plasma of adults who had recently had the disease. γ globulin containing high titre tetanus antitoxin was prepared from the plasma of hyperimmunized donors.

Pharmacologically Active Substances in Human Plasma. Dr. Mackay, Dr. Maycock and Mrs. Silk continued the study of the kininogenetic system of human plasma. G2/1R, a fraction composed largely of α and β lipoprotein globulins, contained the bulk of plasma kininogenases and esterases (Report 1964). G2/1R was subjected to chromatography on DEAE cellulose equilibrated with pH 6.8, 0.02 M phosphate buffer or pH 7.6, 0.01 M phosphate buffer and eluted with NaCl of increasing molarity. Electrophoresis of eluted fractions on acetyl cellulose showed that they were γ globulin, β globulins, α globulins and mixtures of α and β globulins. Plasminogen was adsorbed by DEAE cellulose and not eluted by sodium chloride in concentrations up to 0.3 M. Kallikrein and esterase both appeared in the leading fraction, which behaved electrophoretically as γ globulin. Concentrates of γ globulin prepared by ether fractionation, however, consistently lack kallikrein activity, but contain some esterase.

Kininogenesis was tested in plasma from hypogammaglobulinaemic subjects who had had reactions during treatment with γ globulin concentrates. Addition to intact normal plasma of the γ globulin concentrates associated with clinical reactions in these patients resulted in kinin production; however, addition of the γ globulin concentrates to the corresponding intact plasmas of the patients in whom reactions had occurred was followed by less kinin production than in control plasma.

BLOOD GROUP RESEARCH UNIT

The search for " new " blood groups and the application of the known groups to various problems of human genetics continued throughout the year.

The Xg System. Attempts to improve the gene map of the X chromosome were outlined in the last two Reports (1963, 1964) and subsequent tests on many more families have consolidated the previous results.

The linkage between Xg and glucose-6-phosphate dehydrogenase (G6PD) observed in Israeli and in Greek families was not detected in Sardinian families (Report 1963). More Sardinian families, involving over a thousand members, were tested during the year and the results show clearly that the two genes in the Sardinians are not as close as the 27 map units estimated in the Israelis and the Greeks. Professor M. Siniscalco holds to his opinion, based on previous studies of colour vision and G6PD, that the Xg results are to be reconciled by an inversion within the X, which he suspects to be common in Sardinia. However, the Israeli and Greek evidence now needs support and a second survey is already under way in collaboration with Dr. C. Sheba and Dr. A. Adam of the Government Hospital, Tel Hashomer, and it is hoped that a series of Thessalian families will be tested during the year, in collaboration with Dr. G. Stamatoyannopoulos, of Seattle.

That the gene for angiokeratoma is within measurable distance of Xg continues to seem likely, though more families are needed to make it certain: the best estimate so far is that the genes are about 25 map units apart. (This investigation is in collaboration with Dr. J. M. Opitz, of Madison, Dr. D. Wise, of St.

Thomas's Hospital, and Dr. A. W. Johnston, of University College Hospital.)

Dr. C. Kerr, of the M.R.C. Population Genetics Research Unit, sent samples of blood from 36 families with X-linked ichthyosis and there is good hope that the gene responsible for this condition is also in measurable distance of Xg; the present estimate of the distance between the genes is 23 map units.

The grouping of families with members who have abnormalities of their sex-chromosomes continues (Reports 1963, 1964). One hundred and twenty patients with Turner's syndrome, females who have only one X chromosome, have now been tested with anti-Xg^a: the distribution of the groups is exactly that expected of males. In about one third of the cases the groups of the patient and her parents continue to show whether the single X is from the father or from the mother.

Patients with Klinefelter's syndrome are males who usually have two Xs and one Y: 126 XXY people have been grouped and the distribution of the groups is midway between that expected of females and males. In several families the Xg groups have shown at which stage of cell division the causative non-disjunction of chromosomes has happened: it has been traced to the first meiotic division of spermatogenesis, to the first and second meiotic divisions consecutively and to oogenesis or to an early division in the embryo. (These investigations were in collaboration with Dr. A. Frøland, Copenhagen, Dr. M. A. Ferguson-Smith, Glasgow, Dr. J. H. Edwards, Birmingham, Dr. J. Lindsten, Stockholm, Professor P. E. Polani, London, Dr. A. de la Chapelle, Helsinki, and many others.)

Boys with Klinefelter's syndrome were thought to be born of older than average mothers, but the age effect is obscured by cases due to paternal non-disjunction. By dividing families into those where the Xg groups show the non-disjunction to be paternal and those where they show it to be maternal or post zygotic, the effect of maternal age is becoming clearer. (This work is in collaboration with Dr. Ferguson-Smith.)

Many people with rarer abnormalities of number or of form of the X chromosomes were also tested. A particular study is being made of families in which testicular feminization occurs in attempts to decide whether this condition is an X-linked recessive character or an autosomal dominant character that can be expressed only in males.

The number of unrelated white people of northern European extraction tested in the Unit for the Xg groups has now passed three thousand; as expected, the frequencies have changed hardly at all since the count at two thousand. (Report 1964). In the course of work with Professor Wong Hock Boon, of Singapore, primarily concerning G6PD, a sufficient number of Chinese were tested to show that these people have a significantly lower frequency of the gene Xg^a than have the northern Europeans. The highest frequency of the gene so far observed is in Sardinians.

The testing of animals for the presence of the Xg^a antigen (Report 1964) continued, with the collaboration of Dr. J. K. Moor-Jankowski of Emory University and of the Yerkes and Delta Primate Research Centers of Emory and Tulane Universities. The antigen was not found in 67 chimpanzees, 2 gorillas, 20 orang-utans, 60 baboons, 31 Celebes black apes, 4 drills, 10 rhesus monkeys and 5 pig-tailed monkeys. Only in the gibbon was the antigen found: of 12 males 4 were Xg(a+) and of 6 females 4 were Xg(a+); though not yet statistically significant this distribution gives a strong hint that Xg is X-linked in the gibbon as it is in man. This observation does not appear to have any taxonomic importance, but if the X-linkage is confirmed by further samples there must be some very powerful evolutionary reason why the Xg genes are carried on the X.

But for generous gifts of the original anti-Xg^a plasma from Dr. Amos Cahan of Knickerbocker Biologics (Pfizer), New York, and Dr. J. D. Mann of Butterworth Hospital, Grand Rapids, the work would not have been possible. Dr. I. A. Cook, of Inverness, kindly gave a quantity of the second example of anti-Xg^a which has been invaluable in certain problems. Most of the many colleagues who during the year sent samples for X-linkage and chromosomal upset studies were acknowledged in the last two Reports.

The P System. The work on the antigen P^k, found so far only in Finns (Reports 1961, 1962 and 1964) has resulted in the formulation of a satisfactory theory for the background of the system as a whole: certainly two unlinked gene loci are involved and a third is adumbrated. The theory required the existence of a fifth phenotype in the system and this was subsequently found.

In the last Report (1964) it was stated that a peculiar antibody whose reactions were influenced by the ABO and the P groups of red cells was extraordinary also in not being absorbable by exposure to positive cells. It was later found that some absorption could be achieved if the serum was diluted and the absorbing cells were first treated by enzyme.

The Rh System. Dr. Tippett continued the classification of people whose cells have the Rh antigen D yet whose serum contain anti-D (Reports 1962, 1963, 1964). For some time the number of distinct categories remained at six, suggesting a limit to the diversity, but recently a seventh was recognized.

The Dombrock System. A serum, containing an antibody which defines a new antigen Do^a, was investigated in collaboration with Mrs. Jane Swanson and Dr. H. F. Polesky of the Minneapolis War Memorial Blood Bank. The cells of about 64% of white people have the antigen, which was shown by tests on over 50 northern European families to be a dominant character. Genetic recombination in these families proved that the antigen does not belong to the ABO, MNSs, P, Rh, Lutheran, Kell, Duffy, Kidd or secretor systems and that it is not X- or Y-linked.

The frequencies make the new blood group system a very good autosomal marker; it is indeed sixth in the order of potential usefulness of autosomal blood group systems for application to the genetics of white people.

Autosomal Deletions. Though many genes are known to be carried on the human X chromosome no established gene has yet been assigned to a particular autosome. As an approach to this problem the red cells of people with deletions of part of one of a pair of autosomes are being fully grouped: if such a person can be shown to be heterozygous for a blood group then the locus for that blood group cannot be sited on the deleted portion. In this way negative evidence is slowly accumulating concerning the position of blood group genes on the autosomes. On the other hand if a blood group locus were on the " safe " side of a visible deletion of an inherited kind, positive linkage information would place the blood group locus on the chromosome showing the deletion. This work is in collaboration with a number of cytogeneticists.

Dispermy. Though common in the silkworm this interesting condition was recognized in man only in 1962, at Seattle; since then several other examples have been proved and more suspected. The testing of XX/XY mosaics for corroboratory blood group evidence of a double paternal, and sometimes maternal, contribution is now taking up an appreciable amount of time. On two occasions the condition was first recognized at routine blood grouping and the interpretation later confirmed by karyotype tests. (In collaboration with Dr. J. M. Opitz, Professor J. S. Scott, of Leeds, Dr. R. Mundo, of Barcelona, Dr. W. M. Court Brown, of Edinburgh, and Dr. M. C. Botha, of Cape.)

For routine antisera the Unit is indebted to many colleagues, notably those in the Blood Group Reference Laboratory and the Blood Transfusion Centres at Birmingham, Brentwood, Bristol, Edgware, Sheffield and Sutton. Very useful are panels of fully grouped red cells which come fortnightly from Knickerbocker Biologics (Pfizer), New York, and Laboratorios Grifols Barcelona. Again colleagues at the Institute have kindly given innumerable samples of their blood.

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. 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 blood group antibodies. 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 has continued his investigations into antibodies against leucocytes and platelets. As methods in this field are still not fully standardized, he continued to exchange specimens with Dr. W. J. Jenkins of the North-East Metropolitan Blood Transfusion Centre; they find a steadily improving concordance of results. Tests with leucocyte antibodies continued to be used as possible indicators of incompatibility in cases requiring organ grafts, but other tests of possibly greater value were investigated in order to evaluate them for this purpose. Experiments were continued on the injection of live lymphocyte preparations, in order to elicit the host-versus-graft reaction. Mixed lymphocyte cultures were also performed, and various devices were tried in an attempt to distinguish the reactions of the two sets of cells.

Work was continued by Dr. Goldsmith, in collaboration with a sub-committee of the Council's Blood Transfusion Committee, on the production of anti-7S γ globulin (anti-γG immunoglobulin). Drs. Goldsmith and Kekwick also successfully produced an anti-19S γ globulin (anti-γM immunoglobulin) serum. In collaboration with Dr. J. G. Feinberg they devised and published a new device for immunoelectrophoresis using a perforated plastic mask.

Dr. Ikin, who is in charge of serum production, continued investigations into methods of producing antisera in rabbits, and still further increased the quantities produced. With the collaboration of the Ministry of Agriculture, Fisheries and Food, valuable quantities of seeds containing haemagglutinins or " lectins " were produced.

Dr. Ikin has made provisional standard preparations of " complete " and " incomplete " anti-D serum with a view to their acceptance by World Health Organization as International Standard Preparations. Further standard preparations are also being made.

Dr. Ikin continued her investigations on weak variants of the A antigen and began a comprehensive investigation of variants in the MN system. She continued investigations, in collaboration with Dr. H. Lehmann, Honorary Director

of the Council's Abnormal Haemoglobin Research Unit, on the stability or otherwise of red cells frozen in liquid nitrogen.

Miss Giles devoted most of her time to the detailed investigation of new or very rare antigens and antibodies. She identified the predicted antibody anti-Yt^b and is carrying out a comprehensive investigation of the Yt blood group system. She also identified a new antibody, anti-Cs^a, which appears to define a completely new blood group system.

Mrs. Nunn continued to make highly detailed examinations of large numbers of sera sent in for testing for a variety of clinical reasons, including many from overseas.

Production of anti-human-globulin serum, both experimentally and for routine purposes, is being carried out on behalf of the Blood Group Reference Laboratory at the Medical Research Council's Laboratory Animals Centre at Carshalton, in collaboration with Mr. J. Bleby.

The work begun by Dr. Narayanan on Gm antibodies and antigens continued as part of the routine work of the laboratory.

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.

E. C. DODDS

Chairman.

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THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Accounts 1965

THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Balance Sheet and Accounts

31 DECEMBER 1965

CHELSEA BRIDGE ROAD · LONDON, S.W.1 · 24 MAY, 1966

The Governing Body

Sir CHARLES DODDS, Bt, MVO, MD, D SC, FRCP, FRS, Chairman
Sir HUGH BEAVER, KBE, D ECON SC, Hon. Treasurer
The Rt Hon LORD BRAIN, DM, FRCP, FRS
The Rt Hon LORD BROCK, MS, FRCS
H. P. G. CHANNON, MP
Professor L. H. COLLIER, MD
Professor D. G. EVANS, D SC, FRS
The Rt Hon The EARL OF IVEAGH, KG, CB, CMG, FRS
Professor Sir ASHLEY MILES, CBE, MD, FRCP, FRS

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

Financial Report of the Governing Body

1. The Balance Sheet as at 31st December, 1965, shows balances to the credit of the various funds as follows: Capital Fund £874,271, Specific Funds £218,232 and Bequest Funds £26,097. The balance on the Sinking Fund for Freehold Buildings of £155,896 is after transferring £7,257 from Income and Expenditure Account. During the year donations of £600 have been added to the Re-endowment Fund and there was a loss of £457 on the sale of an investment. The General Fund Investment Reserve has been credited with profits, less losses on sales of investments amounting to £3,728 and the Sinking Fund Investment Reserve has been debited with a loss on sale of an investment amounting to £4,561.

2. The General Fund Income and Expenditure Account shows the Income for the year as £314,548 compared with £280,727 in 1964. Expenditure amounted to £314,217 against £308,665 last year. The surplus for the year is £331 compared with a deficit of £27,938 in1964.

3. The year's surplus of £331 shown by the General Fund Income and Expenditure Account has been transferred to the Capital Fund.

4. Cooper Brothers & Co., the present Auditors, will continue in office pursuant to Section 159 (2) of the Companies Act, 1948.

BRAIN *Acting Chairman of the Governing Body.*

HUGH BEAVER *Hon. Treasurer.*

The Lister Institute of Preventive Medicine
INCOME AND EXPENDITURE ACCOUNT
for the year ended 31 December, 1965

GENERAL FUND

(1964)		Total Expenditure	External Contributions	
£158,165	Salaries and wages	£259,672	£100,458	£159,214
11,340	Emoluments of two members of the Governing Body in an executive capacity	12,098	—	12,098
8,392	Premiums on federated superannuation policies	10,324	3,098	7,226
1,837	Premiums on group pension policy	3,274	1,073	2,201
6,570	Rent, rates and insurance	7,472	600	6,872
17,138	Gas, water, fuel and electricity	20,505	4,044	16,461
5,042	Office expenses, stationery and printing	6,569	1,003	5,566
420	Audit fee	630	—	630
1,791	Travelling expenses	2,649	603	2,046
2,713	Biochemistry expenses	2,392	1,070	1,322
4,800	Microbiology, immunology, experimental pathology and electron microscopy expenses	2,400	1,258	1,142
569	Biophysics expenses	498	—	498
143	Virology expenses	1,096	776	320
40,615	Serum, vaccine and virus vaccine expenses	50,408	2,091	48,317
9,640	Animals	10,142	1,474	8,668
11,612	Animal house expenses and forage	12,135	1,022	11,113
10,540	Buildings, alterations, repairs and renewals (including depreciation £4,400)	14,813	371	14,442
1,968	General apparatus and new installations	3,682	—	3,682
2,214	Library expenses	652	—	652
1,806	General stores	920	—	920
4,368	Staff canteen loss	4,083	513	3,570
—	Blood products laboratory expenses	9,142	9,142	—
6,982	Amount transferred to sinking fund for freehold buildings (including £6,834 interest on investments)	7,257	—	7,257
—	Surplus transferred to Capital Fund after charging to expenditure £16,943 for additions to property and equipment	331	—	331
£308,665		£443,144	£128,596	£314,548

(1964)			
	Interest and dividends on investments:		
£48,708	General fund	£53,874	
6,557	Sinking fund	6,834	
			£60,708
292	Underwriting commission		38
219,556	Sales of sera, vaccines, virus vaccines, &c.		246,712
5,614	Rent		7,090
27,938	*Deficit transferred to Capital Fund after charging to expenditure £12,963 for additions to property and equipment*		—
£308,665			£314,548

For Report of the Auditors and Notes on the Accounts see page 8

The Lister Institute of Preventive Medicine
BALANCE SHEET · 31 December, 1965

(1964)				
	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, 1964	£513,473		
	Add surplus 1965	331		
513,473			513,804	
873,940				£874,271
	Specific Funds			
148,639	Sinking fund for freehold buildings	155,896		
33,589	Pension fund	33,221		
28,972	Re-endowment fund	29,115		
			218,232	
	Bequest Funds			
17,513	Jenner Memorial studentship fund	18,186		
7,771	Morna Macleod scholarship fund	7 911		
			26,097	
236,484				244,329
	Specific Grants and Legacies Unexpended			
272	Cancer research legacies (1937-1950)		96	
4,372	Nuffield Foundation grants (1952-1962)		3,151	
5,826	Guinness Lister research grant (1953-1964)		5,440	
—	Fleming Memorial Fund grant (1965)		243	
10,470				8,930
	Current Liabilities			
46,557	Creditors and accrued charges			41,286

BRAIN *Acting Chairman of the Governing Body.*

HUGH BEAVER *Hon. Treasurer.*

£1,167,451	£1,168,816

(1964)						
	Fixed Assets					
	FREEHOLD PROPERTY (SEE NOTE 1)					
£73,548	Land and Buildings, Chelsea				£73,548	
	Queensberry Lodge Estate, Elstree					
	at cost to 31st December, 1964			£20,455		
	at cost from 1st January, 1965		£15,045			
	Less depreciation		3,000			
				12,045		
20,455					32,500	
2,049	House, Bushey, at cost				2,049	
						£108,097
	FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS (SEE NOTE 2)					
2,472	at cost less depreciation to 31st December, 1963				2,472	
	at cost from 1st January, 1964		7,073			
	Less depreciation		2,873			
5,600					4,200	
						6,672
104,124						114,769

	General, Specific and Bequest Funds. Investments and Uninvested Cash	*Quoted at cost*		*Unquoted*	*Uninvested*		
		In Gt. Britain	*Elsewhere*	*at cost*	*cash*		
697,415	GENERAL	£535,549	£63,299	£32,120	—	630,968	
	SPECIFIC—						
153,637	Sinking fund for freehold buildings	143,081	—	—	13,252	156,333	
33,589	Pension fund	34,217	—	—	996 (Cr.)	33,221	
28,972	Re-endowment fund	27,656	—	—	1,459	29,115	
	BEQUEST—						
17,513	Jenner Memorial studentship fund	14,550	—	940	2,696	18,186	
7,771	Morna Macleod scholarship fund	6,656	—	—	1,255	7,911	
938,897		761,709	63,299	33,060	17,666	875,734	
	LESS INVESTMENT RESERVES						
109,481	General fund				113,209		
4,998	Sinking fund for freehold buildings				437		
						113,646	
824,418							762,088
	Current Assets						
83,576	Debtors and payments in advance					101,798	
155,333	Balance at bankers and cash in hand					190,161	
238,909							291,959
£1,167,451							£1,168,816

For Report of the Auditors and Notes on the Accounts see page 8

Notes on the Accounts

1. Freehold property additions and replacements since 1912 at Elstree and since 1935 at Chelsea, until 31st December, 1964, have been charged to revenue.

2. Additions and replacements to furniture, fittings, scientific apparatus and books between 31st December, 1920 and 31st December, 1963, have been charged to Revenue. No depreciation has been charged between 1920 and 1963.

3. Quoted investments at a cost of £825,008 have a market value at 31st December, 1965, of £1,164,142.

4. Stocks of Sera, Virus Vaccines and Horses on hand at 31st December, 1965, have not been valued in the accounts.

Report of the Auditors to the Members

The accounts set out on pages 4 to 7 are in agreement with the books which, in our opinion, have been properly kept. We obtained the information and explanations we required.

In our opinion the accounts, amplified by the information given in paragraph 1 of the Financial Report of the Governing Body, comply with the Companies Act, 1948, and give a true and fair view of the state of affairs and the surplus of the Institute.

COOPER BROTHERS & Co.

Chartered Accountants.

LONDON, 26th May, 1966.

The Lister Institute of Preventive Medicine

PENSION FUND

(1964)			(1964)		
£2,415	Pensions	£2,458	£33,915	Balance as at 1st January, 1965	£33,589
33,589	Balance carried forward	33,221	2,089	Interest on investments (gross)	2,090
£36,004		£35,679	£36,004		£35,679

JENNER MEMORIAL STUDENTSHIP FUND

(1964)			(1964)		
—	Stipend of student	£182	10,972	Balance as at 1st January, 1965	£17,513
£17,513	Balance carried forward	18,186	957	Interest on investments (gross)	855
			10	Profit on realisation of investment	—
			5,574	Legacy	—
£17,513		£18,368	£17,513		£18,368

MORNA MACLEOD SCHOLARSHIP FUND

(1964)			(1964)		
			£		
—	Stipend of scholar	219	£7,439	Balance as at 1st January, 1965	£7,771
£7,771	Balance carried forward	£7,911	332	Interest on investments (gross)	359
£7,771		£8,130	£7,771		£8,130

NUFFIELD FOUNDATION GRANTS

(1964)			(1964)		
£409	Salaries, wages, laboratory expenses and animals	£1,221	£4,781	Balance as at 1st January, 1965	£4,372
4,372	Balance carried forward	3,151			
£4,781		£4,372	£4,781		£4,372

GUINNESS-LISTER RESEARCH GRANT

(1964)			(1964)		
£12,405	Salaries and wages	£13,142	£5,398	Balance as at 1st January, 1965	£5,826
2,167	Laboratory expenses	2,244	15,000	Amount received	15,000
5,826	Balance carried forward	5,440			
£20,398		£20,826	£20,398		£20,826

THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Report
of the GOVERNING BODY
1966

CHELSEA BRIDGE ROAD · LONDON · SW1

The Governing Body

Sir CHARLES DODDS, Bt, MVO, MD, D SC, FRCP, FRS, *Chairman.*
Sir HUGH BEAVER, KBE, D ECON SC, *Hon Treasurer.*
The Rt Hon LORD BRAIN, DM, FRCP, FRS
The Rt Hon LORD BROCK, MS, FRCS
H. P. G. CHANNON, MP
Professor L. H. COLLIER, MD
Professor D. G. EVANS, D SC, FRS
The Rt Hon The EARL OF IVEAGH, KG, CB, CMG, FRS
Professor Sir ASHLEY MILES, CBE, MD, FRCP, FRS

Clerk to the Governors S. A. WHITE, AACCA

The Council

A. LAWRENCE ABEL, MS FRCS *Representing the British Medical Association*

The Rt Hon Lord BALFOUR of BURLEIGH, DCL, DL *Representing the Members of the Institute*

V. C. BARRY, D SC *Representing the Royal Irish Academy*

Sir HUGH BEAVER, KBE, D ECON SC *Representing the Members of the Institute*

The Rt. Hon Lord BRAIN, DM, FRCP, FRS *Representing the Members of the Institute*

Professor Sir LINDOR BROWN, CBE, MB, CH B, FRCP, FRS *Representing the Members of the Institute*

H. P. G. CHANNON, MP *Representing the Members of the Institute*

Dame HARRIETTE CHICK, DBE, D SC *Representing the Members of the Institute*

Professor P. J. COLLARD, MD, MRCP *Representing the University of Manchester*

Professor R. CRUICKSHANK, MD, FRCP *Representing the University of Edinburgh*

Major L. M. E. DENT, DSO *Representing the Worshipful Company of Grocers*

Sir CHARLES DODDS, Bt, MVO, MD, D SC, FRCP, FRS *Representing the Members of the Institute*

Sir ALAN N. DRURY, CBE, MA, MD, FRCP, FRS *Representing the Members of the Institute*

Professor D. G. EVANS, D SC, FRS *Representing the Royal Society*

Sir PAUL FILDES, OBE, MA, D SC, MB, B CH, FRS *Representing the Members of the Institute*

The Rt. Hon Lord FLOREY, MA, PH D, MB, BS, FRS *Representing the University of Oxford*

Professor R. E. GLOVER, MA, D SC, FRCVS *Representing the Royal Agricultural Society*

Professor R. I. N. GREAVES, BA, MD *Representing the University of Cambridge*

Sir CHARLES HARINGTON, MA, PH D, FRS *Representing the Members of the Institute*

D. W. W. HENDERSON, CB, D SC, PH D, FRS *Representing the Members of the Institute*

The Rt. Hon The EARL OF IVEAGH, KG, CB, CMG, FRS *Representing the Members of the Institute*

Professor Sir ASHLEY MILES, CBE, MD, FRCP, FRS *Representing the Members of the Institute*

Professor J. S. MITCHELL, CBE, MA, MD, FRS *Representing the Members of the Institute*

Professor W. T. J. MORGAN, CBE, D SC, PH D, FRIC, FRS *Representing the Members of the Institute*

Professor Sir RUDOLPH PETERS, MC, MA, MD, FRS *Representing the Members of the Institute*

The President of the ROYAL COLLEGE OF PHYSICIANS *Representing the Royal College of Physicians, London*

The President of the ROYAL COLLEGE OF SURGEONS *Representing the Royal College of Surgeons of England*

The President of the ROYAL COLLEGE OF VETERINARY SURGEONS *Representing the Royal College of Veterinary Surgeons*

MURIEL ROBERTSON, MA, D SC, LL D, FRS *Representing the Members of the Institute*

Professor F. S. STEWART, MD, B CH, BAO *Representing the University of Dublin*

WILLIAM J. THOMPSON *Representing the Worshipful Company of Grocers*

Sir GRAHAM WILSON, MD, B SC, FRCP *Representing the University of London*

The Staff

Director: Professor Sir Ashley Miles
Deputy Director: Professor W. T. J. Morgan
Superintendent of Elstree Laboratories: W. d'A. Maycock

MICROBIOLOGY, EXPERIMENTAL PATHOLOGY AND IMMUNOLOGY

Experimental Pathology and Immunology

†Sir Ashley Miles, CBE, MD, FRCP, FRS (*Professor of Experimental Pathology in the University of London*)
F. R. Wells, MA, BM, B CH
W. E. Parish, MA, PH D, BV SC, MRCVS
Ruth M. Lemcke, B SC, PH D
Brenda Mason, B SC
J. Newsome, MD, DTM & H (*Medical Research Council External Scientific Staff*)

Guinness-Lister Research Unit

†G. G. Meynell, MD (*Guinness Professor of Microbiology in the University of London*)
Vidya K. Gokhale, M SC
Joan M. Walsh, B SC
R. G. Wilkinson, M SC (*Commonwealth Scholar*)

Virology

†L. H. Collier, MD (*Professor of Virology in the University of London and Hon. Director, M.R.C., Trachoma Research Unit*)
C. A. Placido de Sousa, MB, CH B

W. A. Blyth, B SC, PH D
Janice Taverne, BA, PH D
P. Reeve, B SC, PH D
Anne E. Smith, B SC
} **M. R. C. Trachoma Research Unit**

Electron Microscopy Unit

A. M. Lawn, B SC, PH D, MRCVS

BIOCHEMISTRY

†W. T. J. Morgan, CBE, D SC, PH D, MD(*hc*), FRIC, FRS (*Professor of Biochemistry in the University of London*). Principal Biochemist, Elstree
†Winifred M. Watkins, D SC, PH D (*Reader in Biochemistry (Immunochemistry) in the University of London*)
G. M. A. Gray, B SC, PH D
Sheila M. Lanham, B SC (*Trypanosomiasis Research*)
Barbara J. Dod, B SC
G. J. Harrap, B SC
Diane Ziderman, B SC
Anne M. S. Marr, B SC (*Morna Macleod Scholar*)

Medical Research Council Grantees:

E. P. Adams, B SC, PH D
W. P. Aston, MA, B SC
A. S. R. Donald, B SC, PH D
J. Sachs, MB, BS, DIP BIOCHEM
Zeenat H. Gunja Smith, B SC, PH D

British Empire Cancer Campaign Grantees:

Jennifer J. Wells, BA, PH D
G. B. Hay, B SC

D. R. Body, M SC (*New Zealand National Research Fellow*)
Ewa H. Grochowska, PH D (*Poland*)

BIOPHYSICS

†R. A. Kekwick, D SC, FRS (*Reader in Chemical Biophysics in the University of London*)
*J. M. Creeth, B SC, PH D, FRIC
†Professor N. H. Martin, MA, FRCP, FRIC (*Honorary Research Associate*)
J. C. Holt, B SC (*Medical Research Council Grantee*)

HONORARY MEMBERS OF INSTITUTE STAFF (RET'D)

Dame Harriette Chick, DBE, D SC
E. Margaret Hume, MA

PREPARATION and STUDY of THERAPEUTIC SERA (ELSTREE)

*B. G. F. Weitz, OBE. D SC, MRCVS
Angela E. R. Taylor, B SC, PH D (*Trypanosomiasis Research*)
N. Mahoney, DIP TECH

Biochemistry (Elstree)

*D. E. Dolby, B SC, PH D

PREPARATION and STUDY of VIRUS VACCINES (ELSTREE)

*C. Kaplan, MB, CH B, M SC, DIP BACT, MC PATH
H. G. S. Murray, MD
G. S. Turner, B SC, PH D
M. H. Malkinson, BV SC, PH D, MRCVS
L. C. Robinson, B SC
Frances R. Hunter, M SC (*Jenner Memorial Student*)

PREPARATION and STUDY of BACTERIAL VACCINES (ELSTREE)

*A. F. B. Standfast, MA, DIP BACT
Jean M. Dolby, MA, PH D
Delphine C. Miller, MB, CH B, D OBST RCOG
M. P. Banks, B SC
Noreen M. Goggin, B SC

BLOOD PRODUCTS (ELSTREE)

*W. d'A. Maycock, MVO, MBE, MD, FC PATH
L. Vallet, MA
§Margaret E. Mackay, M SC, PH D (*Medical Research Council External Scientific Staff*)
Constance Shaw, M SC, DIP BACT
Shirley M. Slavik, B SC
E. D. Wesley, B PHARM

MEDICAL RESEARCH COUNCIL UNITS HOUSED AT THE INSTITUTE

Blood Group Research Unit

§R. R. Race, MD(*hc*), FRCP, FRS
Ruth Sanger, B SC, PH D
Patricia Tippett, B SC, PH D
E. June Gavin, B SC
Jean E. Noades, B SC

Blood Group Reference Laboratory

K. L. G. Goldsmith, PH D, MD, BS, M C PATH
Toby T. B. Phillips, MB, CH B
Elizabeth W. Ikin
Carolyn M. G. Giles, B SC
Hilary D. Nunn, B SC

ADMINISTRATION

Secretary and Accountant — S. A. White, AACCA
Elstree Secretary and Estate Manager — G. J. Roderick, B COM
Assistant Secretary — Barbara A. Prideaux
Assistant Accountant — E. J. H. Lloyd

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

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

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

Annual General Meeting of the Lister Institute of

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the Institute for the year 1965.

GOVERNING BODY

At a meeting held on 29th June, 1965, the Council reappointed Sir Charles Dodds and Lord Brain as two of its representatives on the Governing Body until 31st December, 1966. In place of Professor J. S. Mitchell, who had resigned, the Council appointed Lord Brock as its other representative.

The Governing Body records with great regret the death of Professor Wilson Smith shortly after he had completed his ten-year term of office as the representative of the Royal Society. His close interest and wise counsel in the affairs of the Institute will be much missed. The Governors welcome Professor D. G. Evans as the Society's new representative.

In accordance with the Articles of Association, Dr. W. d'A. Maycock retired from the Governing Body and was succeeded by Professor L. H. Collier.

The Governing Body takes much pleasure in recording that the honour of Knighthood was conferred upon Professor A. A. Miles in the New Year Honours of 1966.

COUNCIL

At last year's Annual General Meeting the three retiring members of the Council, Dame Harriette Chick, Sir Alan Drury and Professor R. E. Glover, were re-appointed.

The Royal Society appointed Professor D. G. Evans as its new representative on 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 Mr. H. P. G. Channon and Lord Iveagh, each a representative of the Members of the Institute, and Mr. W. J. Thompson, a representative of the Worshipful Company of Grocers.

STAFF AND STUDENTS

The Governing Body records with much pleasure the appointment of Dr. B. G. F. Weitz to the rank of Officer of the Order of the British Empire; and the election this year to the Royal Society of a present member of the staff, Dr. R. A. Kekwick, and of two recent former members, Professor B. A. D. Stocker and Dr. A. E. Mourant.

Professor B. A. D. Stocker, who had been in charge of the Guinness-Lister Research Unit since it started in 1953, resigned in December, 1965, to take up a post as Professor of Microbiology at Stanford University, California. Professor Stocker has been succeeded as Head of the Unit by Dr. G. G. Meynell, who was appointed on 1st January 1966, by the University of London as Guinness Professor of Microbiology.

The University of London conferred upon Dr. L. H. Collier the title of Professor of Virology and appointed Dr. Winifred Watkins a Reader in Biochemistry (Immunochemistry). The Governing Body notes with pleasure that Dr. Watkins received the Oliver Award for 1965 for her work in the field of blood transfusion.

Dr. Delphine C. Miller and Miss N. M. Goggin were appointed to the Bacterial Vaccines Department; Mr. M. Malkinson to the Virus Vaccines Department; Miss M. Inman to the Serum Department; Miss Frances R. Hunter as Jenner Memorial Student; and Miss Anne M. Marr as Morna Macleod Scholar. Dr. W. M. McDonough, Mrs. Elsie Silk and Miss M. Inman resigned during the year.

Sir Ashley Miles visited the Max Planck Institute for Immunobiology at Freiburg in July 1965, where he lectured to the

University Medical Clinic and the Max Planck Institute; he took part in the Lister Centenary Celebrations at Glasgow University in September, 1965, where he presented a paper at the Scientific Symposium; and, as a member of the Royal Society delegation, and at the invitation of the U.S.S.R. Academy of Sciences, visited the U.S.S.R. in October, 1965.

Professor W. T. J. Morgan, in May, 1965, lectured by invitation at the Max Planck Institutes in Freiburg and Tübingen in Germany. In September, 1965, he acted as Chairman at a session of a Conference sponsored by the New York Academy of Sciences on "Bacterial Endotoxins", lectured in a special course on Immunochemistry, at Temple University, Philadelphia and took part in a discussion on "Chemical and Genetic Properties of Bacterial and other Cellular Surfaces" (sponsored by the U.S.A. National Science Foundation) at Woods Hole, U.S.A.

In June, 1965, Professor B. A. D. Stocker took part by invitation in an "Endotoxin Workshop" in Chicago. In September he attended a New York Academy of Sciences Symposium on "Bacterial Lipopolysaccharides".

In June, 1965, Dr. W. d'A. Maycock attended a meeting in Oslo of the Subcommittee of Specialists in Blood Transfusion of the Public Health Committee of the Council of Europe and in September, 1965, he served as a member of the W.H.O. Expert Committee on the Use of Human Immunoglobulins in Geneva.

In August, 1965, Professor L. H. Collier served as a member of the W. H. O. Scientific Group on Trachoma Research. Mr. A. F. B. Standfast attended the Stockholm Round Table Discussion on "Biotechnical Developments in Bacterial Vaccine Production" in June, 1965. As a W.H.O. consultant Dr. C. Kaplan visited India in March and April, 1965, and in May he attended the International Conference on Rabies, at Talloires, France. In May, 1965, Dr. B. G. F. Weitz, under the auspices of the Ministry of Overseas Development, visited Salisbury, Rhodesia, to plan experiments in relation to the feeding habits of tsetse flies.

In September, 1965, at Woods Hole, U.S.A., Dr. Winifred Watkins lectured by invitation at a Symposium on "The Specificity of Cell Surfaces" organised by the Society of General Physiologists and took part in a meeting on "Chemical and Genetical Properties of Bacterial and other Cellular Surfaces". She also lectured by invitation at the University of Michigan, Ann Arbor, at the University of Wisconsin, Madison and at the University of California, Berkeley.

In January, 1965, Dr. Ruth Lemcke went to Australia to work for eighteen months with Professor B. P. Marmion at Monash University, Victoria, where she is continuing her researches on P.P.L.O., and returns to the Institute in June.

Dr. G. M. Gray and Mr. D. R. Body attended the 9th International Congress of Lipid Biochemistry at Noordwijk, Holland, in September, 1965.

In October, 1965, Dr. W. Blyth lectured by invitation at the San Francisco Medical Center, University of California, and at the U.S. Naval Medical Research Institute, Bethesda, Maryland. He also participated by invitation in the Second International Symposium on "The Baboon and its Use as an Experimental Animal", held at San Antonio, Texas.

An extension to the Bacterial Vaccines laboratories and a new office section for the Virus Vaccines department were completed during the year.

For the academic year 1965/6 there are sixteen postgraduate research workers at the Institute registered for higher degrees of the University. Three Ph.D. degrees were awarded to students during 1965.

DONATIONS AND GRANTS

Arthur Guinness, Son & Co., Ltd., continue their generous support of the Guinness-Lister Research Unit and a further £4,000, out of the total grant of £35,000, has been received from the Fleming Memorial Fund for Medical Research for the support of the Electron Microscope Unit.

The Governing Body records its appreciation 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 Medical Research Council for researches on the chemical basis of human blood-group specificity, on the biosynthesis of blood-group specific glycoproteins and red cell antigens, on multiple blood-group specific serological characters associated with simple glycoprotein molecules, on structural analysis of glycolipids and lipoproteins, on allergic reactions in bacterial infection, on the characterization of proteins by the ultracentrifugal steady-state method, on the separation and characterization of the antigenic components of *Bordetella pertussis*, and for studies in immunity in *Bordetella pertussis* infections. Grants were also received from the Ministry of Overseas Development in aid of research on the blood-meals of insect vectors of disease and on the immunology of trypanosomiasis; from the Rhodesian Government for research on the identification of blood-meals; from the Science Research Council for high voltage electrophoresis apparatus; and from the Smith, Kline and French Foundation for research on sensitization to micro-organisms as a cause of generalized eczema.

The Governing Body also gratefully acknowledges donations to the Institute's Re-endowment Fund from The Prudential Assurance Company Limited and The Royal London Mutual Insurance Society Limited.

VISITING WORKERS

The following visitors, in addition to those listed under Staff, worked for short periods in the Institute's Laboratories: Mr. T. Ashiotis, Blood Bank Central Medical Laboratory, Nicosia, Cyprus; Mr. P. F. L. Boreham, Nuffield Institute of Comparative Medicine, Zoological Society of London; Mr. J. Cann, National Blood Transfusion Association, Dublin; Dr. H. Dogal, Beyoglu Ilk Yardim Hastahanesi Kan Bankasi Mütehassisi, Istanbul, Turkey; Dr. M. Fagerhol, National Blood Group Reference Laboratory, Oslo, Norway; Dr. K. Furuhjelm, Finnish Red Cross Blood Transfusion Service, Helsinki, Finland; Dr. P. K. Jain, National Institute for Communicable Diseases, New Delhi; Dr. J. Karrs-Sypesteyn, Academisch Ziekenhuis, Groningen, Netherlands; Dr. P. Kumar, Vaccine Institute, Belgaum, Mysore; Dr. J. Lewin, Centre de Transfusion Sanguine de Strasbourg, France; Dr. F. López Bueno, Escuela Nacional de Sanidad, Madrid; Dr. A. Lundsgaard, The State Serum Institute, Copenhagen, Denmark; Dr. Paul Mattern, Institut Pasteur, Dakar, Senegal; Miss D. Mickerts, Pathologisch-Anatomisches Institut der Universität Wien, Austria; Dr. S-B. Nilsson, Statens Rättskemiska Laboratorium, Stockholm, Sweden; Miss Riitta Nurkka, Finnish Red Cross, Helsinki; Dr. K. E. Sanderson, Brookhaven National Laboratory, New York; Dr. S. Seidl, Universität Frankfurt/Main Blutspendedienst, Germany; Dr. G. L. Timms, Medical Research Laboratories, Nairobi, Kenya; Mr. P. Tulkens, Université de Louvain, Belgium; Dr. J. L. V. Villamarin, Escuela Nacional de Sanidad, Madrid, Spain; Dr. N. Vorias, 1st Regional Centre of Blood Transfusion, Athens, Greece.

Researches in 1965

SUMMARY

The bracketed numbers refer to pages and columns of the report where more detailed descriptions are to be found.

Microbiology

1. Bacterial genetics, physiology and morphology:
   (a) Genetics of "rough" and "semi-rough" mutation in Salmonella (12, ii).
   (b) Characterization of somatic polysaccharides of "rough" mutants of Salmonella (12, ii)) and of flagellar proteins of Salmonella and other enterobacteria (13, i).
   (c) Capsule formation (13, ii) and cell-wall growth of the anthrax bacillus (14, i).
   (d) Electron microscope studies of bacteriophage structure (21, i) and bacterial conjugation (21, ii).

Infective Diseases and Immunity

1. Trypanosomiasis:
   (a) Immunopathology of trypanosomiasis in mice (11, i).
   (b) Biochemical characterization of *T.brucei* antigens (11, i).

2. Virus diseases:
   (a) Growth of slow and fast killing strains of trachoma agent (15, i).
   (b) Antigenic relations of trachoma and related agents (15, ii).
   (c) Immunopathology of trachoma infection of mice and guinea-pigs (15, ii).
   (d) Immunogenicity of trachoma agent (16, ii) and tests of trachoma vaccines overseas (16, ii).
   (e) Chemical inhibitors of virus growth (16, ii).
   (f) Photodynamically inactivated vaccinia virus and its immunogenicity (17, i).
   (g) Purification of vaccinia virus (17, ii) and growth of virus in cell culture (17, ii).
   (h) Purification of rabies virus (17, ii).

3. Bacterial infections:
   (a) Bacterial multiplication in infected animals (14, i).
   (b) Virulence factors of *Escherichia coli* (12, i).
   (c) Immunizing antigens of *Bordetella pertussis* (11, i).
   (d) Toxicity of pertussis vaccines (11, ii).
   (e) Antibodies bactericidal for *B.pertussis* (11, ii) and their mode of action (11, ii).
   (f) Clostridial toxins (12, i).

4. Epidemiology:
   (a) Serological identification of blood meals of insect vectors of infective disease (18, i).

Immunology and Pathology

1. Immunology:
   (a) Auto-degradation of preparations of horse globulins (18, ii).
   (b) Cytophilic antibody and opsonization (19, i).
   (c) Sensitization of tissues to cytotoxic antibody by exogenous antigens in experimental animals (19, i) and in allergic vasculitis of man (19, ii).
   (d) Phagocytosis by human leucocytes (20, i).
   (e) Antisera to human immunogloblins (27, i).

2. Pathology:
   (a) Relation of plasma factors that increase vascular permeability to the kinin-producing system (20, ii).
   (b) Pharmacologically active proteases in human plasma (27, ii).
   (c) Topography of the reactions of the micro-circulation to thermal injury (20, i).

Biochemistry

1. Human blood group substances:
   (a) Characterization of structure by chemical (22, i) and enzymic (23, ii) methods.
   (b) Biosynthesis of blood group substances ((22, ii)).
   (c) Origin of Lewis blood group antigens on the red cell surface (23, ii).

2. Lipids:
   (a) The glycolipids of normal and tumour cells of mammalian tissues (24, i), and of cell membranes (25, ii).
   (b) Characterization (24, ii) and synthesis (25, i) of glycolipids.
   (c) Surface active lipoprotein from mammalian lung (25, i).

Biophysics and Protein Studies

1. Macromolecules:
   (a) Polydispersity of human blood group substances (26, i).
   (b) Refinements of equilibrium centrifuge measurements (27, i).

2. Human plasma proteins:
   (a) Macroglobulins of human plasma (26, ii).
   (b) Preparation of anti-tetanus γ globulin for clinical use (27, ii).
   (c) Anti-Rhesus factor antibody in clinical trials (27, ii).

MICROBIOLOGY

Trypanosomiasis

Trypanosoma vivax Infections. Dr. Taylor studied the course of infection of *Trypanosoma vivax* in mice to assess the value of this organism as an experimental model for immunological studies of the organism. The infectivity and the virulence of the organism was measured by the number of organisms in the blood, the length of the parasitaemia and the death rate. Virulence depended mainly on the stage of infection of the donor mice irrespective of the number of parasites in the inoculum. Successful infection was associated with a destruction of circulating red cells; neither reticulocytes nor white blood cells were affected. Blood from mice at the optimum stage of the *T. vivax* infection remained infective for several months when frozen in liquid nitrogen.

Chemical Analysis of Trypanosomal Antigens. Miss Lanham continued to characterize the antigens in the serum of rats infected with *T. brucei* and compare them with the antigens obtained from homogenized trypanosomes grown *in vivo*. Early fractions from Sephadex G200 columns yielded large-molecular material with no antigenic properties, but most of the later fractions contained antigens. On DEAE columns most of the antigenic components were in fractions corresponding to the γ globulin eluates. The precipitinogens present could not be separated from the protective antigens, contrary to the findings of Seed (1963, J. Protozool., **10**, 380). Most of the antigens appear to be proteins with physicochemical properties like those of the γ globulin of rat serum.

Whooping Cough Bacillus

The Preparation of Bordetella pertussis Protective Antigen. Attempts by Dr. Vincent to produce large amounts of *B. pertussis*, Phase I, in continuous culture, for chemical fractionation (Report 1965) were unsuccessful. One strain, No. 134, containing the protective antigen, which is less exacting nutritionally, grew enough for profitable harvest only after a run of about ten days; but this good growth coincided with the appearance of variants with the growth characteristics of Phase IV and a vaccine of the cells had a low protective potency in mice.

With Dr. Miller, however, a method was devised for producing highly protective bacterial cells in bulk by cultivation in liquid medium rotated in 15-litre bottles. From the cell walls of these bacilli Dr. Vincent prepared an immunogenic particulate fraction like that extracted from the walls of acetone dried cells. The cells were treated by ultrasonic waves to the point when the mixture of cytoplasmic material and fragments of cell wall yielded by differential centrifugation a fraction containing the maximum amount of protective antigen with the minimum amount of other bacterial components. The process was monitored during development by Drs. Miller and Jean Dolby for toxicity, presence of other components, stability, and proportion of protective activity. The investigation of the factors controlling the isolation of the immunogen continues.

Toxicity of Vaccines. Mr. Standfast and Dr. Jean Dolby collaborated in the M.R.C. trial to assess the value of the mouse weight loss as a measure of toxicity of vaccines in children. Using this test, Dr. Miller has re-investigated current and other methods of killing and preserving whole cell vaccine to reduce the mouse toxicity.

Serum Sensitivity of B.pertussis. The killing of mouse virulent *B.pertussis* by antibody and complement can be stopped by coating the organisms with bacterial lipopolysaccharide (Report 1964). Drs. Vincent and Jean Dolby showed that lipopolysaccharide from all the strains tested, whether Phase I mouse-virulent and serum-sensitive, Phase I mouse-avirulent and serum-insensitive, or Phase IV organisms, similarly abolishes the serum sensitivity. It appears that location of the lipopolysaccharide in the cell and not its chemical nature determines serum sensitivity.

The Bactericidal Antibody of B.pertussis Antisera. Drs. Jean and D. E. Dolby continued their investigation of the bactericidal

antibody and the inhibitor of its bactericidal effect, in fractionated antisera. The 7S fraction (Report 1965) which contains both bactericidal antibody and inhibitor was further fractionated on DEAE-Sephadex. Two fractions were obtained, indistinguishable by immune electrophoresis, each with very low inhibitive activity; recombination of the two fractions restored the inhibitive activity almost to that of the most potent inhibitor fraction obtainable.

Escherichia Coli Infection

Dr. Parish continued his investigation of the factors responsible for the pathogenicity of *E.coli* for rabbits and cockerels, using injection of cultures into the ligated small intestine of these animals as a test of virulence (Report 1965). Strains from man maintained at room temperature or 4°C on Dorset egg medium became harmless to rabbits during the year, but most avian strains maintained on the same medium remained pathogenic for cockerels though with diminished ability to penetrate the intestinal wall.

The non-virulent strains and those of diminished virulence had not the salt-agglutinability of rough variants, but were slightly more agglutinable by O somatic antibody, and the non-virulent strains multiplied more rapidly in peptone water than either freshly isolated virulent strains or virulent strains maintained in stock culture.

Attempts continue to find enzymes or antigenic factors associated with pathogenicity.

Clostridial Toxins

Using a small pilot plant, Mr. Mahony investigated the preparation of clostridial toxins by deep fermentation methods. The metabolic requirements and the physical conditions affecting the toxigenicity of *Cl.tetani*, *Cl.welchii* and *Cl.septicum* are being established. Preliminary results indicate that the content of cystine influences the production of inhibitory gases during growth and that the concentration of inorganic iron compounds can be decreased without loss of toxin production.

Inheritance in Bacteria

Genetics of Phage-Resistance and Roughness in Salmonella typhimurium. Mr. Wilkinson continued his study of mutants of *Salmonella typhimurium* strain LT2 resistant to phage P22, which attacks only S (smooth) strains (Report 1965). New classes of mutants were isolated which by their susceptibility to various anti-R (rough) phages and their cultural and serological properties, were broadly divisible into R and semi-R. All had mutant loci located near *xyl*, the locus determining ability to utilize xylose. The R mutants fell into two groups: (1) those with phage patterns similar to those of strains mutated at *rouA*, also located near *xyl* (Reports 1962-64) and (2) galactose-fermenting R mutants lacking the epimerase for uridine diphosphate galactose. A third group, of semi-R mutants, had phage susceptibilities like those of other semi-R strains mutated at *rouB* (Report 1965). Another group of semi-R mutants with the phage pattern associated with mutation at *rouB* (Reports 1962-64) mapped near *his*, the locus determining histidine synthesis

Phage-sensitivity and Somatic Lipopolysaccharide Composition. The second class of R mutant mentioned in the preceding section, which lacks UDP-galactose epimerase, also lacks a sugar-transferring enzyme (Report 1965). Strains of this class differ among themselves in susceptibility to the R-specific phage, Brandis 2. Extracts of several mutants resistant to this phage did not catalyse the transfer of glucose to the somatic LPS (lipopolysaccharide), and extracts of two mutants sensitive to the phage did not catalyse the transfer of galactose to LPS. Strains of a new type of R mutant with distinctive biological properties (e.g. hypersensitivity to sodium deoxycholate, cell-fragility resulting in lysis) and a moderate distinctive phage pattern were isolated by phage selection. One of them was shown by Dr. O. Lüderitz (Max Planck-Institut für Immunbiologie, Freiburg, Germany) to resemble two of his R mutants of *Salmonella minnesota* which had a similar phage pattern and made an abnormal LPS lacking heptose, glucose, galactose and S-specific sugars.

Somatic LPS was extracted from mutants with cultural and serological properties of semi-R strains (Report 1965) but whose phage patterns differed and whose sites of mutation mapped near *xyl*. Chromatographic analysis by Mr. Wilkinson showed that, like the semi-R mutants investigated in collaboration with Dr. H. Nikaido, of Massachusetts General Hospital, Boston, and his colleagues (Report 1965), their LPS had the same qualitative composition as that of wild-type *S.typhimurium*.

Mr. Wilkinson also found that the phages used to classify R and other mutants of *Salmonella typhimurium* strain LT2 fell into several serological groups.

Bacterial Physiology

Properties of Salmonella Flagellar Protein. Dr. McDonough continued his studies with Dr. J. Lowy (Medical Research Council Biophysics Research Unit, King's College, London) on the reaggregation of the sub-units into which flagellin, the bacterial flagellar protein, is separable. Reports elsewhere suggested the existence of two types of flagellar structure: type A, observed in *Salmonella typhimurium* and *Proteus vulgaris* with distinct and presumed spherical sub-units; and type B, observed in *Bacillus subtilis* and *Pseudomonas fluorescens* with five or six lines running the length of the flagellum. Under appropriate conditions sub-units of Salmonella flagellin, obtained by dissociation of whole flagella at pH 2, re-aggregate to form sinusoidal filaments whose structure is identical with that of the original flagella (Report 1965). Proteus flagellin has now been shown to behave in the same way, though the sub-unit is not as distinct as in Salmonella flagella. Similar tests with Bacillus and Pseudomonas type B flagellins could not be made, because, contrary to the reports of others, the flagella not only failed to dissociate, but precipitated at pH 2; these differences perhaps arise because of the peculiarities of the directional strength of bonding between sub-units of the A and B types.

Dr. McDonough analysed the amino-acid composition of Proteus flagellin with results similar to those of others, except that cysteine was not detected. The composition is similar to that of Salmonella flagellins (Report 1964) in having a high content of aspartic and glutamic acids or amides, alanine, threonine, glycine and leucine; a low content of tyrosine, phenylalanine and methionine; and no cysteine or tryptophan. The proline present in all, and the histidine present in most, Salmonella flagellins so far tested were absent from the Proteus flagellin. Tryptic digestion of Proteus flagellin revealed some eighteen peptides compared with the 30-35 peptides obtained from Salmonella flagellin, a finding consistent with a molecular weight of 20,000 for Proteus flagellin compared with 40,000 for Salmonella flagellins calculated from physical data (Report 1964.

Capsule Formation by Bacillus anthracis. Professor Meynell and Dr. Elinor Meynell (Medical Research Council Microbial Genetics Unit, Hammersmith Hospital) completed their work (Reports 1964, 1965) on mutants with new types of CO_2 requirement for capsulation. One class, not previously described, grows without capsules in air, with capsules in 1.2-2.5% CO_2 and is inhibited in 5-10% CO_2, the concentration necessary for maximum capsulation of the wild-type parent.

Exponentially-growing cultures are non-capsulated, and when capsule first appears, it is seen only at the ends of each cell. This may occur because the cells are still growing so that the wall of a given cell is divided amongst its progeny before it has time to form visible amounts of capsule. Later when growth becomes slower, capsule is seen over the whole cell surface. Since it is formed even when protein synthesis, and therefore growth, is inhibited by tetracycline, the capsular material, poly-D-glutamic acid, is presumably not synthesized like protein; in this respect resembling the capsular polypeptide of *Bacillus subtilis* and the peptide antibiotics investigated by other laboratories.

It is possible that the glutamic acid incorporated in capsular polymer is derived from the tricarboxylic acid (TCA) cycle, as in other genera, and that accumulation of glutamic acid induces formation of the

polymerizing system. Bicarbonate ion (HCO^-_3) is presumably fixed in aspartate and related compounds which increase the activity of the TCA cycle, and hence the formation of glutamic acid. However, the cycle does not depend solely on HCO^-_3 for its syntheses and, once active, can produce glutamic acid in air not supplemented with additional CO_2. This model therefore accounts for the observed requirement for HCO^-_3 (Report 1964), the formation of capsules in air by cells with terminal capsular masses which are presumably already committed to capsulation, and the failure of capsulation in anaerobic culture where the TCA cycle is known to be inactive.

(See also Electron Microscopy, p. 21).

Cell Wall Growth in Bacillus anthracis. Professor Meynell observed in stained films that when capsulated cells of *Bacillus anthracis* began to grow, they did not form new capsule until the stationary phase was reached, and that capsular material present on the cells of the original inoculum could be seen many generations later at the ends of growing chains and over occasional intercellular junctions. This was confirmed by electron microscopy in collaboration with Dr. Lawn. The observations suggested that new cell wall was synthesized at the equator of each cell. Cell wall growth was examined by labelling the cells with ferritin-labelled antibody, and observing its distribution amongst the progeny of the labelled cells. The ferritin-antibody was distributed uniformly amongst the progeny, which apparently argued against equatorial growth. However, ferritin was also seen on control cells that had been added to the suspension after removal of free ferritin-antibody; so that the uniform distribution of ferritin-antibody on progeny may therefore represent redistribution of antibody, not simultaneous growth of the whole cell wall.

Bacterial Multiplication in Infected Animals

Professor Meynell continued his analysis of experimental and naturally occurring infections in terms of a birth-death process in collaboration with Professor Peter Armitage, of the London School of Hygiene and Tropical Medicine and Dr. Trevor Williams, of Duke University, North Carolina (Report 1964). The basic model postulates a constant probability per viable micro-organism of dividing and of dying per unit time in its host, and its predictions show a remarkable overall agreement with observations. Nevertheless, serious qualitative discrepancies remain to be explained: in particular, that the mean division rates estimated from the distributions of response times for individual hosts are impossibly short (e.g. 0.2 seconds) for experimental infections but improbably long (e.g. 3,000 hours) for many epidemics. Some of these discrepancies may arise because infection does not progress as postulated by the model. Thus, in the model infection, death is assumed to occur at the moment it becomes inevitable. But in certain experimental infections it is clear that death becomes inevitable some time before it occurs; so that conclusions based on the time of death—usually the only datum available—are misleading.

Another process not accounted for by the present form of the model is the induction of acquired immunity during the infection, with a consequent change in the probabilities per viable organism of dying and of dividing per unit time, so leading to a prolongation of time to death. However, a preliminary analysis of reported death times suggests that this factor is not usually detectable. Acquired immunity may therefore have an all-or-none effect on the infection which provokes it, giving either complete protection or none at all. The extent to which this is true of microbial infection in general cannot be decided without more data and better methods of statistical analysis.

VIROLOGY

Trachoma and Inclusion Blennorrhoea (Conjunctivitis)

For convenience, infective agents of the psittacosis-lymphogranuloma-trachoma group are here referred to as bedsonia; those of the trachoma and inclusion conjunctivitis sub-group as TRIC agents; variants of TRIC agents that for a given dose kill chick embryos more quickly than their parent strains (Reports 1964; 1965) as *f* (fast-

killing) strains, and the parents as *s* (slow-killing) strains.

Growth Characteristics in Chick Embryos and Cell Cultures. Dr. Reeve and Dr. Taverne previously found that the more virulent *f* strains produced the same amount of lethal material whether grown in eggs incubated at 35°C. or 37°C., whereas the less virulent *s* strains multiplied only at 35°C. (Report 1965). Subsequent investigation showed that *s* strains sometimes grew in eggs at 37°C., particularly when a relative humidity of 65% was maintained in the incubator, and the air kept in constant motion. The less virulent strains generally appear to be more exacting in their growth requirements than the more virulent. This work emphasizes the importance of controlling the conditions in incubators within fine limits.

To investigate the adsorption, entry and growth of TRIC agents in the yolk sac cells of chick embryos, Dr. Reeve and Dr. Taverne devised a method for maintaining explanted yolk sac tissue. A culture system in which both fast- and slow-killing strains could be grown *in vitro* might also be adapted for use in a neutralization test, since the usual cell culture systems do not normally support the growth of slow-killing strains. In one series of experiments, yolk sacs were infected in the egg and 24 hours later, when it was known from the results of single-cycle growth curves (Report 1965) that the agent was about to enter its exponential phase of multiplication, but was not yet infective, the yolk sacs were removed from the eggs and maintained in various culture media in flasks for 24 hours, and infectivity titrated in eggs after disruption of the sacs. In some cases multiplication of *f* strains took place *in vitro*, but never as much as *in ovo*.

Dr. Reeve and Dr. Taverne continued to study the growth of TRIC agents in HeLa cell monolayers infected by centrifugation (Report 1965). Although *f* strains grow readily in cell cultures without the aid of centrifugation, they formed 4 to 10 times more inclusion bodies when centrifuged into the cells; in some cases the ratio of total particles to inclusion-forming units approached unity, indicating that every particle was infectious. By contrast, *s* strains formed inclusions only after centrifugation into monolayers, and only about 1 in 1,000 particles formed an inclusion. This ratio is of the same order as that found for killing chick embryos. The non-infectivity of a comparatively large proportion of the elementary bodies in suspensions of *s* strains is under investigation.

Neutralization Tests in Cell Cultures. Miss Graham continued her investigation of antigenic relationships between strains of TRIC agents, using the infectivity neutralization test in HeLa cells. Previously, no differences were detected in cross neutralization tests with unabsorbed rabbit antisera prepared by repeated inoculation (Report 1965). Cross-absorption tests of two *f* strains also indicated antigenic similarity. In an attempt to produce more specific antisera, rabbits were inoculated intravenously with a single large dose of purified elementary bodies, but these sera were no more specific than those from rabbits receiving a series of injections. The titre of complement-fixing antibody increased for 3 to 4 weeks and then slowly decreased; whereas that of neutralizing antibody was detectable after 7 days and had disappeared by the 21st day. This result confirms a suggestion that the antigen responsible for eliciting neutralizing antibody differs from the complement-fixing group antigen (Report 1965).

Immunological Studies in Non-primate Animals. Until recently, study of the immunogenicity of TRIC agents in terms of their ability to protect against infection of the intact animal was confined to primates, which alone were believed to be susceptible to infection. Because the use of small laboratory animals would have great advantages much work was devoted to research on the growth and immunogenicity of TRIC agents in mice and in guinea pigs.

Miss Graham found that all of 14 TRIC agents tested induced pneumonitis after intranasal inoculation in mice. Histologically, there was bronchiolitis and widespread alveolar consolidation; inclusion bodies were present in both bronchiolar epithelium and parenchymal cells, in various forms representing the stages of the developmental cycle of TRIC agents. Other workers have

failed to passage TRIC agents in the lungs of mice with material harvested 7 to 22 days after inoculation. The concentration of micro-organisms is however greatest after 4-5 days, suggesting that passage at shorter intervals might be successful. Strain HAR-2 grew in the lung, but could not be passed; by contrast, its fast-killing variant (HAR-2*f*) was passaged 7 times.

Mice can be protected from death due to the toxin associated with elementary bodies of bedsonia by prior intravenous immunization. Miss Graham showed that the intravenous immunization also protected against the pulmonary infection, and that cross-protection tests with strains representing the two main antigenic groups of TRIC agents gave results similar to those of the toxin protection test. The existence of antigenic differences between *f* and *s* strains (Report 1965) was confirmed. Five *f* strains differed from their parent strains in immunized mice challenged either intravenously or intranasally, but could not be distinguished from each other; two of them differed from a Gambian *s* strain. Dr. Collier and Miss Smith have started to determine the degree of correlation between the results of tests for immunogenicity in mice and baboons respectively.

American workers showed that elementary bodies of bedsonia treated with desoxycholate yield both a soluble group antigen and an insoluble cell wall fraction containing specific complement-fixing antigens. Miss Graham found that mice inoculated with the soluble group antigen were not immune to intranasal or intravenous challenge with the homologous strain; but that mice immunized with the insoluble fraction were protected against intravenous but not against intranasal challenge. This suggests that the cell wall of the elementary body contains an antigen eliciting protection against TRIC agent toxin.

Dr. Blyth extended his work on the use of guinea-pigs for investigating the mechanism of immunity to TRIC agents (Report 1965). The LB4 strain of inclusion conjunctivitis injected intracutaneously induced a papular skin lesion; for 5-6 days after the injection the organisms were isolable from the lesion and regional lymph nodes, but not from the liver and spleen. By contrast, the *f* variant of this strain (LB4 *f*) persisted for 2-3 weeks in the skin, and was also present in the liver and spleen. Intracutaneous injection of live LB4 *f* failed to modify the response to a similar dose given 4 weeks later; but an intravenous injection of killed organisms following the intracutaneous inoculation partly protected against subsequent intracutaneous challenge, in that the agent disappeared from the injection site within 4 days, and was not detectable in the liver or spleen. The degree and duration of the immunity induced in guinea-pigs are now under study.

Dr. Blyth is investigating the culture of various guinea pig cells, including macrophages, with a view to studying their role in immunity to TRIC agents.

Trachoma Vaccine. In a previous experiment, strains LB4 and G187 were cross tested for immunogenicity in baboons (Report 1965). Dr. Collier and Dr. Blyth found that 4 animals immunized with LB4 and 2 immunized with G187, which resisted challenge shortly after immunization, were no longer immune 10 months later; this confirmed an earlier finding that artificially induced immunity in baboons wanes comparatively quickly.

Miss Smith and Dr. Collier tested more than 700 sera from a large series of immunized and control baboons; analysis of the results, which is not yet complete, strongly suggests that serum content of complement-fixing antibody is not closely correlated with the degree of immunity induced by vaccination.

Investigations Overseas. Under Dr. Collier's direction two new field trials of trachoma vaccine were started. A live vaccine prepared from two strains of trachoma is being tested in the Gambia. In Iran, a larger collaborative trial is now in progress (Report 1965) in which the efficacy of live monovalent and live and inactivated bivalent vaccines is being compared.

Shope Fibroma

Dr. Placido de Sousa continued his research on the action of *N*-hydroxy-

urethane and related compounds on Shope fibroma virus growing in rabbits and in cell cultures (Report 1965); as before, the chemical compounds were synthesized by Professor E. Boyland and his colleagues at the Chester Beatty Research Institute.

Studies in Cell Cultures. Ten compounds related to *N*-hydroxyurethane, including a series of esters, all inhibited viral growth in rabbit cell cultures, but none to the same degree as *N*-hydroxyurethane itself; there was no correlation between the degree of inhibition and the size of the *N*-alkyl groups in the esters. Eight other compounds related to *N*-hydroxyurethane had no effect on viral growth.

Viral multiplication was prevented by *N*-hydroxyurethane that had been heated at 100°C. for 30 minutes; since compounds containing nitro groups may be formed by such treatment, sodium nitrite was tested and proved to be inhibitory, but only at high concentration. Like *N*-hydroxyurethane, it inhibited multiplication, and did not affect the virus before it entered the host cell; sodium nitrate was inactive. In tests with adenovirus 12, which affects man and causes tumours in hamsters, *N*-hydroxyurethane, but not sodium nitrite, partially inhibited viral multiplication in human embryo cell cultures.

Studies in Rabbits. Intramuscular injection of both *N*-hydroxyurethane and *n*-butyl-*N*-hydroxycarbamate decreased the size of skin lesions induced in rabbits by Shope fibroma virus. Inhibition by the intramuscular injection of the first named compound was enhanced when the compound was also given at the same site as the virus.

Vaccinia and Variola Viruses

Dr. Turner and Dr. Kaplan investigated the major factors concerned in the photodynamic inactivation of vaccinia virus. Reactivation phenomena observed in viruses inactivated by other methods did not occur with photo-inactivated virus. The immunogenicity for rabbits of virus inactivated by this method was retained after at least four times the minimum illumination needed to kill; thus permitting a very large safety margin in the large scale preparation of inactivated vaccine.

Antibody concentrations in the paired sera provided by Dr. K. Cantell of the Finnish State Serum Laboratory (Report 1965) indicate that one dose of photo-inactivated vaccine was an efficient secondary stimulus in young men previously vaccinated with live vaccine. Clinical trials in unvaccinated children and young adults are planned. Preliminary experiments in mice to determine the relative effectiveness of live and inactivated vaccines in protecting against a strain of vaccinia virus lethal by the respiratory route suggested that similar doses of both photo-inactivated and live virus given subcutaneously are as effective as live virus administered by scarification. The experiments continue.

Dr. Murray continued the investigation of vaccinia virus growth in cultures of calf kidney cells. His results do not encourage the belief that the method will provide a useful source of virus for vaccines.

Mr. Robinson continued his work on the purification of vaccinia virus by stable flow electrophoresis. The amount of virus preparation processed, the rate of flow and the volume purified were greatly increased by installing an appropriate multi-channel pump. Difficulties in adapting the cartesian diver micro-manometric technique were overcome by designing divers which will make possible precise timing of the mixing of reagents and biochemical estimations with microlitre volumes of highly purified virus preparations.

Dr. Malkinson began the study of immediate and delayed hypersensitivity to vaccinia virus in mice and their relation to immunity. He is also investigating reagents for the safety testing of batches of inactivated smallpox vaccine prepared by photo-oxidation.

Rabies Virus

Drs. Turner and Kaplan began a study of rabies virus, as harvested from rabbit neural tissue, the aim of which is to produce a potent vaccine free of the substances in nervous tissue responsible for the demyelinating accidents which may occur

during anti-rabies treatment. Preliminary results in guinea-pigs suggest some success in removing encephalitogenic material from rabbit brain by a process having little or no effect on virus titres.

Thanks to Dr. F. T. Perkins and members of his staff, Miss Hunter studied the cultivation of human embryonic diploid cells and the basic methods of karyology at the M.R.C. Laboratories, Holly Hill. She has begun an investigation of the suitability of diploid cells for virus vaccine production.

IMMUNOLOGY AND EXPERIMENTAL PATHOLOGY

Serological Identification of Blood Meals

Dr. Weitz continued various surveys of the feeding habits of different insects. In co-operation with the World Health Organisation, the source of 125,000 blood meals from 93 species of *Anopheles* of Asia, Africa and Europe has been established in the last 10 years. These studies indicate that some species of mosquitoes change their behaviour after prolonged treatment of domestic houses with " residual" (i.e. persisting) insecticides. For example, *A. gambiae* fed almost exclusively on man in unsprayed areas, but in Zanzibar, where treatment with DDT or Dieldrin was applied, only 25 per cent fed on man and less than 10 per cent of those captured in Rhodesia had fed on man where HCH was applied for some years. Similar results were obtained with *A. funestus*.

The discovery in recent years that a large number of *Anopheles* are infected with malaria parasites of non-human origin has led to a careful study of the feeding habits of forest mosquitoes. Simian malaria is common in Malayasia, India and some parts of Africa and it is necessary to distinguish the mosquitoes feeding on man from those feeding on other primates. The application of the agglutinin-inhibition test with red blood cells sensitized with human and various monkey sera has now been applied to mosquito blood meals. The test was greatly improved by the use of cells sensitized with bis-diazotized-benzidene in place of the tannic acid used with tsetse blood meals.

In Trinidad studies on the *Culicines* transmitting arbor viruses have begun in conjunction with Dr. Brooke Worth of the Rockefeller Foundation. With Dr. A. M. Greenhall of the U.S. Department of the Interior Fish and Wild Life Service, the study continues of the feeding habits of South and Central American bats in relation to the transmission of rabies.

The study of the residual hosts of tsetse flies in areas of Africa where the insect is partially eradicated is to continue for the next few years, after which the results will be assessed.

Miss Inman devised a new technique to measure the antigenic concentration of antigen in blood meals. The saline extract of the blood meal is applied to cellulose acetate membranes impregnated with a polyvalent species antiserum and the membrane, after washing, is dyed. The area of the dyed material, representing the antigen-antibody precipitation, is linearly related to the concentration of antigen in the extract. The test provides a useful check in evaluating the results of inhibition tests as indicators of the identity of blood meals.

Antitoxin Production

It has been known for some time that purified human γ globulin preparations stored at 0-4° undergo limited proteolysis, after which two components are distinguishable by immunoelectrophoresis. Dr. D. E. Dolby found that β globulin preparations from horse sera (Report 1964) undergo a similar process, but to a greater extent. After storage even at —10° for six months up to 90% of them may be converted to a form which behaves on DEAE-cellulose columns like a γ globulin and on a Sephadex column as if it had a lesser molecular weight than that of the original β globulin, while retaining the properties of a β globulin on immunoelectrophoresis. The effects on this process of inhibitors of the proteolytic enzymes of plasma, such as the plasmin inhibitor 6-amino-*n*-hexoic acid, are under study.

The difficulty of obtaining the β globulins free from other plasma protein also led

Dr. Dolby to investigate their further purification by rechromatography on carboxymethylcellulose, as used by Professor M. Raynaud of the Institut Pasteur, Paris. Methods of obtaining pure antitoxic globulins from toxin-antitoxin floccules (Report 1964) were also further investigated.

The "light" and "heavy" polypeptide chains obtained by reductive splitting of antitoxic globulins (Report 1965) were both found to aggregate on storage. The mechanism of this process and methods for its prevention are under study.

Cytophilic Antibody and Opsonins

By further work Dr. Parish confirmed the suggestion that cytophilic antibody has a protective function by acting as a "cell-bound" opsonin, contributing to the removal of bacteria and their toxins from the circulation (Report 1965). The 7S γ globulin cytophilic antibody obtained by injecting protein antigen in mice appeared several days after the first agglutinating antibody, but the 19S γ globulin cytophilic antibody stimulated by bacterial polysaccharides appeared at the same time or soon after the agglutinating antibody. This anti-polysaccharide antibody became firmly attached to macrophages, resulting in specific adherence of many bacteria to the cell surface.

In some cases only a proportion of the particles so attached to the surface of macrophages was ingested. When at the end of the test the cells were no longer flattened on the glass surface it was difficult to distinguish between ingested and adherent particles. Attempts are being made to overcome this by lysing all extracellular particles with specific antisera or bacteriophage.

Exogenous Antigens Acquired by Tissue Cells

An investigation of the effects of antibody reacting with antigen acquired by nucleated cells was begun by Dr. Parish to test the possibility that generalised eczema may result from the incorporation of bacterial or fungal antigens into cells of the skin, which then become liable to damage by specific antibody.

HeLa cells, and primary cultures of human embryo, human and guinea-pig kidney were cultured for varying periods in the presence of pure protein or of serum proteins from a different species. After treatment with trypsin, the cells were exposed, with and without complement, to the appropriate specific antibody. In every test there was more damage, as determined by the dye exclusion method, in cells exposed to antibody plus complement than in those exposed to antibody alone or control sera, though the difference was often small. Cells exposed to specific antibody and complement also extruded part of their cytoplasm, but lost no viability, as determined by subsequent culture *in vitro*.

The protein acquired by the cells was not detectable; most of it being presumably removed by the trypsin treatment. It is probable, however, that only very small amounts of protein need be retained on the cell to make it sensitive to antibody, because only small amounts of antigen, antibody and complement complexes were needed similarly to damage untreated cells when added directly to them.

Allergic Vasculitis

In collaboration with Drs. E. L. Rhodes and G. A. Stirling of the Departments of Dermatology and Pathology, King's College Hospital, Dr. Parish examined the tissues of patients with nodular vasculitis for antigen-antibody complexes that may have caused the lesions. Skin biopsies from 9 patients were tested for plasma γ globulin, and streptococcal and tubercle antigens by the fluorescent antibody technique. Streptococcal polysaccharide and γ globulin were found in the lesions of one patient after a streptococcal infection of the throat and tubercle antigen in the venules of lesions of 2 other patients. In the serum of the patient with streptococcal antigen in the lesions, antibody to streptococcal M protein was detected by agglutination and complement fixation, and its concentration changed little during convalescence. Soluble complexes formed by adding streptococcal

antigen to the patient's serum were ingested by human vascular endothelial cells *in vitro*, and produced local vasculitis in the skin of baboons, suggesting their *in vivo* uptake by endothelial cells.

Phagocytosis

Dr. Newsome investigated the reliability of methods for estimating phagocytosis by human blood leucocytes. Exposure of freshly isolated monolayers of leucocytes to suitable particles in coverslip chambers, and examination of the live cells by phase contrast, appeared to give the most accurate results.

Mechanisms of Inflammation

Vascular Changes in Injury. Dr. F. R. Wells continued his studies of vascular changes induced by injury, especially with regard to the association of phases of exudation with the type of vessel damaged. The earliest reactions of rat cremaster muscle to mild (54° for 20 sec.) and moderate (54° for 40 sec.) thermal injury were analysed in detail. After mild heat, both venular damage and exudation were just detectable from 25–40 minutes after injury and then decreased. After moderate heat, no permeability increase or deposition of circulating colloidal carbon in the walls of damaged vessels was at first observed. This effect proved to be due to immediate vasospasm, lasting for 20 to 60 minutes. The degree and duration of vasospasm was in part determined by the initial state of the vascular bed, being greater when the peripheral circulation of the animal was depressed by anaesthesia. This transient vascular occlusion is distinct from the more permanent occlusion that may occur after 5 or more hours. Relatively transient vasospasm was followed by both venular and capillary damage. However, exudation was maximum at 25 minutes, when the extent of capillary damage was approaching long-lasting maximum, and before any substantial venular damage. There was little exudation at 30 minutes, when the transient phase of venular damage was at its maximum. The capillary damage, judged by the *rate* of carbon deposition in affected vessels, was much greater at 20 than at 25–30 minutes.

In guinea-pigs and rats hypersensitized to tuberculin, intracutaneous injection of antigen elicited an immediate phase of venular damage lasting up to 2 hours. The later mild exudation characterizing the subsequent delayed hypersensitivity reaction was associated in the guinea-pig with a well defined capillary damage, but in the rat no vascular damage was demonstrable by the carbon deposition method. Secondary capillary damage occurred at the periphery of some of the guinea-pig lesions at 60–80 hours.

Activation of Permeability-increasing Plasma Proteins. Miss Mason continued her work on the relation of the kinin-producing system to other permeability factors of the blood plasma. Contact of human and guinea-pig plasma with glass activates two enzymic permeability factors, a γ globulin, kininogenase, and an α or β globulin factor, PF/dil. These reactions are presumably mediated by the activation of the Hageman factor responsible for the initiation of clotting in man, and of its analogue in the guinea-pig. Kininogenases are activated from intact plasma by impure preparations of PF/dil, of the "contact factor" that can be eluted from glass exposed to normal plasma, and of Hageman factor itself. Attempts were made to determine whether the presumed active principles in each of these three preparations are distinct.

In all but one of the tests made, contact factor from human and guinea-pig plasma behaved like human Hageman factor. PF/dil appears to be distinct from contact factor because some activable PF/dil was present in Hageman-trait plasma, which lacks the factor, and both normal guinea-pig and human plasma made to resemble Hageman-trait plasma by removal of contact factor, still contained substantial amounts of PF/dil. It is also unlike contact factor, because plasma could be depleted of the latter without affecting the PF/dil, and because preparations of it were not absorbed to glass, as contact factor is, and its precursor was not activated by glass. Moreover, though preparations of both substances

activated the kininogenase in Hageman-trait plasma, PF/dil, but not contact factor, lost this capacity after treatment with di-isopropylfluorophosphonate. The insusceptibility of contact factor to the drug is at variance with some reports of susceptibility to it of Hageman factor.

Some investigations are in progress of the properties of the inhibitory substance in Hageman-trait plasma that absorbs to glass, and thereby prevents glass-activation of contact factor in normal plasma; and of a substance in normal plasma which appears to absorb to glass already coated with contact factor and inhibits contact factor activity unless it is removed by gentle washing.

Electron Microscopy

Capsule of B. anthracis. In collaboration with Professor Meynell, Dr. Lawn examined the capsule of *Bacillus anthracis* by the negative contrast method of Horne. A number of different contrast agents were used; several interacted with the capsule, giving rise to a coagulated, stranded or rope-like appearance; the remainder gave extremely variable results which could seldom be correlated with the appearances of wet india-ink preparations by light microscopy, which are believed best to demonstrate the natural condition of the capsule. It appears that, because of the low solid content of the capsule and the physico-chemical nature of the constituent polyglutamic acid, negative contrast methods are not applicable. However, nigrosin stained the capsule when applied during drying of the specimens for electron microscopy. This technique was employed to obtain a more detailed picture of inheritance of capsule during cell growth (p. 14).

Other investigations of cell-wall growth of *B. anthracis* treated with ferritin-labelled antibody are noted on p.14.

Structure of Bacteriophage. Dr. Lawn examined the structure of a collection of anti-R salmonella bacteriophages used by Mr. Wilkinson to distinguish classes amongst P22 resistant R mutants of *Salmonella typhimurium* strain LT2 (p. 12). With one exception, the host range and antigenic type of phage were closely related to structure. The phages FO and C21, which were apparently structurally identical, have different host ranges; their structure is being examined more precisely. A new Salmonella S bacteriophage, 9NA, isolated by Dr. P. Gemski proved to be long-tailed; it nevertheless had a terminal base plate like that of the short-tailed, S phage P22.

Bacterial Conjugation: ColI System. In collaboration with Dr. Gemski, an attempt was made to determine whether conjugation of Salmonellae in the colicine I system was associated with specialised pili, as in F^+ mediated conjugation. A non-piliated strain of *Salmonella typhimurium* was infected with *colI* and used to prepare high frequency transfer mixtures (Hfc). In the mixtures of *colI* donors with *colI*$^-$ recipients, no piliated cells were detectable by methods capable of detecting $<1\%$; nevertheless, 5–15% of the donor transferred *colI* to recipient bacilli. The efficiency of transmission is less than that (50–70%) with piliated donors, but it is clear that *colI* can be transmitted by donors which do not bear pili at the time of mixing with recipient bacteria.

Bacterial Conjugation: RTF System. In collaboration with Drs. E. Meynell and N. Datta at the Postgraduate Medical School, Hammersmith, Dr. Lawn provided morphological confirmation that bacteria able to transfer certain drug resistant factors (RTF) to other bacteria by conjugation may carry F^+ type pili, recognisable in the electron microscope by their affinity for the "male-specific" phage MS2. The presence of these pili had already been deduced by Dr. Meynell from her demonstration that some of the bacteria carrying RTF can support multiplication of MS2. This work is now being extended to include other resistance factors and further to explore the relationship between possession of F^+ pili and ability to transfer RTF.

Structure of α Macroglobulin. Dr. Lawn examined a highly purified sample of α macroglobulin, a high molecular weight protein from human plasma, supplied by

Dr. Kekwick. Many of the molecules were rectangular in outline with sides about 16 mμ long. The penetration of the molecule by contrast agents made manifest certain consistently observed structural patterns, particularly one of two or three parallel bars. However, the patterns were not sufficiently defined to permit construction of a three dimensional model.

Dr. Lawn also investigated electron microscopically the following.

For Dr. Gray and Miss Dod; thin sections of a cell membrane fraction from liver, to assess its purity.

For Dr. F. R. Wells; the particle size of materials for use in opaque media for determining the distribution and extent of vascular beds by intravascular injection.

For Dr. Vincent; fragments of *Bordetella pertussis* cell wall, for estimation of particle size.

For Dr. Collier; trachoma virus vaccines for determining the type of particle present.

BIOCHEMISTRY

The Human Blood Group Substances

Structural Studies. Work on the chemical structure of the specific blood-group substances continued and, with the object of obtaining higher yields of the oligosaccharides already identified and of isolating new fragments, much attention was given to the elaboration of more efficient reagents and procedures for the acid hydrolysis and alkaline decomposition of these biologically important substances.

More highly purified and stronger solutions of polystyrene sulphonic acid were prepared and used for the hydrolysis of the specific substances in an apparatus specially designed by Dr. Donald and Professor Morgan. The specific substance was hydrolysed under precisely controlled conditions of time and temperature by being pumped through a heating coil of glass and then through a length of Visking dialysis tubing contained within a larger tube carrying distilled water that flowed in the opposite direction. The rate of flow of the polystyrene sulphonic acid solution of the specific substance, and of the distilled water into which the hydrolysis fragments diffused, were adjusted so that the diffusible fragments were completely recovered before they suffered any further heating with the catalyst. To test the efficiency of this procedure Mr. Aston subjected a large sample (about 40 g.) of group specific substance obtained from hog gastric mucin to partial acid hydrolysis. The satisfactory yield of oligosaccharides and other products obtained indicated that blood-group substances of human origin could be profitably hydrolysed in this way. The diffusible products from the hog gastric mucin were used to devise procedures for the separation of higher oligosaccharides from each other, an indispensible step in the determination of the chemical structure of the specific substances and of associating a particular serological specificity with a relatively small chemical structure.

Procedures for the controlled degradation of the blood-group specific substances by alkali were examined by Miss Marr, and a strongly basic, water-soluble, polystyrene anion exchange resin was prepared from chlormethylated polystyrene through reaction with triethylamine in dioxan-methanol solution. In tests like those with the polystyrene sulphonic acid catalyst the product in its carbonate form proved to be an excellent reagent, and its use for the stepwise degradation of the human blood-group substances is under investigation.

The important problem of identifying the linkage of the carbohydrate chains to the amino acid-containing moiety in each of the specific substances remains to be solved. Dr. Donald and Professor Morgan demonstrated that on treatment with 0.5*N* NaOH at 18–20° for several days the group substances were extensively degraded, and that about 80% of the combined serine and threonine in each substance was destroyed. It is believed that the scission of the linkage joining the carbohydrate chains to the peptide moiety and the fragmentation of the peptide is brought about by a β-elimination mechanism involving an O-glycosidic linkage. Work on this aspect of glycoprotein structure continues.

Biosynthesis of Blood Group Substances. Miss Ziderman and Dr. Watkins continued their search for the enzymes involved in the biosynthesis of human blood group

substances by examining tissue homogenates for glycosyl transferases that will transfer sugar units from nucleotide-bound sugar donor substrates to mono- or di-saccharides, (Reports 1964, 1965). The enzyme, previously found in homogenates of rabbit stomach mucosa (Report 1965), that transfers galactose-C^{14} from uridine diphosphate galactose-C^{14} to the disaccharide 4-*O*-*β*-D-*N*-acetylglucosaminyl-*N*-acetylglucosamine, was found in human stomach homogenates from subjects belonging to groups A, B or O. The chromatographic mobility of the compound to which the galactose was transferred by the human enzyme suggested that it was a trisaccharide. The galactose-C^{14} was released by a *β*-galactosidase but not by an α-galactosidase, demonstrating that the galactose was transferred in a *β*-linkage. On partial acid hydrolysis of the trisaccharide a labelled disaccharide was formed which was chromotographically identical with 4-*O*-*β*-D-galactosyl-*N*-acetyl-D-glucosamine. The enzyme is therefore tentatively characterised as a *β*-(1→4)-galactosyl transferase. The grouping 4-*O*-*β*-D-galactosyl-*N*-acetyl-D-glucosamine occurs in all the blood group specific glycoproteins and is responsible for their cross-reactivity with horse anti-pneumococcal Type XIV serum (Report 1957). Work is in progress to determine whether the galactosyl transferase will add galactose-C^{14} to a macromolecular acceptor containing non-reducing *N*-acetyl-glucosaminyl residues.

Dr. Smith investigated methods for the preparation of uridine diphosphate *N*-acetyl-D-glucosamine, uridine diphosphate-*N*-acetyl-D-galactosamine and guanosine diphosphate fucose; these three sugars are required for experiments on the biosynthesis of A, H and Le^a substances. Radioactive labelled UDP-*N*-acetyl-D-glucosamine-C^{14} was synthesized in three stages; glucosamine hydrochloride was converted chemically to glucosamine-6-phosphate, which was then *N*-acetylated with C^{14} labelled acetic anhydride; the labelled *N*-acetylglucosamine-6-phosphate was converted to the 1-phosphate and thence to UDP-*N*-acetyl-glucosamine by the phosphorylase of Baker's yeast and phosphoglucomutase. Attempts to convert the labelled UDP-*N*-acetylglucosamine to UDP-*N*-acetylgalactosamine with preparations from human stomach were unsuccessful and other sources of UDP-*N*-acetylglucosamine-4-epimerase are being investigated. Enzymic synthesis of GDP-D-mannose-C^{14}, the immediate precursor of GDP-L-fucose-C^{14}, was achieved in two stages: mannose-C^{14} was converted by hexokinase to mannose-C^{14}-6-phosphate, which was then incubated with phosphoglucomutase and the GDP-mannose pyrophosphorylase present in extracts of *Salmonella typhimurium* to give GDP-mannose-C^{14}. The conversion of the GDP-mannose-C^{14} to GDP-fucose-C^{14} by extracts of *Aerobacter aerogenes* is now under investigation.

Enzymic Degradation of Blood Group Substances. Indirect evidence indicated that the destruction of the serological activity of Le^a substance by enzymes of *Trichomonas foetus* occurs because the terminal *α*-L-fucosyl residues are removed (Report 1965). Mr. Harrap and Dr. Watkins demonstrated that preparations of A-destroying enzyme from *T.foetus* contained an Le^a-destroying enzyme. On treatment of Le^a substance with these enzyme preparations, 30% of the fucose and small amounts of galactose and *N*-acetylglucosamine were released. The resulting extensive loss of Le^a specificity supported the suggestion that the Le^a-destroying enzyme is a fucosidase. It now seems that the fucose released from A substance (Report 1965), comes from the Le^a serological determinants in blood group A preparations. Preliminary work indicates that *β*-galactosidase in extracts of *T.foetus* can be separated on Sephadex G.200 from those enzymes destroying blood group activity. It is hoped that this *β*-galactosidase will prove useful for sequential enzymic degradation of the carbohydrate chains of the blood group substances and for the determination of the anomeric linkage of galactose in fragments isolated from the degradation products of blood group substances and of oligosaccharides formed by enzyme biosynthesis.

Acquired Blood Group Substances. The Le^a and Le^b blood group factors, unlike

the other human blood group antigens, do not appear to be an integral part of the red cell surface and are derived from the blood plasma. Dr. Grochowska and Dr. Watkins examined the *in vitro* acquisition of Le^a activity by Le(a-b-) red cells. At least two factors are concerned; a non-specific factor present in ultrafiltrates of serum from either Le(a+) or Le(a–) subjects and a specific factor present in the non-filtrable portion of the Le(a+) but not Le(a-b-) serum. On Sephadex G.200 Le(a+) serum separated into three main fractions of protein, which were tested for their ability to transform Le(a-b-) cells to Le(a+) cells. None of the fractions alone was active but a mixture of the first and third fraction was as active as the original serum.

Phospholipids and Glycolipids

Glycolipids. Dr. Gray continued his studies on the distribution, chemical composition and structure of the glycolipids present in normal and abnormal mammalian cells. With the exception of the CL2/Balb/c (leukaemia) mouse ascites tumours, all the tissues examined, whether normal or tumour, contained the same four classes of closely related glycolipids—the ceramide monohexosides, the ceramide dihexosides, the ceramide trihexosides and the ceramide hexosaminyltrihexosides. Structural studies at the Institute and by others indicate that as a general rule the carbohydrate moiety of the glycolipid molecule is glycosidically linked through glucose to the ceramide portion of the molecule and the hexoses are linked in a $\beta(1\rightarrow4)$ configuration. Some exceptions to these generalisations were found in the ceramide dihexosides isolated from different mouse ascites tumours (Report 1965) and in the ceramide hexosaminyltrihexosides isolated from horse spleen. A structural analysis of the latter compounds is in progress.

Dr. Jennifer Wells continued the investigation of pig lung tissues as a readily available source of glycolipids. Four classes of glycolipid were obtained in good yield (270 mg. total glycolipid/Kg. fresh tissue) and their distribution and chemical compositions determined. The major component was ceramide trihexoside. A complete structural analysis was carried out on the glycolipids of each class by Dr. Adams (see below); they are now in use as standard reference compounds.

Gas Chromatographic Analysis of Glycolipids. Dr. Adams investigated the composition and structure of several glycolipids by gas chromatographic methods. Standardised preparative methods for carbohydrate derivatives suitable for gas chromatography were established. The carbohydrate composition was determined by liberating the hexose units as methylglycosides and converting them to the volatile trimethylsilyl ethers. The methyl esters of the fatty acids produced during the formation of the methyl glycosides were also analysed by gas chromatography. Precise details of the configuration of the glycosidic links were obtained by methylating the glycolipid, releasing the methylated hexoses in the molecule by methanolysis and analysing the various *O*-methyl ethers formed by gas chromatography. A complete analysis can be made with as little as 2–3 mg. of glycolipid; it may prove possible to make one with even less.

The structures of the four glycolipid fractions isolated from pig lung tissue by Dr. Jennifer Wells were determined. The ceramide monohexoside was ceramide glucoside. *O*-β-D-galactosyl-(1→4)-*O*-β-D-glucosyl-(1→1)-ceramide was the major component of ceramide dihexoside fraction but small amounts of *O*-β-D-galactosyl (1→4)-*O*-β-D-galactosyl-(1→1)ceramide and *O*-β-D-glucosyl-(1→4)-*O*-β-D-galactosyl-(1→1)-ceramide were present. This last dihexoside has not previously been reported in mammalian tissues. The ceramide trihexoside fraction and the ceramide hexosaminyltrihexoside fractions were identified as *O*-β-D-galactosyl-(1→4)-*O*-β-D-galactosyl-(1→4)-*O*-β-D-glucosyl-(1→1)-ceramide and *O*-β-D-*N*-acetyl-galactosaminyl-(1→4)-*O*-β-D-galactosyl-(1→4)-*O*-β-D-galactosyl-(1→4)-*O*-β-D-glucosyl-(1→1)-ceramide respectively. The fatty acids in the ceramide moieties of all four classes were lignoceric, nervonic and behenic acids.

Partial Synthesis of Glycolipids. Mr. Hay continued his investigation of methods of synthesis of the simple ceramide glycolipids. The existing methods of complete chemical synthesis of the ceramide monohexosides and dihexosides suffer from the disadvantage of either a large number of chemical stages or starting with products which are difficult to obtain. It appeared possible to exclude the initial stages of synthesis by starting with ceramide—an intermediate product of synthesis—from natural sources. The phospholipid, sphingomyelin, found in most tissues contains the ceramide moiety, which can be quantitatively isolated in a pure state after treatment of the sphingomyelin in aqueous either with phospholipase C from *Cl.welchii.* Pig lung sphingomyelin was chosen as a source because its ceramide fatty acids are similar to those in the ceramide moieties of the glycolipids from tumour tissues. The final stages of synthesis were accomplished by preparing 3-*O* benzoyl ceramide and treating it with the relevant acetobromo-sugar and then removing the acetyl and benzoyl protecting groups.

The synthesis of specific ceramide dihexosides are of particular interest; especially of the ceramide galactosyl-β-(1→4)-galactoside, which was found, as well as the more usual ceramide lactoside, in several different tissues. With this in mind Mr. Hay prepared 4-*O*-β-D-galactosyl-D-galactose by a partial acid hydrolysis of a galactan isolated from lupin seeds.

Surface-active Lipoprotein in Mammalian Lung. Dr. Gray and Mr. Body continued the study of the lipoproteins isolated from the alveoli of mammalian lungs. Lipids consisting of phospholipids (80%) and neutral lipids (20%) accounted for 80% of the dry wt. of the material. The phospholipid composition was phosphatidylcholine (78%) phosphatidyl-ethanolamine (6%, including phosphatidylserine) phosphatidylinositol (4%), phosphatidylglycerol (4%) and sphingomyelin (2%). A large proportion of the fatty acid in the phosphatidylcholine was palmitic acid; the dipalmityl phosphatidylcholine conferred the surface active properties on the lipoprotein. A very interesting finding was phosphatidylglycerol, which is not a common component of tissue phospholipids. Preliminary results suggest that it was present in two different forms, one of which was a fatty acid ester derivative of the other. The relative distribution and structures of the phosphatidylglycerols in the lipoprotein fraction and in whole lung tissue are being studied.

Recently, a method for the isolation of the surface-active lipoprotein (cf. Report 1965) was devised elsewhere, which produces a much higher yield of lipoprotein/g. of fresh tissue than the current method. Mr. Body compared the two procedures and obtained a far better yield by the new method; but the composition of the product differed considerably from that of previous preparations. It had a much higher protein content (60%) and a lipid composition very similar to that of whole lung tissue. The composition and properties of both types of preparation of lipoprotein are being studied in detail.

Mammalian Cell Membranes. The lipid composition of isolated cell membrane preparations from rat liver was studied. The amount of cell membrane material isolated was usually limited to a few milligrams. Miss Dod modified several analytical techniques so that microgram quantities of individual lipid components could be separated from each other and recovered. The lipid compositions of a cell membrane preparation and a cell mitochondrial membrane preparation from rat liver were found to be significantly different. About 55% of the total lipid in the cell membranes was phospholipid as compared with 75% in the mitochondrial membranes. The phospholipids in the cell membranes were phosphatidylcholine (major component) phosphatidylethanolamine, phosphatidylserine, phosphatidylinositide and sphingomyelin; cardiolipin was not detected. In the mitochondrial membranes cardiolipin was present but not sphingomyelin. The ratio of phosphatidylserine to phosphatidylethanolamine appeared to be much higher in the cell membranes than in mitochondrial membranes and whole rat liver. Although the cell membranes contained a higher proportion of neutral lipids than the mitochondrial membranes, there were fewer components. The cholesterol esters and

triglycerides present in the mitochondrial membranes were not detected in cell membrane preparations. Cholesterol, fatty acids and monoglycerides were common to both.

Some indication of the importance of particular lipids in relation to the structural integrity of the lipoprotein complex which constitutes a cell membrane may be obtained by studying the action of enzymes like phospholipases A and C with well-defined lipid substrate specificities, on different cell membrane preparations. As a preliminary, Dr. Jennifer Wells studied methods of purifying phospholipase A of crude snake venoms, and isolated a phospholipase A fraction and a haemolytic fraction from several snake venoms by paper electrophoresis. The action of the purified enzymes on pure phospholipid substrates is being studied.

BIOPHYSICS

Physicochemical Studies of Blood Group Substances

Studies of the sedimentation and viscosity characteristics of blood group substances are described in previous reports (1964, 1965). To supplement the conclusions about macromolecular conformation so obtained, Dr. Creeth and Mr. Knight attempted to measure the extent of polydispersity, and to detect any secondary structure in the peptide part of the molecule.

The extent of polydispersity may throw light on the biosynthesis of these substances. The fewer the steps between the controlling genes and the synthesis of these macromolecules, the more likely are the macromolecules to be homogenous. Where the steps are many, or where the synthesis is virtually uncontrolled, as in starch synthesis by plants, a random distribution of sizes is likely. The B-active preparation previously characterised, giving a single peak in the ultracentrifuge, was studied by sedimentation equilibrium. The z-average and weight average molecular weights so determined define the standard deviation of the distribution. The results indicated a very wide spread of molecular weight, the standard deviation being about 90% of the weight average value of 1×10^6; a strongly skewed distribution must be present, with 5% of the material having a molecular weight above 3×10^6. The implication is, accordingly, that a random synthetic process operates between the stage of peptide synthesis and the addition of terminal carbohydrate residues which confer group specificity, in general agreement with the hypotheses of Morgan and Watkins.

The analytical figures for blood group substances are compatible with a peptide containing 100 or more residues which, uncombined with the carbohydrate moiety, should have a well-defined secondary structure. An attempt was accordingly made to detect such a structure in the glycoprotein. Ultracentrifugal analyses were made of mixtures of the blood group substances with the protein denaturant sodium dodecyl sulphate. The sedimentation coefficient was significantly decreased at fairly high ratios of detergent to glycoprotein, but only to the extent expected from the degree of complexing already established. Moreover, the original sedimentation coefficient, and full biological activity, were restored on removing the detergent by dialysis. It is unlikely, therefore, that a specific secondary structure exists in the peptide part of the molecule.

Macroglobulins of Normal Human Plasma

Dr. Kekwick continued work on the separation and purification of αM (19S α globulin) and γM (19S γ globulin) globulins from the concentrate obtained (Report 1965) from normal human plasma. By molecular-sieve chromatography with Sephadex G.200, followed by ion-exchange chromatography with DEAE-cellulose, immunologically pure αM globulin was obtained which displayed a single peak in the analytical ultracentrifuge under appropriate conditions. Between pH 6.0 and 7.0 this protein proved to be very sensitive to sodium chloride (0.10–0.15M) which induced aggregation that was more extensive at temperatures near to freezing than at room temperature.

This result may explain some difficulties which have occurred in preparing γM

globulin uncontaminated with immunologically detectable traces of αM globulin during purification on DEAE-columns. During preparation, after the bulk of the αM globulin had passed out of the column, the γM globulin was eluted by raising the ionic strength of the initial phosphate buffer by increasing concentrations of sodium chloride. In these circumstances aggregates of αM globulin tending to form would be retained along with the γM globulin on the column until eluted at higher ionic strengths. Modified elution schedules are being devised.

Dr. Creeth and Dr. Kekwick studied the effect of pH on the sedimentation characteristics of the pure αM globulin. Partial dissociation commenced in the region of pH 4 to give a component with a sedimentation coefficient of 12S. This is of considerable interest, for a 12S component would have approximately half the molecular weight of the parent 19S molecule, and is a much larger fragment than can be obtained by conventional denaturing agents. Characterization of the dissociation is now complete, and the isolation of the 12S component is under study.

Animal Antisera to Human Immunoglobulins. Dr. Kekwick has continued to collaborate with Dr. K. L. G. Goldsmith and Dr. T. T. B. Phillips of the Blood Group Reference Laboratory in the production and study of animal antisera to human γG and γM globulins. Immunologically pure human γG globulin was prepared for the production of monospecific antisera. Methods for the removal of heteroagglutinins to human erythrocytes from rabbit, goat and sheep antisera to human immunoglobulins are under examination. The results of chromatographic studies on goat antisera are consistent with the view that these heteroagglutinins are associated with the γM globulin. Other evidence suggests that this is true also of the heteroagglutinins of rabbit and sheep antisera.

Refinements in Equilibrium Ultracentrifuge Measurements

As currently employed, the interference optical system of the ultracentrifuge does not allow the identification of the integral number characterizing a given fringe; hence the absolute values of concentration which are necessary for the precise determination of molecular weights must be obtained by a variety of indirect methods. All these have disadvantages which may become extreme in making measurements on glycoproteins or proteins in denaturing solvents. Dr. Creeth, Mr. Knight and Mr. Holt devised a new method, based solely on the properties of the final interference fringe record, which should be applicable generally to any system. Tests on purified proteins were generally satisfactory and are being extended to mixtures of known composition.

BLOOD PRODUCTS LABORATORY

The laboratory continued to participate actively in the work of the M.R.C. Hypogammaglobulineamia Working Party and to collaborate with the Department of Medicine, Liverpool University and the Regional Transfusion Centre, Liverpool in preparing anti-Rh (D) γ globulin for the clinical trial of its value for preventing haemolytic disease of the newborn.

Dr. Maycock, Mr. Vallet and Mr. Wesley are investigating the problems of obtaining plasma from immunized donors containing adequate amounts of antibody for preparing antitetanus γ globulin.

During the earlier part of the year an I.C.I. team carried out a methods study investigation of the laboratory on behalf of the Medical Research Council, in preparation for the expansion of the laboratory.

Pharmacologically Active Substances in Human Plasma. Dr. Mackay and Mrs. Silk, with the collaboration of Miss Mason, continued their investigation of kininogenetic systems in blood plasma. The effect of solvent on the distribution and activation of esterase, plasma permeability factor (PF/dil) and kallikreinogen was studied with concentrates of fibrinogen and α and β globulins prepared by precipitation with ether, ethanol and acetone. The most striking differences occurred in fibrinogen concentrates precipitated by ethanol and ether. Ethanol-precipitated fibrinogen contained a small amount of esterase and

PF/dil, and less kallikrein than ether-precipitated fibrinogen. Kallikreinogen in ethanol-precipitated fibrinogen was activated by treatment with ether, but not PF/dil or esterase. When ethanol was used, most of the activated plasma enzymes were concentrated in a fraction corresponding to ether-precipitated G2/1R, previously shown to contain these enzymes (Report 1965). Kallikrein, esterase and PF/dil were activated by acetone and were distributed, in approximately similar concentration between fibrinogen and three α and β globulin fractions when acetone was used as a precipitant in plasma fractionation.

BLOOD GROUP RESEARCH UNIT

The year was spent, like the last three, in seeking for "new" blood groups and in applying those already known to problems of human genetics.

The X Chromosome

The two most obvious applications of the X-linked blood group system, Xg (Report 1962), were to attempts to improve the gene map of the X and to the study of people with abnormalities of number (aneuploidy), or form, of the X.

X Mapping. This has been disappointing: about 2,000 families representing 33 of the 60 known X-linked conditions have now been grouped but the distance between the Xg locus and all these other loci appears, with three possible exceptions, to be too great to measure. During this work much has been learnt about the difficulty of measuring linkage.

Early in the work there was strong evidence from Israeli and Greek families, mainly of two generations, that the Xg locus was about 26 recombination units away from the locus for glucose-6-phosphate dehydrogenase deficiency (Report, 1963). On the other hand Sardinian families and a second series of Israeli families, mainly of three generations, indicate that the distance is greater than 26, if indeed it is measurable at all. These conflicting results have rather called in question the efficiency of the mathematical tests necessary to deal with two generation families. The two other possible linkages are with ichthyosis and angiokeratoma.

X Chromosome Aneuploidy. The application of the Xg groups to people with the wrong number of X chromosomes has been very satisfying.

XXY. This is the commonest chromosomal background of Klinefelter's syndrome. One hundred and ninety patients with the karyotype XXY have now been grouped and the Xg distribution differs significantly from that expected both of normal males and normal females. Both parents of 50 of these were tested and, from the Xg groups, it may be calculated, by a method devised for the purpose by G. R. Fraser, that both Xs are maternal, $X^{M}X^{M}Y$, in about 65% of the cases and that one X is paternal, $X^{M}X^{P}Y$, in the rest. That is, about two-thirds of the causative non-disjunctions have happened at oogenesis or at an early division of the zygote and about one-third have happened at spermatogenesis. In those families in which the groups show that a patient has had an X as well as a Y from his father the non-disjunction must have happened at the 1st meiotic division, or earlier. Such families provided the first proof (Report, 1964) that XXY could arise through non-disjunction at spermatogenesis. Analysis of the XXY families gives a strong hint that the Xg locus is a long way from the centromere of the X: this supports a growing impression that Xg is near the end of the short arm.

XX males. Such males are a great rarity: nine were tested and both parents of five of them. Three of the five families were informative and showed that both Xs were of maternal origin. From this it can be very reasonably concluded that such people begin as an XXY and that the Y starts off male differentiation but at some early cleavage division the Y is lost.

XXXY and XXXXY. These abnormal karyotypes are rare causes of Klinefelter's syndrome: four of the former and 19 of the latter were tested. The families of two of the XXXXYs were informative: the Xg groups showed that all the Xs were maternal in both families.

XXYY. Eleven patients were grouped and both parents of five of them. Two families were informative: the Xg groups showed that in both the father must have contributed an X and two Ys. The only easy interpretation is that non-disjunction has happened at both first and second meiotic divisions at spermatogenesis. These families provided the first evidence in man that non-disjunction can happen at both meiotic divisions consecutively.

XO. This is the commonest background to the syndrome named after Turner. Samples from 184 XO females have now been tested in the Unit and the Xg distribution is that expected of males. Both parents of 122 of these patients were also grouped and this allowed the calculation, devised by Dr. Fraser, that about 27% of the cases are caused by the loss of a maternal X and that about 73% are caused by the loss of a paternal X or Y. The Xg groups demonstrated conclusively, for the first time (Report, 1963) that the condition could be caused by the loss of a maternal X. However, the Xg groups can only show, in about one-third of the families, whether a paternal or maternal X has been lost; they cannot show, as they may so neatly in XXY families, at which stage of gametogenesis the accident has happened.

X isochromosome X. This is a less common cause of Turner's syndrome: such patients have one normal X and one X with the short arm missing and replaced by a duplicate of the long arm. Of these, and of the mosaics XO/X iso-X (equivalent as far as the short arm is concerned), 46 examples have been tested so far and they show the male distribution of the Xg groups.

Turner's syndrome due to other karyotypes. This covers a wide range of mosaic karyotypes XO/XXX, XO/X deleted short arm X, XO/X ring X etc., etc. Thirty-four have now been tested and they too show the male incidence of the Xg groups.

XY females. Testicular feminization is a rare condition in which people have the external appearance of females but are, cytologically, males. They have a short vagina but no uterus. The abnormality is clearly inherited, though whether as an X-linked recessive or as an autosomal dominant but sex-limited character is not known: the fact that affected people are sterile prevents the distinction being made from pedigree evidence. Twenty-five families have now been tested in the hope that linkage would be demonstrable either with Xg, which would prove the condition X-borne, or with any of the other blood groups, which would prove it autosomal. So far the work has not been rewarded: if the gene responsible is on the X it is not within measurable distance of Xg: if it is on an autosome it has not shown itself to be a close neighbour of any of the other blood group loci.

A perhaps related condition, male pseudohermaphroditism, presents the same problem, but here again no evidence of linkage has emerged: 16 families were grouped.

The Xg Groups: Frequency and Inheritance. Counts were made of unrelated white people of Northern European extraction tested up to July 1965: 2,082 samples were from Britain, 670 from North America and 666 from the mainland of Northern Europe. Gene frequencies were calculated by the method devised for Xg by the late Professor J. B. S. Haldane. The frequencies were the same in Britain and North America: Xg^a = 67% and Xg = 33%, but, surprisingly, in Northern Europe they are significantly different, 60% and 40%. The gene frequencies may be used to calculate the expected incidence of the four mating types, and the proportion of Xg(a+) and Xg(a–) among the children. Up to July, 1965, 1,339 British, North American and Northern European families had been tested and fitted well with the calculated figures with the notable exception, recorded in the 1964 Report, of two Xg (a-) women who had, between them, five Xg (a+) sons. Of various possible explanations the most attractive is that both fathers, who are Xg(a+), had inherited a Y, or possibly an autosome, on to which a small piece of an X carrying an Xg(a+) gene had been translocated. About 300 large Sardinian families did not show any exception to the known manner of inheritance.

Xg in animals. Since the last Report seven more gibbons were tested and the unequal distribution of the Xg groups in the two sexes is now significant ($p = 1$ in 63) showing that the antigen is X-linked in the gibbon as well as in man.

The Autosomes

Attempts to assign blood group loci to particular autosomes continue (Report 1965) but with no positive success. The negative evidence is increasing and work in the Unit has shown that, for example, the loci for MNSs, Rh, Duffy and Kidd groups are not sited on the short arm of chromosome No. 5; nor are these loci on the short arm of No. 13. The loci for MNSs, Rh, Kell and Kidd are not on half of the long arm of No. 18, though which half it is they are not on is not yet clear.

The steady flow of samples of blood suspected to contain something "new" continues. As in past years the majority come from the States: they often represent cross-matching problems prior to surgery.

The P System. The rare antigen P^k found in Finns (Reports 1961, 1962, 1964, 1965) was found in two more Finnish families: these families were compatible with the theory of inheritance of P^k (Report, 1965).

A puzzling antibody whose reactions are influenced by the P and the A_1A_2BO groups of the red cells was mentioned in several Reports (1962, 1964, 1965). The serum was sent from Oklahoma. Another example was identified in a serum sent from Marseilles and the antigen is being investigated in the large family of the propositus.

The Dombrock System. Families continue to be tested for this new and useful system (Report 1965) and, as a result, Dr. Tippett has accumulated evidence that the Do locus is not closely linked to the locus for any of the following systems: ABO, MNSs, P, Rh, Lutheran, Kell, Duffy, Kidd, Yt or secretor. There is a hint of linkage, not close, with the Duffy genes but more families will have to be tested to settle the point. After the unravelling of this new system two more examples of the antibody, anti-Do^a, were soon found, in Bristol and in Miami: serum from both patients was sent to the Unit for confirmation.

The Rh System. Dr. Tippett continues to classify people whose cells have the antigen D but whose serum contains anti-D (Reports 1962, 1963, 1964, 1965). No recent samples have fallen outside the seven categories mentioned in the last report.

The Colton System. During the course of the year three sera, from Minneapolis, Oxford and Oslo, which had presented transfusion problems proved to contain the same "new" antibody. By tests on families the corresponding antigen, Co^a, was shown not to be a hitherto unrecognized part of most of the established systems. The majority (99.7%) of white people have the antigen, so anti-Co^a is not itself of practical use for genetic application. On the other hand 10% of people are heterozygous Co^aCo, and, if the expected antithetical antibody, anti-Co, does turn up it will be recognizable and will make a genetic distinction of practical use.

Hints of New Antigens. Six examples of an antibody, all sent during the last few years from the States, adumbrate a new antigen related to the MNSs system. The antigen is commoner in Negroes than in Whites. Unfortunately the antibody gives equivocal results with more than half the red cell samples tested and is therefore not yet useful in genetical work.

Eight samples, all but one from the States, of another antibody define an antigen present in more than 95% of white people. The results are, however, consistent only for freshly taken samples of red cells and not for samples sent through the post. Because of this the antibody is for the present, almost useless as a genetic tool, though it does bring into some control yet another hazard of transfusion.

Twins. The Unit is collaborating in various surveys involving twins: the blood groups are done as an aid to establishing the zygosity. Twins or triplets were occasionally grouped in connection with skin grafting for burns.

During the course of the year the Unit worked with more than a hundred cytogeneticists and physicians, only a few of whom can conveniently be thanked here:

Dr. A. Cahan, New York; Professor M. Siniscalca and Dr. L. N. Went, Leiden; Dr. G. Filippi, Rome; Dr. J. M. Opitz, Madison; Dr. C. Sheba and Dr. A. Adam, Tel-Hashomer; Dr. C. Kerr, Oxford; Dr. A. W. Eriksson, Helsinki; Professor P. E. Polani, London; Dr. J. H. Edwards, Birmingham; Dr. A. Frøland, Copenhagen; Dr. J. Lindsten, Stockholm; Dr. M. A. Ferguson-Smith, Glasgow; Dr. A. de la Chapelle, Helsinki; Dr. K. Boczkowski, Warsaw; Professor R. Turpin and Dr. C. Salmon, Paris; Dr. H. F. Polesky and Mrs. Jane Swanson, Minneapolis; Dr. Mary N. Crawford, Philadelphia; Professor B. Broman, Stockholm; Professor J. J. van Loghem Jnr., Amsterdam; Dr. H. Heistö, Oslo; Dr. M. M. Pickles, Oxford; Professor Bruce Chown and Miss Marion Lewis, Winnipeg.

The Unit has also had much help from many of the Regional Blood Transfusion Centres, the Blood Group Reference Laboratory, Pfizer Diagnositics, New York, Spectra Biologicals, East Brunswick and Laboratorios Grifols, Barcelona.

Once again the Unit thanks the Staff of the Institute for giving so many samples of their blood.

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 including those designated as National Reference Laboratories through the World Health Organization. Large numbers of blood and serum specimens from Great Britain and from overseas have been examined for blood-group antigens and antibodies.

On August 31st, 1965, Dr. A. E. Mourant relinquished the Directorship of the Laboratory to take charge of the Medical Research Council's Serological Populations Genetics Laboratory at St. Bartholomew's Hospital, London, E.C.1. In his new post, Dr. Mourant is able to devote his time entirely to the study of blood groups distribution among different populations throughout the world. His place as Director of the Blood Group Reference Laboratory was taken by Dr. K. L. G. Goldsmith, his former Deputy. Dr. T. T. B. Phillips joined the staff of the Blood Group Reference Laboratory as Dr. Goldsmith's assistant.

A notable event was the holding at the Laboratory of the First European Blood Transfusion Course of the Council of Europe. Twelve candidates from twelve different European countries attended the course which lasted two weeks, and they were given both theoretical and practical training by visiting experts and by the laboratory staff. Social activities were also arranged.

Dr. Ikin, who is in charge of serum production, continued her investigations into methods of producing antisera in rabbits with especial reference to antibodies of the MN blood group system. She also identified a second example of the blood group antibody anti-Yt^b, the first example having been discovered in the previous year by Miss Giles (Report 1965).

Dr. Ikin also undertook comprehensive testing of provisional standard preparations of "complete" and "incomplete" anti-D sera which she had earlier produced with a view to their being accepted by the World Health Organization as International Standard Preparations. Samples of the "incomplete" anti-D serum were distributed among 34 specialist laboratories throughout the world, each being asked to examine the material for potency and for specificity. Results have now been received from those laboratories which participated, and in due course their findings will be included in a report to the Expert Committee on Biological Standardisation of the World Health Organization.

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, many samples coming from

overseas. They use as many and varied techniques as possible. Miss Giles also investigated the Rh gene complexes D(C) (e), d (c) (e) and D--, and studied the frequencies of the Yt^b and Cs^a antigens discovered by her in the previous year. She investigated antibodies against stored red cells, that were associated with cirrhosis of the liver and false-positive serological tests for syphilis.

Dr. Goldsmith and Dr. Phillips, with the help of Dr. Kekwick, studied methods of production of anti-gamma-G and anti-gamma-M immunoglobulins and also produced anti-human-complement. They investigated methods for the removal of unwanted heteroagglutinins from anti-human-globulin sera. Dr. Goldsmith continued his investigations into antibodies against leucocytes and platelets, exchanging specimens with Dr. W. J. Jenkins of the North-East Metropolitan Blood Transfusion Centre. Dr. Phillips and Dr. Goldsmith studied methods of lymphocyte culture as one possible means of choosing tissue donors.

The production of anti-human-globulin serum used for routine purposes is being carried out on behalf of the Blood Group Reference Laboratory by Mr. J. Bleby, Director of the Medical Research Council's Laboratory Animals Centre, Carshalton.

Gm-typing and the screening of sera for the presence of Gm antibodies is now part of the routine work of the laboratory.

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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THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Accounts 1966

THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Balance Sheet and Accounts

31 DECEMBER 1966

CHELSEA BRIDGE ROAD . LONDON, S.W.1 . 23 MAY, 1967

The Governing Body

Sir CHARLES DODDS, Bt, MVO, MD, D SC, FRCP, FRS, *Chairman*
H. P. G. CHANNON, MP, *Hon. Treasurer*
The Rt Hon LORD BROCK, MS, FRCS
Professor Sir LINDOR BROWN, CBE, FRCP, FRS
Professor L. H. COLLIER, MD
Professor D. G. EVANS, D SC, FRS
The Rt Hon The EARL OF IVEAGH, KG, CB, CMG, FRS
Professor Sir ASHLEY MILES, CBE, MD, FRCP, FRS

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

Financial Report of the Governing Body

1. The Balance Sheet as at 31st December 1966, shows balances to the credit of the various funds as follows: Capital Fund £826,775, Specific Funds £225,511 and Bequest Funds £25,875. The balance on the Sinking Fund for Freehold Buildings of £164,107 is after transferring £8,211 from Income and Expenditure Account. During the year donations of £605 have been added to the Re-endowment Fund. The General Fund Investment Reserve has been credited with profits, less losses, on sales of investments amounting to £1,952 and the Sinking Fund Investment Reserve has been credited with a profit of £214.

2. The General Fund Income and Expenditure Account shows the Income for the year as £295,886 compared with £314,548 in 1965. Expenditure amounted to £346,265 against £314,217 last year. The deficit for the year is £50,379 compared with a surplus of £331 in 1965.

3. The year's deficit of £50,379 shown by the General Fund Income and Expenditure Account has been transferred to the Capital Fund.

4. Cooper Brothers & Co., the present Auditors, will continue in office pursuant to Section 159 (2) of the Companies Act, 1948.

D. G. EVANS

Acting Chairman of the Governing Body.

The Lister Institute of Preventive Medicine
INCOME AND EXPENDITURE ACCOUNT
for the year ended 31 December, 1966

GENERAL FUND

(1965)		*Total Expenditure*	*External Contributions*	
£159,214	Salaries and wages	£277,168	£101,620	£175,548
12,098	Emoluments of two members of the Governing Body in an executive capacity	13,196	—	13,196
7,226	Premiums on federated superannuation policies	13,800	3,718	10,082
2,201	Premiums on group pension policy	2,543	1,111	1,432
6,872	Rent, rates and insurance	8,253	426	7,827
16,461	Gas, water, fuel and electricity	20,394	4,788	15,606
5,566	Office expenses, stationery and printing	6,555	939	5,616
630	Audit fee	630	—	630
2,046	Travelling expenses	3,715	773	2,942
1,322	Biochemistry expenses	9,779	8,182	1,597
1,142	Microbiology, immunology, experimental pathology and electron microscopy expenses	4,022	1,358	2,664
498	Biophysics expenses	5,053	4,266	787
320	Virology expenses	1,224	619	605
48,317	Serum, vaccine and virus vaccine expenses	43,870	2,042	41,828
8,668	Animals	9,402	1,795	7,607
11,113	Animal house expenses and forage	12,443	1,198	11,245
14,442	Buildings, alterations, repairs and renewals (including depreciation £4,093)	21,979	245	21,734
3,682	General apparatus and new installations	6,008	—	6,008
652	Library expenses	1,842	—	1,842
920	General stores	1,763	—	1,763
3,570	Staff canteen loss	5,187	599	4,588
—	Seventy-fifth anniversary celebrations	5,407	2,500	2,907
—	Blood products laboratory expenses	9,192	9,192	—
7,257	Amount transferred to sinking fund for freehold buildings (including £7,787 interest on investments)	8,211	—	8,211
331	*Surplus transferred to Capital Fund after charging to expenditure £16,943 for additions to property and equipment*	—	—	—
£314,548		£491,636	£145,371	£346,265

(1965)			
	Interest and dividends on investments:		
£53,874	General fund	£54,725	
6,834	Sinking fund	7,787	
			£62,512
38	Underwriting commission		208
246,712	Sales of sera, vaccines, virus vaccines, &c.		225,058
7,090	Rent		8,108
	Deficit transferred to Capital Fund after charging to expenditure £19,671		
—	for addition to property and equipment		50,379
£314,548			£346,265

For Report of the Auditors and Notes on the Accounts see page 8

The Lister Institute of Preventive Medicine
BALANCE SHEET · 31 December, 1966

(1965)				
	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, 1965	£513,804		
	Less deficit 1966	50,379		
		463,425		
513,804	Add profit on house sold	2,883	466,308	
874,271				£826,775
	Specific Funds			
155,896	Sinking fund for freehold buildings	164,107		
33,221	Pension fund	31,684		
29,115	Re-endowment fund	29,720		
			225,511	
	Bequest Funds			
18,186	Jenner Memorial studentship fund	18,302		
7,911	Morna Macleod scholarship fund	7,573		
			25,875	
244,329				251,386
	Specific Grants and Legacies Unexpended			
96	Cancer research legacies (1937-1950)		20	
3,151	Nuffield Foundation grants (1952-1962)		1,783	
5,440	Guinness-Lister research grant (1953-1966)		9,721	
243	Fleming Memorial Fund grant (1965-1966)		—	
8,930				11,524
	Current Liabilities			
41,286	Creditors and accrued charges			61,461
£1,168,816				£1,151,146

D. G. EVANS
G. L. BROWN
} *Members of the Governing Body*

(1965)						
	Fixed Assets					
	FREEHOLD PROPERTY (SEE NOTE 1)					
£73,548	Land and Buildings, Chelsea				£73,548	
	Queensberry Lodge Estate, Elstree					
	at cost to 31st December, 1964			£20,455		
	at cost from 1st January, 1965		27,718			
	Less depreciation		5,693			
				22,025		
32,500					42,480	
2,049	House, Bushey, at cost				—	
						£116,028
	FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS (SEE NOTE 2)					
2,472	at cost less depreciation to 31st December, 1963				2,472	
	at cost from 1st January, 1964		7,073			
	Less depreciation		4,273			
4,200					2,800	
						5,272
114,769						121,300

	General, Specific and Bequest Funds. Investments and Uninvested Cash	*Quoted at cost*		*Unquoted*	*Uninvested*		
		in Gt. Britain	*Elsewhere*	*at cost*	*cash*		
630,968	GENERAL	£552,530	£103,594	£40,587	—	696,711	
	SPECIFIC—						
156,333	Sinking fund for freehold buildings	159,580	—	—	5,178	164,758	
33,221	Pension fund	29,471	—	—	2,213	31,684	
29,115	Re-endowment fund	27,634	—	—	2,086	29,720	
	BEQUEST—						
18,186	Jenner Memorial studentship fund	14,518	—	940	2,844	18,302	
7,911	Morna Macleod scholarship fund	6,631	—	—	942	7,573	
875,734		790,364	103,594	41,527	13,263	948,748	
	LESS INVESTMENT RESERVES						
113,209	General fund				115,161		
437	Sinking fund for freehold buildings				651		
						115,812	
762,088							832,936
	Current Assets						
101,798	Debtors and payments in advance					114,046	
190,161	Balance at bankers and cash in hand					82,864	
291,959							196,910
£1,168,816							£1,151,146

For Report of the Auditors and Notes on the Account see page 8

Notes on the Accounts

1. Freehold property additions and replacements since 1912 at Elstree and since 1935 at Chelsea, until 31st December, 1964, have been charged to revenue.

2. Additions and replacements to furniture, fittings, scientific apparatus and books between 31st December, 1920 and 31st December, 1963, have been charged to revenue. No depreciation has been charged between 1920 and 1963.

3. Quoted investments at a cost of £893,958 have a market value at 31st December, 1966, of £1,145,458.

4. Stocks of sera, virus vaccines and horses on hand at 31st December, 1966, have not been valued in the accounts.

Report of the Auditors to the Members

The accounts set out on pages 4 to 7 are in agreement with the books which, in our opinion, have been properly kept. We obtained the information and explanations we required.

In our opinion the accounts, amplified by the information given in paragraph 1 of the Financial Report of the Governing Body, comply with the Companies Act, 1948, and give a true and fair view of the state of affairs and the surplus of the Institute.

COOPER BROTHERS & Co.
Chartered Accountants.

LONDON, 25th May, 1967.

The Lister Institute of Preventive Medicine

PENSION FUND

(1965)			(1965)		
£2,458	Pensions	£3,161	£33,589	Balance as at 1st January, 1966	£33,221
—	Loss on realisation of investment	476	2,090	Interest on investments (gross)	2,100
33,221	Balance carried forward	31,684			
£35,679		£35,321	£35,679		£35,321

JENNER MEMORIAL STUDENTSHIP FUND

(1965)			(1965)		
£182	Stipend of student	759	£17,513	Balance as at 1st January, 1966	£18,186
—	Loss on realisation of investment	32	855	Interest on investments (gross)	907
18,186	Balance carried forward	18,302			
£18,368		£19,093	£18,368		£19,093

MORNA MACLEOD SCHOLARSHIP FUND

(1965)			(1965)		
£219	Stipend of scholar	702	£7,771	Balance as at 1st January, 1966	£7,911
—	Loss on realisation of investment	25	359	Interest on investments (gross)	389
7,911	Balance carried forward	7,573			
£8,130		£8,300	£8,130		£8,300

NUFFIELD FOUNDATION GRANTS

(1965)			(1965)		
	Salaries, wages, laboratory ex-		£4,372	Balance as at 1st January, 1966	£3,151
£1,221	penses and animals	1,368			
3,151	Balance carried forward	1,783			
£4,372		£3,151	£4,372		£3,151

GUINNESS-LISTER RESEARCH GRANT

(1965)			(1965)		
£13,142	Salaries and wages	£8,848	£5,826	Balance as at 1st January, 1966	£5,440
2,244	Laboratory expenses	4,871	15,000	Amount received	18,000
5,440	Balance carried forward	9,721			
£20,826		£23,440	£20,826		£23,440

The Lister Institute of Preventive Medicine

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

(UNIVERSITY OF LONDON)

75th ANNIVERSARY


LISTER INSTITUTE OF PREVENTIVE MEDICINE

THE LISTER INSTITUTE FROM CHELSEA BRIDGE

Contents

The Governing Body

Sir Charles Dodds, Bt, MVO, MD, D SC, FRCP, FRS, *Chairman*

Sir Hugh Beaver, KBE, D ECON SC

The Rt Hon Lord Brain, DM, FRCP, FRS

The Rt Hon Lord Brock, MS, FRCS

H. P. G. Channon, MP, *Hon Treasurer*

Professor L. H. Collier, MD

Professor D. G. Evans, D SC, FRS

The Rt Hon The Earl of Iveagh, KG, CB, CMG, FRS

Professor Sir Ashley Miles, CBE, MD, FRCP, FRS

Director: Professor Sir Ashley Miles, CBE, MD, FRCP, FRS

Deputy Director: Professor W. T. J. Morgan, CBE, D SC, FRS

Superintendent of Elstree Laboratories: Dr W. d'A. Maycock, MVO, MBE, MD

Secretary and Accountant: S. A. White, AACCA

Elstree Secretary and Estate Manager: G. J. Roderick, B COM

THE PAST

Foundation and Early History

IT IS DIFFICULT, AFTER 75 YEARS, TO REALISE THAT THE LISTER Institute started almost by chance, met at its inception with considerable opposition and overcame the difficulties of its early years by a number of fortunate coincidences.

Early in 1889 the Lord Mayor of London, Sir James Whitehead, visited the Pasteur Institute, where Pasteur was successfully inoculating against hydrophobia people who had been bitten by rabid dogs. He came away determined that some financial recognition of Pasteur's work should be made by the English people and that a centre for the treatment of hydrophobia should be set up in London. Accordingly he called a meeting of influential people to discuss this at the Mansion House.

There was considerable opposition to the Lord Mayor's suggested foundation of a clinic in England. Some felt that a clinic, by lessening the danger in the public mind would make more difficult the task of eradicating rabies by muzzling dogs and by rigid quarantine regulations for animals entering the country. The anti-vivisectionists, who were already against Pasteur because of the alleged cruelty of his experiments on animals, were more vociferous. Demonstrations at the meeting were feared and Ray Lankester wrote to Sir James 'It will be necessary to have a good force of police to guard the entrance, and the stewards must prevent anyone from obtaining admission who has not received an invitation'. The meeting passed without interruptions, but it was decided not to found a clinic.

As a result of this meeting a Committee was formed which raised the sum of £2,000 to be given to the Pasteur Institute. After the final meeting, the Committee took tea in the Mayor's Parlour and the view was strongly expressed by some members that the

idea of establishing an institute should not be given up. A new Acting Committee was formed to carry this into effect. Owing to ill health, the Lord Mayor was Chairman for a short time only and was succeeded by Sir Joseph Lister. Within a few weeks it made a report suggesting that a Jenner-Pasteur Institute be established in Cambridge for 'the preparation and inoculation of material which has been found successful for preventive inoculation and the carrying out of investigations fitted to increase our knowledge of the nature of disease-producing germs'.

In December 1890 an appeal to the public for funds was launched. Contributions did not come in freely although some of the city companies, especially the Grocers' Company, responded generously. Individual people were not easily convinced of the necessity for a research institute; indeed one Harley Street con-

SIR JAMES WHITEHEAD, LORD MAYOR OF LONDON 1889, WHO BEGAN THE PROJECT OF THE INSTITUTE

EDWARD CECIL GUINNESS, 1ST EARL OF IVEAGH, BENEFACTOR

sultant told the Committee's representative he did not approve of preventive medicine and thought a good epidemic was much more satisfactory.

Fortunately at this time a Mr Berridge had left a large sum of money for the furtherance of sanitary science, and the trustees of the fund were persuaded by the Committee to give £40,000 towards the proposed Institute. One condition made by the trustees was that the Institute should be established in London. By the beginning of 1891 about £60,000 had been collected and the Committee decided to incorporate the Institute. The President of the Board of Trade, Sir Michael Hicks Beach, at first declined to grant the application; however, after representations had been made to him by influential people the British Institute of Preventive Medicine was incorporated on 25th July, 1891, though sanction for experimental work on animals was withheld.

A site in Chelsea Bridge Road was obtained on very generous terms from the Duke of Westminster, who had been present at the original Mansion House meeting. It was obvious that some time would elapse before a new building could be completed and the Committee looked round for a suitable organization with which they could amalgamate. The College of State Medicine, a private teaching organization having aims not far removed from those of the proposed Institute was approached, and late in 1893 amalgamation was effected. An important factor was that the College's premises at 101, Great Russell Street were already licensed for animal experiments, and the transfer of this licence to a new building would be relatively easy. Scientific work started at Great Russell Street early in 1894.

Meanwhile the erection of the buildings at Chelsea was meeting with great opposition from local residents, who lodged a petition with the Home Secretary on the grounds that the new laboratories would be a danger to the neighbourhood, because bacteria might escape from the windows and bring disease to their homes. In spite of protests the first half of the building of the Institute was completed in 1898 and scientific work began there during that summer.

As a consequence of a gift of funds raised to mark the centenary in 1896 of Jenner's discovery of vaccination, the Institute was renamed the Jenner Institute of Preventive Medicine. A few years later, however, because a commercial organization supplying smallpox vaccine established its prior claim to the name 'Jenner Institute', the Institute was once again, and finally, renamed the Lister Institute of Preventive Medicine. No happier name could have been chosen, for Lister was not only a pioneer in preventive medicine, but had been from the beginning active in bringing about the foundation of the Institute.

In 1900 the Institute was rescued from a critical financial situation by a gift of £250,000 from the first Earl of Iveagh; this marked the beginning of an association with the Guinness interests

which has continued to the present day. At this time also the constitution of the Governing Body of the Institute was modified and Lord Lister became its first Chairman.

The production of therapeutic sera had begun in 1894 under the direction of Armand Ruffer, the horses being stabled at the Brown Sanatory Institute in Wandsworth, since there was no space for them at Great Russell Street. The first diphtheria anti-toxic horse serum to be made and used in Britain was successfully administered in somewhat dramatic circumstances by Charles Sherrington, then professor-superintendent of the Brown Institution, to a nephew of his who was critically ill. Later in 1894 a farm at Sudbury, near Harrow, was leased by the Institute for stabling animals, but in 1902 these activities were transferred to the Queensberry Lodge Estate at Elstree, which had been bought for this purpose.

The last step, by which the Institute acquired the constitution it has today, was taken in 1905, when it was admitted as a School of London University. Since this time senior members of the staff have held University titles appropriate to their duties, and the research facilities of the Institute have been widely used for the training of postgraduate students proceeding to higher degrees.

The Institute's Work

OVER THE LAST 75 YEARS THE TITLES OF, AND THE KIND OF WORK done in the Institute's various departments have changed with the changing interests and skills of the staff. Physiological and nutritional researches, for example, which loomed large in its earlier days, are no longer pursued.

In a short sketch, it is impossible to highlight past achievements in terms of the many biological disciplines that have gone to their making, and in what follows, they are dealt with, sometimes in a rather Procrustean fashion, as part either of Microbiology, Biochemistry, Biophysics or Experimental Pathology.

MICROBIOLOGY

The study of infections – their pathology and epidemiology, the vaccines and antitoxins for their prevention and cure, and the protozoa, bacteria and viruses that cause them – has been a major part of the Institute's activities. The start to manufacture diptheria antitoxin in 1894 has already been noted. In 1900 a novel attempt was made to produce a plague antiserum. It was abortive, but the disease was tackled as an epidemiological problem by the Institute, in collaboration with the Royal Society, when in 1905-08 the Plague Commission in India, under C. J. Martin's direction, conclusively proved that the rat flea was a carrier of the plague bacillus. This interest in tropical epidemiology was later extended to some distinguished work on fleas and lice, a study of the life cycle of a trypanosome, *T. lewisi*, in fleas and rats and another in Uganda by Muriel Robertson on the life cycle of cattle trypanosomes in the tse-tse fly. In the last 15 years the Institute's interest was renewed in a collaboration in a world-

wide investigation of the feeding habits of the blood-sucking mosquitoes and tse-tse flies that carry malaria, yellow fever and trypanosomiasis, and in work on the immunopathology of trypanosomiasis.

From the 1920's to 1939, there was much pioneer work on the nature of the rickettsial organisms that cause trench fever, and the viruses of vaccinia (the Institute began to make vaccine for smallpox in 1905), fowlpox and psittacosis. In this J. C. G. Ledingham, both before and after he became Director in 1931, was himself a leader, and an inspiration to others in the Institute.

Virus research was resumed after the war, and 1952 saw the establishment of a Virology department at Chelsea, which (in collaboration with the Medical Research Council, whose Trachoma Research Unit was established in 1955 and attached to the department) is chiefly occupied with the development of a prophylactic vaccine for combating trachoma, a crippling eye infection of developing countries.

Among bacteria proper, the emphasis has been on the enterobacteria, especially those responsible for infections of the gut. In the 1920's J. A. Arkwright was the first to describe in the typhoid bacillus a form of variation that has since proved to be of fundamental importance in medical bacteriology, whereby virulence and a certain constituent of the cell-wall are lost together – one indeed being dependent on possession of the other; and A. Felix found a variant of the typhoid bacillus with another cell-wall constituent associated with exceptionally high virulence.

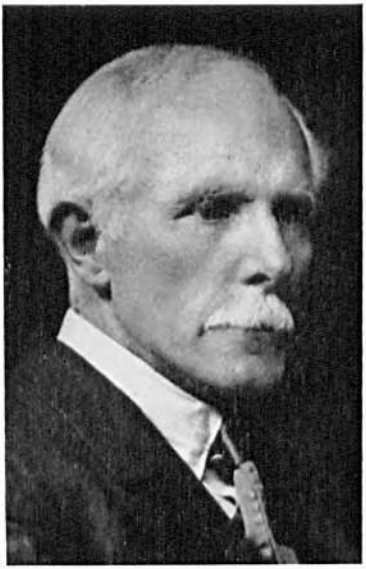
During this decade too, Emmy Klieneberger-Nobel described another important type of variation, in which bacteria lose the power to synthesize their rigid cell wall.

In more recent years, the Guinness-Lister Unit, which was established in 1953 through the generosity of Arthur Guinness Son and Company, among other distinguished work on inheritance in bacteria defined the genes whose mutation determines the variation described by Arkwright. The Unit also revived the study of bacterial physiology, which may be said to have been initiated in the Institute in 1908 by Harriette Chick's and C. J. Martin's classic study of the dynamics of disinfection.

It was in a bacillus of another type of gut infection, dysentery, that W. T. J. Morgan during the 1930's was the first to demonstrate the structure of the cell-wall constituent associated with virulence; a conjugate of lipid, polysaccharide and protein that has since proved to be characteristic for many other bacteria, and of prime significance in the genesis of the infections they cause.

The two World Wars stimulated work on the bacilli of gas gangrene: in 1914-18, on the proper identification of the chief causative organisms, carried out in Paris by a member of the Institute's staff, Muriel Robertson; and in 1939-45 on the main toxin of one of them, *Cl. welchii*, which was proved, uniquely among

SIR CHARLES MARTIN, CMG, FRS
(1866-1955), THE FIRST DIRECTOR



SIR JOSEPH ARKWRIGHT, FRS
(1884-1944), BACTERIOLOGIST

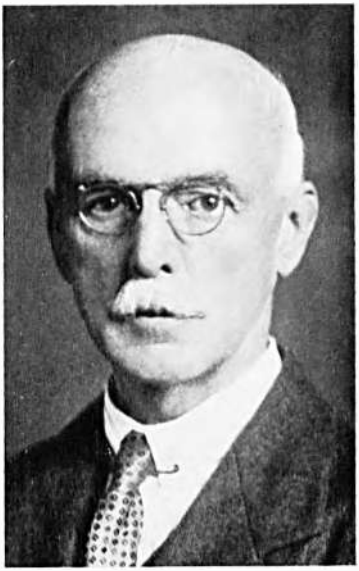
bacterial exotoxins, to be an enzyme attacking lipids, and on the role of another *Cl. welchii* enzyme, hyaluronidase, in promoting the spread of infection in the gas-gangrenous wound.

Equally important are the researches in devising or improving the antitoxins and vaccines made at the Elstree laboratories for clinical use. In post-war years, for example, a smallpox vaccine of high stability – an important property for material used in the tropics – was devised by drying in a form that at temperatures below 50°F is expected to remain active for over 200 years; and the whooping-cough bacillus was analysed to yield a non-toxic substance which immunizes animals as effectively as whole bacilli, and, it is hoped will replace the crude vaccine in current use.

BIOCHEMISTRY

Arthur Harden was appointed as chemist in 1897. At this time Buchner had made a great advance in biochemistry by decomposing sugar with juice extracted from yeast, thereby showing, contrary to current belief, that living cells were not necessary for fermentation. In 1906 with W. J. Young, Harden published his proof that one of the enzymes responsible for this effect was inactive unless a small-molecular substance was also present in the juice. This was the first demonstration of a 'co-enzyme', an outstanding and indeed seminal discovery for which Harden was later awarded a Nobel Prize. They also found that inorganic phosphate stimulated the fermentation, entering the process by forming sugar phosphate compounds, including a hexose diphosphate.

SIR JOHN LEDINGHAM, CMG, FRS (1875-1944), DIRECTOR 1930-1943



SIR ARTHUR HARDEN, FRS (1865-1940); WITH H. VON EULER, NOBEL LAUREATE IN CHEMISTRY, 1929

In parenthesis, there was, over 40 years later, a renewal at the Institute of fundamental work on co-factors in enzyme action, which led to the unambiguous synthesis of another co-enzyme, pyridoxal phosphate, concerned in the bacterial metabolism of amino-acids; and to contributions on the structure and synthesis of co-enzyme A.

R. Robison, who succeeded Young in 1913, discovered a hexose monophosphate, produced during alcoholic fermentation. He followed a later observation, of the breakdown of the calcium salt of hexose monophosphate by the enzyme phosphatase, with a study of calcification in bone-forming tissues. In the presence of this salt, calcium was rapidly deposited in tissue cultures of young bone from healthy animals, but not in those from animals with rickets; both healthy and diseased tissues, however, were rich in phosphatase. Robison's pioneer work in this field had partly unravelled the biochemistry of this difference in behaviour when he died in 1941.

The identification at this time of the main toxin of Welch's gas gangrene bacillus as an enzyme which splits the lipid substance lecithin, stimulated work on the lipids of bacteria, which was later extended to those of mammalian cells. A team is now at work, elucidating the structure of these compounds and their regulatory function in the membranes of the cell and the smaller organelles within the cell – a further problem of great importance in cellular biology.

After the last war, the biochemical pre-occupations under the direction of W. T. J. Morgan shifted from substances in the cell

walls of bacteria to those substances on the walls of human red blood corpuscles which determine the blood group to which an individual belongs. These are glycoproteins, compounds with polysaccharide and polypeptide constituents. During the last two decades, an intensive exploration of the molecular structure of the glycoproteins determining the blood groups within the ABO and the Lewis genetic systems, and the relation of structure to the immunological behaviour of the red cell, was advanced to the point where hypotheses of the gene control of the biosynthesis of these substances can be formulated, and tested experimentally. It appears that all human beings at first synthesize a common glycoprotein; under the influence of his particular blood-group genes each individual then produces 'transferase' enzymes which, by adding chains of sugar molecules in a certain order, transform the common glycoprotein to a characteristic blood-group substance.

Interest in polysaccharide biochemistry was also reflected in researches on the biosynthesis and metabolism of starches and glycogen, especially in relation to the molecular structure of the intermediate products and the enzymes involved in these processes.

BIOPHYSICS AND BLOOD PRODUCTS

The physicochemistry of proteins was long a major interest of the Institute. In the early years of the century, C. J. Martin and Harriette Chick made some pioneer studies of the viscosity and the heat denaturation of proteins in solution. Their investigation of the properties of the globulins separable from horse serum had implications for subsequent work on the refinement of antitoxic sera produced in the horse.

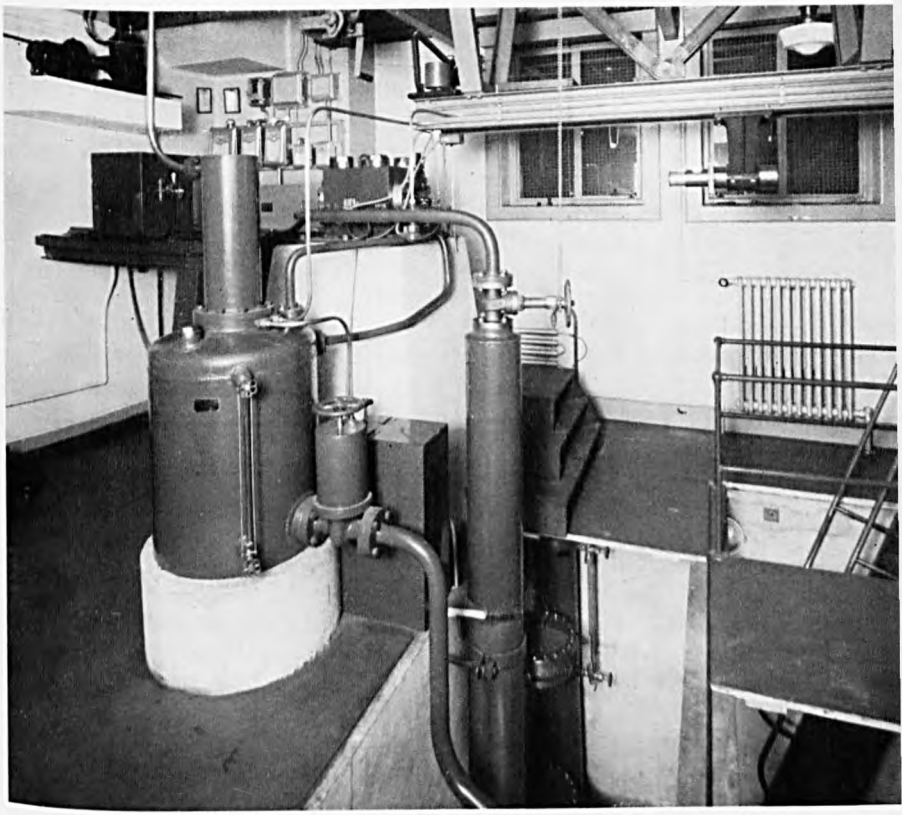
In 1936, the Biophysics Laboratory was built and, through the generosity of the Rockefeller Foundation, equipped with a Svedberg analytical and equilibrium ultracentrifuge. The then recently devised Tiselius apparatus for electrophoresis was added in 1937. These instruments for the exploration of the nature of macromolecules were the first of their kind in Britain and, under A. S. McFarlane, were used in the pre-war years for characterising the viruses of vaccinia and bushy stunt disease, the polysaccharide capsular substance of the pneumococcus, the proteins of human sera in health and disease and of antitoxic horse globulins.

The technical facilities of the department proved to be essential in 1939-45, when the war-time demand for plasma as a substitute for whole blood in transfusions was met by devising a method of providing it, which in R. A. Kekwick's hands was later refined into a system for the separation and purification of a number of the clinically useful proteins of human plasma. This, and the biochemical researches on blood group substances, was the beginning of an emphasis on work with human blood that has

persisted to the present day; an emphasis largely due to A. N. Drury who, on succeeding to the directorship in 1943, brought to the Institute his war-time experience with the Medical Research Council of the organisation of emergency blood supplies and the production of dried plasma. Under his guidance, plasma fractionation began in 1944, and in 1946 he was instrumental in attaching the MRC Blood Group Reference Laboratory and the MRC Blood Group Research Unit to the Institute.

After the war, the increasing demand by clinicians for blood products, which the production capacity at Chelsea was unable to satisfy, led to the establishment, in concert with the Medical Research Council, of the Blood Products Laboratory on the Elstree estate. Here plasma is harvested from blood collected at the Transfusion Centres of the National Health Service, and the products distributed for clinical use. Among the products are whole plasma, freeze-dried in a highly stable form; albumin; fibrinogen and thrombin for use in surgery; gamma globulin for

THE SVEDBERG OIL-TURBINE ANALYTICAL CENTRIFUGE, ERECTED 1936 IN THE BIOPHYSICS LABORATORY



the prophylaxis of measles and other infective diseases; and a concentrate of antihaemophilic factor. The antihaemophilic factor from a human source was the first of its kind to be made available in any country for the treatment of haemophilia.

The biophysical work continues at Chelsea, mainly on biologically important proteins of human plasma, and antibodies of significance in immunology; on other kinds of biological macromolecules, such as glycoproteins and polysaccharides; and on the refinement of the analytical techniques now made possible by the department's newer instrumentation.

EXPERIMENTAL PATHOLOGY

The pathological problems that have been tackled in the Institute are indeed various. Many of them deal with specific infective diseases of man, and are described in the section on microbiology; the others range from detailed studies of the microcirculation in diseased tissues to the nutritional deficiences of children in post-war Europe.

Soon after the start of C. J. Martin's directorship in 1903, the Institute co-operated in an investigation of the physiological adaptation of man to raised and lowered atmospheric pressures, which resulted in a precise definition of the precautions to be taken for the prevention of Caisson disease and other untoward effects during diving operations.

When troops abroad in the 1914-18 war succumbed to scurvy and beri-beri – the latter was recognized by C. J. Martin while on service in the Mediterranean – a team was formed to investigate the nature and the distribution in food of the two vitamins necessary for their prevention. After the war this team, which by then constituted the Nutrition Department of the Institute, collaborated with one from the Medical Research Council in a study of the rickets and scurvy that were affecting the populations of central Europe. In Vienna, Harriette Chick and Margaret Hume contributed substantially, among other things, to a conclusive demonstration that rickets in children, and the related hunger osteomalacia in adults could be cured by Vitamin D, or by exposing the sufferers to sunlight or ultraviolet irradiation. In the interwar years, work continued on various water-soluble vitamins and the diseases due to their deficiency, both at Chelsea and at Chesterton in Cambridge, where C. J. Martin, in his retirement, set up a laboratory. At Cambridge also a nutritional problem posed by the 1939-45 war was examined, that of the nutritive value of bread, to whose solution the Institute contributed with a study of the vitamin content of different parts of the wheat grain; with results that led directly to the 85% extraction rate of grain for the war-time national whole-meal loaf.

The department of Experimental Pathology was re-established

in 1952. Its researches have centred on the nature of the early antimicrobial effects observed in animal tissues, and their relation to the local inflammation that occurs soon after infecting bacteria have lodged there.

As the result of an investigation of how bacterial and other forms of injury to the tissues induce in the nearby vessels the leak of blood proteins that is characteristic of inflammation in general, a system was discovered in the blood and lymphatic fluids, whose activation gave rise to enzymes with leak-promoting properties. The role of these and other leak-promoting factors in natural inflammation remains to be defined.

The foregoing record of some of the Lister Institute's outstanding contributions to medical science omits much of interest and importance, to which only a detailed narrative could do justice. It indicates at least the wide range of the Institute's activities since its foundation, and emphasizes a tradition of work – laboratory, clinical and epidemiological; and advisory as well as research – in both the exploratory and the applied aspects of preventive medicine, often done in association with other institutions and scientistsin Britain and overseas. As will be evident from the outline of present activity that follows on p.24, this tradition is maintained today.

FORMER CHAIRMEN OF THE GOVERNING BODY

1900 Lord Lister, PC, OM, FRS

1903 Sir Henry Roscoe, PC, FRS

1913 Sir John Rose Bradford, KCMG, FRS

1915 Sir Henry Roscoe, PC, FRS

1916 Sir David Bruce, KCB, FRS

1932 Professor William Bulloch, FRS

1941 Lord Moyne, PC, DSO

1942 Sir Henry Dale, OM, GBE, FRS (to 1961)

FORMER DIRECTORS

1903 Sir Charles Martin, CMG, FRS

1931 Sir John Ledingham, CMG, FRS

1943 Sir Alan Drury, CBE, FRS (to 1952)

THE CHELSEA LABORATORIES

1891 Incorporation of 'The British Institute of Preventive Medicine', on funds raised in appeal launched by the Lord Mayor of London.

1893 Purchase of the Chelsea site from the Duke of Westminster.
Amalgamation with the College of State Medicine.

1894 Scientific work starts at 101 Great Russell Street.

1897 First volume of Transactions of the Institute.

1898 First half of Chelsea Laboratories completed and occupied.
Institute renamed 'The Jenner Institute', on accepting funds raised during the 1896 Jenner Centenary.

1900 Gift of £250,000 by the first Earl of Iveagh.
Lord Lister first Chairman of the reconstituted Governing Body.

1903 Institute finally named 'The Lister Institute of Preventive Medicine'.
Director, C. J. Martin.

the History of the Institute

1905 Admission as a School of London University.

1910 Chelsea Laboratory completed (Architects, Alfred and Paul Waterhouse).

1931 Director, J. C. G. Ledingham.

1936 Biophysics Laboratory built, housing the first ultracentrifuge in Britain (Rockefeller Foundation gift).

1943 Director, A. N. Drury.

1952 Director, A. A. Miles.

1953 Guinness-Lister Research Unit for microbiology established.

1965 Electron Microscopy Unit established (Fleming Fund gift).

THE ELSTREE LABORATORIES

1894 Farm rented at Sudbury, Middlesex for stabling animals.

1902 Occupation of Queensbury Lodge estate at Elstree, with house and stabling. Serum Laboratory built.

1903 Vaccine (now Bacterial Vaccine) Laboratory.

1906 'Yersin Block' (now Media Laboratory) built for work on plague.

1910 Cottages and additional stabling built.

1933 Biochemical Laboratory (rebuilt 1938 after a fire).

1947 Smallpox Vaccine Laboratories.

1952 New Animal Houses.

1954 Blood Products Laboratory, Canteen and new Cottages.

1959 Extension of Serum Laboratory.

1961 New Machine Shop.

1963 Virus Vaccine Laboratory.

1964 Extension of Blood Products Laboratory.

1965 Extension of Bacterial Vaccine Laboratory.

1966 Packing Laboratory.

AERIAL VIEW OF THE ELSTREE ESTATE, 1966

THE PRESENT

AT PRESENT (MIDSUMMER 1966) THE INSTITUTE HAS A TOTAL STAFF of 270, of which 56 are graduate scientists. Among these are 5 Professors, one Reader and 7 Recognised Teachers of London University, and 9 students for the degree of Ph D. The staff includes a number of scientists from overseas making prolonged visits; 15-20 scientists a year come for short visits of two weeks or more.

The Institute, though a school of London University, is an independent establishment under the direction of a Governing Body, with no direct financial support from governmental funds. Like other academic research institutions, the Institute has had, and has support from the various Research Councils for special research projects. It derives great scientific benefit too from having three Medical Research Council Units within its walls. Money for special projects has also been forthcoming from private persons and foundations, and from non-governmental public bodies.

The Institute, however, derives its continuing income from endowments and the sale of immunological products – antisera and antitoxins, bacterial vaccines and virus vaccines – made by its production departments at Elstree. These departments, however, are like those at Chelsea, in that members of the graduate staff have the opportunities and facilities for research, which is pursued in addition to the work in devising, developing and producing therapeutic substances, and may be unrelated to production activities. On behalf of the Ministry of Health, the British Council and the World Health Organisation, the staff also devote time to the training of scientists from overseas, particularly from developing countries, in methods of production.

The scope of the Institute's work is illustrated by the following

alphabetical list of the departments, the scientists in charge of them, and the main research in each. For the production departments at Elstree, only the research activities are noted; not the applied research and development of immunological substances and human blood products made for prophylactic and therapeutic use in man.

PLANT FOR FREEZE-DRYING IN THE BLOOD PRODUCTS LABORATORY, ELSTREE

Departments of the Institute

Bacterial Vaccines (Elstree) DR A. F. B. STANDFAST, SC D
The immunopathology of experimental infections by *B. pertussis* (the whooping cough bacillus).
The immunizing substance of *B. pertussis*.

Biochemistry PROFESSOR W. T. J. MORGAN, CBE, D SC, FRS
The molecular structure of human blood-group substances, and the genetic control of their biosynthesis.
The lipid chemistry of mammalian cell membranes, and their relation to the function of normal and tumour cells.

Biophysics PROFESSOR R. A. KEKWICK, D SC, FRS
The isolation, characterisation and structure of biological macromolecules.
The methodology of ultracentrifugal and related physicochemical analytic techniques.

Blood Products (Elstree) DR W. D'A. MAYCOCK, MVO, MBE, MD
Separation and purification of biologically important proteins of human plasma; including proteolytic enzymes, gamma globulins for the therapy of tetanus and antirhesus factor globulins for the prevention of haemolytic disease of the newborn.

Electron Microscopy DR A. M. LAWN, PH D, MRCVS
The correlation of morphological and genetic studies of enterobacteria.
The process of uterine implantation of the mammalian ovum.

Experimental Pathology
PROFESSOR SIR ASHLEY MILES, CBE, M, DFRS
Microcirculatory changes in inflammation, and their biochemical mediators.
Immunology of allergies in man.

Guinness-Lister Research Unit for Microbiology
PROFESSOR G. G. MEYNELL, MD
Physiological and structural studies of bacteria.
The dynamics of bacterial and viral infections.
Antigenic structure in *Mycoplasma*.

Therapeutic Sera (Elstree) DR B. G. F. WEITZ, OBE, D SC, MRCVS
The immunopathology of experimental trypanosome infections.
Blood-feeding habits of African insect vectors of trypanosomiasis.

Virology (to which is attached the MRC Trachoma Research Unit, established 1955) PROFESSOR L. H. COLLIER, MD
Epidemiology of trachoma, immunopathology of experimental trachoma infections, and trachoma vaccine trials in the Gambia and Iran.
Carcinogenic substances affecting viruses.

Virus Vaccines (Elstree) DR C. KAPLAN, MB, CH B
Inactivated vaccinia virus for safe immunization against smallpox.
Purified rabies vaccine free from encephalitogenic impurities.

Besides the Trachoma Research Unit noted under 'Virology', the two following Medical Research Council Units are attached to the Chelsea laboratories, the second in a separate building of its own in the Institute's grounds.

Blood Group Research Unit (established 1946)
DR R. R. RACE, FRCP, FRS
The inheritance of blood groups, and their application in problems of human genetics; chromosome mapping and chromosomal abnormalities.

Blood Group Reference Laboratory (established 1946)
DR K. L. G. GOLDSMITH, PH D, MB, BS
The production of blood-grouping sera. Investigation of clinical blood transfusion problems.

For much of the history, the compilers of this booklet are indebted to Sir Alan Drury's 1948 review of the Institute's past in the Proceedings of the Royal Society, and to Dame Harriette Chick.

MIDSUMMER, 1966

R.A.K.
A.A.M.

PICTURE ACKNOWLEDGMENTS

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THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Report
of the GOVERNING BODY
1967

CHELSEA BRIDGE ROAD · LONDON · SW1

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The President of the ROYAL COLLEGE OF SURGEONS *Representing the Royal College of Surgeons of England*

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Deputy Director: Professor W. T. J. Morgan
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Susan T. Edwards, B SC
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D. G. Godfrey, B SC, PH D (*Trypanosomiasis Research*)

Guinness-Lister Research Unit

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M. R. C. Trachoma Research Unit

Electron Microscopy Unit

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†Winifred M. Watkins, D SC, PH D (*Reader in Biochemistry* (*Immunochemistry*) *in the University of London*)
*G. M. A. Gray, B SC, PH D
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Noreen M. Goggin, B SC
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L. Vallet, MA
§Margaret E. Mackay, M SC, PH D (*Medical Research Council External Scientific Staff*)
D. Ellis, B SC, PH D
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E. D. Wesley, B PHARM

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§R. R. Race, MD(*hc*), FRCP, FRS
Ruth Sanger, B SC, PH D
Patricia Tippett, B SC, PH D
E. June Gavin, B SC
Ann Staley, B SC

Blood Group Reference Laboratory

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Toby T. B. Phillips, MB, CH B
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Annual General Meeting of the Lister Institute of

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the Institute for the year 1966.

75th ANNIVERSARY

To mark the 75th Anniversary of its incorporation in 1891 an Open Day was held at Chelsea on Wednesday 9th November, 1966. Some 200 guests, from this country and from abroad, visited the laboratories and saw an exhibition of historical documents and demonstrations of the current work of the Institute. In the late afternoon a special Anniversary Lecture entitled "Lister and Modern Medicine" was given by the Rt. Hon. The Lord Florey OM, FRS at the Royal College of Physicians.

On the same day an Anniversary Dinner, presided over by Sir Charles Dodds and attended by the Governing Body, Senior Members of the Staff and one hundred and thirty guests, was held at the College. The Guest of Honour was the Rt. Hon. Kenneth Robinson, PC, MP, Her Majesty's Minister of Health. Guests from the United Kingdom included the Lord Mayor of Westminster, Sir Douglas Logan, Principal of the University of London, Professor P. M. S. Blackett, President of the Royal Society, Professor Sir Max Rosenheim, President of the Royal College of Physicians, Mr. A. J. Wright President of the Royal College of Veterinary Surgeons, Sir Solly Zuckerman, Chief Scientific Advisor to H.M. Government, Sir George Godber, Chief Medical Officer, Ministry of Health, the Rt. Hon. The Lord Florey, Provost of Queen's College, Oxford, and Sir Alan Drury; representatives of the Medical Research Council and of private organisations to which the Institute is indebted for financial support, including the Rt. Hon. The Lord Boyd; heads of research institutes and certain University departments of microbiology and pathology; members of Sir Charles Martin's and Sir John Ledingham's families; members of the Council and former members of the staff.

The guests from overseas were Dr. Paul Bordet, Institute Pasteur, Brussels; Dr. O. Girard, Institute Pasteur, Paris; Professor Dr. G. Henneberg, Robert Koch Institute, Berlin; Professor H. Lundbeck, State Serum Institute, Stockholm; Dr. R. de Vicente Jordana, Consejo Superior de Investigaciones Cientificas and Instituto "Jaime Ferran" de Microbiologia, Madrid; Dr. Preben von Magnus, State Serum Institute, Copenhagen; Dr. J. Spaander, National Institute of Health, Utrecht; and Professor Arne Tiselius, Institute of Biochemistry, Uppsala.

As a further part of the anniversary celebrations a Reception attended by members of the Governing Body and over two hundred and seventy past and present staff was held at the Connaught Rooms on 6th December, 1966. To mark this special year presentations were made to fourteen members of the staff, all of whom had completed twenty-five or more years service at the Institute, and who, between them, had completed five hundred and fifteen years service at the Institute.

GOVERNING BODY

At a meeting held on 28th June, 1966, the Council re-appointed Sir Charles Dodds, Lord Brain and Lord Brock at its representatives on the Governing Body until 31st December, 1967. Sir Hugh Beaver resigned as Honorary Treasurer in June, 1966 and was succeeded by Mr. Paul Channon.

The Institute is greatly indebted to Sir Hugh for the time he so valuably devoted to the affairs of the Institute during his eight years as its Treasurer and it is with great regret that the Governing Body records his death in January this year.

The Governing Body also records with great regret the death of Lord Brain, in December, 1966. Lord Brain became a member of the Governing Body in 1960 and

his wide knowledge and kindly interest in the affairs of the Institute will be much missed.

COUNCIL

At last year's Annual General Meeting the three retiring members of the Council, Mr. Paul Channon, Lord Iveagh and Mr. W. J. Thompson were re-appointed.

The three members of Council due to retire this year in accordance with the Articles of Association, but who are eligible for re-appointment, are Major L. M. E. Dent, a representative of the Worshipful Company of Grocers and Sir Lindor Brown and Dr. D. W. W. Henderson, each a representative of the Members of the Institute.

MEMBERS

The Governing Body records with regret the death during the year of Sir Roy Cameron, Professor G. P. Crowden, Professor J. Cruickshank and Sir Philip Manson-Bahr. Professor J. M. Ledingham accepted an invitation to become a member.

STAFF AND STUDENTS

The Governing Body records with pleasure that on 1st October, 1966 the University of London conferred on Dr. R. A. Kekwick the title of Professor of Biophysics. It also notes with pleasure that Dr. Emmy Klieneberger-Nobel, a former member of the Institute staff, was in February 1967 elected an Honorary Member of the Robert Koch Institute, Berlin.

Dr. D. G. Godfrey was appointed as Protozoologist; Dr. Drummond Ellis to the Blood Products Laboratory; Miss J. Walsh and Miss Susan Edwards to the Guinness-Lister Research Unit; Miss Caroline Bronne to the Serum Department; Miss Denise Ward to the Department of Biochemistry, as the Grocers' Company Student; and Mr. A. V. Payne and Miss Beryl Bristow to the Administrative Staff at Elstree. Mrs. Shirley Slavik, Dr. G. J. Harrap and Miss Vidya Gokale resigned during the year and Dr. C. Placido de Sousa completed his appointment.

The Institute was sorry to lose the services of Miss D. M. Whitbourne, who retired in July 1966, and of Mr. A. H. Reynolds who retired in October. Miss Whitbourne had worked in the office at Chelsea for over thirty years and Mr. Reynolds was a member of the maintenance staff for forty-eight years, first at Chelsea and, since 1946, at Elstree. The Governing Body also records with great regret the sudden death in January 1967 of Mrs. R. L. Lickens, the Chelsea storekeeper. Mrs. Lickens had worked at the Institute since 1930 and was due to retire this year.

Sir Ashley Miles, as a member of a Royal Society delegation, visited the National Academy of Sciences in Washington in May 1966. In June he went to Harvard Medical School, Boston, as a Visiting Lecturer.

In September 1966 Professor W. T. J. Morgan attended a symposium on glycoproteins at the IVth International Conference on Cystic Fibrosis at Grundelwald, Switzerland and the Third International Conference on Human Genetics held in Chicago; in October, as 1966 Guest Lecturer, he attended the Annual Meeting of the American Association of Blood Banks in Los Angeles, California and then lectured at the University of Indiana, Bloomington.

Dr. W. d'A. Maycock attended, as Ministry of Health representative, a meeting of the Subcommittee of Specialists on Blood Transfusion of the Public Health Committee of the Council of Europe, held in Berne in June 1966.

During August and September 1966 Professor L. H. Collier visited and lectured by invitation at the Institute for Virus Research, Kyoto University, Japan; at the

U.S. Naval Medical Research Unit, Taiwan; at an international symposium on Trachoma at the University of California Medical Center, San Francisco; and at the University of Washington, Seattle.

Professor R. A. Kekwick represented the Institute and the Royal Society at the 125th Anniversary Celebrations of L'Academie Royale de Médicine de Belgique held in Brussels in October. In December he attended a meeting of the International Committee on Haemostasis and Thrombosis held at Chapel Hill, North Carolina, U.S.A.

In March 1966 Dr. A. F. B. Standfast took part in a Round Table Conference on Adjuvants in Utrecht. In July he attended a conference on Tetanus, in Berne.

In February 1966 Dr. Winifred M. Watkins took part in a Hartford Foundation Conference on Blood Groups and Transfusion at the New York University School of Medicine. In September she attended a Symposium on "Biochemical Genetics" held during the Third International Congress on Human Genetics, in Chicago.

Mr. L. Vallet attended the 14th Colloquium on Protides of Biological Fluids at Bruges in May 1966 and in November a Conference on the Plasma Proteins and Cellular Elements of the Blood, in Cambridge, Mass., U.S.A.

In August 1966, Dr. Margaret Mackay attended the XIth Congress of the International Society of Haematology and the XIth Congress of the International Society of Blood Transfusion, in Sydney.

In May 1966, Dr. Ruth Lemcke acted as co-chairman and lectured, by invitation, at the session on "Immunology" at the Second Conference organised by the New York Academy of Sciences on the Biology of Mycoplasmas. She also lectured by invitation to the N.E. Branch of the American Society of Bacteriologists at the Massachussetts General Hospital, Boston.

Dr. W. A. Blyth, Dr. P. Reeve and Dr. Janice Taverne participated by invitation in a Symposium on Trachoma and Allied Diseases held at the University of California Medical Center, San Francisco. Dr. Reeve also lectured at the University of Washington, Seattle.

For the academic year 1966/67 there are thirteen postgraduate research workers at the Institute registered for higher degrees of the University. Six Ph.D degrees were awarded to students during 1966.

DONATIONS AND GRANTS

Arthur Guinness, Son & Co. Ltd., continue their generous support of the Guinness-Lister Research Unit and a further £4,000 out of the total grant of £35,000 has been received from the Fleming Memorial Fund for Medical Research for the support of the Electron Microscope Unit.

The Governing Body records its appreciation 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 Medical Research Council for researches on the chemical basis of human blood-group specificity, on the biosynthesis of blood-group specific glycoproteins and red cell antigens, on multiple blood-group specific serological characters associated with simple glycoprotein molecules, on structural analysis of glycolipids and lipo-proteins, on the characterization of proteins by the ultracentrifugal steady-state method, on the distribution of serotypes of *Bordetella pertussis*, and on the lipid components of the plasma membranes of mammalian cells. Grants were also received from the Ministry of Overseas Development in aid of research on the blood-meals of insect vectors of disease and on the immunology of trypanosomiasis; from the Rhodesian Government for research on the identification of blood-meals; from the Science Research Council for studies on the nature and structure of biological macromolecules, on the characterization of biological macromolecules by sedimentation analysis, for research on glycoprotein and glycolipid structure and function, and on the amino-acid components of various proteins; and from the Smith, Kline and French Foundation for research on sensitization to micro-organisms as a cause of generalized eczema.

The Governing Body also gratefully acknowledges donations to the Institute's Re-endowment Fund from the Prudential Assurance Company Limited and the Royal London Mutual Insurance Society Limited.

VISITING WORKERS

The following visitors, in addition to those listed under Staff, worked for short periods in the Institute's Laboratories: Dr. Rafia Tawfik Ahmed, Serum Institute, Agouza, Cairo; Miss Wendy Bull, North London Blood Transfusion Centre, Edgware, Middx.; Dr. Sawsan El Tayeb, National Control Department, Serum Institute, Agouza, U.A.R.; Dr. Barbara Jaroszynska-Weinberger, Department of Infectious Diseases in Childhood, Medical Academy, Warsaw, Poland; Dr. Susan Leong, Queen Elizabeth Hospital, Kowloon, Hong Kong; Dr. Eileen Lovett, Department of Genetics, University of Glasgow; Prof. G. V. Marinetti, Department of Biochemistry, University of Rochester School of Medicine, New York; Dr. Zsuzsanna Pusztai, M.D., National Institute of Serobacteriological Production and Research "Human", Budapest, Hungary; Dr. J. M. Reckler, Harvard University Medical School, Boston, Massachusetts; Dr. Trevor Williams, Dept. of Mathematics, Duke University, Durham, North Carolina.

Researches in 1966

SUMMARY

The bracketed numbers refer to pages and columns of the report where more detailed descriptions are to be found.

Microbiology

1. Bacterial genetics, physiology and morphology:
   (a) Antigen conversion by bacteriophages (13, ii).
   (b) Sexual conjugation by pili in colicinogenic bacteria (14, i).
   (c) Cell-wall growth and capsule formation (14, i) of the anthrax bacillus
   (d) Morphology and serology of sex pili (21, ii).
   (e) Ultrastructure of R mutants of Salmonella (22, i).

Infective Diseases and Immunity

1. Trypanosomiasis:
   (a) Immunopathology of trypanosomiasis in mice (12, i).
   (b) Biochemical characterization of *T. brucei* antigens (12, i).

2. Virus diseases:
   (a) Growth of slow and fast killing strains of trachoma agent (15, i).
   (b) Immunopathology of trachoma infection of guinea-pigs and baboons (16, i).
   (c) Immunogenicity of trachoma agent (16, i) and tests of trachoma vaccines overseas (16, ii).
   (d) Chemical inhibitors of virus growth (17, i).
   (e) Mechanism of photo-inactivation of vaccinia virus (17, i).
   (f) Immunopathology of experimental vaccinia infections (18, i).
   (g) Methods of assay of vaccinia virus (18, i).
   (h) Purification, lipid chemistry and biochemical activity of vaccinia virus (18, ii).
   (i) Purification and *in vitro* growth of rabies virus (18, ii).

3. Bacterial Infections:
   (a) Kinetics of bacterial multiplication in infected animals (14, ii).
   (b) Immunizing antigens of *Bordetella pertussis* (12, ii).
   (c) Toxicity of pertussis vaccines (13, i).
   (d) Immunoglobulins of anti-pertussis sera (13, i).
   (e) Clostridial toxins (13, ii).

4. Epidemiology:
   (a) Serological identification of blood meals of insect vectors of infective disease (19, i).

Immunology and Pathology

1. Immunology:
   (a) Autodegradation of preparations of horse globulins (19, i).
   (b) Cytophilic antibody and opsonization (19, ii).
   (c) Sensitization of tissues to cytotoxic antibody by exogenous antigens in experimental animals (20, i) in eczema and allergic vasculitis in man (20, ii).
   (d) Human reaginic antibodies (21, i).
   (e) Antisera to human immunogloblins (29, ii).

2. Pathology:
   (a) Relation of plasma factors that increase vascular permeability to the kinin-producing system (23, i).
   (b) Pharmacologically-active proteases in human plasma and in hydrolysates of fibrinogen (30, ii).
   (c) Topography of the reactions of the micro-circulation to thermal injury (22, ii).

Biochemistry

1. Human blood group substances:
   (a) Characterization of structure by chemical methods (24, i).
   (b) Biosynthesis of blood group substance (25, ii).

2. Lipids:
   (a) The gycolipids of normal and tumour cells of mammalian tissues (27, i) and of cell membranes (28, ii).
   (b) Characterization (27, i) and synthesis (28, ii) of glycolipids.
   (c) Surface active lipoprotein from mammalian lung (28, i).

