BAKER INSTITUTE

RESEARCH

1961

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 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-FIFTH ANNUAL REPORT

of

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

(Including Alfred Hospital Clinical Research Unit)

FIFTH ANNUAL RESEARCH REPORT

of

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

REPORTS

of

ALFRED HOSPITAL RESEARCH FELLOWS

1961

ALFRED HOSPITAL, PRAHRAN
VICTORIA, AUSTRALIA

BAKER MEDICAL RESEARCH INSTITUTE

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	Director of Clinical Research Unit (ex officio)

*Appointed from the University of Melbourne.

ALFRED HOSPITAL RESEARCH FELLOWS, 1961

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"Dr. Henry Laurie":	H. D. BURGER, M.D., M.R.A.C.P.
"Edward Wilson Memorial":	J. B. DAWSON, M.A., B.M., B.Ch. (Oxon.), M.R.C.P. (Edin.). F. H. LUMB, M.B., B.S. (Lond.), M.R.C.P.
"E. H. Flack":	N. McCONAGHY, M.B., B.S., B.Sc., D.P.M.
"Sol Green":	W. M. McDONALD, M.B., B.S., F.R.A.C.S.
"James Richardson":	J. NAYMAN, M.B., B.Ch. (W'srand), F.R.C.S., F.R.C.S. (Edin.).
"Sydney W. Jones Medical Research Foundation":	D. RACE, M.B., B.S.
"Connibere Bequest":	B. B. THOMAS, Dip.Soc.Stud. (Sydney), A.I.H.A. (N.S.W.).

APPOINTED TO RESEARCH FELLOWSHIPS FOR 1962

"Sol Green":	A. BAUMGARTEN, M.B., B.S.
"Sydney W. Jones Medical Research Foundation":	E. COOPER, M.B., B.S.
"Edward Wilson Memorial":	J. B. DAWSON, M.A., B.M., B.Ch., M.R.C.P. (Edin.).
"Dr. Henry Laurie":	GABRIELE MEDLEY, M.B., B.S.
"Alfred Hospital Medical Research Fund":	N. McCONAGHY, M.B., B.S., B.Sc., D.P.M.
"E. H. Flack":	J. NAYMAN, M.B., B.Ch. (W'srand), F.R.C.S.
"R. B. McComas":	D. RACE, M.B., B.S.
"Alfred Hospital Medical Research Fund":	N. KATHLEEN TAYLOR, M.B., B.S.
"Connibere Bequest":	B. B. THOMAS (to 28/2/62).
"Frederick and Esther Michaelis":	G. WAGNER (from 1/9/62).

TRAVEL GRANTS FOR 1962

Medical Research Fund:	A. K. LETHLEAN, M.B., B.S.
"Edward Wilson Memorial Research Fund":	K. N. MORRIS, M.B., M.S., F.R.C.S. F. KINROSS, M.B., B.S.

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INTRODUCTION

The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute was founded under the terms of a Deed of Settlement executed in 1926 between the Settlors and the Board of Management of Alfred Hospital. The Institute was established to provide an efficient hospital laboratory service and facilities for medical research. In the course of time it was found more satisfactory for these routine services to be placed under the control of the Hospital staff, and this transfer was completed in 1948. Since then the Institute staff has been entirely concerned with research, 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 specialties and the organised research facilities of our Hospital, namely, Baker Institute and Clinical Research Unit, are at all times available to them in this work. Such an arrangement is in conformity with our objects—treatment of the sick, training of doctors and nurses, and provision of facilities for research."

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

BAKER MEDICAL RESEARCH INSTITUTE

STAFF

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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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Clerical:	Mrs. I. ROBINSON. Mrs. M. E. AUSTIN (from 17/4/61). Miss B. DOWDELL. Mrs. M. HITCHCOCK (to 28/4/61). Miss J. LOCK (from 4/10/61 to 23/11/61). Miss C. PHILLIPS (to 6/10/61).
Laboratory Assistants:	Mrs. J. BERAN. Miss B. BURKE. Mr. D. BREEN. Miss F. GOON (to 18/9/61). Miss J. HARRIS. Miss J. HOWELLS. Miss D. MAKEPEACE. Miss C. A. MILROY. Miss A. McARDLE. Miss L. PHILLIPS. Miss M. SHIERS. Miss E. WADDY.

WARD STAFF

Registrar:	A. BAUMGARTEN, M.B., B.S.
Resident Medical Officers:	R. A. MEARES, M.B., B.S. N. BETT, M.B., B.S. D. ROBERTSON, M.B., B.S. R. McLELLAN, M.B., B.S.
Sister:	L. STAHR.
Staff Nurses:	D. OWEN (from 4/4/60 to 29/6/61) L. D. BARAGWANATH (from 9/1/61 to 31/7/61). A. van HENNEKELER (from 9/1/61 to 19/6/61). R. KNIGHT (from 19/6/61 to 30/10/61). K. J. BERH (from 31/7/61 to 1/10/61). B. SIMPSON (from 2/10/61). P. FUNSTON (from 30/10/61).

RESEARCH FELLOWS

"Edward Wilson Memorial":	J. B. DAWSON, M.A., B.M., B.Ch. (Oxon.), M.R.C.P. (Edin.). F. H. LUMB, M.B., B.S. (Lond.), M.R.C.P.
"Sydney W. Jones Medical Research Foundation":	D. RACE, M.B., B.S.
"Sol Green":	W. McDONALD, M.B., B.S., F.R.A.C.S.

ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

The term medical research conveys different ideas to different people according to their interests and background and perhaps the following facets listed by Professor D. Mainland, of New York, will serve to illustrate some of these interpretations. He divides medical research into: (a) "real" research which is defined as "an open-minded, objective search for knowledge"; (b) a demonstration designed to test specific hypotheses; (c) an educational programme, and (d) a means of providing improved medical services. There is, of necessity, much overlap between these facets.

Reference to previous annual reports will show that all of these groups have been pursued in the Institute and that the proportion of the effort devoted to and the emphasis placed on each has varied from time to time.

The fundamental studies in the biochemistry of blood coagulation, on the behaviour of the cardiac muscle cell and on scleroderma fall into the first category and embrace both laboratory and clinical studies.

The clinical trials of new drugs for the treatment of high blood pressure and cardiac failure represent the second group, for they demonstrate the validity or otherwise of claims made for these drugs when used under common clinical conditions.

Research as part of an educational programme has been a continuing activity. Reference to the list of Hospital Fellows who have worked in the Institute indicates that many physicians and surgeons have taken the opportunity to include some training in research methods in their experience. Also the attainment of various University degrees by theses on work carried out in the Institute is further indication of research as an educational programme. These degrees include M.D., Ph.D. and M.Sc.

Work which has led to the improvement of services is illustrated by the investigations of open-heart surgery which have materially assisted the Thoracic-Surgical Unit and the investigations of cardiac diagnostic methods which led to the formation of the Cardiovascular Diagnostic Service.

Since the Baker Institute (including C.R.U.) was organised on its present basis some thirteen years ago support for medical research and the arrangements for medical student teaching have changed. There has been a considerable increase in the financial resources available to both Baker Institute and Clinical Research Unit and whereas in 1950 the National Health and Medical Research Council was the major body making to us grants-in-aid of medical research, today there are in addition the Life Insurance Medical Research Fund of Australia and New Zealand, the Anti-Cancer Council of Victoria and the National Heart Foundation of Australia. The availability of finance for worthwhile research projects is in consequence now much greater and more diversified than before.

During this same period the Chairs of Medicine and Surgery of the University of Melbourne were established and they developed teaching and research units in Alfred Hospital. The departure of the medical Unit of the University of Melbourne from the Hospital in 1962 will bring changes and a considerable amount of undergraduate teaching is to be carried out by the clinical staff of

the Institute. Further in 1962 the affiliation of Alfred Hospital with Monash University will become effective and the establishment of the Department of Surgery and some non-clinical medical departments of that University in the Hospital foreshadowed. This again must have some impact on our activities.

Over this same thirteen years there has been marked expansion in the laboratory facilities available for our work but unfortunately no comparable increase in clinical facilities. With the work planned for 1962 both clinical and laboratory facilities will be completely committed so that no further expansion of existing projects or initiation of new ones can be contemplated without an increase in space.

In view of these changed circumstances and the full use of the building it seems appropriate at this time to consider future possible developments. Answers cannot be given but some problems may be stated. Enlargement of clinical facilities to balance the laboratory side is necessary and it is to be hoped that this can be achieved in the Hospital rebuilding programme. Consideration must be given to the question as to whether the Institute has reached an optimal size or should grow further. Although independence of the Institute is desirable, what relationship can be established with Monash University to further the objects of the Institute. Finally, at the present time should some apportionment of effort between the various facets of medical research be made, or should they be followed as opportunity arises.

Summary of Research Projects

The current research projects are described in detail in the scientific section of this report and are summarised in the following paragraphs. Attention should however be drawn to the effective way in which numbers of investigators with very different scientific training have combined into investigational teams and the co-operation which has evolved with workers at Columbia University, New York and National Institute of Neurological Diseases and Blindness, Bethesda, U.S.A. and various Australian Universities.

No new projects have been started in 1961 but all those in being in 1960 have been continued although the emphasis between them has perhaps shifted.

Studies on the control of body fluid volume this year have been largely devoted to observation of the excretion of one of the adrenal hormones (aldosterone) in normal women in relation to their menstrual cycle. This provides important basic data for the hormone is one of the links in the volume control mechanism.

The clotting mechanism of blood continues to provide both clinical and laboratory projects amongst which the mechanism of clot lysis has been studied in detail.

Clinical trials of several new drugs used in the treatment of cardiac failure, hypertension and scleroderma have been carried out.

The long term treatment of high blood pressure states and of occlusive arterial disease is continuing.

Investigations concerning energy production of cardiac muscle have expanded considerably and have emphasised the important role played by cations in the regulation of activity of the cardiac muscle cell. In association with this work the development of techniques for recording the action potentials of cardiac muscle cells has reached a stage where considerable data of importance have been accumulated.

The study of serum proteins of New Guinea natives continues to be an active and extensive programme, and the characterisation of various abnormal proteins such as the macro- and cryoglobulins continues with the aid of newly devised apparatus.

Of the problems of cardiac surgery, that posed by the occurrence of heart block and ventricular fibrillation has received special attention, as has also the use of ventricular fibrillation as an adjunct to surgery. Further study of the problem of coronary arterioplasty has been made.

Studies of certain enzymes of leucocytes and erythrocytes continue and have produced data of clinical value as well as giving an insight into some aspects of lipid metabolism. These studies have shown some differences between leucocytes in certain neoplastic conditions and those of normal individuals.

The clinical survey of patients with scleroderma which was commenced some years ago has been expanded and much data on the disturbance of organ function obtained and biopsy material has been stored for future histological examination.

Shorter and smaller projects which have been conducted or continued during the year include pancreatitis, cerebral injury and the socio-economic problems of cardiovascular disease.

Overseas Visits

Dr. P. Fantl attended a meeting of the International Committee for the Standardisation of the Nomenclature of Blood Clotting Factors held this year at Wiesbaden.

Dr. Kidson attended an Asian and Pacific Congress of Haematology at Manila.

Dr. A. J. Barnett made a study tour in the U.S.A. and England to investigate current work in the fields of hypertension and peripheral vascular disease.

Grateful acknowledgment is made to the National Advisory Heart Council, U.S.A., the Anti-Cancer Council of Victoria, Smith, Kline and French Laboratories Ltd. and the Board of Management of the Hospital for the financial assistance given to them for these visits.

Dr. M. Weiss has been granted leave of absence to work at the University of Utah, U.S.A., during the tenure of a post-doctoral fellowship.

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 Trustees of the Patrick Brennan Trust and Alfred Hospital Medical Research funds, and the assistance given 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 help has been given this year by Professors Davies, King, Lovell, Trikojus and Wright, and the staffs of the Departments of Organic Chemistry, Pathology, Medicine, Biochemistry and Physiology, University of Melbourne, and the staffs of the Commonwealth Serum Laboratories, Commonwealth X-ray and Radium Laboratory and C.S.I.R.O. We thank them and others who have helped for their continuing interest in our work. In reciprocity, clinical and laboratory help has been made available to the Departments of Medicine and Surgery of the University of Melbourne within the hospital.

It is with regret that we record the death during the year of Sir David Rivett. He had been a member of the Advisory Panel of the Institute since 1940 and his wise counsel was on many occasions of considerable assistance and will be greatly missed.

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 of all our activities, including assistance for members to visit other centres, and to thank members of the staff and research fellows for their co-operation during the past year.

T. E. LOWE.

31st December, 1961.

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.
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.
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.
South African Institute of Medical Research.
Strangeways Research Laboratories, Cambridge.
Staten Seruminstitut, Copenhagen.
University of Melbourne.
University of Otago, New Zealand.
University of Sydney.
Universitatis Mariae Curie Sklodowska, Poland.
Walter & Eliza Hall Institute, Melbourne.

ALFRED HOSPITAL RESEARCH FELLOWS IN THE INSTITUTE

1949-61

Anderson, R. McD., 1953-55
Andrew, R. R., 1949-55
Barnett, A. J., 1949-50
Beavis, E. L. G., 1955-56
Boake, W. C., 1958
Breidahl, H. D., 1952-53
Burnside, K. B., 1951
Duffy, D. G., 1952-55
Ferguson, I. A. L., 1957-58
Fowler, R., 1953-54
Francis, J. K., 1956-57
Fraser, J. R. E., 1957
Gardiner, J. M., 1952
Goble, A. J., 1951
Hudson, B., 1952
Jamieson, K., 1954
Kay, H. B., 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., 1959-61
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

Dawson, J. B., 1961 (Oxford)
Emslie-Smith, D., 1955-56 (Dundee)
Hamilton, M., 1954 (London)
Lumb, F. H., 1960-61 (London)
Marshall, R. J., 1957 (Belfast)
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, H. A. Ward, E. C. Osborn and R. J. Sawers[1]

Phospholipids and Blood Coagulation

Continuation of our study of the effects of certain lipids on blood coagulation revealed that egg yolk phospholipids had a very low activity in the clotting process. Fractionation of these phospholipids on a silica gel column yielded a number of components. One minor component which had the highest thromboplastic activity was found to contain phosphatidic acid.

One of the difficulties encountered in the preparation of pure phospholipids is preventing their alteration by the action of oxygen from the air. In order to determine what effects oxygen might have on the phospholipids under consideration total egg yolk phospholipids were heated in air at 100°C for 30-60 min. and their thromboplastic activity was compared with that of the unheated phospholipids. A two- to five-fold increase in activity in thromboplastin generation occurred as a result of heating in air but phospholipids heated while protected by nitrogen showed an insignificant increase in activity. Heating in air reduced the amino-nitrogen : phosphorus ratio of the phospholipids but the degree of unsaturation of the fatty acid portion of the molecule showed little, if any, decrease

Total egg yolk phospholipids which had been heated in air at 100°C for 45 min. were fractionated on a silica gel column and again the fraction with thromboplastic activity had chromatographic and chemical properties similar to that obtained in the previous chromatographic separations of unheated phospholipids.

To determine whether phosphatidic acid itself is active in the generation of thromboplastin the following experiments were undertaken. A fraction of egg yolk phospholipids with a nitrogen : phosphorus ratio of 1.0 choline : phosphorus 0.85, amino-nitrogen : phosphorus 0.16, which showed no activity in thromboplastin formation, was incubated in the presence of ether and calcium ions with an extract of Brussell's Sprouts (a source of phospholipase D). Phospholipase D splits off the base from phospholipids, such as phosphatidylcholine and phosphatidylethanolamine, giving rise to a phosphatidic acid. The product of the incubation of the inactive phospholipids with this enzyme preparation showed activity in clotting tests. Control experiments with phospholipid treated with heat-inactivated enzyme, and with enzyme preparations alone showed no appreciable activity.

The ether phase after the enzyme treatment contained phosphatidic acid as a calcium salt. If the ether solution of the phosphatidate was treated with 0.1 N hydrochloric acid, calcium ions were removed and in several experiments no clotting activity was shown by the free phosphatidic acid. It was possible to reactivate the phosphatidic acid. It appears therefore that whilst phosphatidic acid itself is inactive its salts have thromboplastic activity.

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1 Department of Pathology, Alfred Hospital.

* 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 Patrick Brennan Trust.

Phosphatidic Acid and Ion Transfer

In the previous section it was shown that a hydrophilic salt (calcium chloride) reacted wtih phosphatidic acid in a lipoid solvent and produced a lipophilic calcium salt. Further, when phosphatidic acid dissolved in ether was shaken with magnesium salts or manganese salts in aqueous solution, the corresponding metal salts were obtained in the ethereal phase. These observations suggest that a similar process, namely transfer of cations from an aqueous phase by hydrophobic lipid into the lipid phase, may play some role in the biological transfer of hydrophilic cations into lipophilic salts.

A Comparison of Factor VII Activity of Plasma and Serum

Factor VII is an essential component of the haemostatic mechanism. It is not consumed in the clotting process but is present in serum and could thus continue to be active. To determine whether factor VII is present in plasma as a precursor which is converted during clotting to an active factor, factor VII was separated from human plasma and serum. The isolation procedure consisted of barium sulphate adsorption followed by elution with citrate which gives concentrates containing prothrombin, factor VII, factor IX and factor X from plasma, and prothrombin-free material from serum. These preparations were dialysed against distilled water and adsorbed on diethlyaminoethyl cellulose columns. Elution from these was carried out with sodium chloride solutions of increasing concentration with or without a superimposed pH gradient. The effluent fractions were tested for prothrombin and factor VII by specific tests. Preparations containing 90% factor VII and 10% factor X were obtained.

The factor VII preparations from plasma and serum had the same mobility in agar gel and had similar activities when tested with the euglobulin fraction of normal plasma. Further the clotting times of human serum or plasma added to bovine plasma free of factors VII and X, in the presence of human brain thromboplastin and calcium ions were practically identical.

These experiments indicate that factor VII has the same clotting activity in plasma and serum. No evidence for a conversion of a precursor to an active factor could be found.

In further experiments it was observed that factor VII-containing fractions reacted slowly with a brain component and produced high thromboplastin activity. The reaction was studied by adding the factor VII-containing fractions isolated from either human plasma or serum to human brain suspension and calcium ions. Centrifugation at 61,000 x g produced a sediment which was suspended and tested for activity in prothrombin conversion. The suspension showed as high an activity as expected from the amount of factor VII and factor X added prior to the centrifugation. Plasma factor VII and serum factor VII preparations showed identical behaviour. Brain extract without treatment showed less activity.

From these experiments it is concluded that plasma factor VII and serum factor VII have similar properties.

Plasminogen Activity of Plasma and Serum

In laboratory tests it is desirable to determine the potential fibrinolytic activity of blood under conditions closely approximating those in the circulation. According to current views a precursor (profibrinolysin, plasminogen) present in plasma reacts with an activator or kinase to produce an enzyme (fibrinolysin,

plasmin), which is inactivated by anti-fibrinolysins. The concentration of plasminogen in serum will, in addition, be reduced by any capacity of the blood clot to adsorb plasminogen. Therefore the concentration of plasminogen in plasma should be higher than in serum if these conditions prevail.

In order to obtain information about the difference of plasminogen activity in the two fluids, blood specimens were taken from healthy laboratory personnel who are accustomed to handling blood and do not suffer any apprehensions which might enhance fibrinolysis. In addition blood from patients who were suspected of deficient haemostasis was tested.

Plasmin antagonists were destroyed in cell-free plasma and serum by acidification to pH 2.0. After neutralisation, plasminogen was activated with optimal and with excessive amounts of streptokinase (Varidase, Lederle). Proteolytic activity was measured by addition of casein.

The standard deviation (S.D) of the error of the technique calculated from 17 duplicate estimations was ± 2.90. A linear relationship was established between proteolytic activity and dilution of plasma and serum down to 50% concentration. Unsatisfactory results were obtained with excessive streptokinase concentration. The potential proteolytic activity of plasma and serum was not significantly different (at the 5% level) in 15 out of 21 determinations when optimal amounts of streptokinase were used for activation of plasminogen. In 5 instances serum contained a higher plasminogen activity than plasma and in one serum activity was lower than the plasma activity. The serum concentration of plasminogen was, in the majority of cases, not less than 95% of that in plasma and therefore little adsorption of plasminogen had taken place on the blood clot. Plasminogen determinations carried out with normal human plasma before and after defibrination with bovine thrombin gave similar results.

These results have a bearing on the treatment of thrombosis by fibrinolytic agents. In mixtures of purified plasminogen and iodinated fibrin it has been observed that some plasminogen is adsorbed by fibrin and from this finding it has been assumed that fibrinolysis in the circulation results from the activation of plasminogen supposedly present in a thrombus. Also it has been estimated that approximately 30% of the available plasminogen in plasma is adsorbed onto fibrin during clot formation.

The presented data, however, indicate that a clot prepared from normal blood taken during resting contains no more plasminogen than is present in the plasma included by the clot and less than 5% of plasma plasminogen is adsorbed by the clot. It would appear that therapeutic fibrinolysis will only be successful if the concentration of the agent intended to dissolve fibrin is present in adequate concentration in plasma.

Plasmin an Activator of Fibrinolysis inhibited by Streptokinase

A consideration of the action of fibrinolysin and streptokinase is of importance for both have been proposed for the treatment of thrombosis.

The lysis of human fibrin in the presence of streptokinase depends on the activity of profibrinolysin (plasminogen), anti-fibrinolysins and of streptokinase. At low concentration of the last a long lysis time indicates insufficient formation of fibrinolysin (plasmin). By increasing the streptokinase concentration more fibrinolysin is formed and consequently the lysis time becomes shorter. A further increase of streptokinase beyond the optimal concentration apparently inhibits

fibrinolysin for the lysis time of fibrin is prolonged. Injected fibrinolysin has to be carried in the blood stream to the site of action. Streptokinase is used to form fibrinolysin from profibrinolysin in the circulation. At the present time it is undecided which of the available preparations is preferable clinically for the dissolution of an intravascular thrombus.

In order to determine how the various materials act as fibrinolytic agents experiments were carried out in vitro with the following drugs: 2 streptokinase preparations (Streptokinase, Kinalysin), Thrombolysin (human profibrinolysin activated by streptokinase), and a plasmin preparation obtained by activation of human plasminogen in glycerol. The fibrin gels were observed with little disturbance until complete disappearance.

It was found that streptokinase concentrations greater than 500 units per 0.25 ml. oxalated plasma delayed the lysis time of fibrin gels. In contrast, the Thrombolysin preparation gave, with increasing concentration, shortening of lysis time and no inhibition was observed.

Analysis of Thrombolysin indicated that it had proteolytic activity as determined by breakdown of casein and it contained an activator as determined by plasmin formation after incubation with human plasminogen. It was caluculated that the preparation had an activity corresponding to 20% of a proteolytic enzyme and 80% of streptokinase.

In order to establish whether this behaviour of the Thrombolysin preparation could be imitated by streptokinase mixed with a proteolytic enzyme, mixtures of streptokinase or Kinalysin and a glycerol activated plasmin preparation were tested for fibrinolytic activity. 800-1000 units of streptokinase or 2000 units of Kinalysin per 0.25 ml. oxalated plasma were used and the plasmin preparation was diluted to concentrations which by themselves gave a lysis time greater than 30 minutes. The addition of streptokinase was followed by the addition of plasmin and thrombin.

It was found that the inhibition of excess streptokinase can be overcome by amounts of plasmin which by themselves had little fibrinolytic activity. Similar results were obtained when glycerol activated plasmin was replaced by a preparation made by chloroform activation of bovine profibrinolysin, or by trypsin. It is probable that plasmin acts on the streptokinase inhibited complex. The combination of streptokinase with fibrinolysin may therefore have an advantage in the clinical use as a fibrinolytic agent.

HAEMORRHAGIC DISORDERS[1]

P. Fantl and R. J. Sawers[2]

The Occurrence of a New Type of Specific Inhibitor in the Blood of Haemophilia Patients

It is known that some haemophiliacs who benefit at first by blood transfusion become refractory to further transfusion. An antibody against the factor which is missing in the blood of these patients may be responsible for this phenomenon. We had occasion to study a patient suffering from haemophilia, i.e., congenitally deficient in factor VIII who showed persistent haematuria

1 Mr. E. Mason, Commonwealth Serum Laboratories, collaborated in this study.

2 Department of Pathology, Alfred Hospital.

despite transfusions with fresh frozen plasma which are usually adequate for haemostasis. No indication of an antibody nor of an antagonist could be found with the usual laboratory tests and in particular addition of normal plasma to the patient's plasma gave the expected correction in in vitro clotting tests. However it was found that incubation of 25% normal plasma with the patient's plasma for half an hour resulted in the loss of the added factor VIII of the normal plasma.

We believe that the patient who had received numerous plasma transfusions in the past had become sensitised to factor VIII and producted an antibody which destroyeod added factor VIII.

The unusual feature of this factor VIII destroying principle is the slow reaction. In general antibodies act fairly quickly but in a few cases it has been observed that antigen-antibody reaction can occur over a considerable period. It will be necessary to test plasma of haemophilic patients for the presence of a factor VIII-destroying principles, and reduce plasma transfusions to the absolute minimum in order to avoid occurrence of this factor VIII-destroying material.

CONTROL OF BODY FLUID VOLUME*

T. E. Lowe, A. J. Barnett, A. Baumgarten, M. Weiss and V. Carson

The data collected in this investigation have led to the conclusion that the volume of fluid in the body is controlled by a mechanism of the feedback type in which elements of the types "proportional control", "antagonists" and "time delay" can be identified.

During the past year a study of the urinary excretion of aldosterone in normal women has been made for clinical experience with a case of cyclical oedema suggested that the amount of this hormone excreted varied in relation to the menstrual cycle and that the variations were correlated with variations in fluid content of the body. The excretion has also been measured in a few cases of congestive cardiac failure

As the kidney is the excretory organ for body fluids some studies on the action of diuretics have been made from time to time during this project and during 1960 the effects on renal function of new diuretic Triamterene have been investigated. In the course of this investigation some data were obtained which clearly indicate the part played by dynamics of the renal circulation in the control of fluid excretion.

Urinary Excretion of Aldosterone

Serial collections of 24 hour urine specimens were made from six normal women between the ages of 17 and 30 years during a complete menstrual cycle. Subsequently one or two further groups of specimens were collected at different times in the menstrual cycle. Each subject was weighed daily or on the days of urinary collection.

Aldosterone assays were carried out on a total of 76 specimens from the 6 subjects. The analysis was done on 1/10th of a 24 hour specimen, using the technique described last year. The Na and K concentration of each specimen was also measured.

A day-to-day fluctuation in urinary aldosterone excretion was noted and it had a definite and characteristic pattern throughout the menstrual cycle.

Although of different magnitudes the excretory pattern was similar in each subject and reproducible in subsequent cycles. The range of urinary aldosterone concentration was 6–32μg/24 hrs. The highest levels occurred in all 6 subjects from 1 to 5 days prior to the onset of menstruation with a range of 18–32μg/24 hrs.

During menstruation the aldosterone excretion varied in different subjects, there being a rise in the 2nd or 4th day of menstruation in 3 subjects.

The body weight and urinary Na/K concentration ratio fluctuated during the menstrual cycle with no direct relationship to aldosterone levels. However, specimens with the highest urinary aldosterone concentration had the lowest Na/K ratio. A weight increase 4 to 7 days premenstrually with the maximum 1 to 2 days premenstrually occurred in 4 subjects. Aldosterone concentration was either decreasing or already at a lower level when the weight increase was maximum.

The findings described here provide a base line for normal cyclic variations of aldosterone excretion in women and show the importance of noting the phase of the menstrual cycle when assessing the significance of aldosterone measurements in women.

Clinical Trial of a New Diuretic[1]

Triamterene (SKF 8547–2,4,7-triamino-6-phenylpteridine) is claimed to be an effective diuretic which promotes sodium and water excretion without causing a corresponding loss of potassium which may even be reduced.

The drug is being investigated from several points of view to determine its effect on normal, hypertensive and oedematous subjects, its short and long term effects, its effectiveness compared with other diuretics, when used alone or in combination with others.

Patients under observaton are given a diet with a constant electrolyte content and the daily fluid intake and output, weight, urinary sodium, potassium and chloride excretion measured.

In four normal males a single dose of between 75 and 450 mgms increased the urinary excretion of sodium but no increase or a decrease in potassium excretion during the 12 hours after administration.

Given to three hypertensive nonoedematous patients, daily doses between 225 and 300 mgms. for 7 days produced no significant change in the daily excretion of sodium or potassium and no change in arterial blood pressure.

A permutation trial using a preliminary period of 3 days placebo administration, then chlorothiazide, placebo and triamterene each for 2 days in various combinations, was carried out on 7 patients with chronic oedema. In this trial triamterene produced either no or an insignificant diuresis. It produced more loss of sodium than the placebo but less than chlorothiazide. Excretory loss of potassium was always less than that produced by chlorothiazide and was substantially the same as that of the placebo.

Triamterene was administered over a prolonged period to 6 subjects with chronic oedema in a daily dose of 450 mgms. In two patients it was thought that the triamterene may have contributed to loss of oedema. In general however these patients had already proved refractory to other diuretics.

A study of the effect of the drug on renal clearances is continuing.

1 Kindly supplied by Smith, Kline & French Laboratories (Aust.) Ltd.

ENERGY PRODUCTION IN THE MYOCARDIUM†

W. G. Nayler, M. W. McCulloch, P. Emery, J. E. Wright and T. E. Lowe

Previous investigations into the mechanisms whereby the chemical energy supplied to the heart is converted into useful mechanical work yielded results which suggested that the heart's ability to utilize the available chemical energy for the performance of useful work is largely dependent on the extracellular Ca ion concentration. Those drugs (including ouabain, digoxin, lanatoside C and 9-α-fluorohydrocortisone), which simultaneously improved cardiac contractility and efficiency resembled Ca ions in their mode of action and it therefore seems possible that the effect exerted on the myocardium by such drugs may reflect a Ca ion dependent mechanism. Additional investigations into the enhanced cardiac contractility associated with an augmented rate of stimulation (the classical "staircase" phenomenon and post-stimulation potentiation) further emphasised the importance of the role played by Ca ions in regulating the heart's mechanical activity. In the current year the roles played by Ca ions have been further investigated.

Effect of Ca ions on Anaerobic Glycolysis:

It has recently been shown that the rate of anaerobic glycolysis in skeletal muscle isolated from poikilotherms is regulated, in part, by the Ca ion and K ion concentrations in the extracellular fluid. Because the mechanical activity displayed by cardiac muscle is sensitive to changes in the extracellular cationic composition experiments were carried out to determine whether or not changes in the extracellular Ca/K ionic ratio, which were of sufficient magnitude to evoke profound changes in the mechanical activity of isolated ventricular muscle were accompanied by a changed rate of anaerobic glycolysis such as might be predicted from the recent results of Kaye and Mommaerts. Hearts isolated from poikilotherms, rather than from mammalian animals, were used since it is well established that cardiac muscle which is of poikilothermic origin can withstand prolonged periods of nitrogen induced anoxia.

The rate at which anaerobic glycolysis proceeded in freely suspended strips of toad ventricular muscle was followed by determining the total amount of lactic acid produced during a known time interval (usually one hour). Anoxia was induced by bubbling nitrogen through the perfusion system and the cationic composition of its perfusate changed by varying either the Ca, K or both the Ca and K ionic concentrations. In all more than 150 determinations were performed.

Despite wide variations in the cationic composition of the perfusate no data were recorded to support the hypothesis that the ionic composition of the extracellular fluid modified the metabolic activity of cardiac cells. Since others have shown that the rate of anaerobic glycolysis in poikilothermic skeletal muscle was regulated, in part, by the extracellular concentration of several cations including Ca and K, it must be concluded that the regulation of metabolic activity in cardiac cells differs essentially from that in cells of skeletal origin. Moreover the ability of Ca and K ions to modify cardiac contractility seemingly cannot be attributed to a direct effect of these ions on anaerobic glycolysis.

The Effect of Low Temperature on the Activity of Sympathominetic Amines

The positive inotropic activity of the sympathomimetic amines is abolished at low temperatures. A series of experiments were performed to determine

whether or not the phosphorylase activity of adrenaline is similarly depressed by low temperatures. Both mammalian and cold-blooded hearts were used.

(a) Mammalian hearts (rat):

Adrenaline, in therapeutic doses, augmented the contractile force and stimulated the conversion of phosphorylase 'b' to 'a' (active form) in isolated rat hearts perfused at either 25° or 35°C. At low temperatures (12°C) the response to adrenaline showed a marked change in that in some preparations ventricular contractility was depressed despite a high phosphorylase activity. These results suggest that it is possible to separate the action of the amines on cardiac contractility from their action on the phosphorylase system of mammalian cardiac muscle.

(b) Cold-blooded hearts (toad):

Isolated toad hearts perfused at either 12°C or 22°C produced a positive inotropic response and an augmented phosphorylase activity in response to the administration of adrenaline. At lower temperatures (2-4°C) the results recorded from spontaneously beating preparations were variable in that those hearts which contracted rhythmically at these temperatures displayed an intrinsically high phosphorylase activity even without the administration of exogenous amines. Hearts which were stimulated at the rate of 6 beats/minute but which were perfused at 2°C all displayed a high phosphorylase activity unless the specific amine antagonist, DCI, was present or unless the toads had been pretreated with reserpine. These results have been interpreted to mean that, in cold-blooded animals perfused at low temperatures, spontaneous or electrically induced activity results in the release of endogenous amines; presumably at low temperatures the amines stimulate phosphorylase activity in the cold perfused cold-blooded hearts. However the addition of adrenaline to isolated toad hearts perfused at 8°C did not elicit a positive inotropic response.

The Role played by Ca ions in Regulating Cell Membrane Permeability

During an investigation into the possible role played by Ca ions in regulating the normal events associated with the contraction and relaxation of cardiac muscle, arrhythmias were repeatedly noted following the administration of the disodium salt of EDTA (Na_2 EDTA). These Na_2 EDTA induced arrhythmias were investigated using isolated rat and toad hearts and the results recorded indicated that the arrhythmias originated from the chelation of some of the membrane Ca ions by Na_2 EDTA. Such a finding is in accord with the hypothesis that the selective permeability of cardiac cell membranes is regulated, in part, by a membrane-Ca ion component.

Additional experiments have shown that the role played by Ca ions is specific. That Sr ions cannot replace Ca ions was shown in a series of experiments in which Sr ion enriched Ringer solution replaced Na_2 EDTA Ca-Ringer. Under these conditions contracture developed and the cell membrane resting potential was not maintained, re-introduction of Ca-Ringer restored both contractility and membrane permeability.

Experiments so far suggest that Ca ions play at least two roles: (a) the regulation of cardiac contractility, in which capacity they can be replaced by Sr ions; (b) the regulation of cell membrane permeability, in which role they cannot be replaced by Sr ions.

Throughout these and other experiments seasonal changes in the response of toad cardiac muscle to Ca ions have been repeatedly noted. Thus the response of ventricular muscle to additional Ca ions in the absence of any perfusate K ion undergoes marked seasonal variation. Similarly the response of the isolated toad heart to EDTA and its subsequent removal differs in the summer and winter preparation. Such observations confirm preliminary findings in this study which suggested that the Ca-membrane complex was hormonally regulated.

The Metabolic Basis of the Action of Quinidine on Ventricular and Atrial Muscle

It is generally agreed that quinidine depresses the mechanical and electrical activity of cardiac muscle. Such activity may reflect a changed permeability of cardiac cell membranes and therefore may result from interference by quinidine in either or both of the roles suggested for Ca ions in normal cardiac functioning. Other theories have been postulated to explain the mode of action of quinidine (e.g., an altered efflux of K ions) but in general the mode of action of the drug remains obscure.

A series of investigations have been initiated to study the depressant as well as the antifibrillatory activity of quinidine. Spontaneously beating as well as stimulated isolated rat hearts have been used and the results obtained indicate that high perfusate Ca ion concentrations abolish the depressant action of the drug whilst low Ca ion concentrations potentiate it. By contrast high Ca ion concentrations apparently favour the negative chronotropic activity of the drug whilst low Ca ion concentrations protect against the chronotropic effect.

Membrane Potentials of Cardiac Muscle Cells

As the uneven distribution of ions in the intracellular and extracellular fluids gives rise to electrical changes which can be recorded the following study of the action potentials of cardiac muscle cells has been complementary to the investigation just discussed.

Recording Equipment:

This year, a new pre-amplifier has been incorporated into the apparatus for recording action potentials from toad ventricular cells. It consists of an electrometer tube operating under cathode follower conditions with a feedback device. In this pre-amplifier the input grid current is 10^{-12} amp., i.e., the current flowing through the membrane of the cell into which the microelectrode has been inserted is 10^{-12} amp. under recording conditions. Thus the cell cannot be modified chemically by the ionic current flowing while action potentials are being recorded. The chloride ion concentration in the cell has been calculated to be the ionic concentration most sensitive to input grid current. The net increase of chloride ion concentration in a single cell due to a 10^{-12} amp. current flow for ten seconds is 0.0004mM, i.e., 0.80% change from normal. The input capacitance of the pre-amplifier is (approx) 2pF, giving a time constant of 0.05 m. sec. for a typical microelectrode resistance (25×10^6 ohms).

Also a device has been designed and built to record simultaneously isometric contraction tension and cellular action potentials. A thin strip of ventricle is mounted horizontally on two glass hooks in Ringer solution in a Petrie dish set in a perspex constant temperature bath. The Ringer solution surrounding the ventricle strip is earthed. One glass hook, adjustable in distance from the other hook for the purpose of tensioning the strip of ventricle, is clamped for each recording, while the other hook is attached to an R.C.A. type 5734 transducer

mounted vertically above the hook. The output from the transducer is amplified so that a typical amplified contraction pulse is 2V in magnitude. The action potentials are recorded by an assembly consisting of a microelectrode attached rigidly and connected electrically by platinum wire to the grid of an electrometer tube, which is a part of the pre-amplifier described above. This assembly is mounted on a stage which approaches the ventricle strip at an angle of 45° to the horizontal plane. This stage can also be tilted at any angle to the plane of approach required. Two controls are employed for the approach of the microelectrode assembly to the ventricle strip. These are: (1) Coarse control with 1 mm approach per 360° rotation. (2) Fine control with 5μ approach per 360° rotation.

Stimulation is effected intracellularly by a coarse microelectrode (tip diameter approx. 0.1 mm). A 10V pulse of 10 m.sec. duration from a Tektronix Pulse Generator Type 161 is passed through the stimulation electrode, which has penetrated the outside membrane of the ventricle strip, through the membrane and to ground through the Ringer solution surrounding the strip. Stimulation artefacts initially caused partial obliteration of the action potential upstrokes, and also raised the input grid of the electrometer tube to undesirable voltages above ground. The artefacts were reduced to an acceptably low magnitude by increasing the surface area of the external silver electrode grounding the bath of Ringer solution, and by coating the electrode with a thin layer of silver chloride thereby rendering it nonpolarizable. The effect of these two changes was a reduction of the impedance from the Ringer solution to ground, and hence a reduction in the voltage to which the bath was raised above ground by the stimulus. The outputs from the transducer pulse amplifier and pre-amplifier with microelectrode input are displayed on a double beam Tektronix oscilloscope with a long persistence tube and photographed as time exposures with a reflex camera with coupled close-up lenses loaded with Kodak Tri-X film.

Investigations:

(a) Effect of temperature change on the action potential.

The normal upstroke was found to be logarithmic in form, consisting of two parts, the first of which occupies the first 1.5-2.0 m. sec. of the upstroke and the second a further 1 m. sec. At room temperature (approx. 20°C) during the first part of the upstroke the intra-cellular potential rises by 10 to 15 mV so that the rate of change of $\log_{10}$ (intra-cellular potential) ranges from 48 to 80/m. sec. with an average of 64/m. sec. This variance may be due to cell damage, differences in muscle fibre size, or distance from the stimulated cell. The second part commences suddenly with a decrease in this logarithmic rate to 36 $\pm$ 2/m. sec. (90% confidence limits), and continues until the maximum rate of rise is reached when the intra-cellular potential has risen by approx. 60 mV. The maximum rate of rise at 20°C is 60 $\pm$ 5V/sec. (90% confidence). There follows a gradual decrease in the rate of change of potential until the peak of the action potential is reached between 3.0 and 3.5 m. sec. after the beginning of the upstroke.

The effects of the lowered temperature (15°C) were firstly a 5% drop in the logarithmic rate of the first part of the upstroke per centigrade degree, and secondly an increase in the time required to reach the peak of the action potential once the maximum rate of rise had been reached—the duration of the upstroke became 4.0 to 4.5 m. sec.

At 30°C (10° higher than room temperature) the only significant change from normal was a 1% increase per centigrade degree in the maximum rate of rise of the intra-cellular potential.

(b) Ventricular tachycardia and fibrillation.

The upstrokes of the action potentials of a ventricle beating spontaneously at four times the normal rate were compared with the upstrokes of the same ventricle beating at a normal rate in response to a stimulus. The maximum rate of rise of intra-cellular potential had risen by 42% to 82 V/sec. The form of the upstroke was still logarithmic, the difference from a normal upstroke being that the first part, although its logarithmic rate was normal, extended to twice the normal rise of potential for the first part, and also that the logarithmic rate of the second part had increased by 75%.

(c) The effect of EDTA.

Two concentrations of Na_2 EDTA in the bath were investigated. They were (i) 2.4 mM, (ii) 4.8 mM. The latter proved to be lethal to the mechanism for restoration of resting potential within five minutes of application. The first concentration produced a spike in place of the normal phase 1 of the action potential, i.e., immediately preceding the upstroke, within two minutes. The gradient of phase 1 was at this stage six times the normal gradient, while the duration of phase 1 was one-half of the normal duration. Indications were therefore that the membrane had become much more permeable to K ions and that Ca ions which compete with K ions for entry into the membrane had been rendered inert. The change in the gradient of phase 3 supported these conclusions, for an increase of 25% had occurred. After 30 minutes, phases 1 and 2 had merged into a smooth curve, but phase 3, the rapid repolarization by movement of K ions to the normal resting potential, had shortened by 40% of normal (this had also occurred after two minutes application) and its gradient had increased by 70%. The second concentration also caused an increase in the gradient of phase 3, the magnitude of the increase being 50% after one minute of application. The effect of the second concentration on the upstroke of the action potential after one minute was the loss of the first part of the normal upstroke, and instead a part resembling the second part of the normal upstroke occupied the first three m. sec. but with logarithmic rate of rise 30% lower than normal. The remaining section of the upstroke, i.e., from about 40mV to 100mV, was completed in 14 m. sec. at a gradually decreasing rate.

(d) The effect of low-external Ca ion concentration.

The effect of (1) Ringer solution containing one-half the normal Ca ion concentration, (2) Ringer solution containing no Ca ions, was tested. The gradient of phase 3 after 30 min. beating in the first modified Ringer solution was 50% larger than normal. After one hour, the logarithmic rates of rise of the upstroke were both less than normal, the first part 25%, and the second 20% less. Also after one hour the rise obtained by the first part of the upstroke was only one-half of the normal rise. When the ventricle was immersed in the second solution containing no calcium it could not follow the stimulus rate of 1 beat/2 sec. It responded to every second stimulus by giving a double beat at rate of 1 beat/sec. Phase 1 was not present in these double beats. The gradient of phase 2 after approx. 20 sec. had increased only in the second of each double beat by 0.4%. Also at this time, the gradient of phase 3 had increased by 65% in the first of the double beat, and by 90% in the second, while its duration

decreased by 30% in the first and 50% in the second. After 40 sec. the gradient of phase 3 of each of the beats of the double beat had further increased—the first to 100%, the second to 120%. After 2 min. spiked action potentials appeared. After 30 min. the spikes had disappeared and the gradient of phase 3 was now 160% larger than normal. After one hour, EDTA was added. It caused the gradient of phase 3 to increase still further to four times the normal value, but was lethal after 5 min.

In the light of current hypotheses concerning the production of action potentials these results indicate that a very low concentration of Ca ions causes an increased permeability of the cell membrane to K ions, interferes with the mechanism responsible for phase 1, the initiation of repolarization, and decreases the rate of rise of the upstroke, but has apparently no effect on the maintenance of the resting potential.

(e) Replacement of Ca ions by Sr ions in extra-cellular fluid.

When mechanical activity was arrested by perfusing the ventricle with Ringer solution containing one-half the normal Ca ion concentration for one hour and then adding EDTA to give 2.4mM concentration in the perfusing fluid for another hour, thereby "fixing" all remaining calcium, subsequent immersion of the ventricle in Ringer solution containing no Ca ions for half-an-hour gave a resting potential of 25mV. If the Ringer's solution containing no Ca ions was replaced by one containing Sr ions at a concentration one-half the normal Ca ion concentration, the resting potential after half an hour was 35mV. This value is considerably lower than the corresponding resting potential for a ventricle strip perfused with Ringer solution containing one-half normal Ca ion concentration (80mV). Comparison of ventricle parameters, after perfusion for half an hour in Ringer solution containing (1) one-half the normal Ca ion concentration, (2) Sr at a concentration one-half the normal calcium ion concentration reveals (a) a 40% lower gradient of phase 3, the rapid repolarization, when Sr is substituted for Ca, (b) a 60% drop in the gradient of the plateau, phase 2, (c) no change in the gradient of phase 1, the initiation of repolarization.

(f) The Effect of Aldosterone.

The effect of aldosterone at a concentration in the perfusate of approx. 0.05μg/ml. is being investigated currently by simultaneously recording isometric contraction and action potentials. Within one minute of application of aldosterone the contraction amplitude decreases to one-sixth of its previous value. Recordings of action potentials at this stage indicate, although the figures are not yet statistically significant, (1) a decrease in the duration of the plateau by 60%, (2) a 100% increase in the gradient of phase 1, the initiation of repolarization, (3) no change in the gradient of the plateau, (4) no change in the gradient of phase 3, (5) a considerable decrease in the rate of rise of the upstroke. After 40 min. a partial recovery has occurred with potassium exit from the cell enhanced causing an increase of 80% in the gradient of phase 3.

Plasma Pressor Activity

C. C. Curtain and W. G. Nayler

Nayler and McCulloch have shown that a number of animal and human plasmas contain a dialysable, heat stable factor which has a positive inotropic action on the isolated hypodynamic toad heart perfused with Ringer's solution.

As it is extremely easy to produce pharmacologically active peptides by breakdown of plasma proteins during various isolation procedures it was considered essential to use the mildest possible methods of fractionation. To this end gel diffusion chromatography on 'Sephadex' cross-linked dextran gels was used in the initial process. It was found that the inotropic activity could be separated into two fractions, one with a molecular weight in the range 3500 to 10,000 and the other with a molecular weight over 50,000. Unfortunately 'Sephadex' columns on standing often produced a substance which profoundly depressed the isolated heart preparations, making biological assay of the inotropic activity impossible. This does not happen with the acrylamide gels mentioned in another part of this report and reasonably large-scale preparations of the low molecular weight inotropic factor may be carried out in acrylamide columns. Final separation of the factor was achieved by filter paper electrophoresis at 2°C and 100 V/cm. The material from both the 'Sephadex' and acrylamide gel columns gave 20 ninhydrin reacting bands by this method. Preparative high voltage electrophoresis runs were carried out on wide strips of thick filter paper. After the run the paper was cut into transverse strips, each corresponding to a peptide band, and eluted with Ringer's solution. The eluates from the strips were assayed and only one appeared to have inotropic activity. On concentration and re-running this active fraction migrated as a single band at pH 4.5, pH 6.0 and pH 8.0. Electrophoresis in the presence of radioactive calcium and subsequent autoradiography indicated that the peptide does not bind calcium, and chemical tests for carbohydrate and steroid have so far been negative.

Work is proceeding on the isolation of the activity from the high molecular weight fraction.

HYPERTENSIVE STATES

A. J. Barnett, A. Baumgarten, K. Burnside[1], D. G. Duffy[1], P. Kincaid-Smith, H. A. Luke[2] and F. H. Lumb

Clinical Trial of Hypotensive Drugs

Our study of the treatment of severe hypertension with ganglion-blocking agents, or other sympatholytic drugs such as guanethidine, augmented with other hypotensive drugs such as reserpine and the thiazide group has continued. Prolongation of life and relief of symptoms has already been demonstrated and experience in the past year adds to that already recorded in previous reports. It is considered necessary to continue this trial in the patients' interest and to paint as complete a picture as possible of the prognosis of severe hypertension with strenuous treatment.

The mechanism and nature of hypertension is still not clear, and modern treatment, although valuable, is still not ideal, for complete control of blood pressure cannot usually be achieved without the production of side effects. The patients in this study serve as subjects for assessment of new views on the basis of hypertension, and also for the evaluation of new remedies.

Three new hypotensive drugs have been subjected to clinical trial during the year. They are Bendrofluazide and Cyclopenthiazide which are claimed to reduce high blood pressure without the potassium loss produced by most members of this group and α-methyldopa which is claimed to deplete tissues of noradrenaline.

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Bendrofluazide (Aprinox)[3]

The trial was conducted on 22 patients, all of whom had received previously either chlorothiazide or dihydrochlorthiazide in doses of 1.0 Gm. or 75 mgm. per day respectively. The serum potassium levels of these patients were determined and they were then given bendrofluazide, 5 mgm. per day, for four weeks. Weight and blood pressure were determined weekly during this period and the serum potassium levels were again determined at the end.

Serum potassium concentration increased by 0.5 mEq/1 in three patients, decreased in six, and remained unchanged in nine. Four cases were excluded because of changes in the potassium chloride intake.

The average weight gain was 0.6 Kg., there was no significant average blood pressure change. It was concluded that in the dosages employed bendrofluazide was comparable to the other two drugs in its effects on the blood pressure and serum potassium levels.

Cyclopenthiazide (Navidrex)[1]

A cross-over method was employed in comparing the effects of 0.5 mgm/day of cyclopenthiazide with 1.0 Gm. per day of chlorothiazide in 19 patients. A period of two weeks without either drug at the commencement of the trail and at the cross-over permitted the return of serum potassium concentration to normal values. The effect of the drugs on blood pressure, blood urea and body weight was also determined. Although the final evaluation of the results has not yet been completed, the information at hand indicates that in the dosages employed cyclopenthiazide and chlorothiazide have comparable hypotensive action and a similar tendency to increase the blood urea levels. Cyclopenthiazide may cause a slightly lesser decrease in the serum potassium concentration and in body weight than chlorothiazide.

A-methyldopa (Aldomet)[2]

α-methlydopa has been stated to have marked hypotensive action in animal experiments and a few experiments in man. It is stated to have an inhibitory action on l-aromatic amino acid decarboxylase and to deplete the tissue noradrenaline content.

A trial of this drug has been commenced in 18 hypertensive patients. Ten of these had received no therapy previously or the pre-existing therapy had been discontinued prior to the commencement of α-methyldopa. Eight patients who had not been adequately controlled received α-methyldopa in addition to the previous regime. These patients, with the exception of two who were withdrawn from the trial because of depression, have now received the drug for between eight and twelve weeks.

A satisfactory fall in blood pressure occurred in seven patients on α-methyldopa alone and in four when combined with other drugs at dosages of 0.75 Gm. or less α-methyldopa per day. Two patients had a fall in blood pressure at doses of 4.0 Gm. per day of α-methyldopa alone, while a fall in blood pressure was obtained in the remainder with doses of α-methyldopa above 3.0 Gm. per day in combination with other drugs.

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3 Kindly supplied by Boots Pure Drug Co. (Aust.) Pty. Ltd.

1 Kindly supplied by CIBA Company Pty. Ltd.

2 Kindly supplied by Merck, Sharp and Dohme (Aust.) Ltd.

The toxic effects included depression, dizziness and tinnitus and caused the withdrawal of two patients from the trial. There is some indication that tolerance to the drug may develop.

While no firm conclusion can be drawn as yet, it is suggested that the drug sharply separates the hypertensives into two groups responding to widely different dose levels and this, if confirmed, may be of aetiologic significance. α-methyldopa appears to be an effective hypotensive drug but toxic effects in some patients and developing tolerance in others may limit its use. The trial is being continued to resolve these problems.

Renal Basis of Hypertension

It has been demonstrated that hypertension with the features of essential hypertension may be caused by a renal vascular lesion. In many cases it may be cured or alleviated by nephrectomy or correction of a lesion of the renal artery. Recently the view has been taken that many cases of apparently essential hypertension have a renal vascular basis.

During the year, the majority of patients studied with severe hypertension have been submitted to a test[1] involving the excretion of radioactively labelled para-amino hippurate to determine the proportion of patients with severe hypertension who have a renal vascular lesion revealed by this test. The test is regarded as a screening procedure and its value in indicating a renal vascular abnormality is still under investigation.

Radio-renograms have been performed in 47 of the 79 members of the hypertension group with the following results:

Normal findings		14
Abnormal findings		33
Bilateral symmetrical impairment of function	17	
Bilateral asymmetrical impairment of function	5	
Unilateral impairment of function	11	
		16

Thus in 16 of the 47 cases studied there is an indication of unilateral renal impairment or impairment more marked on one side. Further study is to be undertaken to determine whether the abnormality in these cases is due to a renal vascular lesion, and, if so, surgical treatment is indicated.

The renal hypertension "panel" constituted last year continues to meet and discuss cases of hypertension suspected of having a renal basis. Twenty-six cases of interest seen by members or referred by other physicians or surgeons have been discussed in 1961.

DISEASES OF PERIPHERAL BLOOD VESSELS

A. J. Barnett, A. Baumgarten, V. Carson, A. V. L Hill, K. N. Morris[2] and G. R. Stirling[2]

Arterial Grafting

The treatment of patients with localised obliterative arterial disease of the lower limbs by arterial grafting has continued and 16 grafts have been inserted

1 This test was carried out by courtesy of the Department of Medicine, University of Melbourne, at the Royal Melbourne Hospital.

2 Thoracic Surgical Unit, Alfred Hospital.

during 1961. In all but one the immediate result was good. In the first 4 of these the material used was woven "Teflon" as in the past 2 years. However several cases of late haemorrhage from the anastomosis site have occurred with this material and in the last 12 cases, woven "Dacron" has been used in the hope that a more satisfactory result may be obtained with this material. Although arterial grafting has given worthwhile benefit to many patients the overall results are still below what had been hoped for. Only 40% of grafts remain patent 4 years after operation. Further work is necessary to improve grafting methods and materials.

Sympathectomy

(a) Eight year survey.

The place of lumbar sympathectomy in the treatment of occlusive arterial disease of the lower limbs has remained uncertain. We have reviewed our experience with this method over the past 8 years during which the operation has been performed on 147 patients (245 limbs). The indication for operation was ischaemic symptoms of the skin—mainly of a severe nature such as rest pain, ulceration and localized gangrene. Two-thirds of the patients also suffered from intermittent claudication although this symptom was not used as an indication for operation. Many of the patients were old and feeble, 44 being over 70 years of age.

Five patients (3%) died within 4 weeks of operation. Early relief of symptoms was achieved in all patients with minor symptoms (numbness, paraesthesia, coldness) only, in 60% of those with rest pain without ulceration, and in 25% of those with ulceration. Approximately 50% of patients operated on were receiving benefit in relief of distal ischaemic symptoms one year after operation. This figure was reduced to 30% at 5 years. The relief of claudication was much less frequent, being claimed in about 10% of cases.

The prospect of limb survival after sympathectomy was good—90% of limbs surviving one year after operation and 75% 5 years after operation.

The results were worse the more severe the symptoms and the more extensive the arterial block. The proportion receiving benefit was also less in older people and diabetics.

The survey indicates that sympathectomy is a valuable method of treatment for symptoms of skin ischaemia from obliterative arterial disease, but in the type of patient studied (with severe disease) the proportion obtaining relief was less than was hoped. In view of the low operative risk, and the frequent persistence of benefit in patients obtaining relief, it would seem correct to advise operation at an earlier stage before symptoms have become severe.

(b) Criteria for sympathectomy.

As our survey has indicated it has proved difficult to predict the result in individual cases of sympathectomy performed for the relief of obliterative arterial disease of the lower limbs.

An investigation has been commenced to determine what criteria are of value in predicting improvement from sympathectomy and 20 patients have been investigated so far.

In addition to routine clinical examination, patients have been assessed by postural tests, oscillometry, skin temperature studies and reactive hyperaemia

tests. These tests have not proved very helpful in predicting the outcome as good results were obtained in the majority of patients in spite of unfavourable findings comprising—high level of arterial block, poor oscillometric readings, delayed venous filling time (over 30 sec.), prolonged flushing time (over 30 sec.) and reduced flushing level (under 30 cms.) following reactive hyperaemia.

The tests were repeated 1 week after operation and frequently showed improvement in the nutrition of the skin and decreased venous filling time on dependency in spite of no change in pulse findings and oscillometric readings.

Effect of Triparanol (MER-29)[1] on Serum Cholesterol Levels

Triparanol, a drug which interferes in the biosynthesis of cholesterol has been used in a clinical trial to test its effects on serum cholesterol levels in 12 patients with atherosclerosis of the lower limbs and 10 with coronary atherosclerosis. Assessment of any clinical benefit from the drug was also made.

After establishment of pretreatment serum cholesterol levels (275-526 mgms. per 100 ml.) patients were given MER-29 for 6 months (the dose, initially 250 mg/day was raised in nearly all cases to 500 mg/day after 2 months) and at the end of this time a series of cholesterol determinations were made. The apparent serum cholesterol showed a significant fall ($P < 0.05$) in 17 out of 22 cases. The mean fall (22 cases) was 69 mgms. per 100 ml. or 20%.

A precursor, desmosterol, accumulates as a result of MER-29 therapy and the method of estimation employed does not differentiate between the two sterols. Work in is progress to determine by more involved techniques the actual reduction in total sterols and the relative amounts of each.

The apparent falls in serum cholesterol are of the same order (10%) as those found by other workers.

No side effects or toxic reactions were noted as gauged by a number of haematological and biochemical tests performed before and after treatment. There did not appear to be any real improvement in the patients' clinical condition.

Effect of Nicotinic Acid on Serum Cholesterol Levels

After determining control values, 10 patients with lower limb atherosclerosis were given nicotinic acid (3 g./day) for 6 months. At the end of this time a series of serum cholesterol determinations was made. A significant fall ($p < 0.05$) was obtained in 3 cases and a highly significant fall ($p < 0.01$) in one. In 3 cases where a significant fall was not obtained the patients failed to adhere to the recommended dose, either taking less or at times discontinuing the drug altogether for some weeks.

Patients who failed to get a satisfactory response to either MER-29 or nicotinic acid alone are being given both drugs simultaneously.

Clinical Trial of Segontin[2]

Favourable reports from overseas in 1700 cases prompted the trial of Segontin, a newly synthesised anti-anginal agent with claimed coronary vasodilator and catechol amine depleting activity.

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1 Kindly supplied by Wm. S. Merrell Pty. Ltd.

2 Kindly supplied by Hoechst Pharmaceuticals Pty. Ltd.

Of the 12 patients with stable chronic angina and electrocardiographic evidence of cardiac ischaemia ranging in age from 35 to 66 years, only eight completed the trial. In the first part of the trial a double-blind, cross-over method was employed, and it was found that four of six patients responded favourably to Segontin in a dose of 90 mgms/day. An open method was used in the second part of the trial, over a six-week period with a 180 mgms/day dose of Segontin. Five patients who did not respond to the lower dose were found to have improved on the increased dosage of Segontin the effect was difficult to evaluate in the remaining two patients

The response was considered highly suggestive of the symptomatic effectiveness of Segontin in the treatment of angina pectoris despite the small number of patients. Segontin did not alter the blood pressure, electrocardiographic pattern, haematological findings or the blood urea and serum cholesterol levels.

CARDIAC SURGERY†

G. R. Stirling[1], D. Race, and K. N. Morris[1]

Heart Block

The occurrence of complete dissociation of the atrial and ventricular beats after surgical repair of large defects of the ventricular septum remains as a clinical problem. Advances in surgical technique have reduced its incidence and the use of direct myocardial stimulation has lessened the mortality from it in the early post-operative period. Difficulties in the control of the heart rate in such patients has led us to study the problem in the laboratory.

Heart block was created in dogs by ligature or diathermy of the atrioventricular bundle during a short period of venous occlusion. Flexible insulated braided wire electrodes of stainless steel or silver-coated copper wire were inserted at different points on the myocardium or into the chest wall. A physiological stimulator was then used to stimulate the heart at threshold and optimum levels and a cathode ray oscilloscope displayed the electrical events for measurement and photographic recording. The resistance between the electrodes was determined by substitution with a decade box, and found to be in the range of 50 to 500 ohms. In any particular animal the resistance was found to vary very little over periods up to two weeks. Higher values were recorded when one electrode was placed in the myocardium and the other in the chest wall. Similar studies on 5 human subjects showed that the resistance levels were comparable with those in the dog but on one occasion the resistance was found to be 1000 ohms.

Teflon-coated braided stainless steel and polyethylene-enclosed braided silver-coated copper wire electrodes were both found to be mechanically satisfactory.

The minimum voltage required to stimulate the heart did not vary with the site of the electrodes on the myocardium but a higher voltage was required if one of the electrodes were placed in the chest wall. There was a distinct difference in threshold voltage with reversal of polarity, lower voltages being adequate if the 'heart' electrode were negative.

The influence of the duration and shape of the stimulating current wave were also studied. It was found that higher voltages were required if the pulse

1 Thoracic Surgical Unit, Alfred Hospital.

duration was much less than 2 m.secs. whereas increasing the time of stimulation from 2 to 100 m.secs. had little effect on the threshold voltage. The duration of the stimulating current has both an upper and a lower limit. It was possible to induce ventricular fibrillation by using pulse widths of 30 to 100 m.secs. with voltages within the range commonly employed in clinical practice.

In the light of this information the wave forms generated by 5 commercially available stimulators were studied and several undesirable characteristics were found. An attempt has been made to define the characteristics of a reliable and safe stimulator. These would appear to be:—

1. That the apparatus should be of simple design and rugged construction, capable of functioning without maintenance or loss of efficiency for 200 hours. Isolation from high voltage alternating current sources is desirable.
2. It should be capable of generating a square wave stimulus of 5 to 10 m.secs. duration at 50 to 150 pulses per minute and with voltage regulation from 0 to 10 volts when connected to a patient whose resistance can be expected to lie between 50 and 1000 ohms and whose capacitance will be of the order of 0.3 micro-farads.
3. The two electrodes are best placed in the heart muscle but their precise site is not critical.
4. A switching device for reversal of polarity would seem to be advantageous.

Ventricular Fibrillation and Air Embolism

An extensive experience with the deliberate induction of ventricular fibrillation by electrical stimulation in dogs has demonstrated to us that this state can be initiated and reversed with certainty. It seemed that two advantages might accrue from the application of this knowledge to human open heart surgery. Most importantly the fibrillating heart does not act as a pump so that during cardiotomy with air-filled chambers the risk of air embolism might be reduced. Secondly the absence of a co-ordinated forceful beat facilitates some technical manoeuvres. Deliberate induction of ventricular fibrillation seemed a preferable technique to the induction of asystole since the myocardial blood supply can continue in the former instance.

The functional effects of long periods of ventricular fibrillation have been studied by the myocardial function study technique which has been described in previous reports. Insignificant changes occur after 40 minutes of ventricular fibrillation and after one hour mild to moderate impairment of function is occasionally observed. The deliberate induction of ventricular fibrillation has now been successfully used in 5 patients with encouraging results. The technical problems of avoiding passive distention of the fibrillating heart have been studied and overcome.

Hypothermia

The functional effects of reduction of the body temperature on the myocardium have been studied. Myocardial function is little changed when the temperature is reduced to between 28°C and 30°C, however, progressive reduction below these levels results in gross changes and between 20°C and 25°C the heart fails in the face of a trivial work load. Investigation of the reversibility of these changes with re-warming indicates that there is great individual variation, the degree of recovery being very poor in some instances.

In an attempt to analyse this problem further we have carried out a few pilot studies with an isolated but metabolically supported preparation similar to that described by Sarnoff and Braunwald.

Mitral Valve Replacement

Work continues on the use of autogenous pericardium as a material for replacement of the cusps of the mitral valve. The use of the left-sided approach and of deliberate ventricular fibrillation are significant technical advances.

Coronary Artery Disease

Laboratory studies have now established the techniques for supporting the heart during prolonged operations on the coronary arteries. The insertion of venous autografts as roofing patches has overcome the problem of potential narrowing of the lumen of the artery by direct repair. A considerable experience has now been gained by dissection and 'mock operation' on autopsy material with obliterative coronary arterial disease. The feasibility of extensive endarterectomy in a good proportion of cases has been convincingly demonstrated.

The development of adequate arteriographic display of the coronary arterial tree appears to be the main clinical problem to be overcome in the selection of cases for surgical treatment.

SOCIAL ASPECTS OF CARDIOVASCULAR DISEASE‡

B. B. Thomas[1], E. Rosanove[1], and T. E. Lowe

The study of psycho-social and economic problems associated with cardiovascular diseases has continued on a series of patients aged 30-65 years in 4 diagnostic categories. During the past 18 months, data have been collected about re-employment, economic effects and the effects on rehabilitation of the patient's conception of the consequence of heart disease.

Re-employment

Data show that 42 out of the 60 patients who were considered medically fit to resume work returned to jobs with their former employers. One-half of these resumed their previous jobs while the remaining number were found lighter duties and/or shorter hours. Nine patients found work with different employers and nine have not yet resumed work.

The most notable problems in re-employment have been difficulties in placing patients over 50 years of age; lack of clarity in the definition of light work; patients' lack of training for many categories of sedentary jobs; fears that exertion will produce another heart attack; the availabiltiy of suitable jobs; and the patient's motivation to resume work.

Problems of adjustment experienced by patients who have returned to work included lowered status and prestige, transport difficulties and psychological problems. The more objective problems diminished more readily than the subjective problems of lowered status and psychological difficulties.

The housewife's ability to resume her normal household duties has proved difficult to measure because of the subjective nature of housekeeping standards. It is apparent, however, that there is a need for long-term casual domestic

1 Almoner's Department, Alfred Hospital.

assistance to relieve the sustained pressures both on family members and on the patient. The costs of such a service would be beyond the means of most families and therefore would need to be subsidized.

Economic Effects

Long-term financial stringency creates intense anxiety in the patient and his family and is often a cause of severe family tension. In some cases it has forced patients to return to work, or to unsuitable work, against medical advice. Approximately one-third of the patients previously in gainful employment are receiving less income as a direct result of cardiac illness. A proportion of those patients whose income is derived from Statutory Benefits would receive some relief from their anxieties if part-time and non-competitive employment were readily available.

Effects on Rehabilitation

For return to maximum functioning the patient's conception of the consequences of a heart attack has proved to be the most important factor after the severity of the heart disease *per se*. The patients who were most helped were those with whom a regime was discussed in relation to the illness, to daily activities and to future work prospects. In the absence of such planned discussion many patients limited their activities unnecessarily. By contrast, other patients who were not considered ready to resume work expended as much energy on odd jobs around the home as would have been required in their usual employment.

For many patients, occupation of leisure time has presented considerable difficulties. There has been a tendency to eliminate all normal leisure pursuits for fear of exceeding the limits of "safe" exertion.

Summary

The study clearly indicates that greater help could be given by earlier recognition and treatment of incipient psychosocial problems and by a comprehensive range of rehabilitation services which should include work assessment and selective placement, retraining, sheltered employment and aids for the housewife.

BLOOD PROTEINS*

C. C. Curtain

In previous reports the necessity of adequate instruments for the study of blood proteins has been discussed and the development of new tools described. During 1961 further instruments have been developed and the study of blood proteins continued with their aid.

(a) Instrumentation.

Multiple Column Electrophoresis Apparatus

Paper electrophoresis as a routine method of serum protein analysis has a number of serious disadvantages, particularly it is time-consuming and the staining process lacks reproducibility. In an attempt to overcome these problems an apparatus for carrying out electrophoresis in columns of acetylated cellulose powder has been designed and built. The electrophoresis patterns are eluted

from the columns through a sensitive solid-state ultraviolet photometer which drives an automatic integrating and printing system. Except for loading, the electrophoresis apparatus itself is fully automatic.

Automatic Analysis Apparatus

Previous reports mention a hypothesis relating the high frequency of the haptoglobin Type 1 gene in certain tropical areas to the selective pressure that malaria produces by causing iron deficiency. In the course of an extensive test of this hypothesis it became necessary to carry out a very large number of estimations of serum iron and iron binding capacity.

To enable these to be carried out with the minimum of time and labour an automatic analysis apparatus was designed and built. This incorporates several novel features. Samples and reagents are aspirated into the reaction chamber by a constant pressure venturi pump whose air feed is stabilized by a Beckman flame photometer gas regulator unit. The sample changing unit holds the Wasserman tubes in which our collection of Melanesian sera is stored, obviating the need for handling the sera. The photometer light source is a Beckman monochromator fitted with a beam-splitting device so that sample and blank readings are obtained simultaneously. Solid-state electronic components are used throughout the instrument. At the end of each reaction cycle the photometer current is sampled for 0.1 sec. by a ramp and multiple trigger digitizer and the reading is printed on an electrically operated typewriter.

New Type of Analog Multiplier

In many types of physiological and biochemical investigation it is desirable to multiply two varying quantities together as they are being obtained. If the quantities can be converted to electric currents it is possible to do this with the aid of an analog multiplier. The most common of these is the servo multiplier which is relatively complex, expensive and has a relatively long response time.

As an alternative to the servo-motor and gear box we have investigated the properties of a galvanometer and optical lever as a multiplier. In the complete multiplier the optical lever beams a rectangular highly uniform light spot onto a diffusing screen in front of which is placed a cadmium sulphide photoconductive cell. The resistance of this cell is part of the input resistance of a silicon transistor amplifier of stable operating point and gain. One of the currents to be multiplied is applied to the galvanometer causing the light spot to swing across the photocell changing the input load of the amplifier. The second current is applied across part of this load and as the relative gain of the amplifier varies it is multiplied by the first quantity controlling this gain.

(b) Techniques.

Gel-diffusion Chromatography

For a variety of reasons it was decided to investigate alternatives to the cross-linked dextran gels ("Sephadex"), which have been so far used in the fractionation of proteins by aqueous gel-diffusion chromatography. Of the substances studied the most promising seemed to be a polymer of acrylamide cross-linked with N-N methylene-bis acrylamide. In contra-distinction to the 'Sephadex' gels which have a sponge-like structure, the acrylamide gel resembles a pile of sapling trunks tangled together with their branches (the cross-linking) in contrast

to the dextran gels and the sulphonated-polystyrene ion-exchange resins. Because of this structure the degree of cross-linking within very wide limits has little effect upon the pore size of the gel. Gels prepared with 5% cross-linking appear to have an upper molecular weight limit of exclusion of approximately 10,000. With solutes of molecular weight below 1,000 which can penetrate some distance into the gel grains some interesting steric effects have been noted. For example, it is possible to separate completely methyl green from the very closely related methyl violet in columns of 6 cm. length. There is also some evidence that it is possible to resolve some optical isomers on long columns of the gel.

Electrophoretic Separation of Haemoglobins Lepore, A_1, A_2 and H in Starch Gels

Lepore haemoglobin has been considered particularly difficult to separate from haemoglobin A by any method other than the somewhat cumbersome one of starch-block electrophoresis. It has been found that it is possible to obtain a good separation of Lepore from haemoglobins A, A_2 and H by electrophoresis in starch-gels made from tris-E.D.T.A. buffer. The starch gels have the advantage in that they can be clarified in anhydrous glycerol for photometric quantitation.

(c) Investigations.

Studies on Melanesian Serum Proteins[1]

(i) Serum Protein-genetics of Melanesians.

Haptoglobin and transferrin typing of Melanesians has been continued and directed towards a closer definition of the regional variation of these genes and the influence of various selective pressures, particularly malaria. Radioautographic transferrin typing, carried out by electrophoresis in acrylamide gels, of sera saturated with radioactive iron has confirmed our earlier finding that the major transferrin variants in Melanesia are C and D_0. None of the other six sub-types, or transferrin B have been encountered so far.

(ii) Tropical Hypergamma-globulinaemia.

Studies on the development of tropical hypergamma-globulinaemia are proceeding. Children in all parts of Melanesia under the age of six months appear to have serum globulin profiles similar to those of normal European infants. In the coastal regions however the gamma-globulin level rises after the sixth month and approaches the very high values found in adults by the eighteenth month. This pattern is invariable in holoendemic malarial regions such as Maprik, although many exceptions are found in areas where some measure of malaria control has been attempted or where malaria naturally shows a seasonal incidence. Further studies are being carried out on sera collected by Drs. Kidson and Gorman in an attempt to correlate the development of hypergamma-globulinaemia with infection and nutrition.

(iii) Nature of the Elevated Globulins found in Some Cases of Kuru.

Further immunological studies on the elevated gamma and beta globulins isolated from the sera of some kuru victims have confirmed the identity of these with the gamma cross-reacting globulins of the beta-gamma globulin region. We have concluded from this that the hyperglobulinaemia is of a reactive nature although of still unexplained significance.

1 Carried out in collaboration with Dr. D. C. Gajdusek, National Institute of Neurological Diseases and Blindness, Department of Health, Education and Welfare, Bethesda, Md. U.S.A.

Studies on the "Paraproteinaemias"

(i) Immunocytochemical Localization of an 18S Cryogel Macroglobulin.

Cryogel macroglobulins belong to an extremely rare group of abnormal serum proteins. The availability of a patient suffering from cryogel macroglobulinaemia made possible a study with fluorescent anti-macroglobulin of biopsy sections of lymph node and bone marrow. The macroglobulin appeared to be localized in the nucleus and cytoplasm of large immature plasma cells which were found in slight excess in the bone marrow. None of these cells were found in the lymph node sections.

(ii) Heterogeneity of the Abnormal Globulins in Some Cases of Myeloma.

Immunocytochemical demonstration of heterogeneity:

The development of a satisfactory method for two colour fluorescent antibody staining has made it possible to study at the cellular level the heterogeneity often observed in the abnormal serum globulins. Rabbit antisera to a 7S myeloma globulin and an 18S macroglobulin from the sera of a patient suffering from myeloma were conjugated respectively with fluorescein isocyanate and lissamine-rhodamine. Application of these conjugates to the patient's bone marrow showed two distinct plasma-cell populations, one containing 7S myeloma globulin only, the other 18S macroglobulin only. The cells of the two populations were found to be morphologically identical after conventional haematoxylin-eosin staining.

Electrophoretic and Chromatographic Studies on Heterogeneity:

Nine out of 37 myeloma sera fractionated by starch-gel electrophoresis were found to have two abnormal globulin components, confirming similar findings by Owen and other workers. The abnormal globulin fraction in each of the nine sera was isolated by preparative cellulose column electrophoresis and then submitted to steady state electrophoresis in the Tiselius apparatus. In all cases bimodal heterogeneity was observed. In three of the sera this amounted to a difference of one charge unit as shown by pH mobility curves and potentiometric titration data.

Seven of the nine globulin samples could be fractionated into two components which corresponded to those observed in the starch-gels by ion-exchange chromatography on diethylaminoethyl cellulose.

Immunological Studies on Heterogeneity:

Four of the seven chromatographically fractionable globulins were tested for heterogeneity by the Ouchterlony plate technique. In all cases both of the components gave reactions of identity.

Chemical Studies on Heterogeneity by "Fingerprinting":

Each chromatographically separated component of two of the three globulins which showed heterogeneity of one unit charge was dissociated with mercaptoethanol-urea and performic acid oxidation. The fragments from the pairs of components were run alongside each other in starch gels. One member of each pair was observed to differ from the other in the possession of one fragment with a different mobility. Otherwise the two patterns were identical. The two dissimilar fragments were isolated from the others in each case by electrophoresis and gel diffusion on 'Sephadex' G-75. Tryptic hydrolysates of the separated components were submitted to high voltage electrophoresis on paper

in one dimension and to descending ("finger-printing") butanolacetic acid-water partition chromatography on the other. In one "fingerprint" of each pair a peptide was found which differed in charge and Rf value from its correspondent in the other. The two dissimilar peptides were isolated and hydrolysed and the hydrolysates examined by phenol-water partition chromatography. The peptides were found to differ by one amino-acid only. The more negatively charged of the two possesed a glutamic acid residue in the place of a valine residue in the other. This difference was found in both pairs of proteins. It is hoped to investigate the other heterogeneous proteins by similar methods.

These findings seem to have some relevance to the question of whether the myeloma proteins are produced by mutation, by a process akin to adaptation, or, as we have suggested on the basis of immune tolerance experiments, by hybridization of protein synthesis "templates". It is difficult to reconcile the mutation hypothesis with the production of two chemically distinct abnormal proteins, or with the occurrence of the two distinct cell lines which we demonstrated by the double label technique.

The Precipitation of Cryoglobulins

Some preliminary studies carried out on cryoglobulin precipitation have suggested that inter-molecular hydrogenbonding plays a most important role. Three cryoglobulins were studied. The precipitation in the cold of all three was inhibited by urea in quite low concentrations (1.5M) and by 2.5M potassium bromide. When these inhibitors were removed by dialysis precipitation occurred normally. The temperature at which precipitation occurred increased with decreasing pH and all three proteins were precipitated even at 37°C below pH 3.5. pH values above 9.6 inhibited precipitation at all temperatures. On the basis of this behaviour it was considered that the hydrogen bonding involved carboxyl groups as donors. Carboxyl groups deionise as the temperature and pH fall, thus becoming more able to act as donors. In the search for acceptors high scanning-speed ultraviolet spectrophotometry was used to study the proteins as their solutions were cooled close to their precipitation points. Evidence was found in all three cases that the hydroxyl groups of tyrosyl residues were involved for the hydrogen bonding by these residues was an all-or-nothing phenomenon occurring almost completely over a range of 0.5°C around the precipitation temperature.

It appears that cryoglobulins may differ from normal serum globulins by a peculiar disposition of their tyrosine, glutamic acid and aspartic acid residues which facilitates hydrogen bonding between them when the carboxyls deionize as the temperature falls.

It is hoped to study these differences by chemical methods, including finger-printing.

CELLULAR ENZYMES**

C. Kidson, A. D. McCutcheon and R. G. Wyllie

Leucocyte Lipid Metabolism

Continuing investigations of lipid metabolism in human leucocytes, using radioactive techniques with liquid scintillation counting[1], have provided information bearing on the fundamental nature of leukaemia. These studies have covered several aspects:

1 Carried out at Physiology Department, University of Melbourne.

(a) Lipid Synthesis in Disease States Involving Leucocytes.

A study of lipid synthesis in leucocytes in leukaemia, myeloproliferative states and infection has been completed, thus establishing the metabolic patterns with respect to lipids in these conditions. High rates of leucocyte lipid synthesis in acute myeloid leukaemia were found to be associated with the disproportionate increase in acetate incorporation into phospholipids, whereas in acute lymphatic leukaemia low rates of synthesis were accompanied by a normal fractional distribution. Low rates of total lipid synthesis also showed a normal fractional distribution pattern in both infective leucocytosis and polycythaemia vera. In myelosclerosis, however, the position was found to be less well-defined: a wide range from low through normal to high rates of total synthesis was observed in the patients studied, the majority having a normal distribution in phospholipids and neutral lipids. In one patient with myelosclerosis the pattern of lipid synthesis was similar to that in myeloid leukaemia, suggesting that this metabolic aberration may precede morphological and clinical transition to leukaemia.

(b) Cell Age and Changes in Metabolic Pattern.

The finding of lowered rates of lipid synthesis in the leucocytes from patients with polycythaemia and infection was surprising, in view of the high proportion of band neutrophils present in all instances, indicative of young populations of cells of the granulocytic series. This suggested that ageing of the leucocyte may have a profound effect on its lipid metabolism. In one patient with infection studied until complete resolution, the rate of lipid synthesis was found to rise from very low to normal levels, coincident with a decreasing percentage of band neutrophils and increasing average age of the cell population. These findings suggest that lipid synthesis is age-dependant in the granulocyte, increasing with age in this nucleated cell, the antithesis of the situation in the non-nucleated erythrocyte.

(c) Metabolic Response to Phagocytosis.

By incubating leucocytes in vitro with killed bacteria it has been shown that a marked increase in lipid synthesis occurs, predominantly in phospholipids and that this response is most prominent in the initial 5 to 10 minutes. It is suggested that such a response may be accounted for by the increased requirements for phospholipids at the cell membrane for the phagocytic process and possibly also in the endoplasmic reticulum for increased protein synthesis during this process. Significantly, this type of metabolic response has so far been found to be absent in myeloid leukaemia.

(d) Control Mechanisms.

Transfer of lipids from leucocytes to plasma has been found to vary with the ratio of cells to plasma in the incubation system; the addition of autologous plasma resulted in an increased proportion of labelled lipids appearing in the plasma fraction. Incubation of leucocytes in Ringer solution alone resulted in a 50% reduction in the proportion of lipids appearing in the medium; addition of the albumin fraction of plasma did not, but addition of the globulin fraction did, increase the proportion transferred from the cells. It is suggested that globulin-binding may partially control this transfer process.

It has also been noted that addition of autologous plasma to the incubation system increased the rate of lipid synthesis per cell and that dialysis against

Ringer-phosphate prior to incubation resulted in a decreased synthesis rate. This suggests that a dialyzable factor partially controls intracellular synthesis rate and preliminary studies indicate that this factor(s) is peptide in nature.

(e) Intrinsic and Extrinsic Control of Cell Metabolism.

At the present stage in this study of leucocyte metabolism in human leucocytes it seems evident that both intrinsic and extrinsic control mechanisms are involved in the regulation of this aspect of the cell's function. The extrinsic mechanisms characterised by the effects of plasma factors on synthesis and transfer are common to leucocytes under all conditions studied. The intrinsic mechanism governing the response to the increased demand for phospholipids during phagocytosis appears, on present evidence, however, to be defective in myeloid leukaemia, so that although the resting leukaemic cells exhibit very active lipid synthesis they lack the ability to respond to an increased demand. Such a defect could explain in part the poor response to infection in myeloid leukaemia, and possibly represents one essential aspect of malignant change in leucocytes of the myeloid series.

Neutrophil Alkaline Phosphatase

Throughout the year investigations into the in vitro culture of leucocytes have been made and methods of culturing leucocytes in oxygenated whole blood suspensions and in blood clot preparations have been developed.

Whole blood suspensions were prepared from heparinized venous blood diluted with tissue culture media and placed in a container with a glass covered metal rod. The preparation was incubated on a slowly revolving magnetic stirrer in a 5% CO_2 atmosphere. In these circumstances normal pH and gas tensions can be maintained for 24 hours and the blood sampled readily.

It was found that leucocyte viability and red cell morphology could be maintained for 24 hours but beyond this degenerative changes occur. Neutrophils develop cytoplasmic vacuoles and subsequently disintegrate or the neutrophil nucleus loses its multi-lobed appearance to become round and condensed. Monocytes also show progressive cytoplasmic vacuolation while eosinophils and lymphocytes are still normal at 48 hours.

Blood clots were prepared from venous blood collected into siliconed containers at 4°C and then transferred to shallow chambers made from cover slips and microscope slides. The clot preparation was incubated in tissue culture media with a 5% CO_2 atmosphere. Viability was well maintained and leucocytes adhered to the glass surfaces where they could be examined directly.

These methods have been used to study the reaction of neutrophils to humoral stimuli under controlled conditions. Early results have shown that substrates conventionally used to demonstrate alkaline phosphatase activity do not induce increased enzyme activity.

Zinc Metabolism

Normal granular leucocytes contain a considerable quantity of zinc and leukaemic leucocytes contain approximately half this amount. Specific histochemical stains for zinc have proved disappointing in studying these changes and estimations of white cell zinc content are based on white cell suspensions containing a variety of white cell types.

An attempt is being made to determine the concentration of zinc in individual types of cells using patients with varying degrees of eosinophilia, lymphocytes and polymorponuclear leucocytosis. The zinc determinations have been carried out by Dr. J. Willis (C.S.I.R.O.) using the method of atomic absorption spectroscopy.

Leucocyte Enzyme Genetics

Other studies of leucocyte enzymes have continued, using catalase as a model, in an attempt to understand something of the genetic control of enzymes in the leucocyte in relation to aberrations in leukaemia.

(a) Quantitative Variations of Leucocyte Catalase.

Initial quantitative studies have been completed; normal mature granulocytes were found to have higher catalase activities than normal lymphocytes, while young granulocytes in infective leucocytosis had very low levels of this enzyme. No alteration from the normal pattern was observed in acute or chronic lymphatic leukaemia, but moderately increased activities were found in chronic myeloid leukaemia (CML) and extremely high values in acute myeloid leukaemia (AML).

(b) Possible Mutant Enzyme in Acute Myeloid Leukaemia.

Characterization of catalase in normal and leukaemic cells has been partially achieved. Michaelis constants and optimal temperature of reaction were identical in the normal, CML and AML. Thermal stability and pH optima were identical in the normal and CML, but varied in the cases of AML studied so far. Catalase in AML was much more stable at 60°C than the enzyme from normal controls, while in AML two pH optima at 6.8 and 8.9 were present, as against the single optimum at 6.8 in the normal, suggesting that in AML we are dealing with a mutant enzyme with altered thermal stability and also with a second enzyme with catalase activity which is either absent or unmeasurable in normal cells. Partial purification will be necessary before an exact picture is available.

Metabolic Studies in Thyroid Disease

Initial studies carried out in collaboration with Dr. D. C. Gajdusek, of the National Institutes of Health, Bethesda, U.S.A., on an unusual cretinoid population in Mulia, West New Guinea, who have very large goitres and varying degrees of mental deficiency, have been completed. A proportion of the population had diminished erythrocyte glucose-6-phosphate dehydrogenase activity, suggesting that impairment of carbohydrate metabolism via the pentose phosphate pathway may occur in hypothyroid states. Detailed quantitative evaluation of the state of thyroid function is now under way in Dr. Gajdusek's laboratory and in conjunction with these studies, further investigations of carbohydrate metabolism are being carried out in our laboratory, using radioisotope techniques to determine relative impairment of the glycolytic and pentose phosphate pathway.

Glucose-6-Phosphate Dehydrogenase Deficiency[1]

An extensive study of many aspects of erythrocyte glucose-6-phosphate dehydrogenase (G6PD) deficiency has been carried out, with the main emphasis on problems associated with this inherited trait in New Guinea.

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1 Field survey in New Guinea made possible by a grant from United States Public Health Service.

(a) Geographical Distribution in Asia and the Pacific.

Ceylon. Blood samples were made available by Dr. R. L. Kirk (University of Western Australia). G6PD deficiency was found in 2.5% of Tamils.

Micronesia. Blood samples were made available by Dr. D. C. Gajdusek. G6PD deficiency varied from island to island, from 0 to 8%, a distribution which might be expected in a small scattered population.

Australian Aborigines. In collaboration with Professor O. Budtz-Olsen (University of Queensland) 840 Australian Aborigines were examined, from Cape York Peninsula, Central Australia, the Kimberleys and Port Hedland district in Western Australia. No cases of G6PD deficiency were found, indicating that the trait is either absent or has a very low frequency.

New Guinea. Recent studies in New Guinea have been carried out in collaboration with Dr. J. G. Gorman (Columbia University, New York, U.S.A.). Earlier work had suggested, roughly, a distribution pattern with a very low incidence in the highlands and a high incidence in the lowlands and in New Britain. Closer field examination has revealed exceptions to this generalization; the Sause language group on the Sepik River and the Tolai language group in New Britain have less than 1% of the deficiency, whereas adjacent language groups have up to 15% incidence. A study along the Markham River valley showed an interesting inverse correlation of height above sea level with incidence of the trait, there being a gradation from 29% at sea level to less than 1% above 5000 feet.

(b) Expression in the Heterozygote.

In a large series of family studies carried out in New Britain in collaboration with Dr. L. Champness the trait has been shown to sex-linked as in other populations studied, and the female heterozygote has found to have a varying expression, with enzyme levels ranging from normal to "deficient" values. A variable expression may occur within the one family.

(c) Two Different Chemical Expressions of the Trait.

The majority of cases of G6PD deficiency in New Guinea showed residual enzyme levels of 0 to 4% of normal, representing the Caucasian-type of defect, but an occasional case had a residual enzyme level of 20%, indicative of the Negro type of defect. In the former group, incubation of haemolysates with enzyme-free stroma from normal red cells resulted in activation of the "deficient" enzyme to near normal levels, confirming the work of Bracha Ramot and coworkers in Israel who have shown that the primary defect is a deficiency of this activator. These results suggest that the two types of G6PD deficiency are distinct entities and that both may exist in the same population.

(d) G6PD Deficiency and Anaemias in New Guinea.

An extensive survey of anaemias in three areas of New Guinea is in progress. G6PD is a very significant contributor to problems of anaemia in that country and is of special interest in view of the widespread use of sulphonamides, which not infrequently cause haemolysis in affected persons. Superimposed upon pre-existent severe iron-deficiency anaemia, which has been found to be extremely common in certain areas, the acute haemolysis in infants with G6PD deficiency is sometimes fatal and is a difficult problem in remote villages.

(e) Selection Pressures and Gene Incidence.

Sufficient data have now been accumulated on G6PD deficiency in New Guinea to permit the use of that country as a model for the assessment of selection pressures affecting this gene. The early findings suggested the gene protected against mortality from falciparum malaria, with resulting high incidence of the trait in coastal, high malarious areas. However, the finding of some groups with very low incidence of the gene in highly malarious areas, alongside other groups with a high incidence now suggests that malaria is not the only factor favouring selection of affected persons, if indeed it is a factor at all. Probably social factors, especially dietary habits are of considerable significance in determining the variable incidence.

SCLERODERMA

A. J. Barnett and F. H. Lumb

The observation and investigation of a group of 25 patients suffering from scleroderma has been continued. It has been possible to subdivide these patients into three main groups; (a) localised, where the condition appears to be mainly confined to the extremities; (b) acrosclerotic, where such initial local involvement is later associated with slowly progressive involvement of a more generalised nature; and (c) a third group where the initial manifestations are followed by a more rapid extension, often associated with marked visceral involvement.

A series of investigations, designed to reveal evidence of any underlying auto-immune phenomena has been carried out in all 25 cases with negative results. Serum protein electrophoresis, performed by standard methods, and also using the starch column technique, has shown that the electrophoretic pattern exhibited by these patients does not differ substantially from the normal.

All of the 25 patients in the series have received detailed investigation in an attempt to elucidate the incidence of visceral involvement in scleroderma and a special study of gastro-intestinal involvement has been made with interesting results. A high incidence of abnormality has been revealed, as 22 of the 25 patients showed gastro-intestinal disorder of some kind. The external localisation of scleroderma to the extremities does not therefore preclude the presence of visceral involvement, a high incidence of which was apparent in all three groups described.

A method of mechanically measuring the degree of skin involvement in a given situation has been developed, and applied to observations on the course of the disease, and the efficacy of treatment. Using this test, along with various other criteria for assessment, a double blind trial of the efficacy of potassium para-aminobenzoate in scleroderma has now been completed. The results indicate that such therapy has little if any effect on the gradually progressive course of the disease.

PANCREATITIS

A. D. McCutcheon and D. Race

Acute haemorrhagic pancreatitis is a severe condition, the aetiology of which is ill understood. It sometimes complicates operations in the gastro-duodenal region, especially a Billroth II partial gastrectomy in which the afferent loop is obstructed. The mechanism of its production was investigated in the following ways.

A blind duodenal loop was created in 8 dogs, to simulate an obstructed afferent loop. Post-operatively, 6 dogs developed acute haemorrhagic pancreatitis. Careful examination of the main pancreatic duct in the other two dogs showed that it was blocked.

In another 7 dogs, the same blind duodenal loop was created but in addition the main pancreatic duct was ligated. None of these dogs developed pancreatitis.

As this form of pancreatitis may be prevented by ligation of the pancreatic duct, it is presumably due to reflux of duodenal contents along the pancreatic duct. Active proteolytic enzymes are concerned in the pathogenesis of pancreatitis and these enzymes are normally activated by contact with duodenal secretions containing enterokinase. It would seem therefore that reflux of duodenal contents into the pancreatic duct is the mechanism responsible for some cases of human pancreatitis.

In 10 consecutive fatal human cases of acute pancreatitis, the anatomical relationships between the lower ends of the pancreatic and common bile ducts were studied by dissection, by the injection of contrast medium and X-ray, and by systematic sectioning with histological examination. One popular theory postulates that pancreatitis is caused by reflux of bile from the common bile duct into the pancreatic duct. In 7 of the 10 cases the main pancreatic duct entered the duodenum separately from the common bile duct and in all 10 cases there was no evidence that biliary reflux could have occurred.

In all 10 cases the only way in which material could have entered the pancreatic duct to activate pancreatic proteolytic enzymes, was via the duodenum. This evidence is compatible with the view that pancreatitis in man may be caused by reflux of duodenal contents into the pancreatic duct.

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KINCAID-SMITH, P.—"The Diagnosis and Treatment of Hypertension due to Unilateral Renal Disease". *Med. J. Aust.* Vol. 1 (1961) p. 641.

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LYALL, I. G., and A. J. BARNETT—"A Pilot Trial of 'Persantin'". *Med. J. Aust.* Vol. 2 (1961) p. 435.

McCUTCHEON, A.D.—"Surface Granulation in Myeloid Leukocytes". *Nature* Vol. 192 (1961) p. 684.

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SIMPSON, F. O., and A. J. BARNETT—"Primary Aldosteronism—Conn's Syndrome". *Med. J. Aust.* Vol. 1 (1961) p. 729.

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PAPERS ACCEPTED FOR PUBLICATION

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FANTL, P.—"The Plasminogen Activity of Plasma and Serum". *Science.*

FANTL, P., R. J. SAWERS and H. A. WARD—"Detection of a Self-Inflicted Haemorrhagic Disorder". *Med. J. Aust.*

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KIDSON, C.—"Leucocyte Lipid Metabolism in Myeloproliferative States". *Aust. Ann. Med.*

KIDSON, C., and D. C. GAJDUSEK—"Congenital Defects of the Central Nervous System associated with Hyperendemic Goitre in a Neolithic Highland Society of Netherlands New Guinea. II. Glucose-6-Phosphate Dehydrogenase Activity in the Mulia Population". *Pediatrics.*

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NAYLER, WINIFRED G.—"Ventricular Arrhythmias following the Administration of Na_2EDTA". *J. Pharmac. & Exp. Therap.*

REICH, Magda—"Variations in Urinary Aldosterone Levels of Normal Females during their Menstrual Cycle". *Aust. Ann. Med.*

SIMPSON, F. O., and S. OERTELIS—"Observations on the Fine Structure of Toad Cardiac Muscle". *J. Biophys. Biochem. Cytology.*

PAPERS SUBMITTED FOR PUBLICATION

CURTAIN, C. C.—"A Photoelectric Scanning Microscope". *Aust. J. Sci.*

FANTL, P.—"Plasmin an Activator of Fibrinolysis Inhibited by Streptokinase". *Nature.*

KIDSON, C.—"Some Possible Control Mechanisms of Lipid Metabolism in the Leucocyte". *Biochem. Biophys. Acta.*

NAYLER, WINIFRED G.—"Calcium, Potassium and the Ventricular Staircase". *Amer. J. Physiol.*

NAYLER, WINIFRED G.—"Inotropic Activity in Cardiac Muscle under Isometric and Isotonic Conditions". *J. Gen. Physiol.*

WYLLIE, R. G.—"Neutrophil Alkaline Phosphatase—Response to Acute Inflammation". *Aust. Ann. Med.*

LECTURES DELIVERED DURING 1961

"Gene Markers in Anthropology—Haptoglobins and Transferrins in Melanesians"—*A.N.Z.A.A.S. Brisbane.*	C. C. CURTAIN
"Automatic Microscopy"—*Society of Medical and Biological Electronics, Melbourne.*	C. C. CURTAIN
"Inhibitors in Haemophilia"—*International Congress for Nomenclature of Blood Clotting Factors, Wiesbaden.*	P. FANTL
"Blood Coagulation"—*University of Melbourne.*	P. FANTL
"Red Cell Enzyme Deficiency in Various Populations"—*Victorian Society of Pathology and Experimental Medicine.*	C. KIDSON
"Recent Studies on the Genetics and Distribution of Glucose-6-Phosphate Dehydrogenase Deficiency"—*Blood Club.*	C. KIDSON
"Glucose-6-Phosphate Dehydrogenase Deficiency: Recent Advances In Genetic Aspects"—*Royal Children's Hospital.*	C. KIDSON
"Effects of Plasma on Leucocyte Lipid Synthesis"—*Australian Biochemical Society, Brisbane.*	C. KIDSON
"An Inherited Enzyme Deficiency in New Guinea: Relation to World Distribution" in Symposium on "Genetic Markers in Anthropology"—*A.N.Z.A.A.S., Brisbane.*	C. KIDSON
"Some Metabolic Interrelationships in Leukaemia and Myeloproliferative States"—*Second Asian and Pacific Congress of Haematology, Manila.*	C. KIDSON
"Erythrocyte Enzyme Deficiency as a Factor in Population Selection"—*Second Asian and Pacific Congress of Haematology, Manila.*	C. KIDSON
"The Mechanism of Pancreatitis and its Bearing on Treatment"—*Alfred Hospital Clinical Society.*	A. D. McCUTCHEON
"Experimental Pancreatitis"—*Surgical Research Society.*	A. D. McCUTCHEON
"The Results of Arterial Surgery"—*Royal Australasian College of Surgeons, Brisbane.*	K. N. MORRIS
"Calcium Ions and Ventricular Muscle Activity"—*Australian Physiological Society, Brisbane.*	W. G. NAYLER
"Surgical Aspects of Anomalous Pulmonary Venous Drainage"—*B.M.A. Congress, Auckland.*	G. R. STIRLING
"Total Body Perfusion with a Spinning Disc Oxygenator"—*B.M.A. Congress, Auckland.*	G. R. STIRLING
"Surgical Aspects of Pulmonary Hypertension"—*Symposium on Respiratory Physiology, Melbourne.*	G. R. STIRLING
"Experimental Surgery"—*Australasian Congress of Medical Students, Melbourne.*	G. R. STIRLING
"Blood Coagulation"—*Australian Biochemical Society, Brisbane.*	H. A. WARD
"The Degree of NAP Activity in Various Disease States"—*Royal Australasian College of Surgeons, Melbourne.*	R. G. WYLLIE

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

Revenue Account for the Year Ended 31st December, 1961.

EXPENDITURE.		INCOME.		
Drugs, Chemicals, Provisions, etc.	£2,119 17 11	Donations—		
Fuel and Lighting	289 19 4	Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions	£27,600 0 0	
Instruments and Glassware	2,188 15 11	Other Donations as per attached schedule	1,039 18 0	
Insurance	992 14 6			£28,639 18 0
Library Maintenance	1,319 8 11	Grants in aid of Research—		
Postage, Telephone, Printing and Stationery	1,148 15 9	National Health & Medical Research Council	7,124 14 0	
Repairs and Renewals	1,485 9 4	Anti-Cancer Council of Victoria	8,778 8 0	
Salaries and Wages	34,749 8 4	Life Insurance Medical Research Fund of Australia and New Zealand	2,754 0 0	
Travelling Expenses	668 9 4			18,657 2 0
Provision for Overseas Travel	2,500 0 0	Interest from Investments—		
Sundries	1,136 14 0	Held by the Trustees of the Estate of the late Thomas Baker	849 0 0	
Cost of Animal House	900 0 0	Endowment Fund	1,081 18 0	
				1,930 18 0
		Interest from Commercial Bank of Australia Ltd.		225 14 1
		Deficit for Year		46 1 3
	£49,499 13 4			£49,499 13 4

THE THOMAS BAKER, ALICE BAKER AND ELEANOR SHAW MEDICAL RESEARCH INSTITUTE
Balance Sheet at 31st December, 1961.

LIABILITIES.				ASSETS.				
Current Liabilities—				**Current Assets—**				
Sundry Creditors	£5,460	18	7	Cash at Bank	£4,821	0	11	
Endowment Fund	24,029	6	10	Prepayments	359	19	6	
Capital Grants and Gifts	1,791	2	3	Sundry Debtors	916	19	0	
Accumulated Revenue	2,953	14	9					£6,097 19 5
				Endowment Fund Investments—				
				Inscribed Stock—				
				Commonwealth Government	£13,880	0	0	
				Grain Elevators Board	2,500	0	0	
					16,380	0	0	
				Treasury Bonds—				
				Commonwealth Government	1,010	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				1,791 2 3
				Fixed Assets—				
				Furniture and Fixtures				2,316 13 11
	£34,235	2	5					£34,235 2 5

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

In our opinion the annexed Balance Sheet is properly drawn up to show a true and fair view of the state of the Institute's affairs at 31st December, 1961, 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 (Australia),
Honorary Auditors.

Melbourne,
5th April, 1962.

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, 1961.

CAPITAL GRANTS AND GIFTS.

Balance at 31st December, 1960		£1,780	15 9
Add			
Donations—			
Dr. T. E. Lowe and Associates		57	11 2
For Building Extensions—			
Alfred Hospital	£275 11 9		
Estate of Thomas Baker	275 11 9		
		551	3 6
Anti-Cancer Council of Victoria—			
For Major Items of Equipment		3,000	0 0
Estate of Thomas Baker—			
For Zeiss Microscope	1,000 0 0		
For Alterations to Laboratory No. 15	1,427 0 0		
		2,427	0 0
Haemophilia Society		600	0 0
Transfer from Accumulated Revenue for Portion of Cost of Equipment		660	9 0
		£9,076	19 5
Deduct			
Architect Fees for Building Extensions, New Wing	£551 3 6		
Equipment	5,434 13 8		
Progress Payment for Alterations to Laboratory No. 15	1,300 0 0		
		7,285	17 2
Balance at 31st December, 1961		£1,791	2 3

ACCUMULATED REVENUE.

Surplus at 31st December, 1960	£3,660	5 0
Less		
Transfer to Capital Grants and Gifts for Portion of Cost of Equipment	660	9 0
	£2,999	16 0
Less		
Deficit for Year	46	1 3
Surplus at 31st December, 1961	£2,953	14 9

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

OTHER DONATIONS RECEIVED DURING YEAR TO 31st DECEMBER, 1961

Marian and E. H. Flack Trust	£350	0	0
Geo. F. Little Trust	148	6	6
Mr. and Mrs. Edgar Rouse	105	5	0
Mrs. William Lucey, in memory of Winifred Ann Lucey	100	0	0
Mr. and Mrs. Frank Crane	50	0	0
Kodak (Australasia) Pty. Ltd.	50	0	0
Eagle Star Insurance Co. Ltd.	26	5	0
Mr. J. C. Habersberger	10	10	0
Mrs. Lawrence Simpson	10	10	0
Dr. and Mrs. John Rouse	10	10	0
Mr. J. E. Page	5	5	0
In Memory of Flora Ann Martin	6	0	0
„ „ „ Ralph Thomas	5	7	6
„ „ „ Cornelius Van Niel	5	5	0
„ „ „ J. Ringland Anderson	5	0	0
„ „ „ Sir William Angliss	5	0	0
„ „ „ Ernest E. Blake	5	0	0
„ „ „ George Collie	5	0	0
„ „ „ Arthur Dickinson	5	0	0
„ „ „ Otway Falkiner	5	0	0
„ „ „ Leslie Faulkenberry	5	0	0
„ „ „ Sir Wilfred Fullagar	5	0	0
„ „ „ Wilfred Hassett	5	0	0
„ „ „ Libs Herman	5	0	0
„ „ „ Rev. Rupert P. A. Hewgill	5	0	0
„ „ „ Mary Hordern	5	0	0
„ „ „ Emery Huse	5	0	0
„ „ „ Violet Irving	5	0	0
„ „ „ Essington Lewis	5	0	0
„ „ „ T. Allan McKay	5	0	0
„ „ „ Charles Norman MacKenzie	5	0	0
„ „ „ James Milliken	5	0	0
„ „ „ Sir Angus Mitchell	5	0	0
„ „ „ Phyllis Moore	5	0	0
„ „ „ Walter Morton	5	0	0
„ „ „ Sidney Riley	5	0	0
„ „ „ Sir David Rivett	5	0	0
„ „ „ Marcel Ruot	5	0	0
„ „ „ Archibald Malcolm Scull	5	0	0
„ „ „ Harold L. Shackell	5	0	0
„ „ „ Lieut.-General Edward Kenneth Smart	5	0	0
„ „ „ Mrs. Edward Kivas Tully	5	0	0
„ „ „ Gladys Weir	5	0	0
„ „ „ Rosa Heathcote Wright	5	0	0
„ „ „ Dugald Cameron	1	10	0
„ „ „ Dorothy Mary Gibson	1	10	0
Miss N. E. Cameron	2	2	0
Mr. J. L. Irvine	2	2	0
	£1,039	18	0

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

1961

STAFF

Honorary Physician:	EWEN DOWNIE, M D., F.R.C.P., F.R.A.C.P.
Assistant Physician Scientific Studies:	JOSEPH BORNSTEIN, D.Sc., M.D., F.R.A.C.P.
Assistant Physician Clinical Studies:	BRYAN HUDSON, M.D., Ph.D., M.R.C.P., F.R.A.C.P.
Honorary Assistant Physician:	HARALD BREIDAHL, M.D., M.R.C.P., M.R.A.C.P.
Honorary Clinical Assistant:	A. P. DOREVITCH, M.D., M.R.A.C.P.
Registrar:	B. A. POINTON, M.B., B.S.
Biochemists:	DORA WINIKOFF, M.Sc. JUNE SHEATH, M.Sc. AUSMA DULMANIS, B.Sc. FRANCES WALKER, B.Sc.
Technical Staff:	Mr. W. HUDSON Miss I. EKKEL Mrs. J. DAVIDSON Miss M. VINCENT Miss M. ZWART Miss L. GIBSON Miss P. PEARL Miss W. DAVIES
Secretary:	Miss J. SHARP

DIABETIC CLINIC

Clinical Assistants:	MARGARET SANDERS, M.B., B.S. PAULA PITT, M.B., B.S.
Chiropodist:	MAIDA O'CONNOR, F.Ch.A.V., M.Ch.I.A.

RESEARCH FELLOWS

"Frederick & Esther Michaelis" Scholarship:	HARALD BREIDAHL, M.D., M.R.C.P., M.R.A.C.P.
Wellcome Fellow:	PETER DAVOREN, Ph.D., B.Sc. (on leave).
"E. H. Flack" Medical Research Scholarship:	HENRY BURGER, M.D., M.R.A.C.P.

HONORARY ALFRED HOSPITAL 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.

This Unit was founded five years ago to further the study of problems in Endocrinology. The functions of the Unit were defined as "The treatment of patients, the prosecution of research into Diabetic and Metabolic disorders and the teaching of undergraduate and postgraduate students".

It is now appropriate to review the work of the past five years; to consider the achievements of this group and the influence which the Unit has had, not only in the hospital but elsewhere.

The treatment of many endocrine disorders requires specialised techniques and the development of such investigational methods often commences as a research project. After a period of observation and modification this results in the establishment of a procedure which can become an accurate routine investigation. At this stage it can be passed to the Biochemistry Department of the Hospital, and is then available for general use throughout the Hospital. A typical example of this has been the provision of methods of estimating disturbances of thyroid function and of some aspects of disturbed adrenal activity. Along with this, constant attention is being given to improving these methods with the hope of greater accuracy. The information obtained enables treatment of patients to be undertaken on a rational basis and has resulted in considerable benefit in many instances. A necessary part of this is the analysis of results and the publication of such techniques and their help in the treatment of patients.

Research has also been undertaken into some more fundamental aspects of endocrine disorders, particularly in relationship to the complications of diabetes mellitus and to the behaviour of certain steroids in adrenal diseases.

Yet another activity has been the conduct of controlled clinical trials of new drugs in the management of endocrine diseases. Appreciable work has been done in the assessment of a variety of substances for the oral treatment of diabetes mellitus and of a number of steroid compounds in the treatment of adrenal insufficiency.

Members of the Unit have played a part in the teaching programme of both undergraduate and postgraduate students in the Hospital by seminars and by ward rounds in Ward 1, Caulfield Convalescent Hospital. Lectures have also been given under the aegis of the Melbourne Medical Postgraduate Committee in country centres. Another aspect of Postgraduate training has been the attachment of both Medical and Science graduates for training in various fields. As indicated in last year's report graduates of other clinical schools have worked in the Unit prior to leaving for further study overseas. Three Fulbright Travelling Fellowships and one Nuffield Fellowship have been granted workers in the Unit since its foundation.

Members of the Unit have played a large part in the formation and activities of the Endocrine Society of Australia which was founded in 1958. The Society met this year in conjunction with A.N.Z.A.A.S. in Brisbane where several members of the staff were present and read papers.

During the year Doctor Joseph Bornstein was appointed Professor of Medical Biochemistry in Monash University. Professor Bornstein, who enjoys an International reputation, has been with the Unit since its Foundation and has been closely associated with the Alfred Hospital since his student days. Our congratulations are extended to him and it is pleasing to realise that he will retain association with the Hospital as he has been appointed by the Board

of Management as Consultant Biochemist. This will enable a close link to be maintained between his Department and the Unit.

During the year Doctor Ewen Downie visited medical schools in Singapore, Manila and Hong Kong and later attended the Annual Meeting of the Endocrine Society and the American Diabetes Association in New York. He also represented Australia as an official delegate to the 4th Congress of the International Diabetes Federation in Geneva. Professor Joseph Bornstein also travelled abroad during the year visiting Research Centres in Europe, Great Britain and America. Doctor Peter Davoren, a former Research Fellow, is now engaged in Research in Western Reserve University, Cleveland; Doctor Henry Burger is working with Doctor John Nabarro at Middlesex Hospital, London, and Doctor James Evans, a former Registrar, is studying Gynaecological Endocrinology in Edinburgh.

The Unit continues to attract the attention of visitors both from Australia and abroad. During the year a number of visitors have been welcomed, including: Sir Neil Hamilton Fairley, London; Professor Russell Fraser, Postgraduate Medical School, London; Dr. Ivan Roitt, Middlesex Hospital Medical School, London; Dr. B. de la Cruz, Philippine Atomic Energy Commission; Dr. F. Dudley Hart, Westminster Hospital, London; Mr. R. W. Hill, Ciba Ltd., Basle, Switzerland; Mr. M. H. Knop, Ciba Co. Ltd., Sydney; Professor Douglas Hubble, University of Birmingham; Dr. V. B. Raju, Reader in Pediatrics, University of Madras; Professor A. K. McIntyre, Department of Physiology, University of Otago, New Zealand; Dr. George A. Perera, Columbia University College of Physicians and Surgeons; Dr. Hubert Sissons, Institute of Orthopaedics, London; Sir Derrick Dunlop, Edinburgh University; Dr. I. R. Cox, University of Sydney; Dr. John L. Grove, Launceston General Hospital; Dr. J. B. Brown, Edinburgh; Dr. D. J. Collins, Royal Hospital for Women, Paddington; Dr. J. J. Hobbs, Royal Hospital for Women, Paddington; Mr. A. Hanley, Eli Lilly (Australia) Pty. Ltd.; Mr. J. S. Cameron, Eli Lilly (Australia) Pty. Ltd.

These visits have proved stimulating and much valuable advice and suggestions have been given and are gratefully acknowledged.

Generous assistance has again been provided by a number of individuals and organisations who have provided substantial financial support and gifts in kind. Acknowledgment is made of helpful advice given by the Honorary Medical Staff, by University colleagues and by many others.

In conclusion, it is a pleasure to pay tribute to the loyalty and support of all members of the Unit and to acknowledge the help and co-operation shown by members of the Nursing Staff.

EWEN DOWNIE.

31st December, 1961.

ESTIMATION OF THE TESTOSTERONE IN PLASMA

Miss I. Ekkel, Mr. J. Coghlan[1] and Dr. Bryan Hudson

A method for the estimation of testosterone in plasma using a double isotope technique has now yielded results that indicate the probable levels of this steroid hormone in peripheral plasma. The method involves the addition of a tracer amount of 4-C^{14}-testosterone to 10 ml. of plasma, from which the free steroids are then extracted and, after purification, are acetylated with tritium labelled acetic anhydride. The testosterone acetate is then run on five or six different chromatographic systems until a constant H^3/C^{14} ratio is obtained. When distilled water blanks to which four C_{19}-monohydroxy 17-ketosteroids other than testosterone have been added are taken through the method the residual tritium in the fifth and sixth chromatographic systems is such that the "blank" of the method is less than 0.001 μg per 100 ml. when measured as testosterone. 4-C^{14}-testosterone acetate is added to these samples after acetylation. Likewise, plasma from an elderly female patient who had been hypophysectomized and who had been adrenally insufficient for a number of years showed a peripheral plasma testosterone value of less than 0.001 μg per 100 ml. plasma.

Using this method the values of testosterone in male plasma have ranged from 0.5 to 0.96 μg per 100 ml. : Mean 0.755 $\pm$ 0.125 (s.d.) while in female plasma the levels have ranged from those that are not able to be detected by the method to 0.24 μg per 100 ml. : Mean 0.16 $\pm$ 0.09 (s.d.). A true hermaphrodite phenotypically female but with testicular tissue showed a value of 0.60 μg/100 ml.

These studies are being pursued to determine the range of variation of plasma testosterone in health and disease, and the factors that may affect these levels—such as the response to stimulation with ACTH or gonadotrophic hormones.

THE ESTIMATION OF TESTOSTERONE SECRETION RATE

Miss Ausma Dulmanis and Dr. Bryan Hudson

The method used has been described in the previous report. Following the simultaneous infusion of 7 α H^3-dehydroepiandrosterone (DHEA) and 4-C^{14}-testosterone, urinary metabolites of both compounds are isolated by conventional hydrolytic and chromatographic procedures. The specific activities with respect to tritium in both DHEA and androsterone and with respect to C^{14} in the latter metabolite are measured by colorimetric reactions and liquid scintillation counting. Although this procedure has a number of methodological problems, satisfactory estimations of the secretion rates of DHEA and testosterone have been obtained in a number of subjects. These results are shown in Table 1.

Although these results are biologically reasonable, the precision of the method is subject to some criticism because of the problems associated with subtracting two specific activities in which non-systematic errors may magnify the final answer.

Recently the urine from a number of normal subjects has been examined in order to determine whether testosterone may be present. The method used was essentially that devised for the estimation of testosterone in plasma. Preliminary

1 Department of Physiology, University of Melbourne.

DHEA		TESTOSTERONE	
Males	Females	Males	Females
30.6	26.9	3.5	0.2
15.8	11.1	3.4	0.4
16.2	29.5	1.7†	4.8*
6.2†	23.7	3.1	1.9
43.9		5.4	
18.7		8.9	
11.2		7.16	
10.4		5.9	
16.2		3.8	

TABLE 1.—DHEA and Testosterone secretion rates.
All values are expressed as mg./day.

* This subject was a hirsute female.
† 67-year-old male.

investigations indicate that testosterone is normally present in urine in amounts of from 25 to 50 μg per day although this finding demands further confirmation. As far as is known this steroid has not been previously shown to be present in urine. If these findings can be confirmed then it would be possible to eliminate the use of the indirect procedure (using two isotopes) for the estimation of the rate of secretion of testosterone. Thus, following a tracer dose of 4-C^{14}-testosterone it should be possible to measure the specific activity of urinary testosterone. This has already been performed in two normal male subjects in whom the secretion rate of testosterone has been shown to be between 7 and 11 mg. per 24 hours.

These studies are being pursued not only to study how this may differ from subject to subject but also to prove by chemical, physical and radiochemical techniques the identity of testosterone in urine.

FRACTIONATION OF URINARY 17-KETOSTEROIDS

Miss J. Sheath and Dr. Bryan Hudson

During the past year a satisfactory procedure has been developed for the fractionation of urinary 17-ketosteroids. Urines from normal and abnormal subjects have been examined by this procedure. The method consists of hydrolysis with a mixed β-glucuronidase and sulphatase enzyme preparation (from the Australian limpet cellana Tramoserica) followed by solvolysis using tetrahydrofuran in acid conditions. After purification the urine extract is subjected to gradient elution chromatography on alumina, collection of appropriate fractions and purification by paper partition chromatography. In order to calculate losses through the method a small amount of radioactive steroid (4-C^{14}-DHEA) is carried through the complete method and a recovery factor is established.

The results of this investigation are shown in Table 2.

This method will be used to study conditions in which abnormal androgen secretion is suspected.

TABLE 2.—Urinary excretion of various C_{19}-17-ketosteroids by normal and abnormal subjects.

Subjects	mg./24 hrs. DHEA	ANDRO.	ETIO.	11βOH ETIO.	11βOH ANDRO.	11 KETO ETIO.
Normal females (8) Mean values	0.95	3.04	3.09	0.57	0.70	0.76
Range	0.17-3.67	1.21-5.70	1.80-6.92	0.30-0.84	0.23-0.96	0.19-1.42
Normal males (4) Mean values	3.29	3.27	2.82	0.603	0.86	0.64
Range	0.63-6.03	1.97-4.18	1.38-4.88	0.61-0.85	0.54-0.99	0.34-0.86
Hirsute female	6.40	6.48	6.30	1.76	1.67	1.70
Congenital Adrenal Hyperplasia	1.45	2.99	2.91	1.63	14.92	3.12
Hirsute female	0.73	3.91	4.02	0.66	0.66	0.74
Hirsute female	0.48	5.04	3.90	0.41	1.54	0.72
Congenital Adrenal Hyperplasia	0.33	7.74	1.64	2.36	31.1	2.87
Congenital Adrenal Hyperplasia	0.83	8.73	1.58	0.51	11.34	2.84

ESTIMATION OF PREGNANETRIOL IN URINE

Miss I. Ekkel, Miss P. Pearl and Dr. Bryan Hudson

Because the usual methods for the estimation of pregnanetriol in urine using sulphuric acid reactions were regarded as being non-specific and to estimate levels of pregnanetriol in a somewhat erratic fashion, the method of Cox and Finkelstein has been investigated. After enzymatic hydrolysis, pregnanetriol and other C_{20} methyl steroids are isolated by paper partition chromatography in two different systems. They are identified on paper by phosphoric acid fluorescence and eluted. After the second chromatography the steroid is subject to oxidation with periodic acid and the resulting acidaldehyde is trapped and estimated as such. After some initial difficulties the method is now able to be used routinely. Normal values of pregnanetriol in urine have been found to

range between 0.16–0.44 mg./24 hours : Mean 0.33 ± 0.08 (s.d.). It is of interest that in the urine of patients with "idiopathic" hirsutism levels of pregnanetriol have ranged from 0.6 to 4.3 mg. per day, values that are statistically different from that found in normal subjects. In addition, other C_{20} methyl steroids, pregnanetriolone and pregnanetetrol have been isolated from urine and their levels are currently being determined in this group of hirsute females.

ESTIMATION OF ALDOSTERONE IN URINE

Dr. Bryan Hudson, Miss P. Perl and Miss A. Dulmanis

This involves a modification of the method originally described by Peterson and Kliman in which a known amount of C^{14}-aldosterone is added to urine and the urine extracted after hydrolysis at pH 1 overnight. The extract of the hydrolysed urine is then acetylated with tritium labelled acetic anhydride and the aldosterone diacetate purified by paper chromatography. After two chromatographies a derivative is formed by oxidation which is subjected to further purification by chromatography. The ratio of H^3/C^{14} in the final sample is estimated by liquid scintillation counting. Using this procedure, normal levels of urinary aldosterone have ranged from 2 to 8 μg per 24 hours.

The C^{14}-aldosterone used in this study was prepared biosynthetically, using beef and rat adrenals, with Mr. John Coghlan from the Department of Physiology, University of Melbourne.

DIABETES

Dr. H. D. Breidahl

During 1961, the efficacy of numerous orally active hypoglycaemic agents was investigated. The currently available compounds, Tolbutamide and Chlorpropamide, are generally satisfactory, and are standards to which other compounds can be compared. They are sulphonylurea compounds, and four new substances of similar chemical composition were tried. As the trial were initial clinical trials, it is unlikely that the results will be published. None of the compounds tested was superior to Tolbutamide or Chlorpropamide, and those whose activity was almost comparable were found to have some side effects, so it is unlikely that any of these newer compounds will reach the market.

More extensive trials were undertaken with a substance of a different chemical composition—Phenformin, or D.B.I., which is a biguanide derivative. The results of the first 56 patients were published in the Medical Journal of Australia, and now that over 100 patients have been given D.B.I., a more comprehensive review of its use will be prepared and published in 1962. It would appear that D.B.I. is indicated when there has been secondary failure to the sulphonylurea compounds, or where a brittle diabetes exists, when it is used in conjunction with insulin. Its more general use in diabetes will probably be prevented by a high incidence of gastro-intestinal side effects.

ORAL PROGESTATIONAL AGENTS

Dr. H. D. Breidahl

With the introduction of oral substances with a progestational action, and known to inhibit ovulation, we were approached to try a new compound, and assess its nature as a method of contraception. Trials with this compound are

being performed in conjunction with Dr. Pincus Taft at the Royal Melbourne Hospital, and the patients studied are those in whom there is a medical indication for avoidance of pregnancy. To date, 13 patients have been studied for periods of up to 4 months, and the trial aims at studying 25 patients over 2 years. No preliminary opinion can be given.

MICROIODINE ASSAYS

Mrs. Dora Winikoff

Improvements of techniques used for routine diagnostic work have been achieved. The ashing modification for Protein Bound Iodine has been restandardized by substituting 2N potassium hydroxide for 4N sodium carbonate. This permitted reducing the temperature of ashing from 625°C to 590°C and the time from three to two hours. Iodine losses have been greatly reduced and the reproducibility of assays is now very satisfactory throughout the whole range of normal and abnormal values. The addition of KNO_3 or $KClO_3$ to aid combustion has been eliminated. The normal range of values is between 3.0–7.0 $\mu g\%$.

Globulin Bound Iodine estimation has also been improved by the addition of dry haemoglobin to act as protein carrier. Haemoglobin which has been subjected to long dialysis and freeze dried is practically iodine free. Dry egg albumin can also be used but it contains small amounts of iodine-like substances.

A SURVEY OF INDICES OF THYROID FUNCTION IN DIFFICULT CASES

Mrs. Dora Winikoff

A group of 16 patients with (a) equivocal clinical symptoms with and without exophthalmos, (b) after thyroidectomy or radioiodine (I^{131}) therapy and (c) following the withdrawal of medication, has been studied. The indices used were 4-hour uptake of radioiodine (I^{131}), 48-hour Protein Bound Radioiodine (PBI^{131}), Protein Bound Iodine (PBI) and Globulin Bound Iodine (GBI).

In the exophthalmic group the combination of PBI^{131} and GBI being elevated was the only indication of underlying thyroid abnormality. After radioiodine therapy—(since PBI^{131} is mostly elevated due to a rapid rate of turnover) —GBI was the most reliable index of toxicity. Following medication, the uptake and PBI^{131} were affected for a shorter period than PBI and GBI, which were often depressed (after antithyroid drugs) or elevated (after iodides).

In conclusion, there is definite advantage in carrying out all these measurements of thyroid function simultaneously before deciding on the future management of patients.

PUBLICATIONS DURING 1961

HUDSON, Bryan and Georg W. OERTEL—"Determination of Dehydroepiandrosterone and Total Neutral 17-Ketosteroids in Human Plasma". *Analytical Biochemistry*, 2: 248: 1961.

BREIDAHL, H. D.—"Hypocalcaemia in Childhood. Case Report". *Med. J. Aust.* 1: 139: 1961.

BREIDAHL, H. D.—"Phenformin in the Treatment of Diabetes Mellitus. A Preliminary Report". *Med. J. Aust.* 2: 1041: 1961.

PAPERS ACCEPTED FOR PUBLICATION

HUDSON, Bryan, Ausma DULMANIS and June SHEATH—"Hydrolysis of Steroid Glucuronides with β-Glucuronidase from Preputial Glands of the Female Rat". *Endocrinology.*

HUDSON, Bryan and James EVANS—"Adrenocortical Hyperplasia Associated with Bronchogenic Carcinoma". *J. Clin. Endocrinol. & Metab.*

BREIDAHL, H. D. and B. C. RITCHIE—"Hypercalcaemia due to Malignant Ovarian Tumour. Case Report". *Med. J. Aust.*

PAPERS IN PREPARATION

HUDSON, Bryan, I. EKKEL, A. DULMANIS and J. COGHLAN—"The Estimation of Testosterone in Biological Fluids".

HUDSON, Bryan, A. DULMANIS and J. COGHLAN—"The Estimation of Testosterone Secretion Rate".

HUDSON, Bryan and June SHEATH—"The Fractionation of Urinary 17-Ketosteroids".

LECTURES DELIVERED DURING 1960

"β-glucuronidase from Preputial Glands of Female Rats". Annual Meeting, Endocrine Society of Australia, Brisbane, May, 1961.	BRYAN HUDSON
"Cushing's Syndrome". For the Anti-Cancer Council, June, 1961.	BRYAN HUDSON
"The Measurement of Steroid Secretion Rates by Isotopic Procedures". Alfred Hospital Clinical Society, August, 1961.	BRYAN HUDSON
"The Estimation of Testosterone Secretion by Isotope Dilution". Ordinary Meeting, Royal Australasian College of Physicians, Adelaide, October, 1961.	BRYAN HUDSON
"The Diagnosis and Treatment of Endocrine Disorders". Ballarat, November, 1961—for the Melbourne Medical Postgraduate Committee.	BRYAN HUDSON
"The Use of Isotopic Steroids". Clinical Pathology Section, B.M.A., December, 1961.	BRYAN HUDSON
"Physiological Basis of Steroid Therapy". Prince Henry's Hospital.	H. D. BREIDAHL
"Treatment of Carcinoma of Thyroid". Anti-Cancer Council.	H. D. BREIDAHL

MEETINGS ATTENDED DURING 1961

Annual Meeting, Endocrine Society of Australia—Brisbane, May, 1961.	BRYAN HUDSON DORA WINIKOFF JUNE SHEATH
Ordinary Meeting, Royal Australasian College of Physicans—Adelaide, October, 1961.	BRYAN HUDSON
A.N.Z.A.A.S.—Brisbane, May, 1961.	DORA WINIKOFF

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

STUDIES ON CHEMOTHERAPY[1]

J. C. Tolhurst, G. Buckle, A. Perceval and M. Dorr

Studies on chemotherapy continupe to rank highly in the interests of the Bacteriology Staff. Methods of performing sensitivity tests have been compared and the results are reported in the new edition of "Chemotherapy with Penicillin and Allied Drugs" which is in the press.

Clinical trials on patients have been performed with the new penicillin known as methicillin.

Studies on individual patients have been continued with the object of determining whether chosen drugs in the dosage given can be expected to effect a cure. This is done by testing the bactericidal action of the patient's serum against his particular organism. In some cases of bacterial endocarditis it was found that the organisms were unusual in the long period for which they could survive the effect of penicillin. This led to modifications in the treatment of the patients.

In patients with meningitis refractory to treatment it is now our practice to estimate the degree of diffusion of drugs into the cerebrospinal fluid. This proved enlightening in a case of nocardial meningitis. In torula meningitis work has been done on the diffusion of amphotericin B into the cerebrospinal fluid. This is a highly toxic drug and the dosage should be kept at the lowest effective level, hence, in our opinion such tests are desirable in every patient suffering from this disease.

GENERAL BACTERIOLOGICAL STUDIES[1]

A. Perceval

The case of nocardial meningitis mentioned above was the first recorded in Australia and the first nocardial infection seen in this hospital. The characteristics of the organism in vitro have been examined fully and pathogenicity tests performed.

Work on immunity in torulosis has proceeded satisfactorily.

RENAL DIALYSIS[1]

M. R. Ewing and J. Nayman

The service of haemodialysis instituted in the University Department of Surgery and carried out from the University Medical and Surgical Units in the Alfred Hospital has continued.

During the year 41 patients have been dialysed in the treatment of acute renal failure, and 61 dialyses have been done.

Developments in technique which have been introduced in the department have made it possible to reduce the time of dialysis from about 10 hours to 6 hours, using the same apparatus but more efficiently. These developments are:

(a) Introduction of a weighing bed. This is carried on a simple "standing" weighing machine which has been designed and built. It is now possible to

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1 Department of Pathology.

1 Department of Surgery, University of Melbourne, Alfred Hospital.

monitor changes in weight during dialysis very accurately. This has proved of great value in the treatment of patients admitted with oedema due to over-transfusion subsequent to renal failure.

(b) The development of indwelling arteriovenous shunts has also been achieved this year with a great degree of success. It is possible after the introduction of an arteriovenous shunt between the brachial artery and associated veins to achieve greater clearance rates in the artificial kidney. This technique has enabled repeated dialyses to be performed, where this is necessary, with great ease. This work is not yet completed and continues.

(c) The technique of regional heparinization of the kidney coil has also been introduced. This has the theoretical advantage that the blood coagulation time in the patient's circulation remains unchanged, obviating the problems of haemorrhage which have been reported in other centres as a serious complication of haemodialysis. This has made possible the treatment of patients who have associated coagulation problems.

URAEMIA[1]

M. R. Ewing and J. Nayman

Experimental Induction

Acute uraemia has been produced by the induction of acute tubular necrosis using intravenous injections of anhydrous uranium nitrate. The histological and biochemical changes are predictable and the uraemia follows closely the course seen in acute renal failure in man.

The suppression of tubular function is reversible in some cases. It has been possible to use the group which is recovering to study the problem of wound healing in acute uraemia following the clinical observation that this is not normal in uraemic patients.

Effects of Uraemia on Wound Healing

The effect of uraemia on wound healing has been studied in 20 animals. If uraemia is induced when a wound has healed, no effect on the scar is observed. If a wound is created after the induction of uraemia it does not heal normally and disruption occurs in all cases. If uraemia is induced during the later stages of wound healing its effect becomes progressively less.

ANURIA[1]

M. R. Ewing and Elizabeth Kidd

The bacteriological study of the infections complicating anuria has been instituted. The observation that infection was a very important cause of death in patients with acute oliguric renal failure, led to investigation of the importance of septicaemia and wound infections in relation to uraemia.

The association of urinary tract infection with septicaemia following endoscopic resection of the prostate is also being undertaken and data have been obtained on the association of post operative shock with renal failure and septicaemia, though as yet, the results are inconclusive the work continues into 1962.

1 Department of Surgery, University of Melbourne, Alfred Hospital.

EXPRESSION OF THE HAEMOPHILIC GENE[1]

R. J. Sawers

A partially completed study to detect variability in the expression of the haemophilic gene in families with mild haemophilia A has shown that the plasma levels of factor VIII activity in different members of the same family are similar and frequently identical. A comparison of a semi-quantitative and two quantitative techniques shows that the former gives results which are four times higher than the latter two. These latter tests are modifications of the plasma thromboplastin generation and partial thromboplastin tests which are considered to be specific for the assay of factor VIII activity. Due to occasional variations, it was necessary to use both of the latter techniques on the same day to act as independent checks. It was confirmed by doing repeated studies on the same individual that the error in the result of the assay was ±25% of the value determined. Within this range, it has been found that the expression of the gene in different members of the same family has been constant in the 7 families so far investigated. However, further study is required to show whether some mild differences are consistent or due to the error of the methods used. The overall indications are that the expression of the gene is constant in different members. This finding is consistent with the theory that there is a series of allelic genes for haemophilia.

MORBIDITY PATTERNS IN ALFRED HOSPITAL

F. H. Hocking[1,2]

The pattern of morbidity in the Alfred Hospital was investigated by assessing the incidence of some commonly occurring medical, surgical and psychiatric conditions amongst the total of approximately 15,300 patients presenting for in-patient, out-patient, or casualty treatment during the year 1959. Bronchial asthma (535 cases), appendicitis of all types (439), abdominal pain of unknown aetiology (431), attempted suicide (322), other psychiatric conditions (257), congestive cardiac failure (238), haematemesis and melaena (221), myocardial infarction (216), and acute and chronic alcoholism (164) were the commonest non-traumatic conditions treated. Traffic accidents accounted for almost 10% of the total hospital population.

Overt psychiatric disability (attempted suicide plus other obvious psychiatric conditions) formed the largest single non-traumatic group even without the inclusion of alcoholism and psychosomatic disorders. It is probable that the true incidence of psychiatric illness was greater than that shown because of the difficulty of accurate diagnosis in, for example, patients with somatic depressive equivalents, under the conditions of a busy casualty department. Nevertheless, the figures as they stand underline the importance of psychiatric conditions and, in particular, attempted suicide, to a general hospital and to the community as a whole.

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1 Department of Pathology.

2 This investigation was aided by a grant from the Felton Bequest.

METHYLPHENIDATE AS AN AID TO PSYCHOTHERAPY

F. H. Hocking[1]

The piperidine derivative methylphenidate ("Ritalin") is a stimulant of the central nervous system, probably at both cortical and subcortical levels—particularly the sympathetic centres of the posterior hypothalamus. The drug, injected intravenously, was given to 30 patients judged likely to benefit from drug-assisted psychotherapy: each of them received multiple injections ranging in number from 5 to 32. The result indicated that methylphenidate given intravenously is a useful aid to "ventilation", and that side effects are seldom troublesome for the patient, although the drugs should not be used in the presence of the physical concomitants of anxiety.

In 24 of these patients, the drug was compared with amylobarbitone sodium given intravenously, and it appeared to be superior as a ventilating agent. These 24 patients also received intravenously combinations of amylobarbitone sodium followed by "Ritalin" and vice versa, but the combinations appear to offer no particular advantage over the individual drugs used.

THE NERVOUS TYPE RELATED TO CERTAIN PERSONALITY DIMENSIONS OF HUMAN SUBJECTS

N. McConaghy[1]

The hypothesis that certain personality differences in humans are produced by differences in strength of nervous processes was investigated. Sixty-three subjects, including 28 fourth year medical students at the Alfred were interviewed and classified into allusive and non-allusive thinkers on the basis of the type of abstract thinking they showed. (In brief, allusive thinkers tend to employ vaguer, less relevant associations than do non-allusive thinkers.) The subjects then completed a questionnaire, thereby rating themselves as extraverts or introverts.

In a subsequent session each subject learned in succession two lists of nonsense syllables and three lists of words. In such serial learning tasks it has been established that the middle of the list is learned most slowly. It is hypothesised that this is due to the action of an inhibitory process which accompanies learning. From the results of this experiment it was shown that extraverts had significantly less difficulty in learning the central portions of the work lists. This is interpreted as indicating that extraversion is associated with a weaker inhibitory process in central nervous activity.

In addition it was shown that the performance of non-allusive thinkers on the initial work list was significantly better than that of allusive thinkers, but that of allusive thinkers on the subsequent lists improved at a significantly greater rate than that of non-allusive thinkers. This is interpreted as indicating that allusive thinking is associated with a weaker arousal or attention response which gradually strengthens whereas the arousal response of non-allusive thinkers is initially stronger but does not show a significant increase during learning. This gradual and continuous improvement in performance on a learning task has recently been described in several studies of schizophrenics. The hypothesis that allusive thinking is indicative of a predisposition to schizophrenia is therefore strengthened by the result of this experiment.

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TREATMENT OF PSYCHIATRIC SYNDROMES WITH LEARNING THEORY METHODS

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If an activity is carried out repeatedly over a brief space of time the ability to continue it is weakened and remains weak for some subsequent time. This observation has been employed in the treatment of nervous tics. The patient is encouraged to repeatedly imitate the tic for about half an hour daily. The tendency for the tic to occur at other periods of the day is thereby weakened and successful cure has been reported in some cases. It was decided to employ this method experimentally in the treatment of intractable compulsions and some success has been achieved.

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"The Artificial Kidney"—*British Medical Association, Shepparton.*	J. NAYMAN
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