BAKER INSTITUTE

RESEARCH

1965

ALFRED HOSPITAL

THIRTY-NINTH ANNUAL REPORT

of

THE THOMAS BAKER, ALICE BAKER AND ELEANOR SHAW MEDICAL RESEARCH INSTITUTE

(Including Alfred Hospital Clinical Research Unit)

NINTH ANNUAL RESEARCH REPORT

of

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

REPORTS

of

ALFRED HOSPITAL RESEARCH FELLOWS

1965

ALFRED HOSPITAL, PRAHRAN,
VICTORIA, AUSTRALIA

The Baker Medical Research Institute derives its main financial support from the Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions. It is also dependent upon donations from private sources. The latter may be allocated to an Endowment Fund. Donations of $2 or more are permissible deductions for income tax purposes.

The Diabetic and Metabolic Unit is a department of the Alfred Hospital, part of whose duties is to conduct research in some aspects of endocrinology.

Research Fellowships are awarded by the Appointors for Research Scholarship Funds of the Hospital in consultation with the Research Advisory Committee of the Board of Management.

BAKER MEDICAL RESEARCH INSTITUTE

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	N. E. JONES.
	K. N. MORRIS, M.B., M.S. F.R.C.S., F.R.A.C.S.
	EDGAR ROUSE.
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	DIRECTOR OF CLINICAL RESEARCH UNIT (ex officio).

ALFRED HOSPITAL RESEARCH FELLOWS 1965

"Edward Wilson Memorial": *Medical Research Fund*: *"Collier"*:	MARJORIE BICK, M.Sc.
"Sol Green":	H. D. BREIDAHL, M.D., M.R.C.P., M.R.A.C.P.
"E. H. Flack": *"S. W. Jones"*:	CLINICAL RESEARCH UNIT.
"R. B. McComas": *"J. F. Mackeddie"*:	DIABETIC AND METABOLIC UNIT.
"Sol Green": *"S. W. Jones"*:	B. S. GILLIGAN, M.B., B.S., M.R.A.C.P.
"Dr. Henry Laurie":	A. D. McCUTCHEON, M.D., M.R.A.C.P.
"Dr. Henry Laurie":	J. NAYMAN, M.B., B.Ch. (W'srand), F.R.C.S., F.R.C.S. (Edin.), F.R.A.C.S.
"Dr. Henry Laurie": *"Victor Y. and Margaret Kimpton"*:	R. J. SAWERS, M.B, B.S., F.R.A.C.P., M.C.Path.
"H. M. Black": *"Amelia Haigh Estate"*: *(Heart disease)*:	N. KATHLEEN TAYLOR, M.B., B.S.
"Edward Wilson Memorial":	TAN BOCK YAM, M.B., B.S. (Malaya), M.R.C.P. (Edin.).
"Connibere Bequest":	JEAN TOLHURST, D.Sc.

APPOINTED TO RESEARCH FELLOWSHIPS FOR 1966

"Edward Wilson Memorial":	T. G. JONES, M.B., B.S. (Lond.), M.C.Path.
"James Richardson":	J. M. CALVERT, M.B., B.S., F.R.C.S., F.R.A.C.S.
"Sol Green": *"S. W. Jones"*:	C. C. ENNIS, M.B., B.S., M.R.A.C.P.
"E. H. Flack":	F. W. GURR, M.B., B.S., M.R.A.C.P.
"Dr. Henry Laurie": *"Amelia Haigh Estate"* *(Rheumatoid Arthritis)*:	T. E. LOWE, D.Sc., M.D., F.R.C.P., F.R.A.C.P.
"J. F. Mackeddie": *"R. B. McComas"*:	J. NAYMAN, M.B., B.Ch. (W'srand), F.R.C.S., F.R.C.S. (Edin.), F.R.A.C.S.
"Victor Y. and Margaret Kimpton":	J. A. OWEN, B.Sc., M.D., Ch.B., Ph.D. (Edin.), M.C.P.A.
"Frederick and Esther Michaelis": *"Connibere Bequest"*:	N. KATHLEEN TAYLOR, M.B., B.S.
"Collier": *"George Merriman"*:	JEAN TOLHURST, D.Sc.
"A. A. Swallow": *"H. M. Black"*:	B. J. SAWERS, M.B., B.S., F.R.A.C.P., M.C.Path.
"Amelia Haigh Estate" *(Heart Disease)*: *"Edward Wilson Memorial"*:	A. D. McCUTCHEON, M.D., M.R.A.C.P.

TRAVEL GRANT

"J. H. Patterson Travelling Scholarship":	I. E. COOPER, M.B., B.S., F.R.A.C.S.

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INTRODUCTION

The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute was founded under the terms of a Deed of Settlement executed in 1926 between the Settlors and the Board of Management of Alfred Hospital. The Institute was established to provide an efficient hospital laboratory service and facilities for medical research. In the course of time it was found more satisfactory for these routine services to be placed under the control of the Hospital staff, and this transfer was completed in 1948. Since then the Institute staff has been entirely concerned with research, wih emphasis on the basic medical sciences. This is integrated with projects of the Clinical Research Unit.

This unit was formed in 1949, and as a result the Board of Management set up a Research Advisory Committee in accordance with suggestions made by the National Health and Medical Research Council at the time of formation of a similar unit in a sister State. The purposes of this Committee were to advise the Board on matters of appointment to the Unit and to accept responsibility that the funds allocated by the Council were expended in accordance with the conditions of the grants.

The appointment of Dr. T. E. Lowe as Director of the Clinical Research Unit in 1948 was followed by his appointment as Director of the Baker Medical Research Institute in 1949, and since that time the Committee has become concerned with an increasing interest and responsibility not only for clinical research conducted within the Clinical Research Unit, but also with Research Fellows who work in various departments of the Hospital, supported from specific research funds bequeathed in trust to Alfred Hospital.

The annual reports of the Baker Institute have been published since 1927, and soon after the formation of the Clinical Research Unit it was felt desirable to publish a combined volume entitled "Research". This made its first appearance in 1953, and contained the twenty-seventh annual report of the work of the Baker Institute and the fifth annual report of the work of the Clinical Research Unit and the Alfred Hospital Research Fellows.

In 1956 the Board of Management formed a Diabetic and Metabolic Unit, which is engaged in investigation of endocrine and allied disorders. This has also been placed under the supervision of the Research Advisory Committee.

Because of the increasing importance and diversity of the investigational activities conducted in Alfred Hospital, it has been decided to present this report in several sections, indicating the activities of the Baker Institute (including the Clinical Research Unit), the Diabetic and Metabolic Unit, and the work of the Research Fellows.

This follows the policy expressed by the Board of Management in the Annual Report of Alfred Hospital in 1950:

"It is now generally accepted that research into human disease must be conducted predominantly in close relationship with patients undergoing investigation and treatment. Such research is conducted on two levels. The first is concerned with the basic medical sciences (e.g., at Baker Medical Research Institute), and the second is associated with a study of disease as encountered in the sick person, i.e., clinical research. The organisation of Australian hospitals, which is peculiar to this country, necessitates that the development of the

research function of the Hospital be mainly conducted in separate specially equipped units. In addition, many members of the Honorary Medical Staff devote their valuable time to research in their various specialities and the organised research facilities of our Hospital, namely, Baker Institute and Clinical Research Unit, are at all times available to them in this work. Such an arrangement is in conformity with our objects — treatment of the sick, training of doctors and nurses, and provision of facilities for research."

The Trustees of the Institute and the Research Advisory Committee are fully aware of the necessity of relating fundamental research to clinical problems, and have pleasure in presenting detailed reports of the research activities within the Hospital during the past year illustrating this concept.

BAKER MEDICAL RESEARCH INSTITUTE

STAFF

Director:	T. E. LOWE, D.Sc., M.D., F.R.C.P., F.R.A.C.P.
Associate Directors:	P. FANTL, D.Sc., F.R.A.C.I. A. J. BARNETT, M.D., F.R.A.C.P., M.R.C.P.
Assistant Director:	WINIFRED G. NAYLER, D.Sc.
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Technical:	S. HART (Laboratory Supervisor). J. L. BREMNER. Miss J. IRVINE (from 22/2/65). Mrs. J. OVERBERG. Mrs. R. SABO.
Clerical:	Mrs. I. ROBINSON. Mrs. B. R. ASHTON. Miss R. CROZIER Miss M. GAYTON. Mrs. H. P. MORRIS (Part-time).
Laboratory Assistants:	Mrs. J. BERAN (to 9/7/65). D. BREEN. R. CAMP. Miss J. FINDLAY. K. HARVEY. Miss F. HIRSCH. Miss D. HUGHES Mrs. Y. KNEALE (to 1/6/65). Miss R. KNOWLES Miss V. MACK. Miss E. MINSTER. Miss J. MOYLAN (from 1/11/65). Miss M. SHIERS. Miss R. TURNBULL.

WARD STAFF

Registrar:	ISLA WILLIAMS, M.B., B.S.
Resident Medical Officers:	STEPHANIE TASKER, M.B., B.S. A. MEATHREL, M.B., B.S. I. W. BLACK, M.B., B.S. R. R. CLARK, M.B., B.S.
Sister:	J. W. SIMON.
Staff Nurses:	E. M. BELL (to 31/10/65). S. CARTER (from 8/2/65). H. MONTGARRETT (from 11/10/65). S. H. ROBERTSON (to 7/2/65).

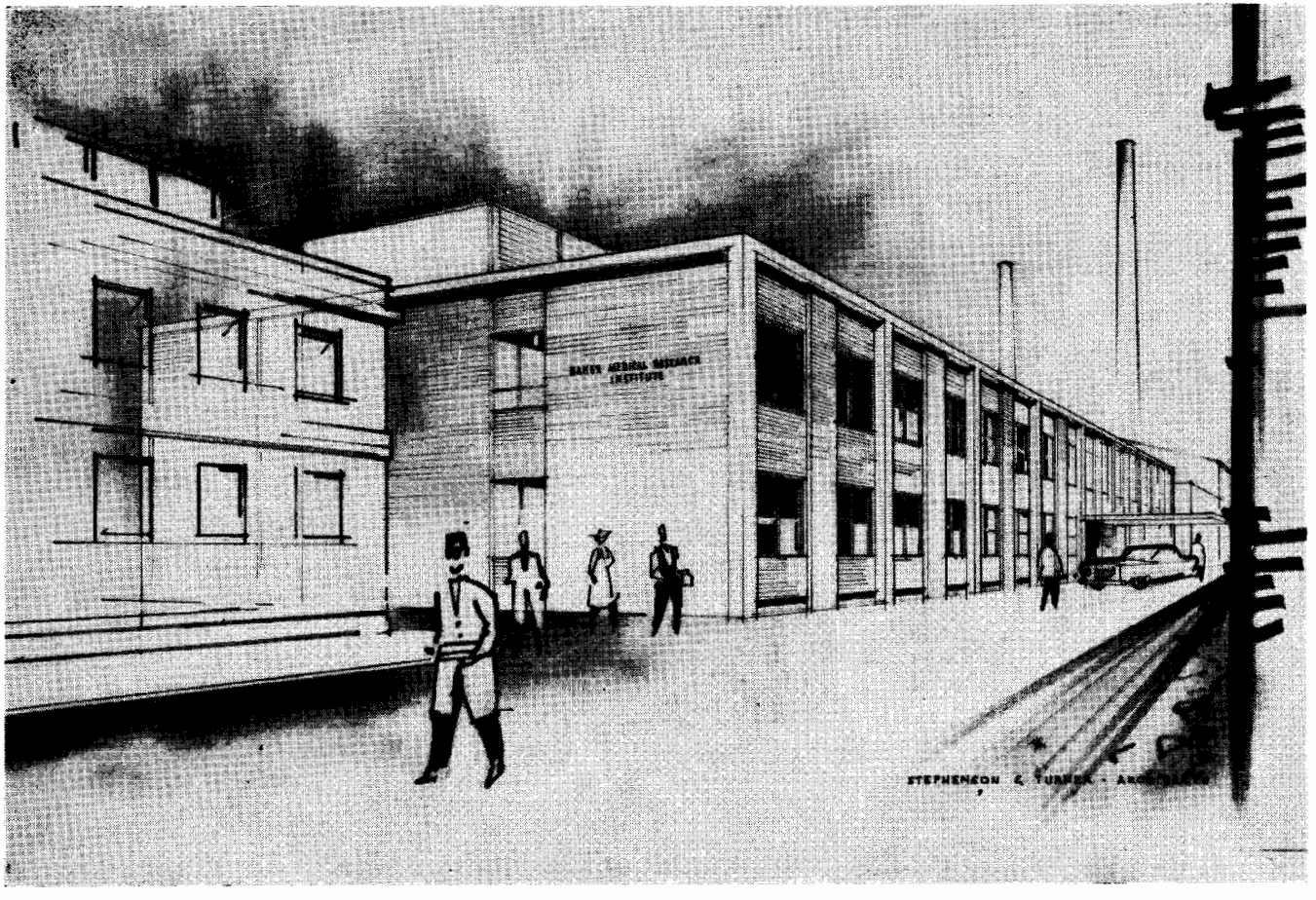
ALFRED HOSPITAL RESEARCH FELLOW

"Dr. Henry Lauric":	A. D. McCUTCHEON, M.D., M.R.A.C.P.

ANTI-CANCER COUNCIL RESEARCH FELLOW

"A. A. *Thomas*":	C. KIDSON, Ph.D. (Lond.), M.B., B.S., B.Sc. (Med.).

PROPOSED NEW BUILDING FOR INSTITUTE



BASEMENT AND TWO FLOORS
Floor Area 30,000 sq. ft. Net.

Estimated Project Cost $1,100,000.

ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

In this section of the report a general review of happenings during 1965 and short summaries of some research projects are presented. Detailed accounts of all investigations are given in the Scientific Section. It is hoped that the summaries will indicate the breadth and fields of research and the techniques we have used.

DEVELOPMENT OF RESEARCH

In last year's report I described in some detail how the development of modern instruments in all branches of science had affected the conduct of medical research and had led to an increased demand for laboratory space and a change in the skills of various workers needed for its conduct. From this survey it was concluded that rebuilding and enlargement of the Institute had become essential and that a formal affiliation between the Institute and Monash University was desirable.

Rebuilding

During 1965 the Trustees decided to rebuild on the present site and the extension made available by the Board of Management of the Hospital. A first sketch of the proposed building is shown on page 12. Construction will be in two stages so that the work of the Institute will not be interrupted. In Stage I, the ward and the other clinical facilities will be moved to very adequate temporary quarters in other hospital buildings and half of the new building erected on the area so vacated. Detailed plans and specifications for this stage are now complete and it is hoped to commence construction in the second quarter of 1966. After completion of Stage I the rest of the existing building will be demolished and the remainder of the new building erected provided finance is available.

One of the features of our research organisation has been the complete physical and functional integration of clinical and laboratory facilities but for some time the physical, but not the functional, unity will be lost. The duration of this separation will be determined by the time before the main ward block of the Hospital is built and occupied for it will then be possible to connect the new clinical facilities directly with the roof of the laboratory building by a bridge and covered way. The difficulties produced by these moves will be ameliorated by the knowledge that they are temporary and are a prelude to much more satisfactory accommodation being available.

Affiliation with Monash University

On 23rd December, at a small ceremony in the Institute, an affiliation agreement with Monash University was signed. The affiliation is "for the purpose of promoting the teaching of undergraduate medical students and post-graduate students of the University and providing facilities therefor and promoting and encouraging medical research amongst such students and providing facilities therefor". It is anticipated that this will facilitate closer collaboration between workers in all branches of science in the Universiy and the Institute. Together with the affiliations which already exist between Alfred Hospital and

the Institute and between the Hospital and University this latest association will complete a powerful instrument for the conduct of modern medical research.

Postgraduate training in medicine and science has always been conducted by members of the Institute but is a lesser known activity. It is therefore of interest to note that, to review only the past fifteen years, eleven postgraduate degrees have been awarded to workers in the Institute for theses on work carried out therein. (D.Sc., 2; M.D., 1; Ph.D., 1; M.Sc., 7.) This training has been supervised by a senior staff which has not exceeded four at any one time. The stimulus to our established research workers of having enthusiastic and able students working in the Institute has been great and it is hoped that the increased space in the new building and the University affiliation will enable this activity to increase in future years. Some clinical instruction of undergraduate medical students has for a long time been undertaken and during the long vacation 1965/66 a medical student sponsored by the National Heart Foundation will come from University of Sydney for some training in research methods.

RESEARCH PROJECTS

Energy Production in the Myocardium

In the simplest terms the heart can be considered as a complex pump which moves blood around the body through a system of blood vessels. The energy required for this purpose is derived from chemicals dissolved in the blood and the energy output useful for the circulation is represented by the pattern of pressure and flow maintained in the circulation. The bulk of the heart is muscle which is made up of millions of tiny cells that perform the energy conversion and by contracting rhythmically in unison move the blood. Each cell, although minute, has a complex internal structure and the protein fibres which alternately shorten and lengthen to produce the pumping action of the heart are deep in the interior of the cell.

The cycle of contractile activity of these cells commences with a change in the electrical state of the cell surface (excitation) and this is followed after an interval by shortening of the protein fibres (contraction). After contraction the processes reverse to complete the cycle.

The way by which the muscle cells of the heart use and control the energy of chemical substances to produce this rhythmic activity have been investigated by physiologists for many decades and much progress in understanding has been made in recent years by the use of modern techniques employing electron microscopy, radio-isotope tracers and electronic equipment. Since 1953 various workers in the Institute have been studying some aspects of this problem and the continuing project has been called "Energy Production in the Myocardium". This commenced with the development of a technique using the toad heart to study the overall metabolic and mechanical energy exchange of the heart. The next step was an investigation of the effects of drugs upon the efficiency of this energy conversion and was in turn followed by studies of the electrical state of the cell membrane and electron microscope observations of the cell structure. Then there evolved studies aimed at elucidating how excitation and contraction are coupled together and others directed at determining the role of the essential calcium ions in the cell.

The investigations also led to the discovery of kinekard, which is a substance normally present in the blood and which has an important action upon the heart muscle cells.

Whilst this project has at the moment no direct impact on clinical medicine its ultimate importance to this branch of medical science is clear when it is remembered that the electrocardiogram derives from the electrical activity of the muscle cell and the circulation of the blood is dependent on the contraction of the protein fibres and that many drugs are known which affect these events. A clear understanding of how the muscle cell works should indicate ways of correcting the mechanism when it goes wrong.

During the present year work has been directed to the role played by calcium in the maintenance and regulation of normal heart muscle function, to the effect of drugs and lowered temperature on the calcium content of various structures within the muscle cells, to the role of lipids in calcium transport across cell membranes and to the effect of lowered temperature on drug action on heart muscle.

Kinekard

The functions of various organs of the body have for a long time been investigated by isolating an organ and perfusing it with a nutrient solution. Because of the difficulties attending the use of blood as the perfusing fluid solutions based on saline have commonly been employed even though it has been known that these are not as effective as blood. Many of our investigations into the behaviour of heart muscle have been based on isolated toad heart preparations perfused with saline solutions and we found some years ago that when a proportion of blood plasma was added to the saline perfusate the ability of the heart to pump was greatly improved.

Since then we have been endeavouring, by the use of methods of physical chemistry on a "micro" scale, to isolate the components of the complex plasma responsible for this action and have separated a fraction in which much of this activity lies. So far study of this fraction indicates that its active substance may be a peptide and that it differs from other known substances which act upon heart muscle. As this action on the heart was the first facet of the substance studied we have named it KINEKARD.

Further studies have shown that kinekard also has a powerful action on the musculature of arteries and smooth muscle generally. It has also been isolated from the blood of all species so far examined and no species specificity of action has been noted. Observations on patients show that its concentration in the blood of man can be raised or lowered in illness.

Much work remains to be done to determine the chemical structure and physiological properties of kinekard and also in which tissue it is made, how it acts and its relationship to various diseases. The activity of minute amounts of kinekard is so great, it is found in such a diversity of creatures and it is so stable that it is believed that it should have a special role in the body economy. This role has yet to be found.

Blood Coagulation

Two years ago in this report a summary of studies made over some twenty years on the processes of blood coagulation was presented and stress was laid on the contribution of the laboratory investigations to the treatment

of patients with bleeding and clotting disorders. This work continues and during 1965 has been largely devoted to a comparative study of various tests and ways of expressing results used in different laboratories. In this project we are co-operating with a number of centres throughout the world, at the request of the International Committee for Haemostasis and Thrombosis. This committee hopes to evolve a standard set of tests and methods of expressing results so that the international traveller being treated for these conditions may be adequately cared for by physicians in the various countries through which he passes. At present this lack of uniformity sometimes produces hazards for these people.

Cybernetics

The term cybernetics was used in 1948 by Nobert Weiner to describe the study of control and communication whether in machines or animals. On the machine side this study has developed greatly and forms the basis of the design of automatic machinery. Many of the ideas so evolved have a fairly direct application to biology and clinical medicine whenever the control of bodily processes is considered. In 1948 a group of our workers had commenced a study of the control of body fluid volume in man and animals and the application of cybernetic theory proved extremely valuable in understanding this particular mechanism. In this project data concerning the intake, output and storage of fluid were collected; then hydraulic models of the envisaged system were made and studied in the laboratory. From the models predictions of behaviour in various circumstances were made and checked against similar naturally occurring events in man and animals. This additional data was then used to modify the models and so advance our understanding of the problem.

Since that time several projects have been carried out in which this pattern of experiment and the ideas of cybernetics have proved of great help. An electrical analogue was built in 1963 which gave some further insight into the mechanism of the heart beat. Recorded later in this report are studies on the control of the distribution of blood to various parts of the body and others dealing with the reproduction of molecules within cells. In both of these an endeavour is being made to produce mathematical models of the processes involved and by the use of computers first to build adequate hypotheses and later to apply the predictions of these models to the real situation.

Gammaglobulin

Gammaglobulins are proteins which are found in the blood in small quantities and are involved in the processes by which the body develops immunity to various infections. In some cases gammaglobulin is injected into patients in an attempt to confer immunity to some particular infection. The production of gammaglobulin molecules, like other proteins, within lymph tissue of the body is under gene control.

A group of workers within the Institute has been investigating for several years now conditions and mechanisms which lead to excessive amounts of gammaglobulin in the blood. Since this condition occurs more commonly in tropical populations than in Australia close co-operation has been maintained with workers, and some field work done, in New Guinea and other islands for population studies and, because of the hereditary element in the problem,

considerable investigation of other genetically controlled blood components is also being carried out in these populations. Parts of these studies are also linked with an associated study which is being carried out by a group from Columbia University, N.Y. These studies rely on immunological and physico-chemical techniques and require considerable use of automatic data processing.

Other projects of 1965 include studies concerned with the production of protein molecules and nucleic acids by cells and with some enzyme systems in various cells. In addition studies based on clinical observation concern the bases of various conditions and their treatment. These embrace high and low blood pressure, obstructive disease of arteries, the problems of amputees, the movement of various parts of the intestines and the biochemical behaviour of the lining cells and the disease of scleroderma.

These short sketches highlight some of the projects being undertaken by members of the Institute, they serve to indicate the range of techniques and skills needed in modern research and although the projects are to some extent in apparently unrelated fields they all interact on each other and are germane to our objective of applying the knowledge of basic science to the problems of clinical medicine.

TRUSTEES OF THE INSTITUTE

In April, Mr. Balcombe Quick and Dr. F. Grantley Morgan regretfully resigned for reasons of health as Trustees of the Institute and Messrs. M. A. Cuming and J. C. Habersberger were appointed in their places.

Mr. Quick, who was deputy-chairman, became a Trustee in 1940 and Dr. Morgan in 1945.

The staff of the Institute wish to thank our two friends of long standing for the help and understanding which they have so readily given to us and to welcome the new members and wish them many years of fruitful association with the Institute.

STAFF

During the year Dr. P. Fantl announced his intention to relinquish the administrative duties of Associate Director on 31st May, 1966 and Dr. Winifred Nayler has been appointed to that position from 1st June, 1966. Dr. Fantl plans to take long service leave and then to return as one of our research team.

In December the degree of Doctor of Science was conferred upon Dr. C. C. Curtain by Melbourne University for the work embodied in his thesis "Studies on the Nature and Origin of Abnormal Serum Globulins".

Dr. D. Race was awarded a Fellowship by the State University of New York to work in their Department of Surgery at Buffalo. He left in June and will return in mid. 1966 to continue his work on factors controlling the distribution of blood in the body.

Dr. M. Rosenbaum has been awarded an Overseas Research Fellowship by the National Heart Foundation of Australia. This provides for two years of study at the Bockus Research Institute, Philadelphia, U.S.A., and for a third year of support on return to the Institute. He will be studying the factors controlling the circulation of the blood.

OVERSEAS VISITS

Dr. P. Fantl made an overseas visit in September to attend a meeting of the International Committee on Haemostasis and Thrombosis in Switzerland and to see colleagues in Europe and England. Whilst away he also made enquiries about the design features of new laboratories of interest to us.

Dr. Winifred Nayler attended the 23rd International Congress of Physiological Sciences in Tokyo to present a paper and discuss our discovery of kinekard with a number of colleagues from other countries.

Drs. C. C. Curtain and C. Kidson visited New Guinea and New Britain in connection with field studies in the project on hypergammaglobulinaemia.

Dr. M. Rosenbaum visited Singapore to take part in Physiology instruction in a course arranged by the Royal Australasian College of Surgeons during August-September.

RESEARCH GRANTS

Many of the investigations recorded in this Report have been supported by funds provided by the Life Insurance Medical Research Fund of Australia and New Zealand, the Anti-Cancer Council of Victoria, the National Heart Foundation of Australia, the Asthma Foundation of Victoria, the Appel Family Bequest, Alfred Hospital Research Funds and this assistance is gratefully acknowledged.

It is a pleasure to record thanks for generous donations from those whose names are listed in the various financial reports.

Many organisations have made gifts to the Institute library and our thanks are expressed to them, to various libraries that have loaned us journals and particularly to the librarians whose assistance is greatly valued.

Considerable assistance has been given to us through the year by Heads and Staffs of various departments of the University of Melbourne, Monash University and the Australian National University, also by members of the Commonwealth Serum Laboratories, Commonwealth X-Ray and Radium Laboratories and C.S.I.R.O. We thank them all for this continuing interest in our projects and their ready help. Such help as we have been able to give in return has been freely availed of.

It is a pleasure for me to thank the Trustees of the Institute and the Board of Management of the Hospital for their continued generous support and to thank members of the Staff and research fellows for their co-operation during the past year.

T. E. LOWE.

31st December, 1965.

LIST OF ORGANISATIONS WHICH HAVE MADE GIFTS TO THE LIBRARY DURING THE YEAR

Australian Medical Association.
Adelaide Children's Hospital.
Anti-Cancer Council of Victoria.
A.N.Z.A.A.S.
Austin Hospital.
College of Physicians and Surgeons, New York.
Commonwealth Department of Health.
Commonwealth X-ray and Radium Laboratory.
Department of Health, New Zealand.
Department of Territories, Canberra.
Halstrom Institute of Cardiology, Sydney.
Instituto de Biologia y Medicina Experimental, Buenos Aires.
Institut Pasteur, Algiers.
Institute of Dental Science.
Institute of Medicine and Veterinary Science, Adelaide.
Kanematsu Memorial Institute, Sydney.
Medical Research Council, London.
Middlesex Hospital Medical School.
National Heart Foundation, Australia.
National Institute of Nutrition, Japan.
New York State Department of Health.
New York University College of Medicine.
New Zealand Medical Research Council.
Ophthalmic Research Institute of Australia.
Queensland Institute of Medical Research.
Rockefeller Institute, New York.
Royal Children's Hospital, Melbourne.
Royal Melbourne Hospital.
Royal Prince Alfred Hospital, Sydney.
Royal Women's Hospital, Melbourne.
St. Vincent's Hospital, Melbourne.
St. Vincent's School of Medical Research, Melbourne.
South African Institute of Medical Research.
Strangeways Research Laboratories, Cambridge.
Staten Seruminstitut, Copenhagen.
University of Melbourne.
University of Otago, New Zealand.
University of Queensland.
University of Sydney.
Universitatis Mariae Curie Sklodowska, Poland.
Walter and Eliza Hall Institute, Melbourne.
Wellington Medical Research Foundation.
World Health Organisation.

ALFRED HOSPITAL RESEARCH FELLOWS IN THE INSTITUTE

1949-1965

Anderson, R. McD., 1953-55
Andrew, R. R., 1949-55
Barnett, A. J., 1949-50
Baumgarten, A., 1962-64
Beavis, E. L. G., 1955-56
Boake, W. C., 1958
Breidahl, H. D., 1952-53
Burnside, K. B., 1951
Cooper, E., 1962
Duffy, D. G., 1952-55
Ferguson, I. A. L., 1957-58
Fowler, R., 1953-54
Francis, J. K., 1956-57
Fraser, J. R. E., 1957
Gardiner, J. M., 1952
Goble, A. J., 1951
Hudson, B., 1952
Jamieson, K., 1954
Kay, H. B., 1959-60
Kincaid-Smith, P., 1959-60
McCutcheon, A. D., 1959, 1965
McDonald, W., 1960-61
McNeur, J. C., 1955
McRae, C. J., 1955
Murfitt, L., 1955
Newman, H. C., 1954
Parsons, P. J., 1951
Quinn-Young, M., 1956
Race, D., 1959-63
Sawers, R. J., 1953-60
St. Clair, W. A., 1955
Silberberg, F. G., 1953
Stern, W., 1954-55
Stirling, G. R., 1955
Wagner, G., 1958

OVERSEAS FELLOWS

1954-1965

Dawson, J. B., 1961-63 (Oxford)
Emslie-Smith, D., 1955-56 (Dundee)
Hamilton, M., 1954 (London)
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REPORT OF SCIENTIFIC INVESTIGATIONS

ENERGY PRODUCTION IN THE MYOCARDIUM † ‡

W. G. Nayler, N. Dorevitch, J. R. Hasker, J. M. Price and T. E. Lowe

IONIC STUDIES‡

Despite the intensive effort applied during recent years to investigations relating to the mechanisms whereby calcium ions maintain and regulate cardiac contractions, the precise factors involved in the regulation of such mechanisms remain obscure. Previous studies in these laboratories have indicated that the role played by calcium ions in the maintenance of cardiac contractility is highly specific, other biologically occurring divalent cations failing to substitute for calcium in this respect. Sufficient data now seem to be available to substantiate an hypothesis that calcium ions entering muscle cells during or in association with membrane depolarization initiate or are in some way associated with the release of ionized calcium from cellular stores. Experiments performed during the past year have been planned to provide some information about such intracellular stores, to identify their intracellular location and to determine under what conditions these stores accumulate or release calcium.

Preparation of Subcellular Fractions

Subcellular fractions have been isolated from amphibian and mammalian cardiac muscle under carefully controlled conditions. Before homogenization the muscle was perfused with a particular physiological saline solution, with and without various inotropically active drugs as required.

Homogenization has been effected with a Potter Elvejehm homogenizer operating at 1425 r.p.m., care being taken to keep the homogenate cold (2°C). Subcellular fractionation was effected by means of differential centrifugation followed by sucrose-gradient purification if required. Mitochondrial and microsomal subfractions were identified (a) by their characteristic appearance in electron micrographs, and (b) in terms of their enzyme activities. Thus the microsomal fraction derived from the fragmented sarcoplasmic reticulum displayed the characteristic $Na^+ - K^+$ dependent, ouabain-inhibited ATPase activity and electron micrographs confirmed that it consisted of an accumulation of small membrane-lined vesicles.

Drugs and Calcium Content

Previous studies have indicated that the xanthines, including caffeine and theobromine, and Na_4EDTA displace ionized calcium, detected as ^{45}Ca, from cellular stores in cardiac muscle.

The immersion of ventricular muscle in physiological saline solutions containing 5mM caffeine or theobromine apparently results in the mobilization of some of the mitochondrial calcium so that the total calcium concentration

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within the mitochondria is significantly reduced. It is concluded that such released or mobilized calcium ions appear in the myoplasm and are thence dispersed into the extracellular fluid.

4mM Na_4EDTA, when added to the physiological saline solutions used to perfuse such cardiac muscle, results in the displacement of ionized calcium from the mitochondria. These and other comparable results provide the basis for an hypothesis that cardiac mitochondria provide one intracellular site from which calcium ions can be mobilized. Their immediate proximity to the myofibrils would be advantageous, the distance through which such released ions must diffuse before reaching the myofibrils being very small indeed.

The xanthine drugs and Na_4EDTA similarly displace ionized calcium from the microsomal fraction.

Extracellular Ionic Environment and Calcium Content

The calcium contained in *in situ* cardiac mitochondria and sarcoplasmic reticulum has been found to be dependent upon the extracellular ionic environment. Thus perfusing hearts with calcium-enriched physiological saline solutions results in an accumulation of calcium in both the mitochondrial and microsomal fractions. The calcium present in the microsomal fraction, but not in the mitochondrial components of this system, similarly is increased if extracellular sodium ions are replaced with sucrose. Lowering the extracellular concentration of calcium results in a loss of ionized calcium from mitochondrial and microsomal fractions alike.

Reduced Temperatures and Calcium Content

It has been known for many years that amphibian cardiac muscle displays a positive inotropic response in association with reduced temperatures. ^{45}Ca studies have shown that this positive response is associated with a reduced efflux of ionized calcium from the muscle, so that the muscle actually accumulates calcium in response to the reduced temperature. Experiments are in progress to determine whether this accumulation of calcium by cold-perfused hearts reflects a change in the ability of the lipids present in cardiac cell membranes to facilitate the transport of ionized calcium from a lipid solvent to an aqueous phase.

