BAKER INSTITUTE

RESEARCH

1962

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-SIXTH ANNUAL REPORT

of

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

(Including Alfred Hospital Clinical Research Unit)

SIXTH ANNUAL RESEARCH REPORT

of

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

REPORTS

of

ALFRED HOSPITAL RESEARCH FELLOWS

1962

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, 1962

"Sol Green":	A. BAUMGARTEN, M.B., B.S.
Medical Research Fund:	J. M. CALVERT, M.B., B.S., F.R.A.C.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. (Oxon.), M.R.C.P. (Edin.).
"Dr. Henry Laurie":	GABRIELE MEDLEY, M.B., B.S.
Medical Research Fund:	N. McCONAGHY, B.Sc , M.B., B.S., D.P.M.
"E. H. Flack":	J. NAYMAN, M.B., B.Ch. (W'srand), F.R.C.S., F.R.A.C.S.
"R. B. McComas":	D. RACE, M.B., B.S.
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, M.B., B.S., B.Sc. (Med.), M R.A.C.P. (from 1/9/62).

APPOINTED TO RESEARCH FELLOWSHIPS FOR 1963

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

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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

Director:	T. E. LOWE, D.Sc., M.D., F.R.C.P., F.R.A.C.P.
Associate Directors:	P. FANTL, D.Sc., F.R.A.C.I. A. J. BARNETT, M.D., F.R.A.C.P., M.R.C.P.
Graduates:	Mrs. V. CARSON, M.Sc. C. C. CURTAIN, Ph.D., M.Sc., F.R.A.C.I. Miss P. EMERY, B.Sc. A. V. L. HILL, M.B., B.S. CHEVIOT KIDSON, M.B., B.S., B.Sc. (Med.) (on leave from 1/9/62). A. D. McCUTCHEON, M.D., B.S., M.R.A.C.P. Mrs. W. G. NAYLER, M.Sc. D. RACE, M.B., B.S. Mrs. H. STROSBERG, B.Sc. H. A. WARD, M.Sc. Mrs. M. WEISS, Ph.D., M.Sc. (on leave). Miss J. WRIGHT, B.Sc. R. G. WYLLIE, M.B., B.S.
Technical:	S. HART (Laboratory Supervisor). J. L. BREMNER. Miss J. HOWELLS. Mrs. R. SABO. Miss A. STANTKE.
Clerical:	Mrs. I. R. ROBINSON. Mrs. M. E. AUSTIN (to 1/10/62). Miss R. A. BELL. Miss L. CORKE (from 24/9/62). Miss B. DOWDELL.
Laboratory Assistants:	Mrs. J. BERAN (to 12/9/62). Mr. D. BREEN. Miss B. BURKE (to 1/10/62). Miss E. DENEREAZ. Miss D. DIXON (from 12/2/62). Miss J. FINDLAY (from 24/9/62). Miss S. FREAME. Miss J. HARRIS (to 31/3/62). Mrs. Y. KNEALE (from 9/4/62). Miss A. McARDLE. Mrs. M. McVICAR (from 30/7/62). Miss D. MAKEPEACE. Miss C. A. MILROY (to 23/3/62). Miss E. MINSTER. Miss L. PHILLIPS. Miss M. SHIERS. Miss E. WADDY.

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Registrar:	M. BRANDSTATER, M.B., B.S.
Resident Medical Officers:	D. S. ROSENGARTEN, M.B., B.S. K. MOUNTAIN, M.B., B.S. A. STEINIGER, M.B., B.S. A. M. KING, M.B., B.S.
Sister:	L. STAHR.
Staff Nurses:	K. G. PALMER (from 22/1/62 to 17/3/62). J. F. McRAE (from 26/2/62 to 23/7/62). S. ROSS (from 30/4/62 to 24/9/62). M. MACFARLANE (from 23/7/62 to 25/12/62). P. M. FUNSTON (from 3/9/62 to 19/11/62). P. J. JEFFERY (from 19/11/62).

RESEARCH FELLOWS

"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.).
"R. B. McComas":	D. RACE, M.B., B.S.

ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

Before reviewing the work of the Institute during the year it may be well to consider briefly the principles which have guided the selection of projects and determined the pattern of our research activities for the past several years. The general theme behind this work has been a desire to apply the principles and techniques of basic science to clinical medicine. This however is so wide a proposition that one segment only of the body—the cardiovascular system—has been chosen as a field of endeavour and the cardiovascular system has been broadly defined as the heart, the blood vessels and the body fluids related to them. To carry out the research programme a number of workers, highly skilled in the disciplines of clinical medicine and surgery, physiology, biochemistry, physical chemistry, biophysics and pharmacology have been brought together from time to time. Such a grouping of highly trained workers poses certain problems: on the one hand it is essential to preserve their intellectual initiative; on the other it is necessary to weld them into a team. The first requirement might easily lead to the Institute becoming merely a collection of isolated separate groups, the second to the development of a team in which the initiative of the members decreases from the time of its formation. In an endeavour to avoid both of these undesirable results it has been the policy to encourage individual workers to pursue such investigations in such fields as they wish within the facilities available whilst at the same time to require them to assist by discussion, advice or technical assistance, each other's project in so far as their special skills and knowledge enable them. The Annual Reports of the past indicate some success in this direction and the detailed report of scientific investigations this year again emphasises the team aspect of many of the projects, the participation of members in more than one team and the individual nature of yet other projects.

Intellectual initiative leading to creative thought is often stimulated in trained persons by apparently non-pertinent reading and discussions and these encounters are fostered by providing occasions for informal discussion and by good library facilities. The Institute is fortunate that generous foresight has provided adequate space for the library and co-operation with other libraries makes it effectively much larger than we could ourselves provide. However the growth in numbers of research workers using the library is placing strain on current methods of journal borrowing. In the coming year it is planned to re-organise this aspect of library service and by the use of photocopying methods to increase the availability of current journals to readers. In this way it should be easier for workers to browse around their subjects and to keep abreast of current happenings in their own special field.

In August the University of Melbourne held celebrations marking the Centenary of the founding of its Medical School. As part of these an open invitation was extended to the participants to visit the Institute and on August 16 a series of demonstrations was arranged to illustrate all facets of our research programme. This was a most successful function and some 120 visitors attended.

As foreshadowed in the last report changes in the association of the Hospital with the medical schools of the two universities has indirectly led to a substantial amount of undergraduate clinical teaching being undertaken by members of the

Staff. A small increase in the number of beds available to us has been very welcome and has greatly helped this facet of our activities.

Summary of Research Projects

Detailed accounts of the current research projects are presented in the scientific section of this report and are briefly summarised here.

Studies on the control of body fluid volume are now largely concerned with the details of this mechanism and progress slowly. The limits of our current techniques of observation appear to have been reached and new techniques are being investigated. This year some progress has been made towards developing a continuously recording device to study the concentration of sodium in the blood stream. If successful this device should enable changes in the osmotic pressure of body fluids to be studied in a manner similar to that which was used to follow volume changes.

Detailed investigation of the mechanism of blood coagulation and of lysis of blood clots continues to provide both laboratory and clinical projects.

Clinical tests of new drugs used in the treatment of cardiac failure, hypertension and arterial disease continue and the great value of hypotensive drugs in the treatment of "malignant" hypertension is emphasised.

A study of the incidence of unilateral renal disease as a cause of hypertension has been commenced and that of surgical treatment of occlusive arterial disease continued and extended.

Investigations concerning energy production by cardiac muscle have followed several lines. The role of calcium ions in the cardiac muscle contraction-relaxation cycle, that of divalent cations in the action of certain drugs, the mode of action of quinidine on cardiac muscle, the characteristics of cardiac muscle cell membrane potentials and the characterisation of a cardio-active substance found in normal plasma are all being investigated.

The study of various serum proteins remains an active and extensive programme and is being associated with genetic studies on the people of New Guinea and related areas.

The effects of various procedures in cardiac surgery, such as bypass, on the function of various organs have received special attention. Some of the problems of carrying out surgical procedures on the mitral and aortic valves have been investigated in various ways.

Considerable work on cellular enzymes has continued and some of it may be grouped as "molecular genetics" for the abnormalities of the enzymes studied in both erythrocytes and leucocytes can best be considered in genetic terms. In particular glucose-6-phosphate dehydrogenase in erythrocytes, catalase and neutrophil alkaline phosphatase in leucocytes and lipid metabolism in both have been studied. This study is giving some insight into clinical problems and the differences between leucocytes in certain neoplastic conditions and those of normal individuals.

Experimental work on the cause of acute pancreatitis has led to the formulation of an hypothesis which has led to a line of treatment which may be of clinical value.

A small project on the toxic action of drugs has this year been directed to phenacetin.

Overseas Visits

During the year the Director and several other members of the Staff have made or commenced overseas visits of varying duration.

My visit to Canada, U.S.A. and England was undertaken with several objectives in view. First, to get an appreciation of the latest lines of investigation into problems of cardiovascular disease; secondly, in view of the association of the Alfred Hospital with the new Medical School of Monash University to learn of new ideas in medical education, and thirdly, to make additional contacts with people who could help in maintaining the high standard of appointees to the Edward Wilson Memorial Fellowship. All these hopes were realised largely through the very generous assistance given me by the heads and members of numerous departments that I visited. These are too numerous to list completely but I thank them all for their unfailing courtesy and advice. In particular I acknowledge the help of Dr. George Griffith (American College of Physicians) in Los Angeles, Dr. E. Braunwald (National Institutes of Health) in Bethesda and Professor J. McMichael in London.

It is difficult and perhaps pointless to endeavour to summarise the experiences of such an extensive trip but one impression must be recorded for it points to necessary future developments in the Institute. In both clinical and basic science aspects of medical research the use of sophisticated apparatus to obtain data is growing apace. This development has had to await modern developments in electronics and nuclear physics but is rapidly becoming established. As a consequence many investigations are expensive in equipment and in many cases depend on the availability of workers trained in physics, mathematics and other sciences in addition to their training in biology and medicine.

It is of some satisfaction that the growing points in cardiovascular research embrace many projects that we have instituted and to note that the work in our laboratories and our senior workers are well known and respected in the appropriate overseas centres.

Dr. P. Fantl attended the Annual Meeting of the International Committee for the Standardisation of the Nomenclature of Blood Clotting Factors held this year in Stockholm.

Dr. M. Weiss completed her studies at the University of Utah, U.S.A., during the tenure of a United States Public Health Service Post-doctoral Fellowship.

Dr. Chev Kidson left late in the year to work for a period at the Chester Beatty Research Institute in London.

Grateful acknowledgment is made to the National Advisory Heart Council, U.S.A., the National Health and Medical Research Council of Australia, the Anti-Cancer Council of Victoria, the Board of Management of the Hospital and the Trustees of the Institute for the financial assistance which has made these visits possible.

Prizes

The David Syme Research Prize of the University of Melbourne was awarded this year to Winifred G. Nayler for her work on "The Role of Calcium Ions in the Normal Functioning of Cardiac Muscle Cells". This prize is given for the best original research work in Biology, Physics, Chemistry or Geology produced in Australia during the preceding two years.

The Baker Institute Prize (1962) for the best research by a recent medical or science graduate employed by the Institute or Alfred Hospital has been awarded to Dr. P. S. Bhathal for a thesis entitled "Some Aspects of Uraemic Myocarditis".

Research Grants

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

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

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

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

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

T. E. LOWE.

31st December, 1962.

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

1949-62.

Anderson, R. McD., 1953-55
Andrew, R. R., 1949-55
Barnett, A. J., 1949-50
Baumgarten, A., 1962
Beavis, E. L. G., 1955-56
Boake, W. C., 1958
Breidahl, H. D., 1952-53
Burnside, K. B., 1951
Cooper, E., 1962
Duffy, D. G., 1952-55
Ferguson, I. A. L., 1957-58
Fowler, R., 1953-54
Francis, J. K., 1956-57
Fraser, J. R. E., 1957
Gardiner, J. M., 1952
Goble, A. J., 1951
Hudson, B., 1952
Jamieson, K., 1954
Kay, H. B., 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., 1959-62
Sawers, R. J., 1953-60
St. Clair, W. A., 1955
Silberberg, F. G., 1953
Stern, W., 1954-55
Stirling G. R., 1955
Wagner, G., 1958

OVERSEAS FELLOWS

1954-62.

Dawson, J. B., 1961-62 (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, R. Sawers[1], H. A. Ward and H. Strosberg

FUNCTION OF FACTORS VII AND X IN THE FORMATION OF BRAIN THROMBOPLASTIN

In continuation of previous work the role of factors VII and X in the formation of brain thromboplastin has been investigated.

Mixtures containing factors VII and X were prepared from human sera. These and Ca^{2+} were added to extracts of acetone-dried human brain to produce thromboplastin. The mixture was then centrifuged at 61,000 x g for 1 hour at 2°C in a Spinco ultracentrifuge. The activity of both factor VII and factor X in the supernatant layer was determined. The recovery of factor VII was 20-46% and that of factor X was 16-40% of the original activity. The concentration of these factors in the original preparations varied by more than sevenfold yet the recovery was not much different in either case.

These observations suggest that 50-70% of both factors are bound by the thromboplastin present in the sediment of ultracentrifuged brain extract.

As factors VII and X are considered to be species specific, a comparison of preparations containing both factors was carried out by the assay of factor VII with the use of human as well as rabbit brain extracts.

The combination of factor VII and factor X with the brain precursor showed no absolute species specificity although a markedly higher activity in the homologous system was apparent.

On the assumption that factor VII is stable in the reaction mixtures containing brain extract a much higher recovery of factor VII would be necessary to support an enzymatic nature of factor VII in tissue thromboplastin formation. In view of the fact that both factors VII and X were recovered to approximately the same degree it is suggested that both these factors are bound by the thromboplastin present in the sediment of the ultracentrifuged brain extract. This might indicate the special case of adsorption when the fraction of adsorbent surface covered is small or the concentration of factors VII and X being adsorbed is low.

PHOSPHORUS CONTENT OF FIBRINOGEN AND FIBRIN

During a study concerned with the chemical changes associated with the retraction of fibrin gels by platelets it became necessary to determine the phosphorus content of fibrin formed in the absence of platelets.

Venous blood was taken from healthy donors and a variety of animals. Plasma with less than 500 platelets/mm^3 was obtained by centrifugation at 32,000 x g at 5°C for 45 minutes. Fibrin was formed with bovine thrombin, washed free of soluble proteins with 0.154 M sodium chloride. The organic

* 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 the Anti-Cancer Council of Victoria and those marked (‡) by grants from the National Heart Foundation of Australia.

1 Department of Pathology, Alfred Hospital.

material was combusted with perchloric acid and phosphorus was determined in the residue.

31 g. phosphorus was present in 385,000-435,000 g. (mean 410,000 g.) human fibrin when coagulation was carried out at concentrations between 8 and 31 mg. fibrinogen per 100 ml in the reaction mixture. For other species the corresponding mean figures were: domestic cattle 220,000; sheep 450,000; rabbit 400,000; dog 970,000.

If fibrin was formed at higher concentrations the protein content was 108-115% of that of fibrin obtained from 17 to 20-fold diluted plasma but the phosphorus content was 133-189% of fibrin from diluted plasma.

Phosphorus was also determined in purified fibrinogen. In some species (domestic cattle, sheep, goat) the phosphorus content of fibrinogen was almost the same as that of fibrin but in the case of human fibrinogen the phosphorus content was approximately twice that of fibrin. Dog fibrinogen had practically the same phosphorus content as human fibrinogen but dog fibrin contained considerably less phosphorus.

As the phosphorus content of the fibrin clot may be influenced by adsorption of non-fibrin material clots were prepared at low plasma concentration in order to minimise such adsorption.

The fibrinogen preparations were made from plasma with low platelet count and were of high purity; their phosphorus content was therefore unlikely to be influenced appreciably by contaminants.

The data suggest that the phosphorus in fibrinogen of cattle, sheep and goat is not released during clotting but in the case of human fibrinogen apparently one atom of phosphorus is present in one of the fibrinopeptides. Dog fibrinogen represents still another type in that most of the fibrinogen phosphorus is released during clotting.

The results give a measure of the minimum molecular weight of fibrinogen and place it about 210,000 for man. Physical methods have given quite a wide range (269,000 to 580,000) of results for the molecular weight of fibrinogen. It may be that the variations in molecular weight estimations by physical methods are due to different degrees of aggregation of a fundamental unit and the chemical estimation should give a more satisfactory result.

THIOL GROUPS OF BLOOD PLATELETS IN RELATION TO CLOT RETRACTION

We have shown previously that reagents which combine with –SH groups (mercuric compounds, cupric sulphate, arsenicals or iodoacetate) added in 10^{-5} to 10^{-4} molar concentration to isolated human blood platelets inhibit retraction of fibrin gels. It was therefore concluded that platelet –SH groups are essential for this process. However, these compounds can react with groups other than –SH so in order to substantiate the presence of –SH groups in platelets they were treated with N-ethylmaleimide (NEM) which binds –SH groups specifically. Platelets were isolated from human blood and the reaction with NEM was carried out in veronal or phosphate buffer pH 6.8, $I = 0.154$, at 2°C for 2 minutes. The suspension was centrifuged at 35,000 x g for 30 minutes at 5°C. The optical density of the clear supernatant was measured at 310 mμ. An equimolar ratio between consumed NEM and –SH groups was assumed. Between 1.9-2.8 μg –SH per 10^9 platelets were found. The amount of reacting

–SH groups was approximately the same in platelets from normal subjects and in those from a patient with myelofibrosis or Glanzmann-Naegeli disease.

These results are influenced by the contamination of platelets with other blood cells for 12-13 μmole of NEM are taken up by 1 ml. packed red cells at 37°C. Freshly isolated red cells tested under similar conditions to those for platelets, consumed 2.4 to 3.7 μmole NEM per 1 ml packed red cells, thus indicating penetration of NEM into red cells even at 2°C although a lower consumption was found. The error due to –SH content of the red cells contaminating the platelet preparations is therefore negligible.

Whereas platelets isolated from normal persons gave fibrin gel retraction before NEM treatment but none after, the platelets from the blood of the patient with Glanzmann-Naegeli disease showed, despite normal –SH content, a diminished fibrin gel contraction even before NEM treatment.

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. 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: two 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 calculated 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. 800-1,000 units of streptokinase or 2,000 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 clinical use as a fibrinolytic agent.

PREPARATION OF STORED HUMAN PLASMA[1]

The provision of stable blood derivatives suitable for transfusion and storage is a concern to both civilian and defence authorities in normal times and during emergencies. It is generally agreed that there is no substitute for human plasma protein except for an occasional and initial small use of artificial nonprotein blood volume expanders in an emergency. Therefore it is essential to have a stable product of human origin available.

A dried plasma preparation can be obtained which, after dissolution in water, gives a product which comes very close to fresh plasma. Such preparations were tested 5 years after production and the activity of their components, including some very labile plasma clotting factors (factor V and factor VIII), were fully preserved. However there are several objections to the use of reconstituted dried plasma. One is the risk of carrying the virus of infective hepatitis which is not destroyed by the drying process. Another is that in certain locations distant from civilisation there is no sterile water available as a solvent for the plasma powder and further, freeze drying is an expensive procedure.

Work was undertaken to find out the best way of preparing a liquid plasma suitable for transfusion. The starting material was citrated blood from which the cellular elements were removed by centrifugation at 2000 x *g* in the cold. This was followed by centrifugation at 35,000 x *g* which separated usually a lipid layer on the top of the plasma and cellular elements and platelets on the bottom. The plasma was stored for several months. Citrate was then removed by filtration through a column of "Sephadex" G50 and the plasma proteins eluted with 0.5% sodium chloride.

This material had the following properties: citric acid, approx. 2.6 μg/ml, total protein, 6.1%; electrophoretic pattern: albumin 56%, α_1 globulin, 4%, α_2 globulin, 9%, β globulin 15%, γ globulin 16%, prothrombin 570 u/ml., fibrinogen practically absent.

SYNERGISM OF DRUGS WITH ORAL ANTICOAGULANTS

Oral anticoagulants of the dicoumarol group depress the prothrombin level of blood and the level of several blood clotting factors (VII, IX and X). Patients who usually stabilise for several months occasionally show unexpected fluctuations to either higher or lower levels of the clotting factors. The first may be due to poor absorption of the drug or higher than normal intake of vitamin K. The

1 This work was carried out in consultation with the late Mr. I. H. Cuming, Resuscitation Officer of the Alfred Hospital.

lowering of the clotting factor activity is dangerous because of the risk of haemorrhages. Several drugs are known to act synergistically with the anti-coagulants. The best known are the salicylates but a new drug caused the following incident.

A female patient, aged 75 years, had a femoro-popliteal artery graft on 20.4.60. She was given EDC (3-3'ethylidene bis 4 hydroxy coumarin) and her prothrombin level was satisfactorily stabilised for two years. She received insulin injections regularly during that period. On 6.6.62 she arrived at Alfred Hospital with severe bruising on both elbows, a haematoma in the tongue, a hoarse voice, a slight epistaxis and a possible deep haematoma in her right leg. The insulin injections had been stopped one week before and replaced by 0.5 g. b.d.s. "acetohexamide".

The results of a blood examination indicated a severe reduction of several blood clotting factors. Two days later the laboratory tests were normal and eight days later the haemorrhages subsided. These results indicate that on 6.6.62 the patient had a severe haemorrhagic disorder, presumably due to synergism between the residual EDC and acetohexamide.

CONTROL OF BODY FLUID VOLUME*

T. E. Lowe, A. J. Barnett, A. Baumgarten, M. Brandstater, J. B. Dawson

Our previous investigations have led to the conclusion that the volume of fluid in the body is controlled by a mechanism of the feedback type and that as part of this mechanism there are receptors which monitor at least two facets of body fluid. These have been postulated to be the volume of the fluid and the osmotic pressure of the body fluid.

The techniques of observation so far employed have required that the unit period of observation is 24 hours and whilst this has been satisfactory so far as volume studies have been concerned the period is much too long for osmotic pressure studies. In an endeavour to reduce this observation period so that the changes in osmotic pressure of body fluids can be followed this year our work has been directed towards the development of a device which can continuously record the level of plasma sodium concentration. As sodium is the major cation in the plasma it is hoped that such a device will enable an adequate assessment of the plasma osmotic pressure changes to be obtained.

This device can also be expected to have considerable application in clinical medicine as, for example, in the control of haemodialysis procedures.

CONTINUOUS RECORDING OF SODIUM CONCENTRATION IN THE BLOOD STREAM

Since Eisenman (1957) demonstrated that the "alkali error" in pH measurement with glass elctrodes was due to impurities in the glass, a glass electrode suitable for single estimations of sodium ions in fluid has been developed by the use of glass of suitable composition and is commercially available.

During this year we have endeavoured to construct a small cannula electrode from Na^+ sensitive glass which can be implanted in a major artery of a pig. From such an electrode it is hoped to evolve a method of continuous recording of pNa. Prototypes of the electrode have been made and with the aid of the

cardiac surgery group inserted into the aorta of a pig and these have revealed problems of blood coagulation and protein deposition within the electrode when in place for many hours. The sodium electrode which works with a miniature calomel half cell is found to be extremely temperature sensitive so that it has become necessary to record the temperature of the electrode with a thermistor bead of appropriate sensitivity so that temperature correction may be applied to the sodium estimations.

CLINICAL TRIAL OF A NEW DIURETIC[1]

In spite of numerous new diuretics, certain patients with oedema remain refractory to treatment with diuretics commonly in use such as mercurials, thiazides, spirolactones or carbonic anhydrase inhibitors.

Triamterene (2,4,7-triamino-6-phenylpteridine) represents a new type of diuretic that has been found to inhibit the sodium retaining and potassium-losing effect of aldosterone and other mineralocorticoids and also to have a diuretic action in adrenalectomised animals and patients without endogenous or exogenous steroids.

Last year we reported some preliminary observations on its action in man and this trial has continued along three lines. First, its effects on subjects without oedema, secondly, a comparison of its effect with that of chlorothiazide over short periods in oedematous patients and thirdly, its clinical value in cases of refractory oedema.

In subjects without oedema orally administered triamterene causes an increased urinary excretion of sodium and chloride, but not of potassium, in the 12 hours following administration.

In a short-term permutation trial of chlorothiazide and triamterene, triamterene was found to have no advantage as a diuretic over chlorothiazide.

In the long-term treatment of refractory oedema, although triamterene alone was ineffective, it had distinct value when combined with chlorothiazide or chlorothiazide plus spironolactone and in six of eight patients a satisfactory diuretic response was obtained, whereas this had not been possible with previous therapy.

ENERGY PRODUCTION IN THE MYOCARDIUM†‡

W. G. Nayler, P. Emery, J. E. Wright, C. C. Curtain, A. Baumgarten and T. E. Lowe

The title "Energy Production in the Myocardium" applied to this project has been retained to indicate the underlying unity in a series of investigations which were commenced some 10 years ago. The purpose of the project, as then defined, was to study the methods by which chemical energy supplied to the heart muscle is converted into the mechanical energy passed to the blood stream by the contraction and relaxation of the myocardial contractile elements. From this study it was hoped to gain an insight into the mode of action of various drugs known to influence cardiac activity and possibly to find methods of enhancing their activity or of discovering new therapeutic agents.

1 Triamterene was supplied by Smith, Kline and French Laboratories (Aust.) Ltd.

Early studies on the efficiency of these energy conversions carried out in a respiratory apparatus using toad hearts indicated that the efficiency could be raised by glycoside drugs, some steroids and by increasing the calcium ion concentration of the perfusion fluid but not by catecholamines. Further investigations were directed to the group of substances enhancing efficiency and these have involved metabolic studies on whole hearts, more detailed observations on the contraction-relaxation cycle of muscle strips and a study of membrane action potentials of individual muscle cells. In addition as the toad is a hibernating animal and its cardiac muscle shows differences between its behaviour in summer and winter some investigations have been directed to this phenomenon.

During the current year investigations have proceeded along five lines:

(i) The role of calcium ions in the regulation of cardiac muscle contraction-relaxation cycle.

(ii) the role of divalent cations in the action of certain cardiac drugs.

(iii) the mode of action of quinidine on cardiac muscle.

(iv) the characteristics of the membrane potential of these muscle cells during activity and the influence of various agents upon it.

(v) the characterisation of a cardio-active substance found in normal plasma.

CALCIUM IONS

Previous investigations have indicated that both the efficiency with which the heart performs its mechanical work and the amount of work it does are regulated in part by the extracellular Ca ion concentration. The availability of the isotope Ca^{45}, together with the facilities for estimating radioactivity, has made possible an investigation into the mode of action of certain cardioactive drugs. The drugs investigated have at least one common action, namely that they alter ventricular contractility, which in this context is used to refer to either the shortening of muscle under isotonic conditions or the tension developed under isometric conditions. The following studies were made in an attempt to establish whether or not the action of the various drugs on ventricular contractility could be accounted for in terms of an altered distribution of calcium ions.

Caffeine

It has long been known that caffeine enhances cardiac contractility. Recently it was shown that this drug altered the distribution of Ca ions in skeletal muscle, a finding which suggested that Ca ions were involved in its effect on cardiac muscle. In a series of investigations it was shown that the positive inotropic activity of caffeine resembled that due to a raised extracellular Ca concentration, for poststimulation potentiation and the classical "staircase" were abolished by the drug. Ca^{45} studies showed that caffeine did alter the distribution of Ca^{45} in cardiac muscle in such a way that, in the presence of caffeine, the muscle accumulated Ca^{45}.

Nicotine

Small concentrations of nicotine enhance cardiac contractility, larger concentrations cause contracture. Investigations into the mode of action of nicotine indicated that it increased both the uptake and release of Ca^{45} from cardiac muscle in such a way that the muscle accumulated Ca^{45}. Further studies showed

that nicotine released Ca^{45} from cardiac muscle even in the absence of any extracellular Ca ions, so that the increased release must represent the mobilisation of Ca ions from an internal site and not simply an increased rate of turnover of Ca ions. These findings were supported by the observation that nicotine evoked contraction in cardiac muscle even in the absence of any extracellular Ca ions and they point to the possible importance of bound Ca ions in the regulation of cardiac activity.

Hypertonic Sucrose Solutions

It was previously shown that hypertonic solutions evoked contractures in ventricular muscle. Again using Ca^{45} it has been possible to demonstrate that this contracture-producing activity of hypertonic sucrose is associated with an increased uptake of Ca^{45} by the muscle from its environment. This finding explains the previously reported dependence of sucrose-induced contractions on the extracellular Ca concentration.

Reserpine

Using isolated toad ventricles it was possible to demonstrate that reserpine can directly evoke changes in cardiac contractility apart from those which can be attributed to the release of stored catecholamines. The direct depressant effect exerted by reserpine on cardiac muscle was shown to be associated with an altered distribution of Ca ions. Thus reserpine accelerated the rate at which Ca^{45} was lost from cardiac muscle and the results were interpreted to mean that the loss of these Ca ions could be associated with the drug's depressant action on contractility.

THE IMPORTANCE OF DIVALENT CATIONS IN THE BINDING OF CERTAIN DRUGS TO THE MYOCARDIUM

Both ryanodine and quinidine are firmly bound by the myocardium. Both drugs depress cardiac contractility and when present in sufficiently high concentrations, produce arrhythmias. In a series of experiments it was shown that these effects on toad ventricular muscle could be reversed by perfusion with EDTA. The results suggest that in either case EDTA chelates a divalent cation at or within the cardiac cell membrane and in so doing it dislodges the bound ryanodine or quinidine. Although it has not yet been established which divalent cation is involved in this process, the findings do point towards the importance of surface acting divalent cations in the mode of action of some cardioactive drugs.

ACTION OF QUINIDINE ON VENTRICULAR MUSCLE

In trying to elucidate the mechanism of quinidine's action work has continued by studying the effect of alterations in the cellular ionic environment on quinidine's action. In experiments in which rat Langendorff heart preparations were used it has been shown that a raised Ca or a lowered Na ion concentration in the extracellular fluid enhanced quinidine's negative chronotropic effect. These findings probably reflect the antagonism between Ca and Na ions described for cardiac muscle. They may also be interpreted to mean that the number of molecules of quinidine which are bound to the myocardium is determined by the extracellular Ca/Na ratio, this in turn being related to the Ca/Na ratio at or in the membrane. Other experiments using the chelating agent, EDTA, sup-

port this view and suggest that the binding of quinidine to the myocardium involves a divalent cation.

The action of quinidine on hearts which were removed from previously reserpinised rats was also investigated and it was found that preliminary reserpinisation reduced both the frequency with which quinidine induced arrhythmias occurred and their intensity. This inter-relationship between quinidine and preliminary reserpinisation is being investigated at a cellular level, using the standard micro-electrode technique.

The ability of quinidine to prevent either fibrillation or the development of arrhythmias during perfusion of isolated rat hearts in glucose-free Tyrode solutions was investigated. 25γ/ml quinidine did reduce but did not abolish such arrhythmias, including fibrillation.

GLYCEROL-EXTRACTED MUSCLE PREPARATIONS

The presence of intact mitochondria in glycerol extracted cardiac muscle fibres has been demonstrated by means of electron microscopy. Enzyme studies on these extracted tissues indicated that both mitochondrial and sarcoplasmic enzymes were extracted together with the contractile proteins. Hence the use of glycerol-extracted tissues during investigations of muscle function will yield unreliable results.

MEMBRANE POTENTIALS OF CARDIAC MUSCLE CELLS

Action of Synthetic Oxytocin

It has been reported that synthetic oxytocin can abolish cardiac arrhythmias and at a concentration of 2 i.u./Kgm can restore normal rhythm in fibrillating ventricles. The action of this antifibrillatory substance on the membrane action potential and/or isotonic contraction was therefore investigated with equipment described last year which enables these two things to be recorded simultaneously. It was found that synthetic oxytocin reduced by 5% the time interval between the upstroke of the action potential and the maximum tension developed which was however itself reduced as was its rate of rise. The contour of the action potential was changed for the gradient of the initial repolarisation (phase I) increased by 40%, the gradient of the plateau (phase II), increased by 20% while the final period of rapid repolarisation (phase III) decreased by 30%. The time between the upstroke of the action potential and the onset of rapid repolarisation decreased some 10-20%.

These findings have been interpreted as indicating a more efficient use of oxygen in phase II and that entry of sodium ions into the cell is impeded and that the exit of potassium ions is initially enhanced but in phase III it is impeded.

Potassium Depolarisation

When toad ventricular muscle strips are soaked in Ringer solution containing 100 mM potassium ion concentration for four minutes the transmembrane potential falls from its normal value of 90mV to about 30mV. This value is higher than would be expected from theoretical calculations based on the Hodgkin-Huxley equations. It is also in contrast to the behaviour of skeletal muscle in these conditions where the membrane fully depolarises within one second.

It is considered that these findings indicate possible saturation of binding sites for potassium ions on the extracellular side of the cell membrane.

Velocity of Conduction of Depolarising Stimuli

Preliminary investigations into the effect of temperature on the velocity with which depolarising stimuli are conducted along strips of ventricular muscle have shown that those processes, which are initiated by stimulation and which result in partial depolarisation of the cell membrane and the influx of sodium ions, are temperature sensitive. This temperature dependence is reflected in the changed rate at which depolarisation stimuli are conducted along the muscle at various temperatures. Changes in the extracellular calcium ion concentration modified the effect exerted by such temperature changes on the velocity of this conduction.

CARDIOACTIVE PLASMA SUBSTANCE

Previously it has been shown that plasma exerts a positive inotropic effect on the isolated toad heart and that this activity could be separated into two fractions, one with a molecular weight in the peptide range and the other in the globulin range.

Initial attempts at large-scale purification by ion-exchange chromatography of this inotropically active peptide met with difficulties owing to the presence of cardiodepressant substances in all the available ion exchange materials. However successful chromatographic isolation was achieved on our dispersed diethylamino ethyl-methacrylate gels and it is now possible to recover 60 micrograms of highly active pure peptide from one litre of human plasma.

Attempts to isolate the inotropically active globulin fraction from plasma by ion exchange chromatography were also frustrated by the presence of cardiodepressant substances in all the ion-exchange materials suitable for protein chromatography. We expect, however, that a low-cross-linked dispersed diethylaminoethylacrylate gel which is free of cardiodepressant substances, will prove of value in the isolation of the inotropically active protein.

Recently plasma has been collected under carefully standardised conditions from normal men and women and from several species of animals and in all cases this cardiac stimulating activity has been demonstrable. When several samples have been collected from the same individual over a period of several weeks the yield of active peptide from the plasma of that individual has remained substantially constant. The level of activity has been the same whether the blood was collected into either a clean glass or a siliconised test tube. Neither storage at 20°C for several weeks nor standing at room temperature for 24 hours affected the activity. The yield was constant, and the shape of the elution diagram remained Gaussian regardless of the speed of separation of the peptide from the protein fraction. With a special apparatus it was possible to achieve complete separation of the peptide from the protein fraction on columns of dispersed polyacrylamide in 0.6 seconds.

In the light of these observations it is considered unlikely that the peptide is produced incidentally to the manipulations involved in the taking of the blood, or by reaction in the gel filtration column.

Some preliminary studies in human disease states indicate that patients with pituitary gland hypofunction have plasma with a low level of activity.

HYPERTENSIVE STATES

A. J. Barnett, M. J. Brandstater, A. Baumgarten, V. Carson and A. Wood[1]

The trial of treatment of severe (complicated) hypertension with hypotensive drugs, particularly the ganglion-blocking and sympatholytic drugs often augmented by other drugs, has continued.

To the present 150 patients have been treated. Relief of symptoms and regression of certain signs of hypertensive disease have previously been demonstrated and modern therapy employing a combination of drugs does not usually produce marked side effects.

The following table shows the numbers of patients surviving after treatment for various lengths of time up to nine years.

Phase of Hypertension	Malignant		Benign	
Survival Time (Years)	Number of Survivors Possible	Actual	Number of Survivors Possible	Actual
1	41	31	105	86
2	38	21	96	71
5	37	15	62	36
9+	27	5	23	5

These data indicate a definite improvement in the expectation of life for patients with "malignant" hypertension and although it is probable that lives of patients with severe hypertension not in the "malignant" phase are also prolonged this cannot be claimed with certainty as the prognosis of this group without treatment is uncertain.

METHYLDOPA (ALDOMET)[2]

A clinical trial of methyldopa in seventeen patients with diastolic hypertension showed that the drug is an effective hypotensive agent and that it reduces blood pressure in the recumbent as well as the standing subject. Its action is additive to that of other hypotensive drugs and combination with a thiazide diuretic may be advantageous because of the observed water retention in some patients.

The effective dosage varies between 0.25 and 4 grams per day. Symptomatic reactions are relatively infrequent and mild but depression was observed in three cases.

The drug had no effect on haematological findings, the liver function tests, the serum protein electrophoretic pattern, the blood urea concentration, the urinary sediment as examined microscopically nor on the protein content of urine.

Previously an impression of tolerance on continued administration was recorded but has not been confirmed.

1 Alfred Hospital Fellow in Urology.

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

Methyldopa has since been used widely by us for the treatment of hypertension and is considered valuable but sometimes less effective than guanethedine in patients with severe hypertension. Some patients are less affected by the side effects of methyldopa than with guanethedine; in others the reverse holds.

RENAL BASIS OF HYPERTENSION

Hypertension due to renal ischaemia, particularly if unilateral, may be curable by removal of an ischaemic kidney or by restoration of its blood flow by arterial surgery. Following recent developments in diagnostic and therapeutic techniques it was considered desirable to re-survey the patients with severe (complicated) hypertension already referred to as the series under study for treatment with hypotensive drugs.

Eighty-four surviving patients were available for survey and were investigated by one or more of the following tests: excretory urogram (I.V.P.), 66; radio-renogram[1] (measurement of radioactivity over each kidney following the injection of radio-actively labelled para-amino hippurate), 70; and aortogram, 22.

Aortography was only performed in patients in whom the excretory renogram or the radio-renogram gave an indication of unilateral renal disease.

Ten cases of probable renal hypertension and four cases of possible renal hypertension were found. However, in each of these cases surgical treatment was regarded as inadvisable because of the patient's general condition (age, overall renal function) or unnecessary because of satisfactory control of the hypertension by drug treatment.

How frequently and how fully hypertensive patients should be investigated for a possible renal basis is still a moot point. Full investigation, including aortography in all cases would subject laboratories to great strain and many patients to disagreeable tests. Cleveland Clinic workers have suggested certain findings as indications for a thorough search, namely: abnormal excretory urogram, "malignant" hypertension, age under 35 years, duration less than 1 year, recent acceleration of hypertension. In our eighty-four cases these indications were present in fifty and absent in thirty-four. All the ten cases of probable renal hypertension were found among the fifty cases in which the Cleveland Clinic indications were present.

In previous years, some patients with suspected renal hypertension were studied by differential renal function tests by the method described by Howard, involving the collection of urine from each ureter. The results were generally unsatisfactory usually because of leak round one or other catheter into the bladder. This year we have followed the modified technique described by Stamey which includes the production of an osmotic diuresis by infusion of urea solution and attention to precise details of ureteric catheterisation. This method has proved much more satisfactory technically, although we have not yet employed it in a a patient with proven unilateral renal hypertension.

DISEASES OF PERIPHERAL BLOOD VESSELS

A. J. Barnett, M. Brandstater, V. Carson, K. N. Morris[2] and G. R. Stirling[2]

ARTERIAL GRAFTING

The study of the value of arterial surgery in occlusive arterial disease continues. Arterial grafting remains the commonest type of direct arterial

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.

surgery and twenty-six grafts have been inserted during this year, bringing the total to 132. A survey of the grafts inserted up to June of this year shows that 85% of the grafts were patent at the time of the patient's discharge from hospital and 40% patent after four years. These proportions are similar to those recorded three years ago.

Because of this somewhat disappointing result sympathectomy has been combined with reconstructive surgery in almost all cases during this year. Endarterectomy is being performed in association with arterial grafting in order to obtain a wide channel at the anastomosis site, and also in association with patch angioplasty in cases with short blocks. It is hoped that, by careful matching of these operative procedures with the lesion, the results of reconstructive arterial surgery will be better than in previous years when the standard procedure was arterial grafting.

HYPOCHOLESTEROLAEMIC DRUGS

The study on the effect of triparanol (MER 29)[2] and nicotinic acid in lowering the plasma cholesterol levels in atherosclerotic patients has been completed. Results reported last year showed that a significant lowering of the serum cholesterol level could be achieved in most patients with either triparanol (0.5 g/day for 6 months) or nicotinic acid (up to 3 g/day for 6 months). In patients for whom a normal serum cholesterol level (under 250 mg/100 ml) was not achieved by these measures, it was found that the addition of nicotinic acid to triparanol treatment gave an additional fall of plasma cholesterol level (mean of 69 mg/100 ml) in 9 patients and the addition of triparanol treatment to nicotinic acid also gave an additional fall (mean of 44 mg/100 ml) in 5 patients. There was no clinical improvement in the patients associated with the fall in plasma cholesterol levels, nor would any be expected in patients who had already gross occlusive arterial disease. It is concluded that it is possible to lower plasma cholesterol levels by drug treatment but this is unlikely to produce apparent clinical benefit in patients with established occlusive arterial disease. Triparanol has been withdrawn from use because of possible toxic side effects (although none were seen in this trial) and large doses of nicotinic acid are associated with unpleasant reactions. Early and long-term treatment with these drugs in patients with hyper-cholesterolaemia in order to forestall atherosclerotic changes is therefore not practicable.

ANGINA PECTORIS

A new drug (Alderlin)[1] (I.C.I. 38,174) claimed to be of value in the treatment of angina pectoris has been submitted to a clinical trial. This drug is a β-adrenergic blocking agent and it was hoped that it would reduce the frequency of angina by protecting the heart against the increased rate and force of beat produced by adrenaline and noradrenaline.

Our observations confirm that "Alderlin" is an inhibitor of certain excitatory effects of the catecholamines and prevents the increased heart rate, rise in pulse pressure and increased cardiac output produced by isopropyl noradrenaline.

However, in a double-blind, cross-over trial in patients with angina pectoris this drug was no more effective than a placebo. Using as a criterion of improve-

2 MER 29 was supplied by Wm. S. Merrell Pty. Ltd.

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

ment a reduction of 25% in the number of attacks of angina per week in fourteen patients, eight showed improvement with both placebo and "Alderlin". In only one of five patients with E.C.G. changes produced by exercise was there improvement during treatment with "Alderlin".

CARDIAC SURGERY†‡

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

ORGAN BLOOD FLOW DURING TOTAL BODY PERFUSION

A preparation which allows the continuous separate measurement of venous drainage from various regions of the dog has been made.

This has been achieved by the separate cannulation of the superior vena cava (draining blood from the brain, the face and the fore limbs); the vena azygos (draining blood from the chest and posterior abdominal walls); the hepatic segment of the inferior vena cava (draining blood from the liver and bowel); the renal segment of the inferior vena cava (draining blood from the kidneys and adrenal glands) and the distal segment of the inferior vena cava (draining blood from the pelvis and hind limbs). The coronary venous return was drained from the right ventricle. The blood from the various regions was collected into calibrated cylinders and the time to collect 100 ml. was determined. From these cylinders the blood passed to the heart-lung machine where it was oxygenated and its temperature adjusted and thence back to the experimental animal via the left common carotid artery.

The preparation has shown long-term stability and the reproducibility of measurements has been good. In some experiments the pH and pCO_2 were measured and found to be within acceptable range of pH (7.30-7.37) and pCO_2 (35 mm. Hg.-57 mm. Hg.).

The Influence of Changes of Total Body Blood Flow Rate on Regional Flow Rate at Normal Temperatures

In eleven successful experiments the following table shows the averaged venous return at two different perfusion rates.

PROPORTION OF VENOUS RETURN IN VARIOUS SEGMENTS OF CIRCULATION

	Perfusion Rate (ml/Kgm Body weight/min.) 100-105	60-65
S.V.C.	26.5%	22.7%
Splanchnic	36.2	37.4
Kidney	14.2	15.1
Distal I.V.C.	13.4	14.2
Vena Azygos	10.2	8.9
Coronary	6.4	6.1

1 Thoracic Surgical Unit, Alfred Hospital.

Under conditions of total body perfusion it seems that the various regions, from which measurements were taken, show very little response to marked changes in flow rate and the consequent change in blood pressure.

The Influence of Temperature on Regional Blood Flow

Using the same preparation the flow was maintained constant at 95-100 ml/Kgm Body Weight/min. for each experiment and the temperature of the perfusing blood was slowly varied from 37° to 15°C, and the resulting flows measured.

The most significant reduction in flow as the temperature fell occurred in the venous drainage from the liver and from the kidneys. The splanchnic circulation showed a decrease from 33.2% of total flow at 37°C to 14.1% at 15°C, and the kidneys from 11.5% to 5.1%. The coronary circulation showed almost no change and the main muscle groups had an increase in venous drainage with a decrease in temperature.

The changes suggest that the acidosis which commonly accompanies hypothermia may be due to a failure of the liver to metabolize organic acids or the kidneys to excrete hydrogen ions since it is difficult to conceive normal function of either of these organs in the face of marked reduction in blood flow. Reports from other workers have shown there is a marked decrease in function of both these organs with reduction in body temperature.

The conclusions which may be drawn from both these series of experiments is that under conditions of total body perfusion the various vascular beds do not react to changing perfusion though they are capable of reacting to changes in temperature of the blood. There is no support for the hypothesis of preferred perfusion of vital organs under conditions of low total blood flow.

METABOLICALLY SUPPORTED ISOLATED DOG'S HEART PREPARATION

This preparation originally described by S. J. Sarnoff of the National Institutes of Health allows study of the performance of the left ventricle independent of the right heart and lungs and the peripheral circulation. The primary circulation of the blood is via a reservoir which controls the filling pressure of the left ventricle thence to the aorta which is cannulated and returned to the left atrium reservoir via a water-filled Starling resistance. The resistance against which the left ventricle ejects its stroke volume can be controlled.

The coronary venous return is collected from the right ventricle by a cannula in the pulmonary artery and returned to a second anaesthetized dog. Any blood losses from the primary circuit are assumed to be due to this coronary return and are made up by donations from this second dog. In this way the supply of fresh blood to the metabolically active heart is ensured.

The heart under examination is isolated from the body by severance of its nervous connections; from its lungs by bypassing them and maintaining oxygen saturation from an anaesthetized but otherwise intact dog and from variations in systemic vascular resistance by substitution with a Starling resistor.

The problems associated with the setting up of this preparation have now been overcome and experience has been gained in 22 experiments and the surgical techniques have been standardized.

These preparations have remained stable up to 2 hours and it is anticipated that with improvement in the mechanics of the blood circuit that longer-term stability will be achieved.

VISUALIZATION AND INVESTIGATION OF MOVING CARDIAC VALVES IN THE POST-MORTEM HEART

The surgery of abnormal cardiac valves has necessitated better methods of investigation of the dynamics of both normal and abnormal cardiac valves.

The main study has been on post-mortem human and animal hearts, placed into systems wherein the characteristics of normal and abnormal circulations can be applied to them under control, and from these systems both visual and hydrodynamic information regarding the valves under investigation can be obtained.

During the year a prototype cardiac pulse simulator was constructed, modelled on both overseas and local ideas, with the aim of furthering cardiac valve study.

The basis of the apparatus consists of intermittent pneumatic compression of a heart which is mounted on a perspex viewing plate, inside an air-tight glass cylinder. A separate closed circulatory system is connected to the heart. The propulsion of fluid (in this case water) through this circulation depends on the force and characteristics of the pulsation applied to the heart and the integrity of the valves.

The mechanism of the system allows us to vary the heart rate, the force of contraction, the peripheral resistance, the filling pressure and the atrial and ventricular filling times, and therefore the stroke volume. The main mechanical limitation of the prototype has been in the allowable cardiac output, which reaches a maximum for normal hearts at 2.5 1/min. However it is expected that with construction of a definitive metal model, this disadvantage will be overcome.

Investigation of both normal, abnormal and artificial cardiac valves has been carried out with the prototype simulator and a cinematographic record made for teaching as well as research purposes.

The recordings made were of pre- and post- valve pressures, total flow and peripheral systolic and diastolic pressures. From this information it is possible to determine whether there is any stenosis or incompetence of the valves being investigated, which up to date have been the mitral and aortic valves.

The absence of active muscular activity, essential for normal mitral valve action, is a limitation which must be overcome before the experimental characteristics of this valve can be accepted. One method to overcome this would be to investigate this valve in the living heart. The methods for doing this are not yet satisfactory, although irrigation of the left ventricle under pressure, with isotonic glucose, and direct visualization of the mitral valve has been carried out at open heart valve surgery, allowing the surgeon an immediate appreciation of the adequacy or not of the valvuloplasty.

Further studies on the above ideas will be pursued into the following year.

SURGERY OF MITRAL INCOMPETENCE

Our initial clinical experience with this lesion was in 1960. Progress was delayed because of several factors relating to the valve itself and to the problem

of prolonged perfusion of poor risk subjects. Increasing clinical experience with total body perfusion has enabled perfusions of 90 to 150 minutes to be successfully managed. Laboratory work had shown that it was possible to conduct total body perfusion for operation through a left thoracotomy using either the right atrium or the outflow tract of the right ventricle for venous drainage.

The exposure from the left side was found to be a great improvement on the original right-sided exposure and was used for a scries of experiments in which patches of autogenous pericardium were in-laid into the anterior leaflet of the mitral valve.

To overcome the possiblity of air embolism the use of deliberately induced ventricular fibrillation was examined in the laboratory. It has proved eminently satisfactory, allowing for continuous myocardial perfusion during repair, but preventing air embolism by the absence of co-ordinated propulsive activity by the ventricle. An open vent placed in the apex of the left ventricle allows for final expulsion of air before sinus rhythm is restored by electrical stimulation.

The Starr Edwards mitral valve prosthesis has been evaluated in both laboratory and clinical experience. This prosthesis, a silastic ball in a rigid tantalum cage, has proved to be a very satisfactory substitute from the haemodynamic point of view. Four complete replacements of the mitral valve have been carried out. In all instances the immediate haemodynamic result has been excellent. Unfortunately one of the patients succumbed two weeks after surgery from factors which were probably unrelated to the valve replacement.

The existence of an adequate prosthesis for the mitral valve has allowed of a great expansion of the group of patients who may now be considered for mitral valve surgery by open techniques.

SURGERY OF AORTIC VALVE DISEASE

Because of the anatomical factors and the necessity to open the aorta to expose this valve the problems involved in its successful repair are much more formidable than they are in the case of the mitral valve. In consequence the clinical situation is still unsatisfactory and our laboratory work will be focused more strongly onto this problem.

The major problem of maintaining the myocardial energy reserves during prolonged periods in which the aortic valve is exposed has been under study for the past two years. In brief it has been concluded that the technique to be adopted must include continuous perfusion of the myocardium by oxygenated blood. Anticipation of surgical difficulties associated with the presence of cannulae in the operation field has suggested that some degree of myocardial hypothermia would be a useful adjunct to the technique—in the event that short periods of complete cessatcn of perfusion might be necessary.

When the myocardium is perfused with oxygenated blood at 20°C for one hour left ventricular function is well preserved. These experiments were conducted by perfusing the root of the aorta below a cross clamp and not by direct coronary artery cannulation as, in our experience, this latter technique is a very uncertain technique in the dog because of anatomical factors. The problem of design of coronary cannulae for human perfusion has exercised us a great deal as there is considerable variation in the local anatomy from case to case. Metal cannulae which were used first were found to be inflexible and dangerous to the intima. The current technique involves direct cannulation of both coronary

arteries with flexible plastic cannulae fitted with inflatable rubber cuffs which both hold them in position and render them blood tight.

The surgical technique most commonly used has been debridement of the calcified plaques in the valve combined with commissurotomy. Some experience has been gained with total replacement of the valve using the Muller prosthesis.

BIOCHEMICAL TECHNIQUES

Determination of arterial pH and pCO_2

Techniques for studying the pH and pCO_2 of arterial blood of dogs during bypass have been studied in some detail. The blood pH was measured in an Astrup apparatus, (which provided means of temperature control) using a combined glass and calomel electrode and a Radiometer pH meter (model 22) with an expanded scale over the pH range 6-8. The accuracy of the meter is of the order of $\pm$ 0.01 pH unit.

pCO_2 was measured by two methods. First, by calculation from the blood pH and the plasma total bicarbonate determined by the method of Van Slyke, using the Henderson-Hasselbalch equation; and secondly, by the interpolation technique of Astrup using the relationship that under standardization conditions pH is proportional to log pCO_2. If the actual pH of the blood is known and the pH measured after equilibration of the plasma with a known tension of CO_2, the pCO_2 of the blood can be derived. It was confirmed that the two methods could give similar results provided that various conditions such as temperature were carefully controlled. It was concluded that the Astrup method was more susceptible to error than the Van Slyke method and offered no advantage in time or laboriousness provided one had the services of a skilled technician to operate the Van Slyke apparatus. (We are able to reproduce the results of the Van Slyke determination within 0.2 meq/l.) Furthermore, where a series of measurements is required at short intervals, the Astrup method becomes impractical unless more than one apparatus and pH meter are available as the specimens cannot be stored and one estimation takes at least half an hour.

On the other hand we have found no change in total CO_2 when plasma is kept anaerobically for 3 days in the refrigerator, so that provided the blood pH is estimated within 10 minutes of sampling, the plasmas may be kept and analysed in batches when convenient.

The acid-base relationships have been studied in several dogs on bypass under varying conditions, including hypothermia.

Bromsulphalein determination in grossly haemolysed or jaundiced blood

In the course of experiments on bypass in dogs, bromsulphalein (BSP) clearance was used as an index of hepatic function. However, in the course of bypass, the dog's blood becomes extremely haemolysed and the usual method of measuring the concentration of dye in the serum cannot be used. A method was worked out whereby the proteins including haemoglobin were precipitated with an acetone-water mixture and the dye determined in the supernatant fluid after centrifugation. Recovery of added BSP was about 95% and although protein removal was not complete the residual haemoglobin from quite grossly haemolysed samples was insufficient to affect the result. Bilirubin is not removed in this procedure but it has negligible absorption at the wave length chosen (580 mμ).

Clark and Collip Method for Serum Calcium Determinations

The Clark and Collip method for serum calcium determination as described by Varley (Practical Clinical Biochemistry) gives two alternative procedures for washing of the calcium oxalate precipitate. First, a single wash with 3 ml. dilute ammonia with removal of the supernatant fluid as completely as possible after centrifuging; and secondly, three washes with 5 ml. of dilute ammonia, centrifuging and pouring off the supernatant fluid.

The second method was used but it was found that our results were consistently about 10% below the expected normal figures. Recovery experiments were then carried out using both methods and it was found that whereas the recovery, using the first method was about 98%, the recovery using the second method was only about 88-90%.

The reason for the low recovery in the second method was thought to be due to the small but appreciable solubility of calcium oxalate in the wash fluid.

A simple calculation based on the solubility of calcium oxalate in water confirms that it is theoretically possible to lose up to 14% of the precipitate in a normal estimation of serum calcium by the second method. By the first method, the gain of oxalate owing to incomplete removal of excess ammonium oxalate is approximately 0.4%. Whilst the loss owing to solubility of calcium oxalate in the wash fluid is approximately 2.9% giving a net loss of 2.5%. This agrees well with our experimental findings.

It is recommended therefore that the "one wash" method be used and that a calcium standard be included in every series of determinations to correct for a less than 100% recovery.

BLOOD PROTEINS*

C. C. Curtain and A. Baumgarten

As in previous reports this project has been divided into sections (a) Instrumentation, (b) Techniques, and (c) Investigations.

INSTRUMENTATION

A NEW TYPE OF ELECTRODE FOR MEASURING CARBON DIOXIDE

In the course of a study of the properties of ionic semi-conductors in relation to the theory of the cell membrane it was found that sheet cellophane impregnated with calcium hydroxide exhibited marked changes in electrical resistance in atmospheres of varying carbon dioxide content. This effect has been used as the basis of a simple carbon dioxide-measuring device. A film of the calcium hydroxide membrane is wrapped around and sealed by its edges to a glass rod with epoxy resin. Two platinum wires are then sealed with resin on opposite sides of the membrane. The whole is then wrapped in a thin film of polyethylene which is sealed to the glass by its edges with resin. In an atmosphere or solution containing carbon dioxide this diffuses through the polyethlyene film into the calcium hydroxide membrane, the polyethylene acting as an effective barrier to the passage of water and electrolytes. The carbon dioxide reacts in the membrane

to form calcium bicarbonate which has a higher solubility product than calcium hydroxide. This increases the density of free ions within the membrane lowering its electrical resistance. These electrodes can be made as small as 0.5 mm. in diameter and could be used in a wide range of physiological experiments where continuous measurement of pCO_2 is required. Unlike other types of carbon dioxide sensor the indicating equipment required is very simple; a 50 cycle AC bridge being used to measure the change in resistance.

A PUNCHED-CARD-PROGRAMMED CHROMATOGRAPHY APPARATUS

A fundamental problem in the design of instruments for automatic analysis is to make provision for future changes in technique. Current design is inflexible in this respect, instrument programming being achieved by motor driven cams and switches. To overcome this inflexibility a prototype punched-card-programmed chromatography apparatus has been designed and built. The basis of the instrument is a reader which translates the pattern of holes on a standard 80 column punched card into electrical impulses which operate a system of uniselectors and relays controlling various chromatographic functions such as electrolytic buffer pumps, column temperature programming and facilities for continuous column effluent analysis.

A PORTABLE ELECTROLYTIC PUMP

A portable electrolytic pump has been developed during the year, whose principle of operation is similar to that employed in the electrolytic gradient generator described in last year's Report. The pump is designed to provide a steady delivery of between 5 and 25 ml of fluid intravenously per day in human subjects and unrestrained animals.

The operation of the pump is based on the electrolysis of water, using a non-decomposing electrolyte such as phosphate, as the current carrier. The current is delivered by platinum electrodes and is provided from a mercury battery, which assures a constant rate of supply. The fluid for delivery is contained in polythene tubing wound around the cylindrical unit containing the electrolytic chamber, the battery and the rheostat. The last permits adjustment in current supplied and so in the rate of fluid delivery. The fluid is driven directly by a column of gas, mainly oxygen for the hydrogen is able to diffuse through the walls of the electrolytic chamber which are made of perspex. Dimensions of the unit are 12.5 cm. long, 2.5 cm. in diameter. Retrograde entry of the blood whose clotting might obstruct the catheter is prevented by a specially constructed polythene valve. The practical applications of this unit, both clinical and experimental, should be very wide and they are to be explored during the year.

TECHNIQUES

CHROMATOGRAPHY OF PROTEINS AND PEPTIDES ON COLUMNS OF SYNTHETIC POLYELECTROLYTE GEL

The currently available ion-exchange materials for chromatography of large molecules are based on modified natural polymers of dextran and cellulose. As these are of uncertain chemical composition and are known to exhibit irreversible adsorption effects, particularly with trace proteins, it was decided to investigate

the properties of polyelectrolyte gel synthesised from monomers of known purity. A series of gels were prepared by co-polymerising diethylaminoethyl-methacrylate, acrylamide and N.N.methylene-bis-acrylamide in various proportions. The gels were dispersed for chromatography by grinding in the dry state and sieving the powder to obtain a uniform particle size. Excellent chromatographic separations were obtained of serum proteins and porcine pituitary peptides on columns fitted with the gel.

MICROTECHNIQUES FOR SERUM PROTEIN STUDIES

In some circumstances the total amount of serum available for investigation is small and further samples from the same source may be difficult or impossible to obtain. This applies particularly to samples collected in field expeditions or from small animals. In order to carry out multiple tests on such samples great economy of material has to be exercised and microtechniques are desirable.

Several such microtechniques have been developed and these have resulted in reduction in the time taken over the tests and the need for storage space.

Microelectrophoresis using narrow strips of cellulose acetate membrane as the supporting medium has permitted satisfactory resolution of proteins in amounts of sera of the order 0.1 to 1.0 microlitre. The electrophoresis is performed with high voltage gradients (typically 60 V per cm.) and separation occurs in 5 to 10 minutes. Using mixtures of peptides instead of sera, separation can be obtained in 2 to 3 minutes. Staining is being carried out by the ninhydrin procedure in the usual way. This assembly can also be used for amino acid separation. The advantages of high voltage electrophoresis thus have been combined with those of economy of material.

Microimmunodiffusion

Two methods of microdiffusion have been adopted, one being a micromethod of double diffusion in agar gels on microscopic slides. The use of glass and perspex templates during the setting of the gel has obviated the need for special cutting of wells and their subsequent sealing. The method permits the use of amounts of sera of the order of 10-20 microlitres. In the other, smaller amounts of serum of the order of 0.1 microlitre are used with cellulose acetate strips as the supporting medium. Results obtained by the two techniques are comparable but the advantage of agar is that it permits the inspection of different patterns during their development.

Microimmunoelectrophoresis

By a combination of the preceding methods using cellulose acetate membranes as the medium for electrophoresis followed by immunodiffusion, it has been possible to obtain immunoelectrophoretic resolution in amounts of sera of the order of 0.1 to 1.0 microlitre. High resolution of the patterns is possible by staining and then clearing the membrane with paraffin, glycerol or "microil". Mounting on glass allows the direct preparation of slides for projection.

INVESTIGATIONS

SERUM PROTEINS IN THE INDIGENES OF NEW GUINEA

Quantitative serum protein data, serum iron levels and haptoglobin and transferrin types have been determined in 3,000 serum specimens collected in

New Guinea and New Britain by Drs. C. Kidson, J. Gorman[1] and D. C. Gajdusek[2]. The results have been listed on punched cards and statistical processing in the IBM 1620 computor[3] is in progress.

Effect of various factors on quantitative serum data

Although statistical processing is incomplete it has been possible to draw some general conclusions from the data in holoendemic malarial regions. There are positive correlations between serum iron level, haptoglobin type, haptoglobin level and parasite count. This is interesting in view of our previous suggestion that the significantly higher frequency of the haptoglobin type 1 gene in holoendemic malarial areas compared with malaria-free highland regions might be due to selective pressures favouring haptoglobin type 1 with its greater haemoglobin binding capacity and therefore favouring its genotypes with better iron conservation. No correlation between the frequencies of the C,D, and B_0 types of transferrin and any environmental factor in the blood parameters could be found, although there is considerable regional variation in their distribution. B_0 for example is missing from many of the areas which we tested although C and D were always present in varying frequencies.

As observed before there was a positive correlation between serum γ-globulin levels and the presence of malaria. In holoendemic malarial areas such as Maprik, γ-globulin levels built up rapidly during infancy, reaching a value of 1.50 gm per 100 ml by the age of 1 year. From then on a steady increase was observed with age and in adults over the age of 30 the γ-globulin level was not uncommonly in excess of 3 gm per 100 ml.

Genetic heterogeneity of the Melanesian peoples

Analysis of the distribution of the haptoglobin and transferrin genes has helped to strengthen the concept of the genetic heterogeneity of the Melanesian people put forward by Simmons et al. and by Kidson and Gorman. In the case of haptoglobin types, however, some selective pressures, presumably due in part to malaria, tend to maintain higher haptoglobin type 1 frequencies in holoendemic malarial areas.

The increase of γ-globulin level and the frequency of the Hp_1 gene in coastal regions over the highlands may not be due solely to the influence of malaria. Marked changes also occur with altitude in the incidence of other parasitic and virus diseases and these may well have a marked effect on the serum protein pattern.

HAEMOGLOBIN ABNORMALITIES IN NEW GUINEA AND NEW BRITAIN

A survey of abnormal haemoglobins in New Guinea and New Britain, which was commenced in 1958, has been completed. 1363 specimens have been examined, of which 333 were collected by Dr. D. C. Gajdusek in the highlands (Eastern-Fore, Keigana, Gadsup, Tairora, Gimi, Usurfa and Kukukuku linguistic groups; Western-Enga linguistic group) and 1030 by Drs. C. Kidson and J. Gorman in the Sepik River District, (Bukawa, Wampur and Mumeng linguistic groups) Markham Valley (Sause and Abelam linguistic groups), and the Gazelle

1 College of Physicians and Surgeons, Columbia University, New York, U.S.A.
2 National Institute of Neurological Diseases and Blindness, Bethesda, Maryland, U.S.A.
3 At the Melbourne Service Bureau of I.B.M. (Australia).

Peninsula of New Britain, (Tolai, Sulka, Baining and Taulil linguistic groups). Haemoglobin electrophoresis has been carried out in starch gels. The frequency of the thalassaemia trait, as estimated from the proportion of samples with haemoglobin A_2 levels exceeding 4%, was found to range from 4 to 10% in the Sepik and Gazelle Peninsula groups. One of the Markham groups, the Bukawa, showed an incidence of 8% and the Wampur and Mumeng had an incidence of 1 and 2% respectively. In the highlands the trait appeared to be absent in the East confirming our observations made in 1956, and of low incidence (2%) in the West. In this regard, thalassaemia tends to follow the pattern observed by Carcassi et al., in Sardinia—a relatively high frequency in proximity to the coast in endemic malarious regions and a fall in incidence associated with increasing altitude and decreasing malarial frequency.

It is tempting to suggest that thalassaemia heterozygotes possess a survival advantage conferred by increased resistance to malaria. It must be reiterated however that changes occur with altitude in diet and nutrition and viral and parasitic disease incidence which are as marked as changes in malaria incidence, and that these may have some influence on the distribution of the thalassaemia genes.

In contrast to the widespread incidence of thalassaemia, abnormal haemoglobins were found to be rare, only four examples of Lepore, one of H and one of E being found in samples from the coastal areas. The Lepore specimens have been found to be identical by "finger printing" to the Lepore of Jonxis et al., the H to a sample of haemoglobin H isolated from a Greek woman in Melbourne and the E to the haemoglobin E of Itano. Since Lepore and H are inseparable from thalassaemia it has been suggested on the basis of the theories advanced by Ingram and Stretton that, as elsewhere, "α" and "β" thalassaemia exist side by side in New Guinea.

SERUM PROTEINS IN KURU

Further studies on the elevated globulins found in some cases of late kuru have suggested that they are identical to "normal" γ-globulin.

COMPONENTS OF HUMAN SERUM PROTEINS

Examination of human sera by immunoelectrophoresis using conventionally prepared antisera to human serum proteins has revealed the existence of some 24 components, four of which show genetically-dependent individual variation. However, under conditions of immunisation with massive amounts of sera, immunological paralysis can be induced to the major protein fractions of the serum and the response to the minor fractions enhanced. Antisera of this type were prepared in sheep and preliminary experiments suggest that using these antisera individual differences among the human serum protein components can be observed. Work is being carried out at present to characterise further these differences.

Using such antisera it has also been possible to observe the appearance of a previously inapparent serum protein component following an incompatible blood transfusion. Attempts to identify this component and to determine whether it develops in other patients under similar circumstances are being made.

FLUORESCENT ANTIBODY

Non-Specific Staining Tissue Component

The development of a chromatographic method for the separation of the non-specifically staining component from fluorescein-conjugated γ-globulin has enabled the component present in normal mesenchymal tissues which reacts with fluorescein conjugated γ-globulin to be isolated.

The highly purified, non-specifically staining fluorescein-γ-globulin fraction is conjugated with azobenzyl cellulose powder. The non-specifically staining component is removed from a physiological saline homogenate of rat liver by passage through a column of the conjugated cellulose. The non-specifically staining component is dissociated from the column material in 5M glycine solution. The isolated component is a basic protein with a molecular weight, by gel filtration, in the 60,000-150,000 range.

This component is of interest because it has been shown by Louis and Hughes that it is either absent, diminished or altered in malignant tissues of mesenchymal origin as these tissues do not stain with fluorescein conjugated globulin. The possible identity of this component with the hepato-carcinogenic azo-dye binding component which is present in normal hepatic tissue, but absent in the dye-induced tumour, is being investigated in collaboration with Dr. P. E. Hughes of the Department of Pathology, University of Melbourne.

Heterogeneity of conjugation of γ-globulin by fluorescein isocyanate

Earlier electrophoretic studies indicated considerable heterogeneity in the conjugation of γ-globulin with fluorescein isocyanate which was considered a reflection of heterogeneity of the general chemical reactivity of γ-globulin. Further investigations carried out on chromatographically isolated fractions of γ-globulin have supported this supposition. Differences in ability to combine with fluorescent isothiocyanate, in lysine content, in "finger printing" of tryptic digests and in N terminal aminoacids, have been found between the different fractions. The implications of these findings are being considered in relation to the hybridisation hypothesis of the origin of the abnormal serum proteins found in neoplastic disease of the lymphoid tissue advanced by us (C.C.C.).

CELLULAR ENZYMES**

Chev Kidson, R. G. Wyllie, C. C. Curtain and A. Baumgarten

MOLECULAR GENETICS

The title "cellular enzymes" has been retained for this section to indicate continuity of these projects with those of previous years. The trend of some of the development of the projects however is such that a general term such as "molecular genetics" might be more suitable. The aim of the investigations is to define in molecular terms the regulatory mechanisms which govern specific cellular metabolic functions with special reference to their aberrations in neoplasia. The genetic control of enzyme protein synthesis and activity has been of special interest.

GENETIC CONTROL OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE[1]

The availability of a number of different human mutants showing deficiency in glucose-6-phosphate dehydrogenase (G6PD) activity in their erythrocytes has rendered possible a partial resolution of the molecular organisation involved in genetic control of this enzyme in man. Comparative analysis of mutants from Melanesian, Negro and European population groups has been achieved by examination of the action of an activator and an inhibitor of G6PD in erythrocyte stroma. In this analysis stromal preparations from normal and mutant erythrocytes were incubated in all possible homologous and heterologous combinations with haemolysate supernatant fractions. These cross-reaction experiments showed that in Melanesians and Europeans mutations may involve the activator or the structure of the G6PD molecule. In Negroes they may rarely involve the inhibitor. In most instances they appear to involve rate control gene(s).

From this comparative analysis a model has been constructed for the genetic control of G6PD in man, involving modification and extension of the Jacob-Monod hypothesis for the genetic control of protein synthesis in bacteria. Both direct (rate control of synthesis of the structural enzyme protein) and indirect (activation, inhibition) mechanisms are thought to be operative. It is considered that at the DNA level these functions may be located in a single *operon*. Rate control of production of the G6PD structural protein appears to be of a different kind to that exerted by bacterial *regulator-operator* systems but complete elucidation has not yet been possible by means of known mutants.

ENZYME INDUCTION AND REPRESSION IN LEUKAEMOGENESIS

Detailed study of leucocyte catalase in normal and leukaemic human cells revealed the presence in the cells of acute myeloid leukaemia of two enzymes with catalatic activity, catalase *a* and *b*. These two enzymes showed properties indicative of different reactive sites; in one instance only starch gel electrophoresis revealed a significant difference in electrophoretic mobility, indicative of a structural mutation in catalase *b* protein. Subsequently it was found that a second catalase occurred occasionally in both normal granulocytes and the cells of chronic myeloid leukaemia; this second enzyme had the same pH optimum as catalase *b* but all other properties identical with catalase *a*. The appearance of a second catalase in granulocytes of the same normal individual was found to be intermittent; a second enzyme does not occur in lymphocytes or erythrocytes of these individuals nor in individuals with acute myeloid leukaemia.

Interpretation of these findings is not yet complete. However, the data in hand suggest catalase *induction* in granulocyte-series leucocytes, probably due to *derepression*. Normally repression of synthesis of the second enzyme can readily occur; but an accompaniment of acute myeloid leukaemogenesis appears to be *permanent derepression*, with subsequent mutation affecting the reactive site and occasionally mutation affecting other points in the enzyme protein molecule.

These findings form an important piece of evidence bearing on the nature of myeloid leukaemogenesis in man. A leukaemogenic agent—carcinogen or virus—might substitute for the normal *inducer* or radiation might mutate the *regulator* gene resulting in loss of its product *repressor*, leading to *permanent derepression*, and subsequent mutation of the structural locus for the enzyme protein.

1 In collaboration with Dr. J. Gorman, Columbia University, New York, U.S.A.

Electrophoresis of Leucocyte Proteins

Methods have been developed to bring into solution granulocyte and lymphocyte proteins by the use of ultrasonic vibration. Preliminary studies of the behaviour of these soluble proteins on starch gel electrophoresis indicate the existence of qualitative similarity in major bands between the two cell types, with quantitative differences in certain bands.

Specific Protein Aberration in Melanesians

A specific protein aberration has been observed in the Tolai linguistic group in New Britain, in the form of an abnormally high incidence of cold agglutinins (> 10%), first noted macroscopically by autoagglutination of blood samples on storage under refrigeration. In neighbouring linguistic groups the incidence is less than 1% although environmental conditions, parasitism and viral infections are almost identical. Tentatively, this aberration is considered to be genetic in origin and investigations to establish the nature of the agglutinins are being carried out.

Peptides regulating Lipid Metabolism

Further studies of plasma factors involved in regulating lipid metabolism in man have resulted in partial characterisation of at least three peptides influencing the incorporation of acetate-1-C^{14} into lipids by leucocytes. Partial purification has been achieved on both Sephadex and polyacrylamide gel columns. An inhibitory peptide has a molecular size in the range 10,000-12,000 and two stimulatory peptides are of molecular size in the ranges 6,000-8,000 and 1,000-3,000 respectively. High voltage electrophoresis of the peptide fractions has indicated that all three fractions are heterogeneous, containing three to six peptides per fraction, so that further purification will be necessary for individual identification. Comparative data indicate that these plasma peptides are different to pituitary peptides with similar activities. Leukaemic leucocytes appear to respond normally to these regulators.

Metabolism of Fatty Acids by Leucocytes and Erythrocytes

Further studies of lipid metabolism by human leucocytes and erythrocytes have shown that, as is the case with synthesis of fatty acids, uptake and oxidation of fatty acids are very much more active in leucocytes than erythrocytes. The cells of chronic lymphoid leukaemia have rates of uptake and oxidation approaching normal values, while the cells of chronic myeloid leukaemia have very low rates, especially of oxidation.

From these and previously reported studies on lipid synthesis and response to phagocytosis the data have been correlated with other known metabolic variations in leukaemic cells to yield a more comprehensive understanding of altered leucocyte metabolism in leukaemic states, in an attempt to find clues to the process of leukaemogenesis. The marked changes in lipid synthesis and oxidation in myeloid leukaemic cells are dependent in part on diminished glycolysis and carbohydrate oxidation, the lowered glycolytic rate being due in turn to specific deficiency in hexokinase. Very many other enzyme aberrations are now established for myeloid leukaemia, suggesting that a great number of mutations are involved in myeloid leukaemogenesis. The cells of lymphoid leukaemia on the other hand show very little variation from normal lymphocytes with respect to lipid and carbohydrate metabolism and there are few, if any, well established aberrations in specific enzymes, suggesting that rather different processes are involved in lymphoid leukaemogenesis.

LEUCOCYTE NEUTROPHIL ALKALINE PHOSPHATASE

Observation of specific cellular components in humans and animals have continued, comparison being made of the findings in normal and leukaemic tissue. Histochemical methods have been used and a number of modifications to these procedures made.

Mouse Leukaemia[1]

Accurate localisation of enzymes and other unstable cell compounds by histochemical procedures demands rapid preparation of tissue at low temperatures. It was found that satisfactory results could be obtained by freezing specimens with CO_2 gas immediately on removal of the tissue. The frozen material could then be sectioned on a freezing microtome and the section placed on a slide directly from the blade and dried quickly with a draught of air at 50°C. This method was economical in time and material and enabled good sections of less than 10 μ in thickness to be cut. The sections were fixed sufficiently to withstand incubation for 2 hours in substrate solutions without distortion or significant loss of enzyme activity. Best results were obtained using mildly hypertonic substrate solutions.

After incubation slides were carefully transferred from the substrate to a suitable fixing solution, after which staining for reaction products and counter staining could be completed in the usual way. Finished sections were comparable in quality with sections prepared from wax embedded material.

Mice of the inbred strain AKR develop an 85-95% incidence of spontaneous lymphoid leukaemia, the first deaths from the disease occurring after the age of six months. Lymphoid cells accumulate first in the thymus, producing a thymic lymphoma, and subsequently they spread to other lymphoid and nonlymphoid organs.

In human chronic myeloid leukaemia the leukaemic cells are notable for their low content of alkaline phosphatase. It has been reported that alkaline phosphatase was low in normal AKR thymuses and the enzyme activity was not localised in the lymphoid cells. Alkaline phosphatase levels were slightly elevated in preleukaemic AKR thymuses. In AKR thymic lymphomata alkaline phosphatase levels were usually elevated, but the levels in individual mice were highly variable. When present alkaline phosphatase activity was localised in the lymphoma cells. Liver alkaline phosphatase became elevated in leukaemic mice only after infiltration of the liver by lymphoma cells.

Other cellular components being investigated include acid phosphatase, adenosine diphosphatase and triphosphatase, glucose-6-phosphatase, esterases and nucleic acids.

Opportunities for examining blocks of human leukaemic tissue occurred too infrequently to be suitable for analysis by the method described above. On the other hand blood, while readily available, has in the past been unsuitable for histochemical procedures because fixing methods inactivate many enzyme systems.

Recently developed reagents of the vinyl-chlorosilane type have been examined and found suitable for histochemical use. The silicon groupings of the chain polymer bind to the glass surface, in the presence of moisture, and leave reactive vinyl groups unengaged. On smearing the surface with biological

1 This work was carried out in collaboration with Dr. D. Metcalf, Cancer Research Laboratory, Walter and Eliza Hall Institute.

fluids the reactive groups on cell surfaces combine with the vinyl group. This bond was sufficiently strong to enable incubation in mildly hypertonic substrate solutions without loss or distortion of the smeared material.

This method is being used to study enzymes in normal and leukaemic blood.

PANCREATITIS

A. D. McCutcheon and D. Race

Work on the aetiology and treatment of acute haemorrhagic pancreatitis has been continued using the blind duodenal loop technique in dogs. A new anti-proteolytic substance, Trasylol, has been available for study. This is a trypsin and kallikrein inhibitor which can be injected intravenously or intraperitoneally with safety. As there is considerable evidence that active proteolytic enzymes are concerned in the pathogenesis of acute pancreatitis, Trasylol might be of value in the treatment of pancreatitis if given early enough. Its effectiveness in preventing experimental pancreatitis in dogs was tested in the following way.

Blind duodenal loops were made in 15 dogs and continuity of the gastro-intestinal tract restored by gastro-jejunostomy. Two groups of dogs were compared.

The control group (7 dogs) was not given Trasylol. All 7 dogs developed haemorrhagic pancreatitis which was severe in four and mild to moderate in three. The other 8 dogs were given a continuous intravenous infusion of Trasylol in saline for 24 hours. None of these dogs had severe pancreatitis and their clinical condition was superior to that of dogs in the control group. In 2 dogs the blind loop had ruptured and these showed mild haemorrhagic inflammation confined to the surface of the pancreas near the perforation. Several dogs which did not develop pancreatitis nevertheless showed extensive neutrophil infiltration of the interstitial tissue of the pancreas and one pancreas contained a few small abscesses.

These results were interpreted as showing that Trasylol is capable of preventing the development of this experimental form of haemorrhagic pancreatitis in dogs; and that there is therefore a theoretical basis for its use in man. Further, that infection in the pancreatic interstitial tissue produces a picture different to that seen in haemorrhagic pancreatitis.

RENAL DAMAGE AND PHENACETIN

A. D. McCutcheon

The belief that phenacetin may cause renal damage is based on numerous reports from overseas which have stressed the association of a particular type of renal disease with a history of excess phenacetin ingestion. However, chronic pyelonephritis commonly complicates the later stages of this disease so it is difficult to evalulate the importance of the various aetiological factors.

Six patients with impaired renal function and a history of prolonged intake of phenacetin were studied. In four patients infection was not a feature. Two appeared to be classical examples of "phenacetin nephropathy" ending in

papillary necrosis and uraemia. The patients appeared to belong to a clinical and pathological syndrome characterised by:

(1) An interstitial (medullary) nephritis producing early effects on tubular function with an impaired ability to concentrate the urine.

(2) The insidious, late development of uraemia.

(3) The common occurrence of papillary necrosis.

(4) Chronic pyelonephritis complicating the later stages.

(5) Absent or mild hypertension.

(6) In Australia, the commonly associated conditions sulphaemoglobinaemia, anaemia and gastro-intestinal ulceration and haemorrhage.

The last are presumed to be due to the associated excess aspirin ingestion.

It is suggested that the renal lesion is produced by a chemical aseptic inflammation which is maximal towards the tips of the renal papillae because of the "counter current system" whereby urine (and any toxic constituents) reaches its maximum concentration in this region.

PAPERS PUBLISHED DURING 1962

BAUMGARTEN, A.—"The Clinical Effects of a New Anti-Anginal Agent, Prenylamine (Segontin)". *Med. J. Aust.* Vol. 1 (1962) p. 181.

BAUMGARTEN, A.—"Clinical Trial of a New Hypotensive Drug, Methyldopa (Aldomet)". *Med. J. Aust.* Vol. 2 (1962) p. 179.

CURTAIN, C. C.—"The Separation of Haemoglobins Lepore and H from A and A_2 in Starch or Acrylamide Gels with tris-EDTA". *J. Clin. Path.* Vol. 15 (1962) p. 288.

CURTAIN, C. C.—"A Photoelectrically Programmed Electrolytic Gradient Generator". *J. Chromatography* Vol 7 (1962) p. 24.

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FANTL, P., R. J. SAWERS and H. A. WARD—"Detection of a Self-Inflicted Haemorrhagic Disorder". *Med. J. Aust.* Vol. 1 (1962) p. 246.

FANTL, P. and H. A. WARD—"The Phosphorus Content of Fibrinogen and Fibrin". *Biochem. & Biophys. Acta* Vol. 64 (1962) p. 570.

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HILL, A. V. L., I. G. LYALL and A. J. BARNETT—"Sympathectomy for Occlusive Arterial Disease of the Lower Limb". *Med. J. Aust.* Vol. 2 (1962) p. 901.

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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* Vol. 29 (1962) p. 364.

KIDSON, C. and D. C. GAJDUSEK—"Glucose-6-Phosphate Dehydrogenase Deficency in Micronesian Peoples". *Aust. J. Sci.* Vol. 25 (1962) p. 61.

KIDSON, C. and J. G. GORMAN—"Mechanisms underlying Glucose-6-Phosphate Dehydrogenase Deficiency: Heterogeneity of Response to Stromal Activation in Erythrocytes". *Biochem. Biophys. Res. Com.* Vol. 7 (1962) p. 268.

KIDSON, C. and J. G. GORMAN—"A Challenge to the Concept of Selection by Malaria in Glucose-6-Phosphate Dehydrogenase Deficiency". *Nature* Vol. 196 (1962) p. 49.

KIDSON, C. and J. G. GORMAN—"Contribution of Red Cell Enzyme Deficiency Trait to an Understanding of Genetic Relationships between Melanesian and Other Populations". *Amer. J. Phys. Anthrop.* Vol. 20 (1962) p. 357.

LOWE, T. E.—"A Changing Scene", in History of Pathology School, *M.U.P.* 1962.

McCUTCHEON, A. D.—"Aetiological Factors in Pancreatitis". *Lancet* Vol. 1 (1962) p. 710.

McCUTCHEON, A. D.—"Renal Damage and Phenacetin". *Med. J. Aust.* Vol. 2 (1962) p. 543.

McCUTCHEON, A. D. and D. RACE—"Experimental Pancreatitis" *Ann. Surg.* Vol. 155 (1962) p. 523.

METCALF, D., N. SPARROW and R. G. WYLLIE—"Alkaline Phosphatase Activity in Mouse Lymphoma Tissue". *Aust. J. Exp. Biol. Med. Sci.* Vol. 40 (1962) p. 215.

MORRIS, K. N., G. R. STIRLING, C. J. O. BROWN and A. J. BARNETT—"A Review of Arterial Replacement". *Aust. & New Zealand J. Surg.* Vol. 32 (1962) p. 1.

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NAYLER, W. G. and P. EMERY—"The Effect of Strontium on Cardiac Contractility and Membrane Resting Potentials". *Amer. J. Physiol.* Vol. 203 (1962) p. 844.

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SIMPSON, F. O. and S. J. OERTELIS—"The Fine Structure of Sheep Myocardial Cells". *J. Cell. Biol.* Vol. 12 (1962) p. 91.

STIRLING, G. R.—"Reflections on Surgical Aspects of Pulmonary Hypertension". *Roy. Melb. Hosp. Clin. Rep.* Vol. 28 (1961) p. 32.

WYLLIE, R. G.—"Neutrophil Alkaline Phosphatase". *Aust. Ann. Med.* Vol. II (1962) p. 118.

PAPERS ACCEPTED FOR PUBLICATION

BARNETT, A. J., A. BAUMGARTEN and M. BRANDSTATER—"Triamterene—A New Diuretic". *Aust. Ann. Med.*

BARNETT, A. J. and Valerie CARSON—"Reduction of Plasma Cholesterol Levels in Atherosclerosis by Diet and Drug Treatment". *Aust. Ann. Med.*

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

CURTAIN, C. C.—"A Multicolumn Zone Electrophoresis Apparatus". *Elect. & Biol. Eng.*

FANTL, P. and E. C. OSBORN—"A Comparison of Plasma and Serum Factor VII Activity and the Formation of Tissue Thromboplastin". *Thrombosis et Diathesis Haemorrhagica.*

FANTL, P. and H. A. WARD—"Phosphorus Content of Fibrinogen and Fibrin". *Biochem. Biophys. Acta.*

NAYLER, W. G.—"A Direct Effect of Reserpine on Ventricular Contractility". *J. Pharm. & Exp. Therap.*

NAYLER, W. G.—"The Effect of Caffeine on Cardiac Contractility and Calcium Flux". *Amer. J. Physiol.*

NAYLER, W. G.—"The Effect of Ryanodine on Cardiac Muscle". *Amer. J. Physiol.*

NAYLER, W. G.—"The Significance of Calcium Ions in Cardiac Excitation and Contraction". *Amer. Heart J.*

RACE, D., G. R. STIRLING and Pauline EMERY—"Electrical Stimulation of the Heart". *Ann. Surg.*

WARD, H. A. and P. FANTL—"Transfer of Hydrophilic Cations from an Aqueous to a Lipophilic Phase by Phosphatidic Acids". *Arch. Biochem & Biophys.*

WYLLIE, R. G.—"Estimation and Evaluation of Neutrophil Alkaline Phosphatase Chemotherapy with Antibiotics and Allied Drugs". *N.H.M.R.C. Special Report Series.*

WYLLIE, R. G. and H. B. KAY—"Relapsing Sub-acute Bacterial Endocarditis". *Alfred Hospital Clinical Reports.*

PAPERS SUBMITTED FOR PUBLICATION

BAUMGARTEN, A.—"A Micromethod for Haptoglobin Typing using Acrylamide Gels". *Nature.*

CURTAIN, C. C. and Winifred NAYLER—"The Isolation from Human Plasma of a Peptide having a Positive Inotopic Activity on the Isolated Toad Heart". *Bioch. J.*

FANTL, P.—"Experimental Fibrinolysis". *Aust. Ann. Med.*

FANTL, P.—"Thiol Groups of Blood Platelets in Relation to Clot Retraction". *Nature.*

KIDSON, C.—"Metabolic Response to Phagocytosis in Human Leucocytes with Respect to Lipid Synthesis". *Aust. J. exp. Biol. & Med.*

KIDSON, C.—"Failure of Metabolic Response to Phagocytosis in Myeloid Leukaemia". *Aust. J. exp. Biol. & Med.*

KIDSON, C.—"Uptake and Oxidation of Long Chain Fatty Acids by Normal Human Leukocytes and Erythrocytes". *J. Lipid Res.*

KIDSON, C.—"Metabolism of Fatty Acids by Human Leukaemic Cells". *Brit. J. Cancer.*

McCUTCHEON, A. D. and D. RACE—"Experimental Pancreatitis, The Use of a New Anti-proteolytic Substance, 'Trasylol'". *Ann. Surg.*

LECTURES DELIVERED DURING 1962

"Renal Hypertension"—*Cardiac Society of Australia and New Zealand, Melbourne.*	A. J. BARNETT
"Microtechniques in Biology"—*Victorian Society of Pathology & Experimental Medicine.*	A. BAUMGARTEN
"Construction and Usage of a Cardiac Pulse Simulator"—*Victorian Society of Pathology & Experimental Medicine.*	E. COOPER
"Cinematograph Film of Normal and Abnormal Mitral Valves in Pulse Simulator"—*Cardiac Society of Australia and New Zealand (Victorian Group)*	E. COOPER
"The Chromatography of Proteins on Diethylaminoethyl-Cellulose Paper"—*Australian Biochemical Society, Melbourne.*	C. C. CURTAIN
"The Isolation from Human Plasma of a Peptide with a Positive Inotropic Actvity on the Isolated Toad Heart"—*Australian Biochemical Society, Sydney.*	C. C. CURTAIN
"The Fundamentals of Electrochemistry" "Reversible Electrodes" "Biological Electrochemistry" —*Royal Melbourne Institute of Technology.*	C. C. CURTAIN
"The Application of Automatic Methods of Analysis and Computation to some Problems in Biology and Medicine" Demonstration: "A Punched Card-programmed Automatic Chromatography Apparatus" —*Second Annual Meeting of Physics in Biology and Medicine.*	C. C. CURTAIN
"The Characterisation of the γ Globulins"—*Victorian Society of Pathology and Experimental Medicine.*	C. C. CURTAIN
"Technological Sophistication and Medical Research"—*Williamstown Apex Club.*	C. C. CURTAIN
"Blood Coagulation"—*University of Melbourne.*	P. FANTL
"Experimental Fibrinolysis"—*Haematological Society, Melbourne.*	P. FANTL
"Function of Factor VII and Factor X in the Formation of Brain Thromboplastin"—*International Congress for the Nomenclature of Blood Clotting Factors, Sweden, July, 1962.*	P. FANTL
"Clinical Significance of Fibrinolysis"—*Alfred Hospital Clinical Society.*	P. FANTL
"International Aspects of Bleeding Disorders"—*Haemophilia Society, Melbourne.*	P. FANTL
"Haematological Problems of New Guinea and South-East Asia"—*Australian Society of Haematology (Victorian Group).*	C. KIDSON
"Aberrations of Leucocyte Metabolism and the Nature of Leukaemia"—*Victorian Society of Pathology and Experimental Medicine.*	C. KIDSON
"Evidence for Complex Mutation Patterns in Leukaemogenesis"—*Australian Society of Haematology, Melbourne.*	C. KIDSON

"Complex Genetic Control of a Single Enzyme in Human Erythrocytes"—*Australian Genetics Society.*	C. KIDSON
"Two Enzymes with Catalase Activity in Human Leucocytes"—*Australian Biochemical Society, Sydney.*	C. KIDSON
"Lipid Metabolism in Relation to Physiological Functions of Leucocytes"—*Australian Biochemical Society, Sydney.*	C. KIDSON
"Control of Erythrocyte Glucose-6-Phosphate Dehydrogenase: Implications in Mammalian Chemical Genetics"—*Australian Biochemical Society, Sydney.*	C. KIDSON
"Complexity of Selection Pressures in Erythrocyte G6PD Deficiency: New Guinea as a Unique Model"—*International Society of Hematology, Mexico City.*	C. KIDSON
"Control of Body Fluid Volume"—*Physiology Society of Philadelphia.*	T. E. LOWE
"Aetiological Factors in Pancreatitis"—*Gastroenterological Society of Australia, Melbourne.*	A. D. McCUTCHEON
"Aetiological Factors in Pancreatitis and the Implications of Treatment"—*The Royal Australasian College of Surgeons, Melbourne.*	A. D. McCUTCHEON
"The Specificity of Calcium Ions in Cardiac Contractility and Excitability"—*Australian Physiological Society, Melbourne.*	W. G. NAYLER
"Arterial Embolism"—*Royal Australasian College of Surgeons, Melbourne.*	G. R. STIRLING
"Phosphatidic Acids in Blood Coagulation and Cation Transfer"—*Australian Biochemical Society, Melbourne.*	H. A. WARD
"A Study of the Activity of Phospholipids in Blood Coagulation"—*Association of Hospital Scientists, Melbourne.*	H. A. WARD
"Neutrophil Alkaline Phosphatase in the Diagnosis of Disease"—*Royal Children's Hospital, Melbourne.*	R. G. WYLLIE
"Fixation for Histochemical Procedures"—*Tissue Culture Society, Melbourne.*	R. G. WYLLIE

DEMONSTRATIONS ARRANGED FOR UNIVERSITY OF MELBOURNE MEDICAL SCHOOL CENTENARY—16th AUGUST, 1962

Advances in the Treatment of Severe Arterial Hypertension	A. J. BARNETT and M. BRANDSTATER
Treatment of Obliterative Arterial Disease of the Lower Limb	A. J. BARNETT, K. N. MORRIS and G. STIRLING
Disturbances in Blood Coagulation	P. FANTL
Cellular Cardiac Physiology	W. G. NAYLER, P. EMERY and J. WRIGHT
Cardiac Pulse Simulator	E. COOPER
Neutrophil Alkaline Phosphatase	R. G. WYLLIE
Biochemical Genetics and Blood Disease	C. C. CURTAIN, C. KIDSON and A. BAUMGARTEN

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

Revenue Account for the Year Ended 31st December, 1962.

EXPENDITURE.				INCOME.						
Advertising	£21	15	1	Donations—						
Drugs, Chemicals, Provisions, etc.	2,851	0	10	Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions	£31,200	0	0			
Fuel and Lighting	517	0	9	Other Donations as per attached Schedule	921	3	6			
Instruments and Glassware	3,201	11	7					£32,121	3	6
Insurance	1,210	3	6	Grants in Aid of Research—						
Library Maintenance	1,770	12	5	National Health & Medical Research Council	3,309	4	0			
Postage, Telephone, Printing and Stationery	1,559	12	4	Anti-Cancer Council of Victoria	9,936	0	3			
Repairs and Renewals	1,581	10	7	Life Insurance Medical Research Fund of Australia and New Zealand	2,925	0	0			
Salaries and Wages	38,230	12	1	National Heart Foundation of Australia	5,921	0	0			
Travelling Expenses	1,042	16	10					22,091	4	3
Provision for Overseas Travel Guest Speaker	1,200	0	0	Interest From Investments—						
Provision for Equipment	1,000	0	0	Held by the Trustees of the Estate of the late Thomas Baker	849	0	0			
Sundries	1,027	8	11	Endowment Fund	1,184	0	11			
Cost of Animal House	1,000	0	0					2,033	0	11
Surplus for Year	351	0	9	Interest from Commercial Bank of Australia Ltd.				319	17	0
	£56,565	5	8					£56,565	5	8

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

Balance Sheet at 31st December, 1962.

LIABILITIES.				ASSETS.					
Current Liabilities—				**Current Assets—**					
Sundry Creditors	£5,302	4	8	Cash on Hand	£10	0	0		
Endowment Fund	24,029	6	10	Cash at Bank	5,411	17	0		
Capital Grants and Gifts	4,021	12	11	Prepayments	871	6	3		
Provision for Travel Expenses	1,200	0	0	Sundry Debtors	368	1	1		
Accumulated Revenue	2,524	7	9					£6,661	4 4
				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				4,021	12 11
				Fixed Assets—					
				Furniture and Fixtures				2,365	7 11
	£37,077	12	0					£37,077	12 0

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

In our opinion the above Balance Sheet is properly drawn up to show a true and fair view of the state of the Institute's affairs at 31st December, 1962, according to the best of our information and the explanations given to us and as shown by the books of the Institute.

FLACK & FLACK,
Chartered Accountants,
Honorary Auditors.

Melbourne,
18th March, 1963.

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, 1962.

CAPITAL GRANTS AND GIFTS.

Balance at 31st December, 1961		£1,791	2 3
Add			
Donations—			
Dr. T. E. Lowe and Associates		87	14 2
For a Refrigerated Centrifuge—			
Estate of H. A. Appel		1,700	0 0
For Major Items of Equipment—			
National Heart Foundation		1,613	17 2
Amount provided from 1962 Revenue Towards Cost of Equipment		1,000	0 0
Transfer from Accumulated Revenue for Portion of Cost of Equipment		780	7 9
		£6,973	1 4
Deduct			
Equipment	£1,954 8 5		
Zeiss Microscope	997 0 0		
		2,951	8 5
Balance at 31st December, 1962		£4,021	12 11

ACCUMULATED REVENUE.

Surplus at 31st December, 1961		£2,953	14 9
Less			
Transfer to Capital Grants and Gifts for Portion of Cost of Equipment		780	7 9
		£2,173	7 0
Add			
Surplus for Year		351	0 9
Surplus at 31st December, 1962		£2,524	7 9

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

OTHER DONATIONS RECEIVED DURING YEAR TO 31st DECEMBER, 1962.

Marion and E. H. Flack Trust	£350	0	0
Geo. F. Little Trust	148	6	6
Mr. and Mrs. Edgar Rouse	105	5	0
Kodak (Australasia) Pty. Ltd.	50	0	0
Mr. S. Meyer	50	0	0
Eagle Star Insurance Co. Ltd.	26	5	0
Mrs. Lenna Hewgill	25	0	0
In memory of: Mr. and Mrs. Richmond Baker; Capt. T. C. Richmond Baker, D.F.C., M.M. and Bar; Lady Campbell Brown; Frances Caroline; Rev. and Mrs. A. C. Webb			
Mr. J. C. Habersberger	10	10	0
Mr. and Mrs. Lawrence Simpson	10	10	0
Dr. and Mrs. John L. Rouse	10	0	0
Miss N. E. Cameron	2	2	0
In Memory of Thomas Jean Hargrave	15	10	0
" " " Sir William Angliss	5	0	0
" " " Emily Louisa Black	5	0	0
" " " Charles Paul Brown	5	0	0
" " " Alexina Bryson	5	0	0
" " " Harry English	5	0	0
" " " Edward S. Farrow	5	0	0
" " " John Foster	5	0	0
" " " Allen William Fox	5	0	0
" " " Alfred Griffiths	5	0	0
" " " Capt. Frank Hurley	5	0	0
" " " Sir William Johnston	5	0	0
" " " Alfred Landucci	5	0	0
" " " Monte Luke	5	0	0
" " " Elsie M. A. Luke	5	0	0
" " " Herbert Luhn	5	0	0
" " " Grace Marshall	5	0	0
" " " Clive Mendelsohn	5	0	0
" " " Mary Harriet Parmenter	5	0	0
" " " Douglas Frencham Vaughan	5	0	0
" " " Charles Roy Walker	5	0	0
" " " Stella Whitehead	5	0	0
" " " Ernest W. Williams	5	0	0
" " " Irene B. Martin	2	2	0
" " " Caroline Schneider	2	2	0
" " " Mervyn Leslie Wade	1	10	0
" " " Dr. William McDermott	1	1	0
" " " Flora Ann Martin	1	0	0
	£921	3	6

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

1962

STAFF

Honorary Physician:	EWEN DOWNIE, M.D., F.R.C.P., F.R.A.C.P.
Honorary Consulting Biochemist:	JOSEPH BORNSTEIN, D.Sc., M.D., F.R.A.C.P.
Physician in Charge, 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 Assistants:	A. P. DOREVITCH, M.D., M.R.A.C.P. K. J. CATT, M.D., M.R.A.C.P.
Registrar:	D. J. B. ST. JOHN, M.B., B.S.
Biochemists:	DORA WINIKOFF, M.Sc. JUNE SHEATH, M.Sc. AUSMA DULMANIS, B.Sc.
Technical Staff:	Mr. W. HUDSON Miss I. EKKEL Miss M. ZWART 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. (on leave)
Chiropodist:	MAIDA O'CONNOR, F.Ch.A.V., M.Ch.I.A.

RESEARCH FELLOWS

Wellcome Fellow:	PETER DAVOREN, Ph.D., B.Sc. (on leave)
"Dr. Henry Laurie":	GABRIELE MEDLEY, M.B., B.S.
Medical Research Fund:	N. KATHLEEN TAYLOR, M.B., B.S.

HONORARY RESEARCH FELLOWS

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

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

*Appointed Professor of Medicine, Monash University, September, 1962.

ANNUAL REPORT

During this year two events have occurred which have necessitated a reorganisation of the direction of the Unit. In April, Dr. Ewen Downie retired as Honorary Physician in Charge and in September Dr. Bryan Hudson was appointed to the Chair of Medicine in Monash University. His Department of Medicine is to be established at Prince Henry's Hospital and it is anticipated that the constructional work will be completed by the middle of 1968. Until that time Professor Hudson, who has been appointed Honorary Consultant Physician to Alfred Hospital, has continued to occupy his office in the Unit and to exercise supervision over the laboratory. Our sincere congratulations are extended to Professor Hudson, the second member of the staff of the Unit to attain Professorial rank in Monash University.

The Board of Management has given careful consideration to the future direction of the Unit and has sought the advice of the Honorary Medical Staff and of the Dean and members of the Faculty of Medicine of Monash University. It is anticipated that applications for the positions of Physician in Charge of the Unit and of Honorary Physician to the Diabetic and Metabolic Unit will be advertised and filled during the coming year.

During the year Professor Hudson attended an International Congress in Milan (by invitation) and delivered a paper on work done in the Unit. En route he visited Universities and Research Institutes in Malaya, India, Europe, England and the United States of America. Dr. Peter Davoren continued his work in Cleveland, U.S.A. under Professor Sutherland and later in the year visited Great Britain for three months prior to his return to Australia. He has been appointed Senior Lecturer in Monash University School of Biochemistry and our congratulations are extended to him on this appointment. Dr. Henry Burger has continued his work with Dr. John Nabarro at Middlesex Hospital, London, and later in the year he proceeded to Washington where he will work under the direction of Dr. Frederic C. Bartter before returning to Australia during 1964.

In May the Endocrine Society of Australia held its Annual Meeting in Melbourne. Members of the Staff attended and participated in presenting papers and in discussion. In May, 1963, the Society is sponsoring the Second Asia-Oceania Congress of Endocrinology. This will coincide with the Twenty-Fifth Annual Meeting of the Royal Australasian College of Physicians in Sydney and a plenary session has been arranged with the College during the Meeting. Members of the Unit have participated in various committees organising the Congress.

During the year the Unit has been visited by a number of colleagues both from Australia and abroad, including: Dr. Hortense Gandy, Department of Obstetrics and Gynecology, New York Hospital-Cornell Medical Center; Dr. G. I. M. Swyer, University College Hospital, London; Dr. Thomas F. Acheson, Medical Director, Schering A.S.; Dr. T. J. Danaraj, Faculty of Medicine, University of Malaya, Kuala Lumpur; Mr. A. C. Taylor, Organon Laboratories Limited, London; Professor Lindsay Davidson, University College of Rhodesia, Nyasaland; Professor Alexander P. Thompson, University College of Rhodesia, Nyasaland.

These visits have provided stimulating discussions on many subjects and valuable advice and suggestions have been received which are gratefully acknowledged.

Generous assistance has been provided, both financial and in kind, by individuals and organisations and this is gratefully acknowledged. The work of the Unit could never have expanded as it has without the support it has received to assist in the development of research projects. In addition assistance and advice has been given by members of the Honorary Medical Staff and by members of Departments of both Melbourne and Monash Universities.

In conclusion, I should like to take this opportunity of expressing my personal thanks and appreciation of the loyal support afforded by all members of the staff of the Unit since its foundation six years ago. It has been a rare privilege to have been associated with you all and the achievements of the Unit in this short space of time are a striking tribute to your efforts.

EWEN DOWNIE.

31st December, 1962.

STUDIES IN TESTOSTERONE METABOLISM

Bryan Hudson, Ausma Dulmanis[1], June Sheath, Pamela Pearl,
John Coghlan[2] and Marelyn Wintour[2]

THE ESTIMATION OF TESTOSTERONE IN PERIPHERAL PLASMA

The double isotope dilution derivative procedure that has been devised for the estimation of testosterone in peripheral plasma has now become a relatively routine procedure. The method has been modified so that following acetylation of the plasma extract and three paper chromatographic purifications of testosterone acetate, the thiosemicarbazone derivative is formed and submitted to one more chromatographic procedure.

Using this method the following range of plasma concentrations have been observed:

Normal Males:	0.45-1.0 μg/100 ml. (0.71)
Prepubescent Males:	0.21-0.35 μg/100 ml.
Normal Females:	0.05-0.31 μg/100 ml. (0.12)
Oophorectomised Females:	0.05-0.15 μg/100 ml.
Hypogonadal Males:	0.05-0.45 μg/100 ml.
Hirsute Females:	0.21-0.68 μg/100 ml.

Figures in parentheses are mean values.

The "blank" of the method is 0.02 μg/100 ml. The sensitivity of the method is 0.05 μg/100 ml. The precision of the method is $\pm$ 9.8% in the male range of values and $\pm$ 15% in the female range.

Further studies are in progress to determine:

(a) Possible changes in plasma testosterone concentration following stimuli such as ACTH infusion, gonadotrophin treatment and adrenocortical suppression with cortisone analogues.

(b) The effect of ovarian activity on plasma levels in normal menstruating females.

(c) The levels of testosterone in plasma at different ages in male children and adolescents.

THE ESTIMATION OF TESTOSTERONE PRODUCTION RATES

Because testosterone has been found in the "free", "pH 1 hydrolyzed" and "β-glucuronidase hydrolyzed" fractions of urine these "metabolites" have been used for the estimation of testosterone production rates by an isotope dilution procedure. Thus, following the administration of 4-C^{14}-testosterone urine is collected for two days and the specific gravity of urinary testosterone is measured by the double isotope dilution derivative procedure that has been developed for plasma. If the testosterone measured in the various urinary fractions was a unique metabolite of the testosterone secreted, then this would represent a true estimate of testosterone secretion rate. However, as a result of experiments in which the isotopes of possible precursors of testosterone were administered to normal subjects it seems clear that urinary testosterone is not uniquely derived

1 Partially supported by Grants from the Australian National Health and Medical Research Council.
2 Department of Physiology, University of Melbourne.

from testosterone secreted. Thus, following the administration of 7α-H^3DHEA 0.5% of the H^3 isotopes administered may be found in association with urinary testosterone; likewise, following the administration of 4-C^{14}-androstenedione to another normal subject 0.6% of the administered C^{14} isotope was found in association with urinary testosterone. Because of this, the estimation of the specific activity of urinary testosterone can only measure the production rate of testosterone. (Production rate = amount endogenous secreted *plus* amount converted from other precursors.) Accepting this limitation, the following values for testosterone production rate have been determined:

Normal Males:	4.5-11.0 mg./day
Normal Females:	0.5- 4.0 mg./day
Hypogonadal Males:	0.5- 3.1 mg./day
Hirsute Females:	0.9-20.0 mg./day

In these latter patients there would appear to be two groups—

(a) Those hirsute females with normal production rates.

(b) Those with abnormally high rates.

Possible factors that may affect the secretion and production of testosterone are being studied.

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

N. Kathleen Taylor and Dora Winikoff

A new modification of the resin uptake of I^{131}-Triiodothyronine (T_3) as a test of thyroid function has been established.

Amberlite resin (CG-50, 200 mesh) and "Triomet" (I^{131}-T_3) are incubated with the patient's serum (or plasma) under standardized conditions.

Following repeated washings with normal saline, the amount of radioactivity left on the resin, which represents the balance of T_3 not bound to the serum proteins is being used as an index of thyroid function.

Normal range:	from 25-35%
Hypothyroidism:	18-25%
Hyperthyroidism:	35-53%
Pregnancy:	16-25%

A significant correlation with the protein bound iodine level (PBI) has been found except when the pH of blood or the electrophoretic protein pattern are abnormal.

The test is particularly useful (a) when iodine contamination causes spurious elevation of PBI and depression of I^{131} uptake by the gland; (b) in children or during pregnancy when administration of radioactive iodine is better avoided.

Some patients, following treatment with therapeutic doses of I^{131} who seem to be euthyroid by clinical standards, have been found to have the T_3-resin uptake in the normal range of values while their PBI level is still in the borderline-toxic range. This aspect is being further investigated.

MICROIODINE ASSAYS

Dora Winikoff and Wanda Davies

In the investigation of more accurate assessment of thyroid function the fractionation techniques on "Dowex" anion exchange resin AG 1 - X_2, 200-400 mesh and also the filtration procedure on "Sephadex"-dextran gel medium G-25 have been studied.

The hormonal iodine attached to thyroxine binding globulin (TBG) of serum is adsorbed on Dowex resin equilibrated with acetate buffer pH 4.0. By the use of a series of buffers with gradually lowered pH the protein is eluted, while the hormones (thyroxine and triiodothyronine) remain attached to the column until pH 1.5 is reached. The eluted hormonal iodine at this pH in most euthyroid and hyperthyroid patients ranges from 75-95% of the protein bound iodine value. Any inorganic iodine present remains on the column.

In certain cases of hypothyroidism with a normal or elevated PBI level, the hormonal iodine value was 50% (or less) of the PBI.

By the use of the filtration technique on Sephadex gel equilibrated with distilled water, inorganic iodine can be separated from organically bound compounds.

A series of fractions taken followed by iodine assays can establish with certainty whether a high total serum iodine is due to organic or inorganic source of contamination.

Studies with radiothyroxine I^{131} and radioactive iodide I^{131} incubated with plasma were carried out in order to select a suitable sized column. By the use of a column 7.5 cm. x 1.5 cm. 97% thyroxine can be separated in the first fraction (2 ml. plasma and 5 ml. H_2O) which will retain only 4% of iodide. The bulk of added iodide can be recovered in subsequent fractions.

If very high levels of inorganic iodine are present the separation might be less successful but the elution pattern gives a clear indication of this fact.

Iodine containing organic substances will be either confined to the first fraction or in the case of X-ray contrast media be evenly distributed throughout the whole series.

By using both fractionation procedures in addition to routine thyroid function tests, obscure cases can often be correctly diagnosed.

EXOPHTHALMOS AND THYROID SURVEY

N. Kathleen Taylor

A follow-up study of those patients presenting at the Thyroid Unit with exophthalmos is being undertaken. Particular note is made of their thyroid state and treatment.

Details of the eye symptoms and signs, and specific treatment for such is noted.

An endeavour is being made to elicit the course run in each patient and determine any relationship to their metabolic disorder.

In conjuction with Drs. C. A. Nugent and G. Brown, Department of Medicine, University of Utah, Salt Lake City, Utah, a survey is in progress on all patients referred to the Thyroid Unit with suspected thyrotoxicosis in order to determine, using computer techniques, the probability of diagnosis based on the *appearance* of symptoms and signs.

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PAPERS IN PREPARATION

TAYLOR, N. K. and D. WINIKOFF—"T_3-Resin Uptake as a Test for Thyroid Function".

TAYLOR, N. K. and D. WINIKOFF—"Hypothyroidism in a New Born and its Mother".

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

LECTURES DELIVERED DURING 1962

"Isotopic Steroids in Clinical Medicine"—Clinical Pathology Section, Australian Medical Association.	BRYAN HUDSON
"The Estimation of Testosterone in Peripheral Plasma"—1st International Congress on Hormonal Steroids, Milan, Italy.	BRYAN HUDSON
"Studies on Testosterone Metabolism"—St. Mary's Hospital Medical School, London.	BRYAN HUDSON
"Isotopic Procedures for Androgen Estimations"—Institute of Clinical Research, Middlesex Hospital, London.	BRYAN HUDSON
"The Measurement of Testosterone Production"—Department of Medicine, Cornell Medical School, New York.	BRYAN HUDSON
"Testosterone Secretion Rates"—Department of Medicine, Vanderbilt Medical School, Nashville, Tenn.	BRYAN HUDSON
"The Estimation of Testosterone Secretion"—44th Annual Meeting, United States Endocrine Society, Chicago.	BRYAN HUDSON
"The Estimation of Androgen Production"—Department of Biological Chemistry, University of Utah, Salt Lake City.	BRYAN HUDSON
"Testosterone Metabolism"—University of California, Department of Medicine.	BRYAN HUDSON
"Isotope Dilution Procedures in the Estimation of Steroid Hormone Secretion Rates"—Alfred Hospital Clinical Society.	BRYAN HUDSON
"Hirsutism due to an Hormonally Active Ovarian Tumor"—Royal Australasian College of Physicians—Ordinary Meeting, Melbourne.	BRYAN HUDSON
"Laboratory Diagnosis of Thyroid Disorders"—Bendigo.	BRYAN HUDSON
"Insulin Treatment"—Austin Hospital Clinical Society.	H. D. BREIDAHL
"Prediabetes"—Queen Victoria Hospital Clinical Society.	H. D. BREIDAHL
"Diabetes and Pregnancy"—Royal Women's Hospital Post-Graduate Course.	H. D. BREIDAHL

"Pituitary Disease"—Prince Henry's Hospital Post-Graduate Course.	H. D. BREIDAHL
"Hyperparathyroidism"—Australian Medical Association Meeting, Alfred Hospital.	H. D. BREIDAHL
"Diabetes"—Alfred Hospital Post-Graduate Course.	H. D. BREIDAHL
"Diabetes"—Melbourne District Nursing Service.	H. D. BREIDAHL
"Thyroid Disease"—Melbourne District Nursing Service.	H. D. BREIDAHL
"Endocrine Disease in Pregnancy"—Royal Women's Hospital Post-Graduate Course.	H. D. BREIDAHL
"A Case of Hypothyroidism with Goitre in a New Born with Associated Hypothyroidism in the Mother"—Endocrine Society of Australia, Melbourne.	N. K. TAYLOR and D. WINIKOFF
"T_3-Resin Uptake as a Diagnostic Tool in Thyroid Disorders"—Annual Research Meeting, Baker Institute.	N. K. TAYLOR

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

STUDIES ON CHEMOTHERAPY[1]

J. C. Tolhurst and G. Buckle

Two new antibiotics—colistin and ampicillin—have come into use during the year, and two more—rifomycin and fucidin—have arrived during the past few weeks. Another—cloxacillin—is expected to arrive shortly. The staff have familiarized themselves with the reported properties of these drugs and sensitivity tests with the first four named have been developed and put into use.

New work in connection mainly with the anuric patients continues to make itself necessary. These patients often suffer infections caused by gram-negative bacilli which are resistant to many antibiotics and the choice of antibiotics with which to treat them is therefore restricted.

Often, drugs which normally are excreted through the kidney must be used and since in these patients they cannot be so excreted they tend to accumulate. Consequently, it is necessary to follow the course of events by determining the amount of drug in the patient's blood in order to guide dosage and dose intervals. The situation is made more complex by the fact that many of these patients were receiving other antibiotics when anuria developed and these other drugs are still present.

Work has begun on the method of determining the probable effect of combinations of antibiotics which is advocated by Chabbert of the Pasteur Institute. With this, as with many other aspects of antibiotic testing, the problem is to produce results in time for them to be of full value.

ANTIBIOTIC DUST[1]

G. Buckle and M. Dorr

It has been shown that dust is scattered in the handling, and administration to patients, of antibiotics. The presence of inhaled antibiotic dust apparently favours the nasal carriage of resistant strains of Staphylococcus aureus for it has been found that there is a close correlation in members of the hospital population between exposure to antibiotic dust and such carriage. Some measures have been taken to reduce the amount of antibiotic dust liberated and others will follow.

WOUND INFECTIONS[1]

A. Perceval

A trial has been in progress for some time of the effect of the reduction of the nasal carriage rate of Staphylococcus aureus in patients in a surgical ward. It has been shown to be a worth while procedure in reducing the incidence of wound infections due to this organism and we hope that it will be introduced throughout the hospital shortly.

HAEMORRHAGIC DISORDERS[1]

R. J. Sawers

An investigation was carried out to determine the relative value of the many tests used to detect deficiency of some coagulation factors in patients with

1 Department of Pathology.

bleeding tendencies. A battery of tests was done on 103 new patients, 23 mild haemophiliacs and some of their female relatives. Deficiencies were found in 15 of the new patients.

As compared with the semi-quantitative tests for specific clotting factors, the so-called screening tests were unreliable as both false positive and false negative results were obtained. It was concluded that accurate diagnosis required the use of a battery of tests. Further, it was found that specimens of serum were unsuitable for detecting mild deficiency of factor IX.

A laboratory study was commenced to determine the potency and *in-vivo* effect of the new C.S.L. concentrate of human antihaemophilic factor. The potency of different batches varied from 200% to 800% of the equivalent volume of plasma. Plasma concentrations obtained after transfusion were as high as 60% of normal and the levels obtained were 50-70% of the theoretically expected concentrations. After plasma transfusion maximum levels obtained were 20% of normal, and 40-50% of the theoretically expected concentration. The plasma levels at 24 hours after transfusion of the concentrate were usually less than 1% of normal. These observations indicate that this material will be of considerable value for haemophiliacs undergoing major surgical procedures but that it should only be used in hospitals with a coagulation laboratory.

HAEMODIALYSIS[1]

M. R. Ewing and J. Nayman

Development of Simplified Arterio-Venous Shunt

A new design of arterio-venous shunt has been developed which differs from previous and current models in that only a single junction is employed and there is no separate by-pass component. This has been specifically designed so that it can be tailored to the specific requirements of each patient.

Technique and Apparatus for Fashioning a Plastic Junction between Cannulae

A technique for the fashioning of a junction between two cannulae without any change in the internal diameter of these cannulae and free from leakage has been developed. The appropriate apparatus to fashion this has been designed and constructed. This has been designed specifically for the manufacture of arterio-venous shunts but can have a wide application in current experimental and clinical procedures.

Serum Protein Paper Electrophoresis in the Dog

A technique for this procedure has been developed and a range of normals established. A total of 100 estimations in 50 unselected mongrel dogs formed the basis for this experiment.

Regional Heparinization

An investigation of heparinization of the extracorporeal circuit of the artificial kidney during haemodialysis has been conducted. A consecutive series of over 100 cases have now been performed and this project is in the final stages of completion.

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

A nomogram which will relate the amount of heparin required for any given flow rate is in the process of completion.

Biochemical Techniques for Monitoring Haemodialysis

(a) A rapid method of estimating blood urea levels has been developed. This method is simple and can be accomplished in 5 to 10 minutes. A total of 886 estimations using this technique have been performed.

(b) A simplified method for dilution of plasma for the estimation of potassium and sodium has been developed.

Dialysing Bath Concentrate

A concentrate of dialysing fluid is under study. This would have the great advantage of being able to prepare requirements of dialysing fluid in bulk.

The main problem in maintaining stability of the concentrate and preventing contamination have been overcome and the concentrate is now ready for a clinical trial.

Open Renal Biopsy

In acute renal failure a technique of open renal biopsy involving the use of an air driven biopsy gun is being elaborated. This is designed so as to be able to obtain a biopsy in those patients with high blood urea levels and in those where percutaneous biopsy is contra-indicated, e.g., unco-operative patients (fitting), obese patients and patients with coagulation anomalies. Biopsies have been performed in a total of 8 such patients. It is planned to modify the present air driven biopsy gun so that it can be made more adaptable for renal biopsy.

Indwelling Peritoneal Cannulae

An apparatus and technique are being developed whereby a peritoneal plug can be inserted by a closed technique and peritoneal cannula inserted into this for regular peritoneal dialysis without subjecting the patient to any further operative procedures.

Peritoneal Dialysis (in conjunction with Dr. Gurr)

This technique is being developed and established as an additional method of treatment in patients with renal failure.

Modifications to Present Travenol Twin-coil Disposable Kidney

Various modifications which include the insertion of the ultra-violet light filter in the circuit, the overflow stop cock of the bath and overhead coil clamp have been constructed.

Wound Healing in Relation to Renal Failure

The study of wound healing in renal failure in the experimental animal has been completed.

Peritoneoscopy

A clinical investigation relevant to the technique and application of peritoneoscopy is under way and a total of 8 cases have been investigated.

Film

A 16 mm. cine film depicting the technique of insertion and construction of an arterio-venous shunt is in the process of completion.

Microscopic Examination of Urine: Technique and an Atlas

This project has been undertaken in order to provide a fairly comprehensive record of the possible findings—normal, contaminants and pathological—when examining a specimen of urine microscopically. There is a special need amongst students and resident staff for such an Atlas.

Most of the material has been collected and photographed. It now remains to correlate this material into the form of an Atlas.

ANURIA[1]

M. R. Ewing and Elizabeth Kidd

The enquiry into the importance of infection in determining both morbidity and mortality during the treatment of patients with acute oliguric renal failure has been continued. Infection in one form or another was found in 85% of the patients studied.

The susceptibility of rabbits rendered uraemic by intoxication with uranium nitrate to intradermal infection with Staph. aureus was investigated. No increased susceptibility was demonstrated.

The study of septicaemia, bacteraemia and intravascular haemolysis occurring during transurethral resection has been completed.

THE RELATION BETWEEN ALLUSIVE THINKING AND LEARNING[2]

N. McConaghy

From previous research evidence has been advanced that people can be divided into two groups depending on whether or not they show allusive thinking. Allusive thinking can be defined as the tendency, when formulating new ideas at an abstract level of thought, to utilise expressions which are over-abstract and imprecise; and to introduce new material which in the context is only partially relevant. It is considered that allusive thinking represents in mild form the thought disorder found in schizophrenia. It was demonstrated that as compared with non-allusive thinkers allusive thinkers show under certain conditions a type of learning performance similar to that reported to be shown by schizophrenics—viz., an initial lower performance but a greater steady improvements as learning continues. This type of learning curve was found with a group of university students of limited age range when they learned three lists of sixteen words after learning two lists of nonsense syllables. However, when this study was replicated with subjects of a wider age range and educational attainment this difference in performance between allusive and non-allusive thinkers did not appear. However, when they learned three lists of sixteen words after learning two lists of nine words the difference was again apparent at a significant level. This was considered to support the theory advanced as to the aetiology of allusive thinking, viz., that it is due to the presence of a less intense arousal mechanism in the subjects showing it and hence a weaker initial exclusion of irrelevant aspects of the learning task. Hence the change from nine to sixteen words produces greater distraction and so greater learning impairment in the

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2 Department of Psychiatry.

allusive thinkers. This compares with Shakow's finding on the performance of schizophrenics that they show an "initial difficulty in focusing on the relevant aspects of a defined situation".

The Effect of Increased Arousal on Learning

It has been suggested that increased learning ability can be related to increased arousal. To provide further evidence for this theory the finding of Sokolov was utilised that administering a foreign or unexpected stimulus increases arousal. A series of experiments have been carried out in which three lists of words are learned, one word in each list being accompanied by either a loud, a soft or no sound. The onset of the sound would be expected to increase arousal. However, the arousal would not be directed to the word being learned but to the foreign sound. To predict the result of this experiment the findings of Pavlov on the simultaneous administration of two stimuli must be taken into account. He found that a weak stimulus summated with a strong one but a strong one tended to have an inhibitory effect. Hence it would be expected that if the sound were weak learning would be improved, but if strong, learning would be impaired. This prediction was verified. This finding strengthens an arousal as opposed to a reinforcement theory of learning.

Non-Verbal Psychotherapy

The treatment of psychiatric patients by non-verbal methods of therapy derived from learning theory has been continued. Three basic methods are employed.

1. Extinction—This has been utilised in the treatment of patients with phobic responses. The patient is repeatedly exposed to a situation sufficiently like the feared one to produce by generalisation some anxiety, but only a minimal amount. The anxiety produced is insufficient to maintain itself as a permanent reaction and it diminishes with regular exposure. When it is negligible the patient is exposed to a more similar situation and can now tolerate this more easily. In this way the patient is gradually able to tolerate more and more similar situations with minimal anxiety until the phobia itself is overcome.

2. Avoidance—This has been utilised to treat patients with obsessive thoughts, torticollis and habit spasms. The patient is instructed to concentrate on the obsessive thought or carry out the motor act which is to be inhibited and a painful electric shock is then administered. The tendency for the thought or act to be repeated in the future is thereby weakened.

3. Inhibition of Reinforcement—This has been used to treat patients with compulsive behaviour or tics. The patient is made to carry out the behavioural act or tic repeatedly over intervals of time and the tendency for the act or tic to persist is thereby reduced.

Fourteen patients have completed treatment, all of whose illnesses were of a very chronic nature and had resisted other forms of treatment. Seven have been greatly improved or recovered, at least two of whom would otherwise have proceeded to leucotomy.

SOCIAL ASPECTS OF CARDIOVASCULAR DISEASE[1]

B. B. Thomas

Fears of Heart Disease

Previous reports have indicated that the chances of rehabilitation for cardiac patients are strongly influenced by beliefs and attitudes about heart disease.

The two-year study now completed showed that one of the most widely held and crippling beliefs was that exertion is harmful and likely to produce further heart damage and probably death. As in other illnesses, patients' reactions were closely related to premorbid personality patterns, but in heart disease the additional and controllable factor was the understanding of the heart attack and its consequences. Public education can play a part in creating healthy and realistic attitudes, but the greater opportunity rests with the physician who can speak with authority in the early stages of illness when patients are most receptive. .

Fears arising from beliefs of this kind prejudice every aspect of social functioning, but are particularly destructive in their effects on re-employment. It was found that in the majority of cases there was no specific discussion with patients about work, yet throughout the study it was strikingly clear that this was a question of paramount importance to most patients with heart disease and one on which reassurance and guidance was needed as soon as it was reasonable to give it.

Employment

Of the 92 patients gainfully employed prior to illness, 61 were assessed as physically fit to resume work. Nine of this group died during the study. Forty-seven were back at work within 6 months and 40 of these were still working one year later. Another nine resumed work in later months. Thirty-four patients, including some who were invalided by unwarranted fears, were not working at the close of the study.

The best chance of re-employment proved to be with the former employer, whether or not the regular job needed to be modified. The most difficult placements were those for men over 50 years who were advised to take lighter work which could not be provided by the former employer.

SUBARACHNOID HAEMORRHAGE[2]

James M. Calvert

An investigation of nontraumatic subarachnoid haemorrhage from aneurysms or other vessel malformations of the central nervous system is being conducted. Cases with aneurysms diagnosed in the absence of haemorrhage are also included. A preliminary but incomplete survey of cases of subarachnoid haemorrhage admitted to the Alfred Hospital during the past ten years has been made and is being continued prospectively.

The data so far gathered indicate a considerable preponderance of this condition in females, and that a larger percentage of females than males have suffered previous haemorrhage. The average age of patients of either sex is

1 Almoner's Department.

2 Neurosurgical Unit.

approximately the same (the late forties) and the age incidence varied from 16 to 71 years in the females and from 16 to 66 years in the males. The same proportion of males as females have a family history of cerebro-vascular or cardio-vascular disease but in only a couple of families did the incidence of subarachnoid haemorrhage appear significant.

In most patients carotid angiography was carried out, but in about 18% it failed to demonstrate the source of haemorrhage. This raises the question whether vertebral angiography should be done in these cases for although many aneurysms of the basilar system are surgically inaccessible it is possible to operate on some. Overall the procedure of angiography for subarachnoid haemorrhage is very safe and very few complications have been noted in this investigation.

Most of the aneurysms have been found on the internal carotid artery, the remainder being equally on the anterior and middle cerebral and anterior communicating arteries.

More than half of the cases so far studied have been treated by operation, in a few cases by carotid ligation in the neck, but usually by direct attack which is favoured by the modern aids of hypothermia and urea shrinkage of the brain. The relative merits of conservative and operative treatment have still to be assessed.

Investigations are continuing on the results and merits of treatment of aneurysms of different sites, and on possible factors predisposing to haemorrhage. However, it is felt that the numbers which can be studied can only be statistically significant over a long period. For this reason it seems that the most valuable findings are likely to be gained by incorporating the data from many centres in a central registry for processing, as is currently being done by the Central Aneurysm Agency of America.

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"Wound Healing in Renal Failure"—*Royal Australasian College of Surgeons, Wellington.*	J. NAYMAN
"A Method for the Treatment of Idiopathic Hydroceles in the Adult"—*Royal Australasian College of Surgeons, Melbourne.*	J. NAYMAN
"Recent Developments and Techniques in Haemodialysis and their Application with Special Reference to Surgical Cases of Renal Failure"—*Victorian Society for Pathology and Experimental Medicine.*	J. NAYMAN
"Treatment of Renal Failure by Dialysis"—*Medical Association of Australia, Shepparton.*	J. NAYMAN
"The Artificial Kidney"—*Royal Victorian College of Nursing, Bendigo.*	J. NAYMAN
"Acute Renal Failure and its Management"—*James Stewart Lecture.*	J. NAYMAN
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