BAKER INSTITUTE

RESEARCH

1963

ALFRED HOSPITAL

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.

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.

THIRTY-SEVENTH ANNUAL REPORT

of

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

(Including Alfred Hospital Clinical Research Unit)

SEVENTH ANNUAL RESEARCH REPORT

of

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

REPORTS

of

ALFRED HOSPITAL RESEARCH FELLOWS

1963

ALFRED HOSPITAL, PRAHRAN
VICTORIA, AUSTRALIA

BAKER MEDICAL RESEARCH INSTITUTE

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ALFRED HOSPITAL RESEARCH FELLOWS, 1963

"Sydney W. Jones Medical Research Foundation":	A. BAUMGARTEN, M.B., B.S.
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Medical Research Fund:	R. D. GORDON, M.B., B.S., M.R.A.C.P.
"Dr. Henry Laurie": *"Frederick and Esther Michaelis":* *"J. F. Mackeddie":* *"R. B. McComas":*	N. McCONAGHY, B.Sc., M.B., B.S., D.P.M.
"Sol Green": *"Victor Y. and Margaret Kimpton":*	J. NAYMAN, M.B., B.Ch. (W'srand), F.R.C.S., F.R.C.S. (Edin.), F.R.A.C.S.
"H. M. Black Estate":	D. RACE, M.B., B.S.
"Edward Wilson Memorial":	P. G. C. ROBERTSON, M.B., B.Ch. (St. Andrew's).
Medical Research Fund:	N. KATHLEEN TAYLOR, M.B., B.S.

APPOINTED TO RESEARCH FELLOWSHIPS FOR 1964

"R. B. McComas": *"Frederick and Esther Michaelis":* *"Amelia Haigh Heart Research Scholarship":* *"Connibere Bequest":*	A. BAUMGARTEN, M.B., B.S.
Medical Research Fund:	J. M. CALVERT, M.B., B.S., F.R.C.S., F.R.A.C.S.
"E. H. Flack":	J. F. MAINLAND, B.Sc., M.B., B.S., F.F.A.R.A.C.S.
"Dr. Henry Laurie":	N. McCONAGHY, B.Sc., M.B., B.S., D.P.M.
"Victor Y. and Margaret Kimpton": *"J. F. Mackeddie":*	J. NAYMAN, M.B., B.Ch. (W'srand), F.R.C.S., F.R.C.S. (Edin.), F.R.A.C.S.
"Sol Green": *"Edward Wilson Memorial":*	P. G. C. ROBERTSON, M.B., B.Ch. (St. Andrew's).
"James Richardson": *"Amelia Haigh Rheumatoid Arthritis Research Scholarship":*	N. KATHLEEN TAYLOR, M.B., B.S.

TRAVEL GRANT

"J. H. Paterson Travelling Scholarship":	A. D. McCUTCHEON, M.D., M.R.A.C.P.

TABLE OF CONTENTS

Investigations by Research Fellows in Other Departments of the Hospital

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, with 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 lems, 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.
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Technical:	S. HART (Laboratory Supervisor). J. L. BREMNER. Mrs. R. SABO. Miss A. STANTKE.
Clerical:	Mrs. I. R. ROBINSON. Mrs. B. R. ASHTON. Miss L. CORKE. Mrs. Robyn ROBINSON.
Laboratory Assistants:	Mrs. J. BERAN. Mr. D. BREEN. Mrs. S. BROOKS (to 31/5/63). Miss E. DENEREAZ. Miss D. DIXON (to 8/2/63). Miss J. FINDLAY. Mr. K. HARVEY (from 15/7/63). Miss D. HUGHES (from 4/3/63). Mrs. Y. KNEALE. Miss R. KNOWLES (from 27/5/63). Miss A. McARDLE (to 1/2/63). Mrs. M. McVICAR (to 2/9/63). Miss D. MAKEPEACE (to 23/6/63). Miss E. MINSTER. Miss L. PHILLIPS. Miss M. SHIERS. Miss M. STUCKEY (from 16/9/63). Miss E. WADDY (to 14/6/63). Miss Y. WILSON (from 11/2/63).

WARD STAFF

Registrar:	P. de V. MEIRING, M.B., Ch.B. (W'srand.), M.R.C.P., M.R.C.P. (Edin.).
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Sister:	L. STAHR (to 16/6/63). J. W. McCORMICK (from 29/7/63).
Staff Nurses:	P. J. JEFFERY (to 25/8/63). A. M. COCK (from 3/6/63). E. K. BRIGDEN (from 26/8/63).

ALFRED HOSPITAL RESEARCH FELLOWS

"Sydney W. Jones Medical Research Foundation":	A. BAUMGARTEN, M.B., B.S.
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"E. H. Flack": *"A. A. Swallow":* *"George Merriman":* *"Connibere Bequest":*	J. B. DAWSON, M.A., B.M., B.Ch. (Oxon.), M.R.C.P. (Edin.).
"H. M. Black Estate":	D. RACE, M.B., B.S.

ANTI-CANCER COUNCIL RESEARCH FELLOW

"A. A. Thomas": CHEVIOT KIDSON, M.B., B.S., B.Sc. (Med.), (on leave).

ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

An awareness of the importance and value of research to the community in general is today well established and has resulted in much more generous financial support than was available ten or fifteen years ago. This is indeed fortunate for, as Sir Howard Florey commented in the David Rivett Memorial Lecture 1963 — ". . . progress in acquiring knowledge depends on progress in devising subtler and more accurate methods of experiment and . . . this frequently means acquiring costly and often bulky machines". Whilst increased financial support has enabled us to keep abreast of these developing techniques, they have created new problems which have not yet been adequately solved. The increase in amount of and space occupied by equipment is increasing so fast that the Institute machines are pushing people out of laboratory space. Further, these instruments which have resulted from the solution of intricate engineering problems usually require the user to have a considerable knowledge of branches of science not directly connected with the research problem in hand. This in turn increases the number of trained workers needed to effectively prosecute many projects in order to have all the requisite knowledge and skills available.

This trend in techniques may be illustrated by considering the changes in chemistry over the past 50 years. At the beginning of this century a chemist interested in the biological field needed reagents, which came in bottles, simple equipment, such as test tubes, flasks, glass tubing, gas burners and a beam balance which would weigh materials accurately to a few milligrams. At that time because of this limitation in accuracy of weighing only the major constituents of body fluids could be studied. Some twenty or so years ago methods for microanalysis of body materials were developed and hailed as a great advance. This was achieved by converting the substance of interest into a coloured compound in solution and comparing the colour of the liquid with that of a known standard. Instruments for comparing the colours of solutions — colourimeters — were developed for this purpose. Inevitably these instruments were improved, prisms and filters were introduced to use light of narrow wavelength bands in both the visible and invisible regions and so spectrophotometers came upon the scene. The price rose from a few tens of pounds to a few thousand pounds per instrument and the bench space occupied rose from one to many square feet. It was now possible to determine substances in microgram quantities and a great deal more knowledge could be gained about biological processes more rapidly and with no loss of precision.

Of recent years these micromethods have been supplemented by radioactive isotope and neutron activation procedures so that amounts of substances ten times smaller than those detected by previous methods are easily assayed. Again cost in money and space has increased as has the knowledge required to operate efficiently the instruments and in the case of neutron irradiation it has become necessary to seek the collaboration of other bodies such as the Australian Atomic Energy Commission for the procedures are no longer feasible in an Institute such as ours.

The biological importance of such traces of substances as can now be detected is shown by a recent experience in which two samples of sucrose

were used in the perfusion fluid of a muscle experiment. Both were claimed by the makers to be analytically pure, with impurities not exceeding one part in a million, yet one sample was completely inhibitory to the muscle and the other was not inhibitory at all.

One of the implications of these recent developments for the Institute is that considerable extensions to or rebuilding of our premises must be undertaken very soon, unless the quantity and quality of our research projects is to fall. The building is overcrowded now.

In last year's report some reorganisation of our library service was forecast and the provision of photocopying methods has been of great value to the staff. The volume of published research work is becoming enormous and any aid to workers in extracting what is of interest to them is extremely valuable. The cost of communicating results between workers is an integral part of any project, for knowledge buried is knowledge largely lost, but like much else this cost is rising and is reflected in the financial statements. One item in this category which is new and tending to increase is the requirement of a growing number of publishers of scientific journals that the author of an article contribute to the cost of publication. Previously, no charge was made to the author except for reprints.

The past year has seen further growth in the medical school of Monash University and considerable co-operation between individual members of its staff and ours has developed on both teaching and research sides. This changing academic scene has led to review of the role of the Institute and its relationship with both Hospital and University, and it is expected that amicable and profitable co-operation between all will evolve.

In February the Anti-Cancer Council of Victoria appointed Dr. Chev Kidson to its Arthur A. Thomas Cancer Research Fellowship and it has been agreed that he will continue his research project in Molecular Genetics in the Institute on his return from overseas at the end of 1964.

Research Projects

A feature of these Annual Reports has for a long time been concise but technical reports of research projects in progress and it is pleasing to record that letters and requests for further information received by many members of the staff indicate that the Reports are being carefully studied by other workers in many parts of the world.

Detailed technical accounts of research in progress are presented in the scientific section of this report and cover studies into the control of body fluid volume, the processes of blood coagulation, the mechanisms of energy production in heart muscle, the role and variation of various proteins in the blood, the behaviour of cellular enzymes in some diseases, the control of blood distribution in the body, the clinical control of high blood pressure states, the treatment of diseases of the peripheral arteries, the surgical treatment of heart conditions and the nature of acute pancreatitis. Instead of attempting to give short summaries of each project as in previous reports a review for laymen of the studies on blood coagulation since their inception is included here.

Process of Blood Clotting

Blood in vessels is normally a fluid (plasma) in which blood cells are suspended but when it escapes from the vessels following injury or is withdrawn into a test tube it changes within a few minutes into a firm jelly (clot). After a further variable time the jelly shrinks and separates from a liquid part (serum). This process is somewhat analogous to the conversion of milk into curds and whey. Within the body in due course the clot, whose normal function is to seal the damaged vessel, is dissolved (lysis). Without an ability to control this clotting process transfusion of blood and the use of machines to temporarily take over the work of the heart, lungs or kidneys – all now standard procedures in treatment – would be impossible. Diseases such as strokes and coronary occlusions are associated with upsets of the clotting process in which blood clots inside blood vessels and various bleeding diseases, e.g., haemophilia, arise because blood fails to clot when it should. A detailed knowledge of the various physical and chemical changes which bring about the clotting of blood are clearly therefore of prime importance in medicine as are methods of controlling this process.

The investigations carried out here have been under the direction of Dr. Fantl whose interest in the problem was aroused in the first place by a request from hospital clinicians to prepare a synthetic Vitamin K for them. Vitamin K, then in short supply because of war, was used to control some bleeding states. Shortly after this the discovery in the U.S.A. that Dicumarol – the causative agent of "Sweet clover" disease in cattle – interfered with the clotting of blood together with his interest in vitamin K led Fantl to commence a study of the processes of blood clotting.

When this work started (1940) all that was known about the clotting process was that a protein (fibrinogen) in solution in plasma was changed into an insoluble one (fibrin) by another substance (thrombin) which could be recovered from clotted blood but not from unclotted blood. Further, some indirect evidence indicated that thrombin was made from a preexisting substance (prothrombin) in blood when an agent was released from damaged tissues. It was also known that calcium, normally present in blood, accelerated the clotting process. In all, six substances were believed to be involved in a series of sequential reactions. Today thirteen substances involved in these reactions have been identified.

One of the difficulties of experiments at this time arose from the lack of a method of isolating prothrombin from plasma. Various methods of doing this were evolved by different workers but one now commonly used was developed in 1945 in these laboratories. Once it was possible to use isolated components in the study of blood clotting it quickly became apparent that plasma contained a substance which accelerates the reactions. Fantl and his colleagues are credited with the first detailed description of this substance, now called factor V. Research into the nature of the substance in tissue extracts (thromboplastin) led to the recognition that an inherited bleeding condition (haemophilia) was due to its deficiency and that two varieties of the deficiency occurred. In this phase of the work Fantl and his colleagues were unfortunate that priority for their discovery was forestalled by publication of similar data in England just at the time they had completed this study.

Of recent years the studies here have been directed to the role of platelets, which are small cell-like structures in blood, in clot formation, to the role of various fatty substances in abnormal clotting and to the mechanism of the ultimate lysis of the clot.

It was previously mentioned that there are now 13 substances recognised as being active in the clotting process and the sequence of their reaction has recently been put in a schematic pattern by the International Committee for the Nomenclature of Blood Clotting Factors and given the name of the Fantl-Koller Scheme. This is a fitting recognition of a long period of exacting work carried out by Dr. Fantl and his helpers.

The contributions of these studies to the clinical practice of medicine have been considerable. The work on dicumarol led to the synthesis of a compound known as E.D.C. which was introduced into the Hospital at an early date for treatment of conditions due to clotting of blood in blood vessels. The work on haemophilia led to methods of measuring thromboplastin deficiency in blood and to a survey of the incidence of haemophilia in Victoria and indirectly to the establishment of the Haemophilia Society of Victoria. The training given to workers in these investigations made possible the formation of an excellent service in the Hospital devoted to clinical investigation of patients with bleeding and clotting disorders. The ready availability of skilled assistance and detailed knowledge of these problems greatly facilitated the establishment of heart-lung machines as an aid to cardiac surgery in the Hospital. Also personnel of both civilian and service authorities have frequently sought and been given advice about the provision of blood and blood products for transfusion.

This brief review, which touches only a few highlights of the project, shows how a question arising in clinical medicine may start a train of basic investigations which in turn may return considerable dividends to the clinician and the community as a whole.

Overseas Visits

Dr. P. Fantl again attended the Annual Meeting of the International Committee for the Standardisation of the Nomenclature of Blood Clotting Factors held in Gleneagles, Scotland. During this trip he took the opportunity to visit workers and laboratories in his field in Los Angeles, Montreal and several centres in the United Kingdom. Grateful acknowledgement is made to the National Advisory Heart Council, U.S.A. and the Trustees of the Institute for the financial assistance for this visit.

Research Grants

Many of the investigations recorded in this Report have been supported by funds provided by the National Health and Medical Research Council, the Life Insurance Medical Research Fund of Australia and New Zealand, the Anti-Cancer Council of Victoria, the National Heart Foundation of Australia, Alfred Hospital Medical Research Funds and Mr. Denis Bottomley 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 the Heads and Staffs of various departments of the University of Melbourne, Monash University and the Australian National University, also by the members of the Commonwealth Serum Laboratories, Commonwealth X-ray and Radium Laboratory 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, 1963.

Addendum:

Since this report was written the University of Melbourne has conferred the degree of Doctor of Science on Mrs. W. G. Nayler and the Trustees have appointed her Assistant Director of the Institute.

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

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.
Hallstrom 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 Foundation, New York.
Royal Children's Hospital, Melbourne.
Royal Melbourne Hospital.
Royal Prince Alfred Hospital, Sydney.
Royal Women's Hospital, Melbourne.
St. Vincent's Hospital Medical Research Unit, 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

1949-63

Anderson, R. McD., 1953-55
Andrew, R. R., 1949-55
Barnett, A. J., 1949-50
Baumgarten, A., 1962-63
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
Frazer, J. R. E., 1957
Gardiner, J. M., 1952
Goble, A. J., 1951
Hudson, B., 1952
Jamieson, K., 1954
Kay, H. B., 1949-53
Kincaid-Smith, P., 1959-60
McCutcheon, A. D., 1959
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

1954-63

Dawson, J. B., 1961-63 (Oxford)
Emslie-Smith, D., 1955-56 (Dundee)
Hamilton, M., 1954 (London)
Lumb, F. H., 1960-61 (London)
Marshall, R. J., 1957 (Belfast)
Robertson, P. G. C., 1963 (Dundee)
Simpson, F. O., 1958-59 (Edinburgh)
Stevenson, M. M., 1957 (Belfast)
Thomson, J. W. W., 1959 (Edinburgh)

REPORT OF SCIENTIFIC INVESTIGATIONS

BLOOD COAGULATION*

P. Fantl, R. J. Sawers[1], H. A. Ward, H. Strosberg and A. Baumgarten

STIMULATION OF FACTOR VIII (ANTIHAEMOPHILIC) ACTIVITY BY TRANSFUSED SERUM

Patients with factor VIII deficiency and prolonged skin bleeding time (von Willebrand's syndrome) who receive fresh blood or plasma from normal donors or from patients with haemophilia A with very low factor VIII activity show a much higher factor VIII activity in their plasma than could possibly be explained by the amounts given. The maximum activity is reached 4-8 hours after transfusion and persists longer than in haemophilia A. The prolonged skin bleeding time may be temporarily shortened.

The component responsible for the factor VIII stimulation has not been distinguished from that associated with the skin bleeding time but the following case suggests that the two factors are different.

A 21 year old male with a bleeding tendency since infancy has approximately 10% of normal factor VIII activity in his plasma and his skin bleeding time is consistently in excess of 20 minutes, except after blood transfusions. He has been transfused successively with plasma, serum and plasma fraction I-0. Factor VIII determinations in plasma and skin bleeding time measurements have been performed at intervals. His plasma factor VIII activity rose to a much higher level than expected following transfusion of plasma or serum and his skin bleeding time was shortened after the transfusions of plasma or of the plasma fraction I-0 but not after the serum transfusion. Factor VIII activity was assayed by two independent techniques (partial thromboplastin time and thromboplastin generation) using plasma with less than 1% factor VIII from haemophilia A patients. Non-specific effects were excluded. It is believed the results represent true factor VIII levels.

The observations suggest that normal plasma contains two components which are lacking in patients with von Willebrand's syndrome. The component which stimulates factor VIII activity in vivo is present both in citrated plasma (pH 7.1) stored at −20°C for 30 days and in serum. Only a low activity is present in lyophilized plasma fraction I-0. The second component which shortens the bleeding time is present in frozen plasma and plasma fraction I-0, but is absent from serum.

As serum transfusions have no influence on the factor VIII level of haemophilia A patients, it is suggested that serum transfusion could be used to differentiate patients who have both a low factor VIII level and prolonged skin bleeding time. This seems necessary since, in our experience, such patients form a heterogeneous group.

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* In this report of scientific investigations those projects marked (*) were supported wholly or in part by grants from National Health and Medical Research Council, those marked (†) by grants from the Life Insurance Medical Research Fund, those marked (**) by grants from Anti-Cancer Council of Victoria and those marked (‡) by grants from the National Heart Foundation of Australia.

1 Department of Haematology, Alfred Hospital.

PHOSPHORUS IN HUMAN FIBRINOGEN AND FIBRIN

Fibrinogen was isolated from cell-free plasma, particular attention being paid to the removal of platelets. The ethanol precipitation technique of Cohn, as modified by Blombäck and Blombäck, was used in earlier work and more recently the ether precipitation technique of Kekwick et al. was employed. The preparations were convertible into fibrin to the extent of 90-96% based on protein content. In the majority of cases normal human fibrinogen contained 31 g. phosphorus in 210,000 g. protein, but in one person 31 g. was present in 310,000 g. protein. This last result was obtained on two occasions three months apart. Thus it may be that at least two groups of humans can be distinguished with regard to the phosphorus content of their fibrinogen. It remains to be established whether we are dealing with isofibrinogens in man which are genetically determined.

LIPIDS AND BLOOD CLOTTING

Although a number of workers have tried to establish that lipids influence the blood coagulation process no unanimity has been reached. It is thought that an increase in the lipid concentration in blood may be related to atherosclerosis and thrombosis. In the following experiments the influence of various lipids on certain phases of the coagulation process was determined.

Blood from fasting persons with normal lipid concentrations was compared with blood obtained from patients whose total plasma lipid concentration was greater than 1000 mg.%. In siliconised apparatus no significant difference in the whole blood clotting times in the two series was seen. In addition, sera were tested for platelet-like activity in the thromboplastin generation test but no acceleration of clotting was observed. In some cases of hyperlipaemia inhibition was noticed.

Several lipid materials were tested with regard to their effect on the clotting process. The addition of cream or butter to whole blood shortened the clotting time and also had a potent platelet-like activity in the thromboplastin generation test. On the other hand, chylomicrons isolated from the blood of patients who had taken either butter or cream orally, had little effect on the coagulation process in vitro tests.

Influence of Heparin on Lipoprotein Lipase Activity in Blood after Fasting and after Lipid Ingestion

The activity of lipoprotein lipase in plasma was measured by the amount of free fatty acids released by the plasma enzyme. A variety of substrates were tested. Aqueous emulsions, with "Tween" as emulsifier, of glyceryltrioleate, glyceryltristearate, glyceryltripalmitate, glyceryldioleate and glyceryl mono-oleate as well as cream and egg yolk were not hydrolysed by plasma lipoprotein lipase. Chylomicrons isolated from lipaemic plasma were a substrate for the plasma enzyme.

Lipoprotein lipase activity was determined in the plasma in two groups of people aged 21-30 years and 55-66 years. Tests were carried out first when fasting, and secondly, following the ingestion of half a pint of cream. On each occasion blood samples were taken immediately before and fifteen minutes after the intravenous injection of 5000 units of heparin.

After a fatty meal and without injection of heparin there was endogenous lipoprotein lipase activity present in the plasma. Further, it was observed that lipoprotein lipase activity was induced even in fasting plasma by the intravenous injection of heparin.

The activity induced by heparin injection following a fatty meal amounted to an average release of 3.5 m.eq. of fatty acids per litre of plasma after 2 hours incubation at 37°C in the group aged 21-30 years, and 1.5 m.eq. of fatty acids per litre of plasma after 2 hours incubation at 37°C in patients aged 55-66 years.

THE USE OF CITRATED BLOOD IN OPEN HEART SURGERY

These experiments were undertaken for the purpose of providing blood for transfusion in emergency cardiac operations, where donors could not be bled at short notice. Because heparinized blood which is used in open heart surgery should be as fresh as possible it was thought advisable to determine whether citrated blood could be used as an alternative. In one series of experiments dogs were infused directly with solutions containing varying concentrations of citrate, dextrose, calcium acetate and magnesium acetate. In the other series dogs were heparinized and then put on by-pass, using whole citrated dog blood collected one day earlier. Prior to the experiments donors' blood was mixed with heparin plus calcium acetate and magnesium acetate. At various times citrate, glucose and calcium were determined in the blood specimens.

It was observed that citrate concentrations of 1250-1560 mg.% in the transfusion fluids were reduced to approximately 6 mg.% in 2 hours and that calcium concentration of 24-74 mg.% were reduced in one hour to 9 mg.%. The concentration of ionised calcium was maintained between 0.5 mM and 2.0 mM.

AN IMMUNOLOGICAL APPROACH TO FIBRINOLYSIS

Using purified human fibrin dissolved in 50% urea as the antigen, a rabbit antiserum has been prepared which forms precipitates with human fibrin, fibrinogen and fractions derived from either by proteolytic processes. The only plasma component with which the antibodies in the antiserum react is fibrinogen, as shown by immunodiffusion and immunoelectrophoresis. Fibrinogen can be detected by the antiserum in concentrations as low as 2 mg.%.

Human sera, prepared from normal human blood obtained from donors at rest and with the least possible trauma, did not contain components precipitable with the antiserum. When difficulties were encountered during the insertion of the needle into the vein, the resultant sera contained low titres of precipitable components. Serum from a patient with carcinoma of the prostate showed a higher titre of precipitable components. In both cases fibrinolysis is considered to have given rise to the precipitable fractions.

Fibrin breakdown products were produced by streptokinase and by ficin acting on a fibrin clot and studied immunoelectrophoretically. When streptokinase was used two independent arcs of precipitation occurred, while the reaction products in the case of ficin gave rise to only one arc. This indicates that fibrin contains at least two immunological determinants, and that the sites of action of the two agents (streptokinase and ficin) on fibrin are different.

A possible interpretation is that streptokinase which produces fibrinolysin in the presence of profibrinolysin present in fibrin, cleaves fibrin into at least two large fragments, each carrying one immunological determinant, while ficin reduces fibrin to smaller fractions. The residual large fragment carrying both immunological determinants.

ENERGY PRODUCTION IN THE MYOCARDIUM†‡

W. G. Nayler, J. E. Anderson, P. Emery, C. C. Curtain, P. G. C. Robertson and T. E. Lowe

This long term project is directed at the methods by which chemical energy supplied to heart muscle is converted into mechanical energy in the blood stream as a result of the contraction and relaxation of the myocardial contractile elements. During the past few years much of the study has been devoted to an elucidation of the role of calcium ions in this process and this aspect of the work continues. Other facets of the study currently being investigated include the negative inotropic action of amines, the action of certain drugs on myocardial enzymes, the nature of cardioactive plasma substances and the electrical activity of the myocardial cell membrane.

CALCIUM IONS

Eighty years have elapsed since Ringer first described the relationship which exists between the ability of the heart to contract and the extracellular calcium concentration, but the possibility that calcium ions participate in the mechanisms responsible for the maintenance and regulation of cardiac contractile activity continues to arouse interest and provoke speculation. Our previous investigations lead us to conclude that both the efficiency with which the heart performs its mechanical work and the amount of work it does are regulated in part by the availability of calcium ions.

Absence of Added Cations

Provided that the extracellular concentration of sodium ions has been greatly reduced, isolated hearts will continue to contract in response to electrical stimulation in the absence from the perfusate of calcium ions. This continued ability to contract in the absence of added extracellular calcium ions has been interpreted by some to mean that calcium ions are not essentially involved in the maintenance of cardiac contractile activity. However, experiments conducted during the current year have shown that calcium ions, detected as Ca^{45}, continued to emerge from cardiac muscle bathed with a calcium-free perfusate. The calcium ions which emerged from the muscle into the perfusate were readily separated into three distinct components, first, the rapidly diffusing fraction, emerging during the initial five minutes immersion in the calcium-free fluid; secondly, a more slowly diffusing fraction emerging during the subsequent fifty-five minutes immersion in the calcium-free fluid; and thirdly, a slowly emerging fraction appearing in the perfusate after one hour's immersion and continuing to emerge throughout the following four hours. For convenience these three fractions have been related as follows; the rapidly appearing fraction with the surface or membrane bound calcium-complex, the second fraction

with the intracellular freely-ionized calcium, and the third fraction with the bound intracellular calcium. Calcium ions, therefore, continue to diffuse from the muscle into the perfusate even after four hours perfusion with a calcium-free fluid, and it seems probable that it is these calcium ions which in the presence of a reduced intracellular sodium concentration participate in the maintenance of electrical excitability and contractile activity. These experimental findings support our previous conclusion, that bound cellular calcium ions may, under certain circumstances, participate in the regulation of cardiac activity.

Nicotine

Previous investigations into the mode of action of nicotine indicated that its effect on cardiac contractile behaviour reflected its ability to increase the exchangeability of calcium in cardiac muscle. Additional studies have shown that the altered exchangeability of calcium ions in nicotine-treated muscles is independent of any change in the transmembrane potential difference and it can be concluded, therefore that nicotine displaces cellular calcium without simultaneously evoking a change in the transmembrane potential difference.

Tetraethylammonium Ions

Tetraethylammonium ions (TEA^+) augment the cardiac contractions of isolated heart-lung preparations. Experiments have shown that concentrations of TEA^+ which evoke a positive inotropic response and prolong the action potential in toad ventricular muscle enhance the exchangeability of calcium ions, detected as Ca^{45}. This observed increase in the exchangeability of calcium ions in tetraethylammonium treated muscle reflects an increased mobility of calcium ions, and seemingly underlies the positive inotropic activity of the drug. No evidence was obtained to suggest that the TEA^+ displace bound intracellular calcium ions; instead it appears to alter only the exchangeability of the surface and the freely-exchangeable calcium fractions described above.

Xanthines

The pharmacological properties of the dimethylxanthines, theophylline and theobromine, closely resemble those of the trimethylxanthine, caffeine. Previous studies indicated that caffeine enhanced the exchangeability of calcium ions in cardiac muscle, and additional studies now have shown that the dimethylxanthines similarly enhance the exchangeability of cardiac calcium. Theophylline, theobromine and caffeine all share at least one other common property, namely, that they can displace some of the cellular bound calcium. This ability to alter the exchangeability of cellular calcium and to displace some of the bound calcium fraction prompted an investigation into the possible effects of these xanthines on quinidine-induced cardiac arrhythmias.

Quinidine and Xanthine Antagonism

It is now well established that quinidine evokes bradycardia and depresses the contractions of isolated spontaneously beating mammalian hearts; moreover it blocks A-V conduction in electrically stimulated isolated hearts. An extensive series of experiments has shown that theophylline, theobromine and aminophylline, another xanthine derivative, all lessen the severity of the bradycardia

and A-V block induced by quinidine in isolated hearts. This antagonism may prove to be clinically useful in the treatment of quinidine-induced arrhythmias. Other experiments have shown that the xanthines reverse the pentobarbital-induced depression of cardiac contractions indicating the possibility that pentobarbital may exert its depressant effect on the myocardium by reducing the exchangeability of calcium ions.

Zinc

Isaacson and Sandow reported recently that zinc potentiates the twitch tension of the frog sartorius muscle by as much as two to three times. As cardiac muscle is known to contain appreciable quantities of zinc, the possibility that zinc might activate the contractile mechanism in cardiac as in skeletal muscle therefore was considered and investigated. In a large number of experiments no evidence was obtained to suggest that zinc does exert a positive inotropic effect on isolated cardiac muscle, both mammalian and amphibian ventricular muscle having been depressed following the addition of 0.005 to 0.05 mM zinc. Other workers have suggested that zinc exerts its effect on skeletal muscle by displacing cellular bound calcium ions but in the present study no evidence was obtained to support this hypothesis for cardiac muscle.

Negative Inotropic Activity of Amines

Although the sympathomimetic amines normally evoke a positive inotropic response from cardiac muscle, in previous studies it was shown that noradrenaline and adrenaline depressed the contractions of isolated hypothermic rat hearts. Recently we have shown that if the extracellular sodium concentration be reduced so that 60% or more of the extracellular sodium be replaced with sucrose, lithium or choline chloride, tonicity being maintained, then noradrenaline, adrenaline and isoproterenol depress ventricular contraction of isolated toad hearts. Other drugs, including caffeine, aminophylline, nicotine and theophylline, which increase the exchangeability of calcium ions, continue to evoke a positive inotropic response when exhibited under conditions of perfusion which reversed the inotropic action of the sympathomimetic amines. The relationship between the negative inotropic response evoked by the amines under these perfusion conditions and the phosphorylase a/b ratio is being investigated.

Cardioactive Drugs and Na-K Dependent ATPase Activity

The functional significance of the endoplasmic reticulum in muscle cells, including those of cardiac muscle, is as yet unknown. Particulate fractions derived from and associated with this reticulum contain a sodium-potassium dependent ATPase enzyme. Because of the changes in sodium and potassium flux which are known to be associated with each membrane depolarisation and repolarisation, and because of the marked effects exerted by calcium ions and by certain drugs, including quinidine and dilantin, on the permeability of cardiac cell membranes to sodium and potassium ions an investigation into the effect of certain cardioactive drugs including quinidine and dilantin, on this particular ATPase enzyme has been undertaken. A reliable method for the assay of this ATPase enzyme in cardiac muscle microsomes has been developed using Tris-ATP as the substrate.

MEMBRANE POTENTIALS OF CARDIAC MUSCLE CELLS

Initiation of Action Potential

The time interval between stimulation of a toad ventricular myocardial cell and the appearance of the upstroke of an action potential in a similar distant cell has been found to depend on the magnitude of the stimulating current, the concentration of Na^+ and Ca^{++} in the extracellular fluid and the temperature of the preparation. When the logarithm of this time interval is plotted against the inverse of the absolute temperature (an Arrhenius plot) a straight line is obtained. The correlation of the experimental points suggests that for almost the whole of the measured time interval a chemical reaction may be taking place with an Energy of Activation given by the gradient of the Arrhenius plot. Preparations perfused with Ca^{++}-free Tyrode solution had an increased Energy of Activation indicating that Ca^{++} may be playing the part of a catalyst in the process of stimulation.

Time Course of Action Potential

Experiments were carried out on toad ventricular muscle to determine the time course of depolarisation of the cell membrane when exposed to 100 mM K^+ concentration in the perfusion fluid. It was found that depolarisation proceeded at a rate much slower (approx. 1/200) than that reported by Hodgkin and Horowicz for skeletal muscle for exposure to 190 mM K^+ concentration. Also, the final constant potential reached was not that expected from the Nernst equation suggesting that saturation of potassium binding sites on the outside of the membrane might have occurred. The maximum possible concentration of K^+ immediately adjacent to the membrane of the ventricle cell appears to be 38 mM, as calculated from the Nernst equation.

CARDIOACTIVE PLASMA SUBSTANCE

Using the techniques previously reported plasma has been collected from a series of normal males and has been fractionated with an acrylamide separation column.

During experiments directed to developing an assay method for these active substances it was necessary to store both plasma and the fractions. It was found that whereas there was no loss of plasma activity in the whole plasma on storage, either in the frozen state or at 20°C, the separated fractions showed some loss of activity. The degree of loss depends on the length of storage.

It has also been incidentally noted that there is an increase in plasma activity in a post-prandial collection compared to that from the same patient when fasting. This phenomenon will need further study.

Only one patient with congestive cardiac failure, not previously taking digitalis, has been tested to date but it is of interest to note that his plasma had a greatly reduced inotropic action. Difficulties in fractionation and assay are making progress slow in these studies.

HAEMODYNAMIC STUDIES‡

T. E. Lowe, A. J. Barnett, D. Race, P. de V. Meiring
and P. G. C. Robertson

For the efficient functioning of the body the supply of an adequate flow of blood to each and every tissue is essential. As the activity of various organs varies greatly from time to time and there is an upper limit to the minute flow of blood which the heart can produce some mechanism for the control of the distribution of blood throughout the body is clearly essential. To elucidate this mechanism it is necessary to be able to record the state of the circulation in various parts of the body at various times and under varying circumstances. In a general way such a record would give the pressure and flow rate at any instant in all parts of the circulation, but such measurements are difficult to make and most of the data available refer to mean pressures and mean flow rates at various points. The ratio of these two measurements is commonly referred to as vascular resistance which is believed to be an index of the state of the blood vessels in the region studied. Variations in this state due to changes in activity of the muscle in the vessel walls is considered to be part of the mechanism controlling the distribution of blood.

The relative ease with which blood pressure can be recorded and the relative difficulty of measuring even mean blood flow has led to clinical emphasis being placed on blood pressure measurements. In the studies being made data concerning blood pressures and flows are being recorded in patients with abnormal states of the circulation, such as postural hypotension, and in animals with such states experimentally produced.

By these methods it is hoped to be able to characterise the pressure-flow patterns in the circulation and to deduce therefrom some of the characteristics of the mechanism controlling blood distribution. If this mechanism is of a negative feedback type then there should be conditions in which the feature or features of the circulation monitored will oscillate in magnitude. This has been recorded for blood pressure.

CLINICAL STUDIES

In a number of subjects blood pressure and flow patterns have been recorded simultaneously at a central and at a peripheral site. Central pressure has been recorded from a strain gauge manometer attached to a Cournand needle inserted into the left brachial artery and central flow by cardiac output calculation by a dye dilution technique. The dye is introduced through a teflon catheter inserted through the right antecubital vein and blood sampled for dye densitometry through the needle in the left brachial artery.

Peripheral pressure is presumed to be indicated by that in the brachial artery and peripheral flow in the forearm is measured by strain gauge plethysmographs applied to the right forearm and right index finger; the former indicating chiefly muscle blood flow and the latter skin flow. Permanent records of data have been made by recording galvanometers and arterial blood pressure and heart rate monitored by a cathode-ray oscilloscope.

Effects of Posture

To study the effect of postural changes on the pressure-flow pattern of the circulation, the subject lay on a tilting bed with his arms extended sideways so that as the axis of rotation of the bed was at head level the recording points in his arms remained at this level during various tilting manoeuvres.

A number of subjects with no clinical evidence of any circulatory disturbance were used as controls. Tilting the bed from the horizontal to 60° produced a fall in cardiac output (central flow) but little or no change in mean blood pressure indicating a general increase in resistance to flow in the circulation. Similar changes were recorded peripherally in both muscle and skin.

Four patients suffering from "postural hypotension" were similarly studied and showed a fall in both central pressure and flow, in 3 of the 4, a decrease in the ratio of these indicating a lower of resistance to flow. Peripherally changes in the ratio suggested some decrease in resistance to flow in the muscle or skin regions studied in 3 of the 4 patients.

Effects of Butyl-sympatol

Ten subjects were given an intramuscular injection of 50 mgm of this drug which is claimed to be a vasodilator. Measurements 15-20 minutes later showed an increase in central blood flow with little or no change in blood pressure indicating a general lowering of resistance to flow. Changes similar in direction were recorded peripherally in most subjects but in 5 patients with vasospastic conditions, this was too small to be of clinical significance.

Effects of Valsalva Manoeuvre

Forced expiration against a closed glottis produces changes in the circulatory pattern. Central blood pressure changes during this manoeuvre were studied in 13 subjects without heart failure, in 2 with congestive heart failure and in 3 suffering from postural hypotension.

In those without heart failure blood pressure fell during expiration and rose excessively ("overshoot") immediately afterwards. In those with heart failure blood pressure rose during expiration and fell immediately afterwards without overshoot ("square wave response"). In those with postural hypotension there was an excessive fall during expiration and afterwards return of pressure slowly to previous levels without overshoot.

Pressure Waves

During these studies phasic variations in the level of blood pressure were frequently observed during periods of circulatory disturbance. These waves were not related to respiratory changes. They were completely absent in a patient with severe postural hypotension.

ANIMAL STUDIES

A dog preparation which allows continuous separate measurement of the venous drainage from various regions and the continuous recording of blood pressure from a number of sites has been previously described and has been used to study the effects of a number of procedures on the distribution of blood in the body.

The Effect of Halothane on Organ Blood Flow During Total Body Perfusion

In eleven experiments, dogs with an extracorporeal circulation of 120-140 cc/Kg. BW/min. were exposed to a halothane concentration of 1% in the oxygenation gases.

Within five minutes the arterial blood pressure fell to 80% of the control value and at the end of 45 minutes it had stabilized to an average value of 38% of the control level. After stopping the halothane the blood pressure slowly began to rise but at the end of 90 minutes it had only returned to 75% of the control value.

Relating the pressure measurements to blood flows through individual organs indicates that the decrease in blood pressure is associated with a fall in resistance to blood flow through the muscle beds — especially those of the hind legs — although there is a slight fall in resistance in the coronary and renal vascular beds. By contrast the measurements indicate a marked increase in resistance to blood flow through the splanchnic circulation associated with a decrease in flow to 20% of control levels.

The Effect of Prolonged Low Flow during Total Body Perfusion

Total body perfusion was carried out in a number of dogs in whom the total blood flow was reduced to 20-30 cc/Kg. BW/min. Organ blood flows were measured frequently over a period of two hours and at the end of this time the total flow was increased to the initial level of 120-140 cc/Kg. BW./min. and organ flows again measured.

In six dogs, during the period of low flow, there was a progressive fall in splanchnic blood flow to 40% of control levels. The muscular vascular beds showed an increase of blood flow to 300% of control levels indicating marked fall in vascular resistance.

The low blood pressure and low cardiac output state of this preparation is similar to the state of haemorrhagic shock, and suggests that the condition of irreversible shock might be causally related to the incongruous increase in blood flow through the muscle vascular beds.

Other observations on these preparations suggest there is a loss of neurogenic vasoconstriction but peripheral stimulation of the relevant nerves will produce vasoconstriction, so that the neurogenic failure is of central rather than peripheral origin.

Blood Pressure Waves

Rhythmical changes in arterial blood pressure have frequently been recorded in these dogs maintained with an extracorporeal circulation. The changes can be induced by both rapid inflation and deflation of the lungs and it has been shown that the reflex path involved does not include the vagus nerve.

The pressure waves have an average period of 50 seconds and by simultaneous study of independently perfused areas it has been shown that the waves have different amplitude and time lags in different regions. Further work in attempting to characterise the control mechanisms indicated by these rhythmic phenomena is proposed.

HYPERTENSIVE STATES

A. J. Barnett, P. de V. Meiring and P. G. C. Robertson

The trial of treatment of severe (complicated) hypertension with hypotensive drugs continues to demonstrate the value of these drugs in prolonging life and relieving symptoms.

One of the main interests of recent years has been to assess whether new hypotensive drugs enable better blood pressure control with fewer side effects to be achieved.

New drugs currently under trial are methyldopa (Aldomet)[1], pargyline hydrochloride (Entonyl)[2] and bethanidine (BW 467 C60)[3].

METHYLDOPA (ALDOMET)

Having previously demonstrated that methyldopa is an effective hypotensive agent without serious toxic effects, this drug has been used more extensively in the hypertension clinic and the results of this treatment over a period of 1-3 years are at present being evaluated.

There were 29 patients with varying initial grades of hypertension in this series (14 female and 15 male). Their ages ranged from 42 to 65 with an average of 53 years.

Twenty-six of them were treated, in addition to other drugs, with methyldopa, added to all or substituted for one or more of the others after previous treatment for periods from 1-10 years. Three patients were treated with methyldopa and a thiazide drug only.

The experience with methyldopa ranges from 1-3 years with an average of 1.5 years. Its dosage ranged from 0.5 gm to 4 gm daily, with an average of 1.3 grams per day and no tolerance developed once an effective dosage was achieved. The mean of 12 blood pressure readings, lying and standing, before and after the change to methyldopa, was obtained in the 26 patients who started after previous treatment and in this way it was possible to compare its hypotensive action with other drugs.

Methyldopa appears to be slightly less potent than guanethidine in most cases, though equally effective in a few. However, in most of the former it exerted its effect with minimal side effects and with a reasonable degree of control of blood pressure, thus justifying its continued use. Compared to other hypotensive agents in common use, methyldopa was found to be superior to reserpine and equivalent in action or superior to hydralazine and pempidine combined. Where methyldopa was added to treatment by several hypotensives, notably combinations already containing reserpine and guanethidine together, response was negligible, a maximum hypotensive response by drugs with roughly similar action having apparently already been obtained.

Thus methyldopa can be said to be an effective hypotensive agent in suitable patients, though for severe grades, combination with other hypotensive agents appears necessary and it does not seriously rival guanethidine as the drug of choice in such cases.

1 Kindly supplied by Merck, Sharpe and Dohme (Aust.) Ltd.
2 Kindly supplied by Abbott Laboratories Pty. Ltd.
3 Kindly supplied by Burroughs Wellcome & Co. (Aust.) Ltd.

PARGYLINE HCl (ENTONYL)

This drug is of interest particularly as it belongs to a different group from the currently used hypotensive agents. In order to assess its value a "double blind" trial has been undertaken on a co-operative basis with Drs. M. C. Davis, H. B. Kay and J. M. Gardiner of the Hospital Honorary Staff.

The trial was designed to compare pargyline with an established hypotensive drug (guanethidine) and with an inert placebo, starting with doses of 25 mgm per diem rising by increments to 75 mgm per diem. Each course lasted six weeks with two weeks between courses during which standard toxicity tests were performed. Each participating unit selected six patients. As this trial is not yet completed no assessment of the value of the drug can be given.

Pargyline has not yet been used in the clinic for severe (complicated) hypertension.

BETHANIDINE

Bethanidine is a new hypotensive drug related structurally to both bretylium and guanethidine and is believed to act by preventing release of transmitter substance from sympathetic nerve endings. Its onset and duration of action are much shorter than with guanethidine (hours instead of days) and this should have certain advantages in allowing more rapid control of hypertension and more rapid correction of effects of overdose. Currently 12 patients who have been receiving guanethidine are now being treated with bethanidine and also a series of patients admitted for control of hypertension are being so treated.

Evidence from our experience to date is that bethanidine is a potent hypotensive agent.

CONTROL OF BODY FLUID VOLUME

T. E. Lowe, A. J. Barnett, J. B. Dawson and P. de V. Meiring

Studies this year have continued to be devoted to the development of an electrode suitable for the continuous recording of pNa in the blood stream and to a clinical trial of another new diuretic drug.

CONTINUOUS RECORDING OF pNa IN THE BLOOD STREAM

Last year we reported the construction of a small cannula electrode made from Na^+ sensitive glass which could be implanted in a major artery of a pig.

The surgical procedure for insertion of such an electrode in the aorta has been further developed using "Halothane and oxygen" anaesthesia and an extraperitoneal approach to the abdominal aorta. The electrode is inserted into the aorta through a longitudinal incision about one inch below the lower renal artery and the electrode leads are brought to surface through a flank incision. With the assistance of Mr. I. A. L. Ferguson, Honorary Surgeon, Alfred Hospital, it has been possible to do this with the aortic flow interrupted for only 6 minutes thereby avoiding spinal cord ischaemic symptoms which have previously been troublesome. With subsequent antibiotic cover the animals have suffered no disability and electrical recording from the implanted electrode has been satisfactory.

Modifications to the electrode have led to a considerable reduction in its size. The reference electrode has been changed from a calomel half-cell to silver-silver chloride and a sodium or potassium agar gel used for the junction between reference and recording electrodes. The reference electrode and junction together with a thermistor are now situated in a side chamber and separated from the fluid stream by a permeable membrane.

The voltages generated by the ion sensitive electrode are applied to an E.I.L. Vibron Electrometer and registered together with temperature, as indicated by the thermistor, on a Kipp and Zonen "Micrograph" recorder.

Electrical stability of this electrode system is good but not perfect. Coagulation problems when blood flows through it have not been adequately solved, so that its practical application is as yet to situations with noncoagulating fluids. It is believed that the electrode will measure sodium ion concentrations correct to 2 m.eq. or less.

CLINICAL TRIAL OF NEW DIURETIC

With the steady development of new types of diuretics over the past 10 years, the treatment of oedema has become more satisfactory.

One of the newest of these agents is ethacrynic acid[1], 2,3 dichloro 4(2-methylene butyryl) phenoxy-acetic acid, which is reputed to act in a manner different from other known diuretics and to have an additive effect when used with the other agents.

Some preliminary observations using ethacrynic acid as a supplementary diuretic in patients with an inadequate response to other diuretic agents show this additive effect.

DISEASES OF BLOOD VESSELS

A. J. Barnett, P. de V. Meiring, V. Carson, K. N. Morris[2] and G. R. Stirling[2]

RECONSTRUCTIVE SURGERY

Suitable patients with obstructive arterial disease continue to be treated surgically and certain modifications in both diagnostic and surgical procedures have been made.

Pelvic arteriography using a Seldinger catheter introduced in a retrograde direction from the common femoral artery is performed more frequently and is done in all patients in whom surgery is contemplated and in whom there is a suspicion of inequality of the femoral pulses or diminution of the oscillometric readings from the upper part of the thighs.

The operative technique has become more varied so that whereas previously the standard procedure for a localized arterial obstruction was to by-pass it with an arterial graft this is now commonly combined with endarterectomy below the graft, angioplasty or lumbar sympathectomy. In cases with relatively short blocks, endarterectomy is more commonly performed and in 38 cases

1 Kindly supplied by Merck, Sharpe and Dohme (Aust.) Ltd.

2 Thoracic Surgical Unit, Alfred Hospital.

operated upon for occlusive arterial disease of lower limbs during 1963 an arterial graft has been inserted in 21 only.

Our impression is that the results of this recent approach are better than those previously obtained but accurate assessment of this may be difficult for many patients now being operated upon would previously have been rejected as unsuitable.

LIPIDS IN ATHEROSCLEROSIS

Because of the demonstrated association of raised plasma cholesterol levels with atherosclerosis and the presumed causative role of increased cholesterol (and other lipid) levels in the development of this disease, attempts have been made to prevent or treat atherosclerosis by measures aimed at lowering the level of plasma cholesterol. We have previously reported on several of these methods and although some success in lowering plasma levels has been achieved all the methods have had disadvantages.

Dietetic measures involving the restriction of saturated fats need to be strict and are irksome to the patients; high dosage of nicotinic acid is associated with severe side effects in a proportion of patients.

MER29 has been withdrawn by the manufacturers because of reported serious toxic effects.

Atromid[1] is another preparation claimed to lower not only the plasma cholesterol level but also the levels of other lipids raised in atherosclerosis. It is a mixture of androsterone and chlorphenisate (ethyl-p-chlorophenoxy-isobutyrate). Although the mechanism of action is obscure, it is suggested by the makers that the active agent is androsterone and that the chlorphenisate serves to maintain this in an active form by preventing binding to plasma proteins.

The effect of Atromid in atherosclerotic patients has been studied with observations on blood levels of total lipids, triglycerides, cholesterol and uric acid.

Total lipids were determined by a gravimetric method, triglycerides by a modification of the method of Van Handel and Silversmit and cholesterol by the method of Abell *et al.*

The following normal levels for our laboratory were established from subjects (from hospital staff and a convalescent hospital) without clinical atherosclerosis.

Fasting total lipids	450-800 mg./100 ml.
„ triglycerides	60-150 mg./100 ml.
„ cholesterol	130-240 mg./100 ml.

1 Kindly supplied by Imperial Chemical Industries of Australia and New Zealand Ltd.

Thirteen men with clinical evidence of atherosclerosis of coronary or peripheral arteries were selected for study. Their fasting serum lipids were first measured weekly for 6 weeks and then they were treated with Atromid 2.25 g./day for 3 months. Fasting plasma lipids were determined fortnightly during the first half of treatment, and weekly during the final 6 weeks. Atromid treatment was then stopped and the fasting plasma lipids again determined after one, two and three months.

The effects of Atromid treatment on plasma lipid levels may be briefly summarized.

Lipid measured	Initial Level Raised		Initial Level Normal	
	Number of Patients		Number of Patients	
	Total	Showing significant[2] fall after Atromid	Total	Showing significant[2] fall after Atromid
Total lipids	10	8	3	0
Triglycerides	10	6	3	1
Cholesterol	8	6	5	0

[2] $p = 0.05$ or less.

In general a fall in the lipid levels occurred only in those patients in whom they were initially raised.

On stopping the "Atromid" treatment, there was a rapid return of lipid levels toward, and sometimes beyond, their initial level.

The serum uric acid level showed a slight fall in most cases.

Determination of Plasma Triglycerides

The method of Van Handel and Silversmit for the spectrophotometric determination of plasma triglycerides has been modified using a locally produced zeolite resin which is cheap and easily obtainable, in contrast to the expensive "Doucil".

In principle the method depends on the extraction of the lipids from plasma by a chloroform-zeolite mixture during which process the phospholipids are retained on the zeolite and the triglycerides and cholesterol move into the chloroform phase. The triglyceride is then estimated by saponifying the ester and subjecting the free glycerol to periodate oxidation. The formaldehyde so formed is estimated colorimetrically using the chromotropic acid reaction.

Optimum conditions for the test have been established and several hundred determinations have been done. The standard error of a single determination ranges from ± 1.7 to $\pm 3.2\%$ corresponding to a range of triglyceride values from 340 to 68 mg.% respectively.

CARDIAC SURGERY†‡

K. N. Morris[1], E. Cooper[1], Fay Kinross[1], D. Race,
G. R. Stirling[1] and V. Carson

The development of techniques for the replacement of diseased valves by prostheses has been responsible for a further change in emphasis in cardiac surgery. Operations for acquired disease of the valves now exceed in frequency those for congenital heart disease. Growing confidence in the new techniques has resulted in the application of open heart surgery to conditions formerly inoperable, such as aortic regurgitation, and to their more frequent application in conditions such as mitral stenosis, mitral regurgitation and aortic stenosis.

PUMP OXYGENATION

To meet the more exacting needs of prolonged total body perfusion in the adult and the problem of continuous coronary perfusion in aortic valve disease a completely new pump-oxygenator has been designed and constructed in the hospital workshops. Mr. F. Kohler and Mr. F. Merree have been principally concerned with construction of the apparatus. The machine is basically a spinning disc oxygenator and four different sizes of oxygenator units are available. Five single roller pumps are mounted on the chassis so that, in addition to the arterial pump, two separate pumps are available for independent coronary perfusion and two to four are available for cardiotomy return. Separate heat exchangers with independent control are available for systemic and coronary perfusion. The venous reservoir, elevated by a power drive, and the cardiotomy return have been developed in our laboratories. Monitoring and control instruments record the flows through each of the pumps, the temperatures in the oxygenator, both heat exchangers and the patient and pressures in the patient's arterial and venous systems and in the lines of the arterial and coronary pumps. We have been very pleased with the performance of the apparatus.

SURGERY OF THE MITRAL VALVE

The application of a satisfactory method of evaluating mitral valve competence after surgical repair has been responsible for greater precision and growing confidence in reparative procedures for mitral regurgitation and stenosis. We have found a complete correlation between the findings noted during valve testing at open cardiotomy and the immediate post-operative result. This has been a significant technical advance.

Valve replacement with a Starr-Edwards prosthesis has proved an acceptable operation in those cases where reconstruction is not feasible due to heavy calcification.

AORTIC VALVE SURGERY

The technical problems of providing effective continuous coronary perfusion during open operation on the aortic valve have been largely overcome. Two separate pumps with metered outputs and line-pressure control are connected to flexible plastic cannulae fitted with a compressible "O" ring which sit snugly

1 Thoracic Surgical Unit, Alfred Hospital.

in each coronary orifice. Uninterrupted perfusion has been the rule and has enabled us to seriously attack the problems of aortic valve repair. The Starr-Edwards prosthesis has been used successfully for the treatment of both calcific aortic stenosis and aortic regurgitation with encouraging results.

In anticipation of the use of homograft aortic valve replacement the problems of valve collection, sterilisation and storage have been studied. Irradiation appears to be the method of choice for homograft sterilisation.

HEART BLOCK

The treatment of complete heart block by the implantation of a transistorised cardiac pacemaker has now become established surgical practice. During 1963, 10 implantations have been carried out with a successful outcome in eight cases. The apparatus used has included the Chardack-Greatback pacemaker and the St. George's Hospital pacemaker. An important advance has been in the use of a bipolar cardiac catheter electrode and an external cardiac pacemaker in the pre-operative phase. The electrode-catheter can be passed quickly and easily under fluoroscopic control and this enables a more planned and definitive operative implantation of an internal pacemaker at a time of election. Electrode fracture has been a problem in one instance after implantation.

PULSE DULPICATOR

The study of normal and diseased cardiac valves using this technique is a continuing project. Cine-film strinps of selected specimens have been compounded into a film on normal and abnormal valve function.

HAEMOLYSIS

The sources of haemolysis during total body perfusion has been analysed. The contributions of the pump-oxygenator, the cardiotomy return system and the donor blood have been separately studied. The outstanding source of high serum haemoglobin levels during perfusion has been related to the former practice of returning to the oxygenator blood which had escaped into the pericardial cavity. The levels of serum haemoglobin in this blood have been consistently high and since discarding this from the system acceptably low levels of serum haemoglobin have been consistently achieved during periods of perfusion of up to 150 minutes. The mechanism of haemolysis under these circumstances is the subject of a study to be elaborated in 1964.

HAEMORRHAGE AFTER TOTAL BODY PERFUSION

Excessive haemorrhage following operations in which total body perfusion has been used has remained as an occasional problem. Drs. P. Fantl and J. McLean have taken part in a joint study with the surgical team to investigate in the broadest terms the demonstrable defects in the coagulation mechanism after total body perfusion. In alternate cases the transfusion of unmodified blood ("direct blood transfusion") has been used immediately after operation. The case groups should be of adequate size for a comparative study to be made early in 1964.

SPECTROPHOTOMETRIC DETERMINATION OF BLOOD OXYGEN SATURATION

The spectrophotometric determination of oxygen saturation by the method of Roos and Rich has been adapted for use with dog blood.

A Beckman DU spectrophotometer with the light path of standard 1 cm. cells reduced to 0.5 mm by the use of perspex blocks of thickness 9.5 mm was used to obtain optical densities which were in the range 0.070-0.700. The ratio of oxyhaemoglobin to total haemoglobin was determined by measuring the optical density of saponin-treated blood at wavelengths of 600 mμ and 503 mμ, the latter wavelength being the isobestic point for dog blood. Optical constants were determined from the optical densities, at the two wavelengths, of fully oxidized and fully reduced blood. These constants varied in samples from different dogs and it was necessary to determine them for each experiment.

The quality of saponin was critical and B.D.H. white saponin gave good results. Optical densities were stable for about 7 minutes giving adequate time to do a number of determinations simultaneously.

Preliminary comparison with results obtained by the gasometric method of Van Slyke indicates that agreement will be satisfactory. However, comparison of the ratio of total haemoglobin to oxygen capacity in different dogs indicates that this ratio varies and the whole question of so-called "inactive-haemoglobin" warrants further investigation.

The perspex blocks were made quite simply and cheaply in our laboratory and the use of standard laboratory equipment and techniques allows the analysis of many times the number of samples that could be estimated by the laborious Van Slyke procedure.

ELECTRODE MATERIALS FOR CARDIAC STIMULATION

Some experiments were carried out to determine the most suitable metal for electrodes to be applied to the heart for direct cardiac stimulation. Indications are that silver-coated copper braid is superior to stainless steel and Surgaloy.

THE EFFECT OF HYPAQUE 85 ON HAEMODYNAMICS

This agent when used in clinical practice for the production of left ventricular angiograms causes a short period of hypotension and a temporary increase in left artrial pressure. Studies on animals using our method for the assessment of myocardial function before and after various procedures show that this radio-opaque substance causes an ephemeral depression of myocardial function and a prolonged decrease in peripheral resistance.

BLOOD PROTEINS*

C. C. Curtain and A. Baumgarten

This report is arranged as in previous years into sections (a) Instrumentation, (b) Techniques and (c) Investigations.

INSTRUMENTATION

HIGH VOLTAGE GEL ELECTROPHORESIS TANK

The micro-method (see below) of serum protein electrophoresis in thin layers of acrylamide gel has two limitations. First, a protein component in the sample may be too low to be detected after staining of the pattern and secondly, the protein pattern may be too short for adequate resolution of components. The first could be overcome by using larger or more concentrated samples in larger gels and the second by using a high voltage gradient to keep the time of electrophoresis short enough to avoid diffusion of components. In both cases dissipation of heat generated becomes difficult. To solve these problems an electrophoretic tank measurying 30 cm by 24 cm by 72 cm has been constructed of "perspex". The gel is sandwiched in the tank between a brass plate and a brass cover, both sealed in epoxy resin and both cooled by refrigerated water or alcohol/water mixtures pumped through copper pipes welded to the brass. The degree of cooling can be adjusted in response to the internal temperature measured by a thermistor attached to the plate. Four buffer tanks around the periphery of the plate provide facilities for two-dimensional electrophoresis to be carried out without disturbing the gel. Platinum strips attached to the sealed surface of the brass plate permit determination of the actual voltage across the gel, while an ammeter in series with the tank allows determination of the current which can be altered by resistances in series. The gels use in the tank are 3 to 6 mm. thick. At pH 9 and 900 volts a 15 cm long serum protein pattern can be obtained in 60 to 90 minutes. Because spreading of components is minimal under such precisely controlled conditions, an excellent resolution of components is achieved. This tank should be particularly useful for the resolution of the peptides produced by the partial enzymatic hydrolysis of proteins in the course of structural studies.

TECHNIQUES

At its present stage this project is largely dependent upon the application of some of the newer methods of protein analysis to the study of sera obtained from large populations, mostly in New Guinea, in an attempt to evaluate the relative importance of environmental and genetic influences on the polymorphism of various blood proteins. As the greatest economy must be exercised in regard to both sample size and time taken for each analysis much effort has been devoted to the development of rapid microtechniques.

MICROMETHOD OF SERUM PROTEIN ELECTROPHORESIS IN ACRYLAMIDE GELS

Electrophoresis of serum in media such as paper, cellulose acetate or agar allows a resolution of between five and eight protein components. In last year's report, techniques were described whereby much analysis could be performed within ten minutes on samples as small as 0.1 microlitre. However, by immunoelectrophoresis or starch-gel electrophoresis each of the zones observed on paper can be resolved into several components. Therefore, although much information

has been gathered on the empirical relationship between disease states and variations of the serum protein patterns on paper, a re-examination of these relationships appears necessary to elucidate the role of individual protein species revealed by more sensitive techniques. To this end, and also to permit the examination of large numbers of small samples, it was necessary to develop a simple method capable of giving at least similar resolution to that of the more cumbersome starch-gel technique.

A micromethod of serum protein electrophoresis in thin layers (1 mm) of acrylamide gels on microscope slides has been developed which is very simple because the gels are connected to open buffer tanks and because of their excellent heat dissipation no special precautions are required to prevent heating or evaporation. Several samples may be simultaneously examined on the one slide for comparative analysis under identical conditions. Less than one microlitre of serum is required per sample and duration of electrophoresis is only 30 to 40 minutes. Other advantages over the starch-gel technique are: uniformity of the acrylamide gels owing to the synthetic nature of the medium, controllable "pore" size related to the concentration of acrylamide, greater mechanical strength, resistance to a wide variety of physical and chemical agents and stability over the entire pH range, stability on prolonged storage before use, complete transparency, absence of endosmosis due to lack of charged groups in the gels, the ability to preserve the gels after use by drying out on glass slides, small size which decreases the cost of expensive enzyme stains, and virtually negligible cost of acrylamide itself per sample as compared with the not inconsiderable cost of hydrolysed starch.

Following the initial application of the method to the resolution of haptoglobins and to the identification of catalase in erythrocyte haemolysates the influence of buffers and other factors on the satisfactory resolution of serum protein was investigated. It was found that an approximate molecular grading of proteins could be made on the basis of their penetration into gels of differing concentrations, since a 5% gel had an approximate exclusion limit of substances of M.W. 500,000, a 7½% one of 300,000 and a 12% one of 100,000. Thus, the entry of an abnormal serum protein into a 5% gel immediately precludes a diagnosis of macroglobulinaemia, whilst a failure to enter confirms the diagnosis. The resolution of serum transferrins was confirmed by autoradiography using Fe^{59}. Caeruloplasmin was demonstrated by the staining procedure described below. Zones containing esterase activity were demonstrated by a staining method using diazotised p-rosanilin. A method was developed to demonstrate zones of amylase activity by incorporating starch in the acrylamide gels. Other enzymes were identified, including lactic dehydrogenase isozymes in the serum and glucose-6-phospate dehydrogenase in red cell lysates. Abnormal haemoglobins, S and Leporc have been resolved successfully from haemoglobin A using the micromethod, just as they have been resolved previously in large starch and acrylamide gels. The micromethod is also being used routinely for the resolution and quantitation of haemoglobin A in cases of suspected thalassaemia minor. The gels are also being used routinely in our studies of population genetics of haptoglobins, transferrins and abnormal haemoglobins. 2000 samples have been examined in 6 months. With the same number of personnel a previous survey of 2000 samples using starch-gel electrophoresis took 2 years. Its simplicity, high resolution and diversity of application suggest that the acrylamide gel microslide will find widespread use.

The availability of thiocholine esters as histochemical reagents prompted an investigation of the possibility of following potentiometrically their hydrolysis by cholinesterases with the aid of a silver-thiol electrode. It was found that a silver-plated gold-on-platinum electrode with a thin surface layer of silver mercaptide (formed by dipping the wire into dilute hydrogen sulphide solution) gave a potential which was proportional to the concentration of thiocholine liberated by the hydrolysis of its esters. This has been made the basis of an automatic method, using 0.05 ml of serum, for the determination of the activity of serum pseudocholinesterase and its inhibition by dibucaine, fluoride and solanidine. The electrode system and potentiometer replaces the photometer normally used in our automatic analysis system. The potentiometer readings are printed by an electric typewriter, then transferred to punched cards and the kinetics of the pseudocholinesterase in the serum sample in the presence and absence of the inhibitors is analysed in an IBM. 1401 computer.

A widespread survey of serum pseudocholinesterase variation in New Guinea is important for two reasons. The serum pseudocholinesterase level may be used as an index of liver function and this is of some importance in deciding whether the extremely high γ-globulin levels found in many of the coastal peoples are a reflection of a high antigenic load or are due in part to liver damage consequent upon protein malnutrition. Individuals with low serum pseudocholinesterase levels and those possessing some rare genetically controlled variants of the enzyme, detectable by an analysis of the enzyme kinetics in the presence of fluoride, dibucaine and solanidine, are dangerously sensitive to the muscle relaxant suxamethonium and to certain agricultural insecticides. As the spread of modern medicine and agriculture will bring new populations in contact with these agents it is important to evaluate the risks.

AN IMPROVED METHOD FOR THE DETECTION OF CAERULOPLASMIN

The serum copper-binding protein caeruloplasmin, may be detected after electrophoresis or chromatography by its ability to oxidise paraphenylenediamine to a dark quinonoid polymer. In the course of attempts to discover a less-toxic reagent amongst the paraphenylenediamine derivatives used as colour photographic developers it was found that the colour produced by the oxidation of paraphenylenediamine could be intensified sevenfold if the reaction were carried out in the presence of the colour photographic coupling agent 2-cyano-acetyl coumarone. Unfortunately, paraphenylenediamine must be still used as the substrate for the oxidase activity of caeruloplasmin as none of its less-toxic derivatives are oxidised to any significant extent. In addition to increasing the sensitivity of detection of caeruloplasmin the method also enables times of incubation with the reagents to be shortened thus reducing diffusion of the caeruloplasmin in the electrophoretic and chromatographic media and enhancing resolution.

A NEW METHOD OF CONCENTRATING SMALL VOLUMES OF PROTEIN SOLUTION

The current methods of concentrating protein solutions are, for a variety of reasons, unsuitable for small volumes (0.4-1.0 ml). The new method consists of equilibrating the protein solution with a rod of dry hydrophilic gel, prepared from acrylamide cross-linked with N,N-methylenebisacrylamide. When the volume of protein solution is reduced to the desired extent the rod is removed. A 90% reduction in volume occurs in 18 hours. No loss of protein occurs and because of the ready diffusibility of salts into the gel the ionic strength remains constant during concentration.

COMPUTATIONAL METHODS

The accessibility of high-speed digital computers has been vastly increased by the development of autocoding programming languages. These languages which presuppose no knowledge of the machine's internal structure enable programmes to be written using a notation closely resembling that of everyday algebra and symbolic logic. The programming language used in our current studies is Fortran (formula translation) which is in use at the Melbourne Service Bureau of IBM (Aust.). We are however, examining other autocodes (Algol, Cobol and Elliott's) for their suitability for biomedical computation. In addition to the specialised programmes for the statistical analysis of our New Guinea blood protein data we have devised two programmes which may be of general utility.

A GENETIC DATA CONCORDANCE PROGRAMME

A tremendous amount of labour is involved in the testing of phenotypic data determined experimentally from haptoglobins, transferrins, glucose-6-phosphate dehydrogenase deficiency, Gc type, haemoglobin types and blood groups against genealogies, often of considerable size. A programme has been developed which allows the correlation of the parental phenotypes with those of the children to be carried out by an electronic computer using a punched card input. For the purpose of the programme each individual is assigned a family number in addition to his individual number. The family status of child or parent is arithmetically defined, a family being defined as consisting only as parents and children. Where an individual is a parent of the second or successive generation his card is duplicated and he is assigned a different family number as a child and as a parent. The same process takes place in case of multiple marriages of the one individual and the ensuing offspring. The genetic parameter is also numerically defined, with provision for variants, and this information is punched on the cards. The latter are then mechanically sorted into families and subsequently read into the computer, which absorbs the data pertaining to each family. It tests the children's phenotypes according to condition of "possibility" or "impossibility" defined by the parental phenotypes. The computer then prints its "decision". Where the data for the child does not "fit" that of the parents, the parental data is printed as well as that of the child and thus it is easy to check the original sources of information.

LOG LIKELIHOOD RATIO TEST PROGRAMME

It has been pointed out by a number of workers that the log likelihood ratio test (G-test) performs better for small numbers than the widely used X^2 test in comparing observations (samples) with hypothetical values (anticipated values). The G-test has been avoided largely because of the tedium of the calculations, although attempts have been made to alleviate this by publication of tables of G-values. With the ready availability of high speed computers with log function sub-routines the calculation of G-values no longer presents a problem. A G-test programme has been written in Fortran for the testing of the significance of the differences between phenotype distribution and gene frequencies in different populations and the significance of their departure from the anticipated values, calculated, e.g., by the Hardy Weinberg law.

INVESTIGATIONS

GENE FLOW PATTERNS IN THE MARKHAM VALLEY REGION OF THE TERRITORY OF PAPUA AND NEW GUINEA

This work was undertaken to improve our knowledge of the roles played by selection and genetic drift and social influences on the genetic heterogeneity of the Melanesian peoples which we have observed in our earlier studies. In this study[1], blood specimens, genealogies and anthropometric data were obtained for 3,600 individuals living in selected communities in the Markham Valley. The blood specimens were examined for the presence of erythrocyte glucose-6-phosphate dehydrogenase deficiency and are being typed for transferrin and haptoglobin. Electrophoresis will also be carried out for the detection of abnormal haemoglobins and thalassaemia trait. The incidence of erythrocyte glucose-6-phosphate dehydrogenase deficiency was found to vary widely from community to community with a decrease in incidence with increasing altitude. This confirms the findings made by Drs. Kidson and Gorman in the Markham Valley in 1961. Data from haptoglobin and transferrin typing, although still incomplete, suggests that, in confirmation of previous findings, considerable genetic differences exist between communities speaking different languages. In particular, a very high incidence of the gene for transferrin D has been found in some populations where up to 30% of the individuals appear to carry it.

Gc TYPING OF NEW GUINEA POPULATION

An investigation of the distribution of the group specific (Gc) component of Hirschfeld has been undertaken. This investigation demands immunoelectrophoretic analysis of sera and no data has been hitherto obtained for either Australia or New Guinea. The genetic polymorphism of this protein is unrelated to other commonly investigated parameters such as blood groups, haptoglobin, transferrin and enzyme differences. It is important to determine its incidence in view of the demonstrated differences in distribution of the other genetic markers, because it may help in clarifying certain difficulties associated with current theories of the origin of such differences. Data from regions of such

1 Carried out in conjunction with Dr. G. Giles, Department of Anthropology, Harvard University.

varying ethnological, nutritional and environmental circumstances as those found in New Guinea may also throw light on the role of this protein in the bodily economy. Preliminary data indicate that differences as wide as those observed in the distribution of haptoglobin and transferrin types also exist in the distribution of the Gc phenotypes.

TROPICAL HYPERGAMMAGLOBULINAEMIA

Our studies of the serum proteins of the peoples of Papua and New Guinea have suggested that the extremely high level of γ-globulin (mean 2.5 gm.%) found in adults in the lowland regions of the territory is due in the main to a high antigenic load. Further study of these γ-globulins from epidemiological, chemical and immunological standpoints may help shed light on autoimmune disease (for instance 10% of sera collected from the Tolai people of New Britain have high cold agglutinin titres) and those neoplasias of the lymphoid tissue which are associated with hypergammaglobulinaemia. Our preliminary chemical approach is based upon that of Edelman and others who found that it is possible to dissociate γ-globulin into light and heavy polypeptide chains by means of reducing agents which break disulphide bonds. Since we would expect the antigenic experience of Melanesians living in New Guinea to be not only much more intense, but also to differ in quality from that of Caucasians living in Australia, we decided to compare the reduction products of γ-globulins isolated from the two populations. No difference could be detected on electrophoresis of the reduction products in acrylamide gel so they were fractionated into light and heavy chains by gel filtration in 1 N-acetic acid on "Sephadex" G75. The separated chains were then digested with trypsin and the peptide fragments separated by ion-exchange chromatography. The heavy chains of both the Caucasian and Melanesian γ-globulins give identical peptide spectra, but the light chains of the two γ-globulins differed sharply in several respects. When more Melanesian material can be obtained it is planned to study these differences at the individual level and to investigate the influence of ecological changes on the peptide composition of the light chains.

THE MOLECULAR BASIS OF COLD PRECIPITATION AND AGGLUTINATION

As a corollary to the studies on tropical γ-globulin structure, similar analyses were carried out on a cryogel-macroglobulin and a cold haemagglutinin obtained from two patients suffering from a proliferation of lymphocytoid cells. It was found that the light chain fraction from the haemagglutinin inhibited cold haemagglutination by the patient's serum and the light chain fraction from the cryogel-macroglobulin inhibited cryogel formation by the intact macroglobulin, either isolated, or in the patient's serum. The inhibitions were specific, i.e., the cryogel light fraction did not inhibit the haemagglutinin nor did the haemagglutinin light chain fraction inhibit the gelling of the cryogel-macroglobulin. It was considered that the light chain fractions contained univalent fragments bearing the specific groupings responsible for cryogel formation or cold haemagglutination. The haemagglutinin-inhibitory fraction also inhibited cold haemagglutinin activity in all the specimens of sera obtained from some members of the Tolai linguistic group in New Britain.

It is possible, in view of the possible role of light chains as bearers of antibody specificity in the normal γ-globulins that cryo-gel formation, cryo-precipitation and cold haemagglutination in some way reflect the general ability of the light chain structure to participate in association reactions. In antibodies these are highly specific; in the cold associations specificity is lower and some prior stabilisation is necessary either of donor or acceptor sites, probably by hydrogen bonding between tyrosyl and carboxyl side chains. The latter is in accord with the spectral changes and heats of precipitation which we have observed previously for a cryoglobulin.

histochemical techniques for the identification of cell types in these diseases. A number of new techniques has been investigated and the results of their employment in exfoliative cytology and mouse leukaemia is recorded.

POST-INCUBATION FIXATION

Last year a method by which cell enzymes could be stained in unfixed histological tissue sections which were subsequently fixed and counterstained was described. Development of this technique has enabled it to be applied to blood smears and tissue imprints. The use of changes in activity of blood cell enzymes as markers of physiological and pathological events thus becomes feasible. Three different physical principles were examined in the attempts to hold cellular material on slides for staining before fixation.

Silicone Reagents

Reagents of the vinyl-chlorsilane type will anchor cells to glass for their silicone groups bind strongly to glass leaving the reactive vinyl groups to join with complementary sites on cell surfaces. However, these reagents are not suitable for routine use with large numbers of specimens because they must be handled with care, glass surfaces must be very clean and after coating the glass must be protected from dust – all time consuming requirements.

Irradiation with Ultraviolet Light

Ultraviolet light has a mild denaturing effect on cell protein and will fix cell to glass but it also inactivates cellular enzymes. By suitable control of the time of irradiation it was found possible to fix cells to glass slides before enzyme inhibition occurred. Lack of a suitable source of U-V light has held up exploitation of this phenomenon.

Dextran Solutions

Incorporation of polyvinyl pyrrolidone (PVP) in substrate solutions to increase their osmotic pressure and so to protect tissue detail has been advocated but experiments showed that PVP is a partial inhibitor of some enzymes and therefore unsuitable for this work although producing satisfactory adherence of cells to glass.

Preliminary experiments indicate that a suitable concentration of dextran in substrates enabled unfixed smears and imprints to be stained for enzymes, fixed post-incubation and then counterstained with a quality of histological detail indistinguishable from that obtained by conventional methods. This dextran technique has enabled many hydrolytic and dehydrogenitic enzymes to be examined and to be shown to possess greater activity than that demonstrated by usual methods.

Partially polymerised acrylamide has also been found suitable for the purpose but has the disadvantage that it produces mild inhibition of activity of some enzymes.

It seems probable that it is the increase in viscosity of solutions rather than increase of osmotic pressure which is the role of these agents. Dextran can be recommended as giving reliable results at minimum cost in time and money.

STAINING METHODS

Periodic acid-Schiff (P.A.S.) staining of carbohydrate materials has been used for many years. While the specificity of the reaction is well accepted the colour intensity of the final product may be weak. Experiments using unfixed frozen sections showed a significant increase in staining intensity but deterioration of section quality. Examination showed that specificity could be preserved and staining intensified considerably by using unfixed frozen sections, oxidising in periodic acid in methanol, staining with Schiff reagent, potentiating the stain with an aldehyde fuchsin solution and then counterstaining.

EXFOLIATIVE CYTOLOGY

Application of exfoliative cytology in the early diagnosis of cancer is now well established. Rapid and certain identification of tumour cells by morphology limits the use of cytology to those with special training and skills. If cytology is to fulfil its promising potential in preventive medicine at reasonable cost it must be translated from the special to the routine laboratory. Several reports, and much basic work remains to be done in this field, have described differences in enzymic constitution between normal and malignant tissue of the lungs, uterus and cervix. Use is being made of enzyme histochemistry on exfoliated cells from these sites in the differential diagnosis of cancer, and in the event of a positive diagnosis, recognition of the type of tumour.

LEUKAEMIA

Alkaline phosphatase is a labile enzyme in neutrophils activated at sites of acute tissue destruction. It is absent in chronic myeloid leukaemia.

A labile nonspecific esterase in monocytes has recently been demonstrated in this laboratory and activation of this enzyme occurs in the peripheral circulation by some stimulus other than acute tissue destruction. The highest levels of activity occur in myeloid leukaemia.

Glycogen aggregates in lymphocytes vary in amount in health and disease. The large amount of glycogen in these cells enables lymphosarcoma to be separated from other types of reticuloses.

Further exploratory work on the histochemistry of mouse thymus has been carried out but so far no cell marker for thymocytes has been identified. However, an esterase of variable occurrence has been found in the thymic medulla. Cells containing this enzyme have large vesicular nuclei and occur singly and in groups. Activity of the enzyme increases as the cells rearrange to form the lining of hollow tubes which contain cells resembling large lymphocytes. Connections of these cells, if any, are currently being traced by frozen serial sections and injection methods.

MOLECULAR GENETICS**

Chev Kidson[1]

Studies have continued on molecular genetics, with investigations concerned with fractionation of DNA, preparation and characterization of mammalian messenger RNA, and genetic regulatory mechanisms at the level of genome transcription. DNA segments have been fractionated by nucleotide base sequence and native DNA by partial separation of the two strands. This latter approach has permitted estimation of the proportion of a genome which is active under given conditions.

Messenger RNAs have been characterized in many tissues and on countercurrent distribution it has been possible to identify specific tissue patterns and changing, altered patterns during tumour progression. In this way it is possible to "read" the expressed genome under varying conditions on a much wider scale than that available by limited enzyme screening.

These investigations have led to the demonstration of selected induction and repression of synthesis of specific groups of messenger RNAs as a regular process in mammalian cells, analogous to that in microorganisms in some respects. Starvation, selective refeeding, high and low protein diets all cause widespread, reversible induction and repression of messenger RNA synthesis. At least several anabolic hormones act by selective alteration of messenger RNA synthesis, with resultant induction and repression of very many enzyme proteins. During the reversible stages of azo-dye induced hepatocarcinogenesis the messenger RNA synthesis pattern has been shown to alter by reversible induction and repression on a selective, progressive basis, before there is any evidence of mutation in liver DNA. These data open up a new approach to the study of gene regulation in mammalian cells generally and in man, in relation to a variety of physiological and disease states.

PANCREATITIS

A. D. McCutcheon and D. Race

PATHOGENESIS

Evidence is accumulating in support of the suggestion made two years ago, that reflux of duodenal contents into the pancreatic duct is an important aetiological factor in pancreatitis. Previous work with blind duodenal loops in dogs had shown that duodenal contents under pressure in vivo, would travel past a normal pancreatic duct papilla into the pancreas and produce haemor-

1 On leave. This report concerns work carried out at the Chester Beatty Research Institute, England.

rhagic pancreatitis. An analogous situation occurs in man where there is an obstructed afferent loop following Polya type partial gastrectomy.

The effect of changes in pressure on normal and abnormal pancreatic duct papillae was then tested in vitro. The papillae are lined by delicate mucosal folds whose apparent function is to prevent regurgitation of duodenal contents. The freshly isolated duodenum was distended with coloured saline until the intraduodenal pressure was equal to 30 mm Hg.; no fluid escaped from the cut ends of the pancreatic ducts. The duodenum was opened and the mucosal portion of the papilla with its contained mucosal "valvules" excised. On raising the intraduodenal pressure to 30 mm Hg. again, coloured fluid escaped from the duct with the damaged papilla. A series of such experiments has shown that atrophic or damaged mucosal folds in duct papillae would allow the reflux of duodenal contents under physiological pressures.

Reflux of duodenal contents into the pancreatic duct may be demonstrable by choledochograms. One hundred choledochograms were studied and the pancreatic duct was seen in 18. In two of these the openings of the pancreatic and common bile ducts were completely separate so that dye had entered the duodenum from the bile duct before passing into the pancreatic duct. Our observations have shown that (1) reflux of duodenal contents into the pancreatic duct can produce typical haemorrhagic pancreatitis, and that (2) reflux of duodenal contents into the pancreatic duct may occur under the following conditions:

(i) Normal intraduodenal pressure and relaxed sphincter of Oddi.

(ii) Increased intraduodenal pressure and normal pancreatic duct papilla.

(iii) Relatively low intraduodenal pressures and an abnormal duct papilla.

TREATMENT

Previous work had shown that the antiproteolytic enzyme substance Trasylol was capable of preventing the development of experimental haemorrhagic pancreatitis in dogs. A small series of human sufferers from pancreatitis has been treated with Trasylol. A firm clinical impression has been gained that this drug is very useful in many cases. A double-blind controlled trial has not yet been done but useful information has been gained concerning the dosage required.

FAT NECROSIS

Widespread intraperitoneal fat necrosis is a characteristic and peculiar feature of acute pancreatitis, and its pathogenesis is uncertain. In view of the success with Trasylol in preventing experimental pancreatitis in dogs, a series of experiments was done to determine whether Trasylol had a similar protective effect under conditions designed to produce fat necrosis only.

Fat necrosis was produced in 2 series of dogs by the technique of Popper and Necheles whereby pancreatic secretions are allowed to drain into the peritoneal cavity after first passing across duodenal mucosa which activates the enzymes. In the control group of 10 dogs, nine showed fat necrosis which was extensive in 8 and more limited in one dog. In the other dog which had little fat necrosis the pancreatic duct had been sealed off by omentum.

In a second group of 10 dogs, in addition to this procedure, a constant intravenous infusion of Trasylol was given. Four of this group showed no evidence of fat necrosis, two had very little fat necrosis and the other four had moderate to marked fat necrosis. The dogs which had little or no fat necrosis also had pancreatic ducts which had become walled off by omentum. Where free drainage of pancreatic juice occurred, fat necrosis was also present, despite the satisfactory infusion of Trasylol.

These experiments provide additional evidence that trypsin and other pancreatic proteolytic enzymes which are inhibited by Trasylol, do not cause fat necrosis.

STEROID PATTERNS IN MARSUPIALS AND MONOTREMES*

M. Weiss

No work has yet been reported on the adrenal secretions of the Australian marsupials and monotremes and this study might reveal significant information regarding the evolutionary development of the biosynthetic systems as we know them in the higher mammals. The study up to now has been confined to the echidna and brushtailed opossum.

Adrenal venous blood was obtained from anaesthetized animals by cannulation of left renal vein after left nephrectomy.

Echidna

Five animals have been studied and the major biologically active steroid in adrenal venous blood was found to be corticosterone secreted at the rate of approximately 1 to 1.5 μg/100 mg adrenal/hour or less. Cortisol was also found in one animal in minute amounts. In up to 70 mls of peripheral plasma no free nor conjugated corticosteroids nor 17-ketosteroids were present in detectable amounts.

The only steroid metabolites detectable in the urine were 17-ketosteroids ranging from 2-5 μg/24 hrs. but they were not detected in the majority of samples. On two occasions 33 and 44 μg/24 hrs. were found. 17-ketosteroids were also detected in the faeces, the range of 7 specimens being less than 1-50 μg/24 hrs. The adrenal weights ranged from 3.3-4.9 mg/100 gm. body weight.

Brush-tailed Opossum

Eleven animals have been studied. Analysis of adrenal venous blood from five animals showed that the major corticosteroid is cortisol secreted at a rate of 10 to 14.6 μg/100 mg. adrenal/hour. Corticosterone was also detectable, the ratio of F/B ranged from 6-11. ACTH injection had no effect on corticosteroid secretion rate in a female and caused a small increase in the secretion rate of cortisol and corticosterone in a male. In the peripheral plasma the only steroid present in detectable amounts was cortisol, the level ranging from less than 0.1 to 2.5 μg/100 ml.

Analysis of urine samples showed a large variation in the excretion rate of steroid metabolites. In most specimens however, the levels were below the detectable range. The steroids found were cortisol ranging from less than 0.1-5.5 μg/24 hrs. tetrahydro-cortisol, and cortisone ranging from 5-118 μg/24 hrs.,

glucuronide conjugated tetrahydro-cortisol and cortisone, less than 1-64 μg/24 hour specimen. The alimentary tract was also a route of steroid excretion but the amounts per 24 hour specimens were considerably less than in the urine. The weight of the combined adrenal glands relative to body weight was small as compared with other mammals. The mean in the male was 5.6 and in the female 13.5 mg/100 gm. body weight.

These findings show that the major corticosteroid in the adrenal venous effluent of the echidna was corticosterone and of the brush-tailed opossum cortisol. Compared with other mammals the adrenal secretion rate of the echidna is extremely low, while that of the opossum approaches that of some mammals but is still of a low order.

CHEMICAL COMPOSITION OF AN ENTEROLITH

P. Fantl and H. A. Ward

A duodenal calculus was removed by Mr. A. Rollo, Honorary Surgeon at Alfred Hospital, from a 68 year-old female patient. Because these calculi rarely occur, this example was analysed chemically and found to consist of organic matter only.

The total N was 0.52%. All but 15% of the material was soluble in ethanol. The alcohol soluble material was acidic and its equivalent weight was calculated to be 374-396. Heating with alcoholic NaOH did not result in further hydroxyl ion consumption.

Thin layer chromatography[1] of the calculus in two solvent systems indicated the presence of two components which, according to their R_F values were cholic and de-oxycholic acid, present in a ratio of approximately 1:3.

There was no indication of the presence of conjugated bile acids.

1 Chromatography carried out by Mrs. M. Malinek, Diabetic and Metabolic Unit, Alfred Hospital.

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CURTAIN, C. C.—"A Structural Study of the Abnormal Haemoglobins Occurring in New Guinea", with an addendum by C. C. Curtain and J. Pye on the "Sedimentation and Diffusion of Haemoglobin H". *Aust. J. exp. Biol. & Med.*

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NAYLER, W. G. and P. F. EMERY—"The Positive Inotropic Activity of Tetraethyl-ammonium Ions and Their Effect on Radio-Calcium Movement". *J. Pharm. exp. Therap.*

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PAPERS SUBMITTED FOR PUBLICATION

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CURTAIN, C. C.—"A New Method for the Detection of Caeruloplasmin". *J. Chromatography.*

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NAYLER, W. G.—"The Dimethylxanthines and Radiocalcium Movement". *J. Pharm. exp. Therap.*

NAYLER, W. G. and N. C. R. MERRILLEES—"Some Observations on the Fine Structure and Metabolic Activity of Normal and Glycerinated Toad Ventricular Muscle". *J. Cell. Biol.*

LECTURES DELIVERED DURING 1963

"A Micromethod of Haemoglobin Separation in Acrylamide Gels"—*Alfred Hospital Clinical Society.*	A. BAUMGARTEN
"Microimmunoelectrophoresis – a Comparison of Several Techniques"—*Australian Biochemical Society, Melbourne.*	A. BAUMGARTEN
"A Micromethod of Serum Protein Electrophoresis in Acrylamide Gels"—*Australian Biochemical Society, Melbourne.*	A BAUMGARTEN
"Fluorescent Antibody Techniques" – *Cell Biology Symposium, Melbourne.*	C. C. CURTAIN
"Automation and Medical Laboratory Technology"—*Australasian Institute of Medical Laboratory Technology, Melbourne.*	C. C. CURTAIN
"The Structure of Abnormal Haemoglobins Occuring in New Guinea"—*Australian Biochemical Society, Melbourne.*	C. C. CURTAIN
"Some Biochemical and Genetic Aspects of Thalassaemia in New Guinea"—*Australian Society of Haematology, Melbourne.*	C. C. CURTAIN
"Electrode Systems in Biology and Medicine"—*Royal Melbourne Institute of Technology.*	C. C. CURTAIN
"Phosphorus Content of Fibrinogen and Fibrins"—*Australian Biochemical Society, Melbourne.*	P. FANTL
"Phosphorus Content of Mammalian Fibrinogens and Fibrins"—*Conference of International Committee for Nomenclature of Blood Clotting Factors, Gleneagles, Scotland.*	P. FANTL
"Determination of Certain Blood Coagulation Factors"—*County General Hospital, Los Angeles.*	P. FANTL
"Laboratory Diagnosis of Haemorrhagic Disorders"—*Queen Victoria Hospital, Montreal.*	P. FANTL
"Blood Coagulation"—Two lectures—*Monash University.*	P. FANTL
"Cardiovascular Research"—*Combined Malvern and Waverly Apex Clubs.*	T. E. LOWE
"Experimental Pancreatitis: The Use of a New Antiproteolytic Substance, Trasylol"—*Royal Australasian College of Surgeons, Melbourne.*	A. D. McCUTCHEON
"A New Treatment for Pancreatitis"—*Royal Australasian College of Physicians, Sydney.*	A. D. McCUTCHEON
"Surgery of Mitral Regurgitation"—*Cardiac Society of Australia and New Zealand.*	K. N. MORRIS
"Recent Advances in Cardiac Surgery" – *Australian Medical Association.*	K. N. MORRIS
"Some Observations on the Fine Structure and Metabolic Activity of Normal and Glycerinated Toad Ventricular Muscle"—*Australian Physiological Society, Canberra.*	W. G. NAYLER and N. C. R. MERRILLEES
"The Action of Halothane on the Ventricular Fibrillation Threshold"—*Victorian Society of Pathology and Experimental Medicine.*	D. RACE
"The Distribution of Blood Flow During Extracorporeal Circulation"—*Society of Experimental Surgery.*	D. RACE
"Induced Ventricular Fibrillation in Open Heart Surgery"—*Royal Australasian College of Surgeons, Melbourne.*	D. RACE
"Principles of Electrocardiography"—*Monash University.*	D. RACE
"Anomalous Pulmonary Venous Drainage"—*Symposium on Cardiac Surgery. University of New South Wales.*	G. R. STIRLING

"Surgical Aspects of Myocardial Infarction"—*National Heart Foundation Symposium, University of Melbourne.*	G. R. STIRLING
"Principles of Left Heart Surgery"—*Royal Australasian College of Surgeons.*	G. R. STIRLING
"The Mechanism of Fibrin Gel Contraction"—*Australian Biochemical Society, Melbourne.*	H. A. WARD
"Histochemistry"—*Cell Culture Society of Victoria.*	R. G. WYLLIE

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

Revenue Account for the Year Ended 31st December, 1963.

EXPENDITURE.		INCOME.		
Advertising	£59 8 9	Donations—		
Drugs, Chemicals, Provisions, etc.	3,120 11 7	Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions	£31,200 0 0	
Fuel and Lighting	357 15 3	Other Donations as per attached schedule	1,878 10 4	
Instruments and Glassware	2,690 11 8			£33,078 10 4
Insurance	1,048 19 10	Grants in Aid of Research—		
Library Maintenance	1,838 10 10	National Health and Medical Research Council	5,589 0 0	
Postage, Telephone, Printing and Stationery	1,493 16 3	National Heart Foundation of Australia	7,183 0 0	
Repairs and Renewals	1,735 12 3	Anti-Cancer Council of Victoria	4,662 0 0	
Salaries and Wages	41,604 15 6	Life Insurance Medical Research Fund of Australia and New Zealand	3,040 0 0	
Travelling Expenses	350 5 0			20,474 0 0
Sundries	930 7 2	Interest from Investments—		
Cost of Animal House	1,000 0 0	Held by the Trustees of the Estate of the late Thomas Baker	849 0 0	
Surplus for Year	397 17 11	Endowment Fund	1,180 11 7	
				2,029 11 7
		Interest from Commercial Bank of Australia Ltd.		261 10 1
		Blood Specimen Tests (Dr. E. Giles)		785 0 0
	£56,628 12 0			£56,628 12 0

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

Balance Sheet as at 31st December, 1963.

LIABILITIES.				ASSETS.					
Current Liabilities—				**Current Assets—**					
Sundry Creditors	£4,719	1	10	Cash on Hand	£10	0	0		
Endowment Fund	24,029	6	10	Cash at Bank	3,564	15	2		
Capital Grants and Gifts	1,986	16	1					£3,574	15 2
Provision for Travel Expenses	1,200	0	0	**Endowment Fund Investments—**					
Accumulated Revenue	672	5	8	Inscribed Stock—					
				Commonwealth Government	£13,380	0	0		
				Grain Elevators Board	2,500	0	0		
					15,880	0	0		
				Treasury Bonds—					
				Commonwealth Government	1,510	0	0		
				Shares in Companies	6,672	2	1		
				Cash at Bank (overdrawn)	32	15	3		
								24,029	6 10
				Restricted Funds (represented by Cash at Bank)—					
				Capital Grants and Gifts				1,986	16 1
				Fixed Assets—					
				Furniture and Fixtures				3,016	12 4
	£32,607	10	5					£32,607	10 5

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

In our opinion the above Balance Sheet is properly drawn up to show a true and fair view of the state of the Institute's affairs at 31st December, 1963, 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,
19th February, 1964.

NOTE: 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.

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

Year Ended 31st December, 1963.

CAPITAL GRANTS AND GIFTS.

Balance at 31st December, 1962		£4,021	12 11
Add			
Donations—			
Dr. T. E. Lowe and Associates		44	4 8
Transfer from Accumulated Revenue for cost of Equipment		2,250	0 0
		£6,315	17 7
Deduct			
Equipment (Scanner)	£2,250 0 0		
Refrigerated Centrifuge	1,979 1 6		
Baker Prize (Dr. P. S. Bhathal)	100 0 0		
		4,329	1 6
Balance at 31st December, 1963		£1,986	16 1

ACCUMULATED REVENUE.

Surplus at 31st December, 1962	£2,524	7 9
Less		
Transfer to Capital Grants and Gifts for Cost of Equipment	2,250	0 0
	£274	7 9
Add		
Surplus for Year	397	17 11
Surplus at 31st December, 1963	£672	5 8

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

OTHER DONATIONS RECEIVED DURING YEAR TO 31st DECEMBER, 1963.

The William Angliss Charitable Fund	£400	0	0
Marion and E. H. Flack Trust	350	0	0
The George F. Little Trust	148	6	6
Estate Edward Naylor	125	3	10
Mr. and Mrs. Edgar Rouse	105	5	0
Kodak (Australasia) Pty. Ltd.	50	0	0
Siegfried Meyer	50	0	0
Mr. and Mrs. Lawrence Simpson	10	10	0
Mr. J. C. Habersberger	10	10	0
Dr. and Mrs. John Rouse	10	0	0
Mr. and Mrs. T. Luxton	3	0	0
Miss N. E. Cameron	2	2	0
In Memory of William S. Lucey	500	0	0
„ „ „ St. John Costello Street	10	10	0
„ „ „ Sir William Angliss	10	0	0
„ „ „ William Sydney Robinson	5	5	0
„ „ „ Dr. Keith Colquhoun	5	0	0
„ „ „ Jessie Connochie	5	0	0
„ „ „ William J. German	5	0	0
„ „ „ Newton B. Green	5	0	0
„ „ „ Frank T. Keon	5	0	0
„ „ „ Dorothy Vaughan Kilburn	5	0	0
„ „ „ James Lindsay Law	5	0	0
„ „ „ Dr. Leslie Love	5	0	0
„ „ „ George Sackett	5	0	0
„ „ „ James Stanley Shaw	5	0	0
„ „ „ Ernest Walter	5	0	0
„ „ „ Sir Rowden White	5	0	0
„ „ „ James Wilson	5	0	0
„ „ „ Jean Agnes Knight	4	4	0
„ „ „ G. S. Norris	4	4	0
„ „ „ Jean Buchanan Sutherland	4	4	0
„ „ „ Dr. Harry Baines	3	3	0
„ „ „ John Bruce Wallace	3	3	0
„ „ „ Antony J. M. Shepherd	2	10	0
„ „ „ Jack Street	1	10	0
	£1,878	10	4

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

1963

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:	J. A. KEIPERT, M.B., B.S., D.Ch., M.R.C.P.E. A. P. DOREVITCH, M.D., M.R.A.C.P. K. J. CATT, M.D., M.R.A.C.P.
Registrar:	C. C. ENNIS, M.B., B.S.
Biochemists:	DORA WINIKOFF, M.Sc. JUNE SHEATH, M.Sc. AUSMA DULMANIS, B.Sc. (to July, 1963).
Technical Staff:	Mr. W. HUDSON. Miss I. EKKEL. Miss P. PEARL (to July, 1963). Miss W. DAVIES. Miss M. O'CONNOR (to July, 1963). Mrs. F. RABOLD (part-time).
Secretary:	Miss J. SHARP.

DIABETIC CLINIC

Clinical Assistants:	MARGARET SANDERS, M.B., B.S. A. P. DOREVITCH, M.D., M.R.A.C.P. R. D. GORDON, M.B., B.S., M.R.A.C.P.
Chiropodist:	MAIDA O'CONNOR, F.Ch.A.V., M.Ch.I.A. (on leave).

RESEARCH FELLOWS

Medical Research Fund:	N. KATHLEEN TAYLOR, M.B., B.S.
Medical Research Fund:	R. D. GORDON, 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

This past year has seen the completion of the re-organisation of the Diabetic and Metabolic Unit. This had become necessary because of the departure within a period of twelve months of three of its foundation members. Dr. Ewen Downie, Honorary Physician in Charge, retired from the active staff of the Alfred Hospital, Dr. Joseph Bornstein accepted the first Chair of Biochemistry at Monash University and Dr. Bryan Hudson, Physician in Charge of Clinical Studies, the first Chair of Medicine, also at Monash University.

Dr. Pincus Taft was appointed on 13th June as Physician-in-Charge and commenced duties in early October. His appointment is a part-time one; however, facilities provided within the Unit enable him to be "geographically" full-time. Dr. Harald Breidahl, formerly Honorary Assistant Physician, was appointed on 13th June to the position of Honorary Physician to the Diabetic and Metabolic Unit. With Professor Hudson's departure, Dr. Richard Gordon and Miss Ausma Dulmanis also went to the Department of Medicine at Prince Henry's Hospital to continue their work begun in the Unit under Professor Hudson's direction.

Members of the Unit presented papers at the Second Asia and Oceania Regional Congress of Endocrinology which was held in Sydney in June, and overseas participants to the Congress later visited the Unit. These included Dr. Kris Eik-Nes of the University of Utah, U.S.A., Drs. Tsuji and Shinko of the Kobe Medical College, Japan and Dr. Kaye Ibbertson of Auckland, New Zealand. Other visitors to the Unit during the year included Professor George W. Thorn, Professor of Medicine, Harvard Medical School, and Dr. John Nabarro of the Middlesex Hospital, London. These visitors are always welcome and are a great stimulus to members of the Unit. They provide the basis for much fruitful discussion during and subsequent to their visit, discussion which often lends assistance in our work.

Dr. Frederick Bartter of the National Heart Institute, Bethesda, Maryland, U.S.A., was Guest Lecturer at the Alfred Hospital in May, spending some four weeks at the Hospital. This visit was supported by a grant from the Felton Bequest and he worked in the Unit undertaking research into Circadian Rhythm with members of the department. He also gave a series of lectures and conducted seminars in the Hospital on those aspects of Endocrinology which are his special interest.

Continued generous assistance has been provided by individuals and organisations. This has taken the form of financial and technical aid and is gratefully acknowledged since without this support the work of the Unit would be considerably restricted. A close association with the Monash University has been developed in the departments of Medicine and Biochemistry. A joint conference in Endocrinology takes place weekly with members of both departments, the special facilities of the Unit and of the University are mutually available to all and the presence in our laboratory of Mr. Ian Parsons of the Department of Biochemistry who is undertaking his research here, is of substantial benefit to the Unit.

This report is my first. It represents the work of my predecessors, for this year has been a transitional one. The future research programme will of necessity

be of a somewhat different nature. The challenge is welcomed and my gratitude is extended to all those in the Diabetic and Metabolic Unit and interested people not directly associated whose assistance is making the task of carrying on its work so much easier.

PINCUS TAFT.

31st December, 1963.

Grateful acknowledgment is made of financial assistance provided by—

Alfred Hospital Research Funds
Burroughs Wellcome & Co. (Australia) Ltd.
Dunklings Pty. Ltd.
Eli Lilly & Co., Indianapolis, U.S.A.
Boots Pure Drugs Co. (Aust.) Pty. Ltd.
Pfizer Corporation.
Difrex Laboratories Pty. Ltd.
Mrs. M. Clark.

Acknowledgment of gifts in kind is acknowledged from—

Ciba Company Pty. Ltd.
Hoechst Pharmaceuticals.
Sandoz Ltd.
The Upjohn Company, Kalamazoo, U.S.A.

THE SECRETION AND METABOLISM OF ANDROGENS[1]

**Bryan Hudson, Ausma Dulmanis and Pamela Pearl,
in collaboration with J. Coghlan and M. Coghlan, Howard Florey Laboratories of Experimental Physiology.**

During the past twelve months the methods that have been previously developed for the estimation of testosterone in peripheral plasma, in urine and of the production rate of testosterone have been used to investigate a number of normal individuals and patients with apparent disorders of androgen production.

LEVELS OF TESTOSTERONE IN PLASMA

More than 50 normal males and 40 normal females have been studied. The mean level of testosterone in the plasma of normal adult males is 0.72 μg/100 ml., the range being 0.42-1.0 μg/100 ml. A number of pre-pubescent and pubescent boys have been studied; in the former testosterone is present in low concentrations in plasma, the values found approximating those in normal adult females (0.05-0.15 μg/100 ml.), while in pubescent boys values that range from these low levels to those of adult males have been found. In general, they appear to correspond with the degree of advancement of puberty.

In normal adult females, values have ranged from less than 0.05 μg/100 ml. (the probable lower level of sensitivity of the method) to 0.17 μg/100 ml. Blood samples taken from normal females at different intervals through the menstrual cycle have shown no consistent differences in level at the different phases of the cycle.

Several male patients with clinical hypogonadism due to "constitutional", pituitary and gonadal causes have been found to have uniformly low levels of testosterone in their plasma. Those patients with hypogonadism as a result of the former two causes have been shown to respond to human chorionic gonadotrophins (HCG) in doses of 1000 I.U. per day i.m. for five days, the response being assessed by an elevation of the level of plasma testosterone to within the normal adult male range. Work is proceeding in order to develop a test of testicular function in which HCG is administered and changes in the level of plasma testosterone measured.

Several females with apparent abnormalities of androgen production have been studied and abnormally high levels of testosterone in plasma have been shown.

LEVELS OF TESTOSTERONE IN URINE

Testosterone has been found in urine in three fractions – free (no hydrolysis), following hydrolysis for 24 hours with mineral acid at pH 1 at 40°C. and after hydrolysis with β-glucuronidase. Although there is a difference in the rate of excretion of testosterone between males and females the degree of overlap is considerable and these estimations do not appear to be of significant value in clinical diagnosis.

The rate of excretion of testosterone in five normal females was estimated

1 This work was begun in the Diabetic and Metabolic Unit, and was continued under its auspices until 30th June, 1963, since which time financial responsibility has been accepted by the Medical Research Centre, Prince Henry's Hospital. It is now being continued at Prince Henry's Hospital with Professor Hudson's departure from the Unit following his appointment to the Chair of Medicine at Monash University.

daily during one menstrual cycle. In all the subjects studied it was found that the rate of excretion of testosterone fluctuated, apparently in relation to the phase of the cycle. Thus in all fractions there was a distinct preovulatory peak occurring between the eighth and tenth days, a lesser peak occurring at the time of ovulation and yet a third and smaller peak occurring in the midluteal phase of the cycle. The signifiacance of these cyclic changes has yet to be elucidated.

ANDROGEN PRODUCTION RATES

The production rate of androgens has been measured using the principle of isotope dilution. Patients are given tracer doses of 4-C^{14}-testosterone by a single intravenous injection and urine is collected for 48 hours thereafter. The procedures developed for the estimation of testosterone in plasma and urine are applied to this specimen and the specific activity of the urinary testosterone is measured. The production rate is determined by the following equation:

$$P_A = \frac{R}{S_A \times t}$$

where:

P_A = production rate (mg./day),
R = radioactivty administered (c.p.m.),
S_A = specific activity of urinary testosterone (c.p.m./mg.),
t = time of collection (days).

Employing this principle, the production rate of testosterone in 10 normal females has ranged from 0.8-3.7 (mean: 1.9) mg./day, and of 15 normal males from 3.0-11.1 (mean: 6.9) mg./day.

A variety of male and female patients with apparent abnormalities of androgen production have been studied and the results of these studies are shown in the accompanying table.

A. Females with hirsutism and other abnormalities of androgen production.

Patient	Androgen Production Rate (mg./day)	Plasma Testosterone (μg/100 ml.)
M.T.	3.1	0.55
J.J.	8.7	0.31
R.R.	6.1	0.1
W.R.	12.5	0.21
B.R.	9.1	0.2
A.M.	8.4	0.49
R.B.	9.3	0.58
D.W.	5.8	—
M.E.	1.7	0.15
L.B.	5.9	0.46
L.C.	6.1	0.75

B. Hypogonadal Males.

Patient	Testosterone Production Rate (mg./day)	Plasma Testosterone (μg/100 ml.)
W.H.	0.8	0.05
D.G.	2.9	0.17
J.J.	3.1	0.07
J.A.	3.4	—
K.S.	3.5	0.12

Due emphasis should be given to the choice of the term "Production" rate of androgens. This term has been chosen advisedly because there is good evidence that the production rate figure cannot be equated with the "Secretion" rate of testosterone. The reasons for this belief are that any procedure that employs the principle of isotope dilution demands that the urinary metabolite examined (in this instance testosterone) must be uniquely derived from the secretory product. It has been observed that at least two other compounds, dehydroepiandrosterone and 4-androstenedione, both androgens and normally secreted, are precursors of urinary testosterone, which is therefore not a unique metabolite of testosterone. The degree to which these compounds contribute to urine testosterone appears to vary from subject to subject so that no general correction can be applied in order to determine the true secretion rate of testosterone.

Attempts to derive this value by kinetic studies in blood and by the direct measurement of testosterone in timed collections of spermatic vein blood are in progress.

STUDY IN CIRCADIAN RHYTHMS

Richard Gordon

Many physiological functions exhibit a 24 hour or an approximately 24 hour periodicity. The term "circadian" is used to include all such periods approximating 24 hours, with "exact" (average) 24 hour periodicity as a special case of circadian rhythm. The numbers of circulating leucocytes and the urinary excretion of water, salt, adrenal corticosteroids and their metabolites are known to manifest such periodicity in normal subjects.

The present study was designed to demonstrate changes in blood and urine thought or known to be due to cyclic changes in adrenal cortical and testicular function in a group of normal subjects. Such information provides temporal reference standards so that changes resulting from disease or treatment can be differentiated from spontaneous fluctuations. It is known, for instance, that blood levels of cortisol are highest between 6 a.m. and 9 a.m. and lowest between 9 p.m. and 2 a.m. These changes may reflect changes in ACTH secretion, adrenal sensitivity or both.

This project was initiated during the stay of Dr. Frederick C. Bartter, Alfred Hospital Guest Lecturer, and aims at demonstrating the normal rhythm

of secretion and excretion of cortisol, corticosterone and testosterone. Variations in blood eosinophil levels and urinary excretion of electrolytes and urea were measured concurrently.

Blood and urine samples were collected every three hours for 30 hours from eight normal male medical students. The hormone levels are measured by a double isotope dilution derivative procedure.

The measurements are not yet completed.

MICROIODINE ASSAY

Dora Winikoff

Further studies on the use of sephadex-dextran gel for the rapid separation of protein bound iodine (PBI) fraction in the presence of iodine containing contaminants have been carried out.

By the use of sephadex 25 medium in columns of 7.5 x 1.2 cm. equilibrated with distilled water, inorganic iodine can be separated from organically bound compounds.

The preparation of a series of fractions and their subsequent analysis by stable iodine assay has in this laboratory given an indication of the amount as well as the nature of the contaminants.

The application of this technique, however, as a preliminary process to a PBI assay requires rigidly controlled conditions. Apart from the dimensions of the column and the particle size of the bed material, the mode and the degree of packing, the flow rate and the age of the column appear to influence results.

A series of experiments was undertaken using a plasma with a very high inorganic iodine content (following intravenous Lugol's drip) added to normal plasma in increasing quantities. With iodine content raised to 100 μg% the PBI value was not affected.

If the flow rate exceeded 3-4 drops/min. or old or loosely packed columns were used there was a considerable amount of iodine present in the PBI fraction. Sephadex gel procedure is therefore not recommended for very high iodine levels. In suspected cases a screening procedure of assaying a total plasma iodine (TPI) level should be carried out.

RESIN UPTAKE OF I^{131}-TRIIODOTHYRONINE AS THYROID FUNCTION TEST

N. Kathleen Taylor, Dora Winikoff and Wanda Davies

Further work is in progress using the resin uptake method in the study of the binding capacity of plasma proteins for the added radioactive triiodothyronine (T_3).

Since beginning this work 1600 patients have been studied (a PBI estimation being done simultaneously). In general good correlation has been observed and the test is routinely applied as an aid to the diagnosis of thyroid disease.

Results are now expressed as a percentage of the T_3 resin uptake of the pooled normal serum tested with each batch of sera studied.

Euthyroid:	85%—114% of pooled serum.
Hypothyroid:	53%— 81% of pooled serum.
Hyperthyroid:	118%—170% of pooled serum.

There exists a slight overlap of 81-85% between hypothyroid and euthyroid subjects, and 114-118% between euthyroid and hyperthyroid subjects.

In this way external factors (variation in temperature or time of incubation, etc.) which alter the T_3 uptake are eliminated.

It has been found that in many patients receiving treatment with I^{131} for thyrotoxicosis the resin uptake reaches normal levels before the PBI. No further treatment has been given to these patients and in all instances, after an interval of months the PBI has returned to euthyroid levels.

The resin uptake results are, therefore, useful in preventing the occurrence of some cases of myxoedema, giving an earlier index of return to normal of thyroid function after such treatment.

Oral contraceptives, and it is thought transquillisers and hypnotics, lower the resin uptake. It is believed that this phenomenon results from an increase in binding sites. These findings and other abnormalities in binding of thyroid proteins will be studied with the aid of electrophoresis.

In hypothyroid children maintained on l-thyroxine very high levels of PBI have been found, while T_3 resin uptake is often in the euthyroid range of values. In most cases there is no clinical evidence of overtreatment. Further studies on this problem are contemplated.

THYROID SURVEY — A STUDY OF THE PROBABILITY OF THE DIAGNOSIS OF THYROID FUNCTION BASED ON SYMPTOMS AND SIGNS

N. Kathleen Taylor

An exchange of findings in the presenting symptoms and signs in suspected thyrotoxicosis and their correlation with the final diagnosis is continuing with the Department of Medicine in the University of Utah, Salt Lake City.

Although it is too early to give statistically the most important combinations of signs and symptoms, a definite pattern is emerging.

Further computer studies are to be made during 1964.

EXOPHTHALMOS AND THYROID DISEASE

N. Kathleen Taylor

Study of many patients has shown no correlation between the course run by the eye condition and the metabolic disorder of the patient. The interval between the appearance of exophthalmos and development of thyrotoxicosis may be as long as five years. Treatment of the thyrotoxicosis does not consistently alter the eye condition.

The use of thyroxine in exophthalmos has been found to be of little value, if any. Cortisone in some cases of severe exophthalmos has been extremely useful in inducing regression sufficient — in conjunction with local treatment of the conjunctiva and with tarsorrhaphy — to protect the cornea.

This study is continuing.

INTERMEDIARY METABOLISM IN DIABETES UNDER TREATMENT WITH BIGUANIDE

June Sheath and Pincus Taft

these changes are induced by a stimulation of glycolysis and an inhibition of cellular oxidation. With a further compound — dimethyl biguanide — few reports are available and studies of the effects of this material on intermediary metabolites are being undertaken. The levels of pyruvic and lactic acids, of glucose and free fatty acids are being measured before and after food, before treatment and with progressively increasing dosage of dimethyl biguanide in order to compare the metabolic effects of this drug with those of phenethyl biguanide.

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PAPERS IN PREPARATION

WINIKOFF, Dora—"The Use of Dowex Resin and Sephadex Gel as Refinements of Protein Bound Iodine Estimation".

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Post-Graduate Course in Medicine conducted for Royal Australasian College of Physicians—*Singapore.*	BRYAN HUDSON
Lectures in Endocrinology delivered at Centenary Scientific Sessions, *Launceston.*	BRYAN HUDSON
"Normal Adolescence"—*School Medical Service.*	H. D. BREIDAHL
"Dietetics"—*Flinders Naval Depot.*	H. D. BREIDAHL
"Testicular Biopsy in Male Hypogonadism" — *Second Asia and Oceania Congress of Endocrinology, Sydney.*	H. D. BREIDAHL
"The Endocrine Aspects of Infertility"—*Queen Victoria Hospital Clinical Society.*	H. D. BREIDAHL
"Diabetes"—(2)—*Melbourne District Nursing Service Post-Graduate Course.*	H. D. BREIDAHL
"Thyroid Diseases"—*Melbourne District Nursing Service Post-Graduate Course.*	H. D. BREIDAHL
"The Resin Uptake of I^{131}-Labelled Triiodothyronine in the Diagnosis of Thyroid Disorders"—*Second Asia and Oceania Congress of Endocrinology, Sydney.*	KATHLEEN TAYLOR and DORA WINIKOFF
"The Use of Dowex Resin and Sephadex Gel as Refinements of Protein Bound Iodine Estimation"—*Second Asia and Oceania Congress of Endocrinology, Sydney.*	DORA WINIKOFF

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

SOFT TISSUE TUMOURS

A. V. Jackson[1] and N. A. Davis[1]

Along with routine surgical pathology of the necropsy service a more detailed recording and investigation of soft tissue tumours is maintained. For some years this has been a continuing study in the department but recently interest has been further stimulated by the establishment by the International Council of Societies of Pathology of a Soft Tissue Tumour Centre in Washington. This Centre has now completed its preliminary work and is issuing a provisional classification which is being submitted to national societies of pathologists for comment and study and we have been asked to co-operate.

GYNAECOLOGICAL CANCER CYTOLOGY**

A. V. Jackson[1] and N. A. Davis[1]

With the co-operation of the Gynaecology Unit we will conduct in 1964 a gynaecological cancer cytology survey of female inpatients in the hospital. It seems likely that this 12-months survey will demonstrate the need for maintaining a regular service. However, even if cancer cytology is offered as a routine diagnostic service very real uncertainties about the degree of the correlation between the statics of cellular morphology and the dynamics of malignant behaviour, and also quite justifiable concern about the risks of increased morbidity and mortality from possibly unnecessary surgery, make it essential that cytology studies be maintained at a very critical "research" level.

CORONARY HEART DISEASE‡

A. V. Jackson[1] and N. A. Davis[1]

One of us (N. A. Davis) spent some time last year with Professor W. B. Wartman at Northwestern University in Chicago developing a technique for objectively measuring the intra-luminar volume of the coronary arteries in hypertrophied and ischaemic hearts.

Radioactive iodinated albumen is added to an injection mass and after dissection the radioactivities of measured lengths of the larger coronary branches, and of measured masses of heart muscle are determined by a gamma counter. With the assistance of a grant from the National Heart Foundation this work will be carried on at Alfred Hospital next year, and we hope, for several years. This is a fairly long-term project but the technique appears to be the best method available for obtaining basic anatomical information about myocardial ischaemia and we believe that the data which will be gathered will serve as a valuable base-line for further studies.

COAGULATION STUDIES

R. J. Sawers[2]

Many new patients have been investigated for bleeding disorders and some old patients re-investigated to determine the value of modifications to tests. The most informative investigations which were carried out are described briefly.

** In this report of scientific investigations those projects marked (**) were supported wholly or in part by grants from Anti-Cancer Council of Victoria and those marked (‡) by grants from the National Heart Foundation of Australia.

1 Department of Morbid Anatomy. 2 Haematology Department.

ACQUIRED INHIBITORS ACTING AGAINST FACTOR VIII

Two female patients were investigated and followed up on account of acquired haemorrhagic disorders characterised by low plasma activities (5%) of factor VIII. The usual tests failed to demonstrate the presence of inhibitors but the clotting time of whole blood in siliconed tubes was grossly prolonged. Correction of abnormal tests was less complete than in the case of patients with complete congenital deficiency of factor VIII. In both cases haemorrhagic symptoms slowly remitted as the factor VIII activity increased in their blood. Despite factor VIII activities of 15-25 per cent. in their blood, the silicone clotting times remained prolonged, but later became normal.

HAEMOPHILIA A

An evaluation of factor 1-0 prepared from fresh human plasma by the Commonwealth Serum Laboratories has been made. Different batches of the freeze-dried material released for clinical use were reconstituted and found to have factor VIII activity equivalent to 2.25 to 5 times that of an equal volume of fresh standard control plasma. No side effects have occurred during transfusion of the material and peak elevation of the factor VIII activity in the patients' plasma was between 50 and 75% of the expected level as calculated from the amount of material transfused and their body weight. After 24 hours the factor VIII activity had dropped to 10% of the peak level; the effective half-life is thus approximately 6 hours.

Multiple transfusions of factor 1-0 were given to a haemophilic boy, completely deficient in factor VIII, for removal of tonsils and adenoids. Bleeding occurred on the sixth post-operative day despite high levels of factor VIII. This appeared to be related to an acquired coagulation defect as revealed by abnormally prolonged clotting times in silicone tubes and prolonged thrombin clotting times. At this time he also developed a severe haemolytic state; fresh frozen plasma was used subsequently to control bleeding. While the success of his operation must be attributed to the use of factor 1-0, it is apparent that prolonged massive use causes unexpected side effects.

HAEMODIALYSIS

J. Nayman[1]

ARTERIOVENOUS SHUNTS AND CANNULATION OF VESSELS

(1) Apparatus for Construction of Junction between Plastic Cannulae

An apparatus that would enable the formation of a right angled flange accurately and efficiently has been constructed.

(2) Silastic-Teflon Shunts

The use of these for the long term treatment of chronic renal failure by intermittent dialysis is being investigated, and a prototype design already made.

(3) Arterio-arterial Shunt

The technique of placing a shunt in the course of an artery has been exploited. Blood is removed from the proximal limb, passed through the extracorporeal circuit, and returned via the distal limb.

1 Haemodialysis Unit, and Department of Surgery, Monash University, Alfred Hospital.

(4) Seldinger Technique

This technique for the cannulation of vessels has been used and the possibility of utilizing an arteriovenous shunt principle explored.

REGIONAL HEPARINIZATION

A Braun Dual Infusion Syringe (Unita 1) has been extensively used for the synchronous infusion of heparin and protamine sulphate. An inbuilt auditory alarm system has been incorporated into the body of the machine.

A syringe carriage has been designed so as to enable standard Luer-Lok 50 ml. and 100 ml. syringes to be used.

An improved needle adaptor has been constructed.

DYNAMICS OF HAEMODIALYSIS

The effect on dialysance of employing a larger pump to increase the flow rate of dialysing bath fluid has been studied.

Comparative values with two different sets of coil clamps, as well as with the original pump have been evaluated.

Weighing Bed

A weighing bed with an improved design is almost completed.

RENAL BIOPSY

J. Nayman[1]

A drill biopsy gun worked by high pressure turbine drive mechanism has been constructed and used in the technique of open renal biopsy in selected cases of acute renal insufficiency.

RENAL TRANSPLANTATION

J. Nayman[1]

A preliminary study of the technique of renal autotransplantation in the dog has been undertaken.

ALLUSIVE THINKING

N. McConaghy[2]

FAMILIAL RELATIONS OF ALLUSIVE THINKING

Professor Lidz of Yale University has published a replication of one of our previous studies and has confirmed the demonstrations of high scores on a test of Allusive Thinking by parents of patients with schizophrenia. However, in a personal communication, he has reported other findings which are dissimilar to ours. Further testing of parents is therefore being carried out.

1 Haemodialysis Unit, and Department of Surgery, Monash University, Alfred Hospital.
2 Department of Psychiatry.

INCIDENCE OF ALLUSIVE THINKING IN PSYCHIATRIC PATIENTS

Now that tests to measure Allusive Thinking have been established they are being used to estimate the frequency of this type of thinking in psychiatric patients and to seek correlations with psychiatric illnesses other than schizophrenia.

NON-VERBAL PSYCHOTHERAPY

Eighteen patients treated with this therapy have been studied. They had all failed to respond to other forms of psychotherapy produced marked improvement and it was possible to avoid leucotomy in five.

SOCIAL AND PHYSIOLOGICAL ASPECTS OF CARDIOVASCULAR DISEASE‡

B. B. Thomas[1] and H. L. Millott[1]

The aim of this study is to define by analysis the social and psychological consequences for the patient suffering from his first coronary occlusion. Comparison is made with the previous pattern of living, assessed in the acute stage of the illness, with that observed at three and six months follow up. A set of independent variables are being systematically examined on the assumption that they have a functional relationship to the dependent variable of rehabilitation, as reflected in the work, leisure and attitude patterns of the patient at three and six months after the coronary occlusion.

Patients are referred to us from two public teaching hospitals, one public non-teaching hospital, and from general and consultative practice covering a thirty mile radius of Melbourne. The sample consists of males, aged 30 to 65 years, suffering from their first coronary occlusion. This is diagnosed on clinical history, electrocardiographic abnormalities and/or biochemical tests.

The data, which are screened by one physician, are gathered from the patients, their families, their doctors and their employers. Thus a series of interviews are conducted in the acute stage (one to four weeks) supplemented by job analyses and home visits and these data are integrated to form a systematic social assessment of the function of the patient and family prior to his illness. A joint interview is conducted at home with the patient and the family at three and six months for comparison of the pattern of living. Analysis is made of the factors leading to the post-occlusion pattern with particular attention to those instances reflecting delayed or absent rehabilitation at three and six months. It is proposed to make a careful study of the formal conditions of work (e.g., Trade Awards) which allow or inhibit the re-absorption in work of a person with physical limitations from a cardiac condition.

This analysis is prefaced by a detailed work history and current job study. Data are being gathered by interview of the patient, his superior and, where relevant, his union and the employers' body. This will establish a body of knowledge about work conditions in general and, in particular, as it relates to a given patient.

1 Medical Social Work Department.

Seventy-two patients have been referred up to 1st December, 1963. The median age is fifty years. The age distribution differs slightly from that usually reported for this illness as the present sample cuts off the upper limits. Up to 1st December, 1963, thirty-four patients and families had been followed to three months, and nine of these had been followed to six months after the onset of symptoms.

The results suggest that, although the majority of these patients are work-rehabilitated at three months, a lesser number are rehabilitated by three months to leisure activities, and similarly reflect a lower level of attitude-rehabilitation. One factor which appears to be significantly related to attitude and leisure-rehabilitation is that of the adequacy of the initial medical information given to the patient and his family.

In the final analysis it is proposed to statistically test the relative weight of the independent variables to the dependent variable of rehabilitation as reffected in work, leisure activities and attitude toward the coronary episode. At the present rate of referral it is expected that one hundred and forty to one hundred and fifty patients will comprise that total sample. In addition to the statistical analysis, it is proposed to make a written report of the detail insights gained in arriving at an understanding of the post-occlusion pattern of living of these patients.

SUBARACHNOID HAEMORRHAGE

James M. Calvert[1]

The investigation of nontraumatic subarachnoid haemorrhage from aneurysms or other vessel malformations of the central nervous system has been continued and 200 cases have now been studied. Common factors in aetiology and symptomatology have been sought as possible guides to diagnosis, prognosis and prophylaxis. The results of treatment of aneurysms at various sites have been analysed in an attempt to establish the merits of different methods. However, when the cases are subdivided into groups the numbers in each group are so small that valid conclusions cannot be drawn. In an endeavour to overcome this smallness of numbers in an individual clinic a co-operative effort is being made by this Unit and others in U.S.A., Great Britain and Sweden to pool results and to use the four recognised treatment regimes in a random fashion. It is hoped that over a few years a large number of cases will be available for study and that computer analysis will enable a satisfactory assessment to be made of the treatment appropriate for each variety of aneurysms. The regimes being used are: (1) regulated bed rest, (2) regulated bed rest with drug-induced controlled hypotension, (3) ligation of a common carotid artery, (4) direct intracranial operation.

In the current series carotid angiography was used for diagnosis in approximately 90% and vertebral angiography in addition in 4%. Vessel aneurysms or angiomata were demonstrated in 80% of this group with the following distribution.

1 Neurosurgical Unit.

Artery	Incidence %
Internal carotid	37
Anterior communicating	25
Middle cerebral	14
Anterior cerebral	9
Multiple	9
Angiomata	6

In those cases with a vascular abnormality demonstrated radiographically the common form of treatment was surgical either by direct approach or by proximal occlusion of vessels intracranially or cervically. The majority of patients treated conservatively had not had an aneurysm demonstrated and this group had a mortality of 22% in comparison to 35% for the group operated upon. In addition to the mortality this latter group incurred neurological deficits in 25%.

Autopsy studies have demonstrated aneurysms of the basilar artery system sufficiently frequent to indicate the necessity to carry out vertebral angiography in all cases where bilateral carotid studies do not reveal any abnormality.

The clinical history in a high proportion of patients indicates that symptoms often precede the bleeding and might lead to diagnosis of aneurysm before rupture occurs. Many of these patients had systemic arterial hypertension but the data so far do not suggest an incidence out of line with that of this age group.

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"Pathology of Myocardial Infarction"—*Symposium on Myocardial Infarction — Melbourne Post-Graduate Committee and National Heart Foundation.*	N. A. DAVIS
"Opportunities in Australia and Training in Pathology"—	
"Structure and Foundation of the College of Pathologists of Australia"—*New Zealand Society of Pathologists, Tauranga.*	A. V. JACKSON
"The Management of Acute Renal Failure in Surgical Practice"—*Royal Australasian College of Surgeons.*	J. NAYMAN
"Monitoring Haemodialysis employing a Modified Hench-Alrich Test for Estimating Blood Urea"—*Australasian Surgical Research Society, Melbourne.*	J. NAYMAN
"The Development of an Arteriovenous Shunt in Facilitating Multiple Dialysis in Acute Renal Failure"—*Royal Australasian College of Surgeons.*	J. NAYMAN
"Technical Developments in Haemodialysis"—*Symposium on Renal Failure, Sydney.*	J. NAYMAN
"The Use of a Simplified Apparatus for Sub-Culture"—*Victorian Society for Pathology and Experimental Medicine.*	J. NAYMAN
"(a) Arteriovenous Shunts (b) Miscellaneous Technical Development (c) Regional Heparinization and Monitoring Techniques"— Artificial Kidney "Workshop".	J. NAYMAN

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