PHARMACOLOGICAL STUDIES†

Reduced Temperature and the Response of Isolated Hearts to Drugs

Additional evidence has been accumulated which substantiates an hypothesis that the response of the myocardium to various drugs, including the catecholamines and the xanthines, varies according to the perfusion temperature. Isolated rat hearts perfused at 25°C were found to be markedly more sensitive to aminophylline than hearts perfused at 35°C; reducing the perfusion temperature even further resulted in a further increase in sensitivity of the heart to aminophylline. The reverse holds true for epinephrine and norepinephrine.

Additional studies have shown that amphibian heart muscle displays a marked seasonal variation in its total phosphorylase activity, the activity being lowest during the winter months.

Lipid Facilitation of Calcium Transport

Preliminary studies have indicated that lipids extracted from mitochondria or sarcoplasmic reticulum isolated from amphibian and mammalian cardiac muscle facilitate the transport of ionized calcium, detected as ^{45}Ca, across an aqueous lipid-solvent interface. Since the efflux and influx of calcium ions across the cell membrane reflects the passage of ions from an aqueous to a lipid-solvent phase, this lipid-facilitated calcium transporting system may provide a useful mechanism whereby the effect of drugs on ion transport can be investigated.

Quinidine sulphate, pronethalol and propanolol, all of which are antifibrillatory drugs, inhibit such lipid-facilitated calcium transport. Epinephrine and norepinephrine, in contrast, potentiate such lipid-facilitated calcium transport. These results may be interpreted to mean that lipids present in cell membranes and other subcellular structures provide the receptor sites for the action of certain drugs.

CARDIOACTIVE PLASMA SUBSTANCES†

T. E. Lowe, W. G. Nayler, V. Carson, D. A. Coventry, N. Dorevitch, J. R. Hasker and J. M. Price

When blood plasma is subjected to gel filtration at least two fractions with positive inotropic activity on cardiac muscle can be isolated. One of these with a molecular weight suggesting that it is a polypeptide has been studied in considerable detail. The other with a molecular weight suggesting a globulin is believed to be the cardioactive globulin described by Hajdu and Leonard in 1958. Some preliminary investigations of the latter have been commenced.

KINEKARD

Investigations have revealed the presence in human blood plasma of a substance of molecular weight within the range 4,000-10,000, which produces a positive inotropic response from isolated cardiac muscle. This substance, now named kinekard, has been isolated from a wide variety of animal species including lampreys, fish, tortoise, rats, dogs, rabbits, domestic fowls and guinea pigs. Isolation of kinekard from these various blood plasmas was effected by gel filtration followed by ion-exchange chromatography on diethylaminoethyl-methacrylate gel.

Effect on the Peripheral Circulation

Dogs on heart-lung bypass and isolated hind-limb preparations have been used to study the effect of kinekard on the peripheral circulation. The intra-arterial or intravenous injection of approximately 1 μg kinekard into approximately 15 Kg. dogs on heart-lung bypass resulted in a raised systemic perfusion pressure associated with increased blood flow through the coronary and splanchnic beds; flow through the hind-limb decreased. Adrenalectomy and nephrectomy failed to abolish these peripheral effects of kinekard. When injected into the isolated hind-limb preparation kinekard caused a raised perfusion pressure, indicating vasoconstriction.

Further experiments have shown that the action of kinekard on the peripheral circulation is blocked by the α-adrenergic antagonist, dibenzylene but not by the β-adrenergic antagonists, propanolol or pronethalol. These findings

have been interpreted to mean that kinekard acts on the peripheral circulation through the α receptors, in which case it resembles noradrenaline and adrenaline. That the effect of kinekard on the peripheral circulation is not mediated by the release of locally stored catecholamines (from nerve endings and peripheral tissues) was indicated by other experiments which showed that the prior administration of either reserpine, guanethidine or bretylium tosylate failed to abolish the pressor response.

The direct constrictor action of kinekard on vascular tissue was confirmed using isolated strips of rabbit aorta suspended isometrically. Here the action of kinekard is blocked by the α-adrenergic antagonists phenoxybenzamine and dihydroergotamine.

Kinekard also is active on non-vascular muscle, producing relaxation and/or cessation of spontaneous contractions of isolated segments of rabbit ileum and inhibiting spontaneous contractions and acetylcholine-induced contractions in isolated uterine segments.

Using a biological assay based on inotropic activity and relating this to the response to norepinephrine the level of circulatory kinekard in the venous blood of man has been established in terms of an arbitrary unit equivalent to 1 μg norepinephrine/litre. In a series of patients with a variety of disease states there were found a group with levels below and a group with levels above the normal range, but no correlation between plasma level and disease state, age, sex or arterial blood pressure was apparent.

CARDIOACTIVE GLOBULIN

In this investigation it has been found technically desirable to use serum instead of plasma. Rat serum has been filtered through a column of Sephadex G.200 by an "upward flow" technique which doubles the rate of elution. In this manner, in accord with the findings of Hajdu and Leonard, two globulin fractions of interest can be obtained. They are a blueish opalescent fraction (cardioglobulin C) and a reddish yellow fraction (cardioglobulin A).

These fractions have been tested for inotropic activity on an isolated toad heart preparation. Individually each fraction is without activity but if the heart is first perfused with plasma and then washed in Ringer-saline it becomes sensitized. Then addition of cardioglobulin C followed by cardioglobulin A to the perfusate produces a definite inotropic action as described by Hajdu and Leonard.

The study so far indicates that the activity of these fractions is quite different to that of kinekard.

HAEMODYNAMIC STUDIES‡

T. E. Lowe, A. J. Barnett, D. Race, M. Rosenbaum and I. Williams

Studies of the pressure-flow patterns in the blood circulation have contined and more data have been obtained which will help to elucidate the mechanism which controls the distribution of blood in the body. In addition the value of two animal preparations for the pharmacological testing of vasoactive drugs has been proven.

CLINICAL STUDIES

In these studies normal subjects and others with disorders of the circulation are being investigated.

Pulse rate and arterial pressure through an intra-arterial needle, central venous pressure through a catheter in the superior vena cava or axillary vein and peripheral venous pressure through a needle in an antecubital vein are recorded with forearm and finger blood flows measured from mercury-in-rubber strain gauges placed around a forearm and index finger. During these measurements the subject lies on a table which may be tilted from the horizontal by 60° and to which can be attached a pedalling device on which the recumbent subject performs measured amounts of work. From these measurements an estimate of central and peripheral pressure-flow patterns can be obtained both at rest and under test conditions.

The standard test procedure which has been evolved from such observations requires the subject first to perform the "Valsalva manoeuvre" (breathing out against a 40 mm column of mercury for 15 seconds) whilst recumbent then to be tilted to 60° and finally to pedal against a measured resistance for 5 minutes.

The Valsalva manoeuvre in normal subjects produces a rise of central venous pressure associated with a fall in arterial blood pressure which is inversely related to the rise of venous pressure. Following release of respiratory pressure the arterial pressure returns to control levels and "overshoots" by an amount related to the previous fall. On tilting to 60° for 2 minutes a normal subject shows little change in arterial blood pressure, a rise in pulse rate, a rise in calculated peripheral resistance and variable (up to 60%) fall in the stroke volume of the heart.

During 5 minutes pedalling exercise a normal person shows a rise in arterial blood pressure, pulse rate, stroke volume and a fall in calculated peripheral resistance.

Eight patients with hypertensive disease satisfactorily controlled with a combination of guanethidine, methyldopa and thiazide were investigated by this standard test. Five of them had qualitatively normal responses but three showed a marked fall in arterial blood pressure on tilting which appeared to be due to a fall in the calculated peripheral resistance for the other responses were normal in contrast to the usual rise. In these three patients the blood pressure fell on exercise for the same reason and in the Valsalva manoeuvre they showed no overshoot of arterial pressure.

ST155

ST155[1] is a new drug 2-(2, 6-dichlorophenyl-1-amino) imidazoline HCl which has been suggested for treatment of hypertension. Its effect on the circulation of six normal volunteers was tested with the standard test, recordings being made before the intravenous injection of 150 μg. ST155, 30 minutes and 60 minutes later. Observations at 60 minutes showed the greatest effects, the changes being summarized thus: ST155 produces a slight to moderate fall in arterial pressure when the subject is recumbent, after tilting 3 subjects showed an increase in blood pressure fall and 3 a rise in pulse rate and during

1 ST155 kindly supplied by Boehringer Ingelheim, Pty. Ltd.

exercise the blood pressure rise was reduced without any change in pulse rate. No changes in forearm blood flow or in response to Valsalva manoeuvre were seen.

Unless a subject had dilated finger blood vessels before the administration of the drug the finger blood flow increased.

ANIMAL STUDIES‡

Drugs and Blood Distribution

Using a preparation with total heart-lung bypass to perfuse a dog with constant inflow of blood the effects of a number of drugs on the blood flow through various vascular beds has continued to be studied.

(a) **Pressor Amines, Angiotensin, Kinekard.** These drugs when added to the perfusate in appropriate doses all produced a marked rise in the perfusion pressure and a redistribution of blood through various vascular beds. There is an increase in coronary flow, a decrease in skeletal muscle and renal flows with little or no change in splanchnic flow. The α-adrenergic blocking agent dibenzylene blocks the effect of adrenaline, noradrenaline and kinekard and partially blocks that of angiotensin. Neither the β-adrenergic blocking agent pronethalol nor ganglion blocking drugs affected the responses.

(b) **Eledoisin, ST155.** Eledoisin is a polypeptide which is a strong vasodilator and ST155 is an antihypertensive agent; each of these on addition to the perfusate produced a fall in perfusion pressure and an increase in flow through skeletal muscles. In the coronary, renal and splanchnic beds the flow was reduced even though the calculated resistance of these beds was lowered. The action of ST155 was blocked by pentolinium.

(c) **Adrenergic Blocking Agents.** When dibenzylene is added to the perfusate the calculated total peripheral resistance falls but the dominant field affected is the skeletal muscle vascular bed. Flow in the coronary field is reduced. In contrast the β-adrenergic blocking agents pronethalol and propanolol produce little change in pressure or regional flows.

Vascular Bed of Skeletal Muscle

As the experiments just described indicate that the blood vessels of skeletal muscle are very responsive to drug action, a preparation has been devised to study these vessels in more detail. In the dog it is possible to isolate a gracilis muscle from all connection with the rest of the arterial system of the body and by cannulating its artery of supply this muscle can be separately perfused. Using a constant infusion rate of between 2 and 4 ml/min. changes in the resistance of this vascular bed to blood flow are reflected by changes in perfusion pressure. The preparation is sensitive enough to drugs added to the perfusate to respond to 1×10^{-9} gram noradrenaline. The pressor amines, kinekard and eledoisin produced the same effects as previously seen in the whole animal but ST155 did not produce the increased flow previously seen in the whole animal.

Oscillations in Arterial Blood Pressure

Studies commenced last year have been continued and quantitative data concerning the damped oscillation in blood pressure produced by inflation and sudden deflation of the lungs have been obtained. These have enabled

estimates of the natural frequency and damping of the vascular system to be made and the effects of blood loss on these parameters to be recorded.

The role of peripheral resistance vessels in the control of the natural frequency of oscillation of the vascular system is being assessed with a preparation in which the vasculature of the one hind-limb of a dog is completely isolated. In this limb vasoconstriction can be produced by sympathetic stimulation.

INSTRUMENTATION

Heart-size Measurement in Closed-Chest Dogs

Following the technique of Rushmer we have placed mercury in rubber strain gauges around the heart or left ventricle in dogs. After closure of the thoracotomy these gauges give good indication of heart size changes in the intact animal. Epinephrine, norepinephrine, angiotensin and kinekard produce an increase in cardiac size in addition to a rise in arterial blood pressure. Isoproterenol causes a decrease in size.

Field Pattern of Inductive Pacemaker

The Lucas inductive pacemaker consists of an external primary coil which transmits energy to a secondary implanted coil connected to the myocardium. The coupling between the two coils is quite critical for satisfactory operation and leads to marked changes in output voltage. High output voltage together with the biphasic wave form of the instrument may be significant in causing ventricular fibrillation.

MOLECULAR BIOLOGY

Our work in molecular biology is centred mainly on a study of the regulation of gene expression. This is central in the understanding of such complex biological phenomena as cell differentiation, neoplasia, immunological specificity and the general organization of biological information. The fundamental question is: how is one gene "turned on" while another gene is "turned off" and how can this be expressed in molecular terms? This problem can be further resolved into two steps: the regulation of RNA synthesis—transcription—and the regulation of protein synthesis—translation. Our approaches to the problem, through nucleic acid and protein chemistry, concern both transcription and translation. Genetic and immunological analyses are being applied where possible.

These studies have involved the development of a number of new techniques and the use of a number of different experimental systems. The latter have been chosen for their suitability for the study of each aspect of the problem and range from micro-organisms to mammals including man. Of the specific projects in this programme, several concern protein synthesis or the characterization of genetically determined protein variants; these projects are concerned particularly with the immunoglobulins and hence with the genetic basis of immunological specificity. One concerns hormone action in the control of gene expression in mammals, a question which is relevant to differentiation, neoplasia and a number of other biological problems. Another on chemical carcinogenesis is proving to be a fascinating source of data on the role of miscoding of genetic information in neoplastic transformation, whilst

that on the regulation of transfer RNA synthesis concerns the general problem of decoding in the translation process, providing at the same time some insight into the genetic relationships between virus and host cell.

Finally, our continuing interest in population genetics in Melanesia represents an attempt to interpret more completely at the molecular level many genetic and paragenetic phenomena in man. These populations continue to be a source of valuable biological material not available elsewhere.

REGULATION OF IMMUNOGLOBULIN SYNTHESIS* **

C. C. Curtain, C. Kidson, N. Lobb and T. Golab[1]

This long term study of the environmental and constitutional factors which influence immunoglobulin production both in the normal subject and in some forms of neoplasia has been continued. This year considerable time has been spent in developing satisfactory methods to enable the genetic markers of the Gm and Inv systems to be used in these investigations. In the past the reagents used in these systems were obtained from human sera but the rarity of their occurrence involved the screening of large donor panels. We have now found it possible to produce anti-Gm(a), anti-Gm(b) and anti-Gm(ax) antisera in rhesus monkeys by using as antigens the isolated heavy chains from human IGG of single Gm specificity. The macroglobulin antibodies so produced may be used in the standard erythrocyte agglutination technique for the Gm typing of sera and other fluids or they may be separated by gel filtration and density gradient ultracentrifugation and conjugated with suitable fluorochromes for fluorescent antibody studies. The development of these reagents has made several investigations possible.

The Localization of Immunoglobulin Markers in Human Lymphoid Tissue

It is well established that antibodies of single specificity isolated from heterozygous Gm(ab) or Gm(axb) individuals need only carry one of the alternate alleles. This has been held to be confirmatory evidence for the one-cell one-antibody hypothesis of Nossal and Lederberg. Recently Colberg and Dray, studying the analogous immunoglobulin allelic systems in rabbits, found that although single antibodies need only carry one of the immunoglobulin alleles, these alleles were localized in the same plasma cells in the lymph nodes of heterozygous animals. This would appear to contradict the one-cell one-antibody hypothesis. However, it is not easy to interpret studies carried out with the rabbit allelic system for the antibodies used are prepared in rabbits carrying the very antigenic markers which one is attempting to localize. Our development of fluorescent anti-Gm reagents from antibodies prepared in monkeys which are Gm(a–, x–, b–) has enabled us to study the immunocytochemical localization of these factors in man without such technical problems. We found that fluorescent antibodies to the human immunoglobulin characters Gm(a) and Gm(b) were localized in different plasma cells in the red pulp of the spleen and the medullary cords of the lymph nodes of heterozygous Gm(ab) individuals. Of the cells which were subsequently shown to contain immunoglobulin after bleaching of sections and restaining with fluorescein-anti-whole human immunoglobulin approximately 45% react with the fluorescent anti-Gm(a), 25% with the fluorescent anti-Gm(b) and 30% did not react at all. Sections already

1 Royal Children's Hospital Medical Research Foundation.

labelled with fluorescein anti-Gm(a) and rhodamine anti-Gm(b) upon staining with fluorescein-anti-Inv(a) showed approximately 60% of the cells with mixed fluorescence and 25% of the previously unlabelled cells fluorescing. In Gm(a+, x+, b+) individuals Gm(a) and Gm(x) seem to be localized in the same plasma cells thus confirming the evidence from population studies that Gm(ax) represents an allele of the Gm system. However, in the germinal centres of the lymph nodes in the white pulp of the spleen Gm(a), Gm(b) and Inv(a) appeared to be contained in the same cells. It is possible that the mixture of characters found in the germinal centres might arise from antibody reacting with antigens localized there. Alternative explanations might be that the one-cell one-antibody hypothesis does not apply in the germinal centres or that the germinal centre cells express one allele as antibody and the alternative allele in non-specific or nonsense immunoglobulin.

Distribution of Gentic Factors in Malaria Antibodies

Most of the studies on the genetic composition of human antibodies have been carried out on antibodies which were directed against single antigens and which had been produced in response to relatively weak antigenic stimuli. The roles of heterogeneity of antigenic determinants and the strength and duration of antigenic stimulus in limiting the genetic heterogeneity of antibody molecules are unknown. We have therefore studied the genetic composition of antibodies against malarial parasites produced by members of Melanesian populations who have lived all their lives in areas of very high rates of malaria transmission. Here one would expect antigenic heterogeneity and prolonged duration and high frequency of antigenic stimulation to operate. The Gm phenotype of malaria antibodies was determined by incubating acid-washed air-dried blood films containing malaria parasites with the sera to be tested. The incubated films were washed and then allowed to react with fluorescent anti-Gm(a), anti-Gm(b) and anti-Inv(a), and examined under the fluorescence microscope. If coated with Gm(a+) or Gm(b+) or Inv(a+) antibody the malaria parasites were seen to fluoresce. The malaria antibody of five of 34 Gm(a+, b+) donors possessed no Gm(a+) or Gm(b+) specificity. In three others the malaria antibody possessed Gm(a+) specificity and in one Gm(b+) specificity only. Eighteen of the 34 donors were Inv(a+) and the malaria antibody possessed no Inv(a+) specificity.

It is suggested that phenotypic restriction of the malaria antibody might occur if the individual's initial experience of malaria in infancy occurs before all the immunoglobulin allotypes have developed.

Immunoglobulin Synthesis in Human Lymphocytes

These studies on human peripheral blood lymphocytes, cultured in the presence of phytohaemagglutinin, are part of an investigation of the genetic regulation of immunoglobulin synthesis in relation to immediate hypersensitivity. The kinetics of RNA and protein synthesis before and after two days culture have been examined using ^{3}H-uridine and ^{3}H-leucine and it is clear that these cells are carrying out active synthesis at both periods. A critical examination of the claims made in the literature that these cells are actually capable of synthesizing immunoglobulins reveals that many of the reports were accompanied by meagre description of experimental techniques. Where fluorescent antibody was used to demonstrate the appearance of immunoglobulin in

the cells after culture, these reports failed to give conclusive evidence that the immunoglobulin arose as a result of synthesis in the cell, and not as a result of absorption of immunoglobulins from the serum used in the culture medium. Similarly, radioactive amino-acid incorporation studies lacked evidence excluding absorption.

We applied a new technique to these problems using the genetically-controlled human immunoglobulin markers of the Gm system. Peripheral lymphocytes from a Gm (a+, b+) donor, for example, are cultured in serum obtained from a Gm(a−, b+) donor and the appearance of the Gm(a) character in the cells or culture fluid is tested by two different methods. Cells treated with phytohaemagglutinin show fluorescence under the fluorescence microscope when treated with fluorescent anti-Gm antibody according to the Gm type of donor from which the cells were obtained. Control cells show no fluorescence. Alternatively, it is possible to test for the presence of the Gm character in the culture fluid by a sensitized-red cell agglutination-inhibition test.

MOLECULAR GENETICS**

C. Kidson and N. Lobb

Control of Mammalian Protein Synthesis

Lymphoid cells from rabbit have been used to establish model systems for studying the regulation of protein synthesis in mammalian tissues. Isolated lymph node cells from rabbits have been cultured for short time periods and the kinetics of protein synthesis characterized using radioactively labelled amino acids. Methods have been developed for the isolation and purification of DNA, ribosomal, transfer and messenger RNA from the cultured cells and for their fractionation. This system permits rapid pulse-label experiments which are not possible with tissues such as liver and heart. Experiments are now being directed to the definitive measurement of the kinetics of altered protein synthesis and qualitative assay of the synthesized proteins, especially the immunoglobulins. Altered protein synthesis is being studied under conditions of induction and repression by agents such as hormones, peptides, polyamino acids, antibiotics.

Hormone Action and Gene Expression

Recently data have been accumulated which indicate that some hormones in animals and plants may function as gene regulators. These observations suggest that the rather diverse physiological actions of a number of steroid and peptide hormones may have a common basis.

Earlier we found that certain hormones could "turn on" or "turn off" the synthesis of different groups of messenger RNA. This phenomenon has been further studied using the steroid hormone, cortisol, in the isolated rabbit lymphoid system.

Whereas whole animal or organ studies suggested that considerable time was required for cortisol-induced alterations in RNA and protein synthesis, kinetic studies with the lymphoid system have shown that the effect on RNA synthesis occurs in seconds and so is analogous in terms of time with the action of inducers and co-repressors in bacteria. The effect on protein synthesis requires a little longer—a few minutes—to become measurable. Whereas in liver cortisol causes a net increase in messenger RNA synthesis, the effect in

lymphoid cells is a net decrease, confirming the inference from the selective RNA patterns that it cannot be due to a general effect on RNA polymerase. The rapid decrease in net RNA synthesis is followed by a "burst" of increased protein synthesis and finally by a gradual decrease in net protein synthesis. The "burst" may be of special significance, e.g., it may be concerned with the making of new "repressors".

The uptake of ^{3}H-cortisol becomes maximal in about 5 minutes in the lymphoid cells and this coincides with the maximal RNA effect. A small proportion of the hormone becomes bound to cytoplasmic protein, following kinetics similar to those of absorption, but only traces are found in nuclei, mitochondria and microsomes. The possible role of the bound cortisol in the regulatory process is being studied. Parallel investigations are being carried out using corticosterone, tri-iodothyronine and thyroxine.

On the basis of the more general implications of hormones as gene regulators in mammalian cells a theory of hormonal programming of genetically mediated disease has been proposed. Essentially this theory conceives the hormone balance relevant to a particular mutant genetic locus or cistron as a factor determining the time and mode of expression of that locus. On this basis may be explained many examples of delayed time of onset and sex imbalance of non-sex-linked genetic disease; one example which has been considered in detail is the central nervous degeneration, Kuru, prevalent in the New Guinea Fore population. Of general importance in genetics is the observation that hormonal programming of gene expression could provide a rational basis for variable expressivity and penetrance.

Molecular Basis of Chemical Carcinogenesis[1]

The general pattern of action of carcinogenic and non-carcinogenic aminoazo-dyes on gene transcription is now reasonably clear. Carcinogenic dyes slowly induce selective changes in the pattern of messenger RNA synthesis which are reversible for a time but later become irreversible. Non-carcinogenic dyes produce slower rates of change and these remain reversible for longer periods. The fact that it takes days or weeks to detect such changes suggests that, in contrast to hormones, these carcinogens do not show analogy with bacterial inducers and that the changes observed are probably secondary to earlier events.

A recent report by the Millers in Wisconsin indicates that a metabolite of a carcinogenic aminoazo-dye is bound to protein via methionine; theoretically the resulting sulfonium compound could act as a methylating agent. Experiments employing (^{14}C-methyl)methionine have shown that in the presence of the carcinogenic dye, increased methylation of ribosomal RNA and transfer RNA occurs in rat liver. Interestingly enough, a non-carcinogenic analogue also increases the degree of methylation of RNA. Methylation alters the structure and coding properties of RNA. Thus the present findings suggest the possibility that these carcinogens alter the ability of liver ribosomes to handle messenger RNA and at the same time alter the fidelity of the translation system for protein synthesis by changing transfer RNAs. The predictable result would be fairly gross errors in protein synthesis with the production of missense or

1 This study was carried out in collaboration with P. E. Hughes and J. Blunck, Department of Pathology, University of Melbourne.

nonsense proteins by affected cells; the production or otherwise of a tumour might well depend on the resulting spectrum of abnormal proteins. Our studies on chemical carcinogenesis are thus now centred around the question of miscoding and its implications.

Virus-Host Relationships and Regulation of Transfer RNA Synthesis[1]

An important question in the regulation of protein synthesis in any system is whether the selective synthesis of messenger RNAs or their translation into protein is accompanied by a selective synthesis of transfer RNAs which perform a critical decoding function in the translation process. The system chosen for this investigation is the infection of *Pseudomonas aeruginosa* by a virulent phage, E79. Questions arising in this system are whether the phage carried in its own genome the code for all its transfer RNA synthesis in the host or whether it uses pre-infection, host-synthesized transfer RNAs. These questions are being studied by the technique of DNA-RNA hybridization, and by fractionating and characterizing transfer RNAs at various stages during the course of infection.

DNA Secondary Structure in Relation to Transcription, Replication and Transformation

The development of a counter-current system suitable for fractionating DNA has provided a useful tool for assessing small changes in DNA secondary structure. Evidence from thermal denaturation and reaction with formaldehyde indicates that separation of DNA molecules by this method depends on the degree of partial denaturation present. This permits a measure of the extent to which the strands of the DNA molecules are separated during their biological functioning. Bacterial DNAs have been examined by this method under conditions suitable for studying replication (the synthesis of a DNA copy), transcription (the synthesis of an RNA copy) and transformation (the incorporation of added autologous DNA).

Newly synthesized DNA has been shown to be more denatured than the bulk of the DNA and so can be partially separated from the latter by countercurrent distribution. Evidence from pulse-chase experiments indicates that considerable strand separation occurs in the region of the replication point, as would be expected on the basis of the Cairns model of fork-replication. Of great interest, however, is the observation that it takes some time for the DNA strands to reform a stable double helix after the synthesis of new DNA strands is completed in a given region. It seems possible that this may reflect a round of transcription following the replication; alternatively it might simply reflect the time required for stabilization of the helix by protein.

For experiments analyzing transcription, DNA has been isolated from bacteria grown in minimal media or in media enriched with amino acids. The underlying concept is that cells which are required to synthesize virtually all their requirements *de novo* will be forced to utilize most or all of their genome during growth whereas cells already provided with many of these requirements will use a significantly smaller proportion of their genome during the same period. Countercurrent distribution has shown that bacteria grown in minimal media have a higher proportion of their DNA in a partially denatured state

1 This study was carried out in collaboration with R. J. Young, Department of Biochemistry, Monash University.

than do bacteria grown in enriched media. This evidence favours partial strand separation of the DNA helix during the transcription process. The next step is to determine the manner in which this separation is permitted to occur in one region of the DNA molecule and not another at a given time.

Similar technical approaches have been employed to study the way in which donor DNA is incorporated into the recipient genome in *Bacillus subtilis* during transformation. It seems clear that only double-stranded DNA gets into the recipient cell, that this is incorporated into denatured regions of the recipient but that the donor DNA is at no time present as free, single-stranded material. These data have led to a model for genetic recombination which suggests that the chances of a piece of donor DNA becoming incorporated into its homologous recipient position may depend on the frequency with which that recipient region is undergoing transcription. This offers one rational explanation of the chance of recombination at a given genetic locus in a number of different systems.

TROPICAL HYPERGAMMAGLOBULINAEMIA[1, 2]

C. C. Curtain and C. Kidson

As part of our interest in the genetic regulation of immunoglobulin synthesis we are continuing the study of hypergammaglobulinaemia in tropical populations in Melanesia, Micronesia and South America. Immunoglobulin levels, antibody titres and various genetic markers are being studied in ethno-environmentally unique populations in these regions in an attempt to assess the antigenic loads and the balance of resistances against infection.

At the end of 1965 Dr. Kidson and Dr. Gorman visited the Gazelle Peninsula of New Britain to undertake a survey of cold haemagglutination amongst the Tolai and adjacent populations. We had previously shown a high incidence of cold haemagglutinins of mixed anti-i and anti-I specificity amongst various New Guinea lowland populations including the Tolai. The aim of the survey was to collect sera for a detailed study of the specificity of the cold haemagglutinins and their possible relationship to immunoglobulin levels and types. Subsequently Dr. Curtain visited the Red Cross Blood Bank at Port Moresby to arrange for the collection of large samples of serum from which it would be possible to isolate the cold haemagglutinins for peptide mapping and immunological studies.

INTERNATIONAL BIOLOGICAL PROGRAMME

(Project D) Human Adaptability

C. C. Curtain and C. Kidson

The Baker Institute is one of the reception laboratories for studies to be carried out by research teams during the International Biological Programme under the auspices of the International Council of Scientific Unions in the South Pacific. The aim of this section of the programme is to study simple

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1 In collaboration with Dr. D. C. Gajdusek, National Institute for Neurological Diseases and Blindness, Bethesda, Maryland, U.S.A.; Dr. P. Brown, Department of Medicine, University of California at San Francisco; Dr. J. G. Gorman, Columbia University, New York, and Dr. C. Rutgers, Cornell University, New York.

2 Supported in part by a grant from Columbia University, HE 10040-01.

communities which are threatened with imminent disintegration and loss of physical identity in the face of advancing civilization. Such groups present both in their size and level of economy the closest approximation we can find to the conditions under which mankind has lived for the greater part of its existence. The relatively small size of these populations and the simplicity of their ecology render them more manageable for intensive studies than large and more complex groups with their problems of sampling. These projected studies represent an opportune extension of the genetic studies we have been carrying out for the past eight years in the Melanesian and Micronesian populations.

We are collaborating in microevolutionary studies of the island population of Tongariki, who live in the type of community envisaged by the programme. We are also engaged in the study of ethnic and environmental variables influencing resistance to disease and immunoglobulin synthesis.

Special emphasis has been laid in the IBP on the collection of data in a form suitable for subsequent processing in a digital computor. For this purpose various techniques of automatic recording and filing of data have been investigated. One development which has eventuated is the production of books of prenumbered, mark-sensed cards with interleaved self-adhesive labels which can be attached to blood films and vials of blood. The details concerning each individual are entered on the cards using a special pencil and the marks are subsequently read in a mark-sensing machine which punches the data into the cards for subsequent processing by a computor. These cards have recently been tested in the field in New Britain and their limitations and advantages are being assessed. In the laboratory an increasing amount of data is being recorded as it is produced on punched paper tape thereby eliminating manual card punching from typed or written records although a great deal of descriptive data must still be handled manually.

BLOOD COAGULATION

P. Fantl, R. J. Sawers[1] and B. Sweet

ORAL ANTICOAGULANTS

Dicoumarol and phenylindanedione derivatives taken orally are extensively used for the prevention and treatment of thrombo-embolic conditions. These drugs reduce the blood concentration of clotting factors and spontaneous bleeding may therefore result as a complication of treatment. The degree of this reduction for a given dose of anticoagulant varies from patient to patient and some are relatively unresponsive. Consequently in order to maintain the concentration of blood clotting factors in the blood at a satisfactory therapeutic level — neither too high nor too low — continuing laboratory control of therapy is essential. Unfortunately a number of techniques for measuring, as well as different ways of calculating, the coagulation activity are currently in use and comparison of results from different laboratories is difficult and sometime hazardous. This frequently poses a problem to the international traveller who is being treated with anticoagulants on a long term basis. In order to resolve these problems the International Committee for Haemostasis and Thrombosis has undertaken to recommend standardized laboratory techniques

1 Haematology Department, Alfred Hospital.

and expression of results. As a first step in their programme a number of laboratories throughout the world have been carrying out comparisons of various techniques and as one of the laboratories concerned we have devoted a considerable amount of time to the project and our findings were discussed at the meeting of the Committee at St. Moritz last September. It is hoped that recommendations for this standardization will shortly be available.

BLOOD PLATELETS

Platelets take part in several phases of the coagulation process and are integral components of the thrombus produced. Our previous studies have indicates another method by which platelets in the circulation are normally inactive in this regard but

AN ANTI-THROMBOPLASTIC AGENT

Formation of a clot is a natural way of preventing bleeding from damaged vessels. Sometimes however the clot "breaks down" and bleeding recommences. This process of dissolution of the clot is known as fibrinolysis or thrombolysis. If this phenomenon is widespread and continuing, bleeding may reach a dangerous degree. Several drugs which counteract fibrinolysis are available and of these the epsilon-amino-caproic acid group are very effective but can only be used for a short period. In the search for more suitable compounds we have tested "Trasylol", a polypeptide present in several organs. This substance has both antithromboplastic and antifibrinolytic activity. It may therefore be of value both in preventing intravenous coagulation and fibrinolysis in any intravascular thrombus which may form.

CONGENITAL HAEMANGIOMA OF LIVER

Over the past seven years a study has been made of an unusual coagulation defect in a young woman. A severe haemorrhagic state developed following the birth of her first child and it was found that her blood was deficient in fibrinogen and had a low platelet count. This was treated with transfusions of blood and concentrated fibrinogen but the haemostatic effect of these was very short lived. Her bleeding condition continued in varying severity and over the period of observation she received several hundred pints of blood and blood products. Recently she died and autopsy revealed that she had a very extensive haemangioma of the liver but the true basis of her haemorrhagic state remains obscure.

HYPER- AND HYPOTENSIVE STATES

A. J. Barnett and I. Williams

HYPERTENSION

A clinical trial of long term treatment of severe hypertension with hypotensive drugs was commenced 15 years ago but few patients have been added this year as severe complicated hypertension (particularly with Grade IV eye signs) is now infrequently seen. Most of the patients are adequately controlled on their treatment with minimal symptoms or side effects and statistical results

remain essentially as set out in last year's report. No important new drugs have been tested in 1965 and the commonly used treatment at present is a combination of guanethidine, methyldopa and a thiazide.

Two interesting cases of secondary hypertension were studied during the year. One, a man of 53 in whom hypertension was discovered after a retinal artery thrombosis was shown to have a renal artery stenosis. Removal of the stenosis was followed by a fall in blood pressure, but recurrence of hypertension led to further investigation and the discovery that the stenosis had also recurred. The other, a young woman aged 30 years with a phaeochromocytoma, presented with a symptomless hypertension. She did not suffer from paroxysmal attacks nor any of the clinical features associated with hyperadrenalism. A pre-operative prediction that the catecholamine in the tumour would be almost entirely noradrenaline was substantiated by post-operative analysis of the tumour removed.[1]

HYPOTENSION

One new patient with severe orthostatic hypotension was studied during the year. She was a 45 year old woman and the severest example we have encountered, the patient having been confined to her bed for several months as she could not stand without fainting. Extensive investigations of sympathetic function indicated a widespread loss of sympathetic activity due to a lesion in the efferent pathways. The response to a variety of sympatheticomimetic drugs was tested and it was found (as in another case of this condition investigated several years ago) that there was an excessive response to certain of these drugs (e.g., noradrenaline) and lack of response to others (e.g., ephedrine). The patient's postural hypotension was dramatically relieved by the administration of fludrocortisone acetate.

DISEASES OF BLOOD VESSELS

A. J. Barnett, V. Carson, K. N. Morris[2], I. A. Ferguson[3], B. J. Aarons[2] and I. Williams

RECONSTRUCTIVE SURGERY

During 1965 one hundred operations for the treatment of occlusive arterial disease have been performed and marked advances in technique achieved.

Grafts using the patient's own long saphenous vein have now practically replaced synthetic materials as grafts for occlusive disease of the superficial femoral artery. The long-term results are not yet available but our impression is that in the short-term the results have been better than when synthetic grafts were used.

Use of the Fogarty balloon catheter has greatly simplified arterial embolectomy. The femoral artery may be approached through an incision in the groin, made if necessary under local anaesthesia, the catheter inserted into the artery and thrombus removed both from above (up to aortic bifurcation)

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1 Analysis of catecholamines was kindly performed by Mr. L. Austin, Department of Biochemistry, Monash University.

2 Thoracic Surgical Unit, Alfred Hospital.

3 Honorary Surgeon, Alfred Hospital.

and below (from the popliteal and tibial vessels) the point of insertion. By this means removal of thrombus and embolus is more adequate and can be performed in very sick patients who would not be suitable for general anaesthesia.

It has become common practice to combine several procedures at one operation in the treatment of arterial obstruction. Thus an endarterectomy may be associated with a vein patch to enlarge the lumen, insertion of a graft may be preceded by a local endarterectomy, a recent thrombus may be removed at the time of operation, or lumbar sympathectomy may be added.

The following table indicates the main procedure done in the 100 operations:

Vein graft		47
Synthetic graft		10
Endarterectomy or removal of old thrombus		25
Embolectomy and removal of recent thrombus		
Traditional	1	
Balloon	6	
		7
Miscellaneous and complicated procedures		11
		100

As 6 vein grafts and 3 balloon endarterectomies are included in the complicated procedures there were in all 53 vein grafts and 9 balloon thrombectomies or embolectomies performed.

The improved techniques and the greater variety of operative procedures now available increase the numbers of patients who are suitable for operative treatment and result in an increase of the proportion of patients to whom relief of symptoms and saving of limbs can be offered.

LIPIDS IN ATHEROSCLEROSIS

A preliminary account and plan of a clinical trial of "Atromid-S" [1] (ethyl-p-chlorophenoxy-iso butyrate, or "CPIB"), "Atheran" [2] (16 α-chloro oestrone 3-methyl ether) and a placebo in patients who had atherosclerosis with raised plasma cholesterol, and in most cases raised triglyceride levels, was included in the 1964 report. This study has now been concluded. Treatment periods were of $3\frac{1}{2}$ months. The dose of "Atromid-S" was 2.25 g./day and of "Atheran" 12 mg./day, as recommended by the makers.

The results of the trial may be summarized as follows: "Atheran" (19 patients) had no effect on either biochemical findings or clinical features. "Atromid-S" (17 patients) produced a mean fall in plasma cholesterol level of 14% for the group and a mean fall in triglyceride level of 25% for the group. In respect to individual patients the results differed according to whether they

1 "Atromid-S" kindly supplied by Imperial Chemical Industries of Australia and New Zealand Ltd.
2 "Atheran" kindly supplied by Difrex (Aust.) Laboratories Pty. Ltd.

were assessed on the basis of a clinically important fall or a statistically significant fall. We considered that the former was more meaningful and used as an arbitrary criterion a 20% fall in the mean value. Such clinically important falls in cholesterol levels occurred in 4 subjects and of triglyceride levels in 13 subjects. There was no clinical benefit during the short period of this study.

These results lead us to advise that the use of "Atromid-S" in patients with established atherosclerosis should be limited to patients with consistently raised lipid levels, not responding satisfactorily to a low-fat, low-cholesterol diet and that the treatment should be continued only if it can be demonstrated that the drug is having an effect.

CIBA 31,531-Ba IN ANGINA PECTORIS

A trial has been conducted to determine the value in angina pectoris of Ciba 31,531-Ba[1] which is chemically 2-[N-methyl-piperidyl-(4)]-3-amino-5-(4[1] pyridyl)-pyrazole hydrochloride. This drug is reputed to have a general vasodilator effect but with a special effect on the coronary circulation so that in spite of a fall in arterial blood pressure the coronary blood flow is enhanced.

Using a double-blind crossover method, Ciba 31,531-Ba in dose of 4 mg. three times/day by mouth (recommended by the makers) and a placebo were administered to 11 subjects with angina pectoris. There was a preliminary observation period of 2 weeks and an interval of 2 weeks between treatment, each of which was given for 4 weeks.

The number of attacks of anginal pain/week and electrocardiograms before and after treatment were recorded. The results are summarized in the following table in which figures refer to number of patients.

Treatment	Effect on E.C.G.			Effect on angina		
	Better	Worse	Unchanged	Better	Worse	Unchanged
Ciba 31,531-Ba	3	3	5	0	2	9
Placebo	5	1	5	0	2	9

It was concluded that Ciba 31,531-Ba, in the dose given orally, was of no value in the treatment of angina pectoris.

OESOPHAGEAL MOTILITY

D. A. Coventry

A system of recording pressure tracings simultaneously from several levels of the oesophagus has been set up chiefly to study a group of patients with scleroderma. As this can be a useful procedure in the diagnosis and study of other oesophageal disorders it is hoped that opportunities will exist to study other patients during the coming year.

1 Ciba 31,531-Ba kindly supplied by Ciba Company, Pty. Ltd.

CELLULAR ENZYMES**

R. G. Wyllie

Daoust has described how ribonuclease and deoxyribonuclease in whole tissue sections may be localized by incorporating a suitable substrate in a gelatin film which is layered on the section, incubated and then stained for nucleic acids. Negative staining showed enzyme containing cells. These observations suggested that by suitably controlling the conditions it should be possible to induce these enzymes to hydrolyse their substrates within the cell, and this was achieved by incubating fresh frozen sections in saturated water vapour. The nucleic acids were hydrolyzed leaving behind the cell membrane, enzyme negative cells and the supporting mesodermal stroma were unaffected. The sections were then stained. Sections treated by this method enabled fine relationships between structures to be appreciated when they could not be determined by conventional staining. With the stain chlorantine fast red it proved possible to show the cytoplasmic granules of fibroblasts and continuity between them and collagen fibres.

MAST CELLS

Work on the origin and function of tissue mast cells was continued.

Red Granular Mast Cells

A survey of elastin fibre networks in developing medullary sinuses in lymph nodes of normal animals showed wide fluctuations in frequency and extent. Sometimes elastin was present in large amounts throughout the gland, on other occasions in only small amounts, and rarely nodes were found where a lot of elastin was present in one part only.

Experiments to resolve this problem showed that maximum elastin fibre formation occurred, following the appearance of large numbers of red granular mast cells, in lymph nodes draining areas in which antigen/adjuvant deposition resulted in persistence of antigen release. This effect was further increased with complex antigens, whole bacterial cells being more effective than γ-globulin.

Blue Granule Mast Cells

It is commonly stated that mast cells discharge their granules during anaphylaxis but it was shown that only some of the mast cells do this in fatal anaphylaxis experiments. As signs of histamine and serotonin intoxication increased the granules when extruded from the cell lost their blue staining reaction and stained red. This indicates a loss of basic amines which are the reason for the blue staining in the intact cells. Almost total discharge of mast cells occurred in lymph nodes draining the site of the sensitizing antigen deposition. Least discharge of granules occurred in lymph nodes far removed from that site. Evidence of this kind has led to the hypothesis that only mast cells with an immunological memory for the sensitizing antigen are disrupted as a primary event in acute anaphylaxis.

MALABSORPTION SYNDROME

D. A. Coventry and R. G. Wyllie

Using the Crosby capsule small bowel biopsy has been performed on 30 occasions in a variety of disorders. The usual clinical indication for biopsy has been some form of intestinal malabsorption but other situations have also been studied including patients with ulcerative colitis, scleroderma and rheumatoid arthritis.

In addition to routine dissecting microscope examination and light microscopy, most specimens have also been examined by histochemical means. In patients with evidence of villous atrophy of the small intestine, such as is found in coeliac disease, there appear to be changes in the enzyme content of epithelial cells. The significance of these changes will be more apparent when patients with coeliac disease who have been on gluten-free diets and who show histological improvement in the small intestine are reviewed by these techniques.

ULCERATIVE COLITIS

D. A. Coventry and A. D. McCutcheon

RECTAL BIOPSY[1]

Using systems of grading sigmoidoscopic findings and histological appearances in rectal biopsies from patients with this disease it has been found that there is a good correlation between these features in almost 80% of combined examinations. The rectal biopsies were all performed using a suction biopsy instrument.

In this series where sigmoidoscopic appearances were normal the histological appearances were also normal. However in several instances where various sigmoidoscopic features consistent with ulcerative colitis were present histological features appeared to be within normal limits.

Interpretation of the histological features in biopsy specimens can be difficult and it is not always easy to grade those observed. Careful examination of several sections is necessary as considerable variation can occur between sections. Rectal biopsy appears to be useful as an aid to following progress in selected patients and as a research tool in the study of ulcerative colitis and experimental forms of colitis. When there is good clinical and radiological evidence for the diagnosis of ulcerative colitis it does not appear to be necessary to take biopsies. Rectal biopsy may be of help diagnostically, but this aspect has not been studied.

HISTOCHEMISTRY

The aim of this investigation is to study the pathogenesis of ulcerative colitis with special reference to vascular changes. A series of histochemical stains for a wide range of enzymes is being used on histological sections obtained from normal large bowel, large bowel with ulcerative colitis and large bowel with carcinoma. The variety of enzyme stains (for dehydrogenase, esterase, phosphatases) and other stains for mast cells, elastic tissue and nervous tissue are being used to compare the normal with the pathological. The present phase is descriptive and useful information is being accumulated.

1 Part of this work was performed at the West Middlesex Hospital, Isleworth, England.

LOWER LIMB AMPUTEES

A. J. Barnett, D. J. Guthrie[1], J. McLean[2] and J. E. Crawshaw[2]

After amputation of a lower limb a patient faces many difficulties and information is lacking in respect to the number of amputees who obtain and are able to use an artificial limb and who are able to return to an active and gainful life. Without this information difficulties associated with rehabilitation could not be assessed nor could measures be taken to improve the lot of the amputee.

A retrospective survey of such amputees was attempted but abandoned due to inability to obtain the required data from the records or to trace many of the patients.

A prospective survey was therefore commenced of patients who had a lower limb amputated in 1964. In the later half of 1965, when the most recent amputation had been performed at least 6 months previously, results were reviewed. Surviving patients were interviewed by a medical social worker who noted any difficulties associated with their rehabilitation training and their present social position, particularly in respect to work, and ability to care for themselves. Except for a few who are in nursing institutions, the patients were brought to the hospital for further interview and examination by the two medical officers. At this interview a further assessment was made concerning their adjustment to their disability, their general health, the state of the stump and their ability to use a prosthesis (if supplied) and the state of the circulation of the remaining limb. The survey is not yet fully completed but the present status of the amputees assessed is shown in the following table.

Total number of amputees in 1964		59
Deaths (one during survey)		27
Number included in present survey		32
Present Survey		
Medical examinations completed	24	
Social reports completed	29	
Not yet seen	3	
		32
Recommended for a limb and using it successfully for 12 hours or more/day		13
Recommended for a limb but still awaiting it		1
Recommended for a limb but not using it successfully		
Abandoned attempt (one a double amputee) due to physical deterioration	4	
Still trying	2	6

1 Rehabilitation Officer, Alfred Hospital.
2 Medical Social Work Department, Alfred Hospital.

SCLERODERMA

A. J. Barnett, D. A. Coventry and W. de Boer[1]

Over the past 10 years a proportion of patients presenting with Raynaud's phenomenon have been found to have the skin changes of scleroderma and some of these have also visceral disturbances that have been described in this condition.

In 1959, 33 of these cases of scleroderma and Raynaud's phenomenon were analysed and classified into clinical types. Observations on the circulatory disturbances indicated structural narrowing and occlusions of small vessels.

This year we decided to review the cases of scleroderma seen over the past 10 years to study the visceral disturbances, and to improve knowledge of the natural history and of the basis of the disease.

The review requires interviewing patients, recording data on special forms and carrying out various tests to detect involvement of internal organs, particularly heart, lungs, gastro-intestinal system and kidneys and as scleroderma is often classified with the "collagen diseases" and there is current a widely held view that these are due to "auto-immunity" we have included a number of tests believed to be pointers to auto-immunity.

A search of our records disclosed 37 patients to whom letters could be sent. Replies were obtained from 28, of whom 23 have been interviewed and investigated. The data have yet to be analysed.

1 Department of Pathology, Monash University.

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BLOOD COAGULATION

FANTL, P.—"Clotting in Heparinized Plasma. II. Underlying Reactions". *Aust. J. exp. Biol. med. Sci.*, Vol. 43 (1965), p. 45.

FANTL, P.—"Accidental Clotting in Heparinized Blood". *Thrombosis et Diathesis Haemorrhagica*, Suppl. 17 (1965), p. 263.

FANTL, P. and K. N. MORRIS—"The Influence of Dextrose on Heparinized Blood". *Thorax*, Vol. 20 (1965), p. 372.

DISEASES OF THE BLOOD VESSELS

BARNETT, A. J., L. DUGDALE and I. FERGUSON—"Disappearing Pulse Syndrome. Report of a Case due to Myxomatous Degeneration of the Popliteal Artery". *Med. J. Aust.* Submitted.

BARNETT, A. J. and A. MEATHREL—"Case Report: Renal Artery Stenosis causing Remedial Hypertension". *A.H. Clin. Rep.* In Press.

CARDIAC SURGERY

LOWE, T. E.—"Cardiac Surgery: Baker Medical Research Institute 1945-1964". *A.H. Clin. Reports.* In Press.

MORRIS, K. N., F. KINROSS and G. R. STIRLING—"Haemolysis of Blood in the Pericardium". *J. Thoracic Cardiovasc. Surg.*, Vol. 49 (1965), p. 250.

CELLULAR ENZYMES**

WYLLIE, R. G.—"Fixation in Enzyme Histochemistry". *Nature*, Vol. 207 (1965), p. 93.

WYLLIE, R. G.—"Enzyme Histochemistry for Unfixed Smears and Imprints". *Aust. J. Sci.*, Vol. 27 (1965), p. 261.

MISCELLANEOUS

BARNETT, A. J.—"Modern Diuretic Therapy". *J. Indian med. Prof.* In Press.

BARNETT, A. J. and D. ROBERTSON—"Treatment of Resistant Oedema with New Diuretics – Ethacrynic Acid and Frusemide". *Med. J. Aust.*, Vol. 2 (1965), p. 531.

FANTL, P., A. J. ROLLO and H. STROSBERG—"Chemical Analysis of an Enterolith". *Gut*, Vol. 6 (1965), p. 384.

FANTL, P. and H. A. WARD—"Molecular Weight of Human Fibrinogen Derived from Phosphorus Determinations". *Biochem. J.*, Vol. 96 (1965), p. 886.

LECTURES DELIVERED DURING 1965

"Digital Computer Models of the Gene Regulation of Protein Synthesis" — *Molecular Genetics Seminar, University of Melbourne.*	C. C. CURTAIN
"Phenotypic Restriction in Immunoglobulin Synthesis"—*Australian Biochemical Society, Melbourne.*	C. C. CURTAIN
"Quantum Cybernetics"—*Control of Biological Systems Seminar, Baker Institute—Monash University.*	C. C. CURTAIN
"On Variation in Men and their Molecules and the Relevance of Systems Engineering" — *Biomedical Computation Symposium, University of Melbourne.*	C. C. CURTAIN
"The Immunocytochemical Localization of the Immunoglobulin Factors Gm(a), Gm(b) and Inv(a) in Human Lymphoid Tissue"—*Australian Society for Medical Research, Melbourne.*	C. C. CURTAIN
"Kinekard — A Hormone in Search of a Syndrome"—*Alfred Hospital Clinical Society.*	D. A. COVENTRY
"Ulcerative Colitis — A Rectal Biopsy Study"—*Royal Melbourne Hospital.*	D. A. COVENTRY
"Rectal Biopsy in Ulcerative Colitis"—*Royal Australasian College of Surgeons, Melbourne.*	D. A. COVENTRY
"Laboratory Techniques in Treatment with Oral Anticoagulants"—*International Society for Haemostasis and Thrombosis, St. Moritz, Switzerland.*	P. FANTL
"Studies on Messenger RNA"—*Molecular Biology Seminar, University of Melbourne.*	C. KIDSON
"Alterations in DNA Secondary Structure in Relation to Replication, Transcription and Transformation"—*Australian Biochemical Society, Adelaide.*	C. KIDSON
"The Mechanism of Genetic Recombination during Transformation in Bacillus Subtilis"—*Australian Genetics Society, Hobart.*	C. KIDSON
"Chemical Approaches to the Study of Gene Regulation in Bacteria and Mammals" — *Commonwealth Serum Laboratories, Melbourne.*	C. KIDSON
"Recent Advances in Genetic Coding"—*Molecular Biology Seminar, University of Melbourne.*	C. KIDSON
"Gene Regulation and Mechanisms of Carcinogenesis"—*A.N.Z.A.A.S., Hobart.*	C. KIDSON
"The Inotropic Action of d-Aldosterone on Papillary Muscle Isolated from Monkeys"—*Cardiac Society of Australia and New Zealand. Melbourne.*	W. G. NAYLER
"Some Properties of a Cardioactive Substance Isolated from Human Blood Plasma"—*23rd International Congress of Physiological Sciences, Tokyo.*	W. G. NAYLER
"The Pathogenesis of Pancreatitis"—*Cornell Medical Center, New York.*	A. D. McCUTCHEON
"Mast Cells — A New Concept"—*Department of Pathology, Melbourne University.*	R. G. WYLLIE
"The Origin and Function of Mast Cells"—*Victorian Society of Pathology and Experimental Medicine.*	R. G. WYLLIE
"Mast Cells"—*2nd Australian Medical Congress, Perth.*	R. G. WYLLIE

THE THOMAS BAKER, ALICE BAKER AND ELEANOR SHAW MEDICAL RESEARCH INSTITUTE

Revenue Account for the Year Ended 31st December, 1965

EXPENDITURE.		INCOME.		
Salaries and Wages	£45,758 16 10	**Donations—**		
Laboratory Supplies	11,166 0 4	Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions		£42,000 0 0
Library Maintenance	2,220 9 7	Other donations as per attached Schedule		826 11 0
Isotopes	226 8 0	**Grants-in-Aid of Research—**		
Postages and Telephone	877 4 9	National Heart Foundation of Australia	£7,918 0 0	
Printing and Stationery	791 14 3	Anti-Cancer Council of Victoria	3,956 8 0	
Light and Power	401 17 8	Anti-Cancer Council of Victoria (A. A. Thomas Fellowship)	2,819 17 0	
Insurances	1,127 14 3	Life Insurance Medical Research Fund of Australia and New Zealand	5,529 0 0	
Repairs and Renewals	1,689 7 2	Asthma Foundation of Victoria	1,273 17 0	
Animal House Contribution	1,000 0 0	National Health & Medical Research Council	26 0 0	
Sundries	1,517 12 1	National Institutes of Health, U.S.A.	1,384 2 9	
Travelling Expenses	392 8 6			22,907 4 9
Data Processing	960 1 10	**Interest from Investments—**		
Surplus for Year	153 14 7	Held by Trustees of the Estate of the late Thomas Baker	850 0 0	
		Endowment Fund	1,261 18 0	
				2,111 18 0
		Interest from Bank Account		308 7 8
		Sundry Sales		129 8 5
	£68,283 9 10			£68,283 9 10

THE THOMAS BAKER, ALICE BAKER AND ELEANOR SHAW MEDICAL RESEARCH INSTITUTE

Balance Sheet as at 31st December, 1965.

LIABILITIES.			ASSETS.		
Endowment Fund	£25,902 17 9		Endowment Fund Investments—		
Capital Grants and Gifts	565 2 7		Commonwealth Inscribed Stock	£10,880 0 0	
Restricted Funds	6,264 6 6		Treasury Bonds	4,010 0 0	
Accumulated Revenue	2,200 17 7		Shares in Companies	11,012 17 9	
		£34,933 4 5			£25,902 17 9
Development Fund		295,275 13 4	Development Fund Investments—		
Current Liabilities—			Commonwealth Inscribed Stock	283,000 0 0	
Sundry Creditors	2,501 0 0		Cash at Bank	12,275 13 4	
Accrued Expenses	2,540 0 0				295,275 13 4
		5,041 0 0	Capital Grants, Gifts and Restricted Funds—		
			Cash at Bank		6,829 9 1
			Fixed Assets—		
			Furniture and Fixtures		3,495 11 10
			Current Assets—		
			Cash at Bank	3,736 5 9	
			Cash in Hand	10 0 0	
					3,746 5 9
		£335,249 17 9			£335,249 17 9

AUDITORS' REPORT TO THE TRUSTEES OF THE THOMAS BAKER, ALICE BAKER AND ELEANOR SHAW MEDICAL RESEARCH INSTITUTE.

In our opinion the above Balance Sheet, together with the Notes thereto, is properly drawn up to show a true and fair view of the state of the Institute's affairs at 31st December, 1965, according to the best of our information and the explanations given to us and as shown by the books of the Institute.

FLACK & FLACK,
Chartered Accountants,
Honorary Auditors.

Melbourne,
14th February, 1966.

NOTES:—

(i) In addition to receiving interest from the Investments as shown on the Balance Sheet, the Institute receives the income from 5% Commonwealth Government Inscribed Stock face value of £17,000, which is inscribed in the name of the Trustees of the Estate of the late Thomas Baker for the benefit of the Institute.

(ii) Expenditure on Laboratory equipment, motor vehicles, building improvements and fittings have been charged against the appropriate Revenue Accounts in the periods when expended.

THE THOMAS BAKER, ALICE BAKER AND ELEANOR SHAW MEDICAL RESEARCH INSTITUTE

Year Ended 31st December, 1965.

RESTRICTED FUNDS

Balance at 31st December, 1964		£1,431 10 3
Add		
Donations—		
Appel Bequest	1,594 0 0	
Anti-Cancer Council of Victoria	381 5 0	
Department of Health	241 0 0	
Dr. T. E. Lowe and Associates	8 16 8	
		£3,656 11 11
Deduct		
Equipment—		
U. V. Recorder (Appel Bequest)	£887 10 0	
Radirack Fraction Collector (Appel Bequest)	706 10 0	
Rotor Spinco Centrifuge (Anti-Cancer Council of Victoria)	381 5 0	
Digital Voltmeter	527 0 0	
Leitz Powerpack	294 19 0	
Magnetic Counter	93 12 0	
		2,890 16 0
		£765 15 11
Transfer to Development Fund—		
Dr. T. E. Lowe and Associates	100 0 0	
Kiddle Estate	100 13 4	
		200 13 4
Balance at 31st December, 1965		£565 2 7

ACCUMULATED REVENUE.

Balance at 31st December, 1964	£2,047 3 0
Add	
Surplus for Year	153 14 7
Balance at 31st December, 1965	£2,200 17 7

THE THOMAS BAKER, ALICE BAKER AND ELEANOR SHAW MEDICAL RESEARCH INSTITUTE

Other Donations received during Year to 31st December, 1965.

Marian and E. H. Flack Trust	£350	0	0
George F. Little Trust	178	12	3
Mr. and Mrs. Edgar Rouse	105	0	0
Mr. and Mrs. Frank Crane	50	0	0
Mr. Siegfried Meyer	50	0	0
Mr. J. C. Habersberger	5	5	0
Eagle Star Insurance Co. Ltd.	5	5	0
Mr. R. Blakemore	2	6	9
Donations in memory of: Sir William Angliss, James Winter Dodds, Jan Kroef, Ernest A. Lloyd, Gertrude Luxton, Cecilia McKernan, James McGhee, Graham Mees, James A. Morris, William A. P. Mounsey, W. Poulsen, Thomas Suckling, Myrtle Thomson, Florence Thurman, Raymond Williams	80	2	0
	£826	11	0

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

1965

STAFF

Honorary Consulting Physicians:	EWEN DOWNIE, M.D., F.R.C.P., F.R.A.C.P. BRYAN HUDSON, M.D., Ph.D., M.R.C.P., F.R.A.C.P.
Honorary Consulting Biochemist:	JOSEPH BORNSTEIN, D.Sc., M.D., F.R.A.C.P.
Physician-in-Charge:	PINCUS TAFT, M.D., F.R.A.C.P.
Honorary Physician:	HARALD BREIDAHL, M.D., M.R.C.P., M.R.A.C.P.
Honorary Clinical Assistants:	K. J. CATT, M.D., M.R.A.C.P. V. S. MEENAKSHI, M.D. (Madras). H. D. NIALL, M.D., M.R.A.C.P.
Registrar:	J. HARRISON, M.B., B.S.
Biochemists:	DORA WINIKOFF, M.Sc. JUNE SHEATH, M.Sc. Doris PAGE, B.Sc.
Technical Staff:	Mr. W. HUDSON. Miss I. EKKEL. Miss R. WITCHELL. Miss W. DAVIES (to 24/9/65). Mrs. Y. GREAVES (from 20/9/65). Mrs. F. RABOLD (part-time).
Secretary:	Miss J. SHARP.

DIABETIC CLINIC

Clinical Assistants:	MARGARET SANDERS, M.B., B.S. IAN BURR, M.B., B.S., M.R.A.C.P.
Chiropodist:	MAIDA O'CONNOR, F.Ch.A.V., M.Ch.I.A.

RESEARCH FELLOWS

Medical Research Fund:	N. KATHLEEN TAYLOR, M.B., B.S.
Burroughs Wellcome Research Fellow:	IAN BURR, M.B., B.S., M.R.A.C.P.

HONORARY RESEARCH FELLOWS

MARGARET SANDERS, M.B., B.S.

E. L. G. BEAVIS, M.B., B.S., D.G.O., M.R.C.O.G., F.R.C.S.

ANNUAL REPORT

During the past year work has continued on the projects detailed in the 1964 report. Two of them—The Estimation of Fatty Acids in Plasma and The Estimation of Plasma Gonadotrophins—have been completed and reports submitted for publication. Further work in these fields is projected applying the methods developed. The work on The Influence of Oral Contraceptives on Thyroid Function Tests and Thyroid Hormone Carriage and Binding Capacity has proceeded now to the stage of the preparation of manuscripts for publication.

Today it is becoming increasingly difficult for a variety of reasons to be "first in the field". Although no prior publication of the topic had been made at the time work was begun on two of our investigations, between the completion of the research and submission of papers to editors, articles on practically identical work appeared in the scientific literature. It is encouraging to feel that these problems we have tackled have interested others and that the results we have recorded have been almost identical, but disappointing in that the rewards of labour have been confirmation of existing information rather than the dissemination of truly new knowledge.

However, achievement in a unit such as the Diabetic and Metabolic Unit is a little more intangible than the striking of a balance between scientific work done and original papers published, for there are a number of hidden assets in the balance sheet. Numbered among these are the dissemination of knowledge and the training of graduates and under-graduates in endocrinology and metabolism, collaborative work with other investigators in the field and the provision of the more exotic diagnostic facilities for colleagues with predominantly clinical interest. The success of former registrars, Dr. G. C. Ennis (1963) and Dr. J. R. Stockigt (1964) in the examination for Membership of the Royal Australasian College of Physicians and of their continuing work in the field of endocrinology and metabolism, the former with the Diabetic and Metabolic Unit and the latter with the Department of Medicine, Monash University can likewise be counted as assets in the Unit ledger.

Dr. Tan Bock Yam from the Medical Unit, General Hospital, Singapore, was awarded the Edward Wilson Scholarship and elected to work in the Diabetic and Metabolic Unit. His project has involved studies of cortisol clearance and he has worked in close association with the Monash University Department of Medicine at Prince Henry's Hospital and with Professor Bryan Hudson in particular. It is hoped that the skills he has acquired in this study and his clinical work in the Unit will assist him in his further development of Endocrinology on his return to Singapore.

During the year Mrs. Dora Winikoff during a four months leave of absence attended the Fifth International Thyroid Conference in Rome and subsequently visited centres in the United Kingdom, Europe and the U.S.A. where she was able to exchange views and information on the thyroid function studies which she has been making during the past 8 years.

Helpful criticism and advice have come from visitors to the Unit. These have included Dr. J. Witte, Secretary of the International Diabetes Federation, Utrecht, Holland; Dr. John Butterfield, Professor of Medicine, Guy's

Hospital, London; Dr. Bryan Leeming of Auckland, New Zealand; Dr. C. Nordin, M.R.C. Unit, Leeds, U.K. and Dr. W. P. U. Jackson, Professor of Medicine, Cape Town, South Africa.

Colleagues of the Honorary Medical Staff of the Alfred Hospital and the Monash University Departments of Medicine and Biochemistry have continued to give generously of their advice, assistance and support in clinical and laboratory matters. Again this year gifts from individuals and organizations of money and in kind are gratefully acknowledged. Their demonstration of interest in the activities of the Diabetic and Metabolic Unit encourages and facilitates our work in a very practical sense and to them we are deeply grateful.

PINCUS TAFT.

31st December, 1965.

Grateful acknowledgment is made of financial assistance and gifts in kind from—

Alfred Hospital Research Funds.
Boots Pure Drugs Co. (Aust.) Pty. Ltd.
Burroughs Wellcome & Co. (Australia) Ltd.
Mrs. M. Clark.
Difrex Laboratories Pty. Ltd.
Dunklings Pty. Ltd.
Eli Lilly & Co., Indianapolis, U.S.A.
Hoechst Pharmaceuticals.
Pfizer Corporation.
Sandoz Ltd.
The Upjohn Company, Kalamazoo, U.S.A.
William R. Warner & Co. Pty. Ltd.

STUDIES OF THE METABOLIC CLEARANCE RATE AND PRODUCTION RATE OF CORTISOL IN HUMANS

Tan Bock Yam

with the assistance of Bryan Hudson, Ausma Dulmanis and the Department of Biochemistry, Alfred Hospital.

The commonly observed finding in obese patients of elevated urinary hydroxy steroids, of elevated cortisol production rate yet normal plasma cortisol prompted this study of cortisol clearance rate and a re-examination of cortisol production rate in a variety of clinical states.

Principle.

Knowing the amount of radioactive cortisol which has to be infused to maintain a constant level and knowing the concentration of this material per litre of plasma during infusion, it is possible to calculate the metabolic clearance of cortisol in litres of plasma per day. Knowing, too, the level of plasma cortisol the daily production rate can be calculated.

Tritiated cortisol (H^3F) is infused at a rate of Rx^F counts per minute per day.

Plasma radioactivity concentration of H^3F is x^F counts per minute per litre.

Therefore metabolic clearance rate (MCR^F)

$$= \frac{Rx^F}{x^F} \text{ litres per day}$$

and production rate of cortisol (PR^F)

$$= MCR^F \times \text{plasma cortisol level.}$$

Method.

A priming dose (15 μCi) of H^3F in 10 ml. saline was given and 30 minutes later a further 15 μCi H^3F in 50 ml. saline was administered by constant infusion at a rate of 0.5 ml. per minute. Blood samples were drawn before the priming dose to measure plasma cortisol level and at 45, 60, 75 and 90 minutes after the priming dose and during constant infusion to measure the levels of radioactive cortisol and confirm the constancy of level. Plasma cortisol levels were determined by fluorimetric and/or double isotope techniques. The radioactivity in the samples drawn during constant infusion was measured after extraction of the plasma with methylene chloride and acetylation with pyridine and acetic anhydride. Separation and isolation of the steroid was achieved chromatographically using F acetate in the first two of three Bush systems and as E acetate in the third. After the final chromatography, samples were eluted and counted in a liquid scintillation counter. A known amount of C_{14} cortisol was added to each plasma sample prior to chromatography to check recovery and to enable correction of the figures for losses prior to calculation.

Patients Studied.

Ten patients have been studied. Three were of normal build, two were suffering from proven Cushing's disease, four were obese and one was a patient with anorexia nervosa.

Results.

The figures for seven patients are tabulated in the order in which they were studied. Extraction of the plasma of the last three obese patients has not yet been completed. The significance of these findings is yet to be assessed.

	Condition	Plasma Cortisol (μgm%)	Metabolic Clearance Rate (Litres/day)	Production Rate (mgm/day)
1.	Control	14.9	228	33.0
2.	Control	45.0	96	43.0
3.	Control	21.4	165	35.3
4.	Cushing's Disease	34.2	235	80.3
5.	Anorexia Nervosa	32.6	186	60.6
6.	Cushing's Disease	17.4	174	30.3
7.	Obesity	17.1	134	23.0
8.	Obesity	17.1		
9.	Obesity	14.3		
10.	Obesity	7.4		

GLUCOSE UTILISATION

Ian Burr, Doris Page and William Hudson

Studies have continued using the intravenous glucose load-infusion technique with the following aims:

(a) To determine whether the test would be useful in detecting diabetes and prediabetes in pregnancy.

(b) To elucidate reasons for the differences noted between normals and diabetics using this technique.

Ten pregnant diabetic women have been infused and the response compared with seventeen normal pregnant women and twelve late onset diabetics. Statistical treatment of results (square root transformation and determination of 95% confidence interval) reveals no significant difference between results in the diabetic pregnant and diabetic non-pregnant patient. However, the differences between the pregnant diabetic and pregnant non-diabetic state are statistically significant. The probably clinical significance of this method is indicated by the finding of abnormality in pregnancy, in response to glucose load-infusion in three patients who by standard oral glucose tolerance testing were normal but whose clinical course in pregnancy was as a diabetic.

In an attempt to determine reasons for the differences noted between diabetics and prediabetics on the one hand and normals on the other with this technique and also to test the hypothesis that the differences are due to abnormalities in the hypothalmic-autonomic control of glucose levels in diabetics the following tests were performed.

Normal volunteers were infused with and without pre-treatment with pempidine, a compound capable of inhibiting transmission across autonomic ganglia. Arterial (via radial puncture) and venous blood sugars and venous growth hormone and insulin levels were measured at five minute intervals over the infusion period. Venous cortisol levels were measured at the following times: fasting, during infusion at 30 minutes and 50 minutes, and 20 minutes after infusion. Analysis of results available reveals the following:

(a) "Swings" in blood sugar levels are observed in both arterial and venous blood in the pempidine treated volunteers and are in the range found in diabetics.

(b) Rapid alterations of blood cortisol levels have been observed with marked depression from fasting level during glucose infusion.

(c) Alterations in plasma insulin do not appear to be responsible for observed fluctuations in glucose levels.

There are as yet insufficient results in this phase of the work for adequate conclusions to be made regarding the mechanism of action of the ganglion blocker in reproducing a diabetic-like response to this form of glucose challenge.

DOUBLE ANTIBODY IMMUNOASSAY OF PLASMA INSULIN

Doris Page

The assay technique has been outlined in the 1964 report. However, we have been able to obtain human insulin to replace beef insulin as a standard, and insulin I^{131} of higher specific activity (> 100 mc/mg) as a tracer.

Standard curves prepared with beef, pig and human insulins were not identical, the divergence becoming greater with increasing insulin concentration. Using a human insulin standard curve, a series of plasma dilutions from zero to 1 in 20 gave identical results when appropriate dilution factors were applied. This does not apply when human plasma is read against a standard curve prepared with either pig or beef insulin.

The reproducibility of the method was ascertained by performing 45 individual assays of a plasma pool over a period of 5 months. The insulin levels ranged from 20 to 27 μUnits/ml. plasma, with a mean of 23 μU/ml.

The mean plasma insulin level in normal fasting individuals was found to be 17 μU/ml. with a range of 0 to 30 μU/ml. The fasting plasma insulin level of two patients with known insulinomas was shown to be in excess of this range, being 48 μU/ml. and 125 μU/ml. respectively.

The assay was primarily employed in determining plasma insulin levels during glucose infusion experiments (see report of Dr. I. Burr). Although the number of patients studied was small, it appears that the mean rise in plasma insulin above fasting levels is approximately equal (means 11-19 μU/ml) in normal controls, normal controls given ganglion blockers, non-pregnant non-insulin requiring diabetics, and some acromegalics; and is higher in pregnant women, non-insulin requiring pregnant women (27 μU/ml.), and some acromegalics (40 μU/ml.). The significance of these findings is yet to be assessed.

INSULIN CARRIAGE BY SERUM PROTEINS

Doris Page

The carriage of insulin in serum of diabetic patients was compared to that of normal subjects. Insulin I^{131} was equilibrated, in vitro, with serum or saline-albumin. Serum proteins were fractionated by electrophoresis on cellulose acetate strips in barbitone buffer pH 8.6 and the radioactivity of each fraction ascertained.

Sample equilibrated with Insulin I^{131}	Location of radioactivity on the strip
Saline-Albumin (control)	Sharp peak at origin
Serum from: Normal controls Pregnant women Untreated diabetic patients Diabetic patients on diet Diabetic patients on oral hypoglycaemics	Broad band in albumin to α_1 globulins
Serum from: Diabetic patients on insulin Insulin resistant patients	Broad band in albumin to α_1 globulin and another in γ globulins extending toward the β globulins.

In Vivo.

Perchlorate treated anaesthetized rats were injected with insulin I^{131} and blood samples taken from the tail 10 to 20 minutes later. The sera were fractionated by electrophoresis together with serum samples obtained prior to injection and equilibrated in vitro, with insulin I^{131}.

On all strips most of the radioactivity was located in the albumin, α_1 globulin region, thus suggesting that the in vitro results obtained would be applicable in vivo.

Conclusions.

The observation that insulin is carried on albumin and α_1 globulin in the serum of normal subjects is in agreement with many published reports. No apparent differences in serum insulin carriage between normal and pre-diabetic or non-insulin requiring diabetic patients was observed. However, the administration of exogenous insulin gives rise to an insulin binding antibody in serum.

THE INFLUENCE OF ORAL CONTRACEPTIVES ON THYROID FUNCTION TESTS

Dora Winikoff and Kathleen Taylor

Further studies of the influence of oral contraceptives on thyroid function tests have been carried out. Ten different preparations commonly used in Australia were investigated. As in the previous year, we used protein bound iodine (PBI), hormonal iodine (HI), I^{131}-triiodothyronine resin uptake (RU) and electrophoretic index (EI) based on electrophoresis with added I^{131}-thyroxine as parameters for the assessment of thyroid function.

Following the intake of the ovulation suppressant a characteristic pattern of changes in thyroid function indices can be seen—i.e., an elevation of PBI, HI, and EI and a depression of RU (usually an elevation of EI and a depression of RU is consistent with the clinical picture of hypothyroidism). These changes are attributed to the increase in binding capacity of thyroxine binding globulin (TBG) for thyroid hormone and are found in the course of a normal pregnancy.

Studies were carried out on normal euthyroid women while taking oral contraceptives, patients suspected of thyroid disorders on similar medication, and pregnant women. Variations during and between cycles and the effect of cessation of medication have been observed. Our findings can be summarised as follows:

(1) The intake of ovulation suppressants invariably affected all thyroid parameters studied although some of them still remained within the normal range of values. The effect of oral contraceptives could be easily demonstrated by repeating the tests after cessation of medication.

(2) The change in parameters was proportional to the amount of oestrogens present in different preparations regardless of the type of the progestogen present in the "pill". Further studies are in progress to verify this point. Three different progestogens (ethynodiol diacetate, norethynodrel and medroxy-progesterone) tested without the oestrogen component produced no effect on thyroid function tests. Furthermore, the influence of the amount of oestrogen was demonstrated by studying the effect of two preparations with a different oestrogen content administered to the same patients. The increase in oestrogen invariably intensified the departure from normal levels in all thyroid parameters.

(3) The change in thyroid indices, particularly PBI and HI, was induced even by one cycle of administration. Morcover, after the withdrawal of medication it was still continuing, reaching its peak between the 3rd and 7th day. Following short term administration PBI and HI were more affected than RU and EI, even by preparations containing small amounts of oestrogen. When the oestrogen content was high all parameters were markedly changed.

(4) After cessation of medication all parameters returned to pretreatment levels between 4 weeks and 2 months depending on the length of administration.

(5) In thyrotoxic patients the intake of oral contraceptives resulted in very high PBI and HI levels while RU and EI were within normal or toxic range of values, a distinct difference from euthyroidism. The same pattern was observed in pregnancy coinciding with thyrotoxicosis.

(6) Hypothyroid patients maintained on thyroxine while taking the ovulation suppressant were controlled by smaller doses of thyroxine than is usually required in such circumstances. Their PBI, and HI levels were also lower. It is our impression that oral contraceptives potentiate the action of thyroid hormone perhaps by providing more binding sites on TBG for exogenous hormone.

Conclusion.

Although the effect of long term administration of ovulation suppressants on thyroid function is uncertain there is no doubt of their influence on thyroid function indices. This creates difficulties in assessing the functional state of the gland. Exact knowledge of the type of medication used and the length of administration is absolutely essential for the correct interpretation of results. Multiple thyroid function parameters should be estimated and when in doubt the tests should be repeated two months after cessation of medication.

ELECTROPHORETIC INDEX

Kathleen Taylor and Dora Winikoff

Further studies on the previously described technique of electrophoresis on cellulose acetate strips using radiothyroxine have been undertaken.

The parameter of thyroid function based on this technique and termed by us "electrophoretic index" (EI) was evaluated in more than 1,000 patients. These included euthyroid, thyrotoxic, hypothyroid and pregnant patients, infants, and those taking thyroid supplements and oral contraceptives.

Normal limits range from 61-75%. The mean for euthyroid subjects was 68.9% ± 3.86, for thyrotoxicosis 54.9% ± 6.03, hypothyroidism (including cretins) 70.8% ± 2.97 and in pregnancy 85.7% ± 2.80.

The test is useful for the following reasons. It is unaffected by organic or inorganic iodine contamination, and is less sensitive to changes in pH of blood than the resin uptake I^{131}-triiodothyronine (RU). Combined with RU it reveals changes in the binding capacity of thyroxine binding globulin for thyroid hormone, and has the additional virtue of demonstrating abnormalities in the protein pattern.

The level in normal pregnancy is invariably greater than 80% as early as 4 weeks gestation. Since the PBI in pregnancy is usually elevated, and may not be of use in diagnosing thyroxicosis, an EI of less than 80% will give a final answer.

For maximal clinical response in thyroxine treated patients the EI ideally should be in the high normal or mildly toxic range.

In women using oral contraceptives or patients having oestrogen therapy an EI of greater than 70% is expected. The combination of lowered RU, elevated PBI and elevated EI may indicate the influence of oral contraceptives despite the lack of clinical history.

EI levels in the newborn are in the hypothyroid range, gradually reaching adult euthyroid levels at about 10 days after birth. However, those infants who have been referred with the diagnosis "failure to thrive" frequently have levels in the subthyroid range due to alteration of the protein pattern.

The test is now used in all cases when the diagnosis of the thyroid state is in doubt.

THE ESTIMATION OF NONESTERIFIED FATTY ACIDS, TRIGLYCERIDE FATTY ACIDS, KETONE BODY CONCENTRATION, GLYCEROL, LACTIC AND PYRUVIC ACIDS IN PLASMA OF DIABETIC PATIENTS AND NORMAL SUBJECTS

June. Sheath

The method developed previously in this laboratory for the estimation of nonesterified fatty acids and triglyceride fatty acids is now being employed routinely for their measurement in the plasma of diabetic patients selected from the outpatient clinic. Fasting and postprandial specimens are assayed to determine whether a correlation exists between the level of these substances and the type of diabetes (juvenile or maturity onset), its duration, and the mode of treatment of the patient (with special regard to obesity). Blood sugar estimations are made simultaneously. In this way eight patients have been studied and the mean fasting value obtained for nonesterified fatty acids was 804 μEq/l (range: 340-2310) and that for triglyceride fatty acids was 84.5 mg/100 ml (range: 46.9-129.2).

By this technique it was found that the mean fasting plasma nonesterified fatty acid value of the diabetics studied was much greater than that of the normal subjects (mean: 240 μEq/l); likewise the triglyceride fatty acids are in some cases elevated above the normal value. Because of the wide variation in the nonesterified fatty acid values of the diabetic patients consideration was given to additional tests which may provide further evidence. It seemed that a more reliable index might be obtained by including the measurement of plasma ketone body and glycerol concentrations. Accordingly methods for the estimation of these substances were investigated.

For ketone body concentration the method described by Hird and Symons (Biochim. Biophys. Acta, 35, 1959, 422) and Hird and Weidemann (Biochem. J., 93, 1964, 423) was selected. The method measures the acetone plus acetoacetate of plasma as a separate fraction from the β-hydroxybutyrate. Acetoacetate is decarboxylated to acetone by heating in acid and β-hydroxybutyric acid is oxsidised and decarboxylated to acetone by heating in an acid medium in the presence of dichromate. The acetone due to these components is reacted with 2,4-dinitrophenylhydrazine to form a hydrazone which is then extracted into carbon tetrachloride. Quantitative colorimetric determination is based on acetone standards receiving the same treatment. Estimations of acetone plus acetoacetate, β-hydroxybutyrate and total ketone body concentration are at present being carried out in this laboratory.

To date, this technique has been used for assay of these substances in normal fasting adults and in two diabetic patients in ketosis. Blood samples were taken from the ketotic diabetics on admission and after treatment. From the results obtained, it appears that in normal subjects β-hydroxybutyrate is the predominant substance, whereas in the two patients studied in diabetic ketosis before treatment, a gross elevation of the acetone plus acetoacetate fraction was observed. Within twenty hours after admission, during which time lactate was infused, the reduction in total ketone body concentration was approximately 80%.

By the use of this technique, it is proposed to establish the normal range for plasma ketone body concentration and investigate the levels found in diabetics and other patients.

For the estimation of plasma glycerol the enzymatic method of Garland and Randle (Nature, *196*, 1962, 987) is employed. At present the plasma glycerol concentration of fasting normal adults is being determined. The assay will then be used for patients on whom studies of plasma nonesterified fatty acids, triglyceride fatty acids and ketone body concentrations are being made.

Lactic acid values have been determined on blood samples of patients in diabetic ketosis, those suspected of lactic acidosis (where grossly elevated values were found) and of normal subjects (together with pyruvic acid estimations) at appropriate intervals during glucose infusions.

THE ESTIMATION OF PLASMA GONADOTROPHINS

Ida Ekkel and Pincus Taft

Acetone precipitates of plasma have been used in the past to measure by bioassay gonadotrophin levels in plasma. This technique has enabled the estimation of high levels since the extract is toxic to the test animal, no more than the equivalent of 10 ml. of plasma being tolerated.

An acetone precipitate of plasma, washed with 90% ethanol, extracted four times with 10% ammonium acetate in 70% ethanol then reprecipitated with 10% ammonium acetate in 90% ethanol was found to be well tolerated by the immature female mouse and extracts equivalent to 100 ml. of plasma have been tolerated.

This method of extraction has allowed measurement, using the mouse uterus test, of total gonadotrophin in the plasma of young women and young and old men as well as in the plasma of post-menopausal females.

Two reference preparations have been used, the Proposed International Working Standard which is equipotent with the newly established 2nd International Reference Preparation and H.M.G. 20A, the latter to make comparison with previously reported work more meaningful.

The mean recovery in four experiments was 97% (range 89%-104%).

The results of assay in two plasma pools in different age and sex groups are reported below.

Group Years	Assay Design*	Potency mg P.I.W.S. 100 ml	Fiducial Limits mg P.I.W.S. 100 ml (P = 0.95)	Potency mg H.M.G. 20A/100 ml	Fiducial Limits mg H.M.G.20A 100 ml (P = 0.95)	λ
Females	3 × 2	.111	0.086–0.135	2.142	1.659– 2.611	0.09
18-42	2 × 2	.105	0.013–0.152	2.016	0.246– 2.913	0.11
Males	2 × 2	.125	0.108–0.148	2.408	2.087– 2.854	0.06
24-44	3 × 3	.124	0.112–0.137	2.379	2.155– 2.620	0.05
Males	3 × 3	.142	0.115–0.177	2.726	2.314– 3.392	0.11
45-60	3 × 3	.148	0.127–0.176	2.847	2.440– 3.390	0.08
Females	3 × 3	1.369	1.134–1.691	26.341	21.826–32.534	0.10
50-60	3 × 2	1.824	1.565–2.130	35.091	30.101–40.969	0.07

* Number of dose levels of unknown and standard.

Since this study was submitted for publication Keller and Rosemberg (J. Clin. Endocr., 25, 1965, 1050) have published practically an identical study. It is of interest to note that their findings are similar, comparative figures (expressed as μg. 2nd I.R.P. per litre) reading:

GROUP	EKKEL and TAFT	KELLER and ROSEMBERG
Young females	1.13	1.31
Males	1.35	1.20
Post-menopausal females ..	15.9	17.88

PAPERS PUBLISHED DURING 1965

SHEATH, June B.—"Estimation of Plasma Non-Esterified Fatty Acids and Triglyceride Fatty Acids by Thin-Layer Chromatography and Colorimetry". *Aust. J. Exp. Biol. & Med. Sci.*, Vol. 43 (1965), p. 563.

LECTURES DELIVERED DURING 1965

"Diabetic Emergencies"—*Alfred Hospital Old Residents Association.*	H. D. BREIDAHL
"A Case of Hyperparathyroidism"—*Royal Australasian College of Physicians.*	H. D. BREIDAHL
"Medical Uses of Radio-Isotopes"—*Victorian Association of Science Teachers.*	H. D. BREIDAHL
"Recent Advances in Endocrinology"—*Victorian Dietitians Association.*	H. D. BREIDAHL
"Diabetes in Children"—*Victorian School Medical Officers.*	H. D. BREIDAHL
"The Growth and Development of Children Born to Mothers with Diabetes"—*2nd Australian Medical Congress, Perth.*	H. D. BREIDAHL
"Current Management of Thyrotoxicosis"—*Royal Melbourne Hospital Graduate Society.*	PINCUS TAFT
"The Diagnosis of Thyroid Disease"—*College of General Practitioners.*	PINCUS TAFT
"Observations on Therapy with Adrenal Steroids at Pharmacologic Dosage"—*Peter MacCallum Clinic.*	PINCUS TAFT
"Diabetes in Pregnancy"—*Department of Obstetrics and Gynaecology, University of Melbourne.*	PINCUS TAFT
"Sex and Intersex"—*Royal Melbourne Hospital Clinical Schoool.*	PINCUS TAFT
"The Role of the Liver in Metabolism"—*Royal Australasian College of Surgeons, Symposia in Basic Sciences.*	PINCUS TAFT
"The Induction of Ovulation with Human Pituitary Gonadotrophin"—*Endocrine Society of Australia.* (With D. Adey, J. B. Brown, J. Evans and W. Johnstone.)	PINCUS TAFT
"The Influence of Oral Contraceptives on Thyroid Function Tests"—*Alfred Hospital Clinical Society.* (With K. Taylor and D. Winikoff.)	PINCUS TAFT

REPORT OF INVESTIGATIONS BY RESEARCH FELLOWS IN OTHER DEPARTMENTS OF ALFRED HOSPITAL

STUDIES ON MYCOBACTERIA[1]

J. S. Tolhurst, G. Buckle and J-A. Barnes

M. ULCERANS

It was mentioned in last year's report that a number of cases of ulceration of the skin resembling *M. ulcerans* infection had occurred in Africa both in the Congo and in Uganda and that the Uganda workers had claimed that the acid fast bacillus which they found in the lesions was not *M. ulcerans* but a new species.

Although we had previously requested cultures so that we could compare them with our Victorian cultures we could not obtain these until this year. We received 4 strains from Uganda and 7 strains from the Congo and we collected 10 strains from Australian cases (Bairnsdale and Colac, including 2 of the 3 strains which we described in 1948, Hamilton, Brisbane and Darwin) making a grand total of 21 strains.

It was considered important to make a thorough study of these cultures as quickly as possible so that we could clearly define the characters of *M. ulcerans*. In 1948 we had seen only 6 cases of infection and cultured only 3 strains of the organism and it was likely that our description made at that time was inadequate.

One of the striking observations in our original study was that *M. ulcerans* had an optimum temperature range for growth of 30° to 33°C, and that it failed to grow at 37°C.

This year we have so far performed optimum temperature experiments on 19 of the strains now in hand. While the majority had an optimum temperature about 33°C some grew better at 35°C and one strain grew equally well over a range from 29° to 37°C.

Animal Experiments

(a) **Rats and Mice.**—Nineteen strains have been inoculated intraperitoneally into rats and subcutaneously into mice.

In our original studies on the first three strains which grew at an optimum temperature range of 30° to 33°C, the lesions in male rats were unusual. Epididymal lesions developed first, followed by ascites and if the animal survived, skin lesions were usually found. Inoculated mice developed gross subcutaneous oedema. The viscera were not involved in rats or mice.

In the current study, which is incomplete because some animals do not develop lesions for many months, 2 of the Uganda strains and one of the Congo strains, and the strain from Brisbane and the one from Darwin have produced the expected picture of ascites, etc., in rats and subcutaneous oedema in mice, thus satisfying us that they are in fact *M. ulcerans*. Some other strains although producing a fatal infection in rats did not induce ascites and possible reasons for this are being examined.

We plan to prepare sections of tissues from all the infected animals and to attempt to correlate the sites of lesions with the optimum growth capacity of the organisms. This could be of considerable biological interest.

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1 Department of Bacteriology.

(b) **Toads.**—The source of the infection in man has never been uncovered but patients are often associated with a natural watering place such as the Mitchell River in Bairnsdale or the Nile in Uganda.

Some time ago we demonstrated that toads and frogs became infected when inoculated with a strain of *M. ulcerans*. Since toads are more readily available than frogs we have set out to find whether infection can be produced by the strains at our disposal.

Sixteen strains have been inoculated into toads. Many have died and the organs are to be sectioned and studied.

STUDIES ON CHEMOTHERAPY[1]

G. Buckle, J. C. Tolhurst, A. K. Percival and D. E. Quance

The relationship between Erythromycin and a new antibiotic, Lincomycin, are under investigation. These two drugs are said not to be chemically related but they act on the same species of organisms and we have been finding that there is not uncommonly a peculiar antagonism between them, such that organisms resistant to erythromycin but sensitive to lincomycin, resist the action of lincomycin while erythromycin is present.

Some investigations have been done on Ayermycin—enough to show that it need not be investigated further. Work has begun on yet another antibiotic, Cephalosporin.

Some work has been done on the the transfer of antibiotics across the peritoneal membrane, both aspects of transfer being of interest; sometimes it may be desirable to remove an antibiotic (as well of course as other substances) by peritoneal dialysis and sometimes it may be desired to instil an antibiotic into the peritoneal cavity without it being absorbed into the body generally. This work is to be continued with some help from a temporary assistant in the Dialysis Unit.

The attachment of antibiotics to proteins prevents them from exerting a chemotherapeutic effect. This protein binding has a marked influence on the comparative usefulness of different antibiotics and is thought to be variable from person to person. The method of determining the degree of protein binding has so far been complex and difficult but we are trying to work out a method which could be used quickly and which would employ only small samples of blood so that it might be possible to give more accurate information on prognosis.

METHAEMALBUMIN[2]

J. A. Owen

The chemical reactions of methaemalbumin have been studied with the aim of devising a procedure for the determination of methaemalbumin in plasma. These investigations have indicated that spectrophotometry before and after addition of sodium hydrosulphite may be used for this purpose. Interference from haemoglobin is minimised by making measurements at a wavelength at which haemoglobin and oxyhaemoglobin have identical absorption coefficients. The method is being used in the study of methaemalbumin metabolism.

1 Department of Bacteriology.
2 Department of Biochemistry.

TOXICITY OF PESTICIDES[1]

M. Bick

The estimation of cholinesterase activity in the group of orchardists was continued early in 1965 during the spraying season, and again in September. The figures obtained show that in 1962-63 there was a significant drop in red cell enzyme activity before and after spraying. This fall decreased in 1964 and was not seen in the 1964-65 season. This may be due to the increased care that the orchardists take in handling the materials, improved packaging for small users, or the use of less toxic compounds. With the co-operation of the Commonwealth Serum Laboratories, the relevant statistics are now being obtained.

By courtesy of the Honorary Surgeons at the Alfred Hospital, fifty specimens of subcutaneous fat have been collected from patients at operation. These specimens have been extracted and submitted to column chromatography prior to the determination of chlorinated hydrocarbon residues by gas liquid chromatography.

An attempt has been made to establish the validity of the assumption that the estimation of erythrocyte cholinesterase activity is an index of the decrease in enzyme activity in other tissues, in non-acute exposure to pesticide. Cholinesterase determinations have been made in various tissues of the mouse, following intraperitoneal injection of organic phosphorus and carbamate compounds in increasing amounts.

Reduction in enzyme activity in the homogenized brain and liver can be obtained with large doses, but as yet, it has not been possible to correlate a reduction at low dosage with a small decrease in red cell activity.

AUTOTRANSPLANTATION OF KIDNEY

J. Nayman[2]

A series of 19 autotransplants in the dog were undertaken in order to develop the technique; an end to end and end to side venous anastomosis was found to be satisfactory. A simple ureterovesical anastomosis proved to be the most efficient.

The other kidney was removed and animals survived for periods up to 9 months before being sacrificed.

HAEMODIALYSIS

J. Nayman[2]

DYNAMICS OF DIALYSIS

The Travenol Twin Coil Dialyser has been fitted with a stronger dialysate circulating pump and dialysance studies comparing the static and dynamic coil volumes with the "old" and "new" circulating pumps were compared.

The efficiency of the Capon Heaton "minicoil" and "twin-coil" dialysers were compared with the Travencol twin-coil.

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1 Department of Biochemistry.

2 Department of Surgery, Monash University.

TECHNIQUE IN DOG

It is difficult to dialyse dogs with the Travenol Twin-Coil dialyser because of the high mortality; techniques using regional heparinization; weighing of the dog and careful replacement of fluid losses; priming of machine with canine blood were developed and haemodialysis was able to be undertaken in dogs in renal failure.

WOUND HEALING

J. Nayman[1]

VASCULAR WOUNDS

A study of tensile strength of venous and arterial anastomoses was started; different surgical techniques were compared.

WOUNDS IN RENAL FAILURE

It has previously been shown that the induction of renal failure in the early phases of wound healing in the dog would result in wound disruption. Control of renal failure by daily haemodialysis prevented this wound breakdown.

The study of the effect of renal failure upon wound healing in the mouse has been completed. Hypoplasia of connective tissue associated with failure of migration of epidermal cells was clearly demonstrated.

A satisfactory autoradiographic technique using tritiated thymidine has been developed for the study of fibroblastic activity in wounds.

CEREBROVASCULAR DISEASE

B. S. Gilligan[2]

All cases admitted to the Alfred Hospital with cerebrovascular disease, occurring under the age of 45 years, have been reviewed from 1954 until 1965.

From this series 43 patients with cerebral thrombosis, 17 patients with cerebral embolism, and 26 patients with intra-cerebral haemorrhage have been studied with regard to clinical features, pathological findings at autopsy, aetiological factors and prognosis. These results are being correlated.

1 Department of Surgery, Monash University.
2 Department of Neurology.

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JOHNSON, D. G., Julie-Anne BARNES and Elizabeth McLEOD—"Subacute Bacterial Endocarditis Caused by Streptococcus faecalis and Successfully Treated with Ampicillin". *Med. J. Aust.*, Vol. 2 (1965), p. 1026.

LYALL, I. C., W. ELRICK, J. BANKS and A. PERCEVAL—"Listeria Meningitis: A Case Report". *Med. J. Aust.*, Vol. 2 (1965), p. 756.

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NAYMAN, J.—"Technical Developments in Haemodialysis". *A.H. Clin. Reports,* Vol. 12 (1964), p. 41.

NAYMAN, J. and R. FREAKE—"A Pre-mixed and Pre-tested Dialysing Bath Concentrate for Use in Haemodialysis". *Med. J. Aust.*, Vol. 1 (1965), p. 752.

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PERCEVAL, A.—"Experimental Cryptococcosis: Hypersensitivity and Immunity". *J. Path. Bact.*, Vol. 89 (1965), p. 645.

PAPERS ACCEPTED FOR PUBLICATION

NAYMAN, J.—"Open Renal Biopsy with Limited Exposure of the Kidney". *Ann. Roy. Coll. Surg., Edin.*

NAYMAN, J.—"Modifications to Lid of Dialyser Bath of Travenol Twin-Coil Dialyser and Attachment of Tap to Dialyser Bath to Measure Ultrafiltration". *Med. J. Aust.*

NAYMAN, J.—"An Artero-Arteriovenous Shunt for Use in Intermittent Haemodialysis". *"Developments in Dialysis".*

NAYMAN, J. and D. BREEN—"Anal Retractors: A Brief Historical Summary and Description of a New Single Multipurpose Instrument". *Aust. and N.Z. J. Surg.*

NAYMAN, J. and H. A. F. DUDLEY—"Improving the Efficiency of an Artificial Kidney: The Use of Increased Dialysate Flow Rates". *J. Biol. med. Engng.*

NAYMAN, J., H. A. F. DUDLEY and J. P. MASTERTON—"The General Effects of Injury". *Chapter for New Edition of Companion Studies.*

NAYMAN, J. and L. M. DUGDALE—"Unilateral Multicystic Kidney: Case Report". *A.H. Clin. Report.*

NAYMAN, J. and R. FREAKE—"The Decreased Inhibitory Effect of Uraemic Serum upon the Isolation of Micro-Organisms". *Am. J. Clin. Path.*

NAYMAN, J. and R. FREAKE—"Examination of Urinary Deposits". *Med. J. Aust.*

NAYMAN, J. and R. FREAKE—"An Improved Design 'Clamp On' Infusion Stand". *Am. J. Med. Tech.*

NAYMAN, J., R. FREAKE and F. W. GURR—"The Incidence of Septicaemia in Renal Failure". *Med. J. Aust.*

NAYMAN, J., C. THOMAS and G. FEINT—"An Arteriovenous Shunt Associated with an Adenocarcinoma of the Kidney". *Brit. J. Surg.*

WELLINGTON, N. A. M. and A. K. PERCEVAL—"Bacillary Haemoglobinuria in Cattle". *Aust. Vet. J.*

PAPERS SUBMITTED FOR PUBLICATION

NAYMAN, J.—"The Effect of Renal Failure Upon Wound Healing: An Experimental Study". *Ann. Surg.*

NAYMAN, J. and R. FREAKE—"Bacterial Aerosols in Urine Collection and Their Prevention". *Am. J. Clin. Path.*

NAYMAN, J. and R. FREAKE—"The Use of Anticoagulants in Blood Culture Estimation". *J. Path. Bact.*

LECTURES DELIVERED DURING 1965

"The Effect of Certain Organic Compounds on Cholinesterase Activity"—*Alfred Hospital Clinical Society.*	M. BICK
"Communication between Operating Theatre and Laboratory"—*Royal Victorian College of Nursing.*	G. BUCKLE
"Wound Healing in Renal Failure: An Experimental Study in the Dog"—*Victorian Society of Experimental Medicine and Pathology.*	J. NAYMAN
"Use of Arteriovenous Shunts in Renal Failure". "Regional Heparinization in Haemodialysis". "Surgery in Acute Renal Failure"—*Singapore General Hospital.*	J. NAYMAN
"Dynamics of Dialysate Flow and Method of Improving Dialysance of Kolff Twin-Coil Dialyser"—*Surgical Research Society of Australasia, Sydney.*	J. NAYMAN
"Anal Retractors: A Brief Historical Review and Description of a Single Multi-purpose Instrument"—*Proctological Section, Royal Australasian College of Surgeons, Sydney.*	J. NAYMAN
"The Association of Renal Failure and Dehiscence of Abdominal Wounds"—*Royal Australasian College of Surgeons, Melbourne.*	J. NAYMAN
"Wound Dehiscence in Acute Renal Failure: Clinical and Experimental Studies"—*Australasian Society of Nephrology, Melbourne.*	J. NAYMAN
"Clerical Biochemistry"—*Victorian Branch, Australian Association of Clinical Biochemists.*	J. OWEN
"Effect of Injury on Protein Metabolism"—*Association of Hospital Scientists in Victoria.*	J. OWEN
"A System Providing Composite Serial Biochemistry Records in Patients' Notes and in the Laboratory"—*Australian Association of Clinical Biochemists.*	J. OWEN
"Effect of Stress on Protein Metabolism"—*A.N.Z.A.A.S., Hobart.*	J. OWEN
"A System Providing Composite Serial Biochemistry Records in Patients' Notes and in the Laboratory"—*College of Pathologists of Australia.*	J. OWEN
'Antibiotics in Renal Failure"—(a) *Australian Society of Microbiology, Melbourne;* (b) *Alfred Hospital Clinical Society.*	A. K. PERCEVAL