Biophysics and Protein Studies

1. Macromolecules:
   (a) Polydispersity of human blood group substances (24, i).
   (b) Reversible denaturations of protein (30, i).

2. (a) Globulins and macroglobulins of human plasma (29, i).
   (b) Anti-Rhesus factor antibody in clinical trials (30, i).

MICROBIOLOGY

Trypanosomiasis

Trypanosoma vivax Infections. Dr. Taylor continued her studies on the infectivity of *Trypanosoma vivax* in mice. Optimum infection, in terms of the time and height of the peak parasitaemia, was obtained with an inoculum from infected donor mice containing 10 to 20 trypanosomes per 100 red blood cells. Similar infections were obtained with inocula stored at −190°C in liquid nitrogen for several months. Tests with specific cytotoxic antibody showed no change in the sensitivity of the trypanosomes of the donor mice at different periods during infection. Transmission of the infection by a given dose of trypanosomes was the more successful the earlier the stage of the parasitaemia at which the parasites were obtained. This phenomenon is under investigation.

The sera of mice infected with *T. vivax* contained complement-dependent haemolysins for sheep and horse but not for rabbit and mouse erythrocytes.

A quantitative agglutination test for *T. vivax* was devised by a modification of the cytotoxic test described by Le Page (personal communication). A haemagglutination-inhibition test, in which rabbit red blood cells treated with bis-diazotized benzidine and sensitized with purified and concentrated antigens derived from *T. brucei* and *T. rhodesiense* are used, was applied to the assay of immune sera.

Dr. Godfrey started the study of the nature and quantity of phospholipids found in different species of trypanosomes obtained from animal parasitaemias and from cultures. These investigations should yield useful information about species differences in the metabolism of this group of organisms.

Chemical Analysis of Trypanosomal Antigens. Miss Lanham continued her investigations on the purification of the exoantigens present in the serum of rats infected with *T. brucei*. The immunogenic components of these antigens were precipitated in ammonium sulphate at concentrations above 65 per cent. only. Chromotographic separation on DEAE-Sephadex yielded a fraction of serum γglobulin which contained both the precipitinogens and immunogenic trypanosomal antigens. Further purification with ammonium sulphate removed practically all traces of serum γglobulins. Electrophoresis on cellulose acetate membrane of the final material gave characteristically thin lines staining with glycoprotein specific stains in comparison with serum γ-globulins which gave diffuse lines and did not stain in this way. Trypanosome suspensions derived from infected rats and washed in saline solution yielded similar antigenic material. Such trypanosome washings were another convenient source of purified exoantigen for the specific immunization of mice.

Whooping Cough Bacillus

Agglutinogens. Drs. Standfast, Miller and Jean Dolby investigated the agglutinogens of *Bordetella pertussis*, as part of a nation-wide survey (organized by the Public Health Laboratory Service Working Party on Pertussis) to determine the significance of the agglutinogens in pertussis vaccines.

(1) It was confirmed that the prevalence of the different serotypes had changed during the last 20 years. Of 94 isolations from 24 areas in England, Scotland and Wales, in Nov.–Dec. 1966, 90 per cent. were serotype 1, 3 and 10 per cent. serotype 1,2,3. During the period 1941–1953, of 107 strains, freeze dried on receipt and stored in the Institute's collection, 9 per cent. belonged to serotype 1; 64 per cent. to serotype 1,2; 20 per cent. to serotype 1,2,3 and 7 per cent. to serotype 1,3. The prevalence of the possibly important serotype 1,3 has thus changed from 7 per cent. to 90 per cent. of isolates.

(2) The work will be continued as a study of the serotype antibodies in vaccinated and unvaccinated children before and after infection. Fifteen convalescent sera from N. Ireland, supplied by Dr. M. Haire, have already been studied. Of these 8 had agglutinins to antigen 1 only: 6 to antigens 1,3: and one to antigens 1,2.

(3) Potency tests by the routine intracerebral challenge method of Kendrick were made on a number of vaccines prepared from different serotypes challenging the animals with strains of four serotypes.

The order of potency of the vaccines was the same, irrespective of the serotype of the challenge strain.

Toxicity of Vaccines. Drs. Standfast and Jean Dolby continued to collaborate with the M.R.C. in seeking for a laboratory measure of the toxicity of pertussis vaccines for children. Dr. Miller continued her investigation of methods of killing and preserving vaccines in order to reduce toxicity and maintain potency during long storage periods (Report 1966).

Active and Passive Immunity in Mice. Dr. Jean Dolby concluded her work on factors influencing the immunity in mice after vaccination (Report 1964) by extending to intravenous vaccination the observations on intraperitoneal and intracerebral vaccination. Of the three methods, intracerebral vaccination was the most efficient; it produced both general immunity and local immunity in the brain. With an intracerebral challenge, the combined effect of general and local immunity was powerful in overcoming infection—the bacterial count falling immediately. In passive immunization, achieved in normal mice by pretreating with antibody the organisms used for intracerebral challenge, the bacterial count rose before it fell.

Immunoglobulins of B. pertussis Antisera. The investigation on 7S and 19S antibodies (Reports 1965, 1966) was extended by Drs. D. E. and Jean Dolby to properties other than their bactericidal power. Some 19S fractions were weakly bactericidal and some not at all; and none protected mice against an intracerebral challenge. In mice challenged by the intranasal route, however, 19S fractions were almost as active as the intracerebrally protective 7S fractions. These differences may be the basis of the differences between the two types of protective antigens and antibodies (Report 1958). Five pure 7S globulin fractions in high concentrations all inhibited the *in vitro* bactericidal antibody; they were bactericidal in very dilute solution and protected mice against lung and brain infections. Antihaemagglutin and agglutinins were present in both 19S and 7S fractions, but to a much higher titre in the 7S fractions; this was true for rabbits immunized by 6, 12 or 30 intravenous injections of vaccine.

Laboratory Assay of Typhoid Vaccines. Mr. Banks continued his investigation of the laboratory assay of typhoid vaccines under trial in the field, as part of the collaborative study by the World Health Organization (Report 1964).

Clostridial Toxins

Mr. Mahony studied the production of clostridial toxins by large-scale deep-fermentation methods. High yields of *Cl. tetani* toxin were obtained by altering the physical conditions for the different growth phases during incubation. During the first phase of active cell multiplication, the toxin is formed intracellularly under strict anaerobic conditions without agitation; removal of waste gases by suction gives optimum results. When the toxin is released into the medium in the subsequent autolytic phase, vigorous stirring of the culture in the presence of oxygen aids the release of toxin. Automatic processes are being developed for the large scale preparation of toxin under such conditions.

Inheritance in Bacteria

Origin of Antigen-converting Genes in Phage P22. When certain temperate phages, like γ or P2, adsorb to bacteria already lysogenized by the homologous prophage, the superinfecting phage genome enters the bacterium but does not multiply, although its existence is detectable after many generations of bacterial growth. However, Miss Walsh and Professor Meynell found that phage P22 became undetectable after adsorbing to P22 lysogenic strains of *Salmonella typhimurium* LT2. The phage presumably injects its genetic component since any bacterial genes it may be carrying express themselves in the recipient salmonella, whether it is a lysogenic or non-lysogenic strain. The phage genome is presumably inactivated after entering the lysogenic cells. The P22 prophage of the lysogenic recipient does not cause it to become a restricting host—i.e. one that rejects all donated genes—because the bacterial plasmids F-lac, F-gal and

colI, are transferred equally readily to non-lysogenic and lysogenic recipients. It is of particular interest that other laboratories have shown that when genes determining synthesis of somatic antigen 1 are transferred to recipient bacteria, they also become inactive in a lysogenic antigen 1 $^-$ recipient; that is, these genes behave as if they are phage and not bacterial in origin.

Bacterial Physiology

The Ib Pilus. Bacteria which have just acquired colicin factor I (*colI*) become able to conjugate with other bacteria and thereby to donate *colI* along with their own genes; but when *colI* has been present for a few generations, conjugation only rarely occurs (Report 1959). Acquisition of *colI* therefore confers a short-lived ability to conjugate. Methods exist for preparing cultures of increased donor ability but that used routinely (Report 1959) failed with strains that did not form pili filaments not concerned with motility). Professor Meynell therefore examined the rate at which *colI* spread in growing cultures of such strains and found that the percentage of *colI*+ cells doubled every 3 hours, as compared with 0.5 hr. for motile piliated strains. When this slow spread was allowed for, cultures of high donor ability could be prepared and examined both by electron microscopy and for the percentage of cells capable of donating *colI*. Professor Meynell and Dr. Lawn found that the number of cells able to donate in 20 min. was correlated with the number that formed a new type of pilus. Yet another type of pilus is formed by cells carrying other sex factors (see p. 21). In both cases, the pili may well be the means by which conjugation and gene transfer occur. It is proposed to call these pili "sex pili" to distinguish them from the "common pili" formed by many enterobacteria. The latter are not directly concerned in gene transfer although, by virtue of their known adhesive properties, they appear to encourage conjugation by holding mating cells together.

Cell Wall Growth in Bacillus Species. B. anthracis forms new wall at the equator of the cell, judging from the mode of segregation of pre-existing capsule (Report 1966). The underlying cell membrane in *B. subtilis* also appears to be formed equatorially, as shown by studies at the Institut Pasteur, Paris, on the segregation of tellurium crystals in *B. subtilis*. Professor Meynell and Dr. Lawn therefore attempted to show that wall and membrane segregated together during growth by studying capsule and tellurium simultaneously, but *B. anthracis*, unlike *B. subtilis*, was killed by tellurium. On turning to other *Bacillus* species, shown in Canada to form new wall equatorially by fluorescent microscopy, Professor Meynell and Miss Gokhale found that the rabbit antisera used for the labelling lysed the cells, although complement had been inactivated. Lysis was prevented, without affecting labelling, by absorbing the sera with kaolin, and it is therefore supposed that lysis was due to serum lysozyme. By this method, new wall appeared to be formed uniformly along the length of the bacillus in these species, not just at the equator.

Bacterial Multiplication in Infected Animals

Kinetics of Microbial Infection. The birth-death model of infection is known to be inadequate, although its main assumptions of random microbial death and division appear reasonable (Reports 1964, 1966). In the basic model, the probabilities per unit time of death and division are assumed constant, and Dr. Trevor Williams and Professor Meynell therefore examined the implications of a "linear" model, in which these parameters are assumed to vary linearly with the time elapsed since inoculation. It follows from this assumption that, when logarithm of viable count is plotted against time, the microbial growth curve will be a parabola whose shape is the same for all doses. This was found to be so for mice given either 0.048 or 0.00012 LD50 of *Salmonella paratyphi B*, possibly because this infection is focal; that is, each inoculated organism sets up an independent focus of infection whose fate is uninfluenced by those of other foci. Increase in dose would then merely increase the number of foci, without causing them to interact. However, interaction is evident in mice

infected with *Bordetella pertussis*. It may be that in all infections, interaction is absent at certain doses, as observed with *Salmonella paratyphi B*, and present at others, according to the pathology of the infection.

Mycoplasma

Dr. Lemcke returned in June 1966 from Monash University, Victoria, where she worked with Professor B. P. Marmion and Dr. P. Plackett (C.S.I.R.O. Animal Health Laboratory, Parkville, Victoria) on the antigenic structure of *Mycoplasma pneumoniae*, the causative agent of one form of primary atypical pneumonia in man. The major serologically reactive components of this organism were present in a crude lipid fraction. Evidence was obtained by chemical and enzymic treatment of the lipid and by chromatographic fractionation that carbohydrate was present in the active components.

On returning to the Institute, Dr. Lemcke began preliminary work for a similar immunochemical investigation of *Mycoplasma hominis*, which is associated with human genital tract infections. As it is undesirable that organisms grown in bulk for immunochemical analysis should be grossly contaminated with precipitates (such as serum proteins) derived from the complex media required by parasitic mycoplasma, the formulation was undertaken of a medium giving maximum growth with a minimum of extraneous precipitation from medium constituents.

VIROLOGY

Trachoma and Inclusion Blennorrhoea (Conjunctivitis)

The trachoma and inclusion conjunctivitis micro-organisms are referred to as TRIC agents. Variants that for a given dose kill chick embryos more quickly than their parent strains are *f* (fast-killing) strains, and the parents are *s* (slow-killing) strains.

Growth Characteristics. Dr. Reeve and Dr. Taverne continued to study the growth of TRIC agents in HeLa cell monolayers infected by centrifugation (Report 1966). They investigated the comparative infectivity of various strains for cell cultures with and without the aid of centrifugation, and for the chick embryo, with the main purpose of determining why the unchanged *s* strains do not readily infect cell cultures. Centrifugation on to monolayers for a constant time at different speeds showed that the number of inclusions varied directly with the force applied. Centrifugation for more than 30 minutes at a force of 1,500 *G* did not greatly increase the number of inclusions formed. Measurement of the rate of heat inactivation of *s* strains at 35°C indicated that most of their infectivity was lost during the long adsorption period required for infection without the aid of centrifugation. Thus the increase in adsorption rate afforded by centrifugation allows inclusions to be formed by particles that would otherwise be inactivated. However, *s* strains differ fundamentally from *f* strains in that the increase in the number of inclusions formed after centrifugation is significantly greater; the difference is not entirely accounted for by thermal inactivation.

Studies on the first cycle of growth of TRIC agents in chick embryos (Report 1965) were extended to obtain sufficient data for a statistical analysis by Dr. I. Sutherland of the M.R.C. Statistical Research Unit. A genuine difference was observed in the rates of growth during the exponential period of multiplication of strains of different virulence, *f* strains increasing at a rate of $10^{3\cdot4}$ egg lethal doses (ELD_{50}) and *s* strains by $10^{1\cdot7}$ ELD_{50} per day. This finding confirmed the inference previously made from multi-cycle growth curves (Report 1964). Statistically significant differences were also found between the duration of the lag phases of *f* and *s* strains, that is, the period between inoculation and the first appearance of infective agent.

Dr. Graham (Report 1966) described the induction of pneumonitis in mice by intranasal instillation of TRIC agents. The growth of slow-killing strains in the mouse lung was apparently limited to one cycle, a result similar to that in chick embryos incubated at 37°C rather than at 35°C. It seemed possible that the infection of mice

by TRIC agents was restricted by the temperature of the host. The infectivity of TRIC agents was therefore titrated in mice kept at room temperature and in mice whose body temperature was lowered by adaptation to existence at 4°C. Preliminary experiments showed that mice adapted to 4°C were slightly more susceptible to the lethal action of some TRIC strains administered intranasally.

Immunological Studies in Guinea-pigs. Dr. Blyth previously showed that a course of immunization partially protected guinea-pigs against cutaneous infection with TRIC agents (Report 1966). More detailed study suggested that absence of the organism from the spleen after challenge is the most sensitive index of immunity. Thus in animals infected intracutaneously, a second injection by this route given up to 6 weeks later was not followed by the appearance of organisms in the spleen, even though there was no diminution in the size of the skin lesion induced by the later injection.

The immunity induced by intracutaneous injection of live TRIC agent followed by an intravenous dose of killed organisms (Report 1966) proved to be very short lived; it was at a maximum 2 days after the intravenous injection and waned within 2 weeks to the level found immediately before this dose. The transient nature of the protection as measured by size of skin lesions suggests that it may not be mediated by circulating antibody; prevention of spread of organisms to the spleen might involve a different mechanism, or it might be merely a less severe test of immunity. Investigation is continuing into the mechanism of these protective effects, and the means by which more prolonged immunity can be induced.

Trachoma Vaccine. In collaboration with Dr. Turner, Professor Collier and Miss Smith inactivated a suspension of TRIC agent (strain MRC-4*f*) by treatment with 10^{-4}M methylene blue and subsequent exposure to light. Like trachoma vaccines inactivated by heat, formalin or ultraviolet irradiation, this preparation proved much less effective than live vaccine for immunizing baboons.

Live vaccines may be more immunogenic because the inactivation process destroys the protective antigen, or because, as with live viral vaccines, the trachoma agent multiplies within the recipient and thus stimulates the apparatus of immunity more effectively. Professor Collier and Miss Smith tested the second possibility, and showed that after subcutaneous injection into baboons, live MRC–4 *f* multiplied in the skin and regional lymph nodes; it was detectable in the spleen, but not in the circulating blood or liver, up to 1 month later. After intravenous injection it was isolated from the blood and liver only within the first 24 hours; its titre in the spleen attained higher values than after subcutaneous injection, and it was present in quantity in the peripheral lymph nodes for 3 weeks after injection. As in previous experiments intravenous inoculation resulted in better protection against conjunctival challenge than subcutaneous injection; but again, immunity waned considerably during the next 8 months. However, the finding that TRIC agent can disseminate and multiply in primate hosts may help to explain the better results with live vaccines.

Miss Smith and Professor Collier completed the analysis of complement fixation tests on sera from immunized and control baboons (Report 1966). In general, the most immunogenic vaccines induced the highest titres of antibody that fixed complement with group antigen; but in individual animals there was no obvious relationship between serum titre and immunity to infection with TRIC agent.

In normal and immunized baboons infected with TRIC agent, the relationship between the number of inclusion bodies in conjunctival scrapings and severity of the physical signs may throw light on the immunopathology of trachoma and inclusion conjunctivitis. In collaboration with Dr. I. Sutherland of the M.R.C. Statistical Research Unit, Professor Collier has begun an analysis of the data from a large series of ophthalmic inoculations.

Investigations Overseas. Field trials of trachoma vaccine were continued under Professor Collier's direction (Report 1966). In the Gambia, a live vaccine prepared from two strains of trachoma (SA–2 *f* and

Asghar *f*) failed over a period of 1 year to diminish the attack rate in young children. In Iran however preliminary analysis suggests that vaccines prepared from the same strains diminished the attack rate by a factor of the order of 20 per cent.; in particular, vaccination significantly reduced the incidence of necrotic follicles in the upper tarsal conjunctiva, active pannus, and keratitis. The analysis of these results by the M.R.C. Statistical Research Unit continues; and it is hoped to undertake a second annual follow-up in 1967.

Shope Fibroma and Adenovirus 12

Dr. Placido de Sousa continued to investigate the action of compounds related to N-hydroxyurethane on Shope fibroma virus and adenovirus 12 (Reports 1965, 1966). Four more compounds (supplied by Professor E. Boyland of the Chester Beatty Research Institute) were tested against Shope fibroma in rabbit kidney cell cultures. One of them (isonicotinylamidoxine) was inhibitory; its similarity to nicotinylamidoxine, which proved inactive, again emphasizes the importance of minor chemical differences in determining antiviral activity.

It has been suggested that chemical carcinogens induce malignancy by acting on the cellular nucleic acid. To test the possibility that N-hydroxyurethane might exert an antiviral action in this way, Dr. de Sousa attempted to reverse its effect on Shope fibroma with compounds related to nucleic acid metabolism. Adenine, adenosine and deoxyadenosine were partially effective in this respect; the results with thymine were less conclusive, and 9 other nucleic acid metabolites had no effect.

Study of the inhibition of Shope fibroma by sodium nitrite was also continued. This salt did not affect the virus *in vitro* at room temperature (Report 1966), but the thermal inactivation rate of the virus was increased in its presence. Following inoculation of cell cultures with Shope fibroma, sodium nitrite required a contact time of 27 to 96 hours to inhibit multiplication; it can act synergistically with N-hydroxyurethane, since the addition of non-inhibitory amounts of both compounds to infected cell cultures interfered with viral growth.

Dr. de Sousa found that the concentration of N-hydroxyurethane needed to inhibit adenovirus 12 in human embryo kidney cells was the same as that inhibiting Shope fibroma in rabbit kidney cultures. Previous tests showed that relatively low concentrations of sodium nitrite did not inhibit adenovirus 12; but concentrations higher than those inhibitory for Shope fibroma interfered with the growth of the adenovirus.

Vaccinia Virus

It had been noted earlier that high concentrations of glycerol and glucose decreased the rate of photo-inactivation of vaccinia virus by methylene blue. Dr. Turner completed the investigation of factors concerned in photodynamic inactivation of vaccinia virus. The effect of high concentrations of glycerol could not be attributed to changes in the viscosity of the medium or to the impairment of the initial dye sensitization of the virus, or to changes in 0_2 concentration. Lyophilization of dye-sensitized virus prevented its subsequent inactivation by exposure to light. The results were interpreted as indicating the involvement of water in the photoinactivation process, although its role is not clear.

Dr. Kaplan and Dr. Turner examined the pathogenicity of vaccinia virus strains for mice by the intranasal route. Only strains neuropathogenic for adult mice were active by intranasal inoculation. In an experiment to determine the usefulness of a mouse protection test for potency assay of inactivated smallpox vaccine, intermediate doses of vaccines were protective although there were deaths among animals given small doses of vaccines and also among those given the largest doses. It is thought that these anomalous results may be related to hypersensitivity in heavily immunized animals and experiments are in progress to test this view.

Dr. Kaplan has begun an experimental study of the aetiology and pathogenesis of post-vaccinial encephalitis.

Dr. Malkinson succeeded in coating sheep erythrocytes with a soluble extract of vaccinia-infected tissues. With the coated cells it is possible to detect antibodies in the sera of animals immunized against,

or recovered from infection with, vaccinia virus. The nature of the antigen is being investigated. Preliminary results indicate that it may be similar to the serum-blocking antigen of Appleyard, and that the erythrocyte agglutinins may be closely related to virus neutralizing antibodies. Dr. Malkinson and Dr. Kaplan are investigating the suitability of the passive haemagglutination system for the measurement of humoral immunity to pox viruses.

Dr. Malkinson fractionated vaccinial antigens and tested them in the skin of appropriately hypersensitized guinea-pigs. He found antigens potent in producing erythema in the hypersensitized guinea-pigs but without activity in the passive haemagglutination system. He also tested the immunity of mice born of actively immunized mothers; the offspring had a substantial passive immunity one week after birth.

Dr. Murray, in collaboration with Dr. A. Krag Anderson of the State Serum Institute, Copenhagen, investigated differences between the Danish and Lister Institute strains of vaccinia virus. Both strains elicit a strong immune response in rabbits. In virus neutralization tests by the plaque inhibition method clear differences were noted in the slopes of dose response curves and in rates of neutralization. These differences, although more pronounced with rabbit than with human antivaccinial sera, were independent of the serum used. It appears that the Danish strain of vaccinia virus, though a good antigen, is less avid than the Lister Institute strain.

Dr. Murray also compared the potency assay of smallpox vaccine by pock count in the chick embryonic chorioallantois with the potency assay by plaque count in monolayers of chick embryonic cells. If the correlation of the two assays is good it may be possible in routine potency assay to replace titration in chick embryos by the less laborious and more economical method. He is devising a suitable suspending agent for the preparation of freeze-dried smallpox vaccine for jet inoculation, since vaccinia virus grown in cultures of leucosis-free chick embryonic cells, needed for this vaccine, is less stable than virus derived from the skins of sheep. At 37°C purified tissue-culture virus dried in the presence of 10 per cent. sorbitol and 20 per cent. sodium glutamate was as stable as virus dried in 5 per cent. peptone.

By partition methods and stable flow electrophoresis Mr. Robinson prepared highly purified suspensions of vaccinia virus, from which he extracted the lipids. The lipids were separated by chromatography on columns of magnesium silicate or silicic acid into phospholipids and neutral lipids; and further separated by thin-layer chromatography on silica gel. To date at least 5 phospholipids and a single neutral lipid have been separated. Lipids from virus obtained from sheep and from chick embryo chorioallantois had identical thin-layer chromatograms. Identification of the lipids is proceeding.

Mr. Robinson also started the biochemical investigation of vaccinia virus, using the cartesian diver micro-manometric technique. Model experiments with flavine adenine dinucleotide and L-amino acid oxidase have established that the technique is sensitive enough for the tests on microlitre samples of highly purified vaccinia virus suspensions.

Miss Hunter grew vaccinia virus in cultures of the human diploid cell strain WI-38. Titres of $>10^8$ pock forming units/ml. were attained. In preliminary karyological studies a small proportion of uninfected cells in infected cultures showed breaks in chromosomes. The investigation continues.

Rabies Virus

Dr. Turner and Dr. Kaplan continued their study of rabies virus and obtained considerable information on heat and pH stability as well as many data on the biological properties of the virus. The purity of the strain was checked by neutralization tests: it is not contaminated with the virus of lymphocytic choriomeningitis, reported to be a common contaminant of strains of fixed rabies virus in the United States of America.

Miss Hunter attempted to adapt rabies virus to growth in WI-38 cells, with indifferent success to date. Although the

virus grows in the cells it multiplies only slightly. Attempts to obtain high yields of virus continue.

IMMUNOLOGY AND EXPERIMENTAL PATHOLOGY

Serological Identification of Blood Meals

Dr. Weitz and Miss Bronne continued the survey of the feeding habits of *Glossina morsitans* in the Zambesi Valley. The first stage of the experiment, the survey of the natural feeding habits of the insects in an undisturbed area for 18 months, is complete. The next stage will be to remove all the warthogs from one control area and the kudus from another in order to assess the ability of the fly to change its feeding preferences with change in host availability.

The work for the world-wide W.H.O. Scheme for the identification of the blood meals of Anopheline mosquitoes in relation to malaria eradication schemes continues.

Antitoxin Production

In further studies on the structure of β and γglobulin antibodies from horse serum (Report 1965), Dr. D. E. Dolby compared the properties of the heavy and light polypeptide chains separated from them and is studying the number and positions of the sulphydryl groups which they contain. He is also investigating the effect, both on the whole molecules and on the separated chains, of cyanogen bromide, which splits them specifically at the methionine group.

Continuation of the work on the pepsin treatment of toxin-antitoxin floccules as a means of preparing highly purified antitoxin (Report 1966) has shown that the yield is dependent not only on the use of fresh toxin—it was known previously that floccules made from toxoid instead of toxin cannot be successfully treated with pepsin—but also on the use of fresh antitoxin. The alterations in antibody on storage which cause this effect are now under study; they seem to be similar in nature to the alterations which occur in βglobulin on storage (Report 1966), and to be due to the action of proteolytic enzymes of the serum.

Dr. Dolby also undertook during the year an investigation of the purification of gonadotrophin from pregnant mares' serum.

Cytophilic Antibody and Opsonins

In further experiments on the protection conferred by cytophilic antibody and opsonin during infections (Reports 1965, 1966), Dr. Parish compared antisera from the I.C.I. and CBA strains of mice. Cytophilic antibody in most fractions of γ2 globulin behaved as a cell-bound opsonin, so that antigen particles adhering to the surface of the sensitized macrophages were ingested when complement was added. Cytophilic antibody in a few serum fractions from I.C.I. mice caused an adherence of antigen that was not followed by ingestion (Report 1965); this occurred more regularly in fractions from CBA mice encroaching upon the γ1 region. In both strains of mice, γM cytophilic antibody to bacterial polysaccharide antigens sometimes caused adherence without subsequent ingestion, depending upon the nature of the immunizing antigen and the interval between injection of the antigen and harvesting of the serum.

It appears that the phagocytosis depends upon the ability of the cytophilic antibody to fix complement, but since the accurate determination of complement fixation in mouse sera is difficult, these phenomena are being examined in guinea-pigs. However, it is already apparent that a new technique is required to detect complement on the cell surface, for though fresh serum is better than heated serum in enhancing phagocytosis by guinea-pig macrophages, its phagocytosis-promoting ability is unrelated to its haemolytic complement titre.

Exogenous Antigens Acquired by Tissue Culture Cells

The possibility that incorporation of bacterial or fungal antigens by cells of the skin may predispose them to damage by specific antibody manifested as a generalised eczema, was further investigated by Dr. Parish (Report 1966).

HeLa cells grown in the presence of pure protein antigens or serum proteins from various animal species were damaged on the

addition of complement and the appropriate antibody. The concentration of the antigen in the growth medium is an important determinant of the susceptibility of the cells. The antibody appears to become cytotoxic by forming a complex with the acquired antigen and complement. The reverse procedure, of adding the antibody before the antigen, or of adding preformed complexes results in similar cell damage, with characteristic extrusion of cytoplasm.

Bacterial polysaccharides are also taken up by cells in culture, and the adsorption is not influenced by protein in the media. However, human γM antibody capable of complement lysis of antigen-coated red cells failed to damage HeLa cells that had taken up the antigen.

The dye exclusion technique is unsuitable for detecting cell damage in these tests, because severely damaged cells may not take up the dye, and transiently damaged cells may not retain it. Acridine orange fluorescence and non-viability of the cells in subsequent culture *in vitro* are more consistent than the dye exclusion technique in detecting cell damage.

Antibodies to Bacteria and to Skin in Generalised Eczema

Dr. Parish is examining sera from patients with generalised eczema, selected by Drs. R. H. Champion and E. Welbourn at Addenbrooke's Hospital, Cambridge, for antibodies to bacteria of the skin. The bacterial antigens may combine with skin cells predisposing them to the cytotoxic effects of specific antibody, as in the tissue-culture system described above.

Antibodies to the polysaccharide and protein antigens of *Staphylococcus aureus*, Micrococci and *Escherichia coli* were detected by agglutination and antiglobulin tests with monospecific antisera. Patients with eczema and normal persons had approximately the same antibody titre to the polysaccharide and protein antigens of *Staph. aureus*, but the γM anti-polysaccharide antibody in the sera of eczematous patients more frequently utilised complement in complement lysis tests, and these sera more frequently contained γA antibody.

Polysaccharide antigens of the micrococci varied in their ability to cross-react with those of *Staph. aureus;* those of *E. coli* were distinct. Two protein fractions of *Staph. aureus* were separable on Sephadex G.200 columns: agglutinating antibody to one of these was present in nearly all sera, but antibody to the other was present in only a small number of sera from patients and normal individuals.

It was not possible to correlate the results of the *in vitro* antibody tests with the results of skin tests on the patients.

During these investigations 2 more patients with autoallergy to skin were studied. The serum of one patient induced an immediate reaction when injected into the skin of the patient or normal individual, and was toxic for explants of human skin *in vitro*. Immunofluorescence tests revealed a weak complement-dependent γG antibody to epidermal cells, and a γM antibody which combined with scattered unidentified cells of the dermis. Both these antibodies were specific for skin; they were not toxic for explants of other tissues and were adsorbed from the serum only by skin cells. The second patient, who is still being investigated, is the first instance of one possessing a γM antibody to epidermal cells.

Allergic Vasculitis

Dr. Parish demonstrated complexes of bacterial antigen and antibody in the lesions of patients with nodular vasculitis (Report 1966). In an experimental study he found that the histological changes in the experimental lesions in guinea-pigs varied with the concentration of the complexes injected. Complexes containing 1.4 to 0.17 mg rabbit γG antibody nitrogen induced neutrophil infiltration, followed by mononuclear cell infiltration, but complexes with 0.017 mg antibody nitrogen induced mononuclear cell changes with few, if any neutrophils. Complexes formed of γM antibody also induced vasculitis with more severe necrosis of the deeper tissues than caused by γG antibody.

In a further study of the properties of antigen-antibody complexes Drs. Reckler and Parish adsorbed rabbit antisera with

guinea-pig tissues to remove all passively transferable sensitizing antibody and found that the remaining precipitating antibody, when combined with antigen, caused an immediate increase of vascular permeability when injected into the skin of guinea-pigs; and generalised shock on intravenous injection, with mast cell disruption, bronchial constriction and death. This anaphylactoid reaction was identical with classical anaphylaxis and it is possible that urticarial-type conditions may sometimes be caused by antibodies of the type that cannot passively sensitize tissues.

Antibodies Sensitizing Human Tissues

In a study of the properties of human reaginic antibodies Dr. Parish passively sensitized the skin of monkeys with sera from individuals sensitive to *Ascaris lumbricoides*, grass pollens, horse dander (albumen), egg albumen and milk, particularly β lactoglobulin and α lactalbumen. The value of this test is limited, because the sensitivity of monkey skin is about one tenth that of human skin. The sensitivity of the test was, however, increased by concentrating the immunoglobulins for injection.

Reagins were adsorbed from the sera by cells of endothelium, skin and kidney but not by leucocytes—despite reports that the antibody has a special affinity for these cells. Agglutination tests with monospecific antiglobulin sera revealed no correlation between the titre of γG, γA or γM antibodies and the degree of passive sensitization of the tissue. Rabbit anti-γA antibody precipitated γA globulins from the sera without removing the reagins.

A rabbit anti-γA serum prepared from colostral γA, however, precipitated the reagins from sera of 2 patients sensitive to Ascaris, though not reagins to other antigens. Irrespective of the specificity of this particular rabbit antiserum, it provides evidence that reagins are probably not confined to a single class of immunoglobulin in all individuals.

Electrophoretic separation of serum globulins in agar gel revealed that the mobility of reagins was greater than that of γA globulin, though there was considerable overlap. The electrophoretic migration of reagins did not correspond consistently with any known globulin, and attempts are to be made to identify them by precipitation with radio-active antigens.

Electron Microscopy

Sex pili Associated with Bacterial Conjugation. Structural studies of sex pili have continued and sex pili of a new type have been discovered.

Col I System. Dr. Lawn and Professor Meynell identified a new pilus (the Ib pilus) formed by bacteria able to donate colicin factor Ib (see p. 14). This pilus is shorter and more variable in diameter than F type sex pili, but, like the F pilus, may have terminal knobs. Some of the pili appear to have a central hole but this is not as clear as with F pili.

RTF System. Dr. Lawn continued to collaborate with Drs. Elinor Meynell and Naomi Datta of the M.R.C. Microbial Research Unit and the Royal Postgraduate Medical School. At Hammersmith a derepressed mutant of an *fi*$^-$ drug resistance factor (R factor) has been produced, which readily produces sex pili and transfers drug resistance at high frequency. These sex pili are morphologically distinguishable from F pili and the sex pili of *fi*$^+$ R factors, but resemble col Ib pili.

F$_o$lac System. Dr. K. Sanderson, of the University of Alberta, Calgary, provided cultures able to transmit the plasmid, F$_o$lac, which was discovered in a strain of *S. typhi* by Dr. L. S. Baron. Sex pili similar to F pili were discovered. They adsorbed the filamentous DNA phage M13 at their tips but did not adsorb the spherical RNA phage MS2, which explains their insensitivity to RNA male-specific phages.

It has been demonstrated that the repression of F functions (conjugation and sensitivity to male-specific phages) in certain *Salmonella* (*pla*$^-$ strains) results from repression of F pilus production. Production of sex pili is normal when F is introduced into other non-repressing (*pla*$^+$) strains of Salmonella.

Antigenic Relationships Between Sex Pili. Dr. Lawn devized a rapid method for labelling structures such as sex pili with rabbit antibody.

The morphologically similar sex pili of F, and of R1 (a *fi*$^+$R factor) are closely related antigenically, but can be clearly distinguished with absorbed sera. Col Ib pili and the pili produced by *fi*$^-$R factors, which belong to a separate morphological group, are antigenically distinct from F and R1 pili.

Thus all the conjugation systems in the strains of *E. coli* and *S. typhimurium* that have been examined morphologically are associated with the presence of sex pili, namely F, three *fi*$^+$ R factors, an *fi*$^-$ R factor, colicin Ib and F_0lac (all, excepting F, first demonstrated morphologically at the Institute). The pili associated with these different systems can be distinguished, these different systems can be distinguished from each other on thebasis of morphology-phage adsorption and immunological reactions. These investigations are being extended to other sex factors.

Bacterial Cell Wall. In collaboration with Dr. R. Wilkinson, of the Johns Hopkins University, Baltimore, thin sections were prepared of S (complete cell wall) *Salmonella typhimurium LT*2 and several R mutants (defective cell wall). The R bacteria differed characteristically in wall structure from the S, but the interpretation of the differences was difficult because of the uncertainty in identifying the morphological layers with the chemical fractions isolable from the bacteria.

Investigation of cell wall growth in *Bacillus anthracis* (Report 1966) was continued, with tellurium deposition as a marker (see p. 00).

Ovo-implantation in Mice. In collaboration with Mr. C. Finn of the Royal Veterinary College, an investigation of the ultrastructural changes in the mouse uterus during early ovo-implantation and artificially induced decidual reactions was started. It is hoped to distinguish local reactions of the uterus to the presence of the blastocyst from hormonally induced structural changes, in order to throw light on the mechanisms concerned in inducing uterine reactions and their relationship to the induction of inflammation.

The contacts between stromal cells of the uterine connective tissue as they become transformed into decidual cells are more intimate than is generally supposed. In many cases, they are so-called "tight" junctions, in which the outer leaflets of the unit membranes concerned appear as a single fused layer. This observation suggests that the decidual cells form a "functional syncitium" which may play an active part in controlling the environment in which the ovum develops.

Other work. Examination of cell fractions for the Department of Biochemistry has continued, and a highly purified preparation of vaccinia virus was examined for Dr. Kaplan and Mr. Robinson.

Mechanisms of Inflammation

Response to Thermal Injury. Dr. Wells completed his detailed studies on vascular changes in thermal injury of the rat cremaster. Mass injection tests (Report 1965) were made after varying degrees of heat injury. In every case there was immediate vascular occlusion but its duration, and the proportion of the heated area affected, depended on the severity of the injury. Even after a very mild injury (10 seconds at 54°C) the vasospasm lasted up to 15 minutes.

In addition, it is now clear that exposure of lesions to circulating dye and carbon for a period longer than 10 minutes masks a number of features of the vascular responses to injury. For example, with a 10 min. exposure single phases were separable into two phases, and it was evident that, although there was continuing deposition in damaged venules with longer exposure to circulating carbon, most of the deposition in damaged capillaries took place in the first 10 minutes. After heating to 54°C from 10 to 40 seconds there was some element of venular damage associated with a transient phase of increased vascular permeability. The later permeability phase from 15 minutes onwards corresponded closely to the degree of capillary damage;

nevertheless after 90 minutes, though there was no increased permeability, the capillaries continued to be the site of carbon deposition for a further 2 hours.

These findings, in so far as pronounced capillary carbon deposition was observed in the latter part of the time course with virtual absence of protein exudation, support those of Spector, Willoughby and Walters (1965). However, the fact that protein exudation was a prominent feature in lesions in which deposition of carbon in the venules was very slight, suggests that protein exudation from the capillaries may be an important factor in the prolonged phase of thermal injury.

The Tuberculin Reaction. The biphasic permeability response reported previously in animals hypersensitised to tuberculin was further studied. Previous tests had been made in animals bearing tuberculin lesions of ages ranging from 0 to 72 hours, so that the intracutaneous tuberculin injected to produce the older lesions might have partly desensitized the animals to the later injections. To eliminate this possibility the tests were repeated in animals, each of which bore lesions of only one age. The results were essentially the same, in both rats and guinea-pigs. In both species there was an initial phase of venular damage in lesions up to 15 minutes old. However, in rats there was no evidence of appreciable carbon deposition at any later stage of the time course, from 15 minutes to 24 hours. In guinea-pigs, pronounced capillary damage occurred in lesions aged from 6 hours to at least 36 hours. In a small number of guinea-pigs an additional peak of the early permeability response phase was detected in lesions one hour old. This phenomenon is being investigated further.

An exploration of quantitative methods for the study of parenteral inhibitors of the biphasic permeability response to friction-injury of the mouse ear was continued.

Activation of Permeability-increasing Plasma Proteins. Miss Mason continued her work on the relation of the permeability proteases to the kinin-releasing system of the blood plasma. Biologically active kinins are formed in dilutions of guinea-pig or human plasma in contact with glass or other negatively-charged surfaces, as the result of the digestion of a protein substrate (kininogen) by the protease (kininogenase). The production of kininogenase from its precursor is dependent upon a sequence of reactions set off by the activation, and adsorption to the glass surface, of a contact factor, which in man contains the Hageman factor responsible for the initiation of blood clotting. The appearance of the permeability protease PF/dil is also dependent upon contact with glass. Previous work with plasma from people deficient in Hageman factor (Hageman-trait plasma), indicated that human PF/dil, though similar to it in many respects, is distinct from contact factor; and that it is activated by contact factor, in turn releasing kininogenase from its precursor state.

Work this year with dilute guinea-pig plasma depleted of contact factor by adsorption demonstrated the same sequence of activations and also that neither PF/dil nor kininogenase were independently activated by glass surfaces. Adsorbed guinea-pig plasma behaves like human Hageman-trait plasma; both are activable by normal contact factor and both contain pro PF/dil.

The response of dilute plasma to glass activation differs from that of neat plasma. In dilute (1/100) plasma, kinin, kininogenase and PF/dil are produced, and with prolongation of the contact, the first two disappear. In neat plasma, there is a transient appearance only of kinins and kininogenase and no kininogenase is subsequently demonstrable. Since it is difficult to demonstrate any production of PF/dil, the question arises whether the kininogenase in neat plasma is activated directly by contact factor, and that in dilute plasma only when PF/dil has been so activated.

The study of the activation of neat plasma continues. Preliminary work revealed a complicating factor, namely a moderately heat-stable inhibitor of contact factor. The inhibitor is demonstrable in glass-treated plasma and is also attached in a loosely bound form to the contact factor adsorbed on the beads during the

glass treatment. Like the inhibitor of PF/dil it is demonstrable only in neat plasma or low dilutions of it.

BIOCHEMISTRY

The Human Blood Group Substances

Preparation. Professor Morgan and Dr. Winifred Watkins developed an additional method, which is both simple and efficient, for the recovery of the blood-group specific substances from ovarian cyst fluids by the technique of molecular sieve chromatography through polyacrylamide gel. A column of Biogel P300 (100-200 mesh) lets through the glycoprotein, which has a molecular weight greater than 300×10^3. Proteins such as human γglobulin, albumin and smaller molecules are retained on the column. Satisfactory preparations of blood-group substances were obtained, and found to be closely similar in chemical composition and serological properties to the specific substance isolated by the well established procedure of extracting the dried cyst fluid with liquid (90–95 per cent.) phenol (see earlier Reports). In the ultracentrifuge Dr. Creeth found them to be essentially homogeneous, and rather less polydisperse than those obtained by phenol extraction. The results indicate that the specific substance obtained by phenol extraction is not appreciably modified by the isolation procedure.

Structural Studies. Dr. Donald and Mr. Aston continued their work on the isolation of small oligosaccharides from hog gastric mucin glycoprotein after hydrolysis with polystyrene sulphonic acid. Two A-active fragments were isolated and identified as: (1) *O*-α-*N*-acetylgalactosaminyl-(1→3)-β-D-galactosyl-(1→3)-*N*-acetyl-D-glucosamine and (2) *O*-α-*N*-acetylgalactosaminyl-(1→3)-β-D-galactosyl-(1→4)-*N*-acetyl-D-glucosamine. Both these trisacharides were obtained earlier from human blood-group A substance. A tetrasaccharide was also recovered from the products of hydrolysis of the hog gastric mucin. This is believed to have a structure similar to (2) above, and also to contain a fucose unit, the exact position of which has not yet been established.

Dr. Donald and Miss Williamson investigated other products in the acid hydrolysate of the hog glycoprotein and recovered a tetrasaccharide containing equimolar amounts of *N*-acetylglucosamine and galactose. The results of the structural investigations on this unit are, however, difficult to interpret and further work is needed to establish the homogeneity of the oligosaccharide and elucidate its structure.

Mr. Aston has recently completed the hydrolysis of a large preparation of human blood-group H substance and is examining the oligosaccharides recovered by charcoal-Celite and Sephadex column chromatography.

Dr. Donald and Professor Morgan continued their work on the rate and extent of degradation of the hydroxy amino acids and galactosamine in the blood-group substances in an attempt to obtain further evidence for the structures proposed for the carbohydrate chains in A, B, H, and Le^a substances (Report 1964). If, as suggested, there is one molecule of *N*-acetylgalactosamine in each chain in B, H, and Le^a substances and two molecules in A substance, then the ratio of moles of galactosamine originally present to moles of threonine and serine together destroyed by alkali should be 1:1 for B, H and Le^a substances, and 2:1 for A substance. The ratios found for B, H and Le^a substances are 1:1.1, 1:1.0 and 1:1.1 respectively, and 2:0.94 for A substance.

Dr. Donald investigated the use of the "Smith" degradation procedure to elucidate the structure of the human blood-group B substance. This procedure consists of oxidation with periodate, reduction with borohydride and subsequent hydrolysis with mild acid. During the first cycle of the degradation extensive loss of all sugar components, especially fucose and galactose, occurred, but after this the residual material was relatively resistant to further oxidation. The oxidation and hydrolysis conditions were varied considerably but the amount of new structural information obtained from the experiments was disappointingly small, and for the time being this line of approach to the elucidation of structure has been abandoned.

The separation and analysis of glucosamine and galactosamine and their alcohols, a technique of considerable importance in the identification and quantitative determination of the reducing end sugar in oligosaccharides containing both amino sugars, was achieved on the amino acid analyser. A good separation of the four sugar bases was obtained with an accuracy of about ± 5 per cent. using 10–30 μg. of each and the high sensitivity unit on the analyser. With larger amounts, 100–300 μg., an accuracy of ± 2 per cent. was achieved.

Miss Marr examined the products of the partial alkaline degradation of a preparation of H-active blood-group specific glycoprotein that also had Le^a and Le^b specificity. The degradation was brought about by a new form of catalyst (Report 1966), a water-soluble, indiffusible polystyrene quaternary ammonium hydroxide resin in its carbonate form (polyvinylbenzyltriethyl ammonium carbonate). The combined diffusible material, collected over a period of 12 weeks' continuous and controlled degradation in the apparatus described in the 1966 Report, was repeatedly passed through a Sephadex (G15) column and resolved into four main fractions, according to size. Charcoal-Celite column and paper chromatography established that the fractions contained materials that varied in size and composition from simple chromogens derived from amino sugars, through di- and oligosaccharides, to fragments that contained both sugars and amino acids and from their rate of chromatographic movement, were considered to be larger than hexasaccharides. Many of the fractions were serologically active for all or some of the specificities that were associated with the original H substance. Since the H and Le^a specific structures had already been isolated from H and Le^a substances and identified (Report 1965), the most urgent problem was to recover a material that was Le^b active. Earlier work in the department had, by means of haemagglutination inhibition studies, indicated that the Le^b specificity arose from the presence of two α-L-fucosyl groups attached to a disaccharide composed of galactose joined to *N*-acetylglucosamine through a 1:3 glycosidic bond. The fucoses were believed to be joined glycosidically by 1:2 and 1:4 linkages respectively to galactose and *N*-acetylglucosamine. The ratio of these sugars in the Le^b active material recovered in the present investigation is close to 2:1:1, which are the proportions to be expected if the structure predicted from the earlier serological inhibition experiments is correct. The serologically active fragment is not yet completely free from Le^a activity, and the work is continuing in an attempt to obtain an homogeneous substance and determine its precise chemical structure.

Biosynthesis of Blood Group Substances. Attempts to identify the enzymes involved in the biosynthesis of the blood-group glycoproteins were continued with stomach mucosa as the source of the synthesising enzymes and low molecular weight compounds of known structure, as well as macromolecular blood-group substances, as acceptor substrates. Miss Ziderman and Dr. Watkins further characterised the α- and β-galactosyl transferases found in homogenates of human, baboon and rabbit mucosa (Reports 1965, 1966). The β-galactosyl transferase transfers D-galactose in β-configuration from uridine diphosphate galactose to an acceptor containing a terminal non-reducing N-acetylglucosamine residue to give the type of disaccharide unit present in the carbohydrate chains of the blood-group substances (Report 1966). The properties of the enzyme from the three different species appear to be essentially similar and, because of its more ready availability, most of the detailed work was done with rabbit tissue. Fractionation of the homogenate by high speed centrifugation gives a particulate residue at 105,000*g*, which contains most of the enzyme activity. Both magnesium and manganese ions activate the enzyme but manganese ions are the more powerful activators and, in the presence of these ions, up to 70 per cent. of the radioactivity from uridine diphosphate galactose-C^{14} is incorporated into the disaccharide formed with N-acetylglucosamine or the trisaccharide formed with N, N^1-diacetyl chitobiose (4-*O*-β-D-*N*-acetylglucosaminyl-*N*-acetylglucosamine). The enzyme has a broad pH optimum from 6

to 8 and maximum incorporation of D-galactose-C^{14} is obtained after incubation of the enzyme and substrate for 6 hrs. at 37°.

N-acetylglucosamine is present in the carbohydrate chains of all the blood-group substances but in the undegraded substances this sugar has not been demonstrated as a terminal unit at the end of the chains. Attempts are being made to degrade the blood-group substances by enzymic methods in order to expose *N*-acetylglucosamine residues and to determine whether these degraded substances function as macromolecular acceptors for the β-galactosyl transferase.

D-galactose is believed to be present in α-linkage in the human blood-group substances only in B substances, where it occurs at the terminal non-reducing ends of the B-active carbohydrates chains. Rabbits have a "B-like" substance in their gastric mucosa and an α-galactosyl transferase was previously demonstrated in homogenates of rabbit mucosa (Report 1965). Miss Ziderman found a similar α-galactosyl transferase in homogenates of group B human and baboon stomachs but has yet to show that this enzyme is specific to tissues from Group B subjects. The enzyme, which again is located in the particulate fraction, is activated by manganese ions, and transfers D-galactosyl-C^{14} from uridine diphosphate galactose-C^{14} to di- and tri-saccharides containing terminal non-reducing galactose residues. *N*-acetylglucosamine does not serve as an acceptor for the α-galactosyl transferase.

Dr. Gunja Smith prepared uridine diphosphate *N*-acetylglucosamine-C^{14} labelled with C^{14} in the acetyl group (Report 1966) and, with the UDP-*N*-acetylglucosamine-4-epimerase present in extracts of *Trichomonas foetus* (Report 1963), converted part of the nucleotide sugar preparation into a mixture of UDP-*N*-acetylglucosamine-C^{14} and UDP-*N*-acetylgalactosamine-C^{14}. The mixture of radioactively labelled UDP-*N*-acetylhexosamines was used in attempts to demonstrate the incorporation of *N*-acetylgalactosamine-C^{14} into blood-group A substance. The enzyme source was a particulate preparation from hog gastric mucosa and hog H substance was used as the acceptor. Greater incorporation of radioactivity was observed with an enzyme preparation from a group A hog than with a preparation from a group O hog, and on fractionation of the labelled product from the group A digest on a column of Biogel P300 the maximum serological activity and the maximum radioactive counts were found in the same fraction. However, although the radioactivity was evidently incorporated into a macromolecular acceptor of similar size to the blood-group A substance, precipitation with a specific anti-A precipitating reagent, *Dolichos biflorus*, failed to carry down the radioactivity. The labelled hexosamine was not, therefore, incorporated in the blood-group A macromolecule.

The presence of *N*-acetylglucosaminyl transferases in human, baboon and rabbit stomach mucosa was also investigated by Dr. Smith. UDP-*N*-acetylglucosamine-C^{14} was used as the sugar donor and a range of low molecular weight compounds were tested as possible acceptors. Transferases were detected in the particulate fraction of mucosal homogenates from all three species; the enzymes were activated by manganese ions and had pH optima around 7. No incorporation of radioactivity was found with D-galactose itself but methyl β-D-galactoside and the disaccharide 4-*O*-β-D-galactosyl-*N*-acetylhexosamine were acceptors for *N*-acetylglucosamine-C^{14}. Only compounds containing terminal non-reducing galactosyl residues served as acceptors. The α-glycoside, methyl α-D-galactoside, was a much poorer acceptor than the corresponding β-glycoside, indicating that the specificity of the enzyme is such that the galactose in the acceptor must be β-linked. Radioactive *N*-acetylglucosamine-C^{14} was released from the enzymically synthesised di-and trisaccharides by a crude enzyme preparation from *T. foetus* which contained a β-, and not an α-, *N*-acetylglucosaminidase. The *N*-acetylglucosamine is therefore transferred in β-configuration from the nucleotide donor to a β-linked D-galactosyl acceptor. Although it has yet to be demonstrated that these transferases will donate the sugar to macromolecular acceptors the specificity of the enzymes is consistent with the requirements for the formation of the *N*-acetylglucosaminyl-gal-

actosyl linkages found in the carbohydrate chains of the blood-group substances.

As a preliminary to *in vivo* experiments on the biosynthesis of blood group active glycoproteins in baboons, in which radioactive precursors will be injected into blood vessels supplying the stomach, Miss B. D. Ward isolated blood-group active materials from the stomach mucosae of baboons of known ABO group. The chemical composition and serological activity of these preparations is being compared with those of blood-group glycoproteins from human sources.

Phospholipids and Glycolipids

Studies on the distribution, chemical composition and structure of the glycolipids, minor lipid components of normal and diseased mammalian cells, are often made very difficult by lack of material. Improving the sensitivity of the analytical methods so that less material is required is one way of reducing the difficulty. Another way is to make the method of isolation of the glycolipids from a tissue as simple and as quantitative as possible. From both aspects existing methods were not entirely satisfactory and a procedure was devised by Dr. Gray for the quantitative isolation of tissue glycolipids in three simple stages. A major improvement on existing chromatographic procedures was made by the use of a solvent system which in one step separated the total glycolipids from at least 85 per cent. of the phospholipids (phospholipid: glycolipid in tissues is usually greater than 20:1). The same solvent system with silicic acid chromatography also made possible the isolation of all the "acidic" phospholipids from a total phospholipid extract, and with this method Dr. Gray and Mr. Body isolated two phospholipids which were not previously known to occur naturally (see below).

Gas Chromatographic Analysis of Glycolipids. Dr. Adams continued his studies on the composition and structure of glycolipids with the aim of reducing further the quantities of compound required for structural analysis (Report 1966). A successful structural analysis of the carbohydrate part of the glycolipid was carried out on only 0.5 mg of glycolipid. The analysis was reproducible provided that critical control of the conditions of methylation and methanolysis were maintained to keep to a minimum contaminating products arising from side reactions.

Some preliminary experiments in which small amounts of glycolipid were analysed in the presence of an inert "carrier lipid" indicated that 0.1 mg of material might be sufficient for analysis.

The analytical procedure was used to study the structures of some glycolipids isolated from the kidneys of normal C3H mice and of C3H mice carrying BP8 ascites tumours. The kidney glycolipids from the latter animals were different from those of the normal animals. Both glycolipid extracts contained ceramide monohexoside and ceramide dihexoside, but whereas the extract from the normal animals contained, as expected, ceramide trihexoside and aminoglycolipid, the major components in the extract from the tumour bearing animals were ceramide galactoside-3-sulphate and ceramide lactoside-3-sulphate, with only traces of ceramide trihexoside and aminoglycolipid present. Only traces of sulphatides were found in normal mouse kidneys and none were detected in previous analyses of the glycolipids isolated from the BP8 tumours (Report 1964). The glycolipid extracts were from two batches of 250 and 500 mice and it is reasonable to assume that they were representative samples from mice in each condition. It therefore appears that the presence of a BP8 ascites tumour in a C3H mouse results in a modification or block of the normal biosynthesis and/or metabolism of the kidney glycolipids.

The composition and structures of kidney glycolipids in normal mice of different strains were examined. All strains contained the same four types of glycolipid, common to most mammalian tissues, but their distribution in each strain was different. Of considerable interest were the structures of the ceramide dihexosides from each strain. That from the C3H strain was ceramide lactoside which is probably the major dihexoside in most mammalian tissues, but the Balb/c, A and C57/BL

strains contained almost pure *O*-β-D-galactosyl-(1→4)-*O*-β-D-galactosyl-(1→1)-ceramide. The exclusive occurrence of a dihexoside of this structure in a normal tissue has not been previously reported.

Partial Synthesis of Glycolipids. The partial synthesis of the simple glycolipid, ceramide glucoside, carried out by Mr. Hay (Report 1966), was not completely satisfactory because of the difficulty in obtaining good yields of the intermediate 3-*O*-benzoyl ceramide. It was found that the use of a large excess of benzoyl chloride in the benzoylation of trityl ceramide caused the formation of a dibenzoyl-trityl ceramide in which one of the benzoyl groups was attached to the amide group of ceramide. Detritylation of this compound did not yield 3-*O*-benzoylceramide. A good yield of the latter compound was obtained with a calculated excess of benzoyl chloride. The product, purified by silicic acid chromatography, was reacted with acetobromoglucose under anhydrous conditions and ceramide glucoside was obtained in satisfactory yield. Its chromatographic properties, infra-red spectra and chemical composition were similar to the naturally occurring *O*-β-D-glucosyl-(1→1)-ceramide. Small quantities of ceramide lactoside were also synthesized. The procedure appears suited to the preparation of milligram amounts of radioactive glycolipids, which are required for future work on the biosynthesis of the natural compounds.

Surface-active Lipoprotein in Mammalian Lungs. Two distinct forms of phosphatidylglycerol were found, as minor components, in the lipids from the surface-active lipoproteins in mammalian lungs (Report 1966). Dr. Gray and Mr. Body isolated larger amounts of both compounds from the lipid extracts of whole lungs (pig) and confirmed the identity of one as phosphatidylglycerol and characterised the other as the structural isomer, lyso-*bis*-phosphatidic acid. Both phospholipids were also found in the lipid extracts from rat and rabbit lungs. Preliminary results from a detailed examination of the "acidic" phospholipids from rabbit lungs indicate that another minor component is probably a bis-phosphatidic acid with one acyl group missing. It is an intermediate product in the degradation of *bis*-phosphatidic acid to lyso-*bis*-phosphatidic acid. A limited survey of other mammalian tissues is under way to see whether or not the lyso-*bis*-phosphatidic acid is a component common to tissues other than lung. The relative concentrations of phosphatidylglycerol and its isomer are much higher in the lipids from the surface active lipoprotein than in the lipid extract of whole lung, but the significance of this is not known.

Mammalian Cell Membranes. Miss Dod continued her studies on the lipid composition of plasma membranes from rat-liver cells. The stability of the lipid structure in the lipoprotein membrane is influenced by the ratio of cholesterol to phospholipid in the structure. This ratio in the plasma membrane (0.6) is considerably greater than that in the corresponding mitochondrial membrane (0.1) and suggests that the structural arrangement of the lipids in the plasma membrane is more rigid than that in the mitochondrial membrane.

Professor Marinetti (University of Rochester, New York) and Dr. Gray examined the action of a fluorescent chemical marker 4-acetamido-4'-isothiocyanostilbene-2,2'-disulphonic acid (Report 1965) on the membranes of intact liver cells and on subcellular fractions. With intact cells only the plasma membrane was labelled; the marker did not penetrate into the cytoplasm. It was found that the fluorescence was greatly enhanced in the presence of strong alkali. The label will be very useful in following the possible breakdown of membrane protein as a result of the action of certain enzymes (see below).

Dr. Jennifer Wells studied the action of several different preparations of phospholipase A on the plasma membranes of intact rat-liver cells. Isolated cells, suspended in a balanced salt solution, were treated with phospholipase A (from cobra venom) partially purified by heat treatment and paper electrophoresis. The increase in permeability of the membrane as indicated by leakage of the soluble cytoplasmic enzyme glutamic oxalo-acetic transaminase (GOT) appears to be the result of an enzymic

hydrolysis of some of the membrane phospholipids. In the early stages of the hydrolysis of phosphatidylethanolamine in the membrane there was very little leakage of GOT from the cell, but when 30–40 per cent. of the cell phosphatidylethanolamine (accounting for the total in the membrane) was hydrolysed there was a rapid increase in the release of GOT. This suggests that the plasma membrane was full of "holes". It was of interest to note however that at that point only a slight hydrolysis of the major lipid in the membrane, phosphatidylcholine, had taken place.

When the cells were stained with vital stains, such as acridine orange, eosin and nigrosine and examined visually there appeared to be no difference between control cells and those whose plasma membrane lipids had been partially hydrolysed, even to the point where the release of GOT was complete. Only when a major portion of the total cell-lipids were hydrolysed did the cells appear "dead". These studies suggest that normal functions of the cell continue and little change occurs in membrane permeability, even though a considerable portion of a phospholipid in the plasma membrane is degraded.

Dr. Jennifer Wells, in collaboration with Dr. Parish, measured the ability of HeLa cells to grow and reproduce after treatment with a phospholipase A fraction and a haemolytic (peptide) fraction from cobra venom. The membrane phospholipids were hydrolysed by phospholipase A plus lytic fraction but not by the enzyme alone. In this respect the HeLa cell plasma membrane is similar to the red blood cell membrane in that a lytic factor must be present for the phospholipase A to attack the membrane lipids. In the case of the plasma membranes of liver cells no lytic factor is required. These results suggest that important differences in membrane structure exist in different types of cell.

BIOPHYSICS

Human Plasma Proteins

Stability of human γG globulin Preparations. Since 1964, the stability of pools of human γG globulin (Fraction G.3) prepared at the Blood Products Laboratory, Elstree, for clinical use, has been routinely examined by comparing the sedimentation analysis of each pool before and after a period of 28 days incubation at 37° (Report 1965), in order to establish freedom from proteolytic enzymes. If such enzymes were present even in small amounts, degradation of the gamma globulin to lower molecular weight fragments would occur during long term storage of solutions at 4°. During 1965 and 1966 a total of 17 pools prepared by the ether method, and one pool and two experimental batches prepared by the ethanol procedure were examined in this way. None of these preparations was contaminated with proteolytic enzymes. There are many reports of such contamination in similar preparations made in other countries.

Structure of α Macroglobulin. Structural studies of the α macroglobulin of normal human plasma have been started by Dr. Jones and Professor Kekwick; a preliminary amino-acid analysis of the protein has been made. The most suitable conditions of electrophoresis in polyacrylamide gels for the characterization of this protein and sub-units derived from it, are under investigation.

Animal Antisera to Human Immunoglobulins. In collaboration with Dr. K. L. G. Goldsmith, Professor Kekwick devised a procedure for the elimination of heteroagglutinin activity towards human erythrocytes in animal antisera to human γG globulins. Since chromatographic studies (Report 1966) indicated that the heteroagglutinins were associated with the animal γM globulins, their inactivation with thiol reagents was examined. With the majority of antisera (sheep, goat), by incubating at 37°C with 0.05M mercaptoethanol for 3 hr., and terminating the reaction by excess iodoacetamide, the heteroagglutinins were inactivated. This treatment caused no significant loss in antibody towards human γG globulin, although the conditions were such that some disulphide bonds in the animal γG globulin carrying the antibody must have been broken. The antibody activity in the treated sera remained unimpaired during storage for 8 months at –25°C.

The procedure is readily adaptable to the large scale production of antisera for the detection of incomplete γG globulin antibodies to human erythrocyte antigens; it is considerably less laborious than the conventional adsorption methods and results in a better recovery of antibody.

Reversible Denaturation of Proteins. Current theories of protein synthesis account for the unique macromolecular configuration in terms of a genetically controlled primary structure and a thermodynamically controlled secondary and tertiary structure. The experimental evidence that the specific structure of a given protein represents a free-energy minimum is scanty, and has been obtained mostly with relatively small proteins of chain length less than 200 residues (approximate molecular weight 20,000). To obtain more information, Dr. Creeth and Mr. Holt studied the reversible denaturation of ovalbumin, which has a single chain primary structure of about 450 residues, the immediate object of the experiments being to determine whether the product obtained on very slow renaturation, conducted at every stage in a thermodynamically reversible manner, is identical with the native material. Among other methods the ultracentrifugal steady-state method was used; it is particularly adapted to the problem because of its sensitivity to small changes in frictional coefficient.

Results so far obtained with sodium dodecyl sulphate as denaturant show that renaturation occurs over a very narrow concentration-range of denaturant, and that aggregation occurs very easily depending on the concentration of protein. The renatured products obtained were undoubtedly different from the native protein; they were, however, fully soluble and free of detergent, and therefore represent conformations possessing at least some tertiary structure. These products are being characterized and further studies on the environment in the critical re-naturation phase are planned.

BLOOD PRODUCTS LABORATORY

The Laboratory continued to collaborate with the Department of Medicine, Liverpool University and the Regional Transfusion Centre, Liverpool in preparing human anti-Rh (D) immunoglobulin for the clinical trial of its value in preventing sensitization of Rh-negative mothers giving birth to ABO compatible Rh-positive infants. The results of this trial and of similar trials in other countries show that this form of immunoglobulin, if given within 36–48 hours of delivery, will remove from the mother's circulation any Rh-positive red cells that may have crossed the placenta from the foetus at delivery. Sensitization of the mother to the D antigen does not then occur. This treatment is most promising but confirmation must await the outcome of second Rh-positive pregnancies in the treated mothers. The widespread use of this treatment will be beset with many problems, not the least of which will be obtaining plasma containing adequate amounts of anti-Rh (D) incomplete antibody and lacking other atypical blood group antibodies. The staff of the laboratory is collaborating with the Ministry of Health and the M.R.C. Working Party on the use of anti-D immunoglobulin for the prevention of iso-immunization of Rh-negative women during pregnancy.

The Laboratory continued to take an active part in the treatment trial being conducted by the M.R.C. Working Party on Hypogammaglobulinaemia.

Pharmacologically Active Substances in Human Plasma. Dr. Mackay, in collaboration with Miss Mason, continued her investigation (Reports 1961 *et seq*) of the kininogenetic system of human plasma, studying in greater detail the components of the globulin fraction G2/1R separable by chromatography.

Kallikrein was identified by immunoelectrophoresis as a γM globulin and confirmatory evidence of this was obtained by studying PF (permeability factors), kinin production and esterase activity of this fraction, chromatographically prepared from G2/1R centrifuged at 50,000 rpm for 60 minutes and G2/1R heated at 60° for 60 minutes. Centrifugation decreased the PF activity of G2/1R by 9.2 per cent. and TAMe esterase by 34 per cent., and, in the γM fraction of G2/1R, PF was reduced by 99 per cent. and esterase by 27 per cent.

Heat decreased PF activity by 99 per cent. and esterase by 97 per cent. High speed centrifugation might therefore be used as an alternative means of separating PF dil from kallikrein. In neither case was kinin formation in intact plasma greatly decreased, suggesting that denatured protein might behave like an electro-positive surface and activate intrinsic kininogenases.

The Effect of the Hydrolysates of Fibrinogen on Guinea-pig Ileum. In previous work (Report 1962) it was observed that the application of fibrinogen hydrolysed by plasmin to the isolated gut increased its sensitivity to bradykinin and histamine. This phenomenon was further investigated by testing fractions of the fibrinogen hydrolysate separated on DEAE cellulose at pH 9.2. Four fractions were obtained which, by electrophoresis on acetyl cellulose. had the mobilities of alpha and beta globulins and of fibrinogen. The substance increasing the sensitivity of the guinea-pig ileum to histamine and bradykinin was found in the peptides with the mobility of alpha globulins; studies to identify this material are in progress.

THE BLOOD GROUP RESEARCH UNIT

The search for new blood groups and the application of those already known to problems of human genetics went on as usual during the year.

The X Chromosome

The Xg blood groups continued to be used in attempts to improve the gene map of the X and in the study of people with abnormalities of number (aneuploidy), or form, of the sex chromosomes (Report 1962 onwards).

X-mapping. The last Report (1966) on this aspect of the work had a despondent air: 2,000 families representing a great variety of X-linked conditions had been grouped for Xg with the adumbration only of two possible linkages—between Xg and ichthyosis and between Xg and angiokeratoma. During 1966, however, the linkage between Xg and ichthyosis became most satisfactorily established as the result of grouping a second series of English families and a series of Israeli families. The English families were ascertained through the records of St. John's Hospital, Lisle Street by Dr. R. S. Wells and Dr. M. C. Jennings: the Israeli families were the result of a wide survey carried out by Dr. A. Adam, Dr. L. Ziprkowski and Dr. A. Feinstein of the Government Hospital, Tel-Hashomer. The results of these two, together with the original Oxford series, combine to show that the locus for the X-linked form of ichthyosis is about 20 recombination units away from the locus for the Xg blood groups.

The present opinion is that the Xg locus is sited near the free end of the short arm of the X-chromosome: if this be correct then the ichthyosis locus is on the short arm too. The locus for glucose-6-phosphate dehydrogenase is out of measurable reach of the Xg locus. Efforts are being made in Israel, where g-6-pd deficiency is fairly common, to measure the distance between g-6-pd and ichthyosis. If this distance proves short enough to measure, then ichthyosis will bridge the gap between Xg and g-6-pd and a fine map of the X will begin to emerge, for g-6-pd is known to be close to the colour vision loci for deutan and protan and to the locus for haemophilia.

X-chromosome Aneuploidy. The Xg groups of people with the wrong number of X chromosomes go on being informative. The main abnormalities were listed in the 1966 Report. During the last year the Xg groups contibuted surprises in two categories:

i. XX males. Such males usually have Klinefelter's syndrome and they had been interpreted as ex-XXYs in whom the Y had been lost early in development (Report 1966). Were this correct, XX males should show very nearly the female distribution of Xg groups: on the contrary, the 14 so far tested have the male distribution and the difference from that of the female is already highly significant. The interpretation now is that XX males have only one X as far as the Xg locus is concerned and that their second X, though

looking normal, had previously interchanged part of its length with part of a Y chromosome. The possibility of rare translocation between the X and the Y, involving the Xg locus, was thought a possible explanation of two outstandingly abnormal Xg famtlies (Report 1966) and was also postulated by Dr. Ferguson-Smith on other grounds.

ii. XXY mosaics. Certain males with Klinefelter's syndrome are mosaics, the commoenst mixture being XY/XXY. The simplest explanation was that the accident had happened at an early mitotic cell division of an embryo that had begun as a normal XY zygote. If this were correct XXY mosaics should have the male distribution of the Xg groups: but it is now quite certain that their Xg distribution is, like that of ordinary XXYs, very close to the female and very significantly different from that expected of the male. Therefore the primary accident must have happened at a prezygotic, meiotic cell division.

The Autosomes

Still no success has come of attempts to assign any blood group locus to any particular autosome (Reports 1965, 1966) though the negative information about where blood group loci are not sited grows gradually.

The testing of many samples of blood suspected of containing something "new" continues. Nearly all these samples come from abroad and provide a great deal of enjoyable work and, every now and again, they do contain something new.

The ABO System. Evidence is now overwhelming that as an extreme rarity a person can inherit from *one* parent an antigen indistinguishable from A_2 together with an antigen indistinguishable from a weak B. We were given the opportunity to test samples from such a family from Japan. A solution to the genetic background would be of considerable theoretical interest for the condition may be showing that the genes *A* and *B* are not allelic in the strict sense but represent mutational sites so close together that crossing-over between them is very seldom observed: as a less exciting alternative the condition may be due to a "new" allele at the *ABO* locus, one which produces the two antigens.

The P System. The phenotype p is very rare (Reports 1956, 1958). However enough families have now been tested to show that it is controlled by an allele at the P_1P_2 locus: that is to say, the phenotype p corresponds to the genotype *pp*. This is not surprising, but previously an independent inhibitor gene offered as likely an explanation. The most recent addition to the data is from Umea in the north of Sweden where Dr. B. Cedergren has found a pocket of p people.

The Rh System. Dr. Tippett continues her work on refinements of this system and is collaborating with Dr. T. E. Cleghorn in a scrutiny of the Rh antigens of Jamaicans.

The Dombrock System. This new system (Reports 1965, 1966) is now being used in routine work. The hint of linkage with the Duffy locus (Report 1966) was dispelled by tests on more families.

Very Rare and Very Common Antigens. Much of Miss Gavin's time is spent on what seems an endless procession of very rare antigens which usually disclose themselves by causing cross-matching difficulty. Such antigens may have an incidence of from 1 in 200 to 1 in 10,000 people. On occasion they can be shown to be hitherto unrecognized parts of established systems; when this happens they are usually very informative about the organisation of the system as a whole.

There is a class, fortunately smaller, of very frequent antigens, possessed by all but perhaps 1 in 200 to 1 in 10,000 people. These antigens can cause acute transfusion problems in the rare people who lack them and have made the corresponding antibody. If traced to a known system such antigens usually afford fundamental information about that system.

During the year the Unit worked with more than a hundred cytogeneticists and physicians whom we thank. The most regular collaboration was with the following: Dr. A. Cahan, New York, Dr. C. Sheba and Dr. A. Adam, Tel-Hashomer, Dr. R. S. Wells, Dr. T. E. Cleghorn, Professor P. E.

Polani and Professor H. Harris, London; Dr. J. H. Edwards and Dr. J. Insley, Birmingham; Dr. W. H. Price, Edinburgh; Dr. M. A. Ferguson-Smith and Dr. J. H. Renwick, Glasgow; Professor R. Turpin, Dr. J. de Grouchy and Dr. C. Salmon, Paris; Dr. H. Nevanlinna and Dr. U. Furuhjelm, Helsinki; Dr. A. Frøland, Copenhagen; Dr. J. Lindsten, Stockholm; Dr. R. A. Pfeiffer, Münster; Dr. K. Boczkowski, Warsaw; Professor M. Siniscalco and Dr. L. N. Went, Leiden; Professor J. J. van Loghem and Miss Mia van der Hart, Amsterdam; Dr. B. Cedergren, Umea; Dr. Mary Crawford, Philadelphia; Professor B. Chown and Miss Marion Lewis, Winnipeg; Dr. T. F. Nikolai, Wisconsin; Dr. E. Shanbrom, Los Angeles; Dr. H. F. Polesky and Mrs. Jane Swanson, Minneapolis.

The Unit also had much help from Regional Blood Transfusion Centres; the Blood Group Reference Laboratory; Pfizer Diagnostics, New York; Spectra Biologicals, East Brunswick; Hyland Laboratories, Los Angeles; Laboratorios Grifols, Barcelona; Laboratorios Knickerbocker, Barcelona.

Once more the Unit thanks the Staff of the Institute for giving so many samples of their blood.

BLOOD GROUP REFERENCE LABORATORY

The Blood Group Reference Laboratory issues ever-increasing quantities of blood-grouping serum to hospitals and to Regional Blood Transfusion Centres in Britain. The serum comes almost entirely from blood donors and patients who live in the United Kingdom, though some of it comes from overseas. Dr. Ikin and her staff examine not only all this blood grouping serum for potency and specificity but also test many samples of grouping serum produced by other laboratories which ask for an independent opinion on their reagents. Certain grouping sera are produced in rabbits or other animals and while some, particularly anti-M and anti-N are produced in rabbits housed on the premises, the bulk of the antiglobulin serum is now produced in sheep and goats kept on behalf of this laboratory by Mr. J. Bleby and the staff of the M.R.C. Laboratory Animals Centre, Carshalton. In order that the output of grouping serum might be increased, changes have had to be made in the methods used for its handling and packaging, labelling and filling of containers now being partially mechanised.

Miss Giles and Mrs. Nunn continue to examine ever-increasing numbers of blood specimens sent in because of complicated transfusion problems. The Laboratory acts as the central reference laboratory for the World Health Organisation so that samples are received both from the United Kingdom and abroad. Not only does the number of samples received continue to rise but, equally, the techniques used for their examination get more and more complex.

In Britain there exists a National Donor Panel comprising over 2,000 blood donors whose red cells have been typed with a wide range of grouping sera. This Panel, which can be used for the selection of donors for patients whose serum contains antibodies likely to make transfusion difficult, is maintained by the scientific and secretarial staff of this Laboratory. During the year under review the Panel was used to find donors for patients in Britain and overseas.

Efforts are being made by the International Society of Blood Transfusion to establish an International Panel of Donors of Rare Types, the organisation of which would be a responsibility of this Laboratory. During 1966, experts from France, The Netherlands, Sweden and Switzerland came here to discuss the scheme, Dr. Mourant being here to take the chair.

Research continues to play an active part in the work of the laboratory, Dr. Ikin continues to study the MN blood group system and has produced anti-M^s in rabbits. She is also working on the production of anti-M^x, an antibody that has hitherto not been reported. She has also studied methods for improving the potency of Rh antisera by adding to them proteolytic enzymes or dextran. Increasing interest is being shown in the possible use of anti-D gamma globulin as a therapeutic substance that may be used in the prevention of Rh-sensitization of D-negative women delivered of a D-positive infant. Dr Ikin has tested

the *in vitro* potency of anti-D gamma globulin produced at the Blood Products Laboratory as well as examining similar material from overseas.

Miss Giles has performed extensive studies on the frequencies of the Cs^a and Yt^b antigens, both of which she was the first to discover. She has made a thorough investigation of the Rh gene complexes D(C)(e), d(c)(e) and D— —. In addition she has studied a number of "new" blood group antibodies, both by their reaction with random blood samples and by family studies.

Mrs. Nunn, in co-operation with Miss Giles has investigated the Kell blood group system, with regard to the antibody anti-Ku which was found in a patient of the rare Kell phenotype K^o.

Not all blood group antigens are carried on red cells, some being present in the plasma or the serum. Of these, antigens of the Gm system are perhaps the best known and research on them has been performed in this laboratory. It is now recognised that under certain circumstances Gm antibodies may cause red-cell agglutination. If present in grouping serum, these antibodies may cause errors in red-cell typing. All routine blood-grouping sera produced in this laboratory are now examined by Miss Brazier to ensure that unwanted Gm antibodies are not present. In addition, certain reagents used in Gm-testing are being made available to those who need them.

Dr. Phillips has continued to study the production of fluorescein-conjugated anti-globulin serum and has received much help from both Dr. E. J. Holborow of the M.R.C. Rheumatism Research Unit and from Professor Kekwick. As a result of her work, Dr. Phillips has produced fluorescein-conjugated anti-gamma-G reagent which is now available for distribution to those who require it.

Dr. Goldsmith and Miss Brazier have undertaken further studies of platelet and leucocyte antibodies. In addition, they have experimented with improved methods of producing anti-human globulin serum. In this work they have co-operated with Professor Kekwick and also with Dr. F. Stratton of the Regional Blood Transfusion Centre, Manchester. Professor Kekwick has continued to supply some of the antigens used to produce the antiglobulin reagents.

In co-operation with Dr. D. R. Bangham of the Division of Biological Standards of the National Institute for the Medical Research, and with Dr. Mourant, Dr. Ikin and Dr. Goldsmith have established the first International Standard of Incomplete Anti-Rh_o (Anti-D). This preparation has now been recognised as a standard by the Expert Committee of Biological Standardisation of the World Health Organisation.

During the last twelve months a number of visitors have come to the laboratory from Britain and abroad. They have been here for periods ranging from one day to that of several weeks and have been taught by a number of the members of the laboratory staff.

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.

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

Accounts 1967

THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Balance Sheet and Accounts

31 DECEMBER 1967

CHELSEA BRIDGE ROAD . LONDON, S.W.1 . 21 MAY, 1968

The Governing Body

Sir CHARLES DODDS, Bt, MVO, MD, D SC, FRCP, FRS, *Chairman*
H. P. G. CHANNON, MP, *Hon. Treasurer*
The Rt Hon LORD BROCK, MS, FRCS
Professor Sir LINDOR BROWN, CBE, FRCP, FRS
Professor L. H. COLLIER, MD
Professor D. G. EVANS, D SC, FRS
The Rt. Hon. the EARL OF IVEAGH
Professor Sir ASHLEY MILES, CBE, MD, FRCP, FRS

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

Financial Report of the Governing Body

1. The Balance Sheet as at 31st December 1967, shows balances to the credit of the various funds as follows: Capital Fund £720,519, Specific Funds £232,292 and Bequest Funds £25,508. The balance on the Sinking Fund for Freehold Buildings of £171,955 is after transferring £8,079 from Income and Expenditure Account and deducting £231 losses on sales of investments. During the year donations of £612 have been added to the Re-endowment Fund. The General Fund Investment Reserve has been credited with profits, less losses, on sales of investments amounting to £55,715 and the balance of the Sinking Fund Investment Reserve of £651 has been extinquished by losses on sales of investments.

2. The General Fund Income and Expenditure Account shows the Income for the year as £236,374 compared with £295,886 in 1966. Expenditure amounted to £342,630 against £346,265 last year. The deficit for the year is £106,256 compared with a deficit of £50,379 in 1966.

3. The year's deficit of £106,256 shown by the General Fund Income and Expenditure Account has been transferred to the Capital Fund.

4. Cooper Brothers & Co., the present Auditors, will continue in office pursuant to Section 159 (2) of the Companies Act, 1948.

E. C. DODDS

Chairman of the Governing Body.

The Lister Institute of Preventive Medicine
INCOME AND EXPENDITURE ACCOUNT
for the year ended 31 December, 1967

GENERAL FUND

(1966)		*Total Expenditure*	*External Contributions*	
£175,548	Salaries and wages	£302,881	£122,343	£180,538
13,196	Emoluments of two members of the Governing Body in an executive capacity	13,851	—	13,851
10,082	Premiums on federated superannuation policies	11,871	4,388	7,483
1,432	Premiums on group pension policy	4,064	1,000	3,064
7,827	Rent, rates and insurance	9,273	475	8,798
15,606	Gas, water, fuel and electricity	23,206	4,919	18,287
5,616	Office expenses, stationery and printing	6,276	836	5,440
630	Audit fee	630	—	630
2,942	Travelling expenses	4,236	1,099	3,137
1,597	Biochemistry expenses	9,687	7,629	2,058
2,664	Microbiology, immunology, experimental pathology and electron microscopy expenses	15,651	12,308	3,343
787	Biophysics expenses	3,454	2,036	1,418
605	Virology expenses	601	531	70
41,828	Serum, vaccine and virus vaccine expenses	36,765	1,722	35,043
7,607	Animals	10,847	2,171	8,676
11,245	Animal house expenses and forage	11,032	1,023	10,009
21,734	Buildings, alterations, repairs and renewals (including depreciation £4,318)	23,417	236	23,181
6,008	General apparatus and new installations	1,635	—	1,635
1,842	Library expenses	3,114	—	3,114
1,763	General stores	1,020	—	1,020
4,588	Staff canteen loss	4,392	636	3,756
2,907	Seventy-fifth anniversary celebrations	—	—	—
—	Blood products laboratory expenses	8,920	8,920	—
8,211	Amount transferred to sinking fund for freehold buildings (including £7,655 interest on investments)	8,079	—	8,079
£346,265		£514,902	£172,272	£342,630

(1966)			
	Interest and dividends on investments:		
£54,725	General fund	£52,606	
7,787	Sinking fund	7,655	
			£60,261
208	Underwriting commission		400
225,058	Sales of sera, vaccines, virus vaccines, &c.		167,528
8,108	Rent		8,185
	Deficit transferred to Capital Fund after charging to expenditure £9,227		
50,379	for addition to property and equipment		106,256
£346,265			£342,630

For Report of the Auditors and Notes on the Accounts see page 8

The Lister Institute of Preventive Medicine
BALANCE SHEET · 31 December, 1967

(1966)				
	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, 1966	£466,308		
	Less deficit 1967	106,256		
466,308			360,052	
826,775				720,519
	Specific Funds			
164,107	Sinking fund for freehold buildings	171,955		
31,684	Pension fund	30,005		
29,720	Re-endowment fund	30,332		
			232,292	
	Bequest Funds			
18,302	Jenner Memorial studentship fund	18,276		
7,573	Morna Macleod scholarship fund	7,232		
			25,508	
251,386				257,800
	Specific Grants and Legacies Unexpended			
20	Cancer research legacies (1937-1950)		—	
1,783	Nuffield Foundation grants (1952-1962)		1,583	
9,721	Guinness-Lister research grant (1953-1966)		8,779	
11,524				10,362
	Current Liabilities			
61,461	Creditors and accrued charges			50,221

E. C. DODDS
H. P. G. CHANNON
Members of the Governing Body

£1,151,146	£1,038,902

(1966)							
	Fixed Assets						
	FREEHOLD PROPERTY (SEE NOTE 1)						
£73,548	Land and Buildings, Chelsea					£73,548	
	Queensberry Lodge Estate, Elstree						
	at cost to 31st December, 1964			£20,455			
	at cost from 1st January, 1965		32,733				
	Less depreciation		8,611				
				24,122			
42,480						44,577	
							118,125
	FURNITURE, FITTINGS, SCIENTIFIC APPARATUS AND BOOKS (SEE NOTE 2)						
2,472	at cost less depreciation to 31st December, 1963					2,472	
	at cost from 1st January, 1964		7,073				
	Less depreciation		5,673				
2,800						1,400	
							3,872
121,300							121,997

	General, Specific and Bequest Funds. Investments and Uninvested Cash	*Quoted at cost in Gt. Britain*	*Elsewhere*	*Unquoted at cost*	*Uninvested cash*		
696,711	GENERAL	£498,796	£122,766	£40,287	—	661,849	
	SPECIFIC—						
164,758	Sinking fund for freehold buildings	163,655	—	—	8,300	171,955	
31,684	Pension fund	31,702	—	—	1,697 (Cr.)	30,005	
29,720	Re-endowment fund	27,634	—	—	2,698	30,332	
	BEQUEST—						
18,302	Jenner Memorial studentship fund	13,528	—	940	3,808	18,276	
7,573	Morna Macleod scholarship fund	6,631	—	—	601	7,232	
948,748		741,946	122,766	41,227	13,710	919,649	
	LESS INVESTMENT RESERVES						
115,161	General fund				170,876		
651	Sinking fund for freehold buildings				—		
						170,876	
832,936							748,773
	Current Assets						
114,046	Debtors and payments in advance					137,799	
82,864	Balance at bankers and cash in hand					30,333	
196,910							168,132
£1,151,146							£1,038,902

For Report of the Auditors and Notes on the Account see page 8

Notes on the Accounts

1. Freehold property additions and replacements since 1912 at Elstree and since 1935 at Chelsea, until 31st December, 1964, have been charged to revenue.

2. Additions and replacements to furniture, fittings, scientific apparatus and books between 31st December, 1920 and 31st December, 1963, have been charged to revenue. No depreciation has been charged between 1920 and 1963.

3. Quoted investments at a cost of £864,712 have a market value at 31st December, 1967, of £1,356,020.

4. Stocks of sera, virus vaccines and horses on hand at 31st December, 1967, have not been valued in the accounts.

Report of the Auditors to the Members

In our opinion the accounts set out on pages 4 to 7, amplified by the information given in paragraph 1 of the Financial Report of the Governing Body on page 3, give a true and fair view of the state of the Institute's affairs at 31st December 1967, and of its deficit for the year ended on that date and comply with the Companies Act, 1948.

COOPER BROTHERS & CO.
Chartered Accountants.

LONDON, 23rd May, 1968.

The Lister Institute of Preventive Medicine

PENSION FUND

(1966)			(1966)		
£3,161	Pensions	£3,361	£33,221	Balance as at 1st January, 1967	£31,684
476	Loss on realisation of investment	334	2,100	Interest on investments (gross)	2,016
31,684	Balance carried forward	30,005			
£35,321		£33,700	£35,321		£33,700

JENNER MEMORIAL STUDENTSHIP FUND

(1966)			(1966)		
£759	Stipend of student	£866	£18,186	Balance as at 1st January, 1967	£18,302
32	Loss on realisation of investment	120	907	Interest on investments (gross)	960
18,302	Balance carried forward	18,276			
£19,093		£19,262	£19,093		£19,262

MORNA MACLEOD SCHOLARSHIP FUND

(1966)			(1966)		
£702	Stipend of scholar	740	£7,911	Balance as at 1st January, 1967	£7,573
25	Loss on realisation of investment	—	389	Interest on investments (gross)	399
7,573	Balance carried forward	7,232			
£8,300		£7,972	£8,300		£7,972

NUFFIELD FOUNDATION GRANTS

(1966)			(1966)		
£1,368	Salaries, wages, laboratory expenses and animals	200	£3,151	Balance as at 1st January, 1967	£1,783
1,783	Balance carried forward	1,583			
£3,151		£1,783	£3,151		£1,783

GUINNESS-LISTER RESEARCH GRANT

(1966)			(1966)		
£8,848	Salaries and wages	£11,676	£5,440	Balance as at 1st January, 1967	£9,721
4,871	Laboratory expenses	4,266	18,000	Amount received	15,000
9,721	Balance carried forward	8,779			
£23,440		£24,721	£23,440		£24,721

THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Report
of the GOVERNING BODY
1968

CHELSEA BRIDGE ROAD · LONDON · SW1

The Governing Body

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Professor L. H. COLLIER, MD
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The Rt Hon the EARL OF IVEAGH
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Clerk to the Governors S. A. WHITE, AACCA

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Deputy Director: Professor W. T. J. Morgan
Superintendent of Elstree Laboratories: W. d'A. Maycock

MICROBIOLOGY, EXPERIMENTAL PATHOLOGY AND IMMUNOLOGY

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Brenda Mason, B SC

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Susan T. Edwards, B SC

Guinness-Lister Research Unit

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M. R. Hollingdale, B SC (*Medical Research Council Grantee*)

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Angela E. R. Taylor, B SC, PH D
Sheila M. Lanham, B SC

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†L. H. Collier, MD (*Professor of Virology in the University of London and Hon. Director, M.R.C. Trachoma Research Unit*)

W. A. Blyth, B SC, PH D
Janice Taverne, BA, PH D
Anne E. Mogg, B SC

M. R. C. Trachoma Research Unit

Electron Microscopy Unit

A. M. Lawn, B SC, PH D, MRCVS

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†W. T. J. Morgan, CBE, D SC, PH D, MD(*hc*), FRIC FRS (*Professor of Biochemistry in the University of London*). Principal Biochemist, Elstree
†Winifred M. Watkins, D SC, PH D (*Reader in Biochemistry* (*Immunochemistry*) *in the University of London*)
*G. M. A. Gray, B SC, PH D
M. A. Chester, M SC, B TECH (*Research Student*)
Anne M. S. Marr, B SC (*Morna Macleod Scholar*)
B. Denise Ward, M SC (*Grocers' Company Research Student*)
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J. M. Jones, B SC, PH D (*Medical Research Council Grantee*)
J. C. Holt, B SC (*Medical Research Council Grantee*)

§Dame Harriette Chick, DBE, D SC (ret'd)

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W. E. Parish, MA, PH D, BV SC, MRCVS, MC PATH
N. Mahony, M SC, B TECH

Biochemistry (Elstree)

*D. E. Dolby, B SC, PH D

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H. G. S. Murray, MD
G. S. Turner, B SC, PH D
L. C. Robinson, B SC
Frances R. Hunter, M SC (*Jenner Memorial Student*)

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*A. F. B. Standfast, SC D
Jean M. Dolby, MA, PH D
M. P. Banks, B SC
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J. Rodican, B SC

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Constance Shaw, M SC, DIP BACT
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PLASMA FRACTIONS LABORATORY (OXFORD)

Ethel Bidwell, B SC, PH D, FRIC
W. H. Ford, B SC

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Blood Group Research Unit

§R. R. Race, MD(*hc*), PH D, FRCP, FRS
Ruth Sanger, B SC, PH D
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E. June Gavin, B SC
Ann Gooch, B SC

Blood Group Reference Laboratory

K. L. G. Goldsmith, PH D, MB, BS, MC PATH
Toby T. B. Phillips, MB, CH B
Elizabeth W. Ikin, B SC, PH D
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Elstree Secretary and Estate Manager — G. J. Roderick, B COM
Assistant Secretary — Barbara A. Prideaux
Assistant Accountant — E. J. H. Lloyd
C. L. Beard
Beryl I. Bristow

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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

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the Institute for the year 1967.

GOVERNING BODY

At a meeting held on 27th June 1967, the Council re-appointed Sir Charles Dodds and Lord Brock as its representatives on the Governing Body until 31st December 1968. Sir Lindor Brown, who had earlier in the year been invited by the Governors to become a member of the Governing Body, was also re-appointed as one of the Council's representatives.

The Governing Body records with great regret the death of Lord Iveagh on 7th September 1967. Lord Iveagh, a member of the Governing Body since 1945, took a keen and generous interest in the affairs of the Institute, and did much to help it, not least during the establishment of the Guinness-Lister Research Unit in 1952.

It is noted with pleasure that the present Lord Iveagh joined the Governing Body this year.

COUNCIL

At last year's Annual General Meeting the three retiring members of the Council, Sir Lindor Brown, Major L. M. E. Dent and Dr. D. W. Henderson were re-appointed.

The three members of Council due to retire this year in accordance with the Articles of Association, but who are eligible for re-appointment, are Professor D. G. Evans, Dr. V. C. Barry and the President of the Royal College of Veterinary Surgeons representing the Royal Society, the Royal Irish Academy and the Royal College of Veterinary Surgeons respectively.

It is with sincere regret that the Governing Body records the death of Lord Florey. Lord Florey, as the representative of the University of Oxford, was a member of the Council from 1935 until his death in February this year.

MEMBERS

The Governing Body also records with regret the deaths during the year of Lord Balfour of Burleigh, a member of the Governing Body from 1950 to 1957, of Dr. D. McClean and of Miss E. M. Hume. Dr. McClean joined the staff in 1930 and was Head of the Smallpox Vaccine Unit from 1936 until he retired in 1961. Miss Hume came to the Institute in 1916 and was for many years a member of the Nutrition Department until her retirement in 1957.

STAFF AND STUDENTS

The Governing Body records with great pleasure that in March, 1968, Professor W. T. J. Morgan was awarded, jointly with Professor Otto Westphal, the Paul Ehrlich Prize for his contributions to immunochemistry; and that in October, 1967, he and Dr. Winifred Watkins shared the Karl Landsteiner Memorial Award of the American Association of Blood Banks. It also notes with pleasure that Dr. D. G. Godfrey was appointed to the rank of Officer of the Order of the British Empire, that Sir Ashley Miles was elected an Honorary Member of the American Society of Microbiology and that the University of London appointed Dr. J. M. Creeth a Reader in Biophysics.

The Governors offer their congratulations to Dr. B. G. F. Weitz, who resigned on 30th September 1967, on his appointment as Director of the Institute of Dairying Research at Shinfield; and to Dr. C. Kaplan, who resigned on 31st December 1967, on his appointment as Professor of Microbiology at Reading University. Dr. W. E. Parish has succeeded Dr. Weitz as Head of the Serum Department and Dr. H. G. S. Murray has succeeded Dr. Kaplan as Head of the Virus Vaccines Department. Dr. G. S. Turner was appointed Deputy Head of the latter department. As the first holder of a new post, that of Head of the Department for the Co-ordination of Production, Mr. J. Rodican took up his appointment at Elstree on 1st February, 1968.

In August 1967 the Institute accepted administrative responsibility, on behalf of

the Ministry of Health, for the Plasma Fractions Laboratory at the Haemophilia Centre, Oxford and welcomes Dr. Ethel Bidwell and Mr. W. H. Ford of that Laboratory as members of the staff of the Institute.

Dr. A. B. Stone was appointed to the Guinness-Lister Research Unit; Mr. A. Hunt to the Bacterial Vaccines Department; Miss V. J. Rowles to the Blood Products Laboratory; Mr. C. L. Beard to the Administrative Staff at Elstree; and Mr. M. A. Chester was awarded a research studentship in the Biochemistry Department. Dr. Delphine Miller, Dr. M. H. Malkinson, Mrs. J. M. Maw and Mr. A. V. Payne resigned during the year.

In April 1967 Sir Ashley Miles attended a meeting in Naples to discuss the international work of the Naples Zoological Station; and in September 1967 visited the National Academy of Sciences in Washington in connection with the preservation of the Aldabra atoll.

In May 1967 Professor W. T. J. Morgan and Dr. Winifred Watkins took part by invitation in a discussion on "Chemical and Genetic Properties of Bacterial and other Cellular Surfaces" sponsored by the National Science Foundation in Boston, U.S.A.; and in October 1967 they were guest lecturers at the Annual Meeting of the American Association of Blood Banks in New York and afterwards lectured by invitation in Toronto and Quebec.

Dr. W. d'A. Maycock attended a special meeting, convened by the Division of Immunology of the World Health Organisation, at Geneva in April 1967. He attended, as Ministry of Health representative, a meeting of the Subcommittee of Specialists on Blood Transfusion of the Public Health Committee of the Council of Europe, held in Vienna in May and also a meeting of the Working Group on the Toxicity of Plastics of this Subcommittee held in Stockholm in October. In November he attended a meeting of the Group of Experts on Immunological Products of the European Pharmacopoeia Commission of the Council of Europe held in Strasbourg.

In February 1967 Professor L. H. Collier participated by invitation in the scientific meeting in Boston, U.S.A., to celebrate the 70th birthday of Dr. John F. Enders. He also visited the School of Public Health at Harvard University Medical School.

Later in February he visited Algeria at the request of the Dean of the Algerian Faculty of Medicine. During this visit, which was sponsored by the British Council, Professor Collier advised the Institut du Trachome on their research programme and gave three lectures.

In April 1967 Dr. A. F. B. Standfast attended an International Union of Biological Sciences Documentation Meeting in Amsterdam.

Dr. C. Kaplan was a member of the Scientific Group on Smallpox Eradication which met at the World Health Organisation, Geneva, in October 1967. In December he visited Madrid as a World Health Organisation Consultant on Smallpox Vaccine Production.

Mr. L. Vallet visited the Central Laboratory of the Swiss Red Cross Blood Transfusion Service in Berne in November 1967.

Mr. L. C. Robinson and Mr. M. P. Banks attended the Tenth International Congress of the Permanent Section of Microbiological Standardization in Prague in September 1967.

For the academic year 1967/68 there are fourteen postgraduate research workers at the Institute registered for higher degrees of the University. Two Ph.D. degrees were awarded during 1967.

DONATIONS AND GRANTS

Wolfson Foundation Benefaction. The Governing Body records with satisfaction that the Trustees of the Wolfson Foundation have given £300,000 to the Institute for new laboratories, to be built as an extension of the present Institute building northwards along Chelsea Bridge Road. The Governing

Body is indeed grateful for this munificent gift, which is intended to promote research in the immunology of infective diseases.

In the new wing will be housed the department of Experimental Immunology which it is proposed to create for this purpose; it will also accommodate the present overcrowded department of Virology. Some laboratories will be available for the extension of other Institute activities. Other facilities in the new wing include animal quarters, workshops and a small lecture theatre.

Other Donations and Grants in 1967. Arthur Guinness, Son & Co. Ltd., continue their generous support of the Guinness-Lister Research Unit and a further £4,000 out of the total grant of £35,000 has been received from the Fleming Memorial Fund for Medical Research for the support of the Electron Microscope Unit.

The Governing Body records its appreciation of the many other bodies whose benefactions and grants support research work in the Institute. These include a grant from the Arthritis and Rheumatism Council for the study of vasculitis due to complexes of bacterial antigens with antibody; a grant from the British Empire Cancer Campaign for research on lipids; grants from the Medical Research Council for researches on the chemical basis of human blood-group specificity, on the biosynthesis of blood-group specific glycoproteins and red cell antigens, on multiple blood-group specific serological characters associated with simple glycoprotein molecules, on the characterization of proteins by the ultracentrifugal steady-state method, on the lipid components of the plasma membranes of mammalian cells, on the macroglobulins of normal human plasma, on the antigenic structure of *Mycoplasma hominis*, on sensitizing antibodies in sera of cot-death cases and milk-sensitive individuals, on the multiplication of bacteriophage, on the separation and characterization of the antigens of *B. pertussis*, on the distribution of serotypes of *B. pertussis* and on immunity to *B. pertussis* infections. Grants were also received from the Ministry of Overseas Development in aid of research on the blood-meals of insect vectors of disease and on the immunology of trypanosomiasis; from the Science Research Council for studies on the structure of biological macromolecules, on the reversibility of formation of specific disulphide bridges in proteins and on the metabolism and growth of micro-organisms, on glycoprotein and glycolipid structure and function, and on the amino-acid components of various proteins; from the Royal Society for the purchase of auto-titration equipment; and from the Smith, Kline and French Foundation for research on sensitization to micro-organisms as a cause of generalized eczema and on the *in vivo* biosynthesis of blood-group specific glycoproteins.

The Governing Body also gratefully acknowledges donations to the Institute's Re-endowment Fund from the Prudential Assurance Company Limited and the Royal London Mutual Insurance Society Limited.

VISITING WORKERS

The following visitors, in addition to those listed under Staff, worked for short periods in the Institute's Laboratories: Mr. Zafar Ali and Mr. Muhammed Yousuf, National Health Centre, Islamabad, Pakistan; Dr. M. Barme, Institut Pasteur de Dakar, Senegal; Dr. Ali Kemal Batum and Dr. Ramazan Sentürk, Central Institute of Hygiene, Ankara, Turkey; Dr. M. Cascardo, Flow Laboratories, Rockville, Maryland, U.S.A.; Mr. A. H. De Cherney, Temple University Medical School, Philadelphia, U.S.A.; Dr. Sawson El Tayeb, Serum Institute, Agouza, U.A.R.; Dr. Wala Hryniewicz, Institute of Rheumatology, Warsaw; Dr. M. Kai, Institute of Infectious Diseases, Tokyo University, Japan; Dr. E. Nnochiri, Lagos University Medical School, Lagos, Nigeria; Dr. K. Pershad, Institute of Preventive Medicine, Narayanguda, Hyderabad, India; Dr. Valentina Petrovskaya, Gamelya Institute, Moscow; Mrs. Anna Pirkola, Finnish Red Cross, Helsinki; Dr. P. K. Raha, Institute of Postgraduate Medical Education and Research, Calcutta, India; Dr. M. A. Rahman, Public Health Institute, Dacca, East Pakistan; Mr. Antonio Siccardi, Institute of Genetics, Pavia; Professor A. F. de Siqueira, Universidade de Sao Paulo, Brasil; Mrs. Kari Sundvall, Statens Institutt for Folkehelse, Oslo.

Researches in 1967

SUMMARY

The bracketed numbers refer to pages and columns of the report where more detailed descriptions are to be found.

Microbiology

1. Bacterial genetics, physiology and morphology:
   (a) Flagellar phages and sex-pili phages in Salmonella (16, i).
   (b) Morphology and serology of sex-pili (24, i).
   (c) Bacterial sex factors (16, ii).
   (d) DNA synthesis in phage infections (17, i).

Infective Diseases and Immunity

1. Trypanosomiasis:
   (a) Harvesting of blood stream trypanosomes (13, i).
   (b) Lipids of trypanosomes (14, i).
   (c) Antigens of *T. brucei* and *T. congolense* (13, ii).

2. Virus diseases:
   (a) Vaccinia virus. Mechanism of photo-inactivation (22, i); chemical composition (22, ii); *in vitro* growth (22, ii); and methods of assay (22, i).
   (b) Rabies virus. Purification and *in vitro* growth (23, i).
   (c) Cytology of distemper virus infection in the dog (24, ii).

3. Chlamydial and bacterial infections:
   (a) Trachoma agent. Inhibition by antimetabolites (19, i); serology (20, i); immunopathology of infections in guinea-pigs and baboons (20, ii); immunogenicity of live agent (21, i); and tests of trachoma vaccines overseas (21, i).
   (b) Mycoplasma. Antigenic analysis of *M. hominis* (18, ii).
   (c) Infectivity of L-forms of streptococci (19, i).
   (d) Kinetics of bacterial multiplication and death in salmonella infections (17, ii).
   (e) *B. pertussis*. Agglutinogens (14, ii) and protective antigens (15, ii). Immunoglobulins of *B. pertussis* antisera (15, ii).
   (f) *V. cholerae* toxin (15, ii).
   (g) Production of *Cl. tetani* exotoxin (15, ii).
   (h) Assay of diphtheria and tetanus vaccines (15, ii).

Immunology and Pathology

1. Immunology:
   (a) Immunoglobulins of horse antitoxins (23, i).

(b) Sensitization of tissues to cytotoxic antibodies by exogenous antigens (23, i).
(c) Immunological infertility in man (24, i).

2. Pathology:
   (a) Topography of microcirculatory reactions to trauma (25, ii) and to antigen in delayed hypersensitivity (25, i).
   (b) Monocytogenic factor in delayed hypersensitivity (25, i).
   (c) The plasma kinin system in man and guinea-pig (25, ii); in experimental trypanosomiasis (26, i); tissue kininases (26, i).
   (d) Ovum implantation in the mouse (24, ii).

Biochemistry

1. Human blood-group substances:
   (a) Characterization of structure by chemical methods (26, ii).
   (b) Hybrid glycoprotein molecules (27, i).
   (c) Biosynthesis of blood-group substances (28, i).
   (d) Relation of human and baboon blood-group substances (28, ii).
2. Lipids:
   (a) Glycolipids of normal and tumour cells of mammalian tissues (30, ii); effect of lipolytic enzymes on cell membranes (29, ii).
   (b) Phospholipids in mammalian lung (29, i).

Biophysics and Protein Studies

1. Macromolecules:
   (a) Structure of human macroglobulins (30, ii).
   (b) Reversible denaturation of proteins (31, ii).
2. Blood products:
   (a) Anti-rhesus factor antibody in clinical trials (32, ii).
   (b) " Plasma Protein Fraction " for clinical use (33, ii).
   (c) Stability of human immunoglobulin preparations for clinical use (33, i).

Medical Research Council Units

1. Report of the Blood Group Research Unit (33).
2. Report of the Blood Group Reference Laboratory (36).

MICROBIOLOGY

Trypanosomiasis

Harvesting of Bloodstream Trypanosomes. Miss Lanham devised a new method for separating trypanosomes from the blood of infected laboratory animals by fractionation on a column of an anion-exchanger. All the blood cells and platelets are adsorbed and the trypanosomes eluted with the dilute plasma, from which they are easily separable by centrifugation. *Trypanosoma congolense*, an organism hitherto difficult to separate, was readily harvested in this way. The method is a considerable advance over previous techniques; it is time-saving, readily adaptable to large-scale work, and the resulting flagellates, having been maintained throughout in a near-isotonic medium, are viable. Drs. Godfrey and Taylor showed that the eluted trypanosomes had normal morphology, and that the infectivity of *T. brucei* was unchanged.

The process depends on the higher negative surface charge on the blood cells than on the trypanosomes. Conditions for exploiting this difference in charge for fractionation, without harming the organisms, are determined by several interdependent factors; the ionic strength of the buffer; the adsorptive capacity of the anion-exchanger, trypanosomes being more readily adsorbed on DEAE-Sephadex than on DEAE-cellulose; and the surface charge of the species of the trypanosome concerned. Adsorption and desorption studies with several anion-exchangers and buffers of different ionic strength revealed that the species tested could be arranged in the following order of negative charge: *T. cruzi*>*T. lewisi*>*T. vivax*>*T. congolense*>*T. brucei*=*T. rhodesiense*=*T. gambiense*=*T. evansi*, a sequence that corresponds closely with the taxonomic relationships within the genus. The high negative charge of *T. cruzi* made it inseparable from rodent blood by this technique. Despite the differences between the other species, a standard procedure was devised for their separation from blood on a column of DEAE-cellulose, equilibriated with a phosphate-saline-glucose buffer at pH 8.0; a tris-saline-glucose buffer at pH 7.9 also proved useful, except for the separation of *T. lewisi*.

An interesting observation was made in *T. lewisi* infections of rats. Fractionation by the standard procedure was easy until the fourth day of infection; thereafter, an increasing proportion of red cells was not adsorbed, owing to an abnormally low negative charge; by the 11th–13th day, however, all the erythrocytes appeared to have the normal charge. Similar changes were observed in the adsorptive properties of the trypanosomes on the highly adsorbing DEAE-cellulose.

Since the charge on the erythrocytes varies with the species of mammal, the investigation is being extended to large animals—such as cattle, pigs and dogs—in Nigeria, especially to those liable to infection with trypanosomes that are non-infective for laboratory rodents.

Immunological Studies on T. brucei and T. rhodesiense. Dr. Taylor studied the agglutinogens of several strains of *T. brucei* and of *T. rhodesiense*. In rabbits infected with *T. rhosesiense*, during the first five weeks high agglutinin titres for all the *T. rhodesiense* strains appeared, but *T. brucei* agglutinins appeared only in low titre or not at all. The converse relationship was observed in rabbits infected with *T. brucei*. In collaboration with Dr. J. R. Baker of the London School of Hygiene and Tropical Medicine, a similar study was attempted in chimpanzees, with one strain of *T. rhodesiense* and two of *T. brucei*; but the antibodies produced were insufficient for detailed investigation. It was established however that, as with other hosts, the surface antigens of the blood trypanosomes changed during the course of infection.

Many more strains must be studied before the agglutinogenic differences between the two species can be accepted as a reliable means of differentiation. They are otherwise distinguishable only by the inability of *T. brucei* to infect man, which means that authenticated species are not readily obtainable. The investigation will continue as strains become available.

Antilymphocytic Serum. Attempts were made by Dr. Taylor to render rabbits and mice more susceptible to certain trypanosomal infections by means of the immunosuppressive agent, antilymphocytic serum. In rabbits given antiserum raised in rats, infections of *T. brucei* developed in a chronic fashion indistinguishable from that in control animals. In mice given the appropriate antilymphocytic serum raised in rabbits, infection by *T. lewisi*, like that in controls, was transient.

Phospholipids of Trypanosomes. Dr. Godfrey continued his investigation of the interspecies differences in the phospholipids of trypanosomes. Samples of the trypanosomes were collected by fractionation on DEAE-cellulose columns and the washed organisms freeze-dried and stored at −20°C. The phospholipids extracted from this material were separated by thin-layer chromatography and estimated quantitatively. Many samples of *T. brucei*, *T. congolense* and *T. lewisi* were examined with results substantially different from those already recorded. In all cases, the ratio of the amounts of lyso compounds derived from lecithin and phosphatidylethanolamine to those of the parent compounds was higher. Recent studies elsewhere suggest that these changes are due to activation of a lipase by freezing and thawing. However, other factors may play a part since freeze-dried material used in earlier work contained little of the lyso compounds.

Studies of freshly harvested material also showed that the phospholipid pattern of a newly isolated East African strain of *T. brucei* was similar to that previously found in strains from West Africa; that of a strain of *T.lewisi* was also like that of one previously studied. In a single sample of *T. simiae*, a parasite of the domestic pig, the phospholipid pattern differed substantially from a closely related species, *T. congolense*.

Permeability of the Trypanosome Cell. As differing phospholipid patterns may reflect differences in the cell membranes, Dr. Taylor began a study of interspecies differences in cell permeability. The fluorescent compound, tetracycline hydrochloride, is reported as penetrating the cell and mitochondrial membranes of *T. lewisi* suspended in saline, and much more slowly in the presence of serum. Dr. Taylor found that the decrease was possibly due to the chemical change in the tetracycline at the high pH of its solution in serum and not to the suggested protective action of serum. In recent work to establish the correct conditions of the test, the tetracycline was observed to penetrate *T. lewisi* more rapidly (30–40 seconds) in a phosphate-saline-glucose buffer than in Locke's glucose (65–80 seconds).

Whooping Cough Bacillus

The Agglutinogens of Bordetella pertussis. Dr. Jean Dolby, Miss Bronne and Dr. Standfast collaborated with the Public Health Laboratory Service Whooping Cough Committee and Working Party (of which Dr. Standfast is a member) in an investigation of the agglutinogens of *B. pertussis* and the antibodies they elicit in man and laboratory animals. Six agglutinogens are known, identified by number.

Agglutinogen 1 is found in all strains of *B. pertussis*. Antibodies to 1 were found in 100 per cent. of adults, 99 per cent. vaccinated children, and 100 per cent. of unvaccinated, infected children.

Agglutinogen 2 was found in about 13 per cent. of strains isolated from infections in children in 1967. Antibodies to 2 were found in 15 per cent. of adults, 16 per cent. of vaccinated children, and 20 per cent. of unvaccinated, infected children. Since the vaccinated children all received a vaccine containing 1 and 2, the 100 per cent. response to 1 and the 16 per cent. response to 2 indicates that the latter is a poor antigen in man.

Agglutinogen 3 was found in 96 per cent. of infecting strains. Antibodies to 3 were found in 3 per cent. of adults, 6 per cent. of vaccinated children, and 42 per cent. of infected children. The high prevalence of 3 in infecting strains, and the moderate prevalence of 3 antibody in infected children suggest that 3 also is a poor antigen in man.

Agglutinogen 4 was found in about 16 per cent. of infecting strains of *B. pertussis*. Only one of 10 unvaccinated children known

to have been infected with a 4-containing strain had 4 antibody, though all 10 children had 1 antibody.

Agglutinogen 5 is relatively rare and was found only in about 2 per cent. of infecting strains. Only one serum was found containing 5 antibody; this came from an unvaccinated, infected child.

Agglutinogen 6 was found in 17 per cent. of infecting strains. Antibody to 6 was found in 1 of 4 unvaccinated children infected with a 6-containing strain; all 4 had antibodies to 1. In 6-containing strains, the agglutinogen varies in detectability, and may disappear transiently, even during one subculture. It seems to be associated closely with agglutinogen 3.

In man, agglutinogen 1 is obviously a good antigen—though it is not known whether it is also an immunogen. On the other hand 2 and 3 seem to be poor antigens; only children recently infected by 3-containing strains had substantial amounts of anti-3. It therefore seems unlikely that the response at least to agglutinogens 2 and 3 has any significance in resistance of the child to pertussis.

Miss Bronne and Dr. Jean Dolby typed about 400 strains of *B. pertussis* collected from November 1966 in the Public Health Laboratory Service survey (Report 1967). About 90 per cent. had 1 and 3 and no 2 agglutinogens. Of these, 101 were further divided according to their content of the minor agglutinogens 4, 5 and 6. 79 were serotype 103000; 10, serotype 103006; 9, serotype 103400 and 3, serotype 103406. Since the autumn of 1967, serotype 123 has become more common and now accounts for 14 per cent. of the strains isolated.

Miss Bronne showed that in mice some 103400 strains elicit antibodies to 2 as well as to 1, 3 and 4. The possibility that some of these strains and some 123400 strains are mixtures of 120400 and 103000 is being investigated.

Miss Bronne and Mr. Hunt have shown by direct agglutination and by agglutinin production in mice that the 3 agglutinogen in vaccines is not labile, as held by some, and that agglutinogens 2 and 3 are present and actively antigenic in 10-year-old vaccines.

Immunoglobulins. Drs. D. E. and Jean Dolby examined the antibody activities of fractions of antipertussis rabbit sera (Report 1967) in terms of the 19S and 7S γ globulin content. As regards *in vitro* bactericidal power in the presence of complement, 7S preparations have 100 times the activity of 19S. The same ratio holds for protective power tested against brain infection. In sublethal lung infections, 19S and 7S are almost equally protective.

Protective Antigens. Drs. Standfast and Jean Dolby resumed work on the preparation of vaccine made of a cell wall fraction of *B. pertussis* (Report 1966). It was stabilized by adsorption to a mineral carrier. Dr. Ackers began fractionating the protective material in supernatant fluid of suspensions of broken cells after high speed centrifugation.

Vibrio Cholerae

Miss Goggin studied the cell-free toxin of *V. cholerae* and the protection it induces, either as toxin or toxoid, in laboratory animals. It was tested with and without preparations of the vibrio cell wall, to determine whether the cell-wall element has an immunogenic or only an adjuvant action in immunization.

Diphtheria and Tetanus Vaccines

Mr. Banks collaborated with the Division of Immunological Products Control of the National Institute for Medical Research, Hampstead Laboratories, on the development of new methods of assay for diphtheria and tetanus vaccine.

Clostridial Toxins

Mr. Mahony continued his study of the large-scale production of *Cl. tetani* toxin for the preparation of vaccine. The physical conditions established as necessary for good small-scale toxin production (Report 1967) proved to be highly successful with fermentation in 250 litre volumes.

Inheritance in Bacteria

Non-excluding Mutants of Phage P22. This phage differs from other phages like λ and P2 in not being recovered after adsorption to bacteria already carrying it as prophage. This exclusion was shown by Mrs. Maw and Professor Meynell to be specifically

directed against the superinfecting phage and not against other foreign genes (Report 1967). The P22 prophage has therefore been termed "excluding" (x^+). Non-excluding (x) mutants have now been isolated for the first time. The bacteria were lysogenized by mutant ($a1$) phage P22 which does not cause the synthesis of somatic antigen 1 (01). They were next superinfected with $a1^+$ phage P22. The majority of bacteria excluded $a1^+$ phage and therefore remained 01^-; but a minority, because they were lysogenized by non-excluding phage mutant, became 01^+ and were isolated by co-agglutinating them with carrier 01^+ bacteria of a different species, by adding anti-01 serum.

Bacteria lysogenised by x P22 no longer exclude super-infecting phage, yet they are still immune since the super-infecting phage does not replicate vegetatively. Immunity conferred by the P22 prophage is therefore an expression of two processes—exclusion and suppression of vegetative replication—whereas with other temperate phages, only repression is thought to occur.

A New Class of Donor-specific Phages. Bacterial strains able to donate genes by conjugating with the recipient are now known to form a specialised filamentous appendage (the "sex pilus"), of which two types are known (p. 24). The second of these, the I pilus identified by Professor Meynell and Dr. Lawn (Report 1967), has now been found on all strains carrying certain de-repressed mutant drug-resistance (or "R") factors isolated by Dr. Elinor Meynell of the M.R.C. Microbial Genetics Research Unit and Dr. Naomi Datta of the Royal Postgraduate Medical School. Phages exist which attack only bacteria carrying the F sex factor, by first adsorbing to the F sex pilus. Professor Meynell and Dr. Lawn have now isolated the prototype of an analogous class of "I" phages which attack strains forming I pili. The isolation was made possible by a new method of selection, in which sewage was incubated with *Salmonella typhimurium* carrying a de-repressed fi^- R factor and the mixture plated on *Escherichia coli* carrying the same R factor and therefore forming I pili. Since the O somatic antigens of the two bacterial species are different, the great majority of phages attacking Salmonella by reason of adsorption to the cell walls, cannot attack Escherichia; so that amongst the only phages to plate on Escherichia are those adsorbing to the I pilus. In this way, two filamentous (but not isometric) I phages were isolated whose host range is distinct from that of filamentous F phages. These have therefore proved of great value in the classification of bacterial sex factors (p. 24). I and F phages nevertheless have features in common, including serological cross-reactivity, thermo-resistance and continuing release from infected cells.

Flagellar Phages. During the isolation of I phages, flagellar phages were also isolated from five of the ten samples of sewage tested, although such phages are generally considered to be rare, since they have been described only twice before, in 1936 and 1941. They must, however, be moderately widespread in sewage but have been overlooked owing to lack of a method of selection. The method used with the I phage also selects flagellar phages because they can adsorb to the flagella of both *Salmonella typhimurium* and *Escherichia coli*, as shown by Dr. Elinor Meynell. The properties of the new flagellar phages were examined by Miss Edwards and shown to be closely related in morphology, host range and antigenic structure to the flagella phage originally described.

Bacterial Sex Factors. Although these genetic elements conferring donor ability occur widely in bacteria, often associated with a variety of characters like colicin production or drug resistance, they are only of two types (p. 24), one typified by F, the other by I, the sex factor of colicin factor I (*col* I). Another colicin factor, *col* E1a (Report 1965), has the unusual characteristic of undergoing epidemic spread like *col* I. This suggested that the sex factor of *col* E1a might be I, as indeed proved to be the case, as shown by the properties of the sex pilus and by mutual inhibition of epidemic spread.

Col E1a resembles *col* I in repressing its own conjugating function, so that it is donated by only a minority of its host cells.

De-repressed (*drd*) mutants cannot be isolated by the replica-plating method used by Dr. Elinor Meynell and Dr. Naomi Datta to obtain *drd* R factors. However, Miss Edwards and Professor Meynell devised a new method of selection with cultures mated in broth as opposed to cultures replica-plated on agar. Two *drd* mutants of *col* E1a were thus obtained which were initially identified by their susceptibility to the I phage (p. 24), since cells carrying the wild-type factor rarely form I pili because the I factor is repressed and they are therefore I phage-resistant. The behaviour of *drd col* E1a showed that the sex factor and the determinants of the colicin did not comprise a single genetic element, as had been supposed, but existed as distinct elements in the host cell. Thus, I was transferred 10 times more frequently than the colicin determinants, to give I^+ col^- recipients. Examination of bacteria carrying wild-type *col* E1a revealed spontaneous col^- segregants which nevertheless still possessed the sex factor, because their hosts failed to support the epidemic spread of *col* 1.

DNA Synthesis in Phage-infected E. coli. Dr. Stone, who joined the Unit in October 1967, continued his study of the single-stranded DNA phage, ØX174. A stock of mutant phage was prepared, which at 40° neither lyses the host nor makes proygen virus DNA. In the *E. coli*, bacterial DNA synthesis is arrested 15 min. after infection with this mutant. The arrest does not involve degradation of the cellular DNA, in contrast to infection with certain T-phages. It depends on the synthesis of protein, presumably virus-specified, for no arrest occurs when protein synthesis is inhibited by chloramphenicol. The rate of DNA synthesis is, however, decreased in the presence of chloramphenicol when higher multiplicities of infecting virus are used, probably by a different phenomenon, owing perhaps to non-specific damage to the cell. Mutants of both the virus and its bacterial host are being studied to determine the phage functions necessary for the arrest of bacterial DNA synthesis.

Bacteria infected with non-lysing mutants of ØX174 continue to make RNA and protein, and grow to 4 to 10 times their normal length. The cell membranes are intact beneath their elongated walls, and lysozyme converts the cells into abnormally large, osmotically-sensitive spheroplasts. During cell elongation the two nucleoids lengthen correspondingly and become more diffuse. By electron microscopy (with Mr. M. S. C. Birbeck, Chester Beatty Research Institute), these elongated bacteria appear to have normal walls, membranes and distribution of ribosomes. Progeny virus particles occur in paracrystalline masses. This study is preliminary to an attempt to determine the site of phage DNA synthesis by autoradiography.

Maaløe and Lark postulated that, provided "initiator proteins" have been made, DNA synthesis in *E. coli* will proceed without protein synthesis until the two daughter chromosomes are completed. The concept is based on experiments with two amino acid-requiring mutants of *E. coli* 15. The generality of this model is being investigated with amino acid-requiring mutants derived from other strains of *E. coli*. Starvation of different strains for the same amino acid, or starvation of the same strain for different amino acids, show that the amounts of DNA made by these strains are indeed different. A comparison of rich and poor culture media is in progress, to test the validity of a model in which, in a non-synchronized culture, the average number of replication points on the chromosome is assumed to be directly proportional to the bacterial growth rate.

Bacterial Multiplication in Infected Animals

The True Rate of Division. Using the newly isolated non-excluding mutants of phage P22 (p. 15), Mrs. Maw and Professor Meynell applied the superinfecting phage technique (Report 1960) to measuring the true division and true death rates of *Salmonella typhimurium* in the spleens of mice infected by intravenous injection. This kind of measurement has hitherto been impossible because conventional viable counts measure only the net result of microbial division and death *in vivo*; and therefore seriously underestimate the true division rate when

killing of the organisms occurs *in vivo* on a relatively substantial scale. *S. typhimurium* divides every 30 min. during growth in broth; in the mouse spleen, it was found to divide only every 12 hours. The true killing rate was also correspondingly small; so small that, in the absence of cell division, the viable count would halve only every 24 hr. These findings establish unequivocally for the first time that the growth rate of bacteria *in vivo* differs substantially from that observed *in vitro*. In particular, they show that attempts to determine the significance of host antibacterial mechanisms by experimentation *in vitro* are likely to be grossly misleading, in the many cases where bacterial resistance to killing is dependent on the division rate. In this instance, the rate proves to be about 24 times less *in vivo* than that usually encountered *in vitro*.

Kinetics of Microbial Infection. In the course of the superinfecting phage experiments, Mrs. Maw and Professor Meynell noticed that the scatter in colony counts on individual mice was maximal within 2 hr. of inoculation and did not increase in the following 8–10 days. Data from other Salmonella infections gave similar results. This pattern of behaviour suggests that Salmonella infections pass through two stages: an initial "decisive" period—which may correspond to that postulated on other grounds by Sir Ashley Miles (Report 1957)—in which a varying fraction of the inoculated organism is killed—followed by a period covering the remainder of the infection—in which the surviving organisms multiply in much the same fashion in each mouse.

Mycoplasma

Dr. Lemcke continued her studies of *Mycoplasma hominis*, which is associated with human genital tract infections; in particular, the chemical structure of the antigens, their location in the cell and significance in the immunological response of infected persons. A liquid medium was devised giving a high yield of *M. hominis*, minimally contaminated by medium constituents (Report, 1967). Pooled outdated human plasma, treated with kaolin to prevent clotting during subsequent sterilisation by Seitz-filtration, was a good substitute for horse serum, which forms undesirable precipitates on incubation.

Antigenic Analysis by Physical Methods. Fractionation of *M. hominis* by differential centrifugation after disruption of the cells by sudden decompression under nitrogen or by sonic treatment, yielded three fractions fixing complement with antisera against whole cells of *M. hominis*: a "membrane" fraction sedimenting at 37,500G, a "soluble" fraction not sedimenting at 100,000G, and a fraction sedimenting at 100,000G whose activity may be due to contamination by the other two fractions. Although both the membrane and soluble fractions fixed complement with antisera against *M. hominis*, the membrane fraction blocked growth-inhibiting antibody at least 32 times better than the soluble fraction. The two fractions also reacted differently in gel-diffusion precipitin and immunoelectrophoresis tests, the membrane fraction reacting only after solution in detergents or dilute alkali. In both types of test, the membrane fraction gave a precipitin pattern distinct from that given by the soluble fraction. Membrane and soluble fractions thus contain distinct antigens, the antigen blocking growth-inhibiting antibody being located in the cell membrane. Fractionation of the serologically active components by chromatography is in progress.

Chemical Extraction of Antigens. Mr. Hollingdale found that a lipid fraction, extracted from whole cells of *M. hominis* with chloroform-methanol, had some reactivity in complement fixation and gel-diffusion tests, but contained none of the main antigenic components. This is in contrast to the human respiratory pathogen, *M. pneumoniae*, from which most of the serologically reactive material can be extracted with lipid solvents (Report 1967).

When *M. hominis* was treated with aqueous phenol, the aqueous phase, into which lipopolysaccharides usually pass, was inactive. *M. hominis* thus differs from *M. mycoides* (the agent of contagious

pleuropneumonia of cattle), from which a serologically reactive lipopolysaccharide complex can be extracted in the aqueous phase. The material active in complement fixation and in blocking growth-inhibiting antibody was in the phenolic phase, and was, therefore, presumably protein. By gel-diffusion tests, it was found to contain components of both membrane and soluble fractions but chiefly the former. Extraction of whole cells with potassium hydroxide at pH 11.7 also yielded serologically reactive components present in the soluble and membrane fractions.

The results suggest that the main antigens of *M. hominis* are proteins and not lipids as in *M. pneumonia*, or polysaccharides as in *M. mycoides*.

L-forms of Streptococci

L-forms of bacteria, i.e. variants that have lost the ability to synthesize a rigid cell wall, can be induced *in vitro* by certain antibiotics. It is possible that such induction occurs *in vivo* during antibiotic therapy. This, and reports of the isolation of L-forms of streptococci from patients with rheumatic fever, endocarditis and septicaemia prompted a study of the pathogenicity of streptococcal L-forms and their induction and survival *in vivo*.

Induction of L-forms in vitro. Dr. Hryniewicz determined the optimal conditions for induction of L-forms from Group A streptococci; the age of the parent culture, the type of inducing agent, the constitution of the solid medium on which the L-forms develop and conditions of aeration were investigated. Phage-associated lysin, obtained by bacteriophage lysis of a Group C streptococcus, induced more efficiently than penicillin, with respect both to the yield of L-form colonies and the number of strains from which L-forms could be induced. The resulting L-forms were stable immediately after induction and did not revert to the bacterial form on sub-culture, even on media containing yeast extract or egg yolk, both of which often encourage reversion. In all, L-forms of strains belonging to seven different types of Group A streptococci were induced.

Immediately after induction, streptococcal L-forms grew on solid but not in liquid media. This appears to be a physical rather than a nutritional effect. Liquid cultures for the *in vivo* experiments were established most quickly by transfer on agar slopes overlaid with 1–2 ml. of broth.

Survival and Pathogenicity of L-forms in vivo. In mice, the various L-forms did not survive long enough to produce any disease. The maximum period of survival in the peritoneal cavity was 8 hours; no L-forms could be recovered from any organ 10 minutes after their intravenous inoculation. Similarly, none survived for more than 16 hours in embryonated hen's eggs, whereas the parent cocci killed the embryos within 2 to 3 days.

Induction of L-forms in vivo. Chronic pyelonephritis was established in rats by the injection of *Streptococcus faecalis*. The animals were then treated with massive doses of penicillin for periods of up to four weeks. No L-forms were recovered from the kidneys or other organs at any stage during treatment. L-forms, however, were grown from the peritoneal fluid of mice up to six hours after the intraperitoneal injection, separately, of group A streptococci and the phage-associated lysin. It remains to determine whether the L-forms were induced *in vivo*, or in the medium used for their recovery.

TRACHOMA AND INCLUSION BLENNORRHOEA (Conjunctivitis)

The trachoma and inclusion conjunctivitis micro-organisms of the *Chlamydia* group are referred to as TRIC agents. Variants that for a given dose kill chick embryos more quickly than their parent strains are *f* (fast-killing) strains, and the parents are *s* (slow-killing) strains.

Inhibition by Antimetabolites. Chlamydia may be divided into two groups on the basis of their sensitivity to sulphonamides. One group comprises agents like those of psittacosis and meningopneumonitis that are resistant to sulphonamides and utilise an exogenous source of folic acid. By contrast, the growth of TRIC and similar agents in the second group is inhibited by sulphonamides, indicating that they synthesize their own folic acid. Drs. Reeve and Taverne in collaboration with Dr. S. R. M. Bushby

(Wellcome Research Laboratories, Beckenham, Kent) investigated the effect of another folic acid inhibitor, trimethoprim, on the growth of TRIC agents. Trimethoprim is a derivative of 2–4, diaminopyrimidine and inhibits the growth of many bacterial species, competing with folic acid and binding to dihydrofolate reductase; it may strongly potentiate the action of sulphonamides. In cell cultures, trimethoprim decreased the number of inclusions formed by TRIC agents and induced morphological changes in the inclusions similar to those caused by sulphafurazole. In eggs, inhibition by trimethoprim was most clearly demonstrated by measuring prolongation of the mean death time of groups of chick embryos inoculated with a single lethal dose. Over a certain range, prolongation was proportional to the ($\log_{10}$) concentration of inhibitor; higher concentrations were toxic for the embryos. On a weight basis, trimethoprim was not as active as sulphafurazole; it slightly potentiated the action of this sulphonamide. A related pyrimidine derivative, the antimalarial drug pyrimethamine, also significantly inhibited the growth of one strain of trachoma. Inhibition of the trachoma agent by trimethoprim was reversed by leucovorin calcium, indicating that, as with bacteria, the drug acts by blocking the folic acid cycle and that TRIC agents most probably contain a dihydrofolate reductase.

These and other studies were made with baby hamster kidney (BHK-21) cells, which proved more suitable for propagating TRIC agents than the HeLa cells mentioned in previous Reports.

Immunofluorescence Studies. Mrs. Mogg (née Smith) and Professor Collier began an investigation of immunofluorescence techniques for studying TRIC agents and their antibodies. Direct staining with fluorescein-labelled antibody and the use of TRIC agents prepared in yolk sacs both had disadvantages; an indirect staining method with TRIC agents propagated in BHK-21 cells was devised and proved satisfactory. Antisera prepared against several *f* strains stained both the homologous and heterologous *f* strains growing in cell cultures; as did antibodies to other *Chlamydia*, namely enzoötic abortion of ewes and lymphogranuloma venereum. This method is being exploited for studying the serological relationship between *f* and *s* strains and their replication in cell cultures.

Professor Collier devised a method for making sets of graded density filters from strips of photographic film; they are used in fluorescence microscopy for estimating brightness quantitatively, and have the advantages of being cheap and easy to make, of minimising observer error, and of facilitating the comparison of readings made on different specimens and in different laboratories.

Immunological Studies in Guinea-pigs. Intracutaneous infection of guinea-pigs induces an immunity expressed by the failure of the trachoma agent to appear in the spleen after subsequent challenge (Report 1967). With a view to using this phenomenon as the basis of a quantitative index of immunity, Dr. Blyth studied the multiplication of TRIC agent in the spleen; after intravenous injection it could be recovered for at least a week, during which several approximately synchronous multiplication cycles were observed. Because of the probable importance of the spleen in immunity, Dr. Blyth is examining the response to infection in spleen cell cultures from normal and immunized animals; for this purpose he devised a method of growing the cells on thin layers of collagen.

In normal guinea-pigs, heat-killed TRIC agent injected intracutaneously induces no skin lesion, but does so in animals previously given live organisms intracutaneously. The specificity of this reaction, which suggests intermediate or delayed hypersensitivity, was established with TRIC agent grown in eggs and in cell cultures as the sensitizing and challenge agents respectively. Hypersensitivity appears 3 days after the preliminary dose, increases to a maximum at 10–14 days, and slowly wanes during the next 2–3 months; this time course clearly distinguishes it from the short lived reaction of immunity described previously (Report 1967). The finding that intravenous injection of killed organisms prevents the skin reaction in sensitized animals—possibly by combining with available antibody—sug-

gests mediation by a humoral factor; but preliminary attempts at passive transfer of hypersensitivity with serum were unsuccessful. By a comparison of time courses of responses to infection in the guinea-pig, it is clear that the hypersensitive state is not associated with specific immunity to dermal infection, but it may be related to a decreased survival of TRIC agent in the spleen.

For these and other investigations, relatively pure suspensions of TRIC agents with a high proportion of viable organisms are needed. Dr. Blyth and Dr. Taverne improved purification by defining the best conditions of centrifugation, and, when desirable, by precipitating contaminating host material with antiserum prepared against yolk sac. The survival of purified TRIC agent during freezing and subsequent storage was increased from 10 per cent. to nearly 100 per cent. by adding 0.25 M sucrose to the suspending medium.

Trachoma Vaccine. Professor Collier and Mrs. Mogg continued to investigate the dissemination and immunogenicity of live TRIC agents in baboons. A fast-killing variant strain (MRC-4 *f*) mutiplied considerably in the skin, lymph nodes and spleen after parenteral injection and induced firm immunity to subsequent challenge by the conjunctival route (Report 1967). The slow-killing parent strain (MRC-4) multiplied to a very limited extent in the skin after subcutaneous injection and in the spleen and lymph nodes after intravenous injection, a result in line with Dr. Blyth's findings in guinea-pigs. By contrast with MRC-4 *f*, MRC-4 was no longer detectable in any tissue two days after injection and the degree of immunity to conjunctival challenge failed to attain statistical significance in comparison with controls. This work suggests that trachoma vaccines prepared by the present conventional methods are insufficiently potent unless their antigenic stimulus is enhanced by multiplication within the host. The antibodies in serum samples from these experiments are being assayed by the complement fixation test as a preliminary to analysis for various immunoglobulins.

Investigations Overseas. Under Professor Collier's direction, researches were continued in West Africa and in Iran.

In the Gambia, Dr. Shiona Sowa and Mr. J. Sowa completed a study of conjunctivitis in infants born in an urban hospital. In 100 of 300 babies examined, overt conjunctivitis was observed within a few days of birth. Most cases were bacterial in origin, and 6 of them gonococcal. Four infants had TRIC agent conjunctivitis and one of the four fathers harboured the TRIC agent in the urethra; it is of great interest that in all 4 babies, the subsequent onset of keratitis, pannus and scarring characterized the syndrome as trachoma rather than inclusion conjunctivitis. The finding that trachoma can apparently be acquired from the maternal genital tract confirms previous observations in a rural community (Report 1964).

Mr. Sowa had previously found neomycin useful for preventing bacterial contamination of conjunctival scrapings inoculated into chick embryos. Although the high isolation rates in the presence of this antibiotic indicate that most strains of TRIC agent are resistant to it, Mr. Sowa recently found that high concentrations interfere with the isolation of a minority of strains; the inhibitory action appeared to be bactericidal rather than bacteriostatic.

Mr. and Mrs. Sowa began a study of immunofluorescence techniques for detecting antibody to TRIC agent in the conjunctival secretions and blood sera of trachoma patients. With a view to improving the diagnosis of trachoma and inclusion conjunctivitis, they are also determining how fluorescein-labelled antibody compares with iodine for staining inclusions in conjunctival scrapings.

In collaboration with the Institute of Ophthalmology, London and Teheran University, the second and final annual follow-up of the trachoma vaccine trial in Iran was completed. One year after vaccination, there was a moderate but statistically significant reduction in the incidence of trachoma in the immunized children (Report 1967). Analysis of results by the M.R.C. Statistical Research Unit showed that by the end of the second year, this beneficial effect had largely disappeared; and the proportion of fresh cases observed in vaccinated subjects was

significantly higher than in the controls. Similar findings have been reported by other investigators, and suggest that the effect of vaccination is to delay rather than to prevent completely the onset of the disease.

VIROLOGY

Vaccinia Virus

Photo-inactivation. Dr. Turner and Dr. Kaplan continued their studies on the photodynamic inactivation of vaccinia virus and compared the effects of the acidic dye rose bengal with those of methylene blue. Photoinactivation with rose bengal rapidly destroyed the antigenicity of inactivated virus and significantly reduced the histidine content of viral protein. The inhibition of methylene-blue-sensitized inactivation by nucleic acid and that of rose-bengal-sensitized inactivation by protein could be correlated with spectrophotometric data, which indicated specific affinities of the dyes for these substrates. Cross-reactivation studies, however, suggested that both viral protein and nucleic acid were attacked by both dyes.

Inactivated Vaccine. The work on the pathogenicity of vaccinia virus strains for mice by the intranasal route was completed by Dr. Turner. The usefulness of a mouse protection test for potency assay of inactivated vaccines using intranasal challenge seemed doubtful from preliminary results.

Dried Vaccine. Difficulties were experienced by World Health Organisation field workers with batches of dried smallpox vaccine prepared by methods different from our own. Dr. Kaplan and Dr. Murray began tests on the effect of post-drying atmosphere on unsealed ampoules. Exposure of as short a time as 5 minutes significantly impaired the stability of the vaccine in accelerated degradation tests and during storage for one month at 37°C.

Virus Assay. Dr. Murray continued work on the potency assay of smallpox vaccine in chick embryo cell cultures as a possible alternative to titration in the chick chorioallantois. The data were suitable for processing by the London University computer; and analyses of variance of the assays, potency ratios and fiducial limits were calculated according to the Medical Research Council's random bioassay programme No. 5. Of 53 vaccines tested, 86.8 per cent. passed the test in eggs and 79.2 per cent. in chick cell cultures. Further efforts to improve the precision of the tissue culture assay continue.

Dr. Malkinson completed his work on the transference of maternal immunity to vaccinia virus in mice (Report 1967). He continued work on the fractionation of the soluble vaccinia virus antigens from a number of strains and from different source material. The fractions were assayed by passive sensitization of erythrocytes and a partial chemical and physical characterization was accomplished. The fractions contained both nucleic acid and polypeptide and were of relatively low molecular weight. Some fractions were strain specific in neutralization tests. Some produced erythema in the skin of appropriately sensitized guinea-pigs. This work ceased when Dr. Malkinson left the Institute for Israel.

Composition. Mr. Robinson continued his work on the analysis of vaccinia virus, with especial reference to origin, viral or host, of the copper present in preparations of the virus. Highly purified preparations of vaccinia virus were analysed for copper and flavine-adenine dinucleotide by the Cartesian diver technique. The apoenzyme of ceruloplasmin was prepared. With N, N′ dimethyl-*p*-phenylene diamine sulphate as the substrate, oxidase activity of the holoenzyme was measured; the holoenzyme is formed only when copper is provided. It is hoped that this new technique may provide a more accurate estimation of the copper content of the virus.

Analysis of the lipids of vaccinia virus by thin-layer chromatography and the subsequent hydrolysis of the separated components continues.

Growth in Tissue Culture. Miss Hunter studied the growth of vaccinia virus in the human diploid embryonic lung cell strain WI-38 and produced two small-scale experimental vaccines. The effects of various drying agents on their stability was investigated over a range of temperatures.

A further large-scale batch of vaccine was produced for test.

Work continues on the development of a bacteriologically sterile smallpox vaccine for jet inoculation. A suitable suspending agent for freeze drying has been found.

Rabies Virus

Dr. Turner and Dr. Kaplan completed studies on the properties of the Pasteur strain of fixed rabies virus. Work proceeds on the preparation of experimental batches of purified rabies vaccine. Using the human diploid cell strain WI-38, Miss Hunter was unable to grow the virus in sufficient quantities to make feasible the production of a vaccine by this method.

IMMUNOLOGY AND EXPERIMENTAL PATHOLOGY

Antitoxin Production

Dr. D. E. Dolby continued his studies (Report 1966) of β and γ globulin antibodies from horse serum, including the preparation of fractions with differing chromatographic and electrophoretic properties. Conditions for the use of cyanogen bromide in the splitting of whole antibody molecules (Report 1967) are under investigation, in an attempt to prepare molecules of smaller size which retain the biological activity of the original material.

Dr. Dolby prepared fairly large quantities of the heavy and light polypeptide chains from γ globulin antibody and from mixed β globulins, as a preliminary to the study of their properties *in vivo*, particularly of the extent to which they retain their antitoxic activity and the immunogenic specificity of the starting material.

The investigation of the purification of gonadotrophin from pregnant mares' serum (Report 1967) and of the stability of the purified material was completed.

Exogenous Antigens Acquired by Tissue Culture Cells

Dr. Parish continued to investigate the possibility that bacterial or fungal antigens are incorporated in cells of the skin, which are thereby predisposed to damage by specific antibody, with a consequent generalised eczema (Reports 1966, 1967).

HeLa cells grown in the presence of pure protein antigens were damaged on the addition of complement and the appropriate antibody. The damage was estimated by measuring the amount of nucleic acids liberated from the cells, thus avoiding the difficulties of determining cell-viability by dye exclusion or acridine orange fluorescence (Report 1967). The results confirmed that the HeLa cells with acquired protein antigen were susceptible to damage by the appropriate antiserum. Cultures varied in susceptibility, either because more antigen was acquired when cells divide, or because the effect of the antiserum was more potent during division. Arresting cell division by temperature changes made cells more resistant to lysis.

Since the non-specific effects of sera of foreign species on HeLa cells were also greater during cell division, further tests are being made with primary cultures of guinea-pig and rabbit kidney, and antibody prepared in the homologous species. However, it appears that the cells in the cultures are a mixed population, with differing susceptibilities; attempts are being made to prepare monoclonal cultures.

Antibodies to Bacteria in Generalised Eczema

Dr. Parish, in collaboration with Drs. R. H. Champion and E. Welbourn at Addenbrooke's Hospital, Cambridge (Report 1967), extended the study of the cytotoxic effects of antibody on cells acquiring antigen *in vitro* to cell damage in generalised eczema. The sera of eczematous patients were examined for antibodies to bacterial antigens, and those which were positive were tested for their cytotoxic action on cells that had been exposed to the bacterial antigens. All the patients with eczema and all the normal persons had approximately the same agglutinin titres to the polysaccharide antigens of *Staphylococcus aureus* and Micrococci, but by antiglobulin tests, only a few normal sera contained γ_M antibodies to the bacterial antigen, whereas most "eczematous" sera contained them. The corresponding γ_G antibodies were also found in association with the γ_M, a surprising finding in view of the rarity with which γ_G antibodies to polysaccharides are reported to occur.

The γM antibodies to bacterial polysaccharides were not cytotoxic to HeLa or freshly isolated human tissue cells that had been exposed to the antigen *in vitro*. In cells exposed to the antigens *in vivo* the antibodies induced inflammatory reactions but no eczematous change.

Human Infertility

Dr. Parish, in collaboration with Dr. A. Ward of St. Mary's Hospital, resumed his investigation of antibodies in cervical mucus that were cytotoxic to spermatozoa (Report 1964). In three couples with unexplained infertility, two types of cytotoxic antibody and three non-toxic antibodies were found. The γG globulins appeared transiently in two of the women; one antibody was complement-dependent, causing sperm lysis; the other was a sperm-immobilizing antibody. Both reacted with intrinsic spermatozoal antigens. The harmless antibodies were γM or γD globulins reacting with seminal plasma antigens that coat the spermatozoal surface.

It is important to use buffered extracts of mucus for tests because usually thick mucus, or mucus with acid pH can immobolise spermatozoa, thus resembling the effect of immobilizing antibody.

Electron Microscopy

Sex Pili Associated with Bacterial Conjugation. Dr. Lawn's work on sex pili continued in collaboration with Professor Meynell, Dr. Elinor Meynell of the M.R.C. Microbial Genetics Research Unit and Dr. Naomi Datta of the Royal Postgraduate Medical School. Two groups of sex pili, F-like and I-like, were defined by morphology, phage receptor type and antigenic structure. The F-like pili are long, many with an axial canal; they adsorb F-specific but not I-specific (see p.16) phages. They cross react serologically with other F-like pili but not with I-like pili. The I pili are usually short, with an indistinct axial canal, and they adsorb only I-specific phages. In cross-reactions they are serologically related to I-like but not F-like pili.

The sex pili formed by bacteria able to transmit the colicin *col* E1a (see page 16) were shown to be I-like in structure; reacting strongly with antiserum to the pili of R144, an fi⁻ drug resistance factor. More strains with de-repressed drug resistance transfer factors isolated by Dr. Elinor Meynell were examined morphologically and serologically. All the pili were clearly I- or F-type. Some of the few strains appearing to form pili with intermediate properties carry two sex factors, each determining its appropriate type of pilus.

I Phages. The filamentous bacteriophages isolated by Professor Meynell and Dr. Lawn (p. 16) that attack bacteria forming sex pili of the I group were compared with those attacking bacteria with F-like pili. Their sizes were measured and their antigenic relationships determined by the electron microscope method (Report 1967). Although the two types differed in length (1.3μ and 0.9μ respectively), the two groups are serologically related.

Canine Distemper Virus. Chorioallantoic membranes of chick embryos inoculated with this large myxovirus were obtained from Drs. S. A. Keeble and G. N. Woode of Glaxo Laboratories. The morphological features of distemper virus replication in this system were compared with those observed by Dr. Lawn and Dr. E. C. Appleby of the Royal Veterinary College in the brains of puppies with so-called nervous distemper (unpublished observations). Sites of virus replication were located, and the process was found to be similar to that with other myxoviruses. In the infected cells filamentous material occurred in inclusions in the perinuclear regions, in protrusions of the apical cell surface and in the mature extracellular virions nipped off from these protrusions. In none of these features was there any resemblance to the crystalline inclusions found in endothelium and glia of the cerebellar white matter of the puppies. The process of distemper virus assembly may differ in the dog and the chick embryo, or another virus may have been responsible for the canine encephalitis.

Ovum Implantation in the Mouse. Dr. Lawn's collaboration with Mr. C. A. Finn of the Royal Veterinary College continued. It was not possible to confirm the observation of

Potts that well developed desmosomes join the foetal trophoblast to the maternal endometrial epithelium: only simple contacts were found. When the uterine epithelium has dispersed, the trophoblast makes extensive contacts with the maternal decidual cells (transformed fibroblasts). These contacts are also simple, without desmosomes or tight junctions, in contrast to those between decidual cells, where tight junctions are formed (see Report 1967).

Occasionally electron dense bodies were found in the region where endometrial epithelium and blastocyst are apposed. They resemble the cells described by Wilson, but are apparently dead epithelial cells. Adjacent normal epithelial cells rearrange themselves to exclude the dead cell and close the potential gap in the epithelial barrier. In the non-pregnant uterus the excluded cell would have entered the lumen, but in this case it is phagocytosed by the overlying trophoblast, in which it forms a dense inclusion. Although these bodies are present at the same stage of implantation and in the same situation as Wilson's cells, they may not be identical: and the cells which, according to Wilson, leave the trophoblast to initiate the decidual reaction in the maternal endometrium may not have been detected in the present investigation.

Mechanisms of Inflammation

The Tuberculin Reaction. Dr. Wells continued his topographical studies of the reactions of the vascular endothelium of the dorsal skin of hypersensitized rats and guinea-pigs to tuberculin or P.P.D., in terms of deposition of circulating carbon in the vessel walls, or exudation of dye. An earlier observation of a biphasic initial phase of vascular permeability in some guinea-pigs was not confirmed.

With large doses of tuberculin (1000 I.U.), the vascular changes were similar to those with low doses, but there was permanent damage to the deeper vessels of the skin. In the guinea-pig, doses of 1 I.U. or more induced uniformly severe delayed reactions. Carbon was deposited in the superficial dermal capillaries, in many cases escaping into the adjacent connective tissue.

Monocytogenic Factor in the Delayed Hypersensitive State. Five mature offspring of two guinea-pigs sensitized to tuberculin during pregnancy were studied to determine whether the monocytogenic factor, thought by Willoughby and his colleagues to be an auto-antigen, passed the placental barrier and induced tolerance to itself in the foetus.

In comparison with tuberculin-sensitized control animals, the five offspring after similar sensitization with Freund's complete adjuvant became fully sensitized to intracutaneous tuberculin. Subsequent injection into the axillary lymph nodes of incomplete antigen containing carbon or latex particles provoked the expected blood monocytosis in the control animals from the first day. The monocytic response was absent in three of the test animals, and only transiently present on the fourth day in the other two.

Friction Injury in the Mouse. In lightly anaesthetized mice, the effect of systemic triprolidine was tested on the vascular permeability changes induced in each animal by injury in the ear and intracutaneous histamine and 5-hydroxytryptamine in the dorsal skin. There was significant inhibition of the reaction to the "stroke" injury in the ear. Earlier tests (Report 1964) had shown that the 5-hydroxytryptamine inhibitor BOL 147 partly inhibited the ear reaction when applied locally. It appears that both histamine and 5-hydroxytryptamine are liberated locally in this type of mild injury.

Standardization of Carbon Suspension. The commercial preparation used for tests of carbon deposition from the circulation on to the walls of damaged blood vessels exhibits great inter-batch variability in performance. Concentration of the carbon by centrifugation, by evaporation of the suspending fluid, or by polyethylene glycol altered the degree of dispersion of the particles. Treatment by continuous recycling in a membrane filtration apparatus yielded reliable suspensions, presumably by decreasing the proportion of soluble substances in the preparation.

Activation of Permeability-increasing Plasma Proteins. Miss Mason continued her study of the *in vitro* mechanism of the release of the polypeptide kinins, and the relation of

the kininogenetic system to the blood-clotting substance Hageman factor. She confirmed that PF/dil—an enzyme that activates the system—is distinct from the clotting factor in both human and guinea-pig systems. The possibility was examined that there are two pathways to kinin release, one in neat plasma in which the kinin-forming enzyme is directly activated by the Hageman factor, and the other in dilute plasma in which the same enzyme is mobilized indirectly by Hageman factor through activation of the permeability factor PF/dil. However, complete discharge of the kinin system in neat guinea-pig plasma was associated with loss of active or activable PF/dil. It was concluded therefore, that PF/dil is an essential intermediary in the *in vitro* discharge of the kinogenetic system. The kinin forming enzyme was the limiting factor in both human and guinea-pig systems.

Plasma and Leucocyte Kininases. The appearance and persistance of free kinin in the blood and body fluids has been reported in many pathological conditions, e.g., acute gouty arthritis, carcinoid syndrome, hereditary angioneurotic oedema and trypanosomiasis; and some features of these diseases have been attributed to the kinin. Because free kinin in normal subjects is very rapidly destroyed by the widely distributed kininases of blood and tissues, persistence of free kinin must depend either on continuous production, or on depressed activity of the kininases. Accordingly Miss Mason attempted to characterise kininases by inhibitor studies. Both guinea-pig and human serum kininases were found to be metal-activated peptidases, susceptible to sulphydryl inhibitors, heavy metal ions and oxidizing agents. As such they differ from the polymorphonuclear leucocyte kininase.

The Kininogenetic System in Infection. In the study of some protozoan infections of mammals it has been proposed that the successive associations of antibody and antigen serve as activators of the plasma kininogenetic system, and are responsible for the maintenance of quantities of kinin in the bloodstream during the course of the infection. Miss Mason investigated the state of the PF/dil-kininogenetic system in the plasma of guinea-pigs infected with a strain of *Trypanosoma brucei* . A low concentration of a vasoactive substance was present in the plasma of moderately infected animals though no free kinin was detected. However, the kininogenetic system was found on activation by a standard method to produce more kinin than normal plasma does. This condition was not due to deficiencies of the inhibitors of the activator enzymes, but it may be due to a deficiency of the kininogenase inhibitor coupled with a decrease in activity of the plasma kininases. The mobilization of such a readily activated system may be responsible for the acute infective shock seen in trypanosomiasis.

BIOCHEMISTRY

The Human Blood-Group Substances

Structural Studies. Miss Marr, Dr. Donald, Dr. Watkins and Professor Morgan continued their work (Report 1967) on the chemical characterization of two Le^b-active fragments isolated from the alkaline degradation products obtained from an HLe^b-active blood-group substance. The fragments were finally identified as (1) a tetrasaccharide, *O*-α-L-fucosyl-(1→2)-*O*-β-D-galactosyl-(1→3)-[-*O*-α-L-fucosyl-(1→4)-]-*N*-acetyl-D-glucosamine, and (2) a pentasaccharide, similar in structure to (1) but with an additional galactose residue attached by a (1→3) glycosidic linkage to the reducing *N*-acetylglucosamine unit. Earlier haemagglutination inhibition studies indicated that the Le^b determinant was composed of two α-L-fucosyl groups attached to a disaccharide composed of galactose and *N*-acetylglucosamine joined through a (1→3) glycosidic linkage. The structures found for the two Le^b-active oligosaccharides (1) and (2) therefore completely confirm the structure predicted from the serological work.

Earlier work had already established that (1→3) and (1→4) glycosidic linkages occur in the determinant structures in each of the specific blood-group substances, and more recently some evidence for (1→6) linkages was obtained. Mr. Aston, Dr. Donald and Professor Morgan isolated two new fragments from the products of acid hydrolysis

of a group H substance, and established their composition and structure as (3) a trisaccharide, *O*-β-D-galactosyl-(1→4)-*O*-(*N*-acetyl-D-glucosaminyl)-(1→6)-D-galactose and (4) a tetrasaccharide, similar in structure to (3) but with an additional *N*-acetylglucosamine at the reducing end. It can therefore now be accepted that (1→6) linkages as well as (1→3) and (1→4) linkages occur in the carbohydrate chains in the group specific substances. The identification of the *N*-acetylglucosamine unit at the reducing end of the tetrasaccharide (4) indicates that at least two molecules of glucosamine occur in a single carbohydrate chain. The recognition of this additional glucosamine unit extends our knowledge of the sugar sequences in the chains and allows a better understanding of the values found for the ratio of galactosamine to glucosamine in the intact blood-group substances. Further evidence that (1→6) glycosidic linkages occur in the group substances was obtained by hydrazinolysis and acid hydrolysis of the products when a disaccharide (5), *O*-β-(*N*-acetyl-D-glucosaminyl)-(1→6)-D-galactose was isolated.

Dr. M. Kai obtained from the acid hydrolysates of a group B-active-glycoprotein a number of disaccharides composed of fucose. These were identified by standard analytical procedures, including gas chromatography of the methylated derivatives, as 1-*O*-α-L-, 2-*O*-α-L-, 3-*O*-α-L- and 4-*O*-α-L-fucopyranosyl-L-fucose.

Hybrid Glycoprotein Molecules. Dr. Watkins and Professor Morgan showed (Report 1955) that the human blood-group alleles *A* and *B*, acting together in "secretor" persons of genotype AB, give a glycoprotein that has A and B specific structures in the same molecule, both serological characters being expressed without the formation of a new specificity. They have now brought to light another type of hybrid molecule by their work on the HLe^b specific blood-group substance. Here it appears that two genes, *H* and *Le*, act independently to elicit their own specific determinant, but because the determinant groups are in close proximity on the same carbohydrate chain, a new structural conformation is formed, which, as shown in oligosaccharides (1) and (2), is responsible for the serological character Le^b. The clarification of the structural groupings within each type of hybrid molecule illustrates clearly the diversity of the structures that can be encountered in products arising from the action of genes within the ABO, Hh, Lele (Lewis) and Secretor systems. Thus the immunochemical study of a normal variable in man, that of blood-group specificity, gives some reason for questioning the correctness of the generally held belief, "one gene, one antigen", and the dictum that a child does not have a blood-group antigen that is absent in both parents; and the results contribute to a clearer understanding, in terms of chemical structure, of individuality in man.

Investigations of the multiple specificities of blood-group active glycoprotein molecules were continued by Dr. Sachs. Earlier the use of animal and plant specific anti-A precipitating reagents gave evidence that in the secretions of group A_2 persons some macromolecules carry both A and H activities and others H only (Report 1957). Dr. Sachs has now shown that when the A substance is precipitated with monospecific anti-H serum, the precipitate contains A as well as H activity.

Production of Le^a and Le^b Antibodies. Dr. Sachs produced antisera in rabbits to artificial antigens composed of purified blood-group specific substances combined with the conjugated protein component of the O somatic antigen of the "smooth" form of *Shigella shigae*, as described earlier (Report 1943), and obtained potent haemagglutinins and precipitins for the blood-group antigens Le^a and Le^b; valuable alternatives to the human sera containing rather weak natural Le^a and Le^b antibodies, which hitherto were the only reagents available. Most naturally occurring Le^b antibody in human serum is neutralised by H substance as well as by Le^b substance and it is of interest that the rabbit anti-Le^b reagent, prepared from the rabbit antiserum after appropriate adsorption, is not neutralised by H substance and is therefore a specific anti-Le^b reagent. High titre anti-Le^a sera were also produced in the goat.

Biosynthesis of Blood-group Substances. Miss Race and Dr. Watkins continued the examination of galactosyl transferases found in homogenates of human, baboon and rabbit stomach mucosa (Report 1967), and investigated a new source of these enzymes in human submaxillary glands. They established that the α-galactosyl transferase that conveys galactose-C14 from uridine diphosphate galactose-C14 to low molecular weight carbohydrate acceptors occurs in tissues obtained from blood-group B or AB donors and is not demonstrable in tissues obtained from group A or O donors. They also confirmed that the acceptor molecule must contain a terminal non-reducing β-galactosyl residue and further established that transfer occurs only when this galactosyl residue is substituted with L-fucose. H-active structures contain terminal non-reducing β-galactosyl residues substituted with L-fucose (Report 1965), and therefore the α-galactosyl transferase found in group B donors fulfils the requirements postulated for the product of the blood-group *B*, gene; namely, an enzyme requiring H specific structures as the acceptor and nucleotide-bound galactose as the sugar donor, and catalysing the transfer of galactose from the donor to the acceptor in α-linkage.

Synthesis of radioactively labelled nucleotide-bound sugars presents many difficulties and is frequently a slow and tedious process with low yields of end-product. Uridine diphosphate *N*-acetylgalactosamine-C14 is needed for experiments on the biosynthesis of A-active structures, and for this purpose Dr. Smith prepared a mixture of uridine diphosphate *N*-acetylgalactosamine-C14 and uridine diphosphate *N*-acetylglucosamine-C14. This mixture was used to test the presence of a specific *N*-acetylgalactosaminyl transferase in the tissues of blood-group A donors (Report 1967). Interpretation of the results obtained, however, was complicated by the presence of the two labelled *N*-acetylhexosamines in the starting material. Dr. Hearn accordingly examined methods of preparing pure UDP-*N*-acetylgalactosamine-C14. Conversion of galactosamine-C14 to galactosamine-C14-1-phosphate with the enzyme galactokinase, followed by acetylation to give *N*-acetylgalactosamine-C14-1-phosphate and condensation with uridine monophosphate morpholidate yielded some uridine diphosphate *N*-acetylgalactosamine-C14. Preliminary experiments with this preparation and particulate fractions from the stomach mucosa of group A baboons indicate that the labelled sugar is transferred to the same type of L-fucose-containing compounds which act as acceptors for the α-galactosyl transferase found in group B donors.

Fucose is the immunodominant sugar in both H and Le^a specific structures and the primary products of the *H* and *Le* genes are postulated as being α-L-fucosyl transferases that catalyse the addition of L-fucose to different acceptor sugars in a precursor glycoprotein or to an intermediate that subsequently becomes incorporated into the glycoprotein. As a first step in testing this hypothesis Dr. Watkins and Mr. Chester are attempting the preparation of guanosine diphosphate fucose-C14 which is the most probable nucleotide-bound donor of L-fucose for this type of reaction.

Baboon Blood-group Substances

The serological relationships of the ABO blood group systems in man and baboons are established but no investigations on the chemistry of baboon blood-group substances have been reported. The use of tissues from these primates for biosynthesis make it important to determine how closely the baboon substances resemble the glycoproteins with corresponding blood-group activity isolated from human secretions. Miss Ward and Dr. Watkins isolated blood-group substances from mucosal stomach linings of baboons grouped either A, B or AB, according to the presence of these blood-group activities in the animals' saliva. Baboons lacking both A and B activity, that is, corresponding to the human blood group O, were found very rarely. Autolysis of the mucosal linings, followed by extraction of the freeze-dried material with cold 95 per cent. phenol, precipitation of the extracted material with 10 per cent. ethanol, and further purification by ethanol fractionation from aqueous solution, yielded A and B substances with serological activities similar to the human A and B glycoproteins isolated from ovarian cyst fluids. The

baboon preparations each had considerable H activity and very much higher Le^a and Le^b activities than those of the purified preparations from human ovarian cysts.

The baboon blood-group substances were closely similar to the human preparations in both qualitative and quantitative composition. The carbohydrate component contained the same four sugars, L-fucose, D-galactose, *N*-acetylglucosamine and *N*-acetylgalactosamine, and the amino acids serine, threonine, proline and alanine comprised more than half the total amino acid moiety (see Report 1962). Enzyme inhibition experiments established that, as in the human glycoproteins (Report 1954), the immunodominant sugars in the baboon A and B blood-group preparations are *N*-acetylgalactosamine and D-galactose respectively, and that L-fucose is the immunodominant component of the H active structures.

Precipitin titrations and double diffusion tests in agar gel revealed some difference between human and baboon preparations with rabbit antisera, prepared by injection of artificial antigen made from human A and B substances. The baboon preparations did not precipitate all the antibodies from the corresponding anti-A, anti-B or anti-H sera and in double diffusion tests gave reactions of partial identity. With rabbit anti-Le^a and -Le^b reagents (see above), reactions of identity between the human and baboon substances were obtained in double diffusion tests, and the baboon preparations precipitated as much antibody from the corresponding sera as did purified preparations of Le^a or HLe^b substances. These results indicate that the baboon blood-group substances are closely similar to the human preparations, but probably differ in the patterns of sugar residues in some of the oligosaccharide side-chains.

Phospholipids and Glycolipids

Phospholipids in Mammalian Lungs. Mr. Body and Dr. Gray continued their studies of the lipid compositions of the surface-active lipoprotein and of whole lung tissue. A minor component isolated from rabbit lungs (Report 1967) was identified as a semi-lyso-*bis*-phosphatidic acid. The structure and fatty acid composition of this compound and of lyso-*bis*-phosphatidic acid and phosphatidylglycerol, also present in lung tissue, suggests a close biological relationship. It is possible that these phospholipids are metabolically "highly active" and are acting as carriers for the palmitic acid required in the biosynthesis of dipalmityl lecithin, the surface-active component of lung lipoprotein.

A survey showed that though phosphatidylglycerol is common to many mammalian tissues, the two phosphatidic acid derivatives occur predominantly in lung tissue. The origin of these two compounds is obscure. Both are derived from *bis*-phosphatidic acid, but this compound has not been detected in mammalian tissues though it has been chemically synthesised.

Lipases and Mammalian Cell Membranes. The prime object of the present studies is to obtain information about the relative importance of the different phospholipid and glycolipid components of the plasma membrane of a mammalian cell, in relation to membrane stability and structural integrity.

Changes in the permeability of the membranes of intact rat liver cells to the cytoplasmic enzymes glutamic oxaloacetic transaminase (GOT) and malic dehydrogenase (MDH) occur as a result of the action of phospholipase A on the membrane phospholipids. Phospholipase A preparations from different snake and bee venoms differ in their action towards phosphadidylethanolamine and lecithin, and Dr. Jennifer Wells compared the actions of some of them on intact rat liver cells. For all of those tested, the curves for the percentage release of either GOT or MDH plotted against the degree of hydrolysis of phosphatidylethanolamine were the same, even though the amounts of lecithin hydrolysed varied from 4 to 40 per cent. of the total cell lecithin. This suggests that, in terms of the permeability of the plasma membrane to protein molecules, intact phosphatidylethanolamine is more important than intact lecithin in maintaining membrane integrity.

The molecular sizes of phospholipase A (from snake venom) and GOT were compared by passage through a column of Sephadex G200, which showed that phos-

pholipase A was considerably smaller than GOT. This means that at a certain stage of membrane permeability, some phospholipase A can enter the cell before GOT can leak out. Since the phospholipid hydrolysed is measurable only in terms of the total phospholipid of the cell and not membrane phospholipid, the assumption that the phospholipids which are hydrolysed in the early stages are only in the plasma membrane is not entirely justified.

Dr. Gray studied the action of phospholipase C (from *Cl. welchii*) on the plasma membranes of intact rat-liver cells. Compared with phospholipase A this enzyme releases GOT more quickly. For example, 20 per cent. of the total GOT is released when 5 per cent. of the total phosphatidylethanolamine is hydrolysed, whereas with phospholipase A the release of GOT is negligible for the same amount of lipid hydrolysed. Thus the removal of a strongly charged group—the phosphorylated base—from a phospholipid molecule in the plasma membrane appears to cause a greater degree of molecular disorganisation than does the degradation of the same molecule to a lysophospholipid and a free fatty acid.

The usefulness of a detailed analysis of the lipid composition of a plasma membrane preparation is dependent on the purity of the preparation. Electron micrographs give some indication of freedom from contamination, but the activity of specific enzymes in the preparation provides a more reliable criterion. Miss Dod confirmed that her membrane preparations were essentially plasma membrane by measuring these activities. She also analysed an alkali-stable lipid fraction from the plasma membranes of rat liver cells and identified ceramide mono-, di- and tri-hexoside, and a small ganglioside. These glycolipids were not present in a similar fraction isolated from the cell mitochondrial membranes. This is the first time that neutral glycolipids have been localised with certainty in a mammalian cell. Their concentration in the membrane is at least sevenfold that in the whole cell and it may be that they are exclusive to the plasma membrane. Preparations of rough and smooth microsomal fractions are being examined to see whether this is so.

Glycolipids. Mr. Hay continued to work on the partial synthesis of some of the naturally occurring glycolipids and has synthesised *O*-β-D-galacotsyl-(1→4)-*O*-β-D-galactosyl-(1→1)-ceramide. The digalactosyl ceramide is of special interest in relation to glycolipid metabolism and biosynthesis, since in certain instances (e.g., kidney glycolipids in Fabry's disease) it completely replaces the lactosyl ceramide that is common to most normal mammalian tissues. Both of the naturally occurring ceramide dihexosides have now been synthesised but, unfortunately, their synthesis represents the limit of the present methods and the synthesis of the more complex trihexosides and aminoglycolipids is not yet feasible.

A BP8 ascites tumour in a C3H mouse induces a change in the composition of the glycolipids of the mouse kidneys (Report 1967). To determine whether strain-specific ascites tumours in general produce this effect in the different strains of mouse and in other organs as well, Mr. Hay examined the glycolipids of lungs from BP8/C3H and normal C3H mice and found that, in contrast to the kidneys, the compositions were similar. A comparative examination of the glycolipids of the spleens and livers of the C3H mice will be made. The metabolism and biosynthesis of the kidney glycolipids are also being studied.

BIOPHYSICS

Human Plasma Proteins

Structure of Human αmacroglobulin. Studies to elucidate the sub-unit structure of α macroglobulin were continued by Professor Kekwick and Dr. Jones. The dissociation of the intact molecule (sedimentation coefficient 19S) in the pH range 4.5–2.5 giving rise to 12S sub-units in increasing proportion with lowering of the pH, was examined. At 4°, although the components are stable at any pH between 4.5 and 2.5, the dissociation is not reversible when the pH is raised relatively quickly by dialysing, either against buffers whose pH lies within the dissociating range or from within this range to pH 6.0. In these circumstances large amounts of polydisperse aggregates form at the expense of both the 12S and

remaining 19S components. At 25° the system is unstable at pH 4.5–2.5 and aggregates form during the course of dialysis. The isolation of the 12S component, which could well be half the size of the native molecule, is therefore difficult, since all operations have to be made at 4° with rigid stabilization of pH.

Chelating agents do not appear to promote dissociation of α macroglobulin in neutral solutions.

Mild treatment with thiol reagents preferentially cleaves interchain disulphide bonds of many proteins, with the liberation of large constituent sub-units. The effect of three thiol reagents, mercaptoethanol, mercaptoethylamine and N-acetyl cysteine on solutions of α macroglobulin at pH 6.0 and 8.0 was tested. The three are respectively uncharged, positively and negatively charged substances, and for this reason their accessibility to disulphide bonds in the molecule might differ. Further at pH 6.0 the histidine residues in the protein molecule would predominantly be ionised carrying a positive charge, and at pH 8.0 unionised and uncharged, so that pH provides another variable which might influence the cleavage of the molecule.

Thiol treatment yields up to four components by sedimentation velocity analysis, with sedimentation coefficients of about 19, 15, 12 and 8.5S, and bands of increasing order of mobility corresponding to each of these were detected by electrophoresis in polyacrylamide gel. The proportions of the components depend on the nature and concentration of thiol reagent, the pH and, to some extent, duration of treatment. For example, after treatment with 0.01M N-acetyl cysteine at pH 6.0 there are three sedimenting components (19, 12, 8.5S), whereas at pH 8.0 there are two only (19 and 8.5S), although traces corresponding to the 15 and 12S components are detectable by the gel electrophoresis. So far the original 19S molecule has not been completely cleaved. Work on the isolation of the sub-units continues.

If after electrophoresis in polyacrylamide, the cylindrical gels are split longitudinally, one half may be stained with dye to locate the protein bands and the other developed by applying antiserum. With a rabbit antiserum to α macroglobulin, the products obtained by cleavage with thiol reagents all proved to have retained the antigenic specificity of the native molecule.

This procedure was also applied advantageously by Dr. Jones to the constituents of normal human serum and, in collaboration with Dr. Lemcke, to the antigens present in the soluble cellular material from *Mycoplasma hominis*.

Reversible Denaturation of Proteins

Studies of the reversibility of denaturation of proteins provide one of the few methods for testing the thermodynamic theory for the origin of the secondary and tertiary structure of proteins (see Report 1967). If the specific structure under physiological conditions represents a free energy minimum, then it should be attainable from any other state, including the fully-denatured random-coil configuration, provided irreversible conditions are avoided and kinetic barriers are not prohibitive. The correctness of the hypothesis is now adequately demonstrated for several fairly short-chain proteins; for those with 200 or more residues, the possibility that folding initiated during synthesis from the amino-end influences the final conformation has recently received some support. Such proteins would not completely regain their native structure on removal of denaturant.

Two approaches to this problem are being investigated. Dr. Creeth and Mr. Holt continued their work on the reversibility of denaturation of ovalbumin by sodium dodecyl sulphate. Because the disulphide bridge structure is retained, only partial denaturation can occur and the system is in principle simple. The extent of denaturation was determined by measurements of the sedimentation coefficient, reduced viscosity and optical rotatory dispersion constants of the product. The first two qualities are sensitive to molecular size and shape and the third to intramolecular organisation; all change in a way characteristic of expansion and loss of secondary and tertiary structure. Steady state ultracentrifuge experiments showed that the protein-detergent system is in rapidly reversible equilibrium; it follows

that this method cannot be used for characterising the extent of polydispersity of the fully-denatured material, though it may be useful at a later stage.

The detergent was removed reversibly; a very small amount of detergent must be allowed to remain if a stable product is to be obtained. This partially-renatured material, containing about 3 detergent molecules/molecule protein, has essentially the same molecular weight as the native protein, and in several other respects has at least partially recovered the native characteristics. Interest in this modified ovalbumin lies particularly in the location of the small number of detergent molecules; these are most likely to be bound by the hydrophobic regions, perhaps including the aromatic residies. Accordingly, ultraviolet absorption difference spectra for the fully-denatured and partially-renatured products are at present under study.

The aggregation occurring when the detergent has a molecular proportion less than 3 : 1 makes it impossible to compare the fully-renatured and native proteins. The aggregation was not due to a sulphydryl-disulphide exchange mechanism, to traces of heavy metals or to weak intermolecular hydrophobic bonds of the kind stabilising intramolecularly the native structure; it appears to be due to an intermolecular micelle formation, which is probably unavoidable since it is a consequence of those properties of detergents that make them useful denaturing agents. The investigation has defined, in part, the limits of applicability of detergents; it continues, with urea or guanidinium chloride as denaturants having a different mode of action.

In many proteins, the native conformation is stabilized by disulphide bonds as well as by the weak intramolecular hydrogen bonds, hydrophobic bonds and electrostatic interactions that are broken by denaturing agents. Nevertheless, the specific disulphide bridge structure may arise as a consequence of the requirement that the total conformational free energy be minimised; accordingly a protein rich in disulphide should, after reduction and slow reversible reoxidation, revert to its native form. Serum albumin has 17 disulphide bonds in a molecule of some 600 residues; the possibilities of random reformation are therefore immense, and this protein presents a critical test-case for the theory. Dr. Sawyer and Dr. Creeth began an investigation on these lines, using human and bovine serum albumin. When the disulphide bridge structure was left intact, denaturation with 6M guanidinium chloride followed by slow reversible renaturation gave a product containing an appreciable proportion of dimer, but consisting mainly of a protein whose sedimentation coefficient and intrinsic viscosity were the same as those of the native protein. The dimer proved to be formed by sulphydryl-disulphide exchange; recovery of monomer from the denatured and fully-reduced condition was therefore likely to be possible only at low protein concentrations. Protein denatured after treatment with concentrated mercaptoethanol to break all the disulphide bonds, was added slowly to a large volume of buffer, producing a low concentration of denaturant. Dialysis and concentration in the presence of a low concentration of mercaptoethanol—which acts as a redox buffer allowing the sorting-out of the disulphide bond structure by a constant re-shuffling of unfavourable pairs—yielded a partially renatured sample with a major component possessing the sedimentation coefficient of the original molecule, and a range of polydisperse aggregates, which are not the products of disulphide interchange, and may be removed by gel filtration. It therefore appears that the procedure is capable of producing a monomeric protein in high yield which is sufficiently similar to the original to warrant the most detailed characterisation.

BLOOD PRODUCTS LABORATORY

Anti-D Immunoglobulin. Confirmatory evidence of the efficacy of anti-D immunoglobulin in preventing the iso-immunization of Rh-negative women during pregnancy is accumulating from the outcome of second Rh-positive pregnancies in treated Rh-negative mothers (Report 1967). Throughout the year the staff of the Laboratory continued to collaborate with the Department of Medicine, Liverpool University and with the M.R.C. Working Party on the use of anti-D immunoglobulin for the

prevention of iso-immunization of women during pregnancy. Mr. Wesley prepared the four special batches of anti-D immunoglobulin for the dosage trial being conducted by the M.R.C. Working Party. He also made the anti-D immunoglobulin required for the introduction of this form of treatment, at first on a limited scale, in the National Health Service early in 1968.

Hypogammaglobulinaemia. The Laboratory continued to take an active part in the treatment trial conducted by the M.R.C. Working Party on Hypogammaglobulinaemia. During the trial, which has now lasted nearly 12 years, a number of anaphylactoid reactions have followed the injection of immunoglobulin. Reactions of this kind were attributed to the anticomplementary action of aggregates in solutions of immunoglobulin. During the year Mr. Vallet examined many of the batches of immunoglobulin (G3) by molecular sieve chromatography (Sephadex 200). Up to 5 components were obtained. The first to leave the column, an excluded fraction containing large aggregates, was also present, but in variable amounts. The second and third components contained 10S and 7S immunoglobulin and the fourth albumin, known to be present in G3 preparations. In older batches, the fourth component contained an increasing proportion of fragmented protein. The fifth component was present only in batches 5 or more years old, a good indication of the stability of the material.

Miss Mackay investigated the anticomplementary activity of the batches. There was no association between the degree of anticomplementary activity, the amount of aggregated material and the reactions noted in the clinical use of these batches. The anticomplementary activity varied widely, and in batches with low activity, traces of plasmin were generally present and the IgG immunophoretic line was split. Skarvil in 1960 reported that this latter phenomenon was an effect of enzymic proteolysis. However, Miss Mackay observed splitting of the line in immunoglobulin preparations free of plasmin when they were subjected to accelerated ageing by incubation at 37° for 28 days.

The work of Miss Mackay and Mr. Vallet established that no significant changes occurred in immunoglobulin preparations during storage at 4°C. for the normal "life" of 4 years.

Plasma Protein Fraction (PPF). Mr. Vallet and Mr. Wesley continued their pilot scale studies (Report 1965). Clinical trials are in progress. Mr. Vallet investigated the possibility of using molecular sieve chromatography (Sephadex G 150) as a method of controlling both PPF and albumin solutions intended for clinical use. The clear separation of aggregates and polymers obtainable makes it a method of choice.

Plasma Fractionation Laboratory

In 1967 a comprehensive Haemophilia Centre was established at Churchill Hospital, Oxford, in succession to the M.R.C. Coagulation Research Laboratory. It comprises a clinical section, a coagulation research laboratory and a plasma fractionation laboratory. After discussions with the Ministry of Health, the Medical Research Council and the Board of Governors of the United Oxford Hospitals, the Institute undertook to administer the last laboratory in conjunction with the Blood Products Laboratory because of the similarity of the two laboratories' work and the mutual benefits of a close association. The Institute assumed administrative responsibility on 1st August, 1967.

The work of the Plasma Fractionation Laboratory, of which Dr. Ethel Bidwell is in charge, will mainly be concerned with the separation and purification of coagulation factors for clinical use, at first particularly with Factors VIII and IX. Although since August 1967 Dr. Bidwell was largely occupied with problems arising from the building of new accommodation for the Plasma Fractionation Laboratory, which will be occupied in June 1968, she continued work on a pilot scale directed towards selecting the best conditions for the separation of anti-haemophilic globulin.

THE BLOOD GROUP RESEARCH UNIT

The search for new blood groups and the application to human genetics of those

already known continues to be the congenial occupation of the Unit.

The X Chromosome

Attempts to improve the gene map of the X and the investigation of people with abnormalities of number (aneuploidy), or form, of the X chromosomes by means of the X-linked blood group Xg go on with all the more confidence as a result of the finding, at Hyland Laboratories, Los Angeles and at the New York Blood Center, of two more fine examples of anti-Xg^a.

X-mapping. In the last Report (1967) the distance between the locus for X-linked ichthyosis and that for Xg was estimated to be about 20 recombination units. The large survey in Israel, in collaboration with Dr. A. Adam, Dr. L. Ziprkowski and Dr. A. Feinstein of Tel-Hashomer, is showing the distance between the two loci to be less than 20 units, and the present estimate is 11 units.

Since the last report Fialkow, Giblett and Motulsky have established that Xg is within measurable distance of the locus for the rare condition ocular albinism and the present estimate of the distance between the two loci is about 18 recombination units. If we are right in thinking that Xg is near the tip of the short arm then ichthyosis and ocular albinism should be on the same side of Xg and fairly close together. Unfortunately the coincidence of ichthyosis and ocular albinism in one family must be too rare an event to allow any hope of a direct measure of the relationship of the two loci.

There are now two known clusters of loci on the X, Xg, ichthyosis and ocular albinism belonging to one and colour blindness, glucose-6-phosphate dehydrogenase (g-6-pd) and haemophilia to the other. The two clusters have yet to be linked by an intervening locus: there were hopes (Report 1967) that ichthyosis might do this by being within measurable distance of g-6-pd but work with colleagues in Israel and Sardinia is showing that the distance is probably beyond measure.

During the last year there were hints, sufficient to encourage further investigation, that Xg may prove within measurable distance of the loci for nuclear cataract, for retinoschisis and for Åland Island eye disease. Angiokeratoma still remains a possible linkage with Xg but owing to its extreme rarity the proof or disproof may take years.

X-linked conditions recently grouped for the first time include choroideremia, retinitis pigmentosa, mental retardation with hyperuricaemia, low thyroxine-binding globulin, oligodontia and dysphagocytosis. In all, 45 X-linked conditions have been involved in the attempt to improve the map of the X chromosome by means of the Xg groups.

Testicular feminization is a rare inherited condition in which those affected appear externally to be females of, it is said, more than average beauty but whose gonads are testes and whose karyotypes are XY. The condition is rare: one estimate is that it occurs once in 75,000 males. The problem of the inheritance was outlined in the Report of 1966. The total number of propositi now grouped in the Unit is 50, together with nearly two hundred of their relatives. The previous conclusions are consolidated: if the locus for testicular feminization be X-borne, its distance from Xg is too great to be measured. On the other hand there is yet no hint of linkage with any of the autosomal blood groups. So the problem rests where it did. It is of interest that these apparent females have, as expected, the male distribution of the Xg groups.

X-chromosome Aneuploidy. Samples of blood from patients with abnormal X chromosomes or the wrong number of X chromosomes continue to be sent for Xg grouping. Nothing startling has happened since the last report (1967) but the gradually mounting figures will soon call for further computer analyses.

The Autosomes

The testing of many samples of blood suspected to contain something "new" continues. The best of the year's catch came from Helsinki.

Karhula. The serum of a Finnish recipient of many transfusions was found by Dr. H. Nevanlinna, Dr. U. Furuhjelm and Miss

Riitta Nurkka to contain an antibody to an apparently "new" antigen, and the Unit was kindly invited to take part in the subsequent genetical investigation. Tests on about 350 members of the families of 18 propositi showed the antigen to be, as expected, a dominant character: segregation in the families showed it not to be a previously unrecognized member of the ABO, MNSs, P, Rh, Lutheran, Duffy, Kidd or Dombrock systems nor to be X- or Y-linked. The antigen, which is called Ul^a, thus probably defines a new blood group system though it has yet to be shown to be genetically independent of Kell, Yt and Diego. During the family tests hopes for a time ran high that the *Ul* locus would prove to be within measurable linkage distance of the *ABO* locus but this is now very much in the balance.

Dr. Nevanlinna and his colleagues found 2.6 per cent. of 2620 Helsinki donors to be Ul(a+), though the incidence was higher in certain Finnish isolates. Only one Ul(a+) was found in 500 Swedish people and none in 140 Lapps, 300 British and 50 Negroes.

Rh_{null} and MNSs. Though Rh and MNSs are known to be genetically independent, the surprising observation was made in the U.S. that samples of blood of the Rh_{null} type (having no CDE antigens—Report 1961) may give abnormal reactions with antisera of the MNSs system. Work in the Unit is showing that only when an antiglobulin test is involved are the reactions of the MNSs antisera peculiar. The significance of this observation is not yet clear.

Lutheran and Auberger. The rare Lutheran phenotype Lu(a-b-) is the only "minus-minus" blood group phenotype known to be a dominant character (Report 1961). Dr. Tippett observed an association between this phenotype and the Auberger groups (Report 1964) and four subsequent families have completely established the association. There is now emerging an even rarer recessive form of Lu(a-b-) which, from the very limited data of Dr. Tippett, is not related to the Auberger groups—at least not in the same way as the dominant form.

Work on the Auberger group (Report 1962) is much handicapped by extreme shortage of the antiserum because the only known maker of the antibody died some years ago: even the primary question, whether the Lutheran and Auberger antigens are controlled by the same locus, has yet to be answered; the present guess is that they are genetically unrelated but require a mutual precursor substance. Eyes are strained for a second example of anti-Au^a.

The Kell System. Miss Mia van der Hart and Professor van Loghem kindly invited the Unit to share in the investigation of a "new" antibody found in Amsterdam. The antibody belongs to the versatile Kell system and is provisionally called anti-KL: its reactions can most easily be understood in terms of the "genetical pathway" model brilliantly worked out some years ago for the ABO, secretor and Lewis systems by Watkins and Morgan, a model which was so aptly to accommodate later observations in the Rh and P systems.

Very Rare and Very Common Antigens. Miss Gavin continues the work of sorting out these many antigens of which the very common ones can present such acute transfusion problems (Report 1967). In collaboration with the Kings County Medical Center and National Bio Serums, both of Brooklyn, a new very frequent antigen was well taped during the year. It is called At^a and is the sixth such very frequent antigen to be defined which may not belong to any of the established systems—it is known not to belong to MNSs, Rh, Kidd or Xg.

Some of the many physicians, cytogeneticists and blood bank technicians who sent the samples on which the work of the Unit depends were thanked in the last report. A list this year would contain practically the same names.

The Unit had much help from the Regional Blood Transfusion Centres; the Blood Group Reference Laboratory; Spectra Biologicals, East Brunswick; Hyland Laboratories, Los Angeles; Laboratorios Grifols, Barcelona; Laboratorios Knickerbocker, Barcelona; Pfizer Diagnostics, New York and the Minneapolis War Memorial Blood Bank.

Once again the Unit thanks the Staff of the Institute for giving so many samples of their blood.

BLOOD GROUP REFERENCE LABORATORY

The Unit is the central reference laboratory for the investigation of blood grouping problems and for the supply of grouping serum for the United Kingdom. In addition, it acts on behalf of the World Health Organization as the world reference centre. The output of grouping serum rises year by year, most of it being supplied in liquid form for use in the United Kingdom but some is freeze-dried for use overseas.

Today more and more people receive multiple blood transfusions and for this reason the number of patients whose serum contains a complex mixture of blood group antibodies is increasing. It can sometimes be difficult to find compatible blood for such people, but Britain is fortunate in this respect for there is available to the National Blood Transfusion Service a list of some fifteen hundred donors whose blood has been very fully typed and who form the National Panel of Donors of Rare Blood Types. Reference to this Panel enables one to locate donors whose blood is suitable to be given to patients sensitized by previous transfusion. It is a duty of the Blood Group Reference Laboratory to maintain this Panel and during 1967 active steps were taken to revise it by removing the names of certain donors whose blood types were relatively common and by adding to it the names of others whose groups were of great rarity.

Encouragement is given in this Unit to transfusion centres overseas to start their own Panels of Donors of Rare Blood Types. Furthermore, with help and advice from Dr. A. E. Mourant, an International Panel of Donors of Rare Blood Types has recently been formed and it contains the names of donors whose blood groups are so rare that too few may be found in any one country. The Panel will be maintained by the Blood Group Reference Laboratory and full details of all donors will be distributed to National Blood Group Reference Laboratories throughout the world. In this way it will be possible to make blood of donors of rare blood types available wherever it may be needed.

An alternative approach to the problem of having enough blood of rare types available when needed is to stockpile it by storing it for years on end at temperatures well below freezing point. The techniques involved are still somewhat experimental and during 1967 the Blood Group Reference Laboratory has been assisting in British research in this field.

During 1967, members of the staff have continued their own researches in various branches of blood group serology. Dr. Ikin continues to interest herself in new developments in the MN system and with Miss Giles investigated a previously undescribed blood-group antibody, anti-Sd^a. Miss Giles examined serum samples for antibodies to low-frequency red cell antigens and produced new information on the Yt^b and Cs^a antigens, both of which she had earlier discovered. Mrs. Nunn studied methods for enhancing Lewis antisera used for cell-typing and also reported on a problem which involved the Ge antigen. Dr. Phillips, who had earlier performed research on the production of fluorescein-conjugated anti-globulin serum, succeeded in making sufficient fluorescein-conjugated anti-gamma-G to supply those who needed it in Britain. Subsequently she looked into the possibility of making fluorescein-conjugated anti-gamma-M. She was helped considerably by Professor Kekwick who also co-operated with Dr. Goldsmith in the production of various antiglobulin reagents. Dr. Goldsmith interested himself especially in the production of potent and specific anti-human-complement reagents as well as, with the help of Miss Brazier, in the study of leucocyte and platelet antibodies.

Finally, the Governing Body would like to praise the scientific, administrative and technical staffs 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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THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Accounts 1968

Accounts
1968

THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Balance Sheet and Accounts

31 DECEMBER 1968

CHELSEA BRIDGE ROAD . LONDON, S.W.1 . 22 MAY, 1969

The Governing Body

Sir LINDOR BROWN, CBE, FRCP, FRS, *Chairman*
H. P. G. CHANNON, MP, *Hon. Treasurer*
The Rt Hon LORD BROCK, MS, FRCS
Professor D. G. EVANS, D SC, FRS
The Rt Hon the EARL OF IVEAGH
Professor R. A. KEKWICK, D SC, FRS
Professor Sir ASHLEY MILES, CBE, MD, D SC (*hc*), FRCP, FRS
Professor A. NEUBERGER, CBE, MD, FRCP, FRS

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

Financial Report of the Governing Body

1. The Governing Body presents herewith the accounts of the Institute for the year ended 31st December 1968.

2. **Results**

The General Fund Income and Expenditure Account shows the Income for the year as £242,111 compared with £236,374 in 1967. Expenditure amounted to £389,904 against £342,630 last year. The deficit for the year is £147,793 compared with a deficit of £106,256 in 1967. The capital fund has been reduced by the year's deficit of £147,793.

3. **Principal Activities of the Institute**

The Institute continued to carry out research work in connection with the prevention of diseases. It produces for sale sera, vaccines, virus vaccines, the profits from which are utilised for its research and experimental work.

4. **Exports**

Sera and vaccines to the value of £107,284 were exported from the United Kingdom during the year.

5. **Changes in Fixed Assets**

The movements in fixed assets during the year are set out in the table in note 1 on the accounts.

6. **Market Value of Interests in Land**

The market value of the Institute's properties is now in excess of the amount at which they are included in the Balance Sheet. In the opinion of the Governing Body such difference is of no significance as the properties are occupied for the purposes of the Institute's activities.

7. **Governing Body**

In June 1968 Sir Charles Dodds resigned; Professor Collier completed his term of office; and Lord Iveagh and Professor R. A. Kekwick joined the Governing Body. On 15th October 1968 Professor A. Neuberger also joined the Governing body. Other members who held office during the year ended 31st December 1968 were as shown on page 2.

8. **Average Number of Employees and their Remuneration**

The average number of persons employed by the Institute in each week during the year ended 31st December 1968 was 310. The aggregate remuneration paid or payable in respect of that year to these employees amounted to £334,396.

9. **Auditors**

The auditors, Cooper Brothers & Co., will continue in office in accordance with Section 159 (2) of the Companies Act, 1948.

G. L. BROWN

Chairman

The Lister Institute of Preventive Medicine
BALANCE SHEET · 31 December 1968

1967			
£		£	£
121,997	FIXED ASSETS (*note 1*)		174,714
	INVESTMENTS AND UNINVESTED CASH (*note 2*)		
661,849	General Fund	608,244	
232,292	Specific funds	204,321	
25,508	Bequest funds	25,109	
919,649			837,674
	CURRENT ASSETS		
137,799	Debtors	93,950	
30,333	Cash and Bank Balances	57,232	
168,132		151,182	
	Less: CURRENT LIABILITIES		
50,221	Creditors	65,105	
117,911			86,077
£1,159,557			£1,098,465
	Represented by		
720,519	CAPITAL FUND (*note 4*)		572,726
232,292	SPECIFIC FUNDS (*note 5*)		204,321
25,508	BEQUEST FUNDS (*note 6*)		25,109
10,362	SPECIFIC GRANTS AND LEGACIES UNEXPENDED (*note 7*)		7,039
170,876	INVESTMENT RESERVE (*note 8*)		289,270
£1,159,557	G. L. BROWN, H. P. G. CHANNON *Members of the Governing Body*		£1,098,465

The notes on pages 6 to 9 form part of these accounts.
Audit report on page 9

The Lister Institute of Preventive Medicine
INCOME AND EXPENDITURE ACCOUNT
for the year ended 31 December, 1968

1967				
£		£	£	£
	INCOME			
167,528	Sales of sera, vaccines, virus vaccines (*note 9*)			181,929
	Investment Income:			
	General fund			
50,201	Quoted	40,687		
2,405	Unquoted	2,804		
			43,491	
7,655	Sinking fund—quoted		8,268	
				51,759
400	Underwriting commission (less income tax £124)			378
8,185	Rent			8,045
236,374				242,111
		Total expenditure	*External contributions*	
	EXPENDITURE			
191,843	Salaries and wages	340,787	134,137	206,650
10,029	Premiums on federated superannuation policies	18,248	5,442	12,806
3,064	Premiums on group pension policy	5,474	914	4,560
8,798	Rent, rates and insurance	9,458	—	9,458
18,287	Gas, water, fuel and electricity	24,856	5,183	19,673
5,440	Office expenses, stationery and printing	9,032	1,072	7,960
630	Audit fee	630	—	630
3,137	Travelling expenses	4,559	1,150	3,409
2,058	Biochemistry expenses	7,805	5,189	2,616
3,343	Microbiology, immunology, experimental pathology and electron microscopy expenses	13,715	9,793	3,922
1,418	Biophysics expenses	4,514	2,992	1,522
70	Virology expenses	4,013	2,378	1,635
—	Blood products laboratory expenses	10,940	10,940	—
35,043	Serum vaccine and virus vaccine expenses	53,614	159	53,455
8,676	Animals	12,220	647	11,573
10,009	Animal house expenses and forage	14,498	1,192	13,306
1,635	General apparatus and new installations	1,489	—	1,489
3,114	Library expenses	3,319	225	3,094
1,020	General stores	945	—	945
3,756	Staff canteen loss	4,375	641	3,734
18,862	Buildings, alterations, repairs and renewals	15,922	291	15,631
	Depreciation			
2,919	Buildings	2,168	—	2,168
1,400	Furniture fittings, scientific apparatus and books	1,400	—	1,400
334,551		£563,981	£182,345	381,636
98,177	Excess of expenditure over income			139,525
8,079	Amount transferred to sinking fund for freehold buildings			8,268
£106,256	Deficit transferred to capital fund			£147,793

The notes on pages 6 to 9 form part of these accounts.

Audit report—page 9

NOTES ON THE ACCOUNTS · 31 December 1968

1. FIXED ASSETS

	Freehold property		Furniture, fittings, scientific apparatus and books	Total
	Land and buildings Chelsea	Queensbury Lodge Estate, Elstree		
	£	£	£	£
Cost				
At 1st January 1968	73,548	53,188	9,545	136,281
Additions at cost	42,235	8,972	5,078	56,285
At 31st December 1968	£115,783	£62,160	£14,623	£192,566
Depreciation				
At 1st January 1968	—	8,611	5,673	14,284
Charged to income and expenditure account	1,350	818	1,400	3,568
At 31st December 1968	£1,350	£9,429	£7,073	£17,852
Net book value at 31st December 1968	£114,433	£52,731	£7,550	£174,714

Depreciation

Freehold property additions and replacements since 1912 at Elstree and since 1935 at Chelsea until 31st December 1964 have been charged to revenue. Additions since that date until 31st December 1967 have been depreciated at the rate of 10%. Since 1st January 1968 buildings shown in the balance sheet have been depreciated at the rate of 2% on a straight line basis.

Additions and replacements to furniture, fittings, scientific apparatus and books between 31st December 1920 and 31st December 1963 have been charged to revenue. The additions since 1st January 1964 have been depreciated on a straight line basis by reference to the useful lives of the assets.

2. INVESTMENTS AND UNINVESTED CASH

	£	£	£	£	£
	Quoted at cost		Unquoted at cost	Uninvested cash	Total
	In Great Britain	Elsewhere			
General	380,715	164,542	62,987	—	608,244
Specific					
Sinking fund for freehold buildings	146,389	—	—	952	147,341
Pension fund	27,827	—	—	968	28,795
Re-endowment fund	24,566	—	—	3,619	28,185
Bequest					
Jenner Memorial studentship fund	13,838	—	940	3,234	18,012
Morna Macleod scholarship fund	6,631	—	—	466	7,097
	£599,966	£164,542	£63,927	£9,239	£837,674
1967	£741,946	£122,766	£41,227	£13,710	£919,649

Market value of quoted investments 1968 £1,579,169 (1967 £1,356,020)

Unquoted investments valued by Institute's investment advisers 1968 £59,014 (1967 £35,457)

3. STOCK

Stocks of sera, virus vaccines and horses on hand at 31st December, 1968 have not been valued in the accounts

4. CAPITAL FUND

Donations etc. have been received to date from the following:—		£
Dr. Ludwig Mond (1893)		2,000
Berridge Trustees (1893–1898)		46,380
Worshipful Company of Grocers (1894)		10,000
Lord Iveagh (1900)		250,000
Lord Lister's Bequest (1913–1923)		18,904
William Henry Clarke Bequest (1923–1926)		7,114
Rockefeller Foundation (1935–1936)		3,400
Other donations and legacies (1891–1954)		22,669
General Fund Income and Expenditure Account		
Accumulated surplus as at 31st December 1967	360,052	
Less: Deficit 1968	147,793	
		212,259
		£572,726

5. SPECIFIC FUNDS

	£	£	£
Sinking Fund for Freehold Buildings			
As at 1st January 1968		171,955	
Amounts transferred from income and expenditure account		8,268	
		180,223	
Less: Losses on realisation of investment	2,691		
Expenditure on reablement of buildings	30,191		
		32,882	
			147,341
Pension Fund			
As at 1st January 1968		30,005	
Interest on investments		1,894	
Profit on realisation of investment		24	
		31,923	
Less: Pensions		3,128	
			28,795
Re-endowment Fund			
As at 1st January 1968		30,332	
Donations		616	
		30,948	
Less: Loss on realisation of investments		2,763	
			28,185
			£204,321

6. BEQUEST FUNDS

	£	£	£
Jenner Memorial Studentship Fund			
As at 1st January 1968		18,276	
Interest on investments		827	
		19,103	
Less: Stipend	509		
Loss on realisation of investment	582		
		1,091	
			18,012
Morna Macleod Scholarship Fund			
As at 1st January 1968		7,232	
Interest on investments		420	
		7,652	
Less: Stipend of scholar		555	
			7,097
			£25,109

7. SPECIFIC GRANTS AND LEGACIES

	£	£	£
Nuffield Foundation Grants			
As at 1st January 1968			1,583
Guinness-Lister Research Grant			
As at 1st January 1968		8,779	
Amounts received		15,000	
		23,779	
Less: Salaries and wages	14,371		
Laboratory expenses	3,952		
		18,323	
			5,456
			£7,039

8. GENERAL FUND INVESTMENT RESERVE

	£
As at 1st January 1968	170,876
Add: Profits on sales of investments	118,394
	£289,270

9. TURNOVER

Turnover has been arrived at after deducting commission due to agents from the invoice value of sales of sera, vaccines and virus vaccines.

10. Emoluments of Members of the Governing Body

	1968	1967
Emoluments of three members of the Governing Body in an executive capacity	£16,043	£13,850

Particulars of emoluments of the governing body in accordance with Section 6 of the Companies Act 1967

	1968	1967
Emoluments of the Chairman of the governing body	Nil	Nil
Emoluments of the highest paid member of the governing body	£7,309	£6,745
Numbers of members of the governing body whose emoluments were within the range		
No emoluments	6	6
£1 — £2,500	2	—
£2,501 — £5,000	—	1
£5,001 — £7,500	1	1

11. Capital Expenditure Schemes

The position at 31st December 1968 was as follows:—	1968	1967
Commitments in respect of contracts	23,943	9,302
Approved by the Governing Body in addition to commitments	67,000	70,000
	£90,943	£79,302

12. Contingent Liabilities

At 31st December 1968 there were contingent liabilities amounting to £34,719 in respect of indemnities issued to third parties.

Report of the Auditors to the Members

In our opinion the accounts set out on pages 4 to 9 give a true and fair view of the state of the company's affairs at 31st December 1968 and of its results for the year ended on that date and comply with the Companies Acts 1948 and 1967.

COOPER BROTHERS & CO.
Chartered Accountants.

London, 22 May 1969

THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Report
of the GOVERNING BODY
1969

CHELSEA BRIDGE ROAD : LONDON : SW1

The Governing Body

Professor Sir LINDOR BROWN, CBE, FRCP, FRS, *Chairman*
H. P. G. CHANNON, MP, *Hon. Treasurer*
The Rt Hon LORD BROCK, MS, FRCS
Professor D. G. EVANS, D SC, FRS
The Rt Hon the EARL OF IVEAGH
Professor R. A. KEKWICK, D SC, FRS
Professor Sir ASHLEY MILES, CBE, MD, D SC (*hc*), FRCP, FRS
Professor A. NEUBERGER, CBE, MD, FRCP, FRS

Clerk to the Governors S. A. WHITE, AACCA

The Council

A. LAWRENCE ABEL, MS, FRCS *Representing the British Medical Association*

V. C. BARRY, D SC *Representing the Royal Irish Academy*

The Rt Hon Lord BROCK, MS, FRCS *Representing the Members of the Institute*

Professor Sir LINDOR BROWN, CBE, FRCP, FRS *Representing the Members of the Institute*

H. P. G. CHANNON, MP *Representing the Members of the Institute*

Dame HARRIETTE CHICK, DBE, D SC *Representing the Members of the Institute*

Professor P. J. COLLARD, MD, MRCP *Representing the University of Manchester*

Major L. M. E. DENT, DSO *Representing the Worshipful Company of Grocers*

Sir CHARLES DODDS, Bt, MVO, MD, D SC, FRCP, FRS *Representing the Members of the Institute*

Sir ALAN N. DRURY, CBE, MA, MD, FRCP, FRS *Representing the Members of the Institute*

Professor D. G. EVANS, D SC, FRS *Representing the Royal Society*

Sir PAUL FILDES, OBE, MA, D SC, MB, B CH, FRS *Representing the Members of the Institute*

Professor R. E. GLOVER, MA, D SC, FRCVS *Representing the Royal Agricultural Society*

Professor R. I. N. GREAVES, BA, MD *Representing the University of Cambridge*

Sir CHARLES HARINGTON, MA, PH D, FRS *Representing the Members of the Institute*

Professor HENRY HARRIS, D PHIL, FRS *Representing the University of Oxford*

Professor B. P. MARMION, MD, D SC, FC PATH *Representing the University of Edinburgh*

Professor Sir ASHLEY MILES, CBE, MD, D SC (*hc*), FRCP, FRS *Representing the Members of the Institute*

Professor J. S. MITCHELL, CBE, MA, MD, FRS *Representing the Members of the Institute*

Professor W. T. J. MORGAN, CBE, D SC, PH D, MD (*hc*), FRIC, FRS *Representing the Members of the Institute*

Professor Sir RUDOLPH PETERS, MC, MA, MD, FRS *Representing the Members of the Institute*

The President of the ROYAL COLLEGE OF PHYSICIANS *Representing the Royal College of Physicians, London*

The President of the ROYAL COLLEGE OF SURGEONS *Representing the Royal College of Surgeons of England*

The President of the ROYAL COLLEGE OF VETERINARY SURGEONS *Representing the Royal College of Veterinary Surgeons*

MURIEL ROBERTSON, MA, D SC, LL D, FRS *Representing the Members of the Institute*

Professor F. S. STEWART, MD *Representing the University of Dublin*

WILLIAM J. THOMPSON *Representing the Worshipful Company of Grocers*

Sir GRAHAM WILSON, MD, B SC, FRCP *Representing the University of London*

The Staff

Director: Professor Sir Ashley Miles
Deputy Director: Professor L. H. Collier
Superintendent of Elstree Laboratories: W. d'A. Maycock

MICROBIOLOGY, EXPERIMENTAL PATHOLOGY AND IMMUNOLOGY

Experimental Pathology and Immunology

†Sir Ashley Miles, CBE, MD, D SC (*hc*), FRCP, FRS (*Professor of Experimental Pathology in the University of London*)
F. R. Wells, MA, BM, B CH

Brenda Mason, B SC
D. J. McConnell, MD (U.S.A.)
H. C. Polk, MD (U.S.A.)

Microbiology

†G. G. Meynell, MD (*Guinness Professor of Microbiology in the University of London*)
A. B. Stone, B SC, D PHIL
Marylyn D. Cooke, B SC, PH D
Eva Aufreiter, B SC

Guinness-Lister Research Unit

*Elinor W. Meynell, BA, MD, DIP BACT
Ruth M. Lemcke, B SC, PH D
J. E. Dowman, MA, PH D (*S.R.C. Grantee*)
M. R. Hollingdale, B SC (*M.R.C. Grantee*)

D. G. Godfrey, OBE, B SC, PH D (*M.R.C. External Scientific Staff*)
Angela E. R. Taylor, B SC, PH D
Sheila M. Lanham, B SC

Trypanosomiasis Research

Virology

†L. H. Collier, MD, D SC, MRCP (*Professor of Virology in the University of London and Hon. Director, M.R.C. Trachoma Unit*)
J. Alwen, B SC, PH D
Lindsey M. Cox, B SC

W. A. Blyth, M SC, PH D
A. J. Garrett, B SC, PH D
Janice Taverne, BA, PH D
Anne E. Mogg, B SC
Andrea Barton, B SC

M. R. C. Trachoma Unit

Electron Microscopy Unit

*A. M. Lawn, B SC, PH D, MRCVS

BIOCHEMISTRY

†Winifred M. Watkins, D SC, PH D, FRS (*Professor of Biochemistry in the University of London*)
*G. M. A. Gray, B SC, PH D
M. A. Chester, M SC, B TECH (*Research Student*)
B. Denise Ward, M SC (*Grocers' Company Research Student*)
R. D. Poretz, BA, MS, PH D (U.S.A.)

Lydia Coles, B SC, D PHIL (*M.R.C. Grantee*)
Barbara J. Dod, B SC (*M.R.C. Grantee*)
A. S. R. Donald, B SC, PH D (*M.R.C. Grantee*)
Veronica M. Hearn, M SC, PH D (*M.R.C. Grantee*)
Caroline Race, B SC (*M.R.C. Grantee*)
Jennifer J. Wells, BA, PH D (*B.E.C.C. Grantee*)
G. B. Hay, B SC (*B.E.C.C. Grantee*)

§Professor W. T. J. Morgan, CBE, D SC, PH D, MD (*hc*), FRIC, FRS (ret'd).

BIOPHYSICS

†R. A. Kekwick, D SC, FRS (*Professor of Biophysics in the University of London*)
†J. M. Creeth, B SC, PH D, FRIC (*Reader in Biophysics in the University of London*)
†Professor N. H. Martin, MA, FRCP, FRIC (*Honorary Research Associate*)

M. A. Denborough, MB, D PHIL, FRACP, MRCP (Australia)
J. M. Jones, B SC, PH D (*M.R.C. Grantee*)
J. C. Holt, B SC (*M.R.C. Grantee*)
Caroline M. Butterworth, B SC (*M.R.C. Grantee*)
W. H. Sawyer, M SC, PH D (*S.R.C. Grantee*)

§Dame Harriette Chick, DBE, D SC (ret'd)

PREPARATION and STUDY of THERAPEUTIC SERA (ELSTREE)

W. E. Parish, MA, PH D, BV SC, MRCVS, MC PATH
N. Mahony, M SC, B TECH
Wendy Smithson, B SC

Biochemistry (Elstree)

*D. E. Dolby, B SC, PH D

PREPARATION and STUDY of VIRUS VACCINES (ELSTREE)

H. G. S. Murray, MD
G. S. Turner, B SC, PH D
L. C. Robinson, B SC, PH D

PREPARATION and STUDY of BACTERIAL VACCINES (ELSTREE)

*A. F. B. Standfast, SC D
Jean M. Dolby, MA, PH D
M. P. Banks, B SC
Noreen M. Wesley, B SC
Caroline J. Bronne, B SC
A. P. Hunt, B SC
J. P. Ackers, MA, D PHIL (*M.R.C. Grantee*)

CO-ORDINATION of PRODUCTION (ELSTREE)

J. Rodican, B SC

BLOOD PRODUCTS (ELSTREE)

*W. d'A. Maycock, MVO, MBE, MD, MRCP, FC PATH
L. Vallet, MA
§Margaret E. Mackay, M SC, PH D (*M.R.C. External Scientific Staff*)
D. Ellis, B SC, PH D
Constance Shaw, M SC, DIP BACT
E. D. Wesley, B PHARM
Valerie J. Stickley, B SC

PLASMA FRACTIONS LABORATORY (OXFORD)

Ethel Bidwell, B SC, PH D, FRIC
W. H. Ford, B SC, PH D

MEDICAL RESEARCH COUNCIL UNITS HOUSED AT THE INSTITUTE

Blood Group Unit

§R. R. Race, MD(*hc*), PH D, FRCP, FRS
Ruth Sanger, B SC, PH D
Patricia Tippett, B SC, PH D
E. June Gavin, B SC
Ann Gooch, B SC

Blood Group Reference Laboratory

K. L. G. Goldsmith, PH D, MB, BS, MC PATH
Toby T. B. Phillips, MB, CH B
Elizabeth W. Ikin, B SC, PH D
Carolyn M. Giles, B SC, PH D
B. J. Dawes, B SC

ADMINISTRATION

Secretary and Accountant	S. A. White, AACCA
Elstree Secretary and Estate Manager	G. J. Roderick, B COM
Assistant Secretary	Barbara A. Prideaux
Assistant Accountant	E. J. H. Lloyd
Administrative Assistant	C. L. Beard
" "	Beryl I. Bristow
" "	B. M. Walcroft

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

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

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

Annual General Meeting of the Lister Institute

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the Institute for the year 1968.

GOVERNING BODY

At a meeting in April 1968, the Governors accepted with much regret the decision of Sir Charles Dodds to relinquish the Chairmanship and to resign from the Governing Body after the Annual General Meeting. Sir Charles, who joined the Governing Body in June 1956, had been Chairman since December 1961 and the Governors take this opportunity of expressing their gratitude to him for his constant interest in the affairs of the Institute; and in particular for his energetic support of the Institute's application to the Wolfson Foundation which led to the munificent gift of £300,000 announced in last year's Report.

The Governing Body unanimously elected Sir Lindor Brown as its new Chairman and also welcomes Lord Iveagh and Professor A. Neuberger as new Governors.

In accordance with the Articles of Association, Professor L. H. Collier retired from the Governing Body and was succeeded by Professor R. A. Kekwick.

COUNCIL

At last year's Annual General Meeting the three retiring members of the Council, Professor D. G. Evans, Dr. V. C. Barry and the President of the Royal College of Veterinary Surgeons were re-appointed and Lord Iveagh was appointed as one of the representatives of the Members.

During the year Professor Henry Harris was appointed as the representative of the University of Oxford in place of the late Lord Florey.

The three members of Council due to retire this year in accordance with the Articles of Association, but who are eligible for re-appointment, are Professor R. I. N. Greaves and Mr. A. Lawrence Abel representing the University of Cambridge and the British Medical Association respectively, and Dr. Muriel Robertson, one of the representatives of the Members of the Institute.

The Governing Body records with great regret the death in August of Dr. D. W. W. Henderson. Dr. Henderson first came to the Institute as a Beit Research Fellow in 1932 and was a member of the staff from 1935 until 1946.

MEMBERS

The Governing Body also records with great regret the death of Sir Henry Dale in July. Sir Henry was Chairman of the Governing Body from 1942 to 1961.

STAFF AND STUDENTS

Professor W. T. J. Morgan retired on 30th September after nearly forty years service, first as a member of the Serum Department at Elstree and later as a member of the Biochemistry Department, of which he became Head in 1944. In 1953 he became Deputy Director of the Institute. During his distinguished career Professor Morgan received many awards and honours and it is with great pleasure that the Governing Body records that the University of London has conferred upon him the title of Emeritus Professor of Biochemistry; and that in December 1968 the Royal Society awarded him a Royal Medal for his outstanding contributions to the knowledge of the chemistry of blood-group substances, with special reference to genetical as well as immunological considerations. On February 11th 1969 he was made an Honorary Member of the Institute Staff.

Professor L. H. Collier has succeeded Professor Morgan as Deputy Director of the Institute.

The Governing Body records with great pleasure that in April 1968 Dr. Winifred Watkins was awarded an "Anschluss" Paul Ehrlich-Ludwig Darmstädter Silver Medal and Prize for her work on "Genetic

and Immunochemical Aspects of Human Blood-group Specificity"; and that in March 1969 she was elected to the Fellowship of the Royal Society. The University has conferred on Dr. Watkins the title of Professor of Biochemistry.

She succeeded Professor Morgan as head of the Biochemistry Department; and Dr. G. M. A. Gray was appointed deputy head of the department.

The department of Co-ordination of Production, instituted last February with Mr. J. Rodican as its head, completed its first year's work. Substantial progress has been made in the organisation of all the relevant aspects of production, and in accounting, costing, and centralisation of services.

In January 1969 Dr. Elinor W. Meynell and Dr. A. M. Lawn were recognised by the University as Teachers in Microbiology and Physiology respectively.

Dr. Elinor W. Meynell was appointed to the staff of the Department of Microbiology, Dr. Marylin D. Cooke and Miss Eva Aufreiter to the Guinness-Lister Research Unit, Miss Wendy Smithson to the Serum Department and Dr. J. Alwen and Miss Lindsey M. Cox to the Virology Department. Miss Frances R. Hunter and Miss Anne M. Marr completed the tenure of their studentships in the Virus Vaccines and Biochemistry Departments respectively, and Miss Susan T. Edwards resigned from the Guinness-Lister Research Unit during the year.

The Institute was sorry to lose the services of Mr. C. Broder, who retired in May 1968, and of Miss E. R. Rawlinson who retired in November 1968. Mr Broder worked at Chelsea for nearly forty-five years and Miss Rawlinson at Elstree for thirty-two years.

The Governing Body notes with pleasure that the University of Newcastle upon Tyne has conferred upon Sir Ashley Miles the degree of Doctor of Science (*honoris causa*). As Biological Secretary of the Royal Society, Sir Ashley visited the Accademia Nazionale dei Lincei, Rome in January 1968 and, as a member of a Royal Society delegation, went to Brazil and Mexico in August. Sir Ashley resigned from the Biological Secretaryship of the Royal Society in November 1968.

In March 1968 Professor W. T. J. Morgan lectured by invitation at the University of Frankfurt, Germany and in July 1968 he and Dr. Winifred M. Watkins gave lectures in a course in immunochemistry at the University of Freiburg, Germany. In December Professor Morgan took part by invitation in the Landsteiner Centennial Conference arranged by the New York Academy of Sciences in New York.

Dr. W. d'A. Maycock attended, as Ministry of Health representative, a meeting of the Subcommittee of Specialists on Blood Transfusion of the Public Health Committee of the Council of Europe, held in Istanbul in May 1968. In October he attended a meeting of the Group of Experts on Immunological Products of the European Pharmacopoeia Commission of the Council of Europe. In September Dr. Maycock and Mr. L. Vallet attended the Eleventh International Congress of the Permanent Section of Microbiolgical Standardization in Milan.

In May 1968 Professor L. H. Collier and Dr. G. S. Turner attended the first International Congress for Virology in Helsinki.

Professor G. G. Meynell lectured at the Institute of Molecular Biology, Geneva and at the International Agency for Cancer Research, Lyon in February 1968.

Professor R. A. Kekwick visited Geneva in December 1968 as a consultant to the Epidemiological Surveillance Division of the World Health Organisation.

In November 1968 Professor Watkins gave the XXI John Gibson II lecture at the College of Physicians and Surgeons, Columbia University, New York, and visited the Rockefeller Institute and the New York Blood Centre. She also attended a con-

ference on The Plasma Proteins and Cellular Elements of the Blood organised by the Blood Research Institute in Cambridge, Mass.

Dr. G. M. A. Gray took part, by invitation, in a Ciba Foundation Study Group on Gas Chromatography in Biology and Medicine.

Miss Sheila M. Lanham spent four months at the Nigerian Institute for Trypanosomiasis Research at Vom in Northern Nigeria.

For the academic year 1968/69 there are fifteen postgraduate research workers at the Institute registered for higher degrees of the University. Two Ph.D. degrees were awarded during 1968.

NEW BUILDINGS

The Governing Body is happy to report that during the year the Institute was able to purchase the freehold of the studio site, adjoining the main Chelsea laboratories, and that building of the new wing began on March 24th. It is hoped that the work will be completed within eighteen months. The architect is Mr. P. B. D. Sutherland, MA., DIP.Arch., ARIBA.

The new laboratories of the Haemophilia Centre in Oxford, of which the Plasma Fractions Laboratory is one of the three constituent units, were officially opened on 30th September 1968, by Dame Albertine Winner, D.B.E.

Six cottages for estate and stable staff are under construction at Elstree, and should be completed during 1969.

DONATIONS AND GRANTS

Arthur Guinness, Son & Co. Ltd. continue their generous support of the Guinness-Lister Research Unit; a further £4,000 out of the total grant of £35,000 has been received from the Fleming Memorial Fund for Medical Research for the support of the Electron Microscope Unit, and the Grocers' Company has most generously contributed £5,000 towards the cost of the lecture theatre in the new wing.

The Governing Body records it appreciation of the many other bodies whose benefactions and grants support research work in the Institute. These include a grant from the Agricultural Research Council for a study of the molecular weight distribution of humic acid fractions by equilibrium ultracentrifuge measurements; a grant from the Arthritis and Rheumatism Council for the study of vasculitis due to complexes of bacterial antigens with antibody; a grant from the British Empire Cancer Campaign for research on lipids; grants from the Medical Research Council for researches on the chemical basis of human blood-group specificity, on the biosynthesis of blood-group specific glycoproteins and red cell antigens, on multiple blood-group specific serological characters associated with simple glycoprotein molecules, on the characterization of proteins by the ultracentrifugal steady-state method, on the lipid components of the plasma membranes of mammalian cells, on the macroglobulins of normal human plasma, on the gamma macroglobulins of normal human plasma, on the antigenic structure of *Mycoplasma hominis*, on sensitizing antibodies in sera of cot-death cases and milk-sensitive individuals, on the multiplication of bacteriophage, on the separation and characterization of the antigens of *B. pertussis*, on the distribution of serotypes of *B. pertussis*, on immunity to *B. pertussis* infections, on the genetics of drug resistance factors and other bacterial plasmids, on the role of adenovirus in the aetiology of infectious hepatitis, on hypersensitivity to smallpox vaccine, on the reversibility of denaturation of ovalbumin and serum albumin and on the ceramide-containing glycolipids in mammalian tissue, with special reference to their biosynthesis.

Grants were also received from the Ministry of Overseas Development in aid of research on the immunology of trypanosomiasis; from the Science Research Council for studies on the reversibility of formation of specific disulphide bridges in proteins, on the metabolism and growth of micro-organisms, on ultrastructural aspects of implantation and on the characterisation of protein conformation during denaturation and renaturation; from the Royal Society for the purchase of a fluorimeter and recycling chromatographic equipment; and from the Smith, Kline and French Foundation for research on the *in vivo* biosynthesis of blood-group specific glycoproteins and for

the purchase of an Olivetti Programma 101 Computer.

The Governing Body also gratefully acknowledges donations to the Institute's Re-endowment Fund from the Prudential Assurance Company Limited and the Royal London Mutual Insurance Society Limited.

VISITORS

The following visitors, in addition to those listed under Staff, worked for short periods in the Institute's Laboratories: Mrs. Chantana Aumpansang, Government Pharmaceutical Laboratories, Bangkok; Dr. H. Bayraktaroglu, Red Crescent Blood Transfusion Centre, Izmir; Miss Pearl Cooper, New York University School of Medicine, New York; Dr. H. Goldfine, Harvard University, Massachusetts; Mr. Roger Harris, Bedford College, University of London; Mr. A. Himoud, Amiry Hospital, Kuwait; Dr. A. M. Khan, Institute of Blood Transfusion, Lahore; Mr. J. S. Kiango, Ministry of Health and Housing, Dar-es-Salaam; Dr. N. Mizan, Kizilay Kan Merkezi, Ankara; Dr. A. M. Posner, University of Western Australia, Perth; Dr. Girdmar Sahai, Central Research Institute, Kasauli; Dr. R. Schneider, Institut Pasteur, Pl. Ch. Nicolle, Casablanca; Mr. Han Sein, Burma Pharmaceutical Industry, Rangoon; Dr. H. Seyfried, Institute of Haematology, Warsaw; Dr. Sharada Shanmugasundaram, King Institute of Preventive Medicine, Madras; Dr. John Stassinopoulos, Athens; Miss Jean Tweedy, Trinity College, Dublin; Dr. Elie Vamos, Institut Pasteur du Brabant, Brussels; Dr. Reinhild Wokatsch, Impfanstalt Hamburg, Hamburg.

Researches in 1968

SUMMARY

The bracketed numbers refer to pages and columns of the report where more detailed descriptions are to be found.

Microbiology

1. Bacterial genetics, physiology and morphology:
   (a) Distinction between I and F types of sex-pili in Salmonella (17, i).
   (b) Bacterial sex factors in Salmonella; in de-repressed mutants (17, i); as agents either excluding, or inducing immunity to, other sex factors (17, ii); and in relation to chromosomal transfer (18, i).
   (c) DNA synthesis in phage-infected *E. coli* (18, ii).

Infective Diseases and Immunity

1. Trypanosomiasis:
   (a) Surface charge of trypanosomes and erythrocytes (14, ii); and its exploitation in harvesting trypanosomes from the blood (14, i).
   (b) Lipids of trypanosomes and their relation to cell permeability (15, i).
   (c) Ultrastructure of the trypanosome cell (15, i).
   (d) Immunopathology of trypanosome infections (15, i).
2. Virus diseases:
   (a) Vaccinia virus. Relation of hypersensitivity and immunity in the guinea-pig (22, ii). Immunogenicity of inactivated vaccines (22, ii); methods of assay of virus (22, ii); chemical composition of vaccinia virus (22, ii); and growth in tissue culture (23, i).
   (b) Rabies virus. Encephalitogenic factors in vaccines (23, i). Relation of virus to myxoviruses (23, i).
   (c) Infectious hepatitis. Relation to adenovirus infections (22, i).
3. Chlamydial and bacterial infections:
   (a) Trachoma agent. Histochemical (20, i), electron microscopic (20, i) and serological (21, i) studies of cell inclusions in infected cells (20, i). Immunopathology of hypersensitivity (21, i). Relation of vaccine-induced immunity to types of antibody (21, i). Genital trachoma infections in the Gambia (21, ii) and fluorescent antibody in the serology and diagnosis of trachoma infections (21, ii).
   (b) Mycoplasma. Antigenic analysis of *M. hominis* (19, i). Identity of *M. histotropicum* and *M. pulmonis* (19, ii).
   (c) *B. pertussis*. Isolation of protective antigens (16, ii); agglutinogens of prevalent strains of *B. pertussis* (16, i); pertussis antibodies in human sera (16, i); and relation of bactericidal and mouse-protective antibody (16, i).
   (d) *V. cholerae*. Isolation of toxin (16, ii).
   (e) Production of *Cl. tetani* exotoxin (16, ii).

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(b) Eosinophil-stimulating factors in anaphylaxis (23, ii).
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MICROBIOLOGY

Trypanosomiasis

Harvesting of Bloodstream Trypanosomes. Miss Lanham spent four months at the Nigerian Institute for Trypanosomiasis Research developing her technique for the separation of trypanosomes from the blood of large animals, especially those infected by tsetse flies. The erythrocytes of the host species, including others studied in London, could be arranged in order of decreasing negative charge, or of their adsorption to DEAE-cellulose in the presence of phosphate-buffered saline-glucose (PSG) of standard ionic strength (Report 1968): namely monkey, dog, donkey>rat, mouse, man>camel>goat, sheep, ox, guinea-pig, rabbit>pig. From the study of more species of trypanosomes, the list of increasing adsorbabilities and negative charge was extended as follows. *Trypanosoma cruzi>T. lewisi>T. vivax, T. congolense>T. simiae, T. brucei, T. rhodesiense, T. gambiense, T. evansi.*

Salivarian trypanosomes were successfully separated from the blood of man, monkey, dog and donkey under the conditions already established for rat and mouse blood. The necessary reduction in ionic strength of the buffered saline-glucose to bring about red cell adsorption with the elution of viable trypanosomes was determined for the remaining animals in the above list.

In Nigeria, the following species of trypanosomes were separated and some of the material brought back to London. *T. brucei* from monkey, goat, donkey, dog; *T. gambiense* from man, monkey, dog; *T. evansi* from camel; *T. congolense* from sheep, goat, ox, donkey, dog, pig; *T. simiae* from pig; *T. vivax* from sheep, goat, ox, donkey.

Field work in Nigeria also showed that infecting trypanosomes that were difficult to find microscopically in blood films, were detectable in the deposit from the centrifuged eluate after removing the blood cells on DEAE-cellulose. Of particular interest was the detection of *T. gambiense* in two human patients, and *T. congolense* in an ox. Another interesting application of the technique was the collection of a pure suspension of *T. brucei* from the blood of a goat doubly infected with *T. brucei* and *T. vivax.*

As previously reported, *T. lewisi* cannot be separated from the erythrocytes of infected rat blood from the 4th to the 9th day of infection because the adsorbability of the red cells decreases progressively during this period. The two are readily separable, however, when centifuged deposits of red cells and trypanosomes, separated from plasma, are fractionated on the column.

Further attempts to separate *T. cruzi* from infected blood were unsuccessful, even from monkey blood at the ionic strength at which the strongly negative monkey blood cells are adsorbed. It was also impossible to separate another stercorarian trypanosome, *T. theileri*, from cattle blood.

Surface Charge of Trypanosomes and Erythrocytes. Although the different behaviour of trypanosomes and blood cells on the columns of anion-exchanger is mainly due to surface charge, their adsorbability is probably modifiable by external factors.

Plasma probably influences the negative charge. The weakly charged (i.e. weakly adsorbing) erythrocytes of rats infected with *T. lewisi* increased in charge after being freed from plasma, but on exposure either to normal plasma or to plasma from a rat infected for less than three days, the charge decreased to the initial level. This suggests a non-specific plasma factor. On the other hand, a specific factor is suggested by the reduction in charge of normal red cells incubated in plasma from a rat infected six days previously. Normal erythrocytes exposed to the enzymes ficin and α-amylase also became weaker in charge. Neuraminidase and α-amylase reduced the surface charge of washed *T. lewisi.*

Other exploratory work with polyelectrolytes showed that certain basic (positively charged) polypeptides immobilized trypanosomes in 10 to 30 minutes and acted more quickly when the ionic strength of buffer was decreased. On the other hand, an acidic polyelectrolyte, polygalacturonic acid, had no obvious effect, yet inhibited the lethal immobilizing action of the basic polypeptides when added to the trypanosomes beforehand.

Immunological Studies. Immunization of mice with a given variant of *T. brucei* protects them against infection only with the homologous variant. A trypanosome homogenate with Freund's complete adjuvant afforded better protection than an alum-precipitated homogenate. Drs. Taylor and Godfrey attempted, without success, to protect mice with a homogenate of five heterologous variants against challenge with a sixth heterologous variant, all isolated during the course of the same infection.

Antilymphocytic serum, raised in rabbits against mouse thymocytes, broke down the immunity of mice immunized against a variant of *T. brucei*. This breakdown of protection is under investigation with other antilymphocytic sera raised in rabbits and goat.

Phosopholipids of Trypanosomes. Extracts made by Miss Lanham during her visit to Nigeria were analysed by Dr. Godfrey by improved techniques.

The results suggest that the earlier estimations of lecithin were slightly too high, owing to contamination with a small amount of lysophosphatidyl ethanolamine not separable from lecithin by the method previously employed. Samples of *T. evansi*, *T. vivax* (fly-passaged) and *T. simiae* from Nigeria, and of *T. rhodesiense* were examined.

Permeability of the Trypanosome Cell. The uptake of tetracycline as a measure of permeability was discontinued in favour of the extrusion of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT). Drs. Godfrey and Taylor found that negligible amounts of GOT were released from washed *T. brucei* in the presence of cobra venom, and large amounts of GPT. As little as 2 per cent. normal rat plasma considerably decreased leakage of GPT induced by cobra venom. From *T. lewisi*, however, cobra venom released similar amounts of GOT and GPT. As with *T. brucei*, rat plasma slowed down the rate of release.

Ultrastructure. The surfaces of the various species of trypanosome were examined electron-microscopically by Drs. Taylor and Godfrey before and after treatment with venom. Normal *T. brucei* had a typical trilaminar plasma membrane and a thick, uniform external surface coat which persisted even after repeated washings. The surface coat was not as compact in *T. vivax* or *T. congolense*, in which, so far, no distinct three-layered plasma membrane is demonstrable. In *T. lewisi*, the plasma membrane was distinctly trilaminar, and the surface coat irregular and loosely packed.

In the presence of rat plasma, cobra venom had little morphological effect on *T. brucei*. With washed trypanosomes, however, the venom usually disintegrated the plasma membrane and surface coat, although in many organisms only the surface coat was affected. The activity of the venom appears to be due to its content of phospholipase A and a lytic factor. Rattlesnake venom, which contains the enzyme but no lytic factor, had no effect.

A hitherto unreported structure was seen in electronmicrographs. In all four species there was, consistently, in the subpellicular ring of microtubules, a special group of four. The quartet was closely associated with an extension of the outer membrane of the nuclear envelope, which was partly wrapped around each of the four tubules in *T. lewisi*, *T. congolense* and *T. brucei;* these invaginations were very shallow in *T. vivax*. The organelle was often out of line with the ring of microtubules, and always occurred to one side of it near where the flagellum was running along the outside of the organism.

Maintenance of Trypanosomes in vitro. It was noticed that trypanosomes remained very active for long periods in the strongly buffered eluate after column separation from blood cells. Drs. Godfrey and Taylor determined the viability of their suspension by infectivity tests on serial dilutions, rather than by counting motile organisms, thereby ensuring that any change to the non-infective phase would be detected.

The results were variable but the trypanosomes usually survived without loss of infectivity in rat blood diluted with buffer for up to 8 hr. at room temperature. Mouse and guinea-pig blood appeared to be better preserved than rat blood. Infectivity was even better preserved when the trypanosomes were separated on DEAE-cellulose,

and held in fresh blood and buffer, and the cycle repeated 3 times in a 12 hr. period. Plasma was usually as good as whole blood in maintaining the organisms, but plasma of rats infected for three days or less, improved viability, especially at 37°.

Whooping Cough Bacillus

The Agglutinogens of Bordetella pertussis. Miss Bronne continued her work on the serological typing of isolates of *B. pertussis* collected through the Whooping Cough Working Party of the Public Health Laboratory Service. Six agglutinogens, designated by number, are known; all strains of *B. pertussis* contain agglutinogen 1 and one or more of agglutinogens 2–6 (Report 1968). During the last few years most isolates contained agglutinogens 2 or 3, whereas since 1967 the number of isolates containing both 2 *and* 3 has risen and is now about 15 per cent. The question arose as to whether serotype 123400 was a mixture or a pure culture. Miss Bronne showed by serial single-colony culture that, although some 123400 cultures were mixtures of 103000 and 120400 serotypes, some were stable cultures of serotype 123400.

Pertussis Antibodies in Human Sera. Sera from normal adults and children, from vaccinated and/or infected adults and children, and from a few adults and children known to have been exposed but who did not have whooping cough, were tested by Dr. Jean Dolby for agglutinins, antihaemagglutinins, complement-mediated bactericidal antibody and antibodies protecting mice against an intracerebral challenge. Although the sera differed in content of the various antibodies, there was no distribution of antibody types characteristic of any of the categories of donor.

The Mouse Potency Test, Bactericidal Antibody and Passive Mouse Protective Antibody. If mouse protection following vaccination is due to antibody it should be possible to demonstrate this passively, although so far attempts by other workers have failed. Following previous work on the fractionation and testing of rabbit antipertussis serum (Report 1968), Drs. Jean and D. E. Dolby showed that the 7S γ globulin fraction of pooled mouse serum 14 days after a minimal protective dose of vaccine given intraperitoneally was both bactericidal *in vitro* and able to inhibit the growth of pertussis in mouse brains. Bactericidal antibody was demonstrated in the unconcentrated sera of mice 17 days after vaccination, that is, at the critical time for infected vaccinated mice. Mr. Hunt established the conditions for a dose-response assay of pertussis vaccine by the bactericidal activity of sera from such mice, and is now carrying out this assay in parallel with the routine active protection assay in mice, to measure the correlation between results obtained by the two methods.

The Protective Antigen of Bordetella pertussis. Dr. Ackers continued the fractionation of supernatants from high speed centrifugation of sonically-disrupted cells by gel-filtration on a column of 4 per cent. agarose. A partial separation of protective activity from heat-stable toxin was achieved. To determine whether the considerable protective activity of this supernatant is due to a cytoplasmic antigen or to very small fragments of cell wall, large-scale liquid culture of *B. pertussis* spheroplasts was undertaken with the hope of preparing a pure fraction of cell membrane and cytoplasm.

Cholera

Mrs. Wesley (née Goggin) continued her work on the Craig type II cholera toxin. She concentrated mainly on the chemistry and purification of four of its antigenic components from crude toxin preparations; a protein and lipid were isolated, both of which protected mice against challenge by living vibrios.

Work has also commenced on the preparation of a cholera cell-wall vaccine. Preliminary studies suggest that formalin-killed vibrios break with ultrasonic vibrations far more easily and satisfactorily than phenol-killed or heat-killed vibrios.

Clostridial Toxins

Mr. Mahony began a study of the factors in Mueller's medium for growth of *Cl. tetani* which are essential for high yields of toxin.

The casein digest component of this medium was separated into acidic, basic and neutral fractions on a cation exchange column as a preliminary to further fractionation on Sephadex gels. Work continues on the identification of the substances affecting toxin production, for use in an optimal synthetic medium.

Inheritance in Bacteria

Bacterial Sex Pili. These are specific filamentous appendages formed by bacteria capable of gene transfer by conjugation and may be the channel by which donated genes pass to the recipient. Only two classes of sex pili are known: F pili identified elsewhere, and I pili first described by Meynell and Lawn (Report 1968). The two classes of pili are serologically distinct (Report 1967) and their determining genetic elements are unrelated by criteria like superinfection immunity (see Bacterial Sex Factors, below). The lack of relationship is further emphasised by the absence of complementation in the synthesis of sex pili. Miss Aufreiter and Professor Meynell isolated 9 independent defective mutants of wild type *Flac*, designated F*, by exposing F+ strains to the F specific phage, MS2; and 25 I* mutants, by exposing strains carrying de-repressed ColIb-P9 to I specific phage (Report 1968). These mutants were phage-resistant because they no longer form sex pili, but this might have been due to the reappearance of repressor. This possibility was excluded with F, by showing that no F* mutant repressed a repressor-sensitive R factor when both were present in the same strain. Wild type F did not complement any of the I* mutants: nor did a de-repressed I factor complement the F* mutants. Thus, by every criterion applied to date, the F and I systems are distinct.

Bacterial Sex Factors. The collective term "sex factor" is applied to a set of genes responsible for conjugation and gene transfer. The conjugating function of most wild type sex factors is repressed, but de-repressed mutants can now be isolated (Report 1968). Professor Meynell and Miss Edwards applied their method to colicin factors Ib-P9 and B, and found, as with ColEla (Report 1968), that about 10 per cent. of clones tested contained mutants. An R factor previously studied by Dr. Elinor Meynell determined colicin synthesis as well as drug-resistance, and the striking feature of its mutant selected for de-repression of conjugation was that colicin production was also greatly increased. Miss Edwards further found this to be true of all of 5 independent de-repressed mutants of ColIb-P9 and 2 of ColEla. It is possible that the mutation increases the expression of all functions associated with the sex factor without increasing the number of gene copies per cell: or that expression is unchanged but the rate of replication increases, leading to a greater number of copies.

Exclusion and Superinfection Immunity. A sex factor largely prevents a bacterial strain from receiving a related factor by conjugation. Two processes are involved: *exclusion*, reflected in a lowered frequency of transfer, and *superinfection immunity*, which prevents the donated sex factor from replicating in the minority of recipient cells it enters. These processes were first demonstrated with F, and Professor Meynell showed them to be equally demonstrable with the I sex factors of ColIb-P9 and ColIa-CA53. Exclusion by an I+ recipient was removed by starvation to produce an "I- phenocopy". Superinfection immunity was manifested by the formation of "abortive recombinants" which, in this system, are minute colonies formed by those recipient cells acquiring a gene, necessary for growth, whose replication is prevented by superinfection immunity. Only a minority of cells in the recipient clone therefore grow, and a minute colony results. The non-replicating gene thus behaves like the incomplete chromosomal fragments responsible for abortive transduction (Report 1955).

Dr. Elinor Meynell and Miss Frydman used superinfection immunity as a taxonomic criterion for several R factors and ColB factors, all known to carry sex factors similar to F. Combinations of one wild type factor and one de-repressed mutant factor were used to distinguish them and to test for cross-repression of pilus synthesis.

Cells containing two R factors were selected by exposure to suitable combinations of antibiotics. With such selection, the two factors can be maintained together, and when this was done, the de-repressed mutant was repressed by the wild type factor. Later, after the pair had come to coexist stably without constant selection, the mutant became de-repressed, indicating that its mutation entailed loss of repressor, as distinct from insensitivity to repression, and that stabilization involved loss of the wild type sex factor with its functional repressor gene. Transduction by phage P1 showed that the drug resistance genes were either joined to the remaining sex factor; or present in two groups, one resembling the original plasmid and the other now constituting a non-transmissible plasmid lacking a sex factor; or that both states were present in different bacteria of a given culture.

Chromosomal Transfer. Chromosomal transfer in some F+ strains of *Escherichia coli* results from preliminary recombination of F and the host chromosome to form a high-frequency (Hfr) donor, as first shown by Jacob and Wollman by the non-random distribution of Hfr cells in replicate donor cultures. Chromosomal transfer also occurs with the I sex factors of ColIb-P9 and ColEla-16 (Reports 1962, 1968), but Professor Meynell and Miss Edwards found the distribution of donors to be purely random, so that Hfr cells are unlikely to be responsible for gene transfer. Conceivably, random breaks occur in the donor's chromosome and enable it to pass to the recipient through the I sex pilus identified by Professor Meynell and Dr. Lawn (1967). All reports of chromosomal transfer in *Escherichia coli* carrying ColIb-P9 involve the supposedly F– strain, 58.161/sp., which carries a defective F factor. True F– derivatives were isolated but no difference was found in the rates of chromosomal recombination.

Whether chromosomal transfer is always specifically related to the sex factor or is a random consequence of conjugation can also be determined by comparing the donor ability of the same bacteria carrying different sex factors. Dr. Elinor Meynell and Dr. Cooke (1) compared the number and kinds of recombinants produced by eight different de-repressed R factors; although all gave broadly the same numbers as F, each displayed minor distinctive features in the relative frequencies of different recomginants; (2) sought to isolate Hfr variants of strains carrying every sex factor giving chromosomal recombination; a few promising clones have indeed been isolated from several R+ strains: and (3) tested the ability of a functional sex factor to restore the original polarity of transfer to a defective Hfr strain carrying a mutant F factor integrated in its chromosome (see Sex Pili, p. 17). Specificity was evident for, although some sex factors behaved as if they were in an F– host, others restored the original polarity of transfer of the Hfr strain. A trivial explanation would be complementation leading to the reappearance of normal F pili; but this was excluded because the sex pili formed were characteristic of the intact sex factor, not of the mutant F.

DNA Synthesis in Phage-infected E. coli. Dr. Stone continued to examine the abrupt arrest of bacterial DNA synthesis which takes place 15 minutes after infection with bacteriophage ØX174, a single-stranded DNA phage.

The arrest is prevented by chloramphenicol up to about the 10th minute, suggesting that the synthesis of a protein involved in the inhibition of DNA synthesis begins at about this time. The preceding delay does not reflect the need to accumulate a pool of 2-stranded phage "replicative" DNA, for, if such a pool is established in the absence of protein synthesis and protein synthesis then allowed to resume, a delay of over 15 minutes is still observed before bacterial DNA synthesis stops. This indicates that the inhibitory protein is made only after one or several other proteins. The abolition of DNA arrest by exposing the phage to nitrous acid suggests that synthesis of the inhibitory protein may be dependent on a phage gene, whose identity is, however, unknown. DNA arrest is not prevented by mutation in five of the six known ØX cistrons: the sixth cistron, which specifies one of the proteins of the phage capsid, has still to be tested. The proteins involved in the arrest of host DNA synthesis

may include a capsid component or a product of a cistron so far unrecognized.

Another cause of inhibition of macromolecular synthesis in ØX-infected bacteria was found. It acts immediately, is independent of protein synthesis, and is more pronounced at increasing multiplicities of infection. Unlike the first process, it is not confined to host DNA, but affects also the formation of RNA, protein and phage DNA, and is produced even by phage killed with nitrous acid or ultraviolet irradiation. The cause is peripheral damage to the cell resulting from penetration of the phage DNA, which is sufficient to alter cell permeability, as demonstrated by a microfluorescence technique.

Mycoplasma

The Antigens of M. hominis. Dr. Lemcke and Mr. Hollingdale continued their studies of the antigenic structure of *Mycoplasma hominis*, a potential pathogen of the human urogenital tract. Of the cell fractions obtained by physical methods, the cell membrane (i.e., cell contents not sedimentable at 100,000G) rather than the soluble fraction is active in sensitizing tanned erythrocytes in the indirect haemagglutination test, and in adsorbing haemagglutinating antibody from antisera against whole cells of *M. hominis*. Moreover, the properties of antisera prepared against membranes purified on sucrose density gradients and against the soluble fraction suggest that components of the cell membrane induce the formation of growth-inhibiting and indirect haemagglutinating antibody. Thus, the antigens associated with indirect haemagglutination as well as with growth-inhibition (Report 1968) seem to be located in the cell membrane.

In view of the serological importance of the membrane, active membrane components were extracted by various methods. Neither *n*-butanol nor *n*-pentanol, used to extract proteins or lipoproteins from mammalian red cell ghosts, was effective with the membrane proteins of *M. hominis*, and the lipids extracted in the organic phase, like those derived from whole cells (Report 1968), had very little serological activity. However, material active in complement-fixation and indirect haemagglutination tests was obtained from the phenolic phase after extraction with cold aqueous phenol. In gel-diffusion tests, it gave a double precipitin line identical with that given by detergent-lysed membrane. It remains to be determined whether the material extracted in this way is still immunogenic; other less drastic methods of extraction are being investigated.

One of the two main precipitating components of the soluble fraction was separated on Sephadex G150; it has still to be characterized. The other has not so far been obtained free from minor components.

Nucleic acid homology tests by other workers suggest that strains classified as *M. hominis* on serological and biochemical grounds are heterogeneous. Moreover, some serological tests, notably agglutination and metabolic-inhibition, indicate that they differ antigenically. Gel-diffusion tests on fractions of the genital strain already analysed antigenically and of two oral strains revealed that intraspecific differences were confined to the membrane fractions; soluble fractions from the three strains gave identical precipitin lines, but detergent-lysed membranes gave substantially different precipitin patterns. This suggests that previously observed serological differences, including those concerned in metabolic-inhibition tests, depend on the membrane antigens.

The Identity of M. histotropicum and M. pulmonis. Dr. Lemcke examined Sabin's murine Type C mycoplasma, *M. histotropicum*, the taxonomic position of which has been in doubt since her earlier observation that it reacted serologically with antisera to *M. pulmonis*, another rodent mycoplasma. Complement-fixation, growth-inhibition and gel-diffusion mirror tests with antisera to Type C and *M. pulmonis* confirmed the close relationship of the two. There is no justification, therefore, for classifying this organism as a separate species.

TRACHOMA AND INCLUSION BLENNORRHOEA (CONJUNCTIVITIS)

The trachoma and inclusion conjunctivitis micro-organisms of the *Chlamydia* group are referred to as TRIC agents. Variants

that in a given dose kill chick embryos more quickly than their parent strains are *f* (fast-killing) strains, and the parents are *s* (slow-killing) strains.

Replication in Cell Cultures. Although TRIC agents undergo a complex reproductive cycle within the host cell, attention has centred mostly on the extracellular elementary body; the biochemical and immunological reactions in the earlier intracellular stages have been largely ignored. Nevertheless, by contrast with the active synthesis within inclusions early in the infective cycle, the low metabolic activities of elementary bodies in the extracellular state suggested that it might be more useful to regard them as analogous to bacterial spores; with this in mind, Dr. Taverne and Dr. Blyth investigated the early intracellular stages of development and their relationship with the host cell. To improve efficiency of infection and synchrony of growth, baby hamster kidney (BHK-21) cells were infected by centrifugation (Report 1968); an alkaline culture medium and an adequate concentration of bicarbonate ion were important for the growth of inclusion bodies.

With Dr. Lawn, the response to infection of acid phosphatases in the host cell was investigated by light and electron microscopy. Many small vesicles surrounded early inclusions; their significance is unknown but it seemed that their contents were being discharged into the inclusions. Acid phosphatase was not detectable within inclusions, but neither was it readily demonstrable in BHK cells by the histochemical technique used. By contrast, macrophages are rich in acid phosphatase; but although they have been reported to support the growth of some chlamydiae, several strains of TRIC agent failed to form inclusions in mouse peritoneal exudate cells. The elementary bodies were destroyed by these macrophages unless more than 100 per cell were inoculated; such concentrations sometimes destroyed the cells. There was no serological evidence that the mice had previously been infected with a chlamydia.

TRIC agent inclusions contain a carbohydrate that stains brown with iodine, and is probably glycogen. Its origin and significance are unknown, but the observations of Dr. Reeve and Dr. Taverne on its time of synthesis make it unlikely that, as has been claimed, it forms an energy reservoir for the synthesis of elementary bodies. Furthermore, its production and that of elementary bodies are independent, since concentrations of penicillin that completely inhibit formation of elementary bodies permit the synthesis of carbohydrate, although possibly in altered form. Continuing this research, Miss Barton is investigating the influence of various inhibitors of TRIC agents.

Dr. Taverne and Mr. Harris of Bedford College, London, studied the influence of penicillin on the developing inclusions. Penicillin added to the medium after adsorption of TRIC agent in concentrations of 0.01–0.1 units/ml. inhibited the formation of elementary bodies, but the number of inclusions formed was not decreased until at least 25 units/ml. were present; above this level, increasing concentrations caused proportionate falls in the numbers of inclusions. During the 8 hr. period after adsorption, the later the penicillin was added, the fewer the inclusions that were formed. This investigation continues.

Mrs. Mogg and Professor Collier continued their research on indirect fluorescent antibody staining for studying the growth of TRIC agent in BHK-21 cells. Initial bodies were identifiable with certainty 10 hr. after infection. Thorough washing removed all stainable material from inclusions containing these early forms—and from the abnormally large initial bodies induced by penicillin—suggesting that antigen formed early in the cycle is readily soluble. By contrast, the elementary bodies formed late in the cycle would still stain after washing, although with somewhat less intensity.

Dr. Blyth and Dr. Taverne are collaborating with Professor R. Nichols (Harvard University) in an investigation, by immunofluorescence techniques, of the development of antigens within the inclusion during the intracellular growth cycle.

These researches were greatly assisted by a weekly supply of BHK-21 cells from Mr. T. Battersby of the M.R.C. Laboratories, Carshalton.

Immunological Studies in Guinea-pigs. Dr. Blyth continued his work on the use of guinea-pigs for studies of immunity to TRIC infection (Reports 1967, 1968). A test was devised in which the doses infecting the spleens of 50 per cent. of inoculated animals were compared in normal and immunized guinea-pigs. For much of the year, the investigation was seriously hampered by a diminution in susceptibility to TRIC agent of the embryonate eggs used to titrate infectivity; the cause of this phenomenon, which has been observed elsewhere, is unknown. With the aim of avoiding assays in chick embryos—normally the most sensitive method for titrating small amounts of TRIC agent—Dr. Blyth is attempting to increase the efficiency of titration in cell cultures by staining the inclusions with fluorescent antibody rather than Giemsa.

Studies on allergy to TRIC agents were extended to show that a live *f* strain injected intracutaneously in guinea-pigs induced a high degree of hypersensitivity; by contrast, 1000-fold greater doses of a live *s* strain induced only a weak hypersensitivity, no more than that induced by heat-killed organisms of either strain. This finding may reflect the greater ability of *f* strains to multiply within the guinea-pig (Report 1966). Both *f* and *s* strains caused similar lesions in animals sensitized with *f* strains. This study is being continued, because induction of hypersensitivity by trachoma vaccines may be related to their efficacy in preventing infection.

Trachoma Vaccine. Mrs. Mogg and Professor Collier began a study of the antibodies induced by immunization or infection with TRIC agents. In sera from immunized rabbits, fluorescing antibody (FA) and complement-fixing (CF) antibody were predominantly associated with the IgG immunoglobulin. Sera from baboons inoculated parenterally with live MRC-4 or MRC-4 *f* (Report 1967; 1968) were assayed for CF antibody; the titres bore little relation to the degree to which these TRIC agents had multiplied within the hosts, or to the state of immunity of individual animals. The association of FA and CF antibodies with various classes of immunoglobulin is now being studied with a view to clarifying their relationship to immunity, and to a qualititative and quantitative determination of the humoral responses to primary and secondary antigenic stimuli.

Aetiology of Crohn's Disease. The aetiology of this syndrome ('regional ileitis') is not known. Infection with a chlamydia has been suggested because the pathological changes have features in common with trachoma, and because some species of *Chlamydia* are known to infect the gut of certain animals. Mrs. Mogg attempted to isolate this micro-organism from 9 patients, using biopsy material provided by Dr. D. Wright (King's College Hospital); the results of chick embryo and cell culture inoculations were all negative. Sera from these patients and from 60 others with Crohn's disease or ulcerative colitis contained no antibody to TRIC agent detectable by immunofluorescence or complement fixation.

Investigations Overseas. Under Professor Collier's direction, researches were continued in the Gambia.

Dr. Shiona Sowa and Mr. J. Sowa previously isolated TRIC agent from the eyes of 4 infants born in an urban hospital, and from the urethra of one of the fathers (Report, 1968). The pair of strains isolated from the father and his baby were typed by the mouse toxicity prevention test by Drs. J. T. Grayston and K. S. W. Kim at the University of Washington, Seattle, U.S.A. Both fell into subgroup F, the members of which have all been isolated from sources other than classical trachoma (i.e. punctate keratitis, neonatal conjunctivitis and the adult urogenital tract). This finding supports the inference that the infant's eye became infected from the parental genital tract at birth. It is also noteworthy that the infant's strain caused lesions characteristic of trachoma rather than of inclusion conjunctivitis.

Mr. and Mrs. Sowa continued their study of immunofluorescence techniques in the serology and diagnosis of trachoma. In tests by the indirect method, IgG antibody to TRIC agent was not found in the conjunctival secretions of 11 normal subjects, but was detected in low titre in secretions

from 21 of 28 trachoma patients; there was a pronounced association between its presence and that of inclusion bodies in the conjunctival epithelium. This antibody was also present in the blood of 26 of the trachomatous subjects. The investigation is now being extended to include tests for IgA antibody to TRIC agent, with the aid of a generous gift of the necessary anti-IgA conjugated globulin from Dr. W. D. Brighton (National Institute for Medical Research); and to a study of the relationship between conjunctival antibody and corneal vascularization in trachoma.

By staining with iodine, conjunctival inclusions are demonstrable in less than 50 per cent. of patients with trachoma, and any method of increasing this proportion would be welcome as a diagnostic aid. The merits of staining with iodine or with fluorescein-labelled antibody were compared on replicate conjunctival scrapings from 36 trachoma patients; each method separately detected inclusions in the same 12 patients and each gave 3 additional positive results, not confirmed by the alternative technique. In this small series, which is being enlarged, the discrepancies could be ascribed to sampling variation; so far, immunofluorescence microscopy holds no advantage over the simpler iodine method.

VIROLOGY

Infectious Hepatitis

The causal agent of infectious hepatitis is probably a virus, but has not been conclusively indentified. Dr. Alwen found previously that in all of a small number of sera from patients with this disease there were high titres of antibodies neutralizing adenovirus type 5, which commonly infects the human upper respiratory tract; these observations are now being extended. It is unlikely that adenovirus is directly responsible for the liver necrosis characteristic of infectious hepatitis; but Dr. Alwen is investigating the possibility that an adenovirus antigen may damage liver cells, and thus induce an autoimmune reaction leading to necrosis. This possibility would be greater if there were an antigenic similarity between liver cells and adenovirus antigen; rabbit antisera to human foetal liver are being investigated from this point of view.

Vaccinia Virus

Hypersensitivity. Inactivated measles vaccine is reported to induce hypersensitivity. Miss Cox and Dr. Alwen began an investigation to determine whether this is true of smallpox vaccine, and if so, whether the allergenic component can be identified. In preliminary experiments, live vaccine prepared in sheep induced skin lesions characteristic of a hypersensitivity reaction in guinea-pigs previously sensitized to the same vaccine. Adaptation of vaccinia virus to growth in guinea-pig cells is now being attempted; the use of such a strain would rule out the possibility that the skin lesions represented a reaction to a sheep component.

Inactivated Vaccine. Dr. Turner continued to study the inactivation of vaccinia virus. A mouse protection test, with a neurotropic strain of virus given intracerebrally, gave more consistent results than the tests with an intranasal challenge used previously (Report 1968); and the immunogenicity of vaccines inactivated by a variety of methods was determined. The results are being correlated with those obtained, with the same vaccines in rabbits, by measuring antibody production and the response to challenge by the cutaneous route.

Virus Assay. Dr. Murray continued his work on methods of assay of vaccinia virus in tissue culture. A micro-method is under investigation; the results will be compared with those obtained by plaque counts in chick cells and pock counts in chick chorioallantoic membranes.

Composition. Dr. Robinson continued his investigations on the chemical composition of vaccinia virus. The absence of both copper and flavin adenine dinucleotide from highly purified preparations of vaccinia virus was established. They were present only in cruder preparations of vaccinia virus and are probably host-tissue contaminants.

Both double- and single-stranded DNA of vaccinia virus were isolated by partition in an aqueous, two-phase polymer system and purified by counter-current distribution. It is not yet clear whether the single-stranded DNA exists in this form in the intact virus particle or is an artefact of the isolation procedure. The infectivity of the DNA preparations is under investigation.

The lipids of the virus were isolated; seven phospholipids and four neutral lipids were found. Of the phospholipids six were provisionally identified as cardiolipin, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl choline, sphingomyelin and lysophosphatidyl choline; no cholesterol was found.

Work has begun on the nature of the protein coat of the virus particle.

Growth in Tissue Culture. Dr. Murray began work on the large-scale cultivation of vaccinia virus in the Human Diploid Cell Strain W138, in chick embryo cell cultures and on the chick chorioallantois.

Rabies Virus

Dr. Turner and Dr. (now Professor) Kaplan completed their studies on the removal of the encephalitogenic factor from rabbit brain preparations of virus. Tests are under way of the antigenic potency, resistance to lyophilisation and storage of two experimental batches of purified rabies vaccine.

Rabies virus is credited with some of the characters of the myxoviruses. Mucoproteins may therefore play a role as virus receptors in the pathogenesis of rabies. Dr. Turner tested the effect of a number of potent myxovirus inhibitors on rabies virus. Some salivary mucoids inhibited infectivity, collocalia mucoid gave variable results and ovarian cyst mucoids were without effect. The most potent inhibitors were commercial samples of equine α globulin. Preparations of similar material from Elstree horses, and commercial samples of human α, β, γ globulin and bovine α globulin, did not inhibit. The inhibitory action of the equine α globulin was not affected by potassium periodate, receptor-destroying enzyme, heat or dialysis. Inhibitory activity was not associated with large particulate material or blood pigment present in the preparation.

IMMUNOLOGY AND EXPERIMENTAL PATHOLOGY

Antitoxin Production

The method of preparing highly purified diphtheria antitoxin by pepsin treatment of toxin-antitoxin floccules (Reports 1967, 1968) was modified by Dr. D. E. Dolby for the purpose of large-scale production. He also began an investigation of the separation of toxin from antitoxin in floccules, by dissolving them in solutions of urea, sodium salicylate and other compounds, and subsequent chromatography on Sephadex or cellulose ion-exchangers.

The attempt to use cyanogen bromide to split antitoxin into fragments retaining biological activity (Reports 1967, 1968) was unsuccessful. The methods for both the separation of antitoxins from the other proteins of horse sera and defining the purity of the fractions obtained were improved. Work also began on the fractionation of the immunoglobulins in mouse sera.

Anaphylaxis

An Eosinophil-stimulating Factor in Anaphylaxis. Dr. Parish resumed his investigation on the factors causing the sudden rise in the number of eosinophils in the peripheral blood and some organs in human anaphylaxis. Eosinophilia in the skin or lungs may be induced non-selectively by substances attracting all leucocytes, when the number of eosinophils infiltrating the tissue reflects that in the peripheral blood, or it may be induced selectively by substances attracting eosinophils only, when the eosinophilia is independent of the initial number in the peripheral blood (Reports 1964, 1965).

In guinea-pigs, the eosinophilotactic properties of antigen-antibody complexes depend on the type of antibody (Report 1965). Complexes containing γ_2 globulins, which do not sensitize homologous tissues anaphylactically, attract both eosinophils and neutrophils *in vivo* and *in vitro* and are therefore non-selective. Complexes containing γ_1 globulins, which sensitize tissues anaphylactically, selectively induce eosinophilia *in vivo*, though their attraction for these cells *in vitro* is weak.

Miss Smithson and Dr. Parish are attempting to identify the eosinophilotactic substance released from the tissues by the active complexes.

Exogenous Antigens Acquired by Tissue Culture Cells

The possibility that incorporation of bacterial or fungal antigens into cells of the skin may predispose them to a damage by specific antibody that is manifested as a generalised eczema (Reports 1966, 1967) was further investigated by Dr. Parish, who used primary cell cultures and antibody prepared in the homologous species.

The age of the primary cultures proved to have an important influence on susceptibility; cells 10-14 days old adsorbed less antigen and were less susceptible than those 5–7 days old to damage by antibody.

Primary cultures of guinea-pig and mouse kidney cells were less susceptible than HeLa cells. The release of acid phosphatase and lactate dehydrogenase was used as a sensitive indicator of cell damage. More enzymes were released from cells which had adsorbed pure protein antigens or whole extracts of *Staphylococcus aureus*, when tested with specific antibody than when tested with normal serum. A surprising number of sera containing no specific antibodies were toxic, even to cells without acquired antigen.

Drs. McConnell and Parish in this way tested mouse macrophages and kidney cells with tetanus toxoid. The cytotoxic changes were slight, and insignificant amounts of nucleic acids and enzymes were released.

Antibodies to Bacteria in Generalised Eczema

Dr. Parish, in collaboration with Drs. R. H. Champion and E. Welbourn at Addenbrooke's Hospital Cambridge, applied *in vitro* tests on the susceptibility to antibody of cells with acquired antigen, to an examination of sera from patients with generalised eczema.

Sera, from normal people and from eczematous patients, containing antibodies to *Staphylococcus aureus* and micrococci (Reports 1967, 1969), were added to primary cultures of human skin and foetuses previously exposed to extracts of the bacteria. The cells were damaged only by sera containing sufficient IgG complement-fixing antibody, irrespective of whether they came from normal or eczematous persons. If a cytotoxic reaction of this type causes eczema, then the susceptibility to the disease of the eczematous subject does not therefore depend on his having a special kind of antibody, but on the presence of the appropriate antigen in his cells—which may or may not depend on an as-yet-undetermined peculiarity of the cells that adsorb the antigen.

Human Infertility

Dr. Parish and Miss Smithson, in collaboration with Dr. A. Ward of St. Mary's Hospital, investigated the antibodies in uterine mucus cytotoxic to spermatozoa (Reports 1964, 1968). The mucus proved to contain IgA, IgM and β_1C globulins more frequently than cervical mucus. The mucus also had full complement activity, as indicated by the lysis of sensitized red cells. Clearly spermatozoa sensitized by cytotoxic antibody in the cervical mucus may be inactivated and possibly lysed on penetrating to the uterine lumen. *In vitro*, sensitized spermatozoa were lysed as readily by uterine mucus as by serum complement.

They confirmed their previous results, that the IgM antibodies to seminal plasma antigens present in serum or cervical mucus were harmless to sperm; and were sometimes present in pregnant women.

Electron Microscopy

Ovum Implantation in the Mouse. With Dr. C. A. Finn of the Royal Veterinary College, Dr. Lawn's work was directed to later stages of implantation, particularly days 6 and 7. At this time the basement membrane of maternal epithelium, *pari passu* with that of the uterine lumen, becomes attenuated and is absent in places. The occasional contacts seen between endothelial cells and decidual cells are similar in structure to those between decidual cells (Report 1967). The trophoblast covering the blastocyst splits into two layers or becomes canalized, particularly laterally. Maternal erythrocytes are found in these trophoblast-lined spaces, as well as in the remains of the uterine lumen (between decidual cells and trophoblast) and sometimes within the blastocyst. Isolated parietal cells and a thickened Reichert's

membrane now cover the thin internal layer of the trophoblast, whereas the outer layer has, in many places, differentiated into primary giant cells, which are invading the uterine wall. The giant cells extend numerous long tenuous processes, which seem to be guided by extensive contacts with decidual cells, eventually forming a complete lining to the spaces between decidual cell processes. The contacts are unspecialized, in contrast to those between decidual cells, which are more elaborate on day 7 than on days 5 and 6. Decidual cells are not destroyed by trophoblast at this stage of implantation.

Decidualization in the Human Endometrium. In collaboration with Dr. C. A. Finn (Royal Veterinary College) and Dr. E. Wilson (Jessop Hospital, Sheffield), the ultrastructure of the human and the mouse decidual cell is being compared. In women predecidual cells are found late in the menstrual cycle. The structure of these transforming endometrial fibroblasts is similar to that of mouse pre-decidual cells on days 4 and 5 of pregnancy. There are some contacts between cells, a few of which are "tight" junctions. In women 8 weeks or 3 months pregnant, decidual cells bore only a slight resemblance to those of mice. Intercellular material (collagen, reticulin and connective tissue matrix) is so abundant, and decidual cells processes although present are so short, that intercellular contact is almost precluded. Nevertheless tight junctions are found between these processes at the surface of decidual cells: serial sections show that some, if not all, are between processes from the same cell. In the mouse, serial sections have confirmed that the majority of such junctions are intercellular. It is difficult to imagine a function for the tight junctions of human decidual cells, but their presence indicates that the surface properties of these cells resemble those of rodent decidual cells.

Replication of TRIC Agent in Cell Cultures. As reported on page 20, in collaboration with Dr. Blyth and Dr. Taverne, some ultrastructural changes in cells infected with trachoma organisms were investigated.

The facilities of the electron microscope unit were made available to the following members of staff.

Dr. F. R. Wells: structural alterations in microcirculatory system after thermal injury and injury by *Clostridium welchii* α toxin.

Dr. Jennifer J. Wells: assessment of various methods for isolating liver cells with minimum damage.

Dr. Taylor and Dr. Godfrey: the structure of trypanosomes.

Dr. Elinor Meynell and Miss Frydman: assessment of antigens used to raise antibody to bacterial sex pili, and investigation of pili produced by bacteria with mutant sex factors.

Professor Meynell and Miss Aufreiter: examination of adsorbtion of sex phages to mutant bacterial sex pili.

Dr. Ackers: structure of the spheroplasts of *Bordetella pertussis.*

Miss Dod: purity of microsomes and other membrane fractions of mammalian cells.

Dr. Lemcke and Mr. Hollingdale: effect of detergents on membrane fractions of *Mycoplasma hominis.*

Mechanisms of Inflammation

The Tuberculin Reaction. Dr. Wells completed his vascular studies of the tuberculin reaction in the dorsal skin of hypersensitized rats and guinea-pigs (Reports 1966–1968). It is now clear that the initial, largely venular, phase of the response in both species is nonspecific except in a small number of guinea-pigs where there is a well-defined immediate increase of vascular permeability. In rats a maximum response in the delayed phase of increased permeability always occurs in lesions about 15 hr. old. Carbon deposition is also at its maximum in the capillaries of the superficial dermis. Little carbon is deposited at 6 hr. and, in some animals, there was a slight deposition at 23 hr. The delayed phase of the permeability response began in rats at about 7 hr. and rather earlier, sometimes at 4 hr., in guinea-pigs.

Standardization of Carbon Suspensions. Commercial preparations used for tests of carbon deposition on the walls of damaged vessels, concentrated as previously described (Report 1968), were standardized for carbon

content by a gravimetric method with an error of 1.5 per cent. In guinea-pigs with skin thermally injured at 54C., it was found that intravenous doses from 150–250 mg. per kg. body weight were required for a maximum labelling of damaged capillaries. At less than 150 mg., the labelling was only half, and at 95 mg., one sixth as effective. The volume of the untreated preparation, which contains some 75–110 mg. carbon/ml., needed to obtain optimum intravenous dosage may thus be too high for use in either rats or guinea-pigs, and in any event is toxic to guinea-pigs unless previously purified by dialysis.

Monocytogenic Factor in Delayed Hypersensitivity. The results of the test made last year by Dr. Wells (Report 1968) of the transplacental passage of the presumably auto-antigenic factor that induces monocytosis in the delayed hypersensitive state, were re-examined. More precise blood film counts were made, of monocytes and small lymphocytes, and of the monocytic Kurloff cells. The poorer response to stimulation by Freund's incomplete adjuvant, of the offspring of tuberculin sensitive pregnant guinea-pigs, compared with that of offspring of normal guinea-pigs, was confirmed. The mean monocyte count on the test animals was 47 per cent. of that in the controls ($P>0.01$). The population of Kurloff cells was similar in both groups. A more extensive experiment is at present under way. The examination of Kurloff cell counts in monitoring the state of the blood in both male and female offspring has confirmed Ledingham's (1940) original observation that these cells are oestrogen-dependent.

Activation of the Kininogenetic System in Plasma. Miss Mason continued her studies of the pathways in the *in vitro* mechanism of the production of the kininogenases and vaso-active kinins which follow the activation of surface factor in mammalian plasma. Previous work supported the hypothesis of a cascade of enzyme reactions set off by contact with glass surface resulting in the digestion of globulin substrates (kininogens).

Investigations of the inhibitors that impose restraints on the system, confirm that human plasma deficient in the clotting enzyme Hageman Factor contains a heat-stable inhibitor in an active form, which adsorbs to foreign surface and effectively prevents the generation of both permeability factors and kinins in normal human and in guinea-pig plasma. The substance is distinct from a heat-stable inhibitor that is rapidly and non-enzymically produced from a precursor during the activation of the kininogenetic system, and inhibits activated surface factor in both human and guinea-pig plasma. Both inhibitors are distinct from the slow-acting inhibitor of the permeability factor, PF/dil. Plasma discharged of kinins by surface activation (termed component-B-depleted plasma by Margolis) does not produce kinin on a second exposure to clean glass, even though it contains surface factor, kinin-forming enzymes and kininogen. To explain this phenomenon, Margolis postulates a kininogen particularly susceptible to kininogenase; and Vogt a multiplicity of independent kininogenetic systems variously affected in depleted plasma; the phenomenon is, however, explicable by the generation of an inhibitor of surface-factor.

The Kininogenetic System in Infection. The presence of free plasma kinin and the relative absence of the kininogen observed in some protozoan infections have been attributed to the activation of the kinin system by antigen-antibody reactions. Miss Mason continued to investigate the plasma kininogenetic system of guinea-pigs infected with *Trypanosoma brucei.* With increasing infection the plasma bradykininase activity decreased; and the content of total kininogen began to drop slightly, whereas that of the surface factor remained normal. Examination of the *in vitro* activability of the system was complicated by the appearance and increasing production of kinin-potentiating substances. Attempts to activate the system with washed trypanosomes were unsuccessful. In moderately infected animals the reactivity of the vascular bed of the dorsal skin to injected kinin and kininogenetic substances was normal, but that to histamine was consistently heightened.

Characterization of the Components of the Kininogenetic System in Human Plasma. To examine in detail the sequence of enzyme reactions in the system, Dr. McConnell and Miss Mason are fractionating human plasma into purified components. A method for isolating the enzymes using DEAE chromatography was devised. Preliminary results confirm the existence of kininogenases travelling with the γ globulins and of substances capable of activating the system, which are associated with the β and α globulins. The kininogenase fractions were successfully purified and concentrated. Since both PF/dil and surface factor are involved in early stages of kinin release, efforts are being made to separate them.

Antihistamine Assay. The use of a selective inhibitor is one approach towards identifying histamine as a mediator of vascular change in inflammation; and identification is more certain if several inhibitors are effective in the ratio of their experimentally-determined potencies. Miss Mason devised measures to estimate the relative antihistamine potencies of several drugs assayed in guinea-pigs and rabbits. Through the courtesy of the Department of Biological Standards of the National Institute for Medical Research, the data are being processed by the University of London Atlas computor; and analyses of variance of the assays, drug ratios and fiducial limits are being calculated according to the Medical Research Council's random bioassay programmes No's 5 and 10. Results available so far indicate that, though in general the drugs are less effective in the rabbit, there is a close similarity in the order of relative potency for both species.

Pharmacologically Active Substances in Human Plasma Fractions. Dr. Mackay and Miss Mason concluded the studies of the effects of solvent and mode of fractionation on the distribution and potency of the enzymes and substrates of the kinin-forming system in human plasma fractions (Report 1966). Though solvent-activated kinin-forming enzymes were found principally in the G2/1R fractions, kininogenases also contaminated all other fractions except for very pure fibrinogen that had been treated to remove plasminogen. Activators of the kinin system were also widely distributed.

BIOCHEMISTRY

Human Blood-Group Substances

Structural Studies. With the isolation of the Le^b specific determinant as the tetrasaccharide, *O*-α-L-fucosyl-(1→2)-*O*-β-D-galactosyl-(1→3)-[*O*-α-L-fucosyl-(1→4)-]*N*-acetyl-D-glucosamine (Report 1968), the nature of the structures responsible for the various specificities defining the ABO, H and Lewis blood group systems are now largely established. However, many of the fine structural details concerning the carbohydrate chains in the molecules of the specific glycoproteins remain to be elucidated. To fill in the picture more completely, the identification of fragments obtained by acid and alkaline degradation of the glycoproteins was continued during the year. Dr. Aston, Mrs. Hague, Dr. Donald and Professor Morgan obtained a tetrasaccharide, *O*-α-D-galactosyl-(1→3)-*O*-β-D-galactosyl-(1→3)-*O*-(*N*-acetyl-β-D-glucosaminyl)-(1→3)-D-galactose, from the products of hydrolysis of B substance with polystyrene sulphonic acid. This unit was B-active and its identification extends by one sugar residue the structure earlier proposed for the B determinant (Report 1965). Similarly, a trisaccharide, *O*-β-D-galactosyl-(1→3)-*O*-(*N*-acetyl-D-glucosaminyl)-(1→4)-D-galactose, isolated from H substance by Drs. Aston, Donald and Morgan, gave additional information on the glycosidic linkages that occur in the carbohydrate chains. Several trisaccharides containing the sugar sequence galactose-*N*-acetylglucosamine-galactose have been isolated and to the three pairs of glycosidic bond sequences previously described in these trisaccharides, namely (1→3) (1→3), (1→4) (1→3) and (1→4) (1→6), must now be added a fourth pair, (1→3) (1→4).

Acid hydrolysis of H substance yielded two other oligosaccharides. The first, a branched tetrasaccharide, had the structure *O*-β-D-galactosyl-(1→3)-*O*-(*N*-acetyl-β-D-glucosaminyl)-(1→4)-[*O*-α-L-fucosyl-(1→6)-]D-galactose. Fucose can thus be joined to sugar units in the carbohydrate chains by 1→6 glycosidic linkages as well as by the 1→2, 1→3 and 1→4 linkages already known. The second, a hexaose, most probably had the structure *O*-β-D-galactosyl-

(1→4)-*O*-(*N*-acetyl-β-D-glucosaminyl)-(1→6)-*O*-β-D-galactosyl-(1→3)-*O*-(*N*-acetyl-D-glucosaminyl)-(1→3)-*O*-β-D-galactosyl-(1→3)-*N*-acetyl-D-glucosamine.

Alkaline degradation of Le^a specific substance gave three interesting new oligosaccharides. The first, *O*-β-D-galactosyl-(1→4)[*O*-α-L-fucosyl-(1→3)-]*O*-(*N*-acetyl-β-D-glucosaminyl)-(1→3)-D-galactose, had no Le^a activity. Since the Le^a determinant is *O*-β-D-galactosyl-(1→3)-[*O*-α-L-fucosyl-(1→4)-]*N*-acetyl-D-glucosamine (Report 1965), the structure of the new fragment emphasizes the profound effect a small change in overall conformation has on specificity. The second fragment obtained by Dr. Marr, Dr. Donald and Professor Morgan was a crystalline trisaccharide, *O*-β-D-galactosyl-(1→4)-(*N*-acetyl-D-glucosaminyl)-(1→6)-*N*-acetyl-D-galactosamine; it is the first fragment from blood-group active glycoproteins in which two amino sugars are joined to each other. It most probably occupies that part of the carbohydrate chain most distant from the specific determinant and is involved in the linkage of the carbohydrate to the peptide moiety of the glycoprotein. A similar oligosaccharide was isolated in which a second molecule of galactose is attached as a branch structure to the *N*-acetylgalactosamine by a 1→3 glycosidic linkage, thus giving a tetrasaccharide.

Dr. Donald devised a quantitative method for the separation of glucosamine and galactosamine and their glycitols on the amino acid analyser. The reducing sugar unit in an oligosaccharide is usually determined by conversion to an alcohol. In oligosaccharides containing glucosamine and galactosamine the composition after reduction is difficult to determine because, although the two amino sugars can be separated on the amino acid analyser, the corresponding glycitols are inseparable from galactosamine. By changing the buffer from pH 5.28, the conventional pH, to pH 5.09 by 0.2*M* boric acid, the two amino sugars and their glycitols are eluted from the column in well spaced fractions.

Biosynthesis of Blood-group Substances. The step in the biosynthesis of blood group A specific structures under the control of the *A* gene is postulated as the addition of *N*-acetyl-D-galactosamine in α-(1→3) linkage to an H-specific structure by an α-*N*-acetylgalactosaminyltransferase. Dr. Hearn and Professor Watkins, who found the enzyme in the stomach mucosa of group A baboons (Report 1968) now find it in human tissues from group A_1, A_2 and AB persons, but not in B or O persons. Preparations of particle-bound enzymes made from homogenates of submaxillary glands and stomach mucosal linings, transferred *N*-acetyl[^{14}C]galactosamine from uridine diphosphate *N*-acetyl-[^{14}C]galactosamine to a number of oligosaccharide acceptors with a terminal non-reducing β-galactosyl residue substituted at C2 with α-L-fucose; as in H specific groupings (Report 1965). β-galactosyl compounds lacking the fucosyl substituent were not acceptors. Hydrolysis of the radioactive oligosaccharides by an α-*N*-acetylgalactosaminidase from *Trichomonas foetus* (Report 1964) confirmed the α-anomeric linkage of the added *N*-acetylgalactosamine.

Miss Race and Professor Watkins continued their investigations on the α-D-galactosyltransferase associated with the blood-group B character (Report 1968). The substrate specificity of the enzyme was more clearly defined with a wider range of low molecular weight oligosaccharide acceptors. Those containing an α-L-fucosyl residue linked (1→2) to galactose, namely 2′-fucosylgalactose, 2′-fucosyllactose, and the pentasaccharide, lacto-*N*-fucopentaose I, were good acceptors, whereas lacto-*N*-fucopentaose II, in which the L-fucose residue is on the subterminal N-acetylglucosamine, and lacto-*N*-difucohexoase I and lactodifucotetraose, which are substituted with two fucose residues on adjacent sugars, were poor acceptors. Evidently the addition of the α-D-galactosyl residue to the H specific structures may precede the addition of further L-fucose units. Miss Race also examined the metal ion requirements of the α-galactosyltransferase and is now investigating the solubilisation of the particle-bound enzyme.

Blood-group A and B substances are absent from the saliva and tissue fluids of persons who are non-secretors. Neverthe-

less the α-*N*-acetylgalactosaminyltransferase associated with the A character, and the α-galactosyltransferase associated with the B character, were found in submaxillary glands of persons of the appropriate group irrespective of their secretor status; observations that accord with the proposal that the secretor gene *Se* controls the biosynthesis of the substrate for the enzymic products of the *A* and *B* genes, namely the H active structures, and does not directly influence the expression of these genes.

The primary products of the *H* and *Le* genes are considered to be α-L-fucosyltransferases that catalyse the addition of L-fucose to different sites in a precursor glycoprotein to give H, Le^a and Le^b serologically active structures. Mr. Chester and Professor Watkins examined human stomach and submaxillary gland tissues for L-fucosyltransferases of defined specificity, using guanosine diphosphate L[^{14}C]fucose as the sugar donor. Particle-bound enzymes transferring L-fucose in α-(1→2) linkage to *O*-β-D-galactosyl-(1→3)-*N*-acetylglucosamine or *O*-β-D-galactosyl-(1→4)-*N*-acetylglucosamine to give compounds tentatively identified as the two H-active trisaccharides (Report 1965) were detected in ABH secretors but not in those of non-secretors. But both secretors and non-secretors had 4-α-L-fucosyltransferases that conveyed fucose to the C4 position of the *N*-acetylglucosamine residue in *O*-β-D-galactosyl-(1→3)-*N*-acetylglucosamine, forming the Le^a-active trisaccharide. With preparations from secretors containing 2- and 4-fucosyltransferases, a tetrasaccharide was formed, chromatographically identical with the Le^b-active tetrasaccharide from an HLe^b substance (Report 1968). Mr. Chester found in all the tissues examined 3-α-L-fucosyltransferases that added fucose to the C3 position of *N*-acetylglucosamine in *O*-β-D-galactosyl-(1→4)-*N*-acetylglucosamine. Although not associated with any blood-group specificity, 3-fucosyl linkages occur in fragments of blood-group specific glycoproteins (see this Report).

The presence has been established, in tissues from donors of the appropriate blood group, of glycosyltransferases with the specificities required for the formation of A, B, H, Le^a and Le^b active structures. The sequence of events in the synthesis of macromolecular blood-group glycoproteins remains to be established. To obtain information on this question Dr. Poretz examined the incorporation of [^{14}C]galactose from uridine diphosphate[^{14}C]galactose into endogenous B substance. Preparations from stomachs or submaxillary glands of group B donors, after incubation with the labelled nucleotide sugar, yielded on treatment with sodium deoxycholate, soluble material precipitating with B antibody. The radioactivity of the precipitate indicated the incorporation of [^{14}C]galactose into endogenous B substance present in the tissue preparation. ATP and a divalent metal ion, such as Mn^{2+}, were required for maximum incorporation of radioactivity. The addition of exogenous H substance did not stimulate the incorporation of radioactivity into material precipitable with anti-B serum. The failure to liberate with α-galactosidase more than traces of labelled galactose from the radioactive B substance indicates that most of the sugar is incorporated in β-linkages.

In another approach to the problem of biosynthesis Miss Ward and Professor Watkins are examining conditions for the incorporation of radioactivity from [^{14}C]-labelled, low molecular weight compounds, such as threonine and glucose, into blood-group active glycoproteins in human and baboon gastric mucosal slices. The most promising results are with tissue slices incubated in Eagle's medium with added [^{14}C]glucose. Radioactive material precipitable with specific blood-group antisera was detected in the culture medium and in the products of the tryptic digestion of various fractions of the tissue.

Blood-group Substances from Stomach Mucosal Linings. Miss Ward and Professor Watkins continued their investigation of the blood-group substances in baboon mucosa (Report 1968). A, B and AB specimens, each from pools of several stomachs, differed serologically from the human blood-group substances in ovarian cyst fluids in that they exhibited high H, Le^a and Le^b activity. The properties of specimens from single

baboons were similar, but high-activity, although common, was not invariably detected; all had very high Le^a activity. Another difference between the baboon and human blood-group substances was revealed by enzymic degradation. With the α-galactosidase from coffee beans, human B substance loses B activity and acquires H activity; baboon B substance also loses B activity but acquires no H activity. The difference might be attributable to the site of synthesis in the body. Accordingly, blood-group substances from human stomach mucosa were tested. They had the high H activity frequently observed in the baboon preparations, regardless of blood group, but only two of eight human specimens had high Le^a activity. H activity was enhanced by enzymic destruction of B activity; the absence of H activity from treated baboon substances indicates a structural difference peculiar to the species. The structures exposed in the baboon B substance when the terminal α-galactosyl residue is removed are under investigation.

Hybrid Glycoprotein Molecules. Dr. Sachs continued his investigations on the multiple specificities of blood-group active glycoproteins (Report 1968). In gel diffusion tests, rabbit antisera raised against an artificial antigen prepared from HLe^b substance gave two lines with the homologous blood substance. One antibody is partly removed by absorption with group O Le(a+b–) red cells, indicating that it is H antibody. It was wholly removed by an H substance lacking Le^b activity, leaving a specific anti-Le^b precipitating serum. This reagent was used, in conjunction with a plant anti-H precipitin from the seeds of *Lotus tetragonolobus*, to determine whether H and Le^b determinants are on the same molecule in an HLe^b substance isolated from an ovarian cyst. The results indicated that three populations of molecules are present; those having predominantly H specificity, those having both H and Le^b specificity and those having predominantly Le^b specificity.

Lipids and Biological Membranes

Though it is accepted that lipids are essential units in the structure of a biological membrane, the role of the lipid components in relation to membrane stability, structural integrity and function is not yet clear.

Work on the preparation of mammalian cell membranes and the analysis of their lipid components was continued by Dr. Gray and Miss Dod. The lipid compositions of the plasma membranes (PM) and endoplasmic reticulum (ER) of rat-liver cells was established. The purity of the ER, a subfraction of a microsomal fraction, was estimated by assaying relevant enzyme markers; the maximum contamination by PM fragments was about 10 per cent. Phospholipids accounted for 72 per cent. of the total lipids in the ER compared with 60 per cent. in the PM and 80 per cent. in the mitochondria. Sphingomyelin, a major component of the PM lipids (33 per cent.), accounted for only 3 per cent. of those in the ER. The cholesterol to phospholipid ratio in the ER was very low (0.05) and it is possible that the cholesterol was in the contaminating PM fragments. The fatty acids in the phospholipids of the PM were in general more saturated (double bonds/mole, 0.5) than those of either the ER (0.9) or the mitochondria (0.85). The high proportions of cholesterol, sphingomyelin and saturated fatty acids in the PM lipids suggest that the structure of the lipid phase in the membrane is more compact and rigid than that in the ER and mitochondria.

The glycosphingolipids in the PM accounted for about 0.4 per cent. of the total lipid. The major components were ceramide monohexoside and sulphatide (molar ratio, 4 : 1). Only traces of glycosphingolipids were found in the ER fraction and could be accounted for by the contaminating PM fragments. These results support the previous suggestion that the glycosphingolipids are exclusive to the plasma membrane of the cell.

Earlier studies indicated that the fluorescent compound 4-acetamido-4′-isothiocyanostilbene 2, 2′-disulphonic acid (SITS) might be a useful chemical marker for the plasma membranes of mammalian cells. Its usefulness depends on the assumption that it does not penetrate the membrane of the cell and label other intracellular proteins. Since recent work has shown that the cells used in the earlier studies were almost

certainly damaged in the course of preparation, the action of SITS on cells was re-investigated. Miss Dod found that cells which were only slightly damaged, in that their membranes were permeable to eosin, were also permeable to SITS. It therefore appears that SITS is useful only with viable cells.

Dr. Jennifer Wells continued her studies on the changes in permeability of the plasma membranes of rat liver cells that were induced by lipases and lipid soluble drugs. The assessment of a change in the permeability is based on the assumption that the membrane is initially intact. Unfortunately the membranes of rat liver cells prepared by accepted methods were invariably damaged, as judged by their permeability to eosin and by their loss (98 per cent.) of soluble enzymes. The preparation of consistently good cells is very much an art. The best method so far found consists of perfusing the liver with trypsin (0.1 per cent.) in a phosphate-buffered saline solution and incubating slices in the same solution, and harvesting the cells as they separate from the mass, over a period of two hours. The cells, when washed and resuspended in culture medium containing calf serum, were intact, impermeable to eosin, contained all their enzymes and were electron microscopically in good condition. A problem still to be solved is the rapid deterioration of the isolated cells, which precludes a study of the time-course of the permeability changes in the plasma membranes.

The concept that the polyene antibiotics act on the lipids in biological membranes and thereby alter membrane permeability has been supported by studies of their action in various artificial lipid systems. Dr. Wells studied the action of filipin, nystatin and amphotericin B on the plasma membranes of human red cells, mouse ascites tumour cells (strains SA1/A and BP8/C3H) and transplanted hepatoma cells (ethionine induced in rats), assessing their response by leakage of soluble enzymes or, for red cells, of haemoglobin, and by dye exclusion. The membranes of the ascites cells and red cells were 300 times as sensitive as hepatoma cells to filipin. But both ascites and hepatoma cells were less sensitive than red cells to nystatin and amphotericin B. In artificial lipid systems these drugs complex with cholesterol, so that the different responses of the cells to them may be related to availability and quantity of the cholesterol in the membranes. Experiments of this type can be exploited in two ways: a drug could indicate differences in the membrane structures of different cell types; or the reaction of a particular cell to a range of drugs could depend on their relative potencies.

Glycolipids

Dr. Gray and Mr. Hay continued their studies on the glycosphingolipids in mouse tissues. Several factors seem to affect the composition and the amount of the glycosphingolipids in mouse kidneys. The amount varies with mouse strain, for C57/BL and C57xA (F′ hydrids) contained 2 to 3 times the amounts in C3H, Balb/c and A strains. A major component in all strains was ceramide monohexoside sulphate (sulphatide). Rather surprisingly the difference in distribution of the four neutral glycosphingolipids was more pronounced between male and female of a particular strain than between different strains. In the C57/BL male the ceramide dihexoside (CDH) was a major, but in the female a very minor, component. The predominance of CDH in the male was reflected to a lesser extent in the other mouse strains examined; though in the C3H mice it was only slight, the major difference being the large amount of ceramide trihexoside (CTH) in the female as compared with the male. Our results suggest that the glycolipid composition may be controlled by sex-linked genes or hormones. The glycosphingolipid composition in the livers of male and female mice of C3H, C57xA, Balb/c and A strains were all similar.

Mr. Hay further investigated an apparently systemic effect of the BP8 ascites tumour on the kidney glycolipids of C3H mice. The composition of kidney glycosphingolipids in C3H males with tumours was similar to that in the normal male, but in females with tumours there was much less CTH than in normal females. There was also more ceramide monohexoside sulphate

and ceramide dihexoside sulphate in females with tumours than in those without, suggesting that sulphatide metabolism might be affected. However, the kidneys of C3H mice with and without tumours had a similar arylsulphatase content. The sulphatide synthesizing enzyme, 3-phosphoadenosine-5'-phosphosulphate (PAPS) is also being studied.

Attempts to relate tumour growth to change in kidney glycolipid pattern gave variable results. The glycosphingolipids in the livers of C3H females with tumours increased up to threefold as the tumour reached optimum size; but only insignificant changes occurred in males with tumours. However, the association between liver glycosphingolipids and tumour was not observed in every batch of mice. Studies on the effect of BP8, EL4, SA1 and FRED tumours on C3H, C57xA, A and Balb/c mice respectively suggest that ascites tumour cells only indirectly change the kidney glycosphingolipids; and the nature of the change appears to depend on the particular combination of tumour and strain and sex of the mouse.

Metabolism and Biosynthesis of Mouse Kidney Glycosphingolipids. Mr. Hay synthesized [^{14}C-U]-glucosyl-(1→1)-ceramide, [^{14}C-1]-galactosyl-(1→1)-ceramide and [^{14}C-1]-lactosyl-(1→1)-ceramide for use as substrates in studies on the biosynthesis of CDH, CTH and sulphatide. In addition, chemically pure tritiated [^{3}H]CTH and aminoglycolipid were prepared as substrates for the catabolic enzymes that may be present in the kidneys.

The occurrence of digalactosyl ceramide as the CDH in C57/BL mouse kidneys is of particular interest in relation to the biosynthesis of CTH and aminoglycolipid, in which glucose and not galactose is linked to ceramide. Lactosyl ceramide (CDH) is synthesised by the addition of galactose from UDP-galactose to glucosyl ceramide and, by analogy, synthesis of CTH could take place by the addition of a further galactose unit to lactosyl ceramide. The fact that digalactosyl ceramide is the only CDH in the tissue appears to contradict this supposition, unless a very rapid turnover prevents the accumulation of any lactosyl ceramide. Possible pathways of biosynthesis are being studied by Dr. Coles.

BIOPHYSICS

Human Plasma Proteins

Potentiation of Agglutination by Serum Albumin. The capacity of various albumin preparations to influence the agglutination of Rh-positive red cells coated with "incomplete" anti-D sera was studied by Dr. Goldsmith, Dr. Jones and Professor Kekwick. The apparent agglutinating titre of an anti-D serum correlated with the amount of albumin polymer present in several preparations of bovine serum albumin, as revealed by electrophoresis in polyacrylamide gel. Precipitation of a serum albumin preparation with ethanol increased both its polymer content and increased the potentiation titre of the albumin preparation. Similar effects were obtained with bovine and human serum albumin. The potentiating properties of the separated polymer are the subject of further study.

Structure of Human α macroglobulin. According to the conditions, treatment of human α macroglobulin with thiol reagents yields up to four components with sedimentation coefficients of 19, 15, 13 and 8.5S approximately (Report 1968). At pH 8.0, with 0.01M N-acetyl cysteine, the predominant products are 19 and 8.5S components. These were further separated by Professor Kekwick and Dr. Jones by molecular sieving through agarose gel monitoring the effluent by polyacrylamide gel electrophoresis.

The 19S component was little affected by retreatment with 0.01M or 0.02M N-acetyl cysteine at pH 8.0, suggesting a limited susceptibility of the disulphide bonds to these thiol concentrations. However, at pH 3.6 the 19S component isolated from the reduction products dissociates into four sedimenting species, unlike the native molecule, which in these circumstances yields only 12 and 19S species.
Fractions containing only 8.5S components, pooled and concentrated by vacuum dialysis, reaggregated into 13, 15 and 19S components. Since the liberated sulphydryl groups had been blocked with iodoacetamide, the reaggregation presumably arises

from hydrogen bond formation or from electrostatic interaction. Urea, added to inhibit hydrogen bond formation, induced some disaggregation of the faster sedimenting components at pH 8.0, but three components were still evident in 4M urea. Iodoacetamide was substituted by iodoacetate and ethylene-imine as a sulphydryl blocking agent, in the hope that they would contribute an extra negative or positive charge respectively for each sulphydryl group liberated during the cleavage. Neither affected the extent of reaggregation.

In order to establish the weight relationship of the 8.5S subunit and the intact macromolecule, it is essential to define conditions in which preparations remain monodisperse.

Some proteins dissociate into subunits when treated with organic acid anhydrides, such as maleic anhydride, which interact with the ϵ-amino group of lysine residues, replacing a positive by a negatively charged group. The change of charge induced is much greater with maleic anhydride than with the reagents mentioned above. The ultracentrifuge pattern of α macroglobulin treated with maleic anhydride showed one or two components that sedimented much more slowly than the native protein. It is not yet clear how the results are to be interpreted in terms of dissociation or uncoiling of the original molecule; the investigation continues.

Other Macromolecules

Blood-group Glycoproteins. Blood-group substances band in a caesium chloride gradient at equilibrium in the ultracentrifuge. Dr. Creeth and Dr. Denborough applied this technique to the preparation and characterisation of these materials. The glycoprotein in ovarian cyst fluids was successfully separated from contaminating proteins and cell debris. The glycoprotein was concentrated in a narrow zone in amounts adequate for subsequent studies. In view of the simplicity of this procedure and the absence of precipitation or freeze-drying steps, preparations so made were compared with those obtained by the phenol-extraction procedure. Two cyst fluids were studied, one B-specific and the other Le^a-specific, from which Professor Morgan, Professor Watkins and their colleagues had prepared a series of sharply-defined fractions. In confirmation of their findings of variable peptide-to-carbohydrate ratios in different fractions, the glycoprotein isolated from adjacent regions in the preparative tube banded at different buoyant densities in analytical density-gradient experiments, thus demonstrating that the different materials exist in the cyst fluid. The ability to define a characteristic buoyant density with high precision has proved intrinsically useful, but the apparent molecular weight is one of the most informative measurements. Since the distribution in the gradient depends both on molecular weight and buoyant density, a comparison of the apparent molecular weight with that determined conventionally gives a direct indication of the dispersity in density. For example, one fraction of weight-average molecular weight 1.2×10^6 gave an apparent molecular weight in the gradient of 0.14×10^6, indicating an unexpectedly large range of densities.

Sixteen cyst fluids were surveyed, four of each specificity (A, B, H, Le^a), the blood-group substances so isolated being analysed in a gradient of caesium sulphate. Some preparations showed only a single band and in others two or more components were evident; there was, however, no association of the number of components with blood-group specificity.

Molecular Weight Determination in Polydisperse Non-ideal Systems: Humic Acid. The interpretation of ultracentrifugal equilibrium measurements on systems which are both polydisperse in molecular weight and thermodynamically non-ideal is difficult because the effects of non-ideality counteract those of polydispersity—so that a substance may give a spurious appearance of homogeneity. One approach to this problem is to seek conditions where the effects are nearly in balance, when the calculation of apparent molecular weight is easy and correction for the non-ideality can be made; this approach was successful with blood-group specific glycoproteins (Report 1966). A second is to use the high-speed equilibrium technique, where the concentration is effec-

tively reduced to zero at some point in the cell: both number- and weight-average values of the molecular weight can then be measured at very low concentrations where non-ideality effects vanish.

Dr. Creeth and Dr. A. M. Posner of the University of Western Australia, Perth, tested the method with well fractionated samples of humic acid, the constituent chiefly responsible for the ion-exchange properties of soil. By taking rather elaborate precautions, the two kinds of average were measured at a concentration of about 0.2 mg/ml. There was a good correlation between the standard deviation of the distribution of molecular weight of the humic acids and both the type of soil and the method of extraction. The method therefore becomes a useful adjunct to the established techniques.

Reversible Denaturation of Proteins

It is now established that protein conformation is determined by the amino-acid sequence, but not that the native conformation universally represents a free energy minimum. For ribonuclease, among several proteins of similar size-range, the adequacy of the thermodynamic hypothesis was already known, so that the correct folding of the synthetic enzyme was in agreement with expectations. Ovalbumin, by contrast, has a chain about 4 times as long, and one of the highest proportions of hydrophobic residues found in monomeric globular proteins; accordingly it represents a very exacting case, where, if re-folding could be accomplished *in vitro*, there would be good grounds for believing the hypothesis to be universally valid. Dr. Creeth and Mr. Holt continued their investigations of ovalbumin with various denaturants (Report 1968); no conditions were found in which denaturation by detergents is reversible. Guanidine hydrochloride in high concentration was investigated. The course of denaturation and its attempted reversal were monitored by optical rotatory dispersion and sedimentation velocity measurements. Denaturation is much more extensive than in detergents, and the configuration of the protein approaches that of the random coil. Removal of denaturant results in aggregation, however, and although a limited degree of renaturation occurs, intermolecular hydrophobic bonds always form at a critical guanidine concentration. Thus, though negative evidence of this kind is not conclusive, ovalbumin may be a case where folding initiated during synthesis influences the final conformation.

Serum albumin has an even longer primary chain than ovalbumin, but a lower proportion of hydrophobic residues. Dr. Creeth and Dr. Sawyer continued their investigation (Report 1968) of its re-folding from the fully denatured and reduced state. The chief point of interest here is whether the 17 disulphide bridges are re-formed in a native configuration. The re-oxidised material described previously, although essentially monomeric, differed from the native protein in its sedimentation characteristics, which indicated a minor component exhibiting a concentration-dependent association-dissociation. One of the steps in the renaturation process entails a 100-fold concentration, which was accomplished by pressure dialysis: this may have a deleterious effect on the protein and other means of concentration, such as absorption on an ion-exchange column, are being investigated.

The Svedberg Ultracentrifuge

During the year the Svedberg analytical oil-turbine ultracentrifuge, installed in 1936 and in operation since, was dismantled. It was the first ultracentrifuge to be used in this country; only seven of these instruments were built. The equipment was presented to the Science Museum, South Kensington where part of it is being re-erected.

BLOOD PRODUCTS LABORATORY

Anti-D Immunoglobulin. The staff of the Laboratory continued their collaboration with the Department of Medicine, Liverpool University and the M.R.C. Working Party on the use of anti-D immunoglobulin for the prevention of iso-immunization of women during pregnancy. Mr. Wesley and Dr. N. C. Hughes-Jones of the M.R.C. Experimental Haematology Research Laboratory began an investigation of the stability of this antibody in solutions of different protein concentration. Preliminary results indicate that anti-D activity is unimpaired in 5 g. to 10 g. per cent. protein solutions

during storage at 4°C for at least 12 to 18 months.

Hypogammaglobulinaemia. During the year the M.R.C. Working Party on Hypogammaglobulinaemia, on which members of the Laboratory staff have served for many years, began to wind up its therapeutic trial. Until other arrangements are made by the Department of Health and Social Security, the Laboratory, in association with the Assistant Secretary of the Working Party, will continue to be responsible for the calculation and preparation of the doses of immunoglobulin for the patients on the trial.

Dr. Mackay and Mr. Vallet continued the investigation of the anticomplementary activity of immunoglobulin preparations, begun in 1967 in relation to reactions observed in patients in the therapeutic trial. In batches of immunoglobulin examined retrospectively (Report 1968), there was no association between the occurrence of reactions and either the presence of aggregated material or the degree of anti-complement activity.

Dr. Mackay and Mr. Vallet confirmed a direct relationship between anticomplementary activity and aggregation measured by molecular sieve chromatography. Freshly isolated immunoglobulin was not anticomplementary and contained no aggregates; nor did it become anticomplementary after freeze-drying or during storage for a period of 4 months in the freeze-dried state at −25°C., 4°C. and room temperature or, in solution, at 4°C. Anticomplementary activity developed slowly in samples during the first 28 days of storage at 37°C.; and rapidly during the subsequent 58 days.

Factor VIII. Dr. Ellis and Mrs. Stickley began an investigation of the various factors affecting the stability of Factor VIII and its isolation from plasma. The amount of Factor VIII obtainable by ethanol fractionation from plasma separated from whole blood and stored at 4°C. for 18 hours, is at least as great as by ether fractionation. Slightly poorer recoveries were obtained by ethanol fractionation of plasma separated and frozen to −35°C. within 4 to 5 hours of blood collection.

Dr. Ellis and Dr. Bidwell investigated the recovery of clotting factors from the supernatant remaining after the removal of anti-haemophilic globulin by cryoprecipitation. Fibrinogen and Factor IX suitable for clinical use were separable from such supernatants. A single volume of fresh blood can for therapeutic use thus yield red cells, anti-haemophilic globulin cryoprecipitate, fibrinogen, Factor IX, immunoglobulin and albumin.

Plasma Fractionation Laboratory, Haemophilia Centre, Oxford

Dr. Bidwell and her staff occupied the new Laboratory in August 1968.

BLOOD GROUP UNIT

The year was spent as usual in looking for new blood groups and applying the known systems in problems of human genetics.

The X Chromosome

X-mapping. Two large investigations, in Israel and in the Netherlands, into the relationship of the *Xg* locus to that for X-linked ichthyosis were finished and analysed during the year: they were in complete agreement in showing that the original estimate (Reports 1965, 1967) of the recombination fraction was too high, and in making the linkage one of the most solidly established in human genetics. Fifty-five Israeli families tested in collaboration with Dr. A. Adam, Dr. L. Ziprkowski and Dr. A. Feinstein of the Government Hospital, Tel-Hashomer showed the best estimate of the recombination fraction to be 0.105 with 90 per cent. probability limits of 0.06 and 0.16 respectively. A large Dutch kindred involving 147 members tested in collaboration with Dr. L. N. Went of the Department of Human Genetics, University of Leiden gave a best estimate of 0.115 with 90 per cent. probability limits of 0.06 and 0.24.

An Oxfordshire kindred with ocular albinism, ascertained by the M.R.C. Population Genetics Research Unit, was tested in collaboration with Mr. W. G. Pearce. The Xg groups segregated informatively and confirmed the linkage first found in Seattle: the

revised estimate of the recombination fraction is 0.165 with 90 per cent probability limits of 0.10 and 0.29.

That the loci for Xg and for angiokeratoma may be within measurable distance of each other has been suspected for some time (Reports 1964, 1965, 1968). Samples of blood from s Swedish family with this rare condition were sent by Dr. J. Lindsten of the Karolinska Sjukhuset, Stockholm and added support for the linkage: the odds are now 8.3 to one that the two loci are within measurable distance of each other. The present estimate of the recombination fraction is 0.24, but the 90 per cent. probability limits are wide, 0.15 and 0.40.

The locus for Duchenne's muscular dystrophy is not within measurable distance of Xg (Report 1964). In collaboration with Professor A. E. H. Emery of the Edinburgh Royal Infirmary and Professor C. A. B. Smith of the Galton Laboratory enough families have now been tested to show that the same can be said of the less severe Becker's type. It is not known whether Duchenne's type and Becker's type represent alleles: measurable linkage of Xg with one but not the other would have ruled out allelism, linkage with both would have made allelism likely. As it is, linkage with neither gives no information, though the problem serves as an example of another potential use of an X-linked marker, such as Xg.

Work with Dr. A. W. Eriksson of the Population Genetics Centre, Helsinki shows that the locus for retinoschisis is probably within measurable distance of *Xg*. For some time it has been a hopeful candidate for linkage (Report 1968).

Families with choroideremia and with a newly described X-linked form of Ehlers-Danlos syndrome showed that these two loci are not at any rate close to *Xg*. Somewhat hopeful of linkage with *Xg* is the locus for an X-linked form of mental retardation with hyperuricaemia.

X-Chromosome Aneuploidy. Samples of blood from patients with an abnormal X chromosome or the wrong number of X chromosomes continue to be sent for Xg grouping because the groups of the patient and parents can often show at just which division of gametogenesis the accident has happened. This aspect of the work was dealt with in the 1966 and 1967 Reports.

This year's collection included a sample of blood from an XXXX girl, a great rarity (sent by Dr. J. de Grouchy, of the Hôpital des Enfants Malades, Paris). The Xg groups of the parents and the girl suggested that the father's contribution had been an X which was rejected at the first mitotic division of the zygote, at which division five Xs may be too much to cope with. Such an interpretation would fit with XXXXX females being almost unknown while XXXXY males are relatively common.

Xg and X-Chromosome Inactivation. Whether Xg is involved in inactivation has been an open question since its recognition. The evidence recently gathered (in collaboration with Professor P. E. Polani of Guy's Hospital) from families with daughters lacking the long arm of one of their Xs neutralises the evidence of families with daughters lacking a short arm (Report, 1963) which appeared to support the short arm location of *Xg*. The two contradictory observations can only be reconciled on the assumption that *Xg*, at any rate when carried on an *abnormal* chromosome, is subject to inactivation.

Xg and Leukaemia. In collaboration with Dr. D. A. G. Galton and Dr. Sylvia Lawler of the Royal Marsden Hospital a survey has begun of the Xg groups of females suffering from chronic granulocytic leukaemia. The belief is growing that in such people all the circulating cells of myloid origin represent a single clone derived from one stem cell. If this is true, and if Xg is subject to inactivation, female sufferers should show a male distribution of the Xg groups, 66 per cent Xg(a+), and not the female, 88 per cent. Xg(a+). The numbers tested so far are too few to deviate significantly from either distribution.

If these female patients do prove to have the male distribution of Xg they will not only confirm the clonal theory but also establish that the *Xg* locus is subject to inactivation on a normal, as well as an abnormal, X.

The Autosomes

The Antigen Ul^a. In the description of this predominantly Finnish antigen (Report, 1968) mention was made that it had yet to be shown to be genetically independent of the Kell system. The difficulty was that variant forms of Kell, needed to show a genetical relationship, are rare in Finland. However, a search extended over the last year, in collaboration with Professor H. Nevanlinna and Dr. U. Furuhjelm of the Finnish Red Cross, Helsinki, disclosed three informative families which leave no doubt that Ul^a is controlled from the *Kell* complex locus or, less probably from a locus very close to it. The indications are strengthened that the *Kell* locus is as complicated as those responsible for the MNSs, the Rh and the P systems and that all four systems are based on the general plan of successive interaction of genes and substances proposed by Watkins and Morgan for the ABO, secretor and Lewis systems.

The Antigen En^a. An antibody, anti-En^a, was first described by Darnborough, Dunsford and Wallace at a meeting of the British Society for Haematology in Sheffield in 1965. The Unit was invited to join in the testing of what proved to be a second example, found in Helsinki in August 1968. A long and interesting investigation followed which confirmed and extended the Sheffield observations.

The antigen En^a is extremely common: no one lacking it was found outside the families of the two propositi, though many thousands were tested. The phenotype En(a–) is a recessive character: the red cells behave as if they had been treated by a proteolytic enzyme, as shown particularly by their peculiar MN and Rh reactions; further, they are agglutinated by a wide range of non-immune animal sera which do not agglutinate normal En(a+) cells, and they are agglutinated preferentially by certain seed extracts (for example, a preparation from *Sophora japonica* agglutinates En(a–) cells strongly, En(a+) heterozygous cells less strongly and normal En(a+) cells not at all). The common background of these strange reactions is, no doubt, to be found in the results of measurements, by Dr. U. Furuhjelm and his colleagues in Helsinki, which show that the sialic acid content and the electrophoretic mobility of En(a–) cells are greatly reduced, compared with normal En(a+) cells.

There is good reason to think that En^a is genetically independent of the ABO, MNSs, Rh and Duffy blood groups and of sex.

The two human examples of anti-En^a were found following transfusion, so were undoubtedly immune. Absorption of the Helsinki example with cells treated with certain enzymes suggests that this anti-En^a contains at least two types of antibody molecule.

With the help of Dr. Sachs, of the Institute, anti-En^a was successfully made in a rabbit by injection of En(a+) cells and subsequent absorption of the immune serum by En(a–) cells. Attempts to produce an antithetical antibody by injecting En(a–) cells into a rabbit were unsuccessful.

The Phenotype M^k. During the En^a work it was noticed that cells of the rare MNSs phenotype M^k gave reactions with Rh antisera and with seed extracts like those of En^a heterozygotes. This led to tests in Helsinki which showed that the M^k cells also had a lowered sialic acid content and electrophoretic mobility. This has opened up a promising field of investigation of MNSs reactions. There is good evidence that M^k and En are serologically and genetically distinct in spite of the peculiar physicochemical properties they have in common.

The physicians, cytogeneticists and blood bank technicians who sent the samples on which the work of the Unit depends are too numerous to be thanked by name. For the supply of reagents and for help in other ways the Unit is greatly indebted to the Regional Blood Transfusion Centres; the Blood Group Reference Laboratory; Spectra Biologicals, East Brunswick; Hyland Laboratories, Los Angeles; Laboratorios Grifols, Barcelona; Pfizer Diagnostics, New York and the Minneapolis War Memorial Blood Bank.

Once again the Unit thanks the Staff of the Institute for giving so many samples of their blood.

BLOOD GROUP REFERENCE LABORATORY

This Laboratory is responsible for produc-

ing, on behalf of the World Health Organization, international standards for certain blood-typing sera. Immunohaematologists, who for many years referred to the potency of blood-grouping antibodies in terms of arbitrary titres rather than in units, now realize the need for greater accuracy when producing anti-D immunoglobulin. This material, used therapeutically by injection to prevent the iso-immunization of Rh-negative women who have borne an ABO-compatible Rh-positive infant, must be administered in a suitable dose. To ensure adequate potency, anti-D sera from which the anti-D immunoglobulin is to be prepared are tested in parallel with the International Standard for Anti-D "Incomplete" Blood Typing Serum or an equivalent preparation. Members of the staff are also working with the British Committee for Standards in Haematology to devise minimum standards for blood-typing sera and to devise simple, rapid and reliable methods by which the reagents can be used.

In 1968 the Laboratory distributed to National Blood Group Reference Laboratories throughout the world copies of the first number of the International Panel of Donors of Rare Blood Types. Already this Panel has proved useful in enabling blood for a patient requiring blood in one country to be obtained from elsewhere. During 1969 copies of an enlarged Panel will be sent out all over the world, to make it even easier to locate blood of rare types when it is required.

Dr. Ikin was in 1968 the recipient of the Oliver Memoral Award, given in recognition of her outstanding work in the field of blood-group serology and she has continued her research into the complexities of the *MN* locus. Working in conjunction with colleagues in Switzerland, she examined 32 MN variants from Belgium, Finland, Papua, Sweden and the United States of America as well as from the United Kingdom. She also checked, using routine serological techniques, the potency and specificity of anti-D immunoglobulin produced in the Blood Products Laboratory and also in certain overseas laboratories.

Dr. Giles, who is assisted by Mr. Dawes, examined the sera of Rh-negative women who, six months previously had been injected with anti-D immunoglobulin, to see if she could detect anti-D therein. Dr Giles also screened sera received for the production of ABO-typing reagents for the presence of antibodies to low-frequency antigens and investigated what may be three "new" low frequency red-cell antigens referred to the laboratory.

Dr. Phillips, in conjunction with colleagues in Britain and overseas, continued her research on the production of fluorescein-conjugated antiglobulin reagents and attended an Immunofluorescence Round Table Conference held in London.

Miss Brazier, continuing her studies of Gm, Inv and ISf antigens discovered what may be three new serum antigens. She screened all red cell typing reagents handled in this Unit to exclude the presence of antibodies to serum antigens. She also screened 257 sera from East Africans known to be suffering from malaria to exclude the presence of platelet and leucocyte antibodies.

Dr. Goldsmith was concerned with the standardisation of blood grouping reagents and techniques by which they are used.

It has not so far proved possible to produce a standard preparation for anti-D "complete" blood-typing serum because the material is unstable. Dr. Goldsmith is examining this problem with a view to the possible stabilization of the antibody so that a standard can be prepared. In addition, Dr. Goldsmith, in conjunction with Dr. Phillips and Dr. Giles, has studied various ways of producing antiglobulin reagents. In this field, as in many others, the Laboratory has benefited greatly by the help that they have received from Professor Kekwick, Dr. Jones, and other members of the Institute staff.

Finally, the Governing Body thanks the whole staff of the Institute, scientific, administrative and technical, for the way in which they have co-operated to produce work of such distinction.

G. L. BROWN,
Chairman.

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THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Accounts 1969

THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Balance Sheet and Accounts

31 DECEMBER 1969

CHELSEA BRIDGE ROAD . LONDON, S.W.1 . 21 MAY, 1970

The Governing Body

Sir LINDOR BROWN, CBE, FRCP, FRS, *Chairman*
H. P. G. CHANNON, MP, *Hon. Treasurer*
The Rt Hon LORD BROCK, MS, FRCS
Professor D. G. EVANS, CBE, D SC, FRS
C. E. GUINNESS
The Rt Hon the EARL OF IVEAGH
Professor R. A. KEKWICK, D SC, FRS
Professor Sir ASHLEY MILES, CBE, MD, FRCP, FRC PATH, FRS
Professor A. NEUBERGER, CBE, MD, FRCP, FRS

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

Financial Report of the Governing Body

1. The Governing Body presents the accounts of the Institute for the year ended 31st December 1969.

2. **Results**

The General Fund Income and Expenditure Account shows the Income for the year as £261,175 compared with £242,111 in 1968. Expenditure amounted to £400,309 against £389,904 last year. The deficit for the year is £139,134 compared with a deficit of £147,793 in 1968. The capital fund has been reduced by the year's deficit of £139,134.

The first of four annual instalments of £75,000 from the Wolfson Foundation and of five annual instalments of £1,000 from the Grocers' Company have been added to the capital fund.

3. **Principal Activities of the Institute**

The Institute continued to carry out research work in connection with the prevention of diseases. It produces for sale sera, vaccines and virus vaccines, the profits from which are utilised for its research and experimental work.

4. **Exports**

Sera and vaccines to the value of £141,566 were exported from the United Kingdom during the year.

5. **Changes in Fixed Assets**

The movements in fixed assets during the year are set out in the table in note 1 on the accounts. The most important feature is the commencement of a new wing at Chelsea, the cost of which is expected to be about £365,000.

6. **Market Value of Interests in Land**

The market value of the Institute's properties is now in excess of the amount at which they are included in the Balance Sheet. In the opinion of the Governing Body such difference is of no significance as the properties are occupied for the purposes of the Institute's activities.

7. **Governing Body**

Mr. C. E. Guinness joined the Governing Body in July 1969. Other members who held office during the whole of the year ended 31st December 1969 were as shown on page 2.

8. **Average Number of Employees and their Remuneration**

The average number of persons employed by the Institute in each week during the year ended 31st December 1969 was 307. The aggregate remuneration paid or payable in respect of that year to these employees amounted to £371,603.

9. **Auditors**

The auditors, Cooper Brothers & Co., will continue in office in accordance with Section 159 (2) of the Companies Act 1948.

A. NEUBERGER
Acting Chairman

The Lister Institute of Preventive Medicine
BALANCE SHEET · 31 December 1969

1968			
£		£	£
174,714	FIXED ASSETS (*note 1*)		285,905
	INVESTMENTS AND UNINVESTED CASH (*note 2*)		
608,244	General	503,700	
204,321	Specific funds	213,621	
25,109	Bequest funds	23,330	
837,674			740,651
	CURRENT ASSETS		
93,950	Debtors	127,330	
57,232	Cash and Bank Balances	52,285	
151,182		179,615	
	Less: CURRENT LIABILITIES		
65,105	Creditors	63,420	
86,077			116,195
£1,098,465			£1,142,751
	Represented by		
572,726	CAPITAL FUND (*note 4*)		509,592
204,321	SPECIFIC FUNDS (*note 5 and 8*)		205,078
25,109	BEQUEST FUNDS (*note 6*)		23,330
7,039	SPECIFIC GRANTS AND LEGACIES UNEXPENDED (*note 7*)		6,445
289,270	INVESTMENT RESERVE (*note 8*)		398,306
£1,098,465	A. NEUBERGER, H. P. G. CHANNON — *Members of the Governing Body*		£1,142,751

The notes on pages 6 to 9 form part of these accounts.
Audit report on page 9

The Lister Institute of Preventive Medicine
INCOME AND EXPENDITURE ACCOUNT
for the year ended 31 December 1969

1968				
£		£	£	£
	INCOME			
181,929	Sales of sera, vaccines, virus vaccines (*note 9*)			209,758
	Investment Income:			
	General fund			
40,687	Quoted	33,233		
2,804	Unquoted	2,460		
			35,693	
8,268	Sinking fund—quoted		7,256	
				42,949
378	Underwriting commission (*less income tax £132*)			38
8,045	Rent			8,430
242,111				261,175
		Total expenditure	*External contributions*	
	EXPENDITURE			
206,650	Salaries and wages	383,802	154,698	229,104
12,806	Premiums on federated superannuation policies	19,580	6,101	13,479
4,560	Premiums on group pension policy	6,058	1,657	4,401
9,458	Rent, rates and insurance	10,158	—	10,158
19,673	Gas, water, fuel and electricity	25,939	6,241	19,698
7,960	Office expenses, stationery and printing	12,105	1,175	10,930
630	Audit fee	1,070	—	1,070
—	Interest on overdraft	803	—	803
3,409	Travelling expenses	5,872	1,787	4,085
2,616	Biochemistry expenses	8,376	6,176	2,200
3,922	Microbiology, immunology, experimental pathology and electron microscopy expenses	9,802	5,921	3,881
1,522	Biophysics expenses	1,674	548	1,126
1,635	Virology expenses	2,359	1,162	1,197
—	Blood products laboratory expenses	10,912	10,912	—
53,455	Serum vaccine and virus vaccine expenses	34,962	138	34,824
11,573	Animals	12,433	1,805	10,628
13,306	Animal house expenses and forage	13,955	2,495	11,460
1,489	General apparatus and new installations	1,196	—	1,196
3,094	Library expenses	4,566	100	4,466
945	General stores	1,181	—	1,181
3,734	Staff canteen loss	4,880	626	4,254
15,631	Buildings, alterations, repairs and renewals	20,629	2,237	18,392
	Depreciation			
2,168	Buildings	2,320	—	2,320
1,400	Furniture, fittings, scientific apparatus and books	2,200	—	2,200
381,636		£596,832	£203,779	393,053
139,525	Excess of expenditure over income			131,878
8,268	Amount transferred to sinking fund for freehold buildings			7,256
£147,793	Deficit transferred to capital fund			£139,134

The notes on pages 6 to 9 form part of these accounts.
Audit report—page 9

NOTES ON THE ACCOUNTS · 31 December 1969

1. FIXED ASSETS

	Freehold property		Furniture, fittings, scientific apparatus and books	Total
	Land and buildings Chelsea	Queensbury Lodge Estate, Elstree		
	£	£	£	£
Cost				
At 1st January 1969	115,783	62,160	14,623	192,566
Additions at cost	70,446	30,636	14,629	115,711
At 31st December 1969	£186,229	£92,796	£29,252	£308,277
Depreciation				
At 1st January 1969	1,350	9,429	7,073	17,852
Charged to income and expenditure account	1,350	970	2,200	4,520
At 31st December 1969	£2,700	£10,399	£9,273	£22,372
Net book value at 31st December 1969	£183,529	£82,397	£19,979	£285,905

Depreciation

Freehold property additions and replacements since 1912 at Elstree and since 1935 at Chelsea until 31st December 1964 have been charged to revenue. Additions since that date until 31st December 1967 have been depreciated at the rate of 10%. Since 1st January 1968 buildings shown in the balance sheet have been depreciated at the rate of 2% on a straight line basis from the date of completion.

Additions and replacements to furniture, fittings, scientific apparatus and books between 31st December 1920 and 31st December 1963 have been charged to revenue. The additions since 1st January 1964 have been depreciated on a straight line basis by reference to the useful lives of the assets.

2. INVESTMENTS AND UNINVESTED CASH

	£	£	£	£	£
	Quoted at cost		Unquoted at cost	Uninvested cash	Total
	In Great Britain	Elsewhere			
General	261,555	202,459	39,686	—	503,700
Specific					
Sinking fund for freehold buildings	126,067	—	—	34,182	160,249
Pension fund	5,345	—	—	15,635	20,980
Re-endowment fund	22,478	—	—	9,914	32,392
Bequest					
Jenner Memorial studentship fund	11,766	—	940	3,623	16,329
Morna Macleod scholarship fund	5,653	—	—	1,348	7,001
	£432,864	£202,459	£40,626	£64,702	£740,651
1968	£599,966	£164,542	£63,927	£9,239	£837,674

Market value of quoted investments 1969 £1,066,315 (1968 £1,579,169)

Unquoted investments valued by Institute's investment advisers 1969 £34,412 (1968 £59,014)

3. STOCK

Stocks of sera, virus vaccines and horses on hand at 31st December 1969, have not been valued in the accounts.

4. CAPITAL FUND

Donations etc. have been received to date from the following:—	£	£
Dr. Ludwig Mond (1893)		2,000
Berridge Trustees (1893–1898)		46,380
Worshipful Company of Grocers (1894 and 1969)		11,000
Lord Iveagh (1900)		250,000
Lord Lister's Bequest (1913–1923)		18,904
William Henry Clarke Bequest (1923–1926)		7,114
Rockefeller Foundation (1935–1936)		3,400
Wolfson Foundation (1969)		75,000
Other donations and legacies (1891–1954)		22,669
General Fund Income and Expenditure Account		
Accumulated surplus as at 31st December 1968	212,259	
Less: Deficit 1969	139,134	
		73,125
		£509,592

5. SPECIFIC FUNDS

	£	£	£
Sinking Fund for Freehold Buildings			
As at 1st January 1969		147,341	
Amounts transferred from income and expenditure account		7,256	
		154,597	
Less: Expenditure on reablement of buildings		2,890	
			151,707
Pension Fund			
As at 1st January 1969		28,795	
Interest on investments		1,637	
		30,432	
Less: Losses on realisation of investments	6,449		
Pensions	3,004		
		9,453	
			20,979
Re-endowment Fund			
As at 1st January 1969		28,185	
Donations		600	
Profit on realisation of investments		3,607	
			32,392
			£205,078

6. BEQUEST FUNDS

	£	£
Jenner Memorial Studentship Fund		
As at 1st January 1969	18,012	
Interest on investments	608	
	18,620	
Less: Loss on realisation of investment	2,291	
		16,329
Morna Macleod Scholarship Fund		
As at 1st January 1969	7,097	
Interest on investments	376	
	7,473	
Less: Loss on realisation of investments	472	
		7,001
		£23,330

7. SPECIFIC GRANTS AND LEGACIES

	£	£	£
Nuffield Foundation Grants			
As at 1st January 1969			1,583
Guinness-Lister Research Grant			
As at 1st January 1969		5,456	
Amounts received		15,000	
		20,456	
Less: Salaries and wages	12,238		
Laboratory expenses	3,356		
		15,594	
			4,862
			£6,445

8. GENERAL AND SINKING FUNDS INVESTMENT RESERVE

		£
General		
As at 1st January 1969	289,270	
Add: Profits on sales of investments	100,494	
		389,764
Sinking Fund		
Profits on sales of investments during year		8,542
		£398,306

9. TURNOVER

Turnover has been arrived at after deducting commission due to agents from the invoice value of sales of sera, vaccines and virus vaccines.

10. Emoluments of Members of the Governing Body

	1969	1968
Emoluments in an executive capacity	£15,923	£16,043

Particulars of emoluments of the Governing Body in accordance with Section 6 of the Companies Act 1967

	1969	1968
Emoluments of the Chairman of the Governing Body	Nil	Nil
Emoluments of the highest paid member of the Governing Body	£7,256	£7,309
Numbers of members of the Governing Body whose emoluments were within the range		
No emoluments	7	6
£1 — £2,500	—	2
£2,501 — £5,000	1	—
£5,001 — £7,500	1	1

11. Capital Expenditure Schemes

The position at 31st December 1969 was as follows:—

	1969	1968
Commitments in respect of contracts	324,071	23,943
Approved by the Governing Body in addition to commitments, for new laboratories at Elstree	120,000	67,000
	£444,071	£90,943

12. Contingent Liabilities

At 31st December 1969 there were contingent liabilities amounting to £14,041 in respect of indemnities issued to third parties.

Report of the Auditors to the Members

In our opinion the accounts set out on pages 4 to 9 give a true and fair view of the state of the company's affairs at 31st December 1969 and of its deficit for the year ended on that date and comply with the Companies Acts 1948 and 1967.

COOPER BROTHERS & CO.
Chartered Accountants.

London, 21 May 1970

THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Report
of the GOVERNING BODY
1970

CHELSEA BRIDGE ROAD : LONDON : SW1

The Governing Body

Sir LINDOR BROWN, CBE, FRCP, FRS, *Chairman*
H. P. G. CHANNON, MP, *Hon. Treasurer*
The Rt Hon LORD BROCK, MS, FRCS
Professor D. G. EVANS, CBE, D SC, FRS
C. E. GUINNESS
The Rt Hon the EARL OF IVEAGH
Professor R. A. KEKWICK, D SC, FRS
Professor Sir ASHLEY MILES, CBE, MD, FRCP, FRC PATH, FRS
Professor A. NEUBERGER, CBE, MD, FRCP, FRS

Clerk to the Governors S. A. WHITE, AACCA

The Council

A. LAWRENCE ABEL, MS, FRCS *Representing the British Medical Association*

V. C. BARRY, D SC *Representing the Royal Irish Academy*

The Rt. Hon Lord BROCK, MS, FRCS *Representing the Members of the Institute*

Sir LINDOR BROWN, CBE, FRCP, FRS *Representing the Members of the Institute*

H. P. G. CHANNON, MP *Representing the Members of the Institute*

Dame HARRIETTE CHICK, DBE, D SC *Representing the Members of the Institute*

Professor P. J. COLLARD, MD, MRCP *Representing the University of Manchester*

Major L. M. E. DENT, DSO *Representing the Worshipful Company of Grocers*

Sir CHARLES DODDS, Bt, MVO, MD, D SC, FRCP, FRS *Representing the Members of the Institute*

Sir ALAN N. DRURY, CBE, MA, MD, FRCP, FRS *Representing the Members of the Institute*

Professor D. G. EVANS, CBE, D SC, FRS *Representing the Royal Society*

Sir PAUL FILDES, OBE, MA, D SC, MB, B CH, FRS *Representing the Members of the Institute*

Professor R. E. GLOVER, MA, D SC, FRCVS *Representing the Royal Agricultural Society*

Professor R. I. N. GREAVES, BA, MD *Representing the University of Cambridge*

Sir CHARLES HARINGTON, MA, PH D, FRS *Representing the Members of the Institute*

Professor HENRY HARRIS, D PHIL, FRS *Representing the University of Oxford*

The Rt Hon the EARL OF IVEAGH *Representing the Members of the Institute*

Professor B. P. MARMION, MD, D SC, FRC PATH *Representing the University of Edinburgh*

Professor Sir ASHLEY MILES, CBE, MD, FRCP, FRC PATH, FRS *Representing the Members of the Institute*

Professor J. S. MITCHELL, CBE, MA, MD, FRS *Representing the Members of the Institute*

Professor W. T. J. MORGAN, CBE, D SC, PH D, MD (*hc*), FRIC, FRS *Representing the Members of the Institute*

Professor A. NEUBERGER, CBE, MD, FRCP, FRS *Representing the Members of the Institute*

Professor Sir RUDOLPH PETERS, MC, MA, MD, FRS *Representing the Members of the Institute*

The President of the ROYAL COLLEGE OF PHYSICIANS *Representing the Royal College of Physicians, London*

The President of the ROYAL COLLEGE OF SURGEONS *Representing the Royal College of Surgeons of England*

The President of the ROYAL COLLEGE OF VETERINARY SURGEONS *Representing the Royal College of Veterinary Surgeons*

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WILLIAM J. THOMPSON *Representing the Worshipful Company of Grocers*

Sir GRAHAM WILSON, MD, FRCP *Representing the University of London*

The Staff

Director: Professor Sir Ashley Miles
Deputy Director: Professor L. H. Collier
Superintendent of Elstree Laboratories: W. d'A. Maycock

MICROBIOLOGY, EXPERIMENTAL PATHOLOGY AND IMMUNOLOGY

Experimental Pathology and Immunology

†Sir Ashley Miles, CBE, MD, D SC, FRCP, FRC PATH, FRS (*Professor of Experimental Pathology in the University of London*)
F. R. Wells, BM, B CH, MA (*Oxon*)
Brenda Mason, B SC, MI BIOL

D. G. Godfrey, OBE, B SC, PH D (*M.R.C. External Scientific Staff*)
Angela E. R. Taylor, B SC, PH D
Sheila M. Lanham, B SC
} **Trypanosomiasis Research**

Microbiology

†G. G. Meynell, MD (*Guinness Professor of Microbiology in the University of London*)
A. B. Stone, B SC, D PHIL
Marylyn D. Cooke, B SC, PH D
K. G. Hardy, B SC
} **Guinness-Lister Research Unit**

*Elinor W. Meynell, BA, MD, DIP BACT
Ruth M. Lemcke, B SC, PH D
J. E. Dowman, MA, PH D (*S.R.C. Grantee*)
M. R. Hollingdale, B SC (*M.R.C. Grantee*)
H. C. Neimark, BA, PH D (U.S.A.)
T. C. Salzmann, BA, MD (U.S.A.)

Virology

†L. H. Collier, MD, D SC, MRCP (*Professor of Virology in the University of London and Hon. Director, M.R.C. Trachoma Unit*)
J. Alwen, B SC, PH D
Lindsey M. Cox, B SC

W. A. Blyth, B SC, PH D
Janice Taverne, BA PH D
A. J. Garrett, B SC, PH D
Andrea Barton, B SC
R. G. Harris, B SC, MI BIOL
} **M.R.C. Trachoma Unit**

Electron Microscopy Unit

*A. M. Lawn, PH D, B SC, MRCVS

BIOCHEMISTRY

†Winifred M. Watkins, D SC, PH D, FRS (*Professor of Biochemistry in the University of London*)
*G. M. A. Gray, B SC, PH D
M. A. Chester, M SC, B TECH (*Research Student*)
Shirley D. Goodwin, B SC
Jennifer J. Wells, BA, PH D (*B.E.C.C Grantee*)
A. S. R. Donald, B SC, PH D (*M.R.C Grantee*)
Veronica M. Hearn, M SC, PH D (*M.R.C Grantee*)
Caroline Race, B SC (*M.R.C Grantee*)
W. Segal, M SC, PH D (Australia)

§ Professor W. T. J. Morgan, CBE, D SC, PH D, MD (*hc*), D SC (*hc*), FRIC, FRS (ret'd).

BIOPHYSICS

†R. A. Kekwick, D SC, FRS (*Professor of Biophysics in the University of London*)
†J. M. Creeth, B SC, PH D, FRIC (*Reader in Biophysics in the University of London*)
†Professor N. M. Martin, MA, FRCP, FRIC (*Honorary Research Associate*)
Caroline M. Butterworth, B SC (*M.R.C. Grantee*)

§ Dame Harriette Chick, DBE, D SC (ret'd)

PREPARATION and STUDY of THERAPEUTIC SERA (ELSTREE)

W. E. Parish, MA, PH D, BV SC, MRCVS, MRC PATH
N. Mahony, M SC, B TECH
Wendy Smithson, B SC

Biochemistry (Elstree)

*D. E. Dolby, B SC, PH D

PREPARATION and STUDY of VIRUS VACCINES (ELSTREE)

H. G. S. Murray, MD
G. S. Turner, B SC, PH D
L. C. Robinson, B SC, PH D
L. V. Runkel, B SC

PREPARATION and STUDY of BACTERIAL VACCINES (ELSTREE)

*A. F. B. Standfast, SC D
Jean M. Dolby, MA, PH D
M. P. Banks, B SC
Noreen M. Wesley, B SC
Caroline J. Shanbury, B SC
A. P. Hunt, B SC
J. P. Ackers, MA, D PHIL (*M.R.C. Grantee*)

CO-ORDINATION of PRODUCTION (ELSTREE)

J. Rodican, B SC

BLOOD PRODUCTS (ELSTREE)

*W. d'A Maycock, MVO, MBE, MD, MRCP, FRC PATH
L. Vallet, MA
§Margaret E. Mackay, M SC, PH D (*M.R.C. External Scientific Staff*)
D. Ellis, B SC, PH D
Constance Shaw, M SC, DIP BACT
E. D. Wesley, B PHARM
Valerie J. Stickley, B SC

PLASMA FRACTIONS LABORATORY (OXFORD)

Ethel Bidwell, B SC, PH D, FRIC
W. H. Ford, B SC, PH D

MEDICAL RESEARCH COUNCIL UNITS HOUSED AT THE INSTITUTE

Blood Group Unit

§R. R. Race, MD (*hc*), PH D, FRCP, FRS
Ruth Sanger, B SC, PH D
Patricia Tippett, B SC, PH D
E. June Gavin, B SC
Joan Whittaker, B SC

Blood Group Reference Laboratory

K. L. G. Goldsmith, PH D, MB, BS, MRCP, MRC PATH
Toby T. B. Phillips, MB, CH B
Elizabeth W. Ikin, B SC, PH D
Carolyn M. Giles, B SC, PH D
B. J. Dawes, B SC

ADMINISTRATION

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Elstree Secretary and Estate Manager — G. J. Roderick, B COM
Assistant Secretary (Academic) — Barbara A. Prideaux
Assistant Secretary and Deputy Accountant — E. J. H. Lloyd
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†*Appointed Teacher of the University of London*
**Recognised Teacher of the University of London*
§ Honorary Member of Institute Staff

Annual General Meeting of the Lister Institute

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the Institute for the year 1969.

GOVERNING BODY

It is noted with pleasure that in June 1969 Professor D. G. Evans was appointed a Commander of the Order of the British Empire.

The Council, at a meeting held on 24th June 1969, reappointed Sir Lindor Brown, Lord Brock and Professor A. Neuberger as its representatives on the Governing Body until 31st December 1970. In July Lord Iveagh appointed Mr. C. E. Guinness as one of his representatives on the Governing Body.

It was with regret that the Governing Body accepted Lord Brock's decision to resign from the Governing Body on 30th June 1970. Lord Brock has been a Governor for the past five years and his wide knowledge and interest in the affairs of the Institute will be much missed.

COUNCIL

At last year's Annual General Meeting one of the three retiring members of the Council, Mr. A. Lawrence Abel, the representative of the British Medical Association, was reappointed. The reappointment of Professor R. I. N. Greaves, the representative of the University of Cambridge, was left in abeyance pending a decision by the University; and the third retiring member, Dr. Muriel Robertson, did not offer herself for reappointment. Professor A. Neuberger was appointed as a representative of the Members.

The three members of Council due to retire this year in accordance with the Articles of Association, but who are eligible for reappointment, are the President of the Royal College of Surgeons, Sir Rudolph Peters and Professor P. J. Collard, representing the Royal College of Surgeons of England, the Members of the Institute and the University of Manchester respectively.

MEMBERS

The Governing Body records with regret the death of Professor E. J. Conway, Sir Allen Daley, Dr. R. T. Leiper and Sir Samuel Bedson. Sir Samuel Bedson was a member of the staff from 1921 to 1926 and represented the Royal Society on the Governing Body from 1944 to 1954.

During the year the following persons became Members of the Institute: Dr. J. M. Creeth, Dr. G. M. Gray, Professor Harry Harris, FRS, Dr. Ruth Lemcke, Dr. H. G. S. Murray, Dr. W. E. Parish, Dr. Anne-Marie Staub, Dr. G. S. Turner and Professor Otto Westphal.

STAFF AND STUDENTS

The Governing Body notes with pleasure that the University of Michigan, U.S.A., has conferred on Professor W. T. J. Morgan the degree of Doctor of Science (*honoris causa*).

Mrs. Shirley Goodwin was appointed to the Biochemistry Department; Mr. K. G. Hardy to the Guinness-Lister Unit; and Mr. L. V. Runkel to the Virus Vaccines Department.

Mrs. Denise Cole (née Ward) completed the tenure of her Grocers' Company Research Studentship in the Biochemistry Department.

The Institute was sorry to lose the services of Mr. E. E. Jackson, who retired in November 1969. Mr. Jackson was in charge of the Animal House at Chelsea and had worked at the Institute for forty-nine years.

Sir Ashley Miles gave The Fifth Michael Cross Memorial Lecture at the Royal College of Surgeons of England in June 1969. In March 1970, at the invitation of the Pakistan Atomic Energy Commission, he visited high-energy physics and public health laboratories in West and East Pakistan.

Dr. W. d'A. Maycock attended, as a representative of the Department of Health

and Social Security, a meeting of the Council of Europe Working Party on Freedom from Toxicity of Plastics used for Transfusion, in Berne in April 1969; and a meeting of the Sub-Committee of Specialists on Blood Problems of the Public Health Committee of the Council of Europe, in Helsinki in June 1969.

In March 1969 Professor Winifred M. Watkins lectured by invitation at the University of Frankfurt. She took part in an international symposium on Blood and Tissue Antigens at the University of Michigan, Ann Arbor, in September.

Dr .H. G. S. Murray attended a symposium on Smallpox in Zagreb in September 1969.

Dr. W. E. Parish participated by invitation in an international symposium on Immune Complex Diseases, at Spoleto, Italy; and presented a paper at a combined British and Dutch Immunological Societies Meeting in Amsterdam in June 1969. In September and October he lectured at universities and hospitals in the United States and Canada and took part by invitation in a symposium on the Biology of Skin, organised by the Oregon Regional Primate Research Center at Portland.

Mr. L. Vallet visited the Central Transfusion Laboratory of the Netherlands Red Cross, Amsterdam, and the Plasma Fractionation Department, State Serum Institute, Copenhagen, in June 1969.

In May 1969, Dr. Ethel Bidwell attended a colloquium on the Protides of Biological Fluids, in Bruges, and visited a number of laboratories in Holland.

Mr. J. Rodican and Mrs. Noreen M. Wesley attended the Permanent Section of Microbiological Standardisation Meeting in Berne in October 1969.

Dr. G. M. Gray lectured by invitation at the 13th International Conference on the Biochemistry of Lipids, in Athens in September 1969.

In July Dr. D. G. Godfrey, Dr. Angela E. R. Taylor and Miss Sheila M. Lanham took part in the 3rd International Congress of Protozoology at Leningrad and visited laboratories in Leningrad and Moscow.

Dr. Elinor Meynell lectured by invitation at the Botanical Institute, University of Berne, in September 1969.

In April 1969 Dr. J. P. Ackers, Dr. Jean Dolby and Mr. A. P. Hunt attended the Pertussis Conference at Bilthoven, Holland.

Miss Brenda Mason attended an international symposium on Cardiovascular and Neuro-actions of Bradykinin and Related Kinins held at Fiesole-Florence in July 1969.

For the academic year 1969/70 there are eighteen postgraduate research workers at the Institute registered for higher degrees of the University. Three Ph D degrees were awarded during 1969.

PRODUCTION AT ELSTREE

The department for the Co-ordination of Production under Mr. J. Rodican, in collaboration with the heads of production departments and the Elstree Secretary, continues to plan production, both for the immediate future and for a programme of expansion during the next 4–5 years, in which it is expected to increase the range of products and erect several new buildings.

The work has included forecasts of sales, revenue, production costs and desirable stock levels; devising production schedules; and the institution of revised methods of budgeting and of the coding and indexing of invoices.

NEW BUILDINGS

Considerable progress has been made on the construction of the new wing at Chelsea but for a number of reasons, including a fire which destroyed the contractor's site office, work is approximately four months behind schedule.

At Elstree, six new cottages for stable and estate staff were completed and are now occupied. The Serum Department packing store was extended to cater for the increased production of vaccines and sera. A large

extension to the Blood Products Laboratory, to provide facilities for increased production of gamma globulin and other blood products, was started in October 1969. It is hoped that the new building will be completed by the end of 1971.

DONATIONS AND GRANTS

Arthur Guinness, Son & Co. Ltd. continue their generous support of the Guinness-Lister Research Unit, and the final £4,000 of the total grant of £35,000 was received from the Fleming Memorial Fund for Medical Research for the support of the Electron Microscope Unit.

The Governing Body records its appreciation of the many other bodies whose benefactions and grants support research work in the Institute. These include a grant from the Arthritis and Rheumatism Council for research on the role of bacterial complexes in vasculitis; a grant from the British Empire Cancer Campaign for research on the role of lipids in the structure and function of the plasma membranes of normal and malignant mammalian cells; grants from the Medical Research Council for research on the genetics of drug resistance factors and other bacterial plasmids; on protein synthesis and the control of DNA replication in bacteria; on the antigenic structure of *Mycoplasma hominis*; on the role of adenovirus in the aetiology of infectious hepatitis; on hypersensitivity to smallpox vaccine; on biosynthesis of blood-group specific glycoprotein and red cell antigens; on the immunochemistry of human blood-group specific glycoproteins; on the lipid components of the plasma membranes of mammalian cells; on the ceramide containing glycolipids in mammalian tissues, with special reference to their biosynthesis; on the gamma macroglobulins of normal human plasma; on the reversibility of denaturation of ovalbumin and serum albumin; and on immunity in *B. pertussis* infections, the distribution of serotypes of *B. pertussis*, and the separation and characterization of the antigenic components of *B. pertussis*.

Grants were also received from the Ministry of Overseas Development for studies on the biology of trypanosomes, with special reference to their surface properties; from the Science Research Council for studies on the replication of bacterial plasmids, on the amino-acid components of various proteins and on the characterization of protein conformation during denaturation and renaturation; from the Smith, Kline and French Foundation for the purchase of a microscope with automatic camera attachment; and from the World Health Organisation for the development of freeze-dried smallpox vaccine from cell cultures.

The Governing Body also gratefully acknowledges donations to the Institute's Re-endowment Fund from the Prudential Assurance Company Limited and the Royal London Mutual Insurance Society Limited.

VISITORS

The following visitors, in addition to those listed under Staff, worked for short periods in the Institute's Laboratories; Miss Afaf Daccak, Ministry of Health, Damascus, Syria; Mr. A. Q. Faruqui, National Health Centre, Islamabad, West Pakistan; Dr. Nina Gordienko, Institute of Virus Preparation, Moscow; Dr. Yves Goueffon, Pasteur Institute, Cambodia; Dr. R. F. Granado, Instituto Espanol de Hematologia, Madrid; Dr. J. Koscielak, Institute of Haematology, Warsaw; Mr. P. E. Lemoine, Institute de Hygiene et Epidemiologie, Bruxelles; Dr. I. Chandra Prahlad, King Institute, Madras; Miss N. Rasool, Blood Bank Institute, Baghdad; Miss Jean Tweedy, Trinity College, Dublin; Dr. D. H. Walker, Karolinska Institute, Stockholm.

Researches in 1969

SUMMARY

The bracketed numbers refer to pages and columns of the report where more detailed descriptions are to be found.

Microbiology

(a) Bacterial sex factors in enterobacteria. Relation to R factors; I and F type pili (15, i), their determination by sex factors (16, i), their serology (17, ii), and biosynthesis (18, i).
(b) Transfer of drug resistance in *V. cholerae* (16, ii).
(c) DNA synthesis in phage-infected *E. coli*. (17, i) and in Salmonella after amino-acid starvation (17, ii).

Infective Diseases and Immunity

1. Trypanosomiasis:
   (a) Surface charge of trypanosomes and erythrocytes (13, ii); its exploitation in harvesting trypanosomes from infected blood (13, i) and in the diagnosis of natural sub-patent parasitaemia (13, ii).
   (b) Permeability studies and ultrastructure of the trypanosome cell (14, i).
   (c) Cyclical transmission (14, ii).

2. Bacterial and Chlamydial Infections:

   (a) *B. pertussis*. Isolation of protective antigens (14, ii); agglutinogens of prevalent strains (15, i); relation of bactericidal antibody to immunity (14, ii).
   (b) *V. cholerae*. Isolation and characterization of toxins (15, ii).
   (c) Production of tetanus exotoxin (15, i).
   (d) Mycoplasma. Antigenic analysis of *M. hominis* (18, ii); electronmicroscopic structure (19, i); simian mycoplasmas (19, ii); *M. pulmonis* infection in the rat (19, ii); and mycoplasmas in plants (20, i).
   (e) Agents of trachoma and inclusion conjunctivitis. Replication in kidney cells and macrophages (20, i) and infectivity in primates (21, i). Immunogenicity at different stages in the reproductive cycle (21, i) and nature of primary and secondary responses to vaccine (21, ii). Fluorescent antibody in the diagnosis of trachoma (22, i).

3. Virus diseases:

   (a) Vaccinia virus. Relation of hypersensitivity and immunity in the guinea-pig (22, ii). Immunogenicity of inactivated vaccine (23, i); chemical composition (23, i); effect of freeze-drying on live virus (23, i).
   (b) Rabies virus. Growth in human diploid cells (23, ii).
   (c) Infectious hepatitis. Relation to adenovirus infection (22, i).

Immunology and Pathology

1. Immunology:
   (a) Immunoglobulins of horse and mouse (23, ii).

(b) Immunogenicity of diphtheria toxoid (25, ii).
(c) Eosinophil-stimulating factor in anaphylaxis (24, i).
(d) Anaphylactic hypersensitivity to horse serum proteins (24, ii).
(e) Sensitization of cells by exogenous antigen to cytotoxic antibodies in tissue cultures (24, ii) and in eczematous patients (25, i).
(f) Bactericidins and cytotoxins in mononuclear cells (25, ii).

2. Pathology:
   (a) Vascular responses to heat and *Cl. welchii* toxin (26, i).
   (b) The monocytogenic factor in delayed hypersensitivity (26, i).
   (c) The human plasma kininogenetic system; mode of activation (26, ii); activation in plasma fractions for therapeutic use (35, i); isolation of prekallikrein (26, i).
   (d) Mechanisms of infection and defence. *E. coli* infections of mouse muscle (27, i); mechanism of enhancement of Klebsiella infection in the guinea-pig by iron salts (27, ii).

Electron Microscopy

1. Bacterial sex-pili; electron microscope studies of serology (17, ii) and biosynthesis (18, i).
2. Other studies summarized (18, ii).

Biochemistry

1. Human blood-group glycoproteins:
   (a) Nature of the peptide moiety (27, i).
   (b) Biosynthesis. Synthesis by sugar transferases (28, i). Distribution of sugar transferases in human tissues (29, i).
   (c) Ultracentrifugal studies (34, i).
2. Lipids and biological membranes:
   (a) Plasma membranes in ascites tumour cells (36, i); permeability of liver cells (31, ii).
   (b) Glycolipids; biosynthesis in mouse kidney cells (32, i); in normal and transformed hamster kidney cells (32, ii).

Biophysics and Protein Studies

1. Macromolecules:
   (a) Structure of human macroglobulins (33, i).
   (b) Microheterogeneity of human gamma globulin glycoproteins (33, ii).
   (c) Reversible denaturation of ovalbumin and serum albumins (34, ii).
   (d) Molecular weight determinations in polydisperse, non-ideal systems (35, i).
2. Blood products:
   (a) Anti-rhesus factor antibody in clinical trials (35, ii).
   (b) Concentration for clinical use of Factors VIII (36, i) and XII (36, i).

Medical Research Council Units

1. Report of the Blood Group Unit (36, ii).
2. Report of the Blood Group Reference Laboratory (39, i).

MICROBIOLOGY

Trypanosomiasis

Harvesting of Bloodstream Trypanosomes. Miss Lanham successfully separated *Trypanosoma cruzi,* the causative organism of Chagas disease, from rodent blood by means of DEAE-cellulose columns. Recovery was greater than 70 per cent with two strains but less than 40 per cent with a third strain (Y). The method depends on differences in negative surface charge between trypanosomes and host blood cells, and the previous failure to separate *T. cruzi* was partly due to the use of the Y strain, whose charge approaches that of mammalian red blood cells and is more negative than that of the other two strains. Nevertheless all three strains of *T. cruzi* are more negative than *T. lewisi,* which had the highest negative charge of all species so far investigated (Report 1969). Another factor in the poor separation of the Y strain was the phosphate buffer routinely used for the separation of other species of trypanosome. With tris and bicine buffers, yields of all strains of *T. cruzi* increased considerably; as yet, the reasons for the difference are unknown.

In collaboration with the Zoology Department, Imperial College, *T. percae* was concentrated from perch blood, using a high ionic strength phosphate buffer. As expected, this highly negatively charged trypanosome was only partially separable from blood cells. The concentrate was, nevertheless, valuable for morphological studies at Imperial College and for electron microscope studies here. Complete separation from blood cells of this trypanosome and other highly negatively charged species may be possible with the buffers now used for *T. cruzi.*

Platelets adhere strongly to trypanosomes and some may remain attached to them during passage through DEAE-cellulose, although none have been seen in the eluates. To investigate the possibility of minor contamination, Dr. Taylor, Miss Lanham and Dr. Godfrey prepared homogenates of separated *T. brucei brucei,* which were subjected to starch-gel electrophoresis by Dr. C. W. Parr and his colleagues at the London Hospital. The samples contained none of the lactic dehydrogenase (LDH) isoenzyme characteristic of platelets. No other LDH was found in the starch-gels but a trace activity was found in the whole homogenate. Platelet contamination of trypanosomes separated from blood by DEAE-cellulose was therefore considered to be negligible.

Diagnosis of Subpatent Parasitaemia. Work in Nigeria (Report 1969) indicated the value of column-separation for demonstrating subpatent trypanosomiasis, and this was confirmed by Dr. Godfrey, Miss Lanham and Dr. Taylor. Trypanosomes were easily found, unobscured by blood cells, in blood from a subpatent infection with *T. b. brucei* in a goat. The original blood had an infectivity of 631 ID63 per ml which probably approximated to 631 trypanosomes per ml; it was estimated that a parasitaemia of less than 2000 trypanosomes per ml is likely to be missed by normal microscopic examination of blood. *T. b. brucei* was also isolated from samples of artificially infected rat blood; all the blood samples initially contained small numbers of trypanosomes, one of them only 4 per ml.

Centrifugation is inconvenient for field work and other methods of concentrating trypanosomes in the eluates were explored; a limited success was achieved only with membrane filtration.

Surface Charge of Trypanosomes. Miss Lanham investigated the elution of trypanosomes with linearly increasing gradients of cellulose columns. *T. b. brucei,* with a weak negative charge, was eluted with the low ionic strength buffer, the more negative *T. vivax* at higher, and the strongly negative *T. lewisi* at yet higher ionic strengths.

During the course of infection in the rat, circulating *T. lewisi* proved to be slightly less negatively charged on the 5th day than on the 2nd; the charge was less still on the 9th and 16th days. Rigorous control of column conditions is being instituted to confirm the reality of these small differences, before infections with other species are examined.

Sialic acid, which contributes to the negative charge of erythrocytes, was present in *T. lewisi* but only as traces in *T. b. brucei* and *T. vivax.* However, the specificity of the test and possible contamination by

strongly adsorbed blood components requires investigation.

Permeability of the Trypanosome Cell. Drs. Godfrey and Taylor found that the release of glutamic pyruvic transaminase (GPT) from *T. b. brucei* exposed to cobra venom was due to the basic (i.e. positively charged) proteins in the venom and not to the phospholipase A; the phospholipid pattern of *T. b. brucei* was unchanged after treatment. Other basic macromolecules, protamine and two polylysines, acted like the venom; the higher molecular weight polylysine was more active than the one of lower molecular weight. The basic dodecyltrimethyl ammonium chloride had no effect.

Like plasma and serum, heparin protected against, but did not reverse, the action of the venom. The effect of EDTA was complex; it afforded some protection against the venom, but it also decreased the motility of the trypanosomes and partly inhibited the activity of the released GPT.

In the presence of the active bases, *T. b. brucei* released less GPT in a higher than in a lower ionic strength of buffer; more enzyme was released in a tris buffer than in the standard phosphate buffer at the same ionic strength.

Much time was spent on devising a suitable buffer containing divalent cations, which are necessary for normal membrane function but are insoluble in the phosphate buffer. A bicine buffer proved to be more satisfactory than a tris buffer.

Surface Ultrastructure. With the co-operation of Dr. Lawn, Drs. Taylor and Godfrey achieved better resolution of the surface ultrastructure of trypanosomes by fixing the organisms on a membrane filter with a mixture of osmium tetroxide and glutaraldehyde in the cold. *T. percae* and *T. cruzi* had loosely packed surface coats like that of *T. lewisi* (Report 1969).

Low concentrations of cobra venom apparently removed the thick compact surface of *T. b. brucei* before disintegrating the plasma membrane. Similar changes occurred in *T. lewisi*; there was a thickening and granulation of the inner part of the plasma membrane before disintegration. However, it is uncertain at present whether these changes are directly due to the venom or whether they are initially more subtle, and are exaggerated during processing for electron microscopy.

Cyclical Transmission of Trypanosomes. T. b. brucei was transmitted to laboratory animals through the tsetse fly, *Glossina austeni*, supplied as puparia from the Tsetse Research Laboratory at Bristol. The low infection rate, about 3 per cent, meant that experiments with large batches of flies were complicated and time-consuming. A system, however, dealing continuously with small batches of flies was devised and is currently under test; two species, *G. austeni* and *G. morsitans* are being studied.

Whooping Cough Bacillus

The Protective Antigen. Filtration through an agarose column was found by Dr. Ackers to be too laborious for large-scale preparation of a mouse protective fraction (Report 1969). An alternative method of fractionation was devised which, when applied to the supernatant of high-speed centrifugates of sonically disrupted cells, yielded a fraction with 80 per cent of the original mouse-protective activity and little of the original toxicity. It was stabilised to withstand two weeks incubation at 37°. The possibility of its use as the pertussis component of combined vaccine is under investigation.

Bactericidal Antibody and Protection. It was hoped to replace the accepted mouse potency assay for pertussis protective antigen by a method based on the induction of bactericidal antibody (Report 1969). Dr. Ackers has shown conclusively, however, that experimental vaccines may protect without inducing bactericidal antibody or induce bactericidal antibody without protecting. Mr. Hunt also showed, in mice used for routine protection tests, that the dose-responses for bactericidal antibody and protection were not identical. There was no lipopolysaccharide in the vaccine that protected mice without inducing bactericidal antibody. The possibility that lipopolysaccharide is the "bactericidal" antigen is under investigation.

Although vaccinated mice may produce bactericidal antibody without being protected against intracerebral challenge, in certain conditions antiserum containing bactericidal antibody protected mice against

this challenge. Drs. Jean Dolby and Ackers began to study the role in passive protection of the bactericidal and other antibodies in rabbit, mouse and child sera and in globulin fractions prepared from them by Dr. D. Dolby (p. 24).

Agglutinogens. The strains isolated from children in 1967 and 1968 that contained agglutinogens 1, 2, 3 and 4 (Report 1969) were investigated further by Mrs. Shanbury (née Bronne). Although on routine typing these strains behave as mixtures of 1,3 and of 1,2,4 strains, most of the component cells are potential producers of agglutinogens 1, 2, 3 and 4 but the expression of each agglutinogen at each generation is variable. This was established for nine of thirteen strains investigated fully; all the constituent cells of the other four strains produced all four antigens strongly and consistently.

Mrs. Shanbury made vaccines from pertussis cells producing only agglutinogen 1 which had been isolated from cultures forced to go through spheroplast formation (Report 1969). These vaccines, though completely free of agglutinogens 2–6, were fully protective in mice.

Cholera

Mrs. Wesley continued her work on the toxin in the culture filtrate of *Vibrio cholerae* serotype Inaba (Craig type II toxin, Report 1969). The toxin causing accumulation of liquid in the ligated ileal loop of rabbit intestine (ILT) and that producing increased permeability in the skins of guinea-pigs (SPF) were not separable chemically, but their stability differed. Both toxins were antigenic when toxoided.

Guinea-pigs immunised intraperitoneally with culture filtrate containing both ILT and SPF were protected against SPF; guinea-pigs vaccinated with whole cells, cell wall fragments or extracts of cell wall fragments were not so protected. Rabbits immunised subcutaneously with the filtrate or with toxoided filtrate produced neutralising antibodies to SPF and to ILT but were not protected against ILT. They were protected against ILT after a course of peroral immunisation with toxic filtrate in the drinking water. The antisera used in these studies were standardised against the Provisional Standard Cholera antitoxin received from Dr. John P. Craig (State University of New York College of Medicine).

Clostridial Toxins

Mr. Mahony continued his investigations of the factors in Mueller's medium which affect formation of toxins by *Clostridium tetani* (Report 1969). Only a partial separation of the components of the medium was obtained on ion exchange columns or by electrodialysis with polyacrylamide gel membranes. Better separation of the casein digest and muscle extract components was achieved by gel filtration on Sephadex G15. The constituents of the fractions essential for high yields of toxin are being identified.

Inheritance in Bacteria

Bacterial Sex Pili. These are specialised filaments found on rod-shaped colon bacteria when they can transfer genes to other strains by conjugation. From the geneticist's point of view, the pili serve as valuable taxonomic characters by which to classify the genes ("sex factors") determining their formation, since the two main classes known as "F pili" and "I pili" differ in their morphology, antigenic structure and ability to adsorb certain phages. These phages take two forms, RNA-containing and spherical or DNA-containing and filamentous, but are of little discriminatory value as they usually attach equally well to naturally occurring pili. However, Miss Aufreiter and Professor Meynell devised an improved method for selecting mutants resistant to RNA phage while remaining sensitive to DNA phage. A mutagenised donor culture was grown with RNA phage for several days so that all sensitive bacteria were killed. The only bacteria then able to conjugate were those forming sex pili which failed to adsorb the RNA phage. The phage-treated culture was therefore mated with a recipient and about 50 per cent of the sex factors transferred shown to be mutants whose pili still adsorb filamentous DNA phage but not the round RNA phage used for selection. The alternative type of mutant, sensitive to RNA but resistant to DNA phage, has never been described, possibly because DNA phage does not immediately

kill infected cells. However, even a mutant DNA filamentous phage which kills instantly after infection, failed to select such mutants. This mutation presumably occurs but is incompatible with pilus synthesis and it is noteworthy that in the mutants isolated, the pili were grossly abnormal in appearance.

Analysis of the pilus antigens of different F-like sex factors (see p. 17) allows individual sex factors to be studied when one or more are present in the same cell. When a bacterium carries two repressor-deficient or two repressor-insensitive factors, both pilus antigens are present. When one factor is repressor-positive and the other repressor-sensitive, the antigen of the latter cannot be found. Dr. Cooke and Dr. Elinor Meynell previously found that a mutant F factor integrated in the genome and unable to produce sex pili is restored by only some de-repressed F-like R factors, leading to recovery of normal polarised Hfr transfer (Report 1969). The reason for the failures became clear with the discovery of their repressor-positive nature, for any restoration would be masked by repression. With the repressor-deficient R factors, restoration occurred with 12/26 mutants, accompanied by renewed production of F pilus antigen. Thus, it probably resulted from restoration of the missing F function by the R factor. Recovery of Hfr transfer also occurred with 2/4 of the I-like R factors, which are functionally unrelated to F. Here, in contrast to restoration by F-like factors, recovery did not depend on the particular mutational defect in the F factor, since it occurred with every F mutant; moreover, it was evidently not due to restoration of F function since only the I pili of the R factor, and no F pili, were produced. The genome of the I-like factor may have recombined with the integrated F and substituted its own origin of transfer.

Bacterial Sex Factors. With the notable exception of the F factor of *Escherichia coli* K12, the donor ability of most wild type R factors is repressed. De-repressed mutants exist of both R factors and Col factors and should theoretically be of two kinds, repressor-deficient and repressor-insensitive, if gene expression in sex factors is controlled in the same way as in other systems. The repression of F fertility by wild type F-like R factors formed the basis for their original classification into *fi*$^+$ and *fi*$^-$ subgroups. ColB-K98 is also *fi*$^+$ and, since there is no superinfection immunity between it and de-repressed mutant R factors (Report 1969), the nature of these mutants was tested by examining the effect of wild type ColB-K98 on the expression of their sex factor genes. In this way, Miss Frydman and Dr. Elinor Meynell found that 5/8 de-repressed F-like mutant R factors were insensitive to repression by ColB-K98, whereas the remaining 3 remained sensitive and thus presumably owed their de-repressed character to lack of repressor. If this were the case, these mutants should not repress F and, to test this, Dr. Cooke and Dr. Elinor Meynell transferred the R factors to an Hfr strain, which they then examined for normal Hfr behaviour. Here again, the same two groups of R factors differed, for whereas those repressed by ColB-K98 did not affect normal Hfr donor behaviour, those insensitive to ColB-K98 abolished normal chromosomal transfer. These findings may mean that the polarised chromosome transfer characteristic of an Hfr donor requires the functioning of the F factor situated at the origin of transfer and that those de-repressed R factors not repressed by ColB themselves continued to produce repressor.

The sensitivity of F to repression by other sex factors shows that its de-repressed behaviour results from failure to produce repressor. Miss Frydman, Dr. Cooke, Dr. Elinor Meynell and Professor Meynell have now isolated repressor-insensitive mutants of F which function normally in the presence of *fi*$^+$ R factors and ColB factors.

Gene Transfer and Drug-resistance in Vibrio cholerae. Chromosomal genes of the cholera vibrio are said to be transferred by conjugation, by a process so far poorly understood but said to be mediated by a sex factor named "P", analogous to the colicin factors present in *Escherichia coli* and other gram-negative enterobacteria. However, Miss Jean Tweedy found that the colicin-like activity was impossible to detect save by plating the test cells and the susceptible indicator strain together in overlays. By electron microscopy, the areas of inhibition

showed numerous rod-shaped structures resembling phage tails: the "P factor" may therefore be a defective phage. As a first step in determining whether sex factors other than P gave the same linkage relationships in genetic recombination experiments, Miss Tweedy successfully introduced each of the three drug-resistance factors, R1, R136 and R163 into a P^- strain of *V. cholerae*. These evidently still possessed their sex factors since they could be transferred to *E. coli*. The success of these experiments suggests that transmissible drug-resistance may eventually be as much of a problem in the antibiotic treatment of cholera as it has become in so many other infections.

DNA Synthesis in Phage-infected E. coli. The inhibition of DNA, RNA and protein synthesis seen in *E. coli* infected with 10 or more particles of phage ØX is caused by peripheral injury to the cell (Report 1969), although lysis does not occur. The cell membrane remained intact, and the cells formed spheroplasts after treatment with lysozyme. Infection with 4 particles per cell induced partial resistance to superinfection 5 minutes later (which required protein synthesis) but not when nitrous acid- or UV-killed phage were used. Capsid protein synthesised by the primary ØX may attach to the DNA of the second phage as it penetrates the cell, so preventing its further entry.

Particles which received up to 16 lethal hits with nitrous acid, or up to 30 lethal hits with UV or hydroxylamine, were not less inhibitory than live ØX. On the other hand, such killed ØX did not arrest bacterial DNA synthesis by the DNA-specific mechanism dependent on protein synthesis (Report 1969). With UV or hydroxylamine, 1 hit was sufficient to abolish the arrest function, whereas with nitrous acid the arrest hardly diminished until the phage had suffered 7-8 lethal hits. Nitrous acid may act more specifically than UV or hydroxylamine. The effect of this mutagen implicates a phage gene as the cause of the specific arrest of host DNA synthesis. Reports to the contrary probably stem from the use of a large number of infecting particles per cell, which cause generalised inhibition due to non-specific injury.

Bacterial DNA Synthesis after Amino Acid Starvation. Each cycle of bacterial DNA replication is generally thought to begin when an "initiator protein" is synthesised, after which the cycle can continue even without protein synthesis. It has been proposed that the maintenance of DNA synthesis, once started, may be under the control of a gene located near the histidine operon, because a particular histidine-requiring mutant of *E. coli* K12 stopped making DNA in minimal medium as soon as histidine was removed. However, there is a simpler explanation. Without histidine, this mutant cannot make adenine, a nucleic acid precursor, and so cannot synthesise DNA or RNA. When adenine was added, or when rapidly-growing cells, which may contain a larger adenine pool, were used, residual DNA synthesis approached that of the wild type.

Histidine is not a precursor of adenine, but exerts its effect indirectly by inhibiting the first step in histidine biosynthesis. It thus prevents the accumulation of an intermediate (which cannot be metabolised further as a result of the mutation) whose presence inhibits adenine synthesis. The histidine analogue, thiazolealanine, also inhibited the early step, preventing accumulation of the intermediate and permitting normal residual DNA synthesis. Five Salmonella strains with mutations in various genes of the histidine operon were tested. Two of these accumulated the inhibitory compound when histidine was removed, and failed to complete the cycle of DNA synthesis. A series of amino-acid-requiring derivatives is being prepared from the *E. coli* K12 strain for similar testing.

Electron Microscopy

Serology of Bacterial Pili. Dr. Lawn and Dr. Elinor Meynell continued their investigation of the serological relationships between the sex pili determined by different sex factors in enterobacteria (Report 1967). The method whereby antigen and antibody were allowed to react on the support grid was replaced by a micro-method employing miniature discs of membrane filter to retain the antigen, and washing away excess antibody. Only bound antibody remains, which is visualised by negative contrast.

The absence of significant cross reactions between the F-like and I-like classes of pili was confirmed with a larger number of sera (15 specific for F-like pili and 7 specific for I-like pili). Thus these two classes of sex pili not only differ in morphology and in their affinity for donor-specific phages (Reports 1967, 1968) but are serologically unrelated. Most of the F-like sex pili cross-reacted with each other but were separable into different serotypes by differences in the intensity of the cross reactions. The I-pili cross-reacted strongly and were not distinguishable with unabsorbed sera. By the use of sera cross-absorbed with a related antigen, the division of F-like sex pili into 4 serotypes (F and ColV; R1; R538-1; R100, R192 and R136) was confirmed. The degree of affinity for the F-specific phage Qβ of different F-like sex pili was not well correlated with the intensity of their serological cross reactions. It was also possible with absorbed sera to distinguish two serotypes of I-like sex factors (R144, R163 and R538-2; R64). Although the sex factors F and ColV have abundant sex pili, most sex factors are naturally repressed and have insufficient pili for serological investigation, so that it was necessary to test de-repressed mutants (Report 1968) for these factors. There is reason to suppose that the pili of such mutants, although more abundant, are identical with those of the parent, because when several independent mutants of a single sex factor were compared they were serologically indistinguishable.

The Biosynthesis of Bacterial Sex Pili. In studying the genetics of sex factor function (p. 16), the serological method was used to determine the types of pili produced by bacteria carrying two different sex factors. When one of the sex factors is I-like and the other is F-like, the result is straightforward; if both sex factors are neither repressed nor defective, separate I-like and F-like pili are produced, and can be distinguished morphologically, serologically or by using the donor-specific phage MS2 which attaches only to F-like pili. However, when both factors are F-like, only the serological method distinguishes their pili and here the result is less simple. Bacteria carrying F (a naturally repressor-deficient sex factor) and a repressor-deficient mutant (de-repressed mutant; p. 16) of the drug resistance factor R1 were treated with a specifically absorbed antiserum shown to react with R1 sex pili but not with F pili (R1—specific antibody); this antibody was bound strongly to all the sex pili. When the same culture was treated with F-specific antibody, this was again bound to most of the pili but to a varying degree and less strongly than to F pili. Thus every sex pilus produced by these bacteria usually contained both F and R1 antigen. The simplest explanation for these results is that the protein molecules from which F and R1 pili are assembled are similar enough to be co-polymerised into a mixed polymer. It is not surprising that the molecules of I-like pili and F-like pili cannot be co-polymerised in view of the major differences between these classes of pili.

TRIC Agent and Macrophages. Dr. Lawn collaborated with Dr. Taverne and Dr. Blyth in determining the fate of TRIC agent phagocytosed by peritoneal macrophages (p. 20).

The facilities of the electron microscope unit were used by the following.

Dr. F. R. Wells: structural alterations in microcirculation after injury by *Clostridium welchii* toxin.

Dr. Jennifer J. Wells: assessment of various methods for isolating liver cells with minimum damage.

Dr. Taylor, Dr. Godfrey and Miss Lanham: the structure of trypanosomes.

Miss Dod, Miss Ward and Miss Caroline Race: characterization of biochemical tissue fractions.

Dr. Lemcke: structure of mycoplasmas.

Mycoplasma

The Antigens of M. hominis. Mr. Hollingdale and Dr. Lemcke continued their work on the membrane antigens that stimulate the production of antibodies active in indirect haemagglutination (IHA) and growth-inhibition (GI) (Reports 1968, 1969). In collaboration with Mr. R. J. Manchee (Clinical Research Centre, Salisbury, Wilts.), it was shown that antibody which inhibits the adsorption of HeLa cells

to colonies of *M. hominis* is also directed against antigens in the membrane.

Although earlier experiments indicated that the major membrane antigens were not lipids, the possibility that they were lipoprotein complexes was not excluded. However, membranes from which lipid had been removed with *n*-butanol, like purified membranes, gave rise to GI and IHA antibody and to antibody inhibiting HeLa cell adsorption. This suggests that the membrane antigens are proteins rather than lipoproteins.

For separation and characterisation of the membrane antigens, it is clearly desirable that they should be in solution; many membrane proteins are, however, strongly hydrophobic. *M. hominis* membranes can be lysed by various detergents and retain their serological activity in immunodiffusion tests, in which they produce three main precipitin bands (1, 2 and 3) all of which are probably double. Polyacrylamide gel electrophoresis of membranes lysed with detergent resolved 10–15 protein bands; those that were serologically active were identified by immunodiffusion, and their immunogenicity is now being tested. In this way, it is hoped to prepare monospecific sera that can be tested in GI and IHA tests. Chemical methods for obtaining the membrane proteins in a soluble form were also explored; extracts obtained from membranes dissolved in cold aqueous phenol or 8M urea absorbed GI and IHA antibody, and antibody inhibiting HeLa cell adsorption. The urea extract was also immunogenic in rabbits. The resulting antiserum had low GI and IHA activity but did not inhibit HeLa cell adsorption. It contained precipitating antibody to component 3 of detergent-lysed membranes and a low concentration of antibody to one line of component 1. The immunogenicity of the phenolic extract, which apparently contains more component 1, is being investigated.

Electron Microscopy of Mycoplasmas. Since the mode of reproduction of mycoplasmas is still controversial, Dr. Lemcke explored with Dr. Lawn the technique of examining mycoplasmas harvested on membrane filters, thus avoiding high-speed centrifugation that may alter the shape of the non-rigid cells. Mycoplasmas filtered under different conditions were examined *in situ* in the membranes; unless they were first fixed, mycoplasma cells larger than the pore diameter could be squeezed through the filters, even under pressures much lower than those usually used to determine filterability. This confirms a long-standing suspicion that filtration is not a suitable method for determining the size of these organisms.

Simian Mycoplasmas. A mycoplasma from a human umbilical lesion was previously identified by Dr. Lemcke as serologically distinct from other species isolated from man. Recently, strains resembling this organism have been isolated from monkeys. Dr. Lemcke collaborated with Dr. M. F. Barile (National Institutes of Health, Bethesda, U.S.A.) in comparing a representative simian mycoplasma with her cultures of the umbilical strain, both serologically and by polyacrylamide gel electrophoresis of the cell proteins. The simian strain closely resembled the umbilical strain, but was not identical with it. As the differences were of the same order as those observed within other species of mycoplasma, the strains probably belong to the same species.

M. pulmonis Infections in Rats. Respiratory infection caused by *M. pulmonis* is still a problem in many stocks of laboratory rats, including specific pathogen-free (SPF) stocks. However, little is known about its route of introduction and transmission in such stocks, or to what extent latency is important in outbreaks of infection. Dr. Lemcke collaborated with Dr. P. Whittlestone (School of Veterinary Medicine, Cambridge) in tracing the progress of infection in groups of mycoplasma-free rats inoculated intranasally with *M. pulmonis* or exposed to rats already infected. The strain was obtained from an outbreak of acute respiratory infection in a commercial SPF colony. The appearance of complement-fixing antibody in the serum was associated with the appearance of lesions in the respiratory tract and middle ears, and isolation of the organism from these sites; this work is still in progress.

Dr. Lemcke also collaborated with Dr. B.

Wells (Institute of Cancer Research, Belmont, Surrey) in investigating the mycoplasma flora in the lungs of rats exposed to tobacco smoke. Histological changes in the tracheobronchial epithelium occurred more frequently in animals exposed to smoke when *M. pulmonis* was present, suggesting synergism between the mycoplasma and the smoke.

Mycoplasma-like Bodies in Plants. Until 1968, mycoplasmas were thought to be confined to animal hosts, but electron microscopy then demonstrated that mycoplasma-like bodies occur in certain plant diseases for which no viral aetiology has been established. In collaboration with Dr. B. D. Harrison (Scottish Horticultural Institute, Invergowrie), Dr. Lemcke attempted to isolate mycoplasmas from plants infected with potato "witches' broom" disease. Several media used for animal mycoplasmas were employed with and without the addition of the plant auxin, indolyl-3-acetic acid. No mycoplasmas were isolated in them, or in a partly defined medium suitable for the non-sterol requiring mycoplasma, *M. laidlawii*.

TRACHOMA AND INCLUSION CONJUNCTIVITIS

Replication in Cell Cultures. Dr. Blyth and Dr. Taverne continued to study the relationship between the trachoma/inclusion conjunctivitis (TRIC) agent and its host cell. This micro-organism grows well in baby hamster kidney cells (BHK-21) propagated continuously, which contain few lysosomes, but less efficiently in certain primary cultures and not at all in macrophages that are rich in lysosomes. Most human conjunctival cells contain many lysosomes, and the small proportion of cells infected in trachoma might be explained on the ground that TRIC agent multiplies only in the minority containing few of these organelles. However, many experiments with various cell lines of high or low lysosome content, including rat fibroblasts obtained from Dr. Carolyn Brown (National Institute for Medical Research), failed to provide conclusive evidence for the idea that growth of TRIC agents is invariably prevented in cells containing many lysosomes.

Additional evidence was obtained that TRIC agents apparently do not multiply in mouse peritoneal macrophages in culture (Report 1969). Electron microscopy by Dr. Lawn, light microscopy, and infectivity titrations all showed that ingested elementary bodies do not grow; and attempts to infect macrophages by intraperitoneal inoculation of mice failed because the inoculum induced a pure polymorphonuclear exudate. The finding that TRIC elementary bodies damage mouse macrophages was confirmed (Report 1969). A cytopathic effect was seen as soon as 6 hr. after ingestion of 10 or more live organisms per cell, but at least 1000 heat-killed elementary bodies were needed to induce the same effect. Both *s* and *f* strains (Reports 1963–1967) killed cells, and guinea-pig peritoneal macrophages were also susceptible. Macrophages were 100 times more susceptible to injury by TRIC organisms than were BHK-21 cells, which may contain at least 30 early inclusions without apparent damage. These findings may, in part at least, account for the pronounced cell necrosis usually seen in the trachomatous conjunctiva. The observation that TRIC agents do not grow in macrophages may explain why trachoma is a much more localised infection than those caused by Group B chlamydiae, some of which, such as psittacosis, are said to multiply in these wandering cells.

Further comparisons were made of wild-type *s* strains with *f* mutants derived from them. In BHK-21 cells the *s* type inclusions grew more slowly and retained their contents for at least 72 hr., by contrast with *f* type inclusions which were almost all empty by 54 hr.; furthermore, the *s*-type inclusions contained 5–50 times fewer infective particles. If this behaviour is paralleled in the eye, it might contribute to the relative inefficiency with which the wild-type trachoma agent parasitizes the conjunctiva.

When BHK-21 cells are infected with more than one elementary body, the resulting loci of growth fuse to form one inclusion. Such inclusions seem to contain more elementary bodies than those derived from single infections. Miss Barton found that with increasing multiplicity of infection

there was a corresponding increase in the yield of infective organisms from a given inclusion; but there was a decrease in relation to the total number of organisms originally inoculated. This apparently competitive effect was more pronounced early in the growth cycle than later. Miss Barton continued her investigations into the optimum conditions for maximum yield of elementary bodies and other components of the inclusion; and devised methods of assaying carbohydrate from inclusions (Report 1969) in order to study its relation to elementary bodies, and the influence of inhibitors.

With the aid of a quantitative micro-complement-fixation test, Miss Barton is attempting to detect differences between antigens formed at various times during the growth cycle of TRIC agents.

Experiments in Primates. Professor Collier found that the T'ang strain of trachoma and its parent strain PK-2 induced little or nothing in the way of physical signs when applied to the conjunctival surface in baboons (*Papio cynocephalus*); when injected under the conjunctiva, T'ang induced a more severe but transient infection. Inclusion bodies were never detected, but 2 weeks after inoculation Dr. Blyth and Dr. Taverne isolated T'ang from 2 of 11 animals and PK-2 from 1 of 5 animals, so that limited multiplication sometimes took place. These strains are thus of low pathogenicity for the baboon eye; the possibility of using them to induce immunity by conjunctival inoculation will now be investigated.

From one intake of baboons, some of whom were suffering from so-called non-specific folliculitis of the conjunctiva, particles morphologically resembling *Chlamydia* were seen in chick embryo yolk sacs inoculated with conjunctival scrapings. Attempts at subculture and at isolation from the eyes of other baboons with this syndrome were unsuccessful.

Professor Collier examined the possibility of using squirrel monkeys and marmosets as alternatives to baboons. A highly pathogenic strain of TRIC agent induced conjunctivitis and characteristic inclusions in both species; but the physical signs resolved rapidly without proceeding to follicular hyperplasia and the use of these monkeys was abandoned.

Trachoma Vaccine. All trachoma vaccines so far tested were prepared from elementary bodies and none of them conferred complete and long-lasting protection. In the hope of finding a more potent immunogen, Dr. Garrett began work on the antigenic composition of TRIC agent at various stages of its reproductive cycle. These studies demand the use of large amounts of highly purified material collected at defined stages of replication. Purification by sucrose gradient centrifugation of TRIC agent propagated in the yolk sac proved unsatisfactory: Dr. Garrett then devised a method of growing it in suspended cultures of a specially adapted line of BHK cells provided by Mr. T. W. F. Pay (Wellcome Laboratory, Pirbright). With an apparatus based on that of Mr. H. M. Smith (Animal Virus Research Institute, Pirbright) it is hoped to produce batches of infected cells amounting to several litres. Inclusion bodies grown in this system are spontaneously extruded from some host cells 40 hr. after infection, and this process is enhanced by storing the cells at 4°C, a finding that may prove useful in purification.

Mrs. Mogg, Mr. Harris and Professor Collier continued their studies of the antibody response to trachoma vaccines; the amount of complement-fixing antibody in the blood and its association with the IgM and IgG globulins were determined in rabbits injected intravenously with formalin-inactivated TRIC agent. In terms of magnitude, time course and distribution between the two classes of immunoglobulin, the responses to primary and repeated doses were very similar; in particular, the antibody induced by a second dose disappeared rapidly. The failure of booster doses to induce a substantially enhanced and prolonged antibody response may be one of the factors contributing to the poor immunogenicity of conventional trachoma vaccines.

Investigations Overseas. Under Professor Collier's direction, Dr. Shiona Sowa and Mr. J. Sowa extended their study of immunofluorescence techniques in the serology and diagnosis of trachoma (Report 1969) to include a group of 100 young Gambian children; tentative conclusions can now be drawn from the results, which are not yet complete.

The indirect method was used to test for antibody to TRIC agent in the IgG globulin of blood and in the IgG and IgA of conjunctival secretion. Dr. Parish gave advice on tests for IgA antibody, and checked the specificity of the anti-IgA reagent. Antibody was not found in significant amounts in people without trachoma; it was present in the blood of about 20% of those with early trachoma (Tr I), and of about 40% of children in the second stage (Tr II). The corresponding proportions of those with conjunctival antibody were less, and the titres were in general lower than in the blood. The finding that specific antibody in the conjunctival secretion was more often associated with IgG than with IgA was surprising; the possibility of leakage from the blood will be examined. There was a strong positive correlation between the presence of conjunctival inclusions and that of serum antibody; this might be explained on the assumption that the finding of inclusion bodies in detectable numbers indicates a relatively severe infection that is more likely to induce an antibody response. There was no evidence for the hypothesis that the onset of corneal vascularisation in trachoma is related to the appearance of antibody in the blood or conjunctival sac.

Further comparative tests on conjunctival scrapings (Report 1969) suggest that for diagnosing trachoma by finding inclusions, an indirect fluorescent antibody stain may be somewhat more reliable than the iodine method used hitherto; but it is slower to perform, and demands much more in the way of skill and technical facilities.

VIROLOGY

Infectious Hepatitis

Dr. Alwen continued his researches into the possible role of adenovirus type 5 in infectious hepatitis (Report 1969). With the cooperation of Sir James Howie (Public Health Laboratory Service), Dr. G. I. Watson (Royal College of General Practitioners) and other physicians, many acute phase and convalescent sera were obtained from patients with infectious jaundice. The tests so far completed confirm that the titres of adenovirus antibodies in sera collected early in the disease are significantly higher than in normal people.

Adult liver cells might be more satisfactory than foetal material for research on hepatitis, but are very difficult to maintain in culture. Nevertheless, Dr. Alwen, in collaboration with Dr. Jennifer Wells, was able to prepare adult rat liver cultures that proved susceptible to infection with adenovirus type 5. Their viability, in terms of capacity to synthesise protein, is being determined by autoradiography. These studies will if possible be extended to cultures of adult human liver.

Dr. Alwen showed that delayed hypersensitivity to adenovirus type 5 can be induced in guinea-pigs. Since this observation may be relevant to the pathogenesis of infectious hepatitis, an attempt will be made to identify the viral antigen responsible.

Vaccinia Virus

Mechanisms of Immunity. Miss Cox and Dr. Alwen continued to study hypersensitivity and immunity to vaccinia virus. Delayed hypersensitivity of at least 3 months duration, transferable to normal animals by peritoneal exudate cells, was established by sensitising guinea-pigs intracutaneously with live smallpox vaccine; reactions were elicited with heat-inactivated virus. The sensitizing virus did not multiply in the guinea-pig skin; increases either in sensitising or challenge doses enhanced the skin response. The finding that delayed hypersensitivity reactions to vaccinia propagated in sheep could also be elicited by challenge with virus grown in Hela cells, chick embryos or primary guinea-pig kidney cultures, eliminated the possibility of response to a host component of the virus.

Resistance to experimental infection with special reference to cell-mediated immunity will be studied in mice inoculated with a

neurotropic strain of vaccinia. Intravenous inoculation into the tail vein causes easily measurable local lesions on the tail, which, not being lethal, can be used for investigating both primary and secondary responses to infection.

Inactivated Vaccine. Dr. Turner continued his studies on vaccines inactivated by various methods (Report 1969). A single batch of virus of high titre was used to prepare vaccines inactivated by heat, formalin, hydroxylamine, β-propiolactone, ultraviolet irradiation and by light after dye-sensitization. In rabbits all the vaccines induced a similar degree of immunity to dermal challenge; there was no evidence from the skin tests that any of the vaccines induced hypersensitivity. Antibody responses estimated by virus neutralisation, haemagglutinin-inhibition and complement fixation discriminated between the effects of the various inactivating agents. They were, however, unrelated to immunity against dermal challenge in rabbits or intracerebral challenge in mice. Large doses of all the killed vaccines were needed to protect the mice against intracerebral challenge. The role of interferon as a factor in vaccinial immunity was confirmed.

Composition. Dr. Robinson continued to investigate the protein coat of vaccinia virus. Non-enzymic cleavage of tyrosyl-peptide bonds by electrolytic oxidation was undertaken in a specially constructed flow cell. The reaction is mild, specific for tyrosyl residues and non-penetrating so that only surface tyrosyl residues are oxidised. Sequential cycles of electrolysis and cleavage followed by centrifugation to remove residual intact particles resulted in reproducible breakdown of the virus protein. The products were isolated by gel filtration on agarose; the investigation of their immunological properties continues.

Effects of Freeze-drying. The effects of freeze-drying highly purified vaccinia virus derived from cell cultures were examined by Dr. Robinson and Dr. Murray. Loss of infectivity was accompanied by a reduction in the total number of sulphydryl groups, as estimated by an amperometric method. Glutathione reduced infectivity losses and prevented oxidation of sulphydryl groups. Accurate monitoring of the product temperature and rate of oxidation during freeze-drying, together with estimations of residual water, may make it possible to define the optimal conditions for freeze-drying virus vaccines derived from cell cultures.

Rabies Virus

Dr. Turner continued work on rabies vaccines. Three pilot-scale batches of purified vaccine were prepared from rabbit brain. After satisfactory potency tests they were freeze-dried; and tests continue for stability and freedom from detectable encephalitogens.

Further attempts were made to prepare rabies vaccine from virus propagated in human diploid cells (Report 1967). Although the yield of virus was increased by passage of a different strain in this substrate, it is still inadequate for vaccine production. Studies of the potent myxovirus inhibitors that show activity against rabies virus (Report 1969) were continued by Dr. Turner. The presence of inhibitor in commercial equine α globulin was confirmed by tests on further samples. The inhibitors do not seem to be antibody, since they failed to fix complement with a rabies antigen known to do so in the presence of antiserum. Their inhibitory properties for rabies virus were retained after treatment that destroyed activity against the influenza virus. These findings corroborate other evidence that rabies is not a myxovirus.

IMMUNOLOGY AND EXPERIMENTAL PATHOLOGY

Antitoxin Production

Extending his work on the purification of immunoglobulins from horse and mouse sera (Report 1969), Dr. D. E. Dolby attempted to prepare pure IgA. It proved possible to prepare mouse IgA of fairly high purity, but not horse IgA. Horse serum either lacks completely an immunoglobulin of this type or contains very little of it. The removal of lipoproteins from serum with dextran sulphate and precipitation of fractions with polyethylene glycol facilitated purification of all the immunoglobulins. Specific antisera to mouse immunoglobulins

produced by the injection of complexes of ferritin and mouse anti-ferritin into rabbits, are proving valuable for identification of the immunoglobulins.

By the above methods, the immunoglobulins in serum of mice immunised with *Bordetella pertussis* by Dr. Jean Dolby and Dr. Ackers (p. 15), were separated and identified. The sera of children recovering from, or vaccinated against, pertussis infection were also fractionated.

The process for pepsin refining of horse antisera, used routinely in the production of antitoxins for therapeutic use, was applied to the purification of goat sera against a snake venom preparation (Arvin) that is currently being tested clinically for use in cases of thrombosis.

Anaphylaxis

An Eosinophil-stimulating Factor in Anaphylaxis. Miss Smithson and Dr. Parish continued the investigation on the factors causing the sudden rise in the number of eosinophils in the blood and some organs in human anaphylaxis. (Reports 1964, 1965, 1969).

Anaphylaxis in rats sensitized passively with rat reaginic antibody was accompanied by an eosinophilia. No eosinophilia occurred in rats treated with rat IgG2 antibody, which does not sensitize the tissues anaphylactically. This confirms results in guinea-pigs (Report 1969). Rat reagin sensitises the mast cells, which degranulate during anaphylaxis. Accordingly, the role of damaged mast cells as eosinophilotropic agents was tested in the absence of reagin. Normal rats treated with the mast-cell-disrupting, histamine-releasing agent 48/80 had local and generalised eosinophilia. Similar eosinophilia was induced by injecting intact or disrupted homologous mast cells. Thus a mast cell substance, not histamine (Report 1965) or 5-hydroxytryptamine, is moderately eosinophilotropic, though fewer eosinophils are evoked than in anaphylaxis.

An attempt is being made to overcome the difficulty of making eosinophil counts of peritoneal fluid without saline lavage, because repeated injection of saline induces an eosinophilia: this may be related to the mast cell changes also occurring after repeated lavage.

Horse Serum Proteins in Anaphylaxis. Anaphylaxis is reputed to be a serious hazard when treating patients with therapeutic antisera prepared in horses, particularly because sensitivity to horse dander is common in atopic persons. To evaluate this risk, Dr. Parish tested, by passive cutaneous anaphylaxis in monkeys, the sera of 16 people who gave positive skin tests with horse dander allergens, 10 of whom also had rhinitis when inhaling the dander; the sensitivity developed spontaneously in all 16, and none had been injected with sera of any species.

Eleven of the sera reacted with extracts of horse dander, and 9 with whole horse serum or an albumin-rich fraction thereof. None reacted with the Lister Institute purified horse globulin antitetanus and antidiphtheria toxins. It appears that the risk from injecting purified horse globulin into people with spontaneous sensitivity to horse dander has been exaggerated. It is intended to compare these results with those from tests on sera of people who have had serum sickness resulting from treatment with horse serum.

Exogenous Antigens Acquired by Tissue Culture Cells

Dr. Parish continued to investigate the possibility that bacterial or fungal antigens are incorporated in cells of the skin, which are thereby predisposed to damage by specific antibody, with a consequent generalised eczema (Reports 1966 - 1969).

Primary cultures of guinea-pig kidney absorbed pure protein antigens and released acid phosphatase when exposed to antibody specific for the adsorbed antigen. The maximum enzyme release occurred within 5 minutes of the addition of antibody to cells previously exposed to the antigen for 2 hours, and 30 minutes after adding antibody to cells exposed to the antigen for from 16 hours to 3 days. This suggests that the antigen may be on the surface of cells exposed for a short time, but is intracellular after long exposures, so that the antibody has to penetrate the cell membrane in order to combine with it.

Serum from animals injected with antigen, or with adjuvants without antigen, contains a factor that releases acid phosphatase from homologous cells, and does not support cell growth as well as normal serum. The nature of this factor is being examined.

Antibodies to Bacteria in Generalised Eczema

Dr. Parish, in collaboration with Drs. R. H. Champion and E. Welbourn at Addenbrooke's Hospital, Cambridge (Report 1969), continued to study the cytotoxic effects of antibody cells acquiring antigen *in vitro*, in relation to the changes in generalised eczema. Samples of skin from patients with eczema were tested for bacterial antigens, and the sera tested for antibodies to these antigens.

Antigens of staphylococci, streptococci and *Escherichia coli* were detectable by immunofluorescence on the surface of the epidermis, in epidermal vesicles and occasionally just above the dermo-epidermal junction in eczematous skin. Immunoglobulins were rarely found associated with these antigens, as occurs in allergic vasculitis. Moreover, IgG and IgA globulins which were observed in samples of skin fixed in cold alcohol, are easily washed out of sections prepared from unfixed skin samples, indicating that they are not bound firmly to antigens acquired by skin cells. It was not possible to improve the sensitivity of the immunofluorescent technique so as to detect bacterial antigens in all samples of skin from which the bacteria had been cultured.

An earlier finding (Report 1968) was confirmed that in patients with generalised eczema the amount of antibody to microorganisms cultured from their skin is not abnormally high; frequently very little antibody is found to some species of bacteria. Antibody to bacterial antigens acquired by skin cells is unlikely to induce the cytotoxic changes of eczema.

During these tests, some of the sera were observed to precipitate with extracts of occasional strains of *Staphylococcus aureus*, and of *Corynebacterium* spp.; but they contained no agglutinating antibody. The precipitation results from a combination of the bacterial extract with serum proteins other than specific antibody. The activity cannot be removed by frequent absorptions with whole organisms. The biological activity of these precipitates is to be examined.

Biologically Active Substances Released from Mononuclear Cells

Dr. Parish examined substances released from lymphocytes and macrophages, specifically by antigen or non-specifically by inflammatory agents, for bactericidal activity and toxicity for tissue cells, as part of a study of the cellular changes which occur during bacterial infection, vaccination and auto-allergies. Lymphocytes from guinea-pigs sensitized to chemical or to bacterial antigens, when exposed to the antigen *in vitro*, released material that inhibited the *in vitro* migration of macrophages from normal animals and was cytotoxic for cultured cells. Both effects appear to be due to the one substance. Unfortunately the chemical and bacterial antigens were mildly toxic to lymphocytes from normal animals; it is hoped that this may be circumvented by combining the antigens with albumin.

Immunogenicity of Fractions of Diphtheria Toxoid

Dr. Parish started to isolate the components of diphtheria toxoid most active in inducing antitoxic antibodies. Several samples of toxoid were separated on Sephadex G200 and the fractions tested for their ability to induce antitoxin in guinea-pigs, and to flocculate with antitoxic antisera. One fraction, which was eluted before the main peak, had more antigen per milligram of protein than the following fraction, which contained the bulk of the protein. Furthermore, when fractions obtained from strongly and weakly antigenic toxoids were compared, the difference in antigenicity was greater in this small leading peak than in the main protein peak. The physical properties of the molecules in the leading peak are to be examined.

Mechanisms of Inflammation

Monocytogenic Factor in Delayed Hypersensitivity. Dr. Wells continued his studies (Report 1969) on the offspring of tuberculin-sensitive guinea-pigs. Male offspring of guinea-pigs sensitized with Freund's complete adjuvant responded more feebly than control animals to the monocytogenic effect of Freund's incomplete adjuvant. This effect is interpretable as an expression of tolerance induced by the transplacental transmission of maternal monocytogenic factor. The tolerance was greater in the offspring of mothers sensitized for 34 days or more before parturition than in those of mothers sensitized for less than 34 days. The female offspring of sensitized mothers appeared also to be tolerant, but the significance of their lesser response to Freund's incomplete adjuvant was difficult to establish because the responsiveness of female guinea-pigs was generally lower than that of males.

Vascular Responses in Injury. The action of sodium salicylate as a suppressor of venular but not of capillary damage in inflammatory tissue was investigated in the lesions of thermal injury (54° for 40 seconds) to the rat cremaster muscle. An intraperitoneal dose of salicylate, 600 mg/kg body weight, given half an hour before injury, decreased the deposition of circulating carbon in the capillaries by 6-fold and the exudation of dyed protein by about 2-fold in lesions aged 50 minutes. There was no significant effect on the slight deposition of carbon in the venules of this lesion-age.

The lesions were exposed to circulating carbon and dye for 10 minutes. When the exposure period was increased to 20 minutes, capillary deposition was equal to that in control animals, suggesting that the reported absence of a salicylate effect on capillary damage is due to a less sensitive indicator technique, in which there is a long period of exposure to circulating carbon.

The effect of short exposures to circulating indicator substances, carbon and pontamine blue, was also studied in the reaction of the rat cremaster muscle to the α toxin of *Cl. welchii*. Compared with the results of 45 minutes' exposure, the immediate venular and permeability responses evident with a 10 minutes' exposure were about halved. In the delayed phase, in which increased permeability is associated with capillary damage, the results with the short exposure were like those of the longer in lesions up to 5 hours old. Thereafter, as in the immediate phase, the shorter exposure gave the lesser response.

Activation of the Kininogenetic System in Plasma. Miss Mason continued to investigate the systems producing vaso-active globulins and polypeptide kinins that are generated by surface factor when it is activated by the exposure of some mammalian plasmas to glass. The succession of enzyme reactions terminates with the digestion of plasma globulin substrates by the enzyme kallikrein and the consequent release of kinin.

Previous studies on human plasma indicated that during activation an inhibitor is generated which checks the process in its early stages. A similar inhibitory mechanism, activated non-enzymically, operates in guinea-pig plasma. Because active surface factor can be adsorbed from the plasma that contains the inhibitor while the other components of the system are retained in precursor forms, it is suggested that the inhibitor prevents the reaction between surface factor and its substrate.

To gain understanding of the interrelationships of enzymes of the kinin-forming system by studying the properties of the enzymes in their precursor forms, Dr. McConnell and Miss Mason attempted to isolate human plasma prekallikrein. Since DEAE chromatography of plasma resulted in fractions containing predominantly active enzyme, and previous work indicated that heparin inhibits the system, heparin was added to batches of human plasma before fractionation. Isolation of prekallikrein was consistently achieved; the preparations contained trace amounts of active kallikrein but were otherwise functionally pure. They were not physico-chemically pure, the major contaminant being immunoglobulin IgG. Yields of prekallikrein from heparinized plasma were considerably lower than the yields of active enzyme from untreated plasma, since a proportion of the kallikrein was retained with heparin on the DEAE.

Miss Mason and Dr. McConnell studied both the activation of prekallikrein and the inhibitory role of heparin in the kinin-releasing system. So far, it appears that the enzyme is not autocatalytic and is directly activated not by the blood clotting substance Hageman factor, but by an esterase. Heparin appears to retard the production of kinin and vaso-active globulin in human plasma exposed to glass by blocking one or more of the initiating enzyme reactions.

Mechanisms of Infection and Defence

E. coli Infection in the Mouse. Dr. Polk, with Sir Ashley Miles, investigated the first few hours of infection of mouse adductor muscle by *Escherichia coli*; with a view to confirming the validity of the early decisive period in the primary lodgement of bacteria in the tissues, postulated on other grounds. The strain of *E. coli* was chosen to represent the relatively non-pathogenic organisms that may infect open wounds. It is a lysogenic strain that can be superinfected with a related bacteriophage which, failing to be incorporated into the bacterial genome, remains living but not replicating in the cytoplasm of the bacterium. As Professor Meynell showed, when the bacillus divides, the superinfecting phage goes to one daughter cell only, so that, at a given time during the multiplication of a culture of these superinfected organisms, the ratio of those carrying superinfected phage to those from which it has disappeared gives a direct measure of the division rate of the bacteria.

Viable counts of the *E. coli* in the muscle indicated a decisive period of about 4 hours, after which most of the inoculum had disappeared. The rate of disappearance was greatly slowed when the animals were shocked, given ferric iron systemically, or received local adrenalin into the muscle; but neither of these modifying agents affected the course of infection when applied in animals with 4-hour-old lesions. In freshly dead muscle the *E. coli* multiplied steadily, with a division rate at the 4th hour of about 40 minutes. In live muscle the division rate at the 3rd hour was about 8 hours and at the 5th hour about 5 hours.

Klebsiella Infection in the Guinea-pig. Sir Ashley Miles began a study of the mechanism of enhancement of bacterial infections in animals receiving large doses of either ferric or ferrous iron. Local infections in the skin of the guinea-pig by a large variety of bacterial species were investigated. Not all the species whose systemic infectivity for mice is reported to be enhanced by iron were enhanced in the guinea-pig skin; moreover, the infectivity of some species was depressed in iron-treated animals.

Among the species enhanced by iron, Klebsiella was selected for further study. Strains were obtained from Dr. Patricia Carpenter of the Central Public Health Laboratory, London, and from Dr. I. W. Sutherland of the College of Agriculture in Edinburgh. Single doses of iron given at the same time as the Klebsiella proved to act largely during the first few hours; that is, during the early decisive period of the local infection, when there is only a transient exudation of plasma and virtually no exudation of phagocytes into the infected tissue. Ten strains were accordingly tested for their skin virulence, susceptibility to iron enhancement, susceptibility to the bactericidal power of fresh guinea-pig serum and their iron requirements in chemically defined media. None of the results so far obtained support the notion that enhanceability by iron is dependent on satisfying the invading microbes' need for that metal, or that it depends on a destruction by the iron of the natural bactericidal power of the blood for the infecting microbe. The investigation of the nature of the enhancement continues.

BIOCHEMISTRY

Human Blood-group Substances

Structural Studies. The blood-group A, B, H, Le^a and and Le^b active substances isolated from human secretions are glycoproteins with a major carbohydrate component. The blood-group determinants are at the non-reducing ends of the carbohydrate chains in the glycoproteins and there is no evidence that the minor peptide moiety (10-15%) plays any direct part in specificity. Nevertheless, the peptide is important in maintaining the structural integrity of the macromolecules because limited degradation

with the proteolytic enzymes ficin or papain results in loss of serological activity without, as far as can be ascertained, any change in the carbohydrate structures (Report 1959). Moreover, the peptide moiety has a very distinctive composition; serine and threonine account for about half the amino acids and aromatic and sulphur-containing amino acids occur only in trace amounts (Report 1962). Dr. Donald and Professor Morgan attempted to devise techniques whereby the peptide moiety of the blood-group specific glycoproteins could be obtained free from, or with a considerably reduced content of, carbohydrate. Degradation at 60° with 0.5 *N* hydrogen chloride in methanol or propanol, or with 0.5 *N* sulphuric acid in glacial acetic acid yields products containing a considerably higher content of peptide than the original glycoprotein; of the three procedures the sulphuric acid-glacial acetic acid treatment is the most promising. Blood-group substances treated for 24 hours by this method yield a residue that is insoluble in the acid solvent but soluble in water. Saponification with weak alkali removes *O*-acetyl groups and the resulting non-dialysable product has an amino acid composition closely similar to that of the parent glycoprotein but a considerably changed carbohydrate composition. The main sugar left in the preparation is *N*-acetylgalactosamine; fucose is completely eliminated and the galactose and *N*-acetylglucosamine contents are much reduced. The products derived from A, B, H and Le^a substances have strikingly similar compositions and behaviour and are essentially *N*-acetylgalactosaminyl-peptides.

Earlier experiments on intact blood-group active glycoproteins indicated that *N*-acetylgalactosamine is the sugar proximal to the peptide chain, linked by *O*-glycosidic bonds to the hydroxyamino acids serine and threonine. By alkaline reductive cleavage of the *N*-acetylgalactosaminyl-peptide from B substance, Dr. Donald obtained direct evidence for this carbohydrate-peptide linkage; the loss of galactosamine was matched by loss of an approximately equimolar amount of serine and threonine, whereas the proportions of the other amino acids were unchanged. Professor Watkins and Professor Morgan established by serological inhibition tests with the agglutinin from *Helix hortensis*, a reagent specific for α-linked *N*-acetylgalactosamine, and by enzymic hydrolysis with an α-*N*-acetylgalactosaminidase from *Trichomonas foetus* (Report 1964), that the *N*-acetylgalactosamine in the glycopeptide, and hence in the glycoprotein from which this fragment is derived, is joined in α-anomeric linkage to the hydroxyamino acids.

Attempts to eliminate by chemical means the remaining sugars adhering to the peptide have so far proved unsuccessful. Mrs. Goodwin and Professor Watkins are therefore investigating enzymic methods for the removal of these residues. Treatment of the glycopeptide with α-*N*-acetylgalactosaminidase from *T. foetus* releases up to 75% of the *N*-acetylgalactosamine and the added enzyme can be separated from the glycopeptide by fractionation of the dialysed reaction mixture on a column of Sephadex G200. A second treatment with the α-*N*-acetylgalactosaminidase does not result in the liberation of more *N*-acetylgalactosamine, but the apparent resistance to attack may result from the presence on the sugar of galactose or *N*-acetylglucosamine substituents that prevent the action of the enzyme. Suitable enzymes for hydrolysing the remaining galactose and *N*-acetylglucosamine residues are being sought and it is hoped to obtain a sugar-free peptide by the sequential action of the different glycosidases.

Biosynthesis of Blood-group Substances. Dr. Hearn, Miss Race and Professor Watkins continued their investigations of the α-*N*-acetylgalactosaminyltransferase and α-D-galactosyltransferase associated respectively with the A and B blood-group characters (Report 1969). The finding that acetone powders prepared from particle-bound enzyme preparations from human stomach linings or submaxillary glands retain activity for at least three months has greatly facilitated the work on these transferases. With the range of low molecular weight oligosaccharide acceptors so far tested the acceptor-substrate requirements of the two enzymes were identical. Considerable similarity in properties and structure are to be expected if, as has been predicted, the

two transferases are the primary products of the allelic *A* and *B* blood-group genes. To allow more detailed comparisons, the enzymes must first be isolated in a soluble form. Miss Race found that treatment with ultrasonics or extraction of the particles with sodium deoxycholate resulted in total loss of α-galactosyltransferase activity. Lipid solvents, such as ether, n-butanol and tetrahydrofuran, tested by Dr. Hearn on the particle-bound α-*N*-acetylgalactosaminyltransferase, did not release the enzyme although the activity of the extracted particles was enhanced. The most effective method so far investigated for solubilisation of either transferase is extraction of the acetone powder of the particulate enzyme with the non-ionic detergents Triton X-100 or digitonin. Attempts to purify the soluble enzymes are now being made.

In these biosynthesis experiments the amount of a radioactive oligosaccharide formed by enzymic transfer of [^{14}C]-labelled sugars to low molecular weight acceptors is limited by the amounts of enzyme and of labelled nucleotide-sugar available. The yield, calculated from the amount of radioactivity incorporated, is often less than 1 μg and the only sugar in the compound that is identifiable is the one carrying the radioactive marker. To facilitate characterisation of these compounds, Dr. Hearn devised a method for identifying the other sugar components. The labelled oligosaccharides are hydrolysed with acid under conditions that break them down to the free sugars, and are then reduced with tritium-labelled potassium borohydride. The sugar alcohols formed on reduction are thereby labelled with tritium and may be separated by a combination of paper chromatography and electrophoresis, and identified by comparison with authentic specimens of the alcohols. The radioactive tetrasaccharide formed by incubation of the α-*N*-acetylgalactosaminyltransferase from group A tissues with uridine diphosphate *N*-acetyl-[^{14}C]-galactosamine and 2′-fucosyllactose (α-L-fucosyl-(1→2)-β-D-galactosyl-(1→4)-D-glucose) was subjected to this treatment and from the products tritium-labelled fucitol, galactitol, glucitol and galactosaminitol were separated and identified.

Previous experiments established that the α-*N*-acetylgalactosaminyl- and α-galactosyl-transferases convey the sugars from the appropriate nucleotide donors to the β-galactosyl residue in the acceptor molecules. However, the positional linkage, that is, the precise carbon atom in the acceptor sugar to which the glycosidic bond is made, was not known. To fulfil the requirements for the formation of serologically active A and B structures the linkage must be to the carbon-3 position. The α-galactosyltransferase from group B tissues has a high affinity for both uridine diphosphate D-galactose and 2′-fucosyllactose, and with this system Miss Race synthesised an unlabelled tetrasaccharide in sufficient quantities for serological examination. The tetrasaccharide specifically inhibited the agglutination of group B red cells by a human anti-B serum to the same extent as the B-active tetrasaccharide isolated from the alkaline degradation products of human B substance (Report 1965); this is strong evidence that in the enzymically synthesised product the galactose is attached to the carbon-3 position of the β-galactosyl residue in 2′-fucosyllactose.

The distribution and properties of the fucosyltransferases occurring in human tissues synthesising blood-group substances (Report 1969) were further examined by Mr. Chester and Professor Watkins. On homogenisation and fractionation of submaxillary gland and stomach mucosal linings the 2-, 3- and 4-fucosyltransferases were located in the 100,000 *g* pellet and to a lesser extent in the 100,000 *g* supernatant. The particle-bound enzymes are stable in the form of acetone powders, and digitonin extraction of these powders in part solubilises the enzymes. The digitonin extract contains 3- and 4-fucosyltransferases but very little of the 2-fucosyltransferase that forms H-active structures (Report 1969).

By contrast with the α-galactosyl- and α-*N*-acetylgalactosaminyl-transferases that have a very specific requirement for Mn^{2+} ions, none of the fucosyltransferases appear to have an absolute requirement for a divalent metal cofactor.

Experiments with different low molecular weight oligosaccharide acceptor-substrates indicated that the fucosyltransferases do not act in any specific order; 2′-fucosyllactose (α-L-fucosyl-(1→2)-β-galactosyl-(1→4)-D-glucose) and 3-fucosyllactose (β-galactosyl-(1→4)[α-L-fucosyl-(1→3)-]-D-glucose) were both converted to lacto-difucotetraose (α-L-fucosyl-(1→2)-β-galactosyl-(1→4)-[α-L-fucosyl-(1→3)-]-D glucose) by enzyme preparations from the tissues of secretor persons. Similarly lacto-*N*-fucopentaose I, and lacto-N-fucopentaose II, which differ only in that one has fucose on the carbon-2 position of the terminal β-galactosyl residue whereas the other has fucose on the carbon-4 position of the subterminal *N*-acetylglucosaminyl residue, are both converted to lacto-*N*-difucohexaose I in which the terminal and subterminal sugars are substituted with L-fucose. The B-active tetrasaccharide synthesised by Miss Race, (α-D-galactosyl-(1→3)-[α-L-fucosyl-(1→2)-]-β-D-galactosyl-(1→4)-D-glucose), also accepted a second fucose residue, presumably at the carbon-3 position on the terminal reducing glucose unit. These results explain why oligosaccharides containing *N*-acetylgalactosomine or galactrose attached to β-galactosyl-*N*-acetylglucosamine units, in which both sugars are substituted with fucose, have been isolated from A and B active glycoproteins although the compounds containing two fucosyl residues are very poor acceptor-substrates for the A and B gene specified transferases (Report 1969).

Miss Ward and Professor Watkins continued their work on the incorporation of radioactivity from [^{14}C]-labelled low-molecular weight compounds into blood-group glycoproteins in human and baboon gastric mucosal slices (Report 1969). The radioactive products were solubilised by tryptic digestion of the tissue and the labelled blood-group substances were isolated by precipitation with specific antisera. Incubation with [^{14}C]glucose, [^{14}C]glucosamine or [^{14}C]fucose gave radioactive blood-group substances, but there was little incorporation when [^{14}C] threonine was used as the labelled precursor. Known pathways exist in mammalian tissues for the conversion of D-glucose into the four sugar components of blood-group substances—galactose, fucose, *N*-acetylglucosamine and *N*-acetylgalactosamine. [^{14}C]Glucose was incubated with mucosal lining from a human group O stomach; the radioactive H substance recovered from the H-anti-H precipitate was hydrolysed and examined chromatographically. All the component sugars of the H substance were labelled with [^{14}C]. The absence of other labelled sugars, such as glucose or mannose, proved that the radioactivity did not arise from non-specific adsorption to the blood-group substance and that the material precipitated by the anti-H reagent was not contaminated by other glycoproteins. Galactosamine was the least labelled sugar in the H-substance; a result that accords well with the belief that this sugar occurs only at the distal end of the carbohydrate chains at the point of attachment to the peptide backbone.

Homogenisation of the tissue incubated with the labelled precursor, followed by fractionation at 10,000 and 100,000 *g.*, revealed that most of the radioactive blood-group substance was associated with the 100,000 *g.* pellet and supernatant. The 100,000 *g.* pellet, examined in the electron microscope by Dr. Lawn, consisted almost entirely of smooth membranes. This finding is consistent with the implication of the smooth membrane system, especially the Golgi complex, in the biosynthesis of the carbohydrate moiety of glycoproteins but, as these tissues were incubated at 37° for 16 hours before fractionation, the rough membranes may have been destroyed by the action of ribonucleases.

Biosynthesis studies of macromolecular blood-group substances were continued by Dr. Poretz (Report 1969). [^{14}C]-Galactose from uridine diphosphate [^{14}C]galactose was incorporated into exogenous H substances by particulate preparations from group B tissues and about 30% of the radioactivity was released by α-galactosidase. When group A or O tissues were used as the enzyme source [^{14}C]galactose was transferred to exogenous H substance but negligible amounts were released by α-galactosidase. Digitonin treatment of the particulate preparation partially solubilised the galacto-

syltransferases and the amount of α-linked [^{14}C]galactose incorporated into H-substance increased to 60–75% of the total. These results demonstrate an α-galactosyltransferase in group B tissues that uses macromolecular H substance as an acceptor, and does not occur in group A or O tissues. Addition of the α-linked galactose does not convert the H substance into B substance, since it is not precipitable to any extent by an anti-B serum; and therefore the galactose may not be added in the (1→3) linkage that completes the B determinant. However, the total number of D-[^{14}C]galactose residues added to the H substance, calculated from the specific activity of the UDP-D-[^{14}C]galactose, is very small compared with the probable number of carbohydrate chain-endings with available H-active structures. The failure to obtain increased precipitation of radioactive material by antiserum may be because too few new B determinants are formed on the H-active glycoprotein to give the surface configuration of active groupings necessary for firm combination and precipitation with the B antibody.

Lipids and Biological Membranes

The object of this work is to define the roles of certain lipid components of the plasma membrane of a mammalian cell in relation to the structural integrity—especially permeability—and function of the membrane.

The Plasma Membranes of BP8 Ascites Tumour Cells. Dr. Gray and Miss Dod continued their work on the preparation of cell membranes and the analysis of their lipid components. There is no general technique for preparing plasma membranes from different tissues and each type of cell presents special problems. A method whereby plasma membranes of cells in suspension were stabilised by treatment with fluorescein mercuric acetate, and were isolated in the form of cell ghosts after homogenizing the suspension, was modified by Miss Dod to prepare membranes of BP8 mouse ascites tumour cells. The plasma membranes were separated from the cell contents and unbroken cells by density gradient centrifugation on sucrose. Electron microscopy showed that the final preparation consisted mainly of membranous sheets with fibrils attached to one side. There was slight contamination by whole cells, mitochondria and small particles.

The phospholipid components of the membrane contained 26% of sphingomyelin whereas those of the whole cell contained only 12%; and in terms of double bonds per phospholipid molecule, were also approximately three times as saturated. There was also a tenfold increase in the molar ratio of glycosphingolipid to phospholipid (105×10^{-3} compared with 9.55×10^{-3} for the whole cell). The glycosphingolipids accounted for 10% of the total lipid in the membrane and the major compound was diglycosylceramide. These findings agree well with those for the plasma membranes of rat liver cells (Report 1969), and support the idea that in general the lipids in the plasma membrane of a cell have a more rigid structure than those in the membranes of other subcellular organelles.

The Action of Drugs on Intact Mammalian Cells. Dr. Jennifer Wells continued her attempts to prepare a suspension of rat parenchymal liver cells for use in membrane permeability studies. The culture of these cells is difficult and has not been reported. With Dr. Alwen, Dr. Wells showed that cells obtained by perfusing the liver with 0.1% trypsin in modified phosphate-buffered saline containing 0.3% hyaluronidase and 0.3% collagenase survived in culture for up to 72 hours. The cells were viable and suitable for biochemical studies.

Work was continued on the action of the polyene antibiotics (filipin, etruscomycin, amphotericin B and nystatin) on different mammalian cells. These drugs first caused the cells to become flatter, and their contents to become more easily visible by phase contrast microscopy; they then become more permeable to eosin, and finally lyse. Filipin and etruscomycin lysed red cells, rat liver cells and BP8/C3H ascites cells but nystatin and amphotericin B lysed only red cells. Eosin was taken up by liver cells treated with nystatin, but not with amphotericin B. Only the initial changes were observed in BP8/C3H ascites cells treated with either of these drugs. It

is noteworthy that the diminishing order of susceptibility to nystatin of red cells, liver cells and BP8/C3H cells is the same as that for the concentrations of cholesterol in their plasma membranes. In model lipid systems the polyene antibiotics form complexes with cholesterol.

Glycosphingolipids

Metabolism and Biosynthesis of Mouse Kidney Glycosphingolipids. There is a conspicuous difference between the glycosphingolipid composition in the kidneys of male mice and of female mice (strains C3H/He, C57/BL, C57xA (F_1 hybrid), A and BALB/c). In males diglycosylceramide is a usual component of the glycosphingolipids whereas in females it is present in at most trace quantities.

Dr. Gray and Mr. Hay showed that the kidney glycosphingolipids of female mice (C3H/He and C57/BL strains), previously injected with testosterone daily for two weeks, contained a proportion of diglycosylceramide similar to that usually found in the kidneys of the normal male. It was concluded that testosterone was a controlling factor in the biosynthesis of the diglycosylceramide. In relation to the influence of testosterone, it is interesting that the glycospingoliphids in BP8 ascites cells grown in male C3H/He mice contained a greater proportion of diglycosylceramide than those grown in females.

The difference in the glycosphingolipid compositions of male and female mouse kidney may explain the low content of triglycosylceramide in the kidneys of C3H/He females carrying BP8 tumours (Report 1969) compared with normal females. Mr. Hay studied the biosynthesis of of the glycosphingolipids in the C3H/He mouse kidney and showed that the rate of synthesis of diglycosylceramide in the female kidney was only 20% of that in the male kidney. Furthermore, the presence of a BP8 tumour in the host depressed the rate of synthesis of diglycosylceramide in both male and female kidneys so that in the female kidney synthesis of diglycosylceramide virtually ceased. As this compound is the precursor of the triglycosylceramide, the concentration of the latter in the tissue cannot be maintained; and while the mouse is carrying the tumour the triglycosylceramide in the kidney is reduced to a trace.

Dr. Coles continued her studies of the biosynthesis of the diglycosylceramides in the kidneys of the C57/BL mouse. Kidney homogenates of male and female mice synthesised lactosylceramide from UDP-galactose and monoglucosylceramide. The finding that triglycosylceramide was synthesised, by further galactose transfer from UDP-galactose to lactosylceramide, faster than the diglycosylceramide compound, explain why lactosylceramide does not accumulate in the kidneys of either sex. Kidney homogenates from both sexes could synthesise digalactosylceramide from UDP-galactose and monogalactosylceramide. Although the female kidney contained only a small amount of digalactosylceramide (less than 5% of total glycosphingolipid) compared with that of the male (29% of total) the rate of synthesis of this compound from added substrate by homogenates of female kidney was similar to that in homogenates of male kidney. A possible explanation of this anomaly was that *in vivo* there was a lack of the substrate, monogalactosylceramide, in the female kidney; and further studies showed that the rate of synthesis of this compound was indeed much lower in homogenates of female kidney than in those of male kidney.

Glycosphingolipids in Normal and Transformed Cells. The glycosphingolipid compositions of cells transformed by viruses are different from those of normal cells and the difference may be connected with the changes that occur in the surface properties of the cell on transformation.

In collaboration with Dr. I. A. Macpherson, Imperial Cancer Research Fund, Lincoln's Inn, Dr. Gray examined the glycosphingolipids of two lines of hamster kidney cells (BHK21-C13 and NIL-2E), and compared them with those of the same lines of cells transformed by polyoma viruses, strains of Rous sarcoma virus, adenovirus and murine sarcoma virus. In general, different viruses induced different changes in the glycosphingolipid compositions of the cells. In some instances (e.g. polyoma-transformed derivatives of the

NIL-2E line) the transformation caused a significant change in the total negative charge associated with the glycosphingolipids. These changes are of considerable interest in relation to the properties of the malignant cell membrane.

BIOPHYSICS

Human Plasma Proteins

Structure of Human Alpha Macroglobulin. In the study of the structure of human α macroglobulin the tendency of the "8.5S" sub-units, obtained by treatment with N-acetyl cysteine and iodoacetamide, to reaggregate after purification restricted progress (Report 1969). Continuing their attempts to overcome this difficulty, Professor Kekwick and Dr. Jones established that reduction with 0.01M N-acetyl cysteine at pH 8.0 in the presence of 1M urea followed by carboxymethylation with iodoacetamide provided a mixture from which stable solutions of the purified "8.5S" sub-unit could be prepared. Reduction in the presence of 1M urea also improved the yield of "8.5S" sub-unit from 40% to about 55%. Purified samples of sub-unit were obtained initially by passing the reduction mixture through a column of 10% agarose beads equilibrated with sodium phosphate buffer containing 1M urea; but the method had to be abandoned because the molecular sieving characteristics of the agarose deteriorated progressively, probably because of prolonged contact with urea.

In collaboration with Dr. Creeth, separation of the "8.5S" component from the mixture, dissolved in phosphate-urea buffer, was accomplished by high-speed centrifugation followed by displacement fractionation. The "8.5S" component was characterised by equilibrium molecular weight measurements, by determining the optical rotatory dispersion parameters and the buoyant density in a gradient of caesium chloride. Corresponding measurements were made on the native α macroglobulin. The native (19S) protein has a molecular weight of about 720,000, a significantly lower figure than that found by other workers (820,000). It possesses a reasonably compact structure of low helix content. The "8.5S" component has a molecular weight of about 180,000 with essentially the same optical rotatory dispersion and buoyant density characters, and serological specificity of the native molecule. It must therefore be a sub-unit corresponding to one quarter of the native molecule. The identity of the buoyant densities of the native molecule and the "8.5S" sub-unit implies that the carbohydrate that constitutes 8.4% of the molecule is similarly distributed in each of the sub-units.

Microheterogeneity of Normal Human Gamma Immunoglobulin. Among the five groups of human immunoglobulins so far identified, gamma macroglobulin IgM is quantitatively a major component, although normal human plasma contains on average only 75 mg/100 ml. For this reason our knowledge of this protein comes predominantly from material in the plasma of patients with Waldenström macroglobulinaemia, where it occurs in concentrations of up to 4%, and is accordingly obtainable in relatively large amounts. In the large-scale fractionation of normal human plasma for clinical purposes at the Blood Products Laboratory, gamma macroglobulin (IgM), with alpha macroglobulin (α_2M), is segregated into a sub-fraction. From this source it is possible to prepare rather larger amounts of normal human γM than are generally available.

Immunological evidence from observations on Waldenström γ macroglobulins, which are probably of monoclonal origin, suggests that two sub-types of IgM may exist and should be present in normal human IgM. Professor Kekwick and Mrs. Butterworth began an investigation of the microheterogeneity of intact normal human IgM, and of the sub-units and peptide chains that may be derived from it by controlled reduction with thiol reagents, by studying their behaviour during isoelectric focussing in pH gradients established in polyacrylamide gel.

Normal IgM, separated from the α_2M-IgM concentrate at pH 6.8, I 0.01 was purified by further precipitation but still contained traces of IgM that induced antibody in rabbits but were not detectable by immunodiffusion, immunoelectrophoresis or

by electrophoresis in polyacrylamide gel. Because of the tendency of normal IgM to aggregate, satisfactory analysis by isoelectric focussing has not yet been attained with the intact molecule. Preparations of the 7-8S sub-unit, obtained by limited reduction with mercaptoethylamine followed by purification by molecular sieve chromatography, have a single sedimenting peak in the ultracentrifuge and resolve into four main and some minor bands when subjected to isoelectric focussing in a gradient extending from pH 5 to pH 8. Heavy (μ) and light polypeptide chains were prepared from normal IgM by more extensive reduction with mercaptoethanol followed by chromatographic separation. The isoelectric focussing pattern of the μ chains, with four main bands, was closely similar to that of the 7-8S sub-units. The light chains showed 7 or 8 bands and, as might be expected, the pattern resembled that of light chain preparations derived from normal IgG globulin.

The work continues and comparative studies will be made with Waldenström macroglobulins as they become available.

Other Macromolecules

Blood-group Glycoproteins. In collaboration with Professor Watkins, Professor Morgan and Dr. Donald, Dr. Creeth investigated the physical properties of a macromolecular glycopeptide isolated from a blood-group B glycoprotein (see p. 28). The glycopeptide contained all the amino acids of the original glycoprotein but had lost almost all the carbohydrate except those N-acetylgalactosamine residues linked by glycosidic bonds to serine and threonine; since it was produced by a degradation that is thought to leave peptide bonds largely unattacked, its molecular weight and extent of heterogeneity are of great interest. Both sedimentation velocity and sedimentation equilibrium measurements suggested substantial homogeneity, with a molecular weight in the region of 12,000. Two-thirds of the remaining N-acetylgalactosamine could be removed enzymically without loss of amino-acids, giving a product presumably rather close to the postulated structural peptide; in agreement with expectation from the analytical figures, the molecular weight was about 10,000. The frictional ratio of 1.7 obtained for the larger glycopeptide indicates a moderately expanded or asymmetric conformation. Experiments in progress should decide whether the small peptide is indeed a true structural sub-unit or whether it is itself partly degraded.

Studies of the native glycoproteins by the density-gradient equilibrium procedures described previously (Report 1969) were continued, attention being chiefly directed towards the theoretical interpretation of the patterns recorded. These patterns are much broader than would be expected from the known average molecular weight, because of the spread in density arising from variability in composition; comparison of the expected and observed patterns provides information about the glycoprotein that is not otherwise obtainable. However, the breadth of the distributions prevents determination of the mean buoyant density with the precision expected from the density gradient method, and the use of density gradient-forming salts other than caesium chloride or sulphate was explored. The need is for a salt with a very steep distribution at a given speed, and with ions differing greatly in size; so far caesium bromide has proved the most useful.

Reversible Denaturation of Proteins

Dr. Creeth and Mr. Holt continued their investigation (Report 1969) of the reversibility of denaturation of ovalbumin, a protein that has proved extremely recalcitrant in this respect. In previous work, moderately high concentrations were used, with the emphasis on ensuring strictly reversible thermodynamic pathways; since these attempts proved abortive, experiments were performed with very low concentrations (approx 20 μg/ml), to simulate conditions under which many enzymes recover their native conformation. These experiments confirmed the findings previously reported, precipitation of the protein occurring before the native characteristics were more than half restored. Recent developments suggest that ovalbumin may not be unique in its lack of ability to recover its native conformation; emphasis is now placed on the existence, in some cases, of insuperable kinetic barriers at all realisable

concentrations. Thus the concept that the native conformation simply and universally represents a free-energy minimum may require qualification.

In work with human and bovine serum albumin (Report 1969), renaturation was achieved relatively easily, at least to the extent of preparing a globular protein with many of the properties of the original. Some puzzling features remained, however, in particular an anomalous concentration-dependence of sedimentation coefficient and rather low values of the molecular weight. Further work on this protein was prompted by the reported preparation of sub-units of bovine-albumin by the action of the powerful reducing agent dithiothreitol. The molecular weight of the denatured protein was measured in the presence of mercapto-ethanol, to simulate the conditions at the start of the reversal of denaturation. The finding that the molecular weights of the non-reduced denatured protein and of the corresponding mercaptoethanol-treated substance were respectively 74,000 and 36,000 confirmed the existence of a two-chain structure for this protein. The anomalous features referred to in the renatured material thus result from incomplete recombination of sub-units as denaturant and reducing agent are removed; suggesting the interesting possibility of preparing a stable renatured sub-unit.

Methodology of Ultracentrifugal Analysis

A New Method for Molecular Weight Measurement. Globular proteins can be characterised by many well established methods; however, in the analysis of mixtures, polydisperse substances, or substances with pronounced thermodynamic non-ideality (all of which are commonly encountered in the everyday work of the department) many problems arise (Reports 1966, 1969). Chief among them is the difficulty of measuring the concentration at the meniscus in low-speed sedimentation equilibrium experiments. Dr. Creeth and Mr. Holt overcame this difficulty by using a new method of separating variables. It calls for a numerical integration procedure that is tedious by hand calculation; but use of the Olivetti desk-top computer considerably increased the number of systems that could be measured. In assessing the limits of the new method, computer-simulated distributions are essential; the multi-component distributions needed were calculated on a Wang 360 computer with the assistance of Dr. P. A. Charlwood (National Institute for Medical Research).

BLOOD PRODUCTS LABORATORY

Anti-D Immunoglobulin. The staff of the Laboratory continued their collaboration with the Department of Medicine, Liverpool University, and the M.R.C. Working Party on the use of anti-D immunoglobulin for the prevention of iso-immunisation of Rh-negative women in pregnancy. Continued investigation by Mr. Wesley and Dr. N. C. Hughes-Jones of the M.R.C. Experimental Haematology Research Laboratory of the stability of anti-D activity in immunoglobulin solutions (Report 1969) suggested that activity had diminished during storage in a few preparations, without detectable evidence of degradation of the immunoglobulin. As a result of these observations a more extensive investigation of the stability of this specific immunoglobulin has begun.

Pharmacologically active Substances in Human Plasma. Dr. Mackay continued her study of kininogenase in human plasma (Report 1968). The substrates for plasma kallikrein and plasmin in human kininogen can be separated by molecular sieve chromatography through Sephadex G-200. The order in which the substrates are eluted indicates that the kininogen of plasma kallikrein is a larger molecule than plasmin substrate. The molecular weights of the kininogens of plasma kallikrein, trypsin and glandular kallikrein are in this descending order. Since plasmin kininogen and trypsin substrate separate from plasma in the same chromatographic fraction, it is concluded that the molecular weight of plasmin kininogen is intermediate between those of the kininogens of plasma kallikrein and glandular kallikrein.

Dr. Mackay and Mr. Vallet continued to collect data, by molecular sieve chromatography and measurement of anti-complementary activity, about the effect of storage at 4°C on immunoglobulin prepared for the treatment of hypogammaglobulinaemia (Report 1969). The fractions obtained by molecular sieve chromatography were also examined by electrophoresis and iso-electric focussing in polyacrylamide gel. Changes were revealed in the smaller molecular zone of immunoglobulin that had been incubated for 28 days at 37°C, which were not detectable by ultracentrifugation or molecular sieve chromatography.

Extension of Blood Products Laboratory. Building of the extension began on 6th October. The planning of this building and the design, specification and testing of its equipment occupied a large amount of time. Among the equipment under test by Mr. Vallet and Mr. Wesley is apparatus for low pressure evaporation from the liquid phase which may prove satisfactory as an alternative method to freeze drying for the removal of ethanol and water from albumin solutions.

Plasma Fractionation Laboratory, Haemophilia Centre, Oxford

Factor VIII. The more concentrated a preparation of Factor VIII is, the more advantageous it is for clinical use, because a large dose can be given in a small volume, small enough, possibly, to be given by syringe, and because the concentration of Factor VIII in the patient's blood can be raised rapidly towards a normal level.

Dr. Ford assessed the method described by Dr. Alan Johnson of New York, of preparing anti-haemophilic globulin concentrated 12–30 times, or even 150–400 times, with respect to normal plasma. The weaker concentrate has been used clinically with success. Freeze drying, however, may be accompanied by a loss of activity, and Dr. Ford is investigating means of avoiding this loss. Small amounts of the stronger concentrate are being accumulated for clinical trial.

Factor IX. Mr. Dike reviewed methods of separating and concentrating Factor IX with the aim of devising a method which would give a better yield of a more stable final product, which might be given by syringe. A method involving absorption on DEAE cellulose is now under investigation.

BLOOD GROUP UNIT

The Unit has again spent the year looking for new red cell antigens and applying the blood group systems already established to various aspects of human biology.

The X Chromosome

X-mapping. Many families with X-linked conditions, often abnormalities of the eye, were tested. However, the linkage work has of necessity slowed down because the relationship of the *Xg* locus to most of the relatively common conditions is now established.

X-Chromosome Aneuploidy. Samples of blood from patients with abnormalities of number or form of the X chromosome continue to be sent because the Xg groups can often show at which division in gametogenesis the causative accident has happened (Reports 1966, 1967, 1969). The accumulating results were analysed again early in the year and they consolidated knowledge previously gained by this method, with one exception. In the 1967 Report surprise was recorded that the 14 examples of the very rare XX males had a male rather than the expected female distribution of the Xg groups, and this carried important implications; however, the total is now 25 and the deviation from the female distribution is no longer significant.

Xg and Chronic Granulocytic Leukaemia. Since its recognition (Report 1962) there has been doubt whether or not the *Xg* locus, when carried on a normal X, is subject to lyonisation (the irreversible genetic inactivation of one or the other X in each somatic cell at an early stage of embryonic development of mammals). An investigation which was hoped to settle this was begun more than a year ago in collaboration with Dr. D. A. G. Galton and Dr. Sylvia Lawler of the Royal Marsden Hospital (Report 1969). If the theory, rather

widely accepted, that all the myelocytic and erythrocytic cells of sufferers from chronic granulocytic leukaemia represent a clone descended from a single stem cell were correct, and if *Xg* were subject to lyonisation, then the Xg groups of female patients should have the distribution expected of males. Forty-eight female patients, all having the diagnostic Ph^1 chromosome, were grouped: their Xg distribution was just that expected of females and differed significantly from that of males (90 times more likely female than male).

Had the Xg distribution of these females been that of males the clonal theory would have been confirmed and the lyonisation of *Xg* established. In the event, the distribution being female, it can only be concluded that either the clonal theory is incorrect or *Xg* on a normal X is not inactivated.

Xg Gene Frequencies. At the end of 1969 a count was made of the groups of unselected unrelated people tested in the Unit since the last count, in 1965. The 1965 count included 3,418 people of northern European origin and the gene frequencies, calculated by the formula devised for the purpose by Haldane, were Xg^a 0.659 and *Xg* 0.341: the present count adds a further 3,366 such people who, rather satisfyingly, leave the gene frequencies exactly as they were.

From the male and female phenotype frequencies it can be calculated that if *Xg*, when on a normal chromosome, were subject to inactivation the inactivation could not have happened as early in embryonic life as the four to eight cell stage calculated for that event from observations on another X-linked character, glucose-6-phosphate dehydrogenase in red cells.

The 1969 count of northern European families tested with anti-Xg^a adds a further 1,200 to those counted in 1965 and brings the grand total to 2,539 families with 5,819 children. Both matings and issue therefrom fit well with the expected incidence calculated from the gene frequencies and again make inactivation of the *Xg* locus on a normal X unlikely, at least at a stage of development as early as that estimated from g-6-pd.

Autosomes

Families in which visible abnormalities of autosomes are segregating continue to be grouped in the hope of building up evidence for the assignation of blood group loci to particular autosomes. The negative evidence, showing where the loci are not placed, continues to grow.

The En work of last year led incidentally to the recognition of the peculiar serological and physicochemical state of cells representing the MNSs allele M^k (Report 1969). With the help of Dr. M. Metaxas of Zurich the Unit was able to show that the MNSs allele M^s has the same effect on the red cell, though to a lesser degree.

As in past years, much time was spent on testing samples, mostly from abroad, suspected to contain a "new" antigen or antibody.

From the Finnish Red Cross at Helsinki (Prof. H. Nevanlinna and Dr. U. Furuhjelm) came an antibody to an infrequent antigen which is well on the way to recognition as the herald of a new system: segregation in informative families has so far shown the new antigen not to be controlled by the loci for the ABO, MNSs, P, Rh, Duffy, Kidd or Dombrock systems nor to be an X- or Y-borne character. Though the antigen is rare the corresponding antibody is, rather surprisingly, not uncommon in the serum of normal people.

From the Red Cross at Brisbane Mr. J. A. Albrey sent a sample containing an antibody which he had found to react with the cells of all Australian donors tested but not with some U.S.A. negro samples known to be of the phenotype Fy(a-b-). Miss Gavin tested the serum with samples of red cells from 160 West Indians, provided by Dr. T. E. Cleghorn, and confirmed that the only negative reactions were given by cells of the phenotype Fy(a-b-). The phenotype was recognised 15 years ago (Report 1956): of Negroes nearly three quarters are Fy(a-b-) but no European example was found until this Brisbane patient. It is striking that an Australian of European descent should be the first to make an antibody the like of which had been sought for years in the serum of Negroes but never found. The background to the

phenotype Fy(a-b-) in the Brisbane patient must differ fundamentally from that in the Negro: there is very good reason to think that Fy(a-b-) Negroes are homozygous for a third allele at the Duffy locus, but the Australian is probably homozygous for a rare allele at a locus further back on the assembly line of the Duffy antigens, analagous to, for example, KL in the Kell system (Report 1968).

From various places Dr. Tippett continues to be sent samples from the rare Rh positive people who have a D antigen on their red cells yet have anti-D in their serum. Most of them fall into one of six well defined categories (Reports 1962–1966). It was assumed that the D antigen had a certain number of parts any one of which might as a great rarity be missing or altered to allow the owner of the peculiarity to make antibody to that part of the normal D of a donor or foetus. However, an absence of part of the D can no longer be considered as an explanation of at any rate two of the categories for Dr. Tippett has now identified two "new" Rh antibodies, found in Canada and in the States, as being directed against the "missing" parts of categories V and IV respectively. Antibodies to the other categories of D will no doubt turn up some day; they are being looked for in the class of antibodies to very rare antigens.

To the enzymes traditionally used in blood grouping Dr. Tippett has added a new one, α-amylase, prepared from *B. subtilis* which gives a pattern of reactions so far unique. Exposure of red cells to this enzyme had the extraordinary effect of abolishing their S: anti-S but not their s:anti-s interaction. This observation may contribute to thoughts about the chemistry of the Ss antigens of which little is yet known. The enzyme also abolishes the Xg^a:anti-Xg^a, the Lu^a:anti-Lu^a and the Lu^b:anti-Lu^b interactions but leaves practically unaffected all the other blood group interactions tried.

Polyagglutinable Red Cells

Polyagglutinability is a fairly rare and usually transient property acquired by the red cells during the course of acute or chronic infections; it is attributed to the exposure of a hidden antigen called T, believed to be present in all human red cells. The exposure of T can be simulated *in vitro* by treatment of normal cells with neuraminidase.

The Unit's interest in polyagglutinability was awakened by the arrival of two examples of persistent mixtures, in healthy people, of polyagglutinable and not-polyagglutinable cells, a condition only once before reported. One example came from Dr. P. Sturgeon in Los Angeles and the other from Dr. U. Furuhjelm in Helsinki. The polyagglutinable fraction of the Helsinki sample had been found to have two of the properties associated with En(a-) cells (Report 1969), reduced sialic acid content and reduced electrophoretic mobility; however, in spite of these physicochemical similarities the two conditions, En(a-) and polyagglutinability, were shown serologically to have essentially different backgrounds.

This persistent type of polyagglutinability was not due to exposure of the T antigen. The polyagglutinable portion of the cells had a certain affinity for anti-A reagents which could be distinguished from that of true A cells and, perhaps not surprisingly, inhibition tests showed that N-acetyl-D-galactosamine was playing an essential part in the reaction.

A third type of polyagglutinability, distinguished by being an inherited character, was discovered in a Mauritian family by Professor P. Cazal of Montpellier. Professor Cazal sent blood from the propositus and this kind of polyagglutinability proved to be serologically distinct from the other two.

The serological differentiation of the three types of polyagglutinability, the infective, the persistent and the inherited, was made with extracts of certain seeds strikingly, with extracts of certain seeds and of several species of snails.

Later in the year a sample of the persistent type came from Dr. H. Chaplin of St. Louis: it gave the serological reactions of the examples from Los Angeles and Helsinki but presumably represented a more severe form of the condition because all the cells were polyagglutinable.

The physicians, cytogeneticists and blood bank technicians who sent the samples on which the work of the Unit depends are too numerous to be thanked by name. For the supply of reagents and for help in other ways the Unit is greatly indebted to the Regional Blood Transfusion Centres; the Blood Group Reference Laboratory; Spectra Biologicals, East Brunswick; Hyland Laboratories, Los Angeles; Laboratorios Grifols, Barcelona and Pfizer Diagnostics, New York.

Once again the Unit thanks the Staff of the Institute for giving so many samples of their blood.

BLOOD GROUP REFERENCE LABORATORY

National and International Committees

This Laboratory fulfils the functions of the British National Blood Group Reference Laboratory and of the World Health Organization Blood Group Reference Laboratory. It is, therefore, in a unique position in being able to collect information regarding the whereabouts of donors of rare blood types or blood typing sera of specificities that are in short supply.

International Panel of Donors of Rare Blood Types. In the report for 1967, mention was made of this International Donor Panel. A list of donors, prepared by this Laboratory, was circulated to National Blood Group Reference Laboratories throughout the world and during the last year increasing use of it was made to locate compatible blood for patients for whom none could be found in their own countries. Blood donations were sent from transfusion centres in Canada and Western Europe for the treatment of patients in both Eastern and Western Europe, Asia and South America. Dr. Giles, while investigating some of these transfusion problems, found one patient of the Kell phenotype Ko whose serum contained anti-Kp^b and another whose serum contained anti-c, anti-E, anti-Ve and anti-Do^a. Without international co-operation it would be very difficult to find suitable blood for patients such as these. During the XII Congress of the International Society of Blood Transfusion, held in Moscow in August 1969, Dr. Goldsmith called a meeting of those interested in the International Panel of Donors and over 100 people were present.

International Exchange of Blood-typing Sera. For many years the Laboratory has acted as a clearing house for blood-typing sera used in Britain and, during 1969, launched a similar scheme on behalf of WHO for the international exchange of grouping reagents. Already the scheme has met with considerable success, grouping sera being exchanged between laboratories in America, Australia and Europe. Serologists in developing countries whose resources are small can now obtain grouping reagents free of charge whereas before they often had to rely very largely on purchase of foreign commercial reagents, most of which are very expensive.

Standardization of Blood-grouping Reagents. In the Report for 1968, reference was made to the increasing need for the standardization of blood-grouping reagents. This Laboratory has always shown a lead in this respect as it was responsible for the preparation of international standards for anti-A, anti-B and "incomplete" anti-D blood typing sera. Dr. Ikin, who herself prepared these international standards, during 1969 performed accelerated degradation studies on material which may form a standard for anti-c. It was as a result of studies on the standard for "incomplete" anti-D that investigations were commenced by Professor Kekwick, Dr. Jones and Dr. Goldsmith on the causes of the varying capacities of individual batches of albumin to influence the agglutination of Rh-positive red cells by Rh antibodies. Further details of this work are to be found in the report from the Biophysics Department for 1968.

Blood-grouping reagents must reach certain minimum standards, both with regard to potency and specificity. Dr. Goldsmith is Chairman of a Working Party, established on behalf of the British Committee for Standards in Haematology to advise on the control and certification of blood grouping reagents. This Working Party includes, among others, some directors of British blood transfusion centres and hospital

pathologists and they are concerned with seeing that hospital pathologists are provided with blood grouping reagents in a form most suitable for their needs. In this connection it may be mentioned that the output of grouping reagents from the Laboratory continues to rise, and in 1969 894 litres were issued, this being 4% more than in the previous year. Dr. Ikin is responsible for controlling all the Laboratory's serum production and she is an active member of the Working Party just mentioned. Dr. Goldsmith was at the XII Congress of the International Society of Blood Transfusion elected Chairman of an expert panel to advise the International Committee for Standards in Haematology and the International Society of Blood Transfusion on problems of standardisation in blood transfusion.

Problems of Blood-group Serology

Mention has already been made of complicated transfusion problems for which use was made of the International Panel of Donors of Rare Blood Types. In addition, Dr. Giles discovered the first example of anti-Co^b, the first pure example of anti-M^v, a "new" MN variant of the Mi^a series and a "new" Rh gene complex against which there is a specific antibody. In addition, she investigated the inheritance of three "new" low frequency antigens, one of which may be linked to Rh and the other to MN. She also investigated a Canadian family which possessed unusual Lewis phenotypes, the propositus being a chimera whose serum contained the second example of the Siedler antibody. Quite apart from her heavy commitments in the production of blood-grouping reagents, Dr. Ikin has continued her research on MN variants.

A function of this Laboratory is to determine the reliability of new techniques and in this connection Dr. Phillips, who continues to prepare fluorescein-conjugated immunoglobulin reagents, is constantly trying out methods which may be used not only in her own field but in other branches of blood-group serology. Mr. Dawes, who has been associated with Dr. Giles in a number of her investigations, has been experimenting with the use of Pronase to see if it is suitable for routine use in blood-group serology as a proteolytic enzyme.

Investigation of Immunoglobulin Preparations. Dr. Ikin examined anti-D immunoglobulin produced in the Blood Products Laboratory for potency and specificity and she also examined other blood products for the presence of red cell antibodies. Miss Brazier and Dr. Goldsmith showed that certain batches of anti-D immunoglobulin contained also cytotoxic leucocyte antibodies. Dr. Giles tested serum samples from Rh-negative women who some months previously had received injections of anti-D immunoglobulin, to see whether they contained Rh antibodies. Dr. Goldsmith and Miss Brazier, in an effort to study the *in vivo* survival of an immunoglobulin preparation in a normal individual, used Gm and Inv markers for the purpose. The possibility that Gm or Inv antibodies might develop following the injection of the immunoglobulin was also explored.

The Governing Body would like to praise the scientific, administrative and technical staffs 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.

G. L. BROWN,
Chairman.

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BLOOD GROUP REFERENCE LABORATORY

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THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Accounts 1970

THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Balance Sheet and Accounts

31 DECEMBER 1970

CHELSEA BRIDGE ROAD . LONDON, S.W.1 . 18 MAY, 1971

The Governing Body

Professor A. NEUBERGER, CBE, MD, FRCP, FRC PATH, FRS, *Chairman*

R. A. McNEILE, MBE, *Hon. Treasurer*

Professor D. A. K. BLACK, M SC, MD, FRCP

Professor D. G. EVANS, CBE, D SC, FRC PATH, FRS

C. E. GUINNESS

Professor HENRY HARRIS, MB, D PHIL, FRS

The Rt Hon the EARL OF IVEAGH

Professor R. A. KEKWICK, D SC, FRS

Professor Sir ASHLEY MILES, CBE, MD, FRCP, FRC PATH, FRS

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

Financial Report of the Governing Body

The Governing Body presents the accounts of the Institute for the year ended 31st December 1970.

1. Results

The General Fund Income and Expenditure Account shows the Income for the year as £351,077 compared with £261,175 in 1969. Expenditure amounted to £502,346 against £400,309 last year. The deficit for the year is £151,269 compared with a deficit of £139,134 in 1969. The capital fund has been reduced by the year's deficit of £151,269.

The second of four annual instalments of £75,000 from the Wolfson Foundation and of five annual instalments of £1,000 from the Grocers' Company have been added to the capital fund.

2. Principal Activities

The Institute continued to carry out research work in connection with the prevention of diseases. It produces for sale sera, and bacterial and virus vaccines, the profits from which are utilised for its research and experimental work.

3. Exports

Sera and vaccines to the value of £160,258 were exported from the United Kingdom during the year.

4. Fixed Assets

The movements in fixed assets during the year are set out in the table in note 1 on the accounts. The most important feature is the erection of a new wing at Chelsea, the cost of which is expected to be about £365,000. The Governors have transferred £60,000 from the Sinking Fund, first set up in 1901 for the replacement and repair of buildings, towards the cost of this new wing.

5. Interests in Land

The market value of the Institute's properties is now in excess of the amount at which they are included in the Balance Sheet. In the opinion of the Governing Body such difference is of no significance as the properties are occupied for the purposes of the Institute's activities.

6. Governing Body

Sir Lindor Brown, Lord Brock and Mr. H. P. G. Channon resigned in June 1970. Professor D. A. K. Black joined the Governing Body in June 1970; Professor Henry Harris in October; and Mr. R. A. McNeile in December. Other members of the Governing Body shown on page 2 held office during the whole of the year ended 31st December 1970.

7. Employees

The average number of persons employed by the Institute in each week during the year ended 31st December 1970 was 328. The aggregate remuneration paid or payable in respect of that year to these employees amounted to £422,354.

8. Auditors

The auditors, Cooper Brothers & Co., will continue in office in accordance with Section 159 (2) of the Companies Act 1948.

A. NEUBERGER
Chairman

The Lister Institute of Preventive Medicine
BALANCE SHEET · 31 December 1970

1969 £		£	£	£
285,905	FIXED ASSETS (*note 1*)			546,238
	INVESTMENTS AND UNINVESTED CASH (*note 2*)			
503,700	General		406,406	
213,621	Specific funds		164,587	
23,330	Bequest funds		24,471	
740,651				595,464
				1,141,702
	Deduct			
	CURRENT LIABILITIES			
63,420	Creditors		84,512	
—	Bank Overdraft (*note 13*)		185,666	
			270,178	
	Less:			
	CURRENT ASSETS			
127,330	Debtors	171,545		
52,285	Cash and Bank Balances	86,918		
179,615			258,463	
(116,195)				(11,715)
£1,142,751				£1,129,987
	Represented by			
509,592	CAPITAL FUND (*note 4*)			494,323
205,078	SPECIFIC FUNDS (*note 5 and 8*)			148,477
23,330	BEQUEST FUNDS (*note 6*)			24,471
6,445	SPECIFIC GRANTS AND LEGACIES UNEXPENDED (*note 7*)			6,396
398,306	INVESTMENT RESERVE (*note 8*)			456,320
£1,142,751				£1,129,987

A. NEUBERGER, R. A. McNEILE — *Members of the Governing Body*

The notes on pages 6 to 9 form part of these accounts.
Audit report on page 9

The Lister Institute of Preventive Medicine
INCOME AND EXPENDITURE ACCOUNT
for the year ended 31 December 1970

1969				
£		£	£	£
	INCOME			
209,758	Sales of sera and bacterial and virus vaccines (note 9)			303,664
	Investment Income:			
	General fund			
33,233	Quoted	29,971		
2,460	Unquoted	2,444		
			32,415	
7,256	Sinking fund—quoted		6,941	
				39,356
38	Underwriting commission (*less* income tax £42)			64
8,430	Rent			7,993
261,175				351,077

		Total expenditure	*External contributions*	
	EXPENDITURE			
229,104	Salaries and wages	434,478	157,190	277,288
17,880	Superannuation premiums	26,203	7,726	18,477
10,158	Rates and insurances	12,261	—	12,261
19,698	Gas, water, fuel and electricity	28,047	7,522	20,525
10,930	Office expenses, stationery and printing	14,725	3,097	11,628
1,070	Audit fee	950	—	950
803	Interest on overdraft	5,689	—	5,689
8,404	Chelsea research	22,323	13,128	9,195
34,824	Elstree research and production	75,656	3,073	72,583
22,088	Animals and forage	39,699	5,012	34,687
18,392	Alterations, repairs and renewals	17,237	2,013	15,224
15,182	General expenses	12,939	2,652	10,287
	Depreciation			
2,320	Buildings	3,239	—	3,239
2,200	Furniture, fittings, scientific apparatus and books	3,372	—	3,372
393,053		£696,818	£201,413	495,405
131,878	Excess of expenditure over income			144,328
7,256	Amount transferred to sinking fund			6,941
£139,134	Deficit transferred to capital fund			£151,269

The notes on pages 6 to 9 form part of these accounts.
Audit report—page 9

NOTES ON THE ACCOUNTS · 31 December 1970

1. FIXED ASSETS

	Freehold property		*Furniture, fittings, scientific apparatus and books*	*Total*
	Land and buildings Chelsea	*Queensbury Lodge Estate, Elstree*		
	£	£	£	£
Cost				
At 1st January 1970	186,229	92,796	29,252	308,277
Additions at cost	227,414	28,164	11,366	266,944
At 31st December 1970	£413,643	£120,960	£40,618	£575,221
Depreciation				
At 1st January 1970	2,700	10,399	9,273	22,372
Charged to income and expenditure account	1,350	1,889	3,372	6,611
At 31st December 1970	£4,050	£12,288	£12,645	£28,983
Net book value at 31st December 1970	£409,593	£108,672	£27,973	£546,238

Depreciation

Freehold property additions and replacements since 1912 at Elstree and since 1935 at Chelsea until 31st December 1964 have been charged to revenue. Additions since that date until 31st December 1967 have been depreciated at the rate of 10%. Since 1st January 1968 buildings shown in the balance sheet have been depreciated at the rate of 2% on a straight line basis from the date of completion.

Additions and replacements to furniture, fittings, scientific apparatus and books between 31st December 1920 and 31st December 1963 have been charged to revenue. The additions since 1st January 1964 have been depreciated on a straight line basis by reference to the useful lives of the assets.

2. INVESTMENTS AND UNINVESTED CASH

	£	£	£	£	£
	Quoted at cost		*Unquoted at cost*	*Uninvested cash*	*Total*
	In Great Britain	*Elsewhere*			
General	206,400	160,749	39,257	—	406,406
Specific					
Sinking fund for freehold buildings	88,565	—	—	23,576	112,141
Pension fund	17,471	—	—	1,983	19,454
Re-endowment fund	28,608	—	—	4,384	32,992
Bequest					
Jenner Memorial studentship fund	11,766	—	940	4,269	16,975
Morna Macleod scholarship fund	5,653	—	—	1,843	7,496
	£358,463	£160,749	£40,197	£36,055	£595,464
1969	(£432,864)	(£202,459)	(£40,626)	(£64,702)	(£740,651)

Market value of quoted investments: 1970 £801,468 (1969 £1,066,315)

Unquoted investments valued by institute's investment advisers: 1970 £34,397 (1969 £34,412)

3. STOCKS

Stocks of sera and bacterial and virus vaccines on hand at 31st December, 1970 have not been valued in the accounts in accordance with long established practice. It is the present intention of the Governing Body to change this basis of accounting with effect from 1st January, 1971 and in future to value these stocks.

4. CAPITAL FUND

Donations etc. have been received to date from the following:—	£	£
Dr. Ludwig Mond (1893)		2,000
Berridge Trustees (1893–1898)		46,380
Worshipful Company of Grocers (1894 and 1969/70)		12,000
Lord Iveagh (1900)		250,000
Lord Lister's Bequest (1913-1923)		18,904
William Henry Clarke Bequest (1923–1926)		7,114
Rockefeller Foundation (1935–1936)		3,400
Wolfson Foundation (1969–70)		150,000
Other donations and legacies (1891–1954)		22,669
Amount transferred from Sinking Fund		60,000
		572,467
General Fund Income and Expenditure Account		
Deficit 1970	151,269	
Less: Accumulated surplus as at 31st December, 1969	73,125	
Accumulated deficit		78,144
		£494,323

5. SPECIFIC FUNDS

Sinking Fund for Freehold Buildings			
As at 1st January 1970		151,707	
Interest on investments		6,941	
		158,648	
Less: Expenditure on reablement of buildings	2,617		
Amount transferred to Capital Fund	60,000		
		62,617	
			96,031
Pension Fund			
As at 1st January 1970		20,979	
Interest on investments		1,328	
		22,307	
Less: Pensions		2,853	
			19,454
Re-endowment Fund			
As at 1st January 1970		32,392	
Donations		600	
			32,992
			£148,477

6. BEQUEST FUNDS

	£	£
Jenner Memorial Studentship Fund		
As at 1st January 1970	16,329	
Interest on investments	646	
		16,975
Morna Macleod Scholarship Fund		
As at 1st January 1970	7,001	
Interest on investments	495	
		7,496
		£24,471

7. SPECIFIC GRANTS AND LEGACIES

	£	£	£
Nuffield Foundation Grants			
As at 1st January 1970			1,583
Guinness-Lister Research Grant			
As at 1st January 1970		4,862	
Amounts received		15,000	
		19,862	
Less: Salaries and wages	11,539		
Laboratory expenses	3,510		
		15,049	
			4,813
			£6,396

8. GENERAL AND SINKING FUNDS INVESTMENT RESERVE

	£	£	£
General			
As at 1st January 1970	389,764		
Add: Profits on sales of investments	50,446		
		440,210	
Sinking Fund			
As at 1st January 1970	8,542		
Add: Profits on sales of investments	7,568		
		16,110	
			£456,320

9. TURNOVER

Turnover has been arrived at after deducting commission due to agents from the invoice value of sales of sera, vaccines and virus vaccines.

10. Emoluments of Members of the Governing Body

	1970	1969
Emoluments in an executive capacity	£16,556	£15,923

Particulars of emoluments of the Governing Body in accordance with Section 6 of the Companies Act 1967

	1970	1969
Emoluments of the Chairman of the Governing Body	Nil	Nil
Emoluments of the highest paid member of the Governing Body	£7,256	£7,256
Numbers of members of the Governing Body whose emoluments were within the range		
No emoluments	10	7
£1 — £2,500	—	—
£2,501 — £5,000	—	1
£5,001 — £7,500	2	1

11. Capital Expenditure Schemes

	1970	1969
The position at 31st December 1970 was as follows:—		
Commitments in respect of contracts	86,332	324,071
Approved by the Governing Body in addition to commitments, for new laboratories at Elstree	136,000	120,000
	£222,332	£444,071

12. Contingent Liabilities

At 31st December 1970 there were contingent liabilities amounting to £14,985 in respect of indemnities issued to third parties.

13. Bank Overdraft

The overdraft is secured by the Institutes' investments.

Report of the Auditors to the Members

As explained in note 3 stocks of sera and vaccines have not been valued in the accounts. With this reservation in our opinion the accounts set out on pages 4 to 9 give a true and fair view of the state of the company's affairs at 31st December, 1970 and of its deficit for the year ended on that date and comply with the Companies Acts 1948 and 1967.

COOPER BROTHERS & CO.
Chartered Accountants.

London, 18th May 1971

THE LISTER INSTITUTE
OF PREVENTIVE MEDICINE

Report

of the GOVERNING BODY

1971

CHELSEA BRIDGE ROAD : LONDON : SW1

The Governing Body

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R. A. McNEILE, MBE, *Hon. Treasurer*

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The Rt Hon the EARL OF IVEAGH

Professor R. A. KEKWICK, D SC, FRS

Professor Sir ASHLEY MILES, CBE, MD, FRCP, FRC PATH, FRS

Clerk to the Governors S. A. WHITE, AACCA

The Council

A. LAWRENCE ABEL, MS, FRCS *Representing the British Medical Association*

M. L. CONALTY, MD, MRC PATH, DPH, MRIA *Representing the Royal Irish Academy*

Professor D. A. K. BLACK, M SC, MD, FRCP *Representing the Members of the Institute*

The Rt. Hon Lord BROCK, MS, FRCS *Representing the Members of the Institute*

H. P. G. CHANNON, MP *Representing the Members of the Institute*

Dame HARRIETTE CHICK, DBE, D SC *Representing the Members of the Institute*

Professor P. J. COLLARD, MD, MRCP *Representing the University of Manchester*

Major L. M. E. DENT, DSO *Representing the Worshipful Company of Grocers*

Sir CHARLES DODDS, Bt, MVO, MD, D SC, FRCP, FRS *Representing the Members of the Institute*

Sir ALAN N. DRURY, CBE, MA, MD, FRCP, FRS *Representing the Members of the Institute*

Professor D. G. EVANS, CBE, D SC, FRC PATH, FRS *Representing the Royal Society*

Professor R. E. GLOVER, MA, D SC, FRCVS *Representing the Royal Agricultural Society*

Professor R. I. N. GREAVES, BA, MD *Representing the University of Cambridge*

C. E. GUINNESS *Representing the Members of the Institute*

Sir CHARLES HARINGTON, MA, PH D, FRS *Representing the Members of the Institute*

Professor HENRY HARRIS, MB, D PHIL, FRS *Representing the University of Oxford*

The Rt Hon the EARL OF IVEAGH *Representing the Members of the Institute*

Professor B. P. MARMION, MD, D SC, FRC PATH *Representing the University of Edinburgh*

Professor Sir ASHLEY MILES, CBE, MD, FRCP, FRC PATH, FRS *Representing the Members of the Institute*

Professor J. S. MITCHELL, CBE, MA, MD, FRS *Representing the Members of the Institute*

Professor W. T. J. MORGAN, CBE, D SC, PH D, MD (*hc*), FRIC, FRS *Representing the Members of the Institute*

Professor A. NEUBERGER, CBE, MD, FRCP, FRC PATH, FRS *Representing the Members of the Institute*

The President of the ROYAL COLLEGE OF PHYSICIANS *Representing the Royal College of Physicians, London*

The President of the ROYAL COLLEGE OF SURGEONS *Representing the Royal College of Surgeons of England*

The President of the ROYAL COLLEGE OF VETERINARY SURGEONS *Representing the Royal College of Veterinary Surgeons*

Professor F. S. STEWART, MD *Representing the University of Dublin*

WILLIAM J. THOMPSON *Representing the Worshipful Company of Grocers*

Sir GRAHAM WILSON, MD, FRCP *Representing the University of London*

The Staff

Director: Professor Sir Ashley Miles
Deputy Director: Professor L. H. Collier
Superintendent of Elstree Laboratories: W. d'A. Maycock

MICROBIOLOGY, EXPERIMENTAL PATHOLOGY AND IMMUNOLOGY

Experimental Pathology and Immunology

†Sir Ashley Miles, CBE, MD, D SC, FRCP, FRC PATH, FRS (*Professor of Experimental Pathology in the University of London*)
F. R. Wells, BM, B CH, MA

D. G. Godfrey, OBE, B SC, PH D (*M.R.C. External Scientific Staff*)
Angela E. R. Taylor, B SC, PH D
Sheila M. Lanham, B SC
Trypanosomiasis Research

Brenda Mason, B SC, MI BIOL

Microbiology

†G. G. Meynell, MD, D SC (*Guinness Professor of Microbiology in the University of London*)
K. A. Chandrabose, MS, PH D
K. G. Hardy, B SC
Valerie M. Harden, B SC
Guinness-Lister Research Unit

*Elinor W. Meynell, BA, MD, DIP BACT
*Ruth M. Lemcke, B SC, PH D
J. E. Dowman, MA, PH D (*S.R.C. Grantee*)

Virology

†L. H. Collier, MD, D SC, MRCP (*Professor of Virology in the University of London and Hon. Director, M.R.C. Trachoma Unit*)
J. Alwen, B SC, PH D
Lindsey M. Hutt, B SC

W. A. Blyth, B SC, PH D
Janice Taverne, BA, PH D
A. J. Garrett, B SC, PH D
Andrea Barton, B SC
R. G. Harris, B SC, MI BIOL
Margaret J. Harrison, M SC
M. R. C. Trachoma Unit

Electron Microscopy Unit

*A. M. Lawn, PH D, B SC, MRCVS

BIOCHEMISTRY

†Winifred M. Watkins, D SC, PH D, FRS (*Professor of Biochemistry in the University of London*)
Shirley D. Goodwin, B SC
M. A. Chester, M SC, B TECH (*Beit Memorial Fellow*)
J. R. Stealey, MI BIOL (*Grocers' Company Research Student*)
Hilary M. Simpson, B SC (*Research Student*)

A. S. R. Donald, B SC, PH D (*M.R.C. Grantee*)
Caroline Race, B SC (*M.R.C. Grantee*)
Helene T. Cory, B SC, PH D (*M.R.C. Grantee*)

§Professor W. T. J. Morgan, CBE, D SC, PH D, MD (*hc*), D SC (*hc*) FRIC, FRS (ret'd).

BIOPHYSICS

†R. A. Kekwick, D SC, FRS (*Professor of Biophysics in the University of London*)
†J. M. Creeth, B SC, PH D, FRIC (*Reader in Biophysics in the University of London*)
†Professor N. H. Martin, MA, FRCP, FRIC (*Honorary Research Associate*)

Caroline M. Butterworth, B SC (*M.R.C. Grantee*)

PREPARATION and STUDY of THERAPEUTIC SERA (ELSTREE)

*W. E. Parish, MA, PH D, BV SC, MRCVS, MRC PATH
Wendy Jeffery, B SC

Biochemistry (Elstree)

*D. E. Dolby, B SC, PH D

PREPARATION and STUDY of VIRUS VACCINES (ELSTREE)

*H. G. S. Murray, MD
G. S. Turner, B SC, PH D
L. C. Robinson, B SC, PH D
L. V. Runkel, B SC

PREPARATION and STUDY of BACTERIAL VACCINES (ELSTREE)

*A. F. B. Standfast, SC D
Jean M. Dolby, MA, PH D
M. P. Banks, B SC
Caroline J. Shanbury, B SC
A. P. Hunt, B SC
J. P. Ackers, MA, D PHIL
S. T. A. Gilligan, B SC

CO-ORDINATION of PRODUCTION (ELSTREE)

J. Rodican, B SC

BLOOD PRODUCTS (ELSTREE)

*W. d'A. Maycock, CBE, MVO, MD, MRCP, FRC PATH
L. Vallet, MA
Margaret E. Mackay, M SC, PH D (*M.R.C. External Scientific Staff*)
D. Ellis, B SC, PH D
Constance Shaw, M SC, DIP BACT
E. D. Wesley, B PHARM
Valerie J. Stickley, B SC

PLASMA FRACTIONS LABORATORY (OXFORD)

Ethel Bidwell, B SC, PH D, FRIC
T. J. Snape, BA

MEDICAL RESEARCH COUNCIL UNITS HOUSED AT THE INSTITUTE

Blood Group Unit

§R. R. Race, CBE, MD (*hc*), PH D, FRCP, FRS
Ruth Sanger, B SC, PH D
Patricia Tippett, B SC, PH D
E. June Gavin, B SC
Phyllis W. Teesdale, B SC

Blood Group Reference Laboratory

K. L. G. Goldsmith, PH D, MB, BS, MRCP MRC PATH
Toby T. B. Phillips, MB, CH B
Elizabeth W. Ikin, B SC, PH D
Carolyn M. Giles, B SC, PH D
B. J. Dawes, B SC

ADMINISTRATION

Secretary and Accountant — S. A. White, AACCA
Elstree Secretary and Estate Manager — G. J. Roderick, B COM
Assistant Secretary (Academic) — Barbara A. Prideaux
Assistant Secretary and Deputy Accountant — E. J. H. Lloyd
Administrative Assistant — C. L. Beard
" " — Beryl I. Coussens

Solicitors:
Field Fisher & Co.,
296, High Holborn, W.C.1.

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

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

REPORT OF THE GOVERNING BODY

The Governing Body has the honour to present its report of the Institute for the year 1970. The scientific section takes a new form. Brief summaries are given of the various researches; and from now on each Annual Report will also contain an article reviewing the activities of one department. This year, Professor G. G. Meynell describes some of the work of the Department of Microbiology.

GOVERNING BODY

The Governing Body records with great sorrow the death of Sir Lindor Brown in February 1971. Sir Lindor joined the Governing Body in May 1967. He succeeded Sir Charles Dodds as Chairman in June 1969 and resigned in June 1970 because of ill health. At the same time the Governors accepted with much regret the decision of Mr. Channon to relinquish the Honorary Treasurership and to resign from the Governing Body. Mr. Channon, who became a member of the Governing Body in 1957, had been the Institute's Honorary Treasurer since June 1966, but his appointment to a ministerial post in the Government made it impossible for him to continue. The Governing Body takes this opportunity of expressing its gratitude to Mr. Channon for his valuable services to the Institute.

In October 1970 the Governing Body elected Professor A. Neuberger as its new chairman.

The Council, at a meeting held on 30 June 1970, re-appointed Professor A. Neuberger and appointed Professor D. A. K. Black as its representatives on the Governing Body until 31st December 1971. At the same meeting the Governors were authorised to appoint a third representative of the Council and have pleasure in announcing that Professor Henry Harris accepted their invitation to join the Governing Body.

More recently, the Earl of Iveagh appointed Mr. R. A. McNeile as one of his representatives and the Governors are pleased to announce that Mr. McNeile has agreed to be the Institute's new Honorary Treasurer.

DIRECTORSHIP OF THE INSTITUTE

Sir Ashley Miles, Director of the Institute since 1952, retires on 30th September 1971. The Governing Body have appointed Professor D. G. Evans, C.B.E., F.R.S., as Director-designate.

Professor Evans is at present Director of the Department of Bacteriology and Immunology at the London School of Hygiene and Tropical Medicine. He was appointed Head of the Medical Research Council's Biological Standards Control Laboratory in 1955 and in 1958 became Director of the Department of Biological Standards. From 1965 to 1969 Professor Evans served on the Medical Research Council. He is a member of the Panel of the Expert Committee on Biological Standardisation of the World Health Organisation.

COUNCIL

At last year's Annual General Meeting two of the three retiring members, the President of the Royal College of Surgeons and Professor J. Collard, were re-appointed; the third retiring member, Sir Rudolph Peters, did not offer himself for re-appointment. Mr. C. E. Guinness and Professor D. A. K. Black were appointed to the Council as 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 Sir Graham Wilson and Professor R. E. Glover, representing the University of London and the Royal Agricultural Society respectively, and Sir Charles Dodds, a representative of the Members of the Institute.

The Governing Body records with great regret the death of Sir Paul Fildes, a Member since 1931 and a member of the Governing Body from 1941 until 1956.

MEMBERS

The Governing Body records with regret the deaths of Professor F. W. Rogers Brambell, Dr. R. A. O'Brien and Dr. C. J. Virden during the year.

STAFF AND STUDENTS

The Governing Body notes with pleasure that the Vice-Chancellor of the University of London, acting on behalf of the Senate, elected Professor R. A. Kekwick a Fellow of University College, London in February 1971; and approved the recommendation of the Scholarships Committee that the William Julius Mickle Fellowship for 1970 be awarded to Professor Winifred Watkins.

Dr. G. M. A. Gray, deputy head of the Biochemistry department, resigned in January 1971. Dr. Gray had worked at the Institute since 1954 and the Governing Body wishes him every success in his new post on the Headquarters Staff of the Medical Research Council.

Dr. A. B. Stone of the Guinness-Lister Unit, Dr. W. H. Ford of the Plasma Fractionation Laboratory, Mr. N. C. Mahony of the Serum Department, and Mr. B. M. Walcroft, an administrative assistant, also resigned during the year.

Dr. K. A. Chandrabose and Miss V. M. Harden were appointed to the Guinness-Lister Unit; Mr. S. T. A. Gilligan to the Bacterial Vaccines Department; and Mr. T. J. Snape to the Plasma Fractionation Laboratory.

Mr. J. Stealey was awarded the Grocers' Company Research Studentship and Miss H. M. Simpson a Research Studentship in the department of Biochemistry.

Sir Ashley Miles represented the Royal Society at the Jules Bordet centenary celebrations in Brussels in November 1970.

Dr. W. d'A. Maycock attended, as the representative of the Department of Health and Social Security, a meeting of the Council of Europe Working Party on Freedom from Toxicity of Plastics used for Transfusion, in Rome in April 1970; and a meeting of the Sub-committee of Specialists on Blood Problems of the Public Health Committee of the Council of Europe, in Dublin in May 1969. In October he attended, in Strasbourg, a meeting of Group of Experts No. 15 of the European Pharmacopoeia Commission. In November he attended a conference on the Plasma Proteins and the Cellular Elements of the Blood at the Blood Research Institute, Boston and visited the Division of Biologic Standards, National Institutes of Health, Washington, D.C.

In August Professor L. H. Collier, Dr. W. A. Blyth, Dr. Janice Taverne and Dr. J. Garrett participated by invitation in an International Conference on Trachoma held in Boston, U.S.A.

Professor Winifred M. Watkins lectured by invitation at the Fifth International Symposium on Carbohydrate Chemistry in Paris in August 1970. In September, with Miss Caroline Race and Mr. M. A. Chester she attended the Eighth International Congress of Biochemistry in Switzerland.

As a WHO consultant and at the invitation of the governments concerned, Dr. A. F. B. Standfast visited the Government Laboratories in Burma and Thailand in February 1970. Again as a WHO consultant, he spent four weeks in November and December at the Central Research Institute, Kasauli, India.

In July 1970 Dr. Ethel Bidwell took part by invitation in the first meeting of the International Society on Thrombosis and Haemostasis, at Montreux.

Dr. A. M. Lawn attended the Septième Congrès International de Microscopie Électronique in Grenoble in September 1970.

Dr. G. M. A. Gray lectured by invitation at the Sixteenth FEBS Summer School on "Glycolipids in Membranes" at the University of Helsinki in July 1970.

Dr. G. S. Turner attended the Symposium on Human Diploid Cells in Zagreb, Yugoslavia in September 1970.

In October 1970 Dr. Elinor Meynell spoke by invitation at a meeting on Bacterial Replicons held at Lunteren, the Netherlands, which was also attended by Professor Meynell, Dr. J. E. Dowman and Mr. K. G. Hardy.

Dr. Jean Dolby attended, by invitation, the commemoration of the centenary of the birth of Jules Bordet in Brussels in November.

Dr. Ruth Lemcke attended, by invitation, the Fifth International Congress of Infectious Diseases in Vienna, in September 1970 and also lectured at the State Institute of Hygiene, Warsaw.

Early in the year Dr. D. G. Godfrey and Miss Sheila Lanham spent five weeks at the Nigerian Institute for Trypanosomiasis Research, and in the field, evaluating their new method for diagnosing Gambian trypanosomiasis in man. In September Dr. D. G. Godfrey and Dr. Angela Taylor took part in the Second International Congress of Parasitology held in Washington and afterwards visited laboratories in New York and Philadelphia.

For the academic year 1970/71 there are eighteen postgraduate research workers at the Institute registered for higher degrees of the University. Five Ph D degrees were awarded during 1970.

DONATIONS AND GRANTS

Arthur Guinness, Son & Co. Ltd. continue their generous support of the Guinness-Lister Research Unit.

The Governing Body records its appreciation of the many other bodies whose benefactions and grants support research work in the Institute. These include a grant from the Arthritis and Rheumatism Council for research on the role of bacterial complexes in vasculitis; a grant from the British Empire Cancer Campaign for research on the role of lipids in the structure and function of the plasma membranes of normal and malignant cells; grants from the Medical Research Council for research on the genetics of drug resistance factors and other bacterial plasmids; on the antigenic structure of *Mycoplasma hominis*; on the characterization of blood-group specific glycoproteins by density-gradient methods; on the gamma macroglobulins of normal human plasma; on the biosynthesis of blood group specific glycoproteins and red cell antigens; on the characterization of the human blood group P-active substance in hydatid cyst fluid; on the distribution of serotypes of *Bordetella pertussis;* and on immunochemical investigations of human blood group substances; biochemical investigations on the products of the blood group H, Lewis and Secretor genes; and quantitative studies on mammalian cells, trypanosomes and micro-organisms.

Grants were also received from the Overseas Development Administration of the Foreign and Commonwealth Office for studies on the biology of trypanosomes with special reference to their surface properties; from the Science Research Council for studies on the replication of bacterial plasmids; from the Royal Society for research on the characterization of glycoproteins by the method of sedimentation equilibrium in a density-gradient; and for travelling expenses; from the Wellcome Trust for research on the lipid composition of the plasma membranes of different mammalian cells and for travel expenses; from the World Health Organisation for studies on the parasitological diagnosis of Gambian trypanosomiasis; and from the Institute of the Diseases of the Chest, Brompton Hospital for an investigation of human tissue anaphylactic-sensitizing antibodies.

The Governing Body also gratefully acknowledges donations to the Institute's Re-endowment Fund from the Prudential Assurance Company Limited and the Royal London Mutual Insurance Society Limited.

PRODUCTION AT ELSTREE

This has been an encouraging year. Most of the systems for budgeting, forecasting, costing and calculating efficiency and profitability that were introduced two years ago have been consolidated. There was a substantial increase in the contribution of sales revenue to the Institute's research activities. The erection of new buildings for expanding production facilities is planned to commence in the Spring of 1971.

NEW BUILDINGS

The new wing at Chelsea, largely paid for by a very generous grant from the Wolfson Foundation, was completed early in the year and the Virology Department and the Medical Research Council Trachoma Unit now occupy the third and fourth floors. It is expected that by the time this report is published the building will have been officially opened by Mr. Leonard Wolfson.

At Elstree additional stabling, together with a sick bay and forage store, has been provided for twelve more horses and a start has been made on a Pyrogen Test Rabbit House which will be used mainly by the Blood Products Laboratory.

Work on the large extension to the Blood Products Laboratory continues.

VISITORS

The following visitors, in addition to those listed under Staff, worked for short periods in the Institute's Laboratories; Dr. G. del Real y Gomez, National School of Health, Madrid, Spain; Miss Gale Herman, Department of Microbiology, Trinity College, Dublin; Mr. A. Kumar, Central Research Institute, Kasauli, India; Dr. G. M. Levis, University of Athens School of Medicine, Greece; Mr. Pedro Lizardo, Bureau of Research Laboratories, Alabang, Philippines; Dr. Kekadu Makonnen, Imperial Central Laboratory and Research Institute, Addis Ababa, Ethiopia; Mr. W. Maw and Mr. T. M. Saw, the Burma Pharmaceutical Industry, Rangoon, Burma; Mr. Smarn Pongpairoj, Department of Medical Sciences, Ministry of Public Health, Bangkok; Dr. P. V. Ramana Rao, Institute of Preventive Medicine, Narayanaguda, Hyderabad; Dr. Kamal Geryes Rayyan, Jordan Vaccine Institute, Amman, Jordan; Mrs. A. Ruangchan, the Government Pharmaceutical Organization, Bangkok, Thailand; Mr. E. C. T. Ssebabi, Uganda Blood Transfusion Service, Kampala.

The Governing Body wishes to record its thanks and deep appreciation to the scientific, administrative and technical staffs for their wholehearted devotion to the work of the Institute. The very successful results, which are recorded in this Report and in the following pages, could not have been obtained without full co-operation at all levels.

A. NEUBERGER,

Chairman.

REVIEW ARTICLE

IMPLICATIONS OF BACTERIAL SEXUALITY

G. G. Meynell

Guinness-Lister Research Unit

'Is sex infectious?' a headline once asked in the early days of bacterial genetics. "Yes, indeed," a school child could answer to-day. And so are antibiotic-resistance, toxin formation and a host of other bacterial characters with equally disturbing effects. Twenty-five years have passed since bacterial mating first appeared as a laboratory curiosity and, in the interval, more and more transmissible bacterial genes have been discovered until transmission is now thought to be constantly occurring in nature. At the same time, more has been learnt of sexuality in bacteria and has allowed us to bring some order into the multitude of naturally occurring genes which bring about their own transfer from bacterium to bacterium. Two of these "sex factors" are of outstanding importance: F, the first to be identified, and I, whose behaviour has been extensively studied in the Unit.

Looking back, it may be difficult to understand why the genetics of bacteria lagged behind that of other organisms for so long. Diagnostic bacteriology has thrived for decades and relies entirely on the consistency of bacterial behaviour. If a patient has diphtheria, one expects to isolate diphtheria bacilli, not staphylococci, and, if these are subcultured in the laboratory, they are expected to produce replicas of themselves, not of another species. Thus, bacteria (and viruses, for that matter) are assumed in everyday life to breed true; they must, in other words, possess some form of genetic apparatus. It is not that the older literature ignores genetic phenomena. Quite the contrary; it is filled with exceptions to the rule that bacteria generally breed true. There are endless descriptions of "variants" and "variation" without emphasising what now appears the important generality: that like almost always produces like throughout the biological world, in bacteria no less than in other beings.

The difficulties appear to have been twofold. First, an organism's appearance was frequently taken as a direct reflection of its genetic constitution, much as if rickets were thought of as an inherited not an acquired disease; and second, a lack of characters suitable for genetic experiment. This phase was ended largely by workers who were not professional bacteriologists: geneticists who propounded the concept of one gene specifying one enzyme, and biochemists who proved it. Biochemical defects arising from mutation thus came to be recognised as possible genetic markers for micro-organisms of all kinds, just as colour or height had long been used for plants. In the event, these biochemical markers were to prove enormously more powerful as genetic tools, partly because lack of a given enzyme usually corresponds directly to mutation in the corresponding gene in a simple one-to-one fashion, whereas characters like height depend in a complex fashion on the working of many genes; and partly because they

hold out the possibility of selecting the progeny of a mating with a rigour previously unthought of. The first demonstration of bacterial mating by Lederberg and Tatum in 1946 set the pattern for all later work. The two parental strains are each deficient in a different function, depicted as $a^- b^+$ and $a^+ b^-$, and are placed on a culture medium such that only their $a^+ b^+$ progeny will grow. It is easy to choose mutations in each parent that virtually never revert spontaneously to $a^+ b^+$ so that vast numbers of parental organisms can be cultured to isolate progeny occurring as rarely as 1 in 10,000,000,000. This enormous selective power enables rare events like the formation of bacterial hybrids to be detected by a laboratory technique of extreme simplicity. In fact, the method turned out to be potentially far more sensitive than was needed, owing to the biochemical structure of genes. Initially, sets of enzymically defective mutants were prepared from the now classic strain of *Escherichia coli* known as K12 which allowed crosses, $a^- b^- c^+ d^+ \times a^+ b^+ c^- d^-$, to be made. Recombinants such as $a^+ b^+ c^+ d^+$ were found and a mating system in bacteria thus discovered for the first time.

This was not at first thought to involve the transfer of genes from a donor to a recipient, for the two mating cells were believed to fuse completely and only later to grow and divide, leading to the appearance of their recombinant progeny as individuals. The true nature of mating, in the sense of transfer from one cell which acted as donor to a second acting as recipient, only came to light through a succession of later observations. Thus, the number of hybrid progeny was greatly affected by exposing one but not the other of the two parents to streptomycin or to ultra-violet light, a finding that was unexpected if they fused and were equivalent. Moreover, a few mutants of strain K12 were found to be infertile. Fertility thus appeared to be under control of a gene termed F which was present in some mutants of strain K12 but not in others. This F gene was soon found to have remarkable properties quite unlike those of a gene in the bacterial chromosome proper. For one thing, when F^+ bacteria carrying the F gene were crossed with F^- bacteria by mixing their broth cultures for an hour, most of the F^- cells became F+, whereas only 1 in 1,000,000 acquired chromosomal genes from the F^+ donor. In genetic parlance, F appeared "unlinked" to the chromosome and, on the genetic principle that linkage reflects a physical connection and *vice versa*, F was therefore shown to be physically distinct from the chromosome. In other words, these F^+ bacteria owed their donor ability to carriage of a second supernumerary chromosome, their "sex factor", separate from that maintaining the life of the cell as a whole, which could bring about its own transfer very efficiently and also that of the chromosome, albeit far less often (Cavalli, Lederberg, and Lederberg, 1953; Hayes, 1953). In the Lederbergs' phrase, F appeared as an "ambulant gene": in another sense, F was "infectious", because it could be acquired by recipients who themselves became donors a few minutes later.

Considering that this was the state of the subject about 1953, it is remarkable that some salient features of F passed so long unnoticed and that, even to-day, we do not know for certain how the donor's genes are transferred to the recipient. There was, first, the fact that the conjugating ability of F was always active, which is unusual among bacterial functions inessential for life, which are almost always shut-off ("repressed") until the cell has need of them. This property of F was entirely overlooked until another sex factor named I was examined. Like F, I is unlinked to the chromosome and brings about chromosomal transfer but, unlike F, I is normally transferred by only 0.1% of I+ cells at a given time. Nevertheless, immediately after I is transferred, 100% of its new hosts transfer it for a few generations. Thereafter, the figure gradually returns to the usual 0.1% (Stocker, Smith and Ozeki, 1963). This discovery proved to have a far more general application than the continually de-repressed state of F. At this time, many aspects of the regulation of function were becoming clearer from studies on the induction and repression of enzyme synthesis and phage development in bacteria and, by analogy, it appeared that the I factor was self-repressible (Clark and Adelberg, 1962).

Subsequently, this has been found to be true of nearly all naturally occurring sex factors other than F (Meynell, Meynell and Datta, 1968). When such factors first enter a cell, two processes evidently begin: the appearance of conjugating ability and synthesis of the corresponding repressor. For a time, the first process outstrips the second and, as long as this state lasts, all the bacteria can donate. Eventually, repression supervenes in 99.9% of cells, leaving only a minority able to conjugate.

The event which took these observations out of the laboratory and into the field was the discovery of transmissible drug-resistance (Watanabe, 1963; Mitsuhashi, 1969). About 1957, Japanese bacteriologists noticed that strains of *Shigella* isolated from patients suffering from bacillary dysentery were resistant to an unusually large number of chemotherapeutic agents, like the antibiotics and sulphonamides then in use. At the same time, strains of *Escherichia coli* isolated from these patients were also found to be multiply resistant. It was therefore suggested that drug-resistance was being acquired in one step, by transfer of all the resistances together by mating, rather than by the then conventional mechanism of successive rare chromosomal mutations occurring at random in a given strain. Laboratory studies immediately showed this to be so. These multiply resistant strains were acquiring sex factors at the same time as their resistance and were becoming donors in their turn, although the nature of their sex factors remained unclear. In general, they behaved like I rather than F, inasmuch as the percentage of cells capable of transfer depended on how long the factors had been in their current host, indicating that they, like I, were self-repressible. At the same time, however, certain R factors (as these complexes of sex factor and drug-resistance genes are called) were found to interfere with the functions of F (Watanabe, 1963). The key to this problem lay in the recognition that the "interference" with F exerted by these R factors was in fact another instance of repression (Egawa and Hirota, 1962). That is to say, the repression of the R factor which prevented the functioning of its own sex factor must also be acting on the F factor carried in the same cell (Meynell and Datta, 1965). This hypothesis rapidly proved to have widespread implications, extending far beyond this particular class of R factors. If the R factor specified a repressor protein which recognised the appropriate region in F as well as in itself, the two regions must be extremely similar. At the limit, might not the sex factor in R be identical with that in F, with the sole difference that R specified a repressor whereas F did not (Meynell and Datta, 1965)? That this is broadly true was made clear by studying other functions of these sex factors which had meanwhile come to light in experiments concerned with the anatomy of gene transfer.

Transfer in F^+ matings had been shown to depend on physical contact of donor and recipient almost as soon as it was discovered. Fusion was unlikely from the physiological experiments using streptomycin and u.v.-irradiation, and also from direct examination of mating cultures by light microscopy. This showed donor and recipient cells towing each other through the medium in pairs and small clumps, although they were not necessarily lying side by side and often seemed to be joined by an invisible strand. Broadly similar observations were made on I^+ and R^+ matings, though I seemed to offer particularly promising material for determining the nature of the conjugation bridge, since the mixed parental cultures often contained massive clumps formed from thousands of mating cells (e.g. Meynell, 1961) which could be more readily examined by electron microscopy than the pairs and trios usually found with F. In the event, the nature of F conjugation was first demonstrated by a fortuitious observation. Certain bacterial viruses (phages) were known to attack only cells carrying F, and electron microscopy showed their particles attached, not to the bacterial body, but to filaments extending from the body outwards into the surrounding medium (Crawford and Gesteland, 1964). These filaments would have gone unnoticed without having the phage as a label on account of the numerous flagella and other filaments formed by both F^+ and F^- bacteria, although, with foreknowledge, they can now be identified on sight alone. The filaments specified by F,

generally known as "sex pili", were evidently the link seen with the light microscope. It was immediately suggested (Brinton, 1965) that the pili were hollow tubes, which is extremely plausible, down which the donor's genes passed to the recipient, which even now has yet to be proved. With the knowledge that F specified sex pili, an intensive examination of I^+ cultures was begun. By using mutant strains not forming flagella or other filaments and by careful control of the spread of I through I^- cultures so as to convert as many as 50% of the cells to donors, a second type of pilus, the "I pilus", was found, which differed from the F pilus in shape as well as in other properties like adsorption of F phage (Meynell and Lawn, 1967). There were thus two types of sex pilus, each corresponding to one of the two types of known sex factor, F and I. The properties of the pili formed by an unknown donor strain could therefore be used as genetic markers to characterise its sex factor as "F-like" or "I-like" (Lawn, Meynell, Meynell and Datta, 1967). How could these findings be applied to R factors and other sex factors present in bacteria isolated from patients or animals? Recall that some, but certainly not all, R factors exhibited a strong functional relation to F in that both were susceptible to the R repressor. Might not the remaining parts of these R sex factors be the same as F? In particular, might not the sex pili of these R factors be F pili? This was soon proved to be so, by the susceptibility of R^+ cultures to phage known to adsorb only to F pili (Meynell and Datta, 1966) and by the shape and antigenic specificity of the pili formed by cultures with de-repressed R factors (Datta, Lawn and Meynell, 1966; Lawn, 1966). Analysis on precisely the same principle showed that many other naturally occurring sex factors, associated not with drug-resistance but with a diversity of functions like the synthesis of haemolysin, somatic antigens or the antibiotics known as "colicins", were all "F-like". Despite the variety of properties by which these extra-chromosomal factors first reveal themselves, they are all virtually identical in the sex factor genes that enable them to be transmitted by conjugation (Lawn *et al.* 1967; Meynell *et al.* 1968).

If so many sex factors are "F-like", what of the rest? These were known not to repress F although repressing themselves, which argued against a relationship with F. Their nature became easier to establish when mutant sex factors were isolated that were no longer repressed (Meynell and Datta, 1967; Edwards and Meynell, 1968*a*). These failed to form repressor or had become resistant to its action. As expected, the "F-like" factors then caused their hosts to form F pili continuously, just like an F^+ bacterium. The unexpected finding was that the "non-F-like" group nearly always produced I pili, whether the sex factor was associated with drug-resistance, colicin synthesis or, in one case, no other identifiable function. There is therefore a large group of "I-like" sex factors which, taken with those known to be related to F, accounts for perhaps 95% of all naturally occurring extra-chromosomal agents which are self-transmissible (Lawn *et al.* 1967; Meynell *et al.* 1968).

The identification of these factors by isolating de-repressed mutants is laborious, and a considerable advance was a selective technique for the isolation of donor-specific phages from sewage. This yielded I-specific phages, analogous to those that are F-specific (Meynell and Lawn, 1968). With a phage of each class, it became possible to screen naturally occurring strains for the transmissible agents they carried, a technique which immediately showed that many strains carried at least two different R factors, one I-like, the other F-like (Lawn *et al.* 1967; Romero and Meynell, 1969).

In all these experiments, electron microscopy played an outstanding part. One had always supposed that it would reveal the conjugating bridge between mating cells but, in the event, it did not only this but much more. Thus, the presence of F-like pili on cells carrying R factors could have been deduced solely from the growth of F phage in their cultures; but with microscopy, it was possible to see the pili with phage particles attached and so, simply by their size and structure, to confirm their identity with the pili specified by F itself (Datta *et al.* 1966). Similarly, when an improved method was introduced for isolating mutant

sex factors whose hosts were no longer sensitive to one of the two major classes of F phage, microscopy immediately showed that although the cultures were resistant, the phage particles could nevertheless adsorb to the pili, albeit less often, so that a later stage in infection was defective (Valentine *et al.* 1969; Meynell and Aufreiter, 1969*a*). This would have been extremely laborious to establish by biochemical methods but took about 15 minutes by microscopy. At the same time, the mutant pili were shown to be physically unstable, in that their structure frequently collapsed. The same enormous saving in time and effort, accompanied by a remarkable gain in information, was always apparent whenever microscopy was used. To isolate the I phage, drops of suspected phage suspension were spotted on layers of susceptible bacteria which subsequently failed to grow if a phage was present, so producing small areas of lysis or "plaques". To identify a phage is troublesome by purely microbiological techniques but, by microscopy, the contents of a plaque can be examined within a few minutes and show not only the shape of the phage particles but also how they attach to the bacteria. As a result, in addition to the two I phages which were seen attached tip-to-tip to the I pili, we isolated several phages that attached to bacterial flagella although such phages had previously been thought of as rare (Edwards and Meynell, 1968*b*).

Perhaps the other outstanding application of electron microscopy to sex pili has been in the study of their antigenic structure. In the first attempts to identify the I pilus, some supposedly specific antisera were prepared in the hope of detecting the pilus by its reaction with antibody conjugated to ferritin, an electron-dense protein. Although this procedure was never satisfactory, it became clear that even single unconjugated antibody molecules could be seen if they were stained by conventional negative-contrast techniques in which the object remains largely unstained and is seen in relief against a stained background (Lawn, 1967). Pili were particularly favourable material because they extend outwards from the bacterial wall across the background and any alterations to their shape are easily seen. The introduction of this elegant technique therefore allowed the antigenic structure of pili to be determined without using conventional serological methods like agglutination, which were already proving unsatisfactory because of non-specific reactions. It immediately confirmed the division of sex pili into the two major F-like and I-like classes, since the antigenic specificities were as distinct as their phage susceptibilities (Lawn *et al.* 1967). Even then, however, it was apparent that each major class was heterogeneous and, as more de-repressed mutants were isolated, it became clear that the F-like and the I-like groups could be subdivided (Lawn and Meynell, 1970). An intriguing consequence of this work relates to the pili formed by cells carrying F and an R factor simultaneously. Certain strains known as "Hfr" for "high frequency of recombination" carry F integrated at one point in their chromosome, so causing the neighbouring region to be transferred far more frequently than the remainder. R factors are also known to bring about chromosomal transfer, even if F is absent; and provided their sex factors are de-repressed, transfer is as frequent as with F (Cooke and Meynell, 1969). Unlike an Hfr donor, however, every chromosomal gene is transferred equally frequently. An Hfr strain can become unable to form sex pili following mutation in its F factor and consequently becomes unable to donate its chromosome in its characteristic fashion. If, then, an intact R factor is introduced, one can ask if the pattern of chromosomal transfer characteristic of that particular Hfr strain reappears (Cooke, Meynell and Lawn, 1970). As regards pilus synthesis, F-like and I-like sex factors are known to be unrelated (Meynell and Aufreiter, 1969*b*). It turns out that if the R factor is I-like, chromosomal transfer sometimes reappears, although microscopy then shows the sex pili to be purely I-like in their antigens. If the R factor is F-like, transfer again often reappears but each pilus now has two antigens, one due to F, the other due to the R factor (Lawn, Meynell and Cooke, 1971), just as had already been found with a normal F factor in an R^+ host (Meynell and Cooke, 1969). The explanation lies in the bio-

synthesis of sex pili. Like many biological structures, such as viral coats or bacterial flagella, pili do not consist of uniform material like glass but of individual monomers of protein, here called "pilin", which are stacked together to produce a filamentous structure, much as bricks are laid to form a chimney. Bacteria apparently contain a reserve or "pool" of pilin, since they are able to form pili after protein synthesis has been stopped (Brinton, 1965; Meynell and Lawn, 1967). The mutant F factor of the defective Hfr strains can evidently still cause pilin to be produced but not whole pili. The F pilin therefore enters the pool with that from the R factor. If the two sorts of pilin are sufficiently similar, like those of F and an F-like R factor, the bacteria can mix them together in forming its pili; but if they are distinct, like F and I pilin, only pure I pilus is formed.

If, then, this is broadly the stage we have reached to-day, on what lines is the subject likely to develop in the future? Here, what is perhaps most difficult to convey is that although much is known, even more is unknown and is often forbidding in extent and in the intrinsic nature of the problems. Consider the transfer of genes (that is, of deoxyribonucleic acid or DNA) from donor to recipient. The idea of a sex pilus as a hollow conducting tube was first proposed in 1964 but even now has been neither proved nor disproved. One might suppose it a simple matter to demonstrate isotopically labelled DNA within the pilus; yet this is not so, owing to the small amount of DNA involved, the scarcity of sex pili and the relatively high background radioactivity of the medium. One school has little faith in the pilus-conduction model and believes that the pilus only provides an initial link between donor and recipient which is followed by their fusion, perhaps as a result of retraction of the pilus. The pilus is certainly essential for conjugation, since adsorption of phage prevents gene transfer (Valentine *et al.* 1969). Moreover, F-like and I-like sex factors in the same cell do not use each other's pilus, since transfer of either is specifically stopped by the homologous phage or antibody (Salzman, 1971; E. Meynell, unpublished). It is also clear that the synthesis of sex pili has other effects on the host, since many de-repressed factors render bacteria extremely susceptible to lysis, suggesting an unsuspected effect on cell wall structure (Dowman and Meynell, 1970). One major field is therefore the functional and structural analysis of sex pili.

The regulation of pilus synthesis probably offers fewer problems: it conforms to the pattern for negative control by repression established in other systems. Thus, repressor-insensitive mutants of F named Fo^c have been isolated (Frydman *et al.* 1970); and, no doubt, R factors forming exceptionally high quantities of repressor could be isolated in their turn, and so on. Most of the analysis has been done with F-like factors because it requires two sex factors which can co-exist; the factor whose properties are to be determined and a second factor to act as an indicator. This can be done with an F-like R factor and F, or with R factors and colicin factor B (Frydman and Meynell, 1969), but on turning to the I-like R factors, there is no sex factor analogous to F with which they co-exist naturally. There is, then, the question of how many other plasmid-determined functions are repressible. Drug-resistance is not necessarily amongst them, but many other plasmid-determined functions are known and, now that the occurrence of repression has been recognised, one may expect precisely the same form of analysis to be applied to all the known functions of F, R factors and the like.

If the anatomy of gene transfer is still hazy, little more can be said of its physiology and genetics. Even with F, let alone other sex factors, it is still not certain how transfer is initiated. When donor and recipient meet, the donor senses in some way that a copy of its sex factor should pass to the recipient. What signal it receives and how it acts are still a mystery. Still more so is the transfer of genes not physically linked to a sex factor: this is true even of the bacterial chromosome proper which usually appears to be transferred unlinked to F. Many more genetic markers need to be discovered in all sex factors before their function can be understood: for this reason alone, the analysis is primitive compared

with that attained with many phages. Another most difficult yet fundamental problem concerns the replication of sex factor genes during growth of the bacteria. To take but two points: in some way, sex factor and chromosome replicate in step so so that neither outstrips the other. They cannot be dividing independently by chance or the plasmid would soon be lost (Dowman, 1971): there must be a mechanism co-ordinating their replication, as has often been suggested. This is still far from understood: if it were, we should understand one of the outstanding problems of cell biology, the co-ordination of all DNA replication with cell growth. However, one can at least approach this problem experimentally, using biophysical techniques and isotopic labelling of plasmid and chromosomal DNA to identify when and where replication occurs. At a different level, it may be asked if the replication of sex factors exhibits specificities corresponding to the division between "F-like" and "I-like" in sex pili. In fact it does, as shown by the general inability of two R factors specifying the same class of pilus to co-exist readily in the same cell: one inhibits the replication of the other (Wantanabe, 1963). Thus, although replication is largely a mystery, one can in a sense determine its specificity by seeing which pairs of sex factors are able to co-exist. The inhibition observed between two related factors may be a manifestation of the normal control of replication. One might then hope to supplement these studies by looking for a similarity in the mechanism of replication, the process which actually produces new copies of the sex factor. Here there is not so much a conceptual block as the practical difficulty of selecting appropriate mutants.

A topic not yet mentioned is transmission in nature, which might be regarded as being of overwhelming interest in view of the perpetual problem of drug-resistance in treatment and the abundance of R factors in bacteria isolated from patients, animals, sewage and even rivers. Although transmission in nature is a danger to health, it is, strange to say, not really a problem at the experimental level. If we cannot understand the fundamentals of transfer under controlled conditions in a test tube, we shall not understand it any better in an animal. If, however, one accepts transfer as a fact and ignores its mechanism, the question of transfer between enteric bacteria is no more than a particular aspect of the general behaviour of bacteria in the mammalian gut. This is already reasonably understood, for it became of great significance some years ago when broad-spectrum antibiotics came into use. Patients under treatment often suffered from a new disease, an overwhelming infection of their gut by organisms resistant to the antibiotic administered. As these organisms were normally present in only small numbers, the antibiotic had evidently eliminated some natural means of regulating their numbers in the gut contents. This proved to be the metabolic activity of the gut anaerobes which normally preponderate within the gut and render it unsuitable for aerobic species like *E. coli* and other enterobacteria in which R factors occur. These aerobic organisms are often absent from humans and animals, presumably for this reason, and when present, their numbers are remarkably low for organisms referred to as "enterobacteria". A complete transformation takes place on giving a broad-spectrum antibiotic, if the enterobacteria are resistant and the rest of the gut organisms are sensitive so that they are killed and their inhibitory effect removed. The enterobacteria increase vastly in numbers, by perhaps 10,000 fold (Meynell and Subbaiah, 1963; Meynell, 1963). With this in mind, it is not surprising that R factors are transferred in experimental infections with remarkable slowness, for not only are the organisms scanty but the conditions are unfavourable. If an antibiotic is given, transfer occurs much more quickly. Giving an antibiotic may actually encourage the spread of an R factor to otherwise sensitive organisms. The main problem of natural transmission whose explanation is far less clear is why virtually every sex factor is self-repressible. One would suppose that the more frequently a sex factor was transferred, the more widespread it would become. The reasons appear to be several: de-repressed factors cause sex pili to be formed, so making their hosts liable to be killed by the corres-

ponding phages; they make their hosts grow more slowly; they also make them susceptible to lysis. Changes such as these evidently outweigh the disadvantages of a low rate of transfer.

In this field, the pure and applied aspects of research are unusually close. Although one is dealing with small chromosomes in a small organism, the fundamental problems are those of any living cell and have yet to be solved. At the same time, the growing awareness amongst medical and veterinary bacteriologists of the prevalence of gene transfer is already producing many reports of new transmissible genes. Taxonomic criteria like lactose fermentation and phage type are being upset, and other characters will no doubt be involved. Perhaps the most alarming prospect for the future is that genes conferring pathogenicity will become transferable and pass to new hosts with unusual powers of survival. Sex factors have already been transferred from *E. coli* to cholera vibrios or to plague bacilli in the laboratory. Given the far greater scale in time and number that exists in nature, will we eventually find typical coliforms producing cholera or plague; will plague become a water-borne disease; and will all these organisms resist chemotherapy? After all, the phenomenon of transmissible drug-resistance would have seemed far fetched only a short time ago, Indeed, it would still be a mystery were it not for concurrent developments in microbial genetics at the fundamental level.

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RESEARCHES IN 1970

MICROBIOLOGY

Trypanosomiasis

Column-separation. Temperature and the presence of plasma markedly affected the yields and purity of stercorarian trypanosomes harvested from blood (Lanham). Subpatent Gambian trypanosomiasis in Nigerians was diagnosed by column-separation, and with an improved technique a subpatent *Trypanosoma avium* was demonstrated in a rook (Godfrey, Lanham, Williams).

Action of basic macromolecules. DEAE-dextrans, like poly-L-lysines, caused the release of glutamic and pyruvic transaminase from trypanosomes *in vitro*. Adhesion of DEAE-dextran to the surface was the only ultrastructural change observed. Three species, more negatively charged than *T. brucei brucei*, were the less susceptible; the only intraspecies variation in *T.b.brucei* was that organisms from a highly parasitized rat were more susceptible than those from an animal with few organisms (Godfrey, Taylor).

Soluble antigens. Purified soluble antigens of *T.b.brucei* were highly variant-specific immunogens; their antisera partially inhibited trypanosomal GPT activity (Taylor, Lanham, Godfrey).

Whooping Cough Bacillus

Bactericidal antibody and its antigen. The antigen of *B. pertussis* that elicits bactericidal antibody (Report 1970) proved to be a lipopolysaccharide; it was distinguishable from lipopolysaccharide antigens of *B. bronchiseptica* and an atypical *B. pertussis* strain. Research continues on the mode of action of bactericidal antibody (J. Dolby, Ackers). The pyrogenicity and adjuvant effect of the pertussis cell were not attributable to "bactericidal" antigen (Ackers).

Immune response. The immunoglobulin patterns of three strains of normal mice were related to their immune response to *B. pertussis* vaccine. Two antibodies protecting mice against brain infections with *B. pertussis* were present in different proportions in these two strains after immunization (J. Dolby, D. E. Dolby).

Clostridial Toxins

In a continued study of *Clostridium tetani* toxin production, certain fractions isolated from Mueller's medium enhanced toxin formation whereas others inhibited it; the active substances are being identified (Mahony).

Inheritance in Bacteria

Bacterial DNA. Lysis of bacteria by tris buffer, a chelating agent, lysozyme and detergent is often unsuccessful with certain enterobacteria. Inclusion of trace amounts of *n*-dodecylamine causes instantaneous lysis without damaging the DNA released (G. G. Meynell).

Angle rotors are less successful than swing-out rotors in separating plasmid from chromosomal DNA by isopycnic density gradient centrifugation, probably because the gradient is necessarily twice disturbed, when the rotor stops and when the tube is removed to a vertical position (G. G. Meynell, Dowman).

Detecting multiple colicin factors. Provided the assay agar is adequately buffered, polyacrylamide gel electrophoresis can be used for separating mixtures of colicins and greatly simplifies the detection of multiple colicin factors in a single host (Hardy, Neimark).

Bacterial sex factors and sex pili. Transfer of F-like and I-like drug-resistance factors is specifically blocked by F or I filamentous phage, or by F or I anti-pilus antibody, thus confirming the specificity of pilus transfer.

The F factor is only transferred to F^+ recipients from F^+ donors carrying an R factor if this is F-like, not I-like. The difference lies in the sex pili the plasmids determine: F with an F-like R factor give mixed pili, mostly consisting of R pilin, which transfer F; F and an I-like R factor give discrete F and I pili, and the latter cannot transfer F. Exclusion of F by the recipients' F factor is therefore specifically directed against pili with pure F pilin (Salzman, E. Meynell, Harden).

Colicin factors with de-repressed sex factors cause their hosts to form small colonies although more colicin is released. This is due to colicins being largely cell-bound and to de-repressed sex factors rendering the cells prone to lysis (Dowman, G. G. Meynell).

Inactivation of a plasmid gene. Recombination of the plasmid, F'13, and the chromosome of *Salmonella enteriditis* may occur within both plasmid and chromosomal genes which are thereby inactivated (G. G. Meynell).

Implications of stochastic inheritance. Plasmid genomes cannot be distributed at random at cell division, or they would not be stably inherited. This follows from a theoretical study which also covers the important case of non-chromosomal particles incapable of replication (Dowman).

Electron Microscopy

Ultrastructural features of antigen-antibody combination. Examination of the reaction of the sex pili of enterobacteria and of filamentous phages with their respective antisera revealed details of the ultrastructural features of antigen-antibody reactions. In particular, closed IgG molecules (combining sites together) and open IgG (combining sites maximally separated) were almost equal in length. These studies are being extended with purified immunoglobulins (Lawn).

Mycoplasma

Antigens of M. hominis. The separation and characterisation of membrane antigens was continued (Reports 1968-70). Each of the three components identified gave rise to growth-inhibiting and indirect haemagglutinating antibodies. At least two of the components are proteins (Lemcke, Hollingdale).

M. pulmonis infections in rats. Experimental *M. pulmonis* infections simulated the natural chronic respiratory disease of rats. There was no evidence that another pathogen was involved (Lemcke with Dr. P. Whittlestone, School of Veterinary Medicine, Cambridge).

Lactic dehydrogenases in mycoplasmas. The lactic dehydrogenase enzymes of several glucose-fermenting mycoplasmas differed in their properties (Neimark, Lemcke).

TRACHOMA AND INCLUSION CONJUNCTIVITIS

Replication in cell cultures. Whole TRIC (trachoma/inclusion conjunctivitis) inclusions were separated from baby hamster kidney cells (Blyth, Taverne, Garrett) and the carbohydrate matrix was studied (Garrett). The conditions for obtaining maximum yields of elementary bodies were defined, and the amounts of inclusion carbohydrate at various stages of the growth cycle were determined (Barton). Large quantities of TRIC organisms can now be grown in cell suspensions for analytical studies (Garrett).

Interactions with macrophages. In macrophages that had ingested TRIC organisms protein synthesis was depressed; release of acid phosphatase from lysosomes also occurred and might be useful for studying some aspects of immunity to infection. The finding that TRIC elementary bodies lost infectivity but retained their toxicity after ingestion may help to explain the conjunctival cell damage seen in trachoma (Blyth, Taverne).

Immune responses. Intravenous injections of formalin-inactivated TRIC agents induced neutralizing antibody in both IgM and IgG globulins of rabbit sera. Study of the use of an attenuated TRIC agent for local immunization of the eye was continued, and researches on cell-mediated responses were begun (Collier, Harris).

Attempted isolation of a chlamydia from mosquitoes. An attempt to isolate a chlamydia from female *Anopheles stephensi* was unsuccessful (Collier, with Mr. D. Venters, Liverpool School of Tropical Medicine).

Investigations overseas. Continued observation of a group of 100 Gambian children showed that the presence of trachoma antibody in the serum IgG was closely related to the severity of the disease and to the degree of conjunctival follicular hyperplasia, but not to the corneal lesions.

TRIC agents isolated from 53 trachoma patients in 2 widely separated Gambian villages all belonged to the 2 main trachoma serotypes elicited by immunofluorescence;

the communities differed considerably in terms of the relative proportions of types 1 and 2 (S. Sowa, J. Sowa and Collier).

VIROLOGY

Infectious Hepatitis

Researches on the possible role of adenovirus type 5 in infectious hepatitis continued. Methods of assaying neutralizing antibodies and of culturing adult rat liver cells were improved. The cytotoxicity of some infectious hepatitis sera is under investigation (Alwen).

Vaccinia Virus

Mechanisms of immunity. The cellular and humoral aspects of immunity to vaccinia are being explored by various techniques including induction of lesions on the mouse tail by intravenous injection as a test of immunity, assay of neutralizing antibody and the macrophage inhibition test (Hutt).

Smallpox vaccine. Studies on inactivated smallpox vaccine were continued in terms of antigenic differences between extracellular and intracellular vaccinia virus (Turner). The relationships between the potency and stability of freeze dried vaccine and its content of residual water, sulphydryl groups and total nitrogen were investigated (Robinson). Examination of strain differences and the evaluation of assay methods were continued (Murray).

Haemagglutinin. The suitability of different sources of vaccinial haemagglutinin was examined and its purification and characterization were begun (Runkel).

Rabies Virus

The long term stability of pilot batches of purified rabies vaccine was demonstrated (Turner). Their immunogenicity in rabbits compared favourably with that of current vaccines (Turner, Runkel). Attempts to produce potent vaccine from cell culture were continued and the role of interferon in vaccine-induced immunity was examined (Turner).

IMMUNOLOGY AND EXPERIMENTAL PATHOLOGY

Antitoxin Production

Diphtheria toxin was polymerized to improve its antigenicity and attached to an insoluble matrix to act as a specific adsorbent for antitoxin.

Anaphylaxis

An eosinophil-stimulating factor. Work on the eosinophil response in anaphylaxis continued. Mast cell substances contribute to it, but are not the only stimuli (Jeffery). Lymphocytes from nodes draining a site recently injected with antigen induce eosinophilia in normal animals (Parish).

Horse serum proteins. Further research on anaphylactic reactions to therapeutic horse sera showed that atopic persons spontaneously sensitive to horse dander can be safely treated with refined horse globulins; but people who have had serum sickness due to horse globulins cannot (Parish).

Exogenous Antigens Acquired by Tissue Culture Cells

Research continued on the possible role of foreign antigens in eczema (Reports 1966-70). Studies were made on the influence of such antigens on cultured cells (Parish) and on their presence in eczematous lesions (Parish, with Dr. R. H. Champion and Dr. E. Welbourn, Addenbrooke's Hospital, Cambridge).

Mechanisms of Inflammation and Infection

Maternal transfer of monocytosis-promoting factor. The relative insensitivity of the offspring of tuberculin sensitive guinea-pigs to a monocytosis-promoting stimulus was confirmed for male and established for female offspring. Some of these offspring were also relatively insensitive to a variety of substances that induced a delayed inflammatory response (F. R. Wells).

Vascular change in delayed hypersensitivity reactions. In this reaction, the phase of margination and emigration of mononuclear cells coincided with that of vessel damage as indicated by carbon labelling and the exudation of plasma proteins (F. R. Wells).

Plasma kinin systems. The reactions of the kinin-releasing system in human plasma were studied with Hageman-trait plasma and preparations of human prokallikrein (Mason, McConnell). In this system Hageman factor activated human prokallikrein, not directly, but through a surface-active esterase (Mason).

Klebsiella infection in the guinea-pig. The study of the mechanism of the enhancement of Klebsiella infections by ferric iron was continued (Miles).

BIOCHEMISTRY

Human Blood-Group Substances

Structure. Continued research on blood-group specific glycoproteins (Report 1970) indicated that they comprise one region rich in carbohydrate and in serine, threonine and proline; and another that is rich in acidic amino acids (Donald). A method yielding peptides suitable for sequence studies was devised (Donald, Goodwin, Watkins).

Biosynthesis. Structural analysis of B-active tetrasaccharide prepared by biosynthesis established that the enzyme found only in tissues from blood-group B or AB subjects (Reports 1969, 1970) is an α-3-galactosyltransferase; its distribution in different human and animal tissues was examined (C. Race, Watkins). The α-N-acetylgalactosaminyl transferase associated with the blood-group A character was found in soluble form in ovarian cyst fluids and attempts are being made to purify it (Hearn).

A second α-*N*-acetylgalactosaminyl transferase which occurs in all individuals irrespective of blood group was detected in human submaxillary gland and stomach mucosa; it catalyses the transfer of *N*-acetylgalactosamine to the hydroxyamino acids in the peptide chains of the blood group glycoproteins (Hearn, Goodwin, Watkins).

Investigations of the distribution and acceptor specificity of α-L-fucosyltransferases in the tissues of ABH secretor and non-secretor subjects were continued (Chester, Watkins).

Purification of glycosidases. The 2- and 4-α-L-fucosidases in extracts of *T. foetus* were separated by zone electrophoresis (Stealey, Watkins). The pH optima, temperature stability and Km values were established for two α-*N*-acetylgalactosaminidases from *T. foetus* (Goodwin).

Lipids and Biological Membranes

Subcellular organelles of BP8 ascites tumour cells. Continued research on the subcellular organelles of BP8 mouse ascites tumour cells (Report 1970) indicated that loss of differentiation was confined only to the fatty acid components of the phospholipids and did not affect their general composition (Gray, Segal).

Action of polyene antibiotics on intact mammalian cells. The interaction of polyene antibiotics with mammalian cells (Report 1970), although broadly similar to that with model lipid systems, was more complex. In a range of cells the rankings of susceptibility to threshold concentrations of drugs and to concentrations causing severe damage were different (J. Wells).

Glycosphingolipid hydrolases. In a study of the role of glycosphingolipids in membrane function, two galactosidases were isolated from mouse intestine and purified, and a third was detected in crude homogenates (Chandrabose, Gray).

Biosynthesis of mouse kidney glycosphingolipids. The biosynthesis of galactosylceramide in the kidneys of C57/BL mice from galactose and ceramide was controlled by testosterone, probably through its influence on a specific galactosyl transferase (Gray).

BIOPHYSICS

Human Plasma Proteins

In further studies with isoelectric focussing (Report 1970), IgM and IgG from pathological sera resolved into multiple components. The heterogeneity is related to the H-chains, the L-chains being homogeneous. The findings are difficult to reconcile with the view that such pathological immunoglobulins are monoclonal in origin (Kekwick, Butterworth).

Blood-group Glycoproteins

Density gradient studies were extended (Reports 1969, 1970). Fractions from a single ovarian cyst fluid showed wide variations in buoyant density, correlated with the proportion of amino-acids. Density heterogeneity was pronounced in fractions of low amino-acid content, but minimal in those rich in amino-acids (Creeth).

Methodology of Ultracentrifugal Analysis

A two-component analysis for low-speed sedimentation equilibrium was devised and programmed for the Olivetti desk-top computer (Creeth).

BLOOD PRODUCTS LABORATORY

Anti-D Immunoglobulin

Although anti-D immunoglobulin, stored at 37°C for one month, remained stable and retained its potency, some batches kept at 4°C for up to 4 years showed some loss of potency without any detectable change in the protein (Wesley, Kekwick with Dr. N. C. Hughes-Jones, M.R.C. Experimental Haematology Research Unit).

Factor VIII

The addition of 5mg calcium per 100 ml solution of purified Factor VIII diminished the loss of activity caused by incubation at 37°C (Ellis, Stickley).

Pharmacologically Active Substances in Human Blood

Growth of certain cold-growing gram-negative organisms in whole blood (but not in plasma or citrate) is accompanied by formation of histamine, but in amounts insufficient to account for the changes following injection of such infected blood in animals (Mackay, Shaw).

Extension of Blood Products Laboratory

The building progressed according to plan and certain trials of equipment, some specifically designed for use in the extension, were completed satisfactorily (Maycock, Vallet, Wesley).

Plasma Fractionation Laboratory, Oxford

Factor VIII. High purity Factor VIII, which was stable on freeze-drying, was prepared both by Johnson's method and a modified Blomback method; it proved of great clinical value (Ford, Snape).

Factor IX. A more stable and highly purified Factor IX was prepared by a new method; it is more concentrated than earlier preparations so that a clinically effective dose can be given by syringe (Dike).

Factor VII. A method of preparing a Factor VII concentrate was begun (Dike).

BLOOD GROUP UNIT

Search for New Antigens and Antibodies

A new infrequent antigen was found which is genetically independent of other systems. One new antibody belongs to the Duffy and two to the Lutheran systems (Tippett, Gavin, Sanger).

Polyagglutinability of Red Cells

Three types of this state were distinguished (Tippett, Gavin, Sanger).

Application of Blood Groups to Human Genetics

The Xg groups were again applied to the mapping of the X and to problems of sex-chromosome aneuploidy (Sanger, Tippett, Gavin, Whittaker, Race).

The testing of women with chronic myeloid leukaemia and families with X-linked sideroblastic anaemia strongly suggested that the *Xg* locus escapes inactivation and consequently that lyonization of the X is not total (Sanger, Tippett, Gavin).

Tests on a series of patients with polycythaemia vera gave evidence that the *Rh* locus may be sited on the long arm of chromosome No. 4 or No. 5 (Sanger, Tippett).

The Xg groups of the family of an XO/XX mosaic mother showed that her marrow, as opposed to her lymphocytes, must be predominantly XO and this was confirmed by marrow culture. The different

distribution of the two cell lines in different tissues thus demonstrated has wider implications in the interpretation of mosaic karyotypes and of the rare Xg families that appear to break the rules of sex-linked inheritance (Sanger, Tippett, Gavin).

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BLOOD GROUP REFERENCE LABORATORY

Standardization and Control of Blood-Grouping Reagents

British Committee for Standardization in Haematology. In collaboration with a Working Party on Blood-Grouping Reagents, a draft specification of anti-D for rapid typing was devised (Goldsmith). The Working Party also studied the suitability of dyes used in mechanical blood-grouping (Giles, Ikin, Goldsmith).

Bovine albumin used as a diluent in Rh testing. Research continued on the effect of polymerizing bovine albumin used for agglutinating Rh-sensitized cells (Goldsmith, Kekwick).

Red Cell Serology

Several examples of the *OFF* phenotype were discovered in blood samples from Thailand (Giles). Investigation of new variants of the MN system continued (Ikin, Giles). Studies of Mz and Hill antigens were made (Giles) and an immune anti-Mt^a antibody was shown to have caused haemolytic disease of the newborn (Dawes). Antibodies to low and high frequency antigens were studied and a sub-division of the Swann group was revealed (Giles). New examples of rare antibodies, anti-Di^b and anti-K1, were identified (Giles).

Antibodies to Serum Antigens

Screening of red-cell antisera for antibodies to Gm, Inv and ISf antigens continued (Goldsmith, Brazier). The first known human example of anti-Gm (17) was found (Brazier). Red cells coated with gamma-G4 anti-D were used to screen random sera in an attempt to find agglutinin for antigens carried by gamma-G4 protein. No positively reacting sera have so far been found.

Proposed Automation of Screening of Blood-Grouping Sera

Preliminary work on the introduction of an auto analyzer system for initial screening is being undertaken (Dawes).

Processing of Human Plasma to Provide Blood-Grouping Serum

A technique was evolved for the necessary recalcification of plasma and removal of excess calcium ions (Moghaddam, Goldsmith, Kekwick).

Preparation and Standardisation of Fluorescein-conjugated Anti-human IgG

Apart from undertaking routine production of reagents, the Laboratory collaborated in an international field trial of commercial conjugates (Phillips).

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