BAKER INSTITUTE

RESEARCH

1959

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-THIRD ANNUAL REPORT

of

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

(Including Alfred Hospital Clinical Research Unit)

THIRD ANNUAL RESEARCH REPORT

of

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

REPORTS

of

ALFRED HOSPITAL RESEARCH FELLOWS

1959

ALFRED HOSPITAL, PRAHRAN
VICTORIA, AUSTRALIA.

BAKER MEDICAL RESEARCH INSTITUTE

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EWEN DOWNIE, M.D., F.R.C.P., F.R.A.C.P.

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*E. S. J. KING, D.Sc., M.D., M.S., F.R.C.S., F.R.A.C.S., F.R.A.C.P.

Director of Clinical Research Unit (ex officio).

*Appointed from the University of Melbourne.

ALFRED HOSPITAL RESEARCH FELLOWS, 1959

Honorary:	E. L. G. BEAVIS, M.B., B.S., D.G.O., M.R.C.O.G., F.R.C.S.
"Victor Y. and Margaret Kimpton":	H. D. BREIDAHL, M.D., M.R.C.P.
"Alfred Hospital":	P. KINCAID-SMITH, B.Sc., M.B., B.Ch. (W'srand), M.R.C.P., D.C.P.
"Frederick and Esther Michaelis":	V. C. MARSHALL, M.B., B.S., F.R.A.C.S.
"E. H. Flack":	A. D. McCUTCHEON, M.D., M.R.A.C.P.
"Sydney W. Jones Medical Research Foundation":	D. RACE, M.B., B.S.
Honorary:	M. SANDERS, M.B., B.S.
"Sol Green":	R. J. SAWERS, M.B., B.S., M.R.A.C.P.
"R. B. McComas Memorial":	F. O. SIMPSON, M.B., B.Ch. (Edin.), M.R.C.P. (Edin.).
"A. A. Swallow":	I. McN. SMITH, B.A., M.B., B.Chir., F.R.C.S.
"Edward Wilson Memorial":	J. W. W. THOMSON, M.B., Ch.B. (Edin.), F.R.C.S.
Senior Research:	J. C. TOLHURST, M.Sc.

APPOINTED TO RESEARCH FELLOWSHIPS FOR 1960

"Connibere Bequest Research Scholarship":	P. J. ARMSTRONG, M.B., B.S., D.A., F.F.A.R.A.C.S., F.F.A.R.C.S.
"Frederick and Esther Michaelis":	H. D. BREIDAHL, M.D., M.R.C.P.
"E. H. Flack":	H. D. BURGER, M.B., B.S.
"J. F. McKeddie":	B. W. FOX, M.B., B.S., F.R.C.S., F.R.C.S. (Edin), F.R.A.C.S.
"A. A. Swallow":	G. L. GROVE, M.B., M.S., F.R.A.C.S.
"Sydney William Jones Medical Research Foundation":	P. KINCAID-SMITH, B.Sc., M.B., B.Ch. (W'srand), M.R.C.P., D.C.P.
"Edward Wilson Memorial":	F. LUMB, M.B., B.S. (Lond.), M.R.C.P.
"R. B. McComas":	W. McDONALD, M.B., B.S., F.R.A.C.S.
"Dr. Henry Laurie":	D. RACE, M.B., B.S.
"Sol Green":	R. J. SAWERS, M.B., B.S., M.R.A.C.P.
"Victor Y. and Margaret Kimpton":	I. McN. SMITH, B.A., M.B., B.Chir., F.R.C.S.
"George Merriman":	B. B. THOMAS, Dip.Soc.Stud. (Sydney), A.I.H.A. (N.S.W.).

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 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.
Graduate:	Mrs. V. CARSON, M.Sc. (from 1/7/59).
	C. C. CURTAIN, Ph.D., M.Sc., F.R.A.C.I.
	Miss P. EMERY, B.Sc. (from 31/8/59).
	D. McKELVIE, B.Sc. (to 15/5/59).
	Mrs. W. G. NAYLER, M.Sc.
	G. R. STIRLING, M.B., B.S., F.R.A.C.S.
	H. A. WARD, B.Sc.
	R. G. WYLLIE, M.B., B.S.
Technical:	S. HART (Laboratory Supervisor).
	Mrs. J. BATHIE.
	Miss N. BRAIN.
	J. L. BREMNER.
	Mrs. J. GREEN.
Clerical:	Mrs. L. DEMPSTER.
	Mrs. J. DYE (from 9/11/59).
	Miss E. ORR.
Laboratory Assistants:	Miss P. CLIFTON.
	Miss J. EDYVANE.
	Miss J. HARRIS.
	Miss J. HOWELLS.
	Miss W. JENKINS.
	Miss M. KING (to 11/6/59).
	Miss B. LENNIE.
	Miss J. NICHOLS.

WARD STAFF

Registrar:	E. A. DODGE, M.B., B.S., M.R.A.C.P.
Resident Medical Officers:	D. KENNETT (from 12/2/59 to 3/5/59).
	A. ZERFAS (from 5/4/59 to 26/7/59).
	A. V. HILL (from 27/7/59 to 18/10/59).
	A. BODEY (from 19/10/59 to 17/1/60).
Sister:	P. D. CALLINAN (from 12/1/59).
Staff Nurses:	J. R. MORRIS (to 29/3/59).
	J. HUDSON (from 30/3/59 to 13/9/59).
	M. CROPLEY (from 10/8/59).
	M. ROMANES (from 19/10/59 to 1/11/59).
	P. MATENSON (from 2/11/59 to 29/11/59).
	C. R. DODD (from 30/11/59).

RESEARCH FELLOWS

"Alfred Hospital":	P. KINCAID-SMITH, B.Sc., M.B., B.Ch. (W'srand), M.R.C.P., D.C.P.
"E. H. Flack":	A. D. McCUTCHEON, M.D., M.R.A.C.P.
"Sydney W. Jones Medical Research Foundation":	D. RACE, M.B., B.S.
"Sol Green":	R. J. SAWERS, M.B., B.S., M.R.A.C.P.
"R. B. McComas Memorial":	F. O. SIMPSON, M.B., B.Ch. (Edin.), M.R.C.P. (Edin.).
"Edward Wilson Memorial":	J. W. W. THOMSON, M.B., Ch.B. (Edin.), F.R.C.S.

ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

Frequently since its inception the Baker Institute has been fortunate in receiving generous gifts from various benefactors. A number of these benefactions have been commemorated by associating the name of the donor with some feature of the Institute's building or activities. The title name of the Institute commemorates the very generous endowment made by Thomas Baker, his wife and her sister, which not only created the Institute, but has enabled it to develop and carry on research to the level which it has now reached. The Rouse unit and the Lang unit likewise perpetuate the names of benefactors, as does the Nyulasy Scholarship.

On 11th November this year an additional wing, consisting of laboratories and offices, was opened and named "The Catherine Anne Smith Wing" by Professor A. H. Ennor, of the Australian National University. This title also commemorates a generous bequest made to the Institute by Miss Smith, and the wing completes the physical union of the laboratory buildings with those of the ward and clinical facilities of the Clinical Research Unit. In his address Professor Ennor stressed the importance of this union between clinical and laboratory work and the need for both aspects of medical research to be developed to similar high levels.

In addition to the new laboratory facilities provided by the new wing and remodelling of some of the existing laboratories, it is expected that, in 1960, some additional space will be returned for the Institute's purposes by the move of the Cardiovascular Diagnostic Service to its new quarters, on which building has commenced. It is hoped that this will enable extension, small but useful, to the number of beds available to the clinical research group. The present limitation in the number of beds greatly restricts the opportunities which can be offered to medical graduates for research or for training in research methods. This imbalance between clinical and laboratory facilities needs early correction if the Institute as a whole is to continue a balanced development and serve medicine in this community to the fullest extent.

The previous paragraphs record a steady growth of the Institute buildings since 1928. The value of grants-in-aid of research made to us by various State and National bodies to enable specific projects to be carried out show a similar picture of steady growth and reflect the confidence of these organisations in the work of the Institute. These have risen, in round figures, from £5000 in 1957, through £7000 in 1958 and £9000 in 1959, to £17,000 which has been allocated for 1960.

Buildings and money do not, of themselves, produce research work, but without both of them facilities could not be provided for the research workers whose projects for this year are detailed in the scientific section of this report and are summarised in the following paragraphs.

The main aspect of the control of body fluid volume which has been studied this year is the concept that a volume receptor must exist somewhere in the body. From the experimental data which have been collected, it is concluded that such a receptor must exist, although at present it is unidentified.

Further, some of its properties and the way in which it is integrated into the control mechanisms have been postulated.

As for some years past, problems associated with the processes involved in the clotting of blood were a major project in both clinical and laboratory spheres. On the one hand, the action of metal salts and organ extracts in the clotting process and, on the other, bleeding diseases and the coagulation problems associated with open heart surgery have been studied.

The clinical trials of drugs used for the treatment of severe hypertension have included several new drugs, and it is pleasing to record that the prognosis and well-being of these patients improves each year.

Studies of occlusive arterial disease, which have been in progress for many years, continue, and have been extended by investigations into the effect of variations in diet on the patients with these diseases.

Another long-term major project concerns the production of energy by cardiac muscle and some new techniques for investigation have been introduced. Attention has been directed mainly to the part played in cardiac muscle activity by anions and cations both in the presence and absence of glycosides. In addition, evidence pointing to the presence of an unidentified pressor substance in plasma has been obtained. In association with these studies further observations on the histology of cardiac muscle by means of the electron microscope have been carried out. This last investigation was commenced with the help of the Department of Pathology, University of Melbourne, and its continuation has been made possible by the help received by the Commonwealth Serum Laboratories, Melbourne.

Investigations of the serum proteins and haptoglobins of New Guinea natives has continued and data are being collected to reach a statistically significant level. A study of the heterogeneity of human haemoglobin is also being carried out with electrophoretic techniques. Collection of data concerning the serum proteins in Kuru is also being continued.

The study of the problems of open heart surgery continues, and this group of investigators have been working on total body perfusion with the aid of an extra-corporeal pump oxygenator and biventricular by-pass techniques. In addition their observations of ventricular function during various procedures associated with cardiac surgery continue. A study of the problems of surgical treatment of aortic valve disease has been commenced.

A new field of investigation pursued during this year will be expanded next year. This concerns biological studies at the cellular level. At present studies on the cellular structure, cellular electrical activity and cellular enzymes of cardiac muscle are being carried out using histological, electrical and histochemical techniques. Investigation of the cellular enzymes of leucocytes and erythrocytes are being made in connection with inflammations and leukaemia and drug induced anaemias.

In addition to these well-defined projects, a number of smaller investigations have been conducted. These include observations on scleroderma, Conn's syndrome, a preliminary survey of the socio-economic impact of cardiovascular disease on patients and their families, the value of renal biopsy, the chemotherapy of malignant disease, and the causation of biliary calculi. A seminar on The Uses of Computors in Biology was held, and was very well attended.

Last year the institution of a Baker Institute Prize (1958) to encourage younger graduates to attempt research projects of their own was commented upon. This prize was awarded to Dr. R. G. Wyllie for a thesis entitled: Alkaline Phosphatase in Blood Cells. This year the Laura Nyulasy Research Scholarship has become available for competition on similar terms. This scholarship will be open for competition every second year.

In 1952, when the Board of Management created the Edward Wilson Memorial Fellowship, the hope was expressed that this would be awarded each year to a Fellow from overseas to enable him to work in the Alfred Hospital for a year. This hope has been fulfilled and, to date, six graduates from the United Kingdom have been appointed, and have contributed greatly to our medical activities.

During the year Dr. Fantl was invited to attend a meeting of the International Committee for the Standardisation of the Nomenclature of Blood Clotting Factors in Montreux, and he was accompanied by Dr. R. J. Sawers, who returned to Australia via England and the United States of America for the purposes of visiting haematological clinics and laboratories. Acknowledgment is made of the financial assistance given to them by the National Advisory Heart Council, U.S.A.

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, and Alfred Hospital Medical Research Funds. All of these bodies have allocated larger grants for work in 1959, and the assistance granted is gratefully acknowledged.

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 this year by Professors Davies, King, Trikojus and Wright and the staffs of the Departments of Organic Chemistry, Pathology, Biochemistry and Physiology, University of Melbourne, and the staff of the Commonwealth Serum Laboratories. We thank them and others who have helped for their continuing interest in our work. In reciprocity clinical and laboratory help has been made available to the Departments of Medicine and Surgery of the University of Melbourne within the hospital.

It is a pleasure for me to thank the Trustees of the Institute and the Board of Management of the Hospital for their continued generous support of all our activities, including assistance for members to visit other centres, and to thank members of the staff and research fellows for their co-operation during the past year.

T. E. LOWE.

31st December, 1959.

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

Adelaide Children's Hospital.
Anti-Cancer Council of Victoria.
A.N.Z.A.A.S. Congress, Perth.
Commonwealth Department of Health.
Commonwealth X-ray and Radium Laboratories.
Department of Health, New Zealand.
Institutio de Biologia y Medicina Experimental, Buenos Aires
Medical Research Council, London.
Middlesex Hospital Medical School.
National Institute of Nutrition, Tokyo.
New York University College of Medicine.
New Zealand Medical Research Council.
Ophthalmic Research Institute of Australia.
Rockefeller Foundation, New York.
Royal Children's Hospital, Melbourne.
Royal Prince Alfred Hospital, Sydney.
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.

ALFRED HOSPITAL RESEARCH FELLOWS IN THE INSTITUTE 1949-1959

Anderson, R. McD., 1953-55
Andrew, R. R., 1949-55
Barnett, A. J., 1949-50
Beavis, E. L. G., 1955-56
Boake, W. C., 1958
Breidahl, H. D., 1952-53
Burnside, K. B., 1951
Duffy, D. G., 1952-55
Ferguson, I. A. L., 1957-58
Fowler, R., 1953-54
Francis, J. K., 1956-57
Fraser, J. R. E., 1957
Gardiner, J. M., 1952
Goble, A. J., 1951
Hudson, B., 1952
Jamieson, K., 1954
Kay, H. B., 1949-53
Kincaid-Smith, P., 1959
McCutcheon, A. D., 1959
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
Sawers, R. J., 1953-59
St. Clair, W. A., 1955
Silberberg, F. G., 1953
Stern, W., 1954-55
Stirling, G. R., 1955
Wagner, G., 1958

OVERSEAS FELLOWS

Emslie-Smith, D., 1955-56 (Dundee)
Hamilton, M., 1954 (London)
Marshall, R. J., 1957 (Belfast)
Stevenson, M. M., 1957 (Belfast)
Simpson, F. O., 1958-59 (Edinburgh)
Thomson, J. W. W., 1959 (Edinburgh)

BAKER INSTITUTE PRIZE

R. G. Wyllie (1958)

REPORT OF SCIENTIFIC INVESTIGATIONS

BLOOD COAGULATION

CLOTTING ACTIVITY OF MATERNAL AND FOETAL BLOOD*

P. Fantl and H. A. Ward

Deficiency of antihaemophilic factor (Factor VIII) or of beta-prothromboplastin (Factor IX) in parents gives rise to an inherited haemorrhagic tendency. In determining the blood clotting factors in the blood of pregnant ewes and their foetuses at varying stages of gestation particular attention was therefore paid to Factors VIII and IX.

It was found that while Factor VIII activity is present at a low level in foetal sheep plasma at an early stage of gestation, it rises to adult levels towards the end of gestation, but that Factor IX activity of foetal sheep serum was at all foetal ages at a low level compared with that of the maternal serum.

A comparison of these results with the findings in man indicates that the respective activities of the clotting factors in the blood of mother, foetus and normal adult are similar in both species. Determination of Factors VII and IX, prothrombin and fibrinogen in the blood of a newborn infant does not permit one to predict whether a haemorrhagic tendency due to a deficiency of one or more of these factors will develop later in life, since the activity of these factors is low in the normal newborn and increases with age. On the other hand, the activity of Factor VIII in the blood of the normal newborn is as high as in the normal adult, so that it would seem that a low level of Factor VIII activity in the blood of a newborn infant would indicate a permanent Factor VIII deficiency.

As evidence suggests that the foetus cannot form antibodies, it might eventually be possible to graft into it cells which produce Factor VIII. If such grafting should be successful in newborn male infants of mothers who are haemophilia carriers it should then be possible to prevent them from becoming haemophiliacs.

INFLUENCE OF METAL SALTS ON BLOOD COAGULATION*

P. Fantl and H. A. Ward

This work was carried out as a preliminary to investigations of the influence of tissue extracts on the clotting mechanism. Since any compound isolated from organs may contain metal salts, it was essential to determine the effect of pure metal salts on several phases of blood coagulation.

Hg^{2+} was a weak inhibitor of plasma thromboplastin formation when compared with Cu^{2+}, Cd^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}. In view of the great affinity

*In this report of Scientific Investigations those projects marked (*) were supported wholly or in part by grants from the National Health and Medical Research Council, those marked (**) by grants from the Life Insurance Medical Research Fund, and those marked (***) by grants from the Anti-Cancer Council of Victoria.

of Hg^{2+} for SH-groups, this observation indicates that SH-groups are either not present in any of the components of the thromboplastin complex or that they are not essential for the coagulation process.

Cu^{2+}, Cd^{2+} and Zn^{2+} ions inhibited thromboplastin formation, suggesting that these ions react with essential groups in one or more of the clotting factors of the thromboplastin complex. The inhibitory effect of these ions could be counteracted by E.D.T.A., cysteine, histidine, serine and glutamatic acid. Therefore it can be concluded that the metal salts have not caused any denaturation of the clotting factors.

The iron compounds occupy a special place among the inhibitory salts. The activity of Fe^{2+} is of the same order as that of Fe^{3+}, and, in contrast to Cu^{2+}, the inhibitory effect of iron salts is not readily reversed by E.D.T.A. It is likely that the iron is strongly bound by plasma components.

Quantitative considerations showed that more than one metal-combining component of plasma or serum is involved in the inhibition of blood coagulation.

The action of the metal salts is not uniform on the different phases of the coagulation process. Whereas Hg^{2+} is a weak inhibitor of blood thromboplastin formation, it is equally as effective as Fe^{2+} in inhibiting the conversion of prothrombin in presence of brain extract. In contrast, in the presence of Russell's viper venom a similar concentration of Hg^{2+} was not inhibitory at all, but even accelerating.

The inhibitory effect of the metal salts on certain phases of the coagulation process may explain spontaneous bleeding occasionally seen in metal poisoning.

INFLUENCE OF ORGAN EXTRACTS ON BLOOD CLOTTING FACTORS*

P. Fantl and H. A. Ward

Organs damaged by trauma or pathological changes have a profound influence on blood. Damaged tissues often induce rapid clotting, and serum factors are produced, some of which are very active in promoting clotting. In thrombotic conditions blood is in contact with damaged tissues, and it is of importance to know what changes in the clotting factors can be expected under these conditions. Therefore model experiments were carried out in which venous blood or sera from healthy donors was incubated with organ extracts. It was found that brain or lung extracts produced sera which showed a diminished activity in the production of blood thromboplastin. A detailed study indicated that Factor VII and probably Prower-Stuart Factor and Factor IX had been inactivated. In order to find the organ component responsible for the destruction of these clotting factors, a number of derivatives of the brain extracts were prepared. The active component was found to be heat stable and was present in a readily sedimentable brain fraction. This activity was independent of the thromboplastic activity of brain. The results suggest that a lipid or lipoprotein is the factor responsible for the destruction of the clotting factors. The tissue component which inactivates Factors VII and IX might act as an antagonist to the clotting factors, for it could prevent a thrombus from spreading.

BLOOD COAGULATION PROBLEMS IN OPEN-HEART SURGERY*

P. Fantl and H. A. Ward

In the past three years we have, in conjunction with members of the Thoracic-Surgical Unit, studied the blood coagulation problems arising in open heart surgery.

Seitz filtration was found to be the most reliable procedure by which to produce sterile heparin solutions of constant potency. Steam sterilisation cannot be recommended because heating produces loss of heparin potency from these solutions which should have a pH below 7.0.

A technique for the rapid determination of heparin based on the thrombin clotting time of whole oxalated blood was developed. The result is available approximately five minutes after receipt of the blood specimen. This enabled frequent monitoring of blood heparin levels during the period of extra-corporeal circulation.

The phenomenon of reappearance of heparin in the post-operative period after the inactivation of heparin by protamine was investigated in in-vitro experiments. It was found that the reappearance of heparin from the inert heparin-protamine complex is due to the action of an enzyme (protaminase) in blood, which liberates active heparin from the complex.

Coagulation studies were carried out in the post-operative period and special attention was paid to blood platelets. In this connection a number of workers consider that it is essential for all parts of the apparatus with which blood comes in contact during the extra-corporeal circulation to be coated with a water-repellent surface in order to preserve clotting factors, including the platelets. "Silicones" are usually used for this purpose. However, the "silicones" produce a removable film on glass and metal surfaces which may be carried with the blood and lodged in the reticulo-endothelial system. "Silicones" are high molecular substances which are not metabolised and can produce a foreign body reaction. Indeed, there are already reports of granuloma formation after injection of "silicones" into experimental animals. For this reason we have advised against such coating of apparatus used in extra-corporeal circulation. In order to show that "silicone" coating is unnecessary for preservation of platelets, at least in a disc oxygenator, platelet counts were carried out in the pre-operative and post-operative periods. The results indicate that the platelet numbers are practically unaltered in heparinised blood which is in contact with uncoated glass and stainless steel surfaces.

HAEMORRHAGIC DISEASES

R. J. Sawers and P. Fantl

The investigations on patients presenting with haemorrhagic symptoms and of the effect of transfusion of blood products have continued.

Of the new patients examined during the year seventeen have been found to have haemophilia. Unusual observations have been made in two families in which bleeding has been evident in only the most recent generation. In the first, two young brothers have been shown to have the dual deficiency of Factor VIII (A.H.G.) and Factor IX (P.T.C.); in the second an only child,

who is a girl, was found to have deficiency of P.T.C. of moderately severe degree. From the genetic viewpoint she must be regarded as a carrier for beta-haemophilia (haemophilia B).

Other patients with blood coagulation disorders examined during the year include a young woman with a chronic fibrinolytic disorder, a girl with congenital afibrinogenaemia, and two boys with Hageman trait; both parents of the latter patients were shown to have severe deficiency of Hageman factor. thus supporting existing evidence of the recessive mode of inheritance of the trait.

The results obtained from the blood transfusion experiments have not greatly assisted in understanding the behaviour in the body of many of the transfused blood clotting factors or of the blood platelets, but have provided data of value for future work.

A familial haemorrhagic disorder, characterised only by the demonstration on occasional tests of a prolonged skin bleeding time, is the most common bleeding disease encountered. The great variability of the skin bleeding time in most individuals affected has not been explained. It has been shown that major surgical procedures may be carried out in the absence of prior transfusion or corticosteroid therapy if the operation is delayed until the skin bleeding time returns to normal.

LEUKAEMIA

R. J. Sawers

An investigation incorporating the determination of the daily output of uric acid by patients with leukaemia and other blood disorders was commenced. On a low purine containing diet, it was found that the uric acid excreted by control subjects, when related to body surface area, was constant within a narrow range (235 mgm. ± 28 mgm./sq. meter/day). Only four of ten patients were found to exceed the normal range; a number of patients who were expected to excrete an excessive amount of uric acid failed to do so.

CONTROL OF BODY FLUID VOLUME*

T. E. Lowe, A. J. Barnett and F. O. Simpson

Over several years studies of the behaviour of patients with abnormalities in the fluid content of their bodies have enabled the overall general behaviour of the physiological mechanism controlling the volume of fluid in the body to be characterised. An analogue of this system appears to be a storage divided into many compartments, in some places by semi-permeable membranes, in others by impermeable membranes and the various parts connected by the vascular system. Through this system is a continuous flow of fluid (electrolyte solution) controlled hy inflow and outflow mechanisms which are regulated by the volume of fluid in some portion of the storage. In this system a volume receptor appears to be necessary, as are connections between the receptor and the inflow and outflow mechanisms.

During this year much consideration has been given to the receptor and its connections with the inflow and outflow mechanisms. Previous work on the study of renal clearance studies and the assay of antidiuretic hormone has been continued to this end, and previous data relevant to the indentification of the site of the receptor reviewed. As most of the renal clearance studies have been carried out on patients with hypertension, this work is reported under that heading.

From these studies it is to be concluded that available data indicate that regulation of the fluid volume of the body exists independently of the regulation of other aspects of the body fluid such as osmotic pressure. The mechanism by which this regulation is effected is only known in part, and it is necessary to postulate a volume receptor in that mechanism. If, as seems likely, the physics of open systems can be applied to this physiological regulation, then the receptor need be sensitive to change of volume only.

The volume receptor controls the inflow (thirst) and outflow (urine) mechanisms through little known pathways which have both nervous and hormonal components. Further, the evidence to date suggests that some portion of the vascular volume is monitored, but the location of this region has not been conclusively fixed.

HYPERTENSIVE STATES

CLINICAL TRIAL OF HYPOTENSIVE DRUGS

A. J. Barnett, F. O. Simpson, P. Kincaid-Smith

This clinical study, commenced some nine years ago, to investigate the effectiveness of ganglion-blocking drugs in severe hypertension, continues. The value of these drugs is now well established, and with new additions may have reached their peak of usefulness. The value of other drugs used as adjuvants to the ganglion blocking drugs, particularly reserpine and chlorothiazide, is also well established. Most patients with severe hypertension are now treated with a combination of these drugs with fairly good blood pressure control and slight to moderate side effects.

The dangers and side-effects of ganglion-blocking drugs, mostly due to the unwanted action on the parasympathetic nervous system, however, still hamper treatment of hypertension. The recent development of drugs inhibiting sympathetic nervous activity without affecting parasympathetic nervous activity therefore offers hope for safer and more pleasant control of hypertension. Supplies of two of the new compounds—"Ismelin" (Ciba Pty. Ltd.) and "Darenthin" (Burroughs Wellcome) have been obtained for clinical trial.

In preliminary investigations with "Ismelin" a dose of 100 mg. per day has been found to produce lowering of blood pressure, particularly in the erect posture, occurring after treatment for several days and marked after one to two weeks. Tests of autonomic reflexes have been performed before and after treatment for two weeks in six hypertensive patients. The effect of exercise on the blood pressure and pulse rate, the pressor response to cold, and sweating from body heating are diminished, whereas the effect of carotid sinus pressure on the blood pressure and pulse rate is unaltered. The rise in blood pressure

following atropine is increased during treatment with Ismelin, whereas the rise in pulse rate is decreased. The explanation of these latter effects is not clear.

With continued treatment in these patients, blood pressure control has been comparable to that previously obtained with ganglion-blocking drugs, but without side effects other than mild diarrhoea and occasional faintness. Patients prefer the new drug.

DESERPIDINE—A RESERPINE ANALOGUE

F. O. Simpson

Deserpidine (10-methoxy-reserpine) has been claimed to cause fewer side-effects than reserpine and to be equally satisfactory as a hypotensive agent.

This claim has been tested in a clinical trial involving the use of a double blind technique in which sixteen hypertensive patients were each given reserpine 0.25 mg. t.d.s., deserpidine 0.25 mg. t.d.s., and a placebo tablet in varying order for periods of eight weeks each with a rest period between courses. The patients were selected on account of previous intolerance to reserpine, which usually had taken the form of mental depression.

The patients were closely questioned about side-effects, such as mental depression, lethargy, daytime somnolence, nocturnal insomnia, nasal stuffiness, increased appetite and looseness of the bowels. This unavoidably led to a fairly high incidence of these subjective symptoms, even during the periods when the patients were receiving the placebo tablets, but the incidence of side-effects was highest during the period when reserpine was being administered. There was considerable increase in weight in nearly all the patients while the two active drugs were being administered.

Although deserpidine caused fewer side-effects than reserpine, its effect in lowering blood pressure was also rather less than that of reserpine in the same dosage.

It seems likely therefore that deserpidine is a less powerful drug than reserpine both in causing side-effects and in reducing blood pressure. The trial does not, however, exclude the possibility that a larger dose of deserpidine might produce a larger fall in blood pressure without increase in side-effects.

RENAL FUNCTION STUDIES

A. J. Barnett, F. O. Simpson, P. Kincaid-Smith, V. Carson and M. Bick†

Hypertensive Patients

Determinations of sucrose clearance (a measure of glomerular filtration rate) and P.A.H. clearance (a measure of effective renal blood flow) in normotensive subjects and hypertensive patients have been continued with the aim of comparing these functions in the two groups. Concurrently, conventional renal function tests—concentration test, dilution test, water excretion test, urea clearance and creatinine clearance have been studied to discover

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whether there is a significant difference between the findings in hypertensive and normotensive persons, and whether there was a correlation between the urea and creatinine clearances and the sucrose clearance.

These data revealed a marked decrease in sucrose and P.A.H. clearance in hypertensives compared with normotensives. Also the filtration fraction (sucrose clearance/P.A.H. clearance) was higher in the hypertensive than normotensive group; this is probably due to the higher age of the hypertensives, as in the normal subjects the sucrose clearance did not alter with age, whereas the P.A.H. clearance was decreased in older people, giving a higher filtration fraction.

Neither the creatinine clearance nor the urea clearance correlated well with the sucrose clearance although there was a somewhat better correlation, in hypertensive subjects, between the creatinine and sucrose clearance than between the urea and sucrose clearance, suggesting that the creatinine clearance is a somewhat better index of glomerular filtration than the urea clearance.

There was a wide variation in water excretion in both normotensives and hypertensives. Concentration and dilution tests showed no appreciable differences between hypertensives and normotensives (in spite of the marked differences shown in the clearance tests). These tests therefore are not useful as a measure of renal function in hypertensive patients.

The Effect of Newer Hypertensive Agents

Acute and chronic observations on the effect of Darenthin and Ismelin on renal function as estimated by sucrose, P.A.H. and creatinine clearances suggest that these drugs may have a different effect on renal function from previous hypotensive agents. Acute experiments with Darenthin have consistently shown an increase in renal clearances after 2½-3 hours in spite of a marked hypotensive effect at this time. No previous hypotensive agent has been shown to increase renal clearances in this fashion, and if the results are confirmed in long term observations this may make an important contribution to the treatment of uraemic hypertensive patients.

DISEASES OF THE PERIPHERAL BLOOD VESSELS

ARTERIAL GRAFTING FOR OCCLUSIVE ARTERIAL DISEASE OF THE LOWER LIMBS

K. N. Morris† and A. J. Barnett

Difficulty in obtaining human artery grafts has frequently delayed treatment of patients with the procedures which have been used in the past six years. With the recent availability of "Teflon" (a synthetic, plastic material) this material has been used for grafts in sixteen patients to date. In only one of the sixteen cases was the graft non-functioning on discharge from the ward. In one patient the graft has ceased to function after discharge, leaving fourteen patients with functioning grafts. In the previous forty-six patients in whom human grafts were used there was an immediate failure in eight

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cases. In this respect "Teflon" grafts are not inferior (and probably superior) to human grafts. Since most of the "Teflon" grafts have been inserted less than six months ago, it is not possible to compare the long term results with those obtained using human grafts.

An analysis is in progress of the overall results from arterial grafting and a comparison of different methods. Since December, 1953, sixty-two grafts have been inserted in fifty-two patients. One patient has died as a result of the operation (from infection with an antibiotic resistant organism). The present position in respect to establishment and persistence of functioning grafts is as follows:

Grafts blocked before discharge	9
Grafts blocked since discharge	16
Grafts patent until time of patient's death	4
Grafts patent until patient lost from supervision	2
Grafts still patent	31
	62

Before the institution of "Teflon" grafting, two methods of insertion of human grafts were used: end-to-end and side-to-side (shunt) suturing.

A comparison of grafts inserted prior to December, 1956, allowing a possible three year survival, shows:

Method	Total Grafts	Grafts surviving three years
End-to-end	15	4
Side-to-side	20	10

Owing to the effect of possible increase in technical skill and variability in subject material, it is not possible to draw firm conclusions from the figures presented, but they suggest that the use of "Teflon" grafts is superior to that of human grafts in respect to immediate success, and a shunt technique is superior to an end-to-end technique in respect to graft survival.

Although the overall success rate (50% of grafts inserted over a period of six years patent at present) is less than hoped for, it is sufficiently great, in view of the relief afforded in successful cases, to warrant the continued performance of the operation.

DIET IN ATHEROSCLEROSIS

A. J. Barnett, C. C. Curtain and V. Carson

In the previous report an investigation to study the effect of diet in patients with occlusive arterial disease of the lower limbs was outlined. One group of twelve patients was given a normal diet and another group a diet low in animal fat and cholesterol with a supplement of maize oil. These patients have now completed their three months control period and first six months treatment period, and a preliminary assessment of the effects of treatment can be made.

For the first three months of the study both groups remained on their every day diet, and baseline values were obtained for performance in the step test, serum cholesterol, beta/alpha lipo-protein ratio and serum turbidity. One group was then given a diet low in saturated fat, the other was given a placebo. No significant difference has been observed so far between the diet and control groups with respect to clinical condition, serum cholesterol level or beta/alpha lipo-protein ratio. The control group, however, has shown a considerable reduction in serum turbidity, as assessed both by measurement of right angle scattering of white light and by measurement of serum absorption over the range 400-1000 mμ. The absorption measurements suggested that there had been a reduction in both the size and number of fat particles in the sera of a number of the diet group.

Chylomicron Sizing and Counting

Because it appeared that the serum chylomicrons might play an important role in this study, an investigation of chylomicron sizing and counting was made. As the chylomicrons are in constant Brownian motion, visual or photographic sizing is almost impossible. It was decided, therefore, to adapt a microphotometer originally constructed for the photoelectric measurement of interference fringes, for this purpose. A dark ground condensor was fitted to this instrument, and the chylomicrons were viewed in a capillary, similar to the hydro-blood cell propulsion chamber of Crosland-Taylor.

The output from the photometer was displayed on a double beam cathode ray tube which was photographed with an oscilloscope camera. The second beam of the cathode ray tube displayed pulses from a time base whose frequency remained constant only if the thermal expansion advance of the capillary counting chamber was operating correctly.

Chylomicron size and count was determined by measurements on the photographed cathode ray tube trace.

The size distribution of chylomicrons in individuals of all ages was found to be bimodal. In younger individuals the maxima were at 600 mμ and 800 mμ. In older individuals they were at 750 and 1400 mμ.

EVALUATION OF THE SYNCARDON†

E. A. Dodge

The Syncardon is an electronic instrument with a pneumatic pulsating pump which is claimed to increase peripheral blood flow. The principle of action is the application to the limb of an intermittent positive pressure coinciding with the pulse wave. The pulse wave is picked up in the area to be treated by a partially inflated cuff. The time interval between the R wave of the E.G.C. and the pulse impact on the cuff is adjusted on a video screen.

It is claimed that this instrument will increase skin temperature and peripheral circulation.

The skin temperature was measured by means of a thermocouple and spot galvanometer, before and after application of the machine, in six normal patients and four patients with peripheral vascular disease. The cuff was

†The Syncardon was kindly made available by Watson Victor Ltd.

applied to the ankle, calf and thigh in turn, and the pressure adjusted according to instructions.

No increase in skin temperature was found after treatment applied to one leg at room temperature for 20 minutes. Both limbs were then cooled in iced water and the temperature of the treated limb rose at the same rate as the untreated limb.

The same methods were employed in patients with peripheral vascular disease and the cuff applied alternately to the more severely affected and less severely affected limb. Again, after 20 minutes, there was no significant rise in skin temperature of the treated limb, and heat gain after cooling proceeded at the same rate when the cuff was not applied to that limb.

It has, therefore, been concluded that the Syncardon was ineffective in increasing skin temperature in both the patients and the normal controls.

ENERGY PRODUCTION IN THE MYOCARDIUM**

W. G. Nayler, M. W. McCulloch and T. E. Lowe

Data obtained in this study have yielded some information relating to cardiac failure induced to isolated toad hearts by prolonged recycling perfusion. The onset of failure appeared to be independent of the substrate available and was characterised by decreased mechanical efficiency. Whereas the sympathomimetic amines augmented the useful work output of the failing heart without changing the overall mechanical efficiency, the cardiac glycosides induced a sustained increase in the total work done by the heart which was paralleled by a marked rise in efficiency. Certain metabolic inhibitors, including D.N.P. and sodium salicylate, as well as ionic changes, inhibited the positive inotropic activity of the glycosides.

During the past year these observations have been extended, emphasis being placed on the mechanism whereby the glycosides improve the efficiency of the failing heart.

Two additional techniques have been used in this study, and enable isotonic and isometric contractions to be recorded.

POSITIVE INOTROPIC ACTIVITY OF CYTIDINE TRIPHOSPHATE

W. G. Nayler

Cytidine triphosphate (10 ug/ml) when added to a series of isolated hearts perfused at 25.0° C. produced a positive inotropic response resembling that previously recorded following the administration of glycosides and 9 alpha-fluorohydrocortisone to similar preparations. Both responses involved increased work output, raised efficiencies and augmented rates of oxidative metabolism. This similarity raises the question as to whether or not these other nucleotides may play a part in the normal transfer of energy during contraction and relaxation.

The effect of cytidine triphosphate was immediately apparent, indicating the probability that surface activity is involved. This activity may involve the phospholipid component of the membrane or alternatively may be associated with the binding of calcium at the membrane by the cytidine containing compound.

PLASMA—PRESSOR ACTIVITY

W. G. Nayler

It was previously noted that heparinised toad plasma displayed pressor activity when tested against the isolated perfused heart from the donor toad. It has now been shown that qualitatively this pressor response is identical with that recorded during glycoside activity, the raised level of work output being associated with an augmented rate of oxidative metabolism and an overall increase in efficiency. However, pretreatment of hearts with D.N.P. or quinidine sulphate which abolish the activity of strophanthin-G did not interfere with the pressor activity of plasma. It seems unlikely that strophanthin-G and plasma compete for sites of action since the action of plasma was not modified in any way by the presence of the glycoside.

The pressor activity of plasma showed seasonal variation in that the response was greatly lessened or absent during the winter months. Since pre-treatment of the toads (two days before use) with anterior pituitary extract partially restored the plasma activity, it seems probable that plasma contains a substance under hormonal control whose function it is to regulate the activity of the myocardium. The rapidity with which the plasma effect is evident indicates, as with cytidine triphosphate, that a surface binding mechanism governs to some extent the force of cardiac contraction.

IONS AND GLYCOSIDE ACTIVITY

W. G. Nayler and M. W. McCulloch

Anions

It is well established that anion substitution can markedly alter the force of contraction in skeletal muscle. It therefore seemed possible that substitution of NO_3, Br and I for the Cl normally contained in Ringer's solution might modify the glycoside response. In a series of experiments these substitutions were effected and no significant change in the response of the heart to Strophanthin-G was noted. It was therefore concluded that anions play no part in the mechanism of the inotropic response.

Cations

Ca/Na: Recent reports indicate that the tension produced in isolated ventricular strips is directly related to the calcium-sodium ratio at the cell membrane. It has been suggested that calcium and sodium compete for a common site on the membrane, the binding of calcium leading to enhanced contractility and the binding of sodium to depression. Experiments were carried out in the perfusion apparatus to determine whether or not the action of the glycosides, strophanthin-G and lanatoside C, could be fitted into this hypothesis.

Thus, in a series of spontaneously beating hearts varying proportions of sodium in the perfusate were replaced by lithium or sucrose so that constant tonicity was maintained and the inotropic activity of strophanthin-G (10 ug/ml) and lanatoside C (20 ug/ml.) accurately determined. As the sodium concentration was reduced the activity of the glycosides and associated changes in oxidative metabolism decreased so that when 50% of the sodium was replaced either by sucrose or by lithium the glycoside activity was almost abolished.

In another series of experiments the reduction in sodium concentration was paralleled by similar reductions in the calcium content. Normal glycoside activity was recorded in "low sodium–low calcium" perfusion fluids.

The force of isotonic contraction has been studied in isolated toad ventricles perfused with Ringer solutions containing varying concentrations of sodium. The sodium was replaced with isotonically equivalent amounts of lithium or sucrose. Records were made by the P.E. method. The effect of strophanthin-G in these Ringer solutions was recorded, and it was found that lowering the sodium concentration diminished the positive inotropic response of strophanthin-G.

These results suggest that the activity of the glycosides may lie in their ability to influence the relative proportions of sodium and calcium bound at the membrane. Since lithium was not utilised by the membrane as a substitute for sodium the binding of this latter ion must be specific. It is possible that this calcium sodium mechanism may not effect contractility directly, i.e., a secondary permeability factor related to K may be involved.

METABOLIC ACTIVITY OF GLYCOSIDES

W. G. Nayler

During these studies the respiratory quotients have remained unchanged during glycoside activity. To confirm this observation, estimation of lactic acid, pyruvic acid, phosphorylase a and b, and bound lipids have been made after perfusion with normal and with strophanthin enriched Ringer's solution. No significant changes were detected in this series. Similarly perfusion of hearts in the presence of strophanthin failed to alter significantly the phosphocreatine, inorganic phosphate, total acid soluble phosphate or phospho-lipid concentrations. In agreement with other workers, it was found that strophanthin-G (10ug/ml.) caused loss of K from the myocardium during two hours perfusion at 25.0° C.

RECORDING ISOTONIC AND ISOMETRIC CONTRACTION

M. W. McCulloch and J. L. Bremner

Isotonic

A photo-electric (P.E.) method of recording ventricular isotonic contractions in toad hearts has been used in preference to classical methods where the friction of the lever against the drum causes damping of the lever excursion. A thin rigid wire with a bead at one end is introduced into a cannula placed in the aorta, so that the bead rests against the endocardium. At the free end of the wire a light shutter lies directly between a constant light source and a P.E. cell, so that during ventricular contraction and relaxation the shutter is displaced and the light entering the P.E. cell varied. The fluctuating voltage so produced is fed into a D.C. amplifier and recorded on a direct inking recorder.

This method has been used for spontaneously beating and stimulated preparations. With the former care is taken not to damage the pacemaker, with the latter the pacemaker is destroyed by cauterisation and the ventricle stimulated electrically.

Isometric

Isometric contractions have been measured in strips of toad ventricle. The strip is attached to an electro-mechanical transducer, and the tie made taut hy an adjustable tension device. The strip is stimulated electrically and the tension produced fed from the transducer through a D.C. amplifier to a direct inking recorder.

THE ACTION OF ANIONS ON ISOTONIC CONTRACTION

M. W. McCulloch

Previous investigators have studied the action of anions in skeletal muscle. They reported that the tension and duration of a twitch in isolated frog skeletal muscle was increased in the order Bromide < nitrate < iodide when these anions were substituted for chloride in Ringer's solution. Since these anions have such a marked effect on skeletal muscle it was interesting to study their effect on the contractible behaviour of the myocardium. Isotonic force of contraction in spontaneously beating and stimulated toad hearts was measured by the P.E. method.

Replacement of chloride by hromide, nitrate and iodide in both spontaneously beating and stimulated preparations failed to significantly increase the force of contraction. In a high percentage of preparations perfused with iodide Ringer toxic effects were noted. This toxicity could be reversed by the addition of strophanthin-G, plasma or calcium chloride, and made worse by the addition of potassium chloride.

The effect of anions on the contracture produced in toad ventricular muscle by Ringer solution containing 100mM KCl was also studied. Contractures induced in chloride Ringer solution were compared with those found in bromide, nitrate and iodide Ringer. There was no significant difference in the contractures.

The results imply that the anions play only a minor part in regulating cardiac activity.

MEMBRANE POTENTIALS OF CARDIAC MUSCLE

P. Emery

Techniques of recording membrane potentials with microelectrodes have continued to be used in conjunction with other techniques in these investigations of cardiac muscle activity.

HISTOLOGY OF CARDIAC MUSCLE

F. O. Simpson and S. Oertelis†

The fine structure of heart muscle has been studied in two species, the sheep and the toad. In the sheep, the appearances described in mammalian hearts by other workers have been confirmed. In the toad, the myocardial fine structure has not previously been extensively studied, and it is seen to differ in some respects from that of mammals. The differences are partly due to the sinusoidal blood supply of the amphibian ventricular myocardium, but in addition the M bands appear to be absent in the myofibrils of the toad, and the intercalated discs have a much simpler structure in toads than in mammals.

Some applications of electron microscopy to histochemistry have also been studied and the presence of respiratory enzymes (dehydrogenases) in mitochondria has been confirmed.

HISTOCHEMISTRY OF CARDIAC MUSCLE

M. W. McCulloch

An attempt was made to study the effect of drugs histochemically at the enzyme level in cardiac muscle. It was hoped to correlate these studies with physiological and metabolic changes recorded in the various types of perfusion apparatus.

Toad (Bufo marinus) and mouse hearts were used and sections cut of both auricle and ventricle. The hearts were frozen in isopentane surrounded by dry ice, and sections cut at –13° C. (toad) and –8° C. (mouse), using a Cambridge-rocker microtome mounted inside a cryostat. The sections were freeze dried and then stained.

The frozen sections were compared with paraffin sections in which the hearts were fixed in formalin, embedded in paraffin, and cut using a Cambridge-rocker microtome at room temperature. The two methods were compared in order to ensure that the tissue was not destroyed by the frozen technique. Paraffin sections are better than frozen, but enzymes are destroyed by the former technique.

The enzyme, succinic dehydrogenase, was stained by the method of Rosa and Velardo in toad and mouse ventricular sections. A comparison was made between sections incubated with no drug, with strophanthin-G or with nor-adrenaline. No difference in the degree of staining was detected. The effect of the above drugs on the degree of glycogen staining was similarly studied. No difference was detected.

Ventricular sections from toad hearts perfused with Ringer's solution for one hour were compared with those cut from hearts frozen immediately they were removed from the animal. Strophanthin-G was added to some of the perfused hearts. These sections were stained with the standard haematoxylin and eosin stain, and no histological difference was detected.

†Commonwealth Serum Laboratories, Melbourne.

In an attempt to localise potassium at the cell membrane further toad ventricular sections were stained for potassium, but specific localisation of this ion was not found practical.

Sections from toad auricles were stained with osmic acid to illustrate pacemaker tissue. The pacemaker area is small, and it was found difficult to get consistent results.

Rabbit adrenals were used to demonstrate a stain for nor-adrenaline and adrenaline by the method of Hillarp and Hokfelt. Although this stain was successful in adrenals, the amount of nor-adrenaline and adrenaline in cardiac tissue is sub-threshold for this staining technique.

It was not found possible to obtain the localisation of enzymes as we had hoped, mainly due to diffusion difficulties. It would appear from these results that strophanthin-G has little metabolic effect.

CARDIAC SURGERY**

G. R. Stirling, D. Race, K. N. Morris† and F. Kinross†

EXTRA-CORPOREAL PUMPS

Total Body Perfusion

During 1959 a further twenty-six cases were subjected to operation using total body perfusion. The pump-oxygenator used was a Kaye-Cross spinning disc type which has been somewhat modified. The oxygen tension in arterial blood has been monitored directly with a Clarke polarograph electrode and the rate of rotation of the discs controlled so as to maintain an oxygen tension of 100 to 180 mm. of mercury in the arterial blood leaving the unit.

In the first fifty cases submitted to total body perfusion total body oxygen consumption and mean arterial blood pressure are among the variables intensively studied. There is a considerable individual variation in different age groups of the total oxygen consumption during perfusion, and the mean values have varied from 128 ml. oxygen per minute per square metre body surface area in subjects under five years of age to 100 ml/min/m^2. in subjects over 20 years of age. Oxygen consumption was found to be influenced by the rate of perfusion so that optimum rates of oxygen consumption were not achieved when the perfusion index was less than 2.4 litres/min/m^2. The oxygen consumption during perfusion is usually 10 to 25 per cent less than that recorded in similar subjects under basal conditions. This reduction is probably produced by general anaesthesia, reduction in temperature and a considerable reduction in myocardial energy requirements.

The mean arterial blood pressure recorded during total body perfusion is considerably less than normal, and shows no constant relationship to the rate of perfusion. It is suggested that the major determinant of arterial blood pressure during total body perfusion is the level of the systemic vascular resistance.

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Bi-Ventricular By-Pass and Deep Hypothermia

The technique evolved by Drew of London has been investigated. In summary this technique involves the use of two independent extra-corporeal circuits, one acting as an accessory left ventricle and the other as an accessory right ventricle. A simple blood heat exchanger is incorporated in the left heart by-pass circuit to allow rapid cooling or warming of the blood. Drew's technique of cooling dogs to 15° C. was carried out and, at this temperature, the circulation was arrested for 40 minutes. There were no survivors in eight such experiments. Observations revealed an increase in blood volume at low temperature and a fall in oxygen consumption linearly related to the fall in oesophageal temperature. A 10° C. drop in temperature is associated with a 40 to 50% reduction in total body oxygen consumption.

The relative rates of fall in temperature of the oesophagus, rectum and brain were studied. A disturbing finding was that not infrequently there was a significant time lag between cerebral and oesophageal or rectal cooling. In some experiments there was 10° C. differential between the oesophagus and cerebral temperatures. The basis for the variations observed was not fully analysed, but an important factor was the flow rate maintained through the extra-corporeal circuit.

STUDIES ON VENTRICULAR FUNCTION

The technique previously described for studying ventricular function has been used to determine the effects of the following on this factor:

1. The anaesthetic agent Halothane.
2. Hypothermia.
3. Cardiac asystole—
   a. induced by potassium citrate.
   b. induced by anoxia.

Halothane uniformly depressed ventricular function. The magnitude of the effect was proportional to the dosage of halothane employed and was rapidly reversed after halothane inhalation was discontinued.

Reduction in cardiac temperature from 37° C. to 28-30° C. was not associated with any significant impairment of ventricular performance.

Studies on the effects of the various manoeuvres for arresting the heart on ventricular performance are still in progress and remain incomplete. It is clear, however, that periods of more than 20 minutes of asystole produced by the Melrose technique are followed by a significant reduction in ventricular function. Preliminary studies on the effects of short periods of simple anoxic arrest indicate that this procedure may be less deleterious. The injury produced by asystole induced by either technique may be greatly reduced by very careful decompression of the heart during the period of asystole and by the use of an optimal period of cardio-pulmonary by-pass after cardiac activity has resumed. It would seem that the currently used techniques for the production of asystole in clinical open heart surgery are still far from

the ideal. It has been previously shown that prolonged cardio-pulmonary by-pass itself has no deleterious effect on ventricular function.

AORTIC VALVE DISEASE

Studies on the Pathology and Surgery of Aortic Valve Disease

Over 50 specimens of aortic valve disease, both congenital and acquired, were studied. Congenital aortic stenosis may be due to fusion of the commissures of the valve, or to the presence of a diaphragm immediately below or immediately above the valve. The majority of cases with acquired aortic stenosis show fusion of the commissures and rigidity of the cusps due to calcification is evident. In some cases there may be massive calcification in the cusp without commissural fusion. Current techniques for the surgery of aortic stenosis are ineffective in the management of this type of valve.

A series of aortic valves obtained after surgical intervention was studied. In these cases surgical relief failed for one of three reasons: firstly, failure to relieve adequately the aortic stenosis, usually in cases with heavy calcification; secondly, the production of aortic incompetence by laceration or fracture of the valve cusps; and, thirdly, left ventricular failure due to an intolerable stress placed on the massively hypertrophied left ventricle.

The Use of Left Ventricular By-pass and Hypothermia in the Treatment of Aortic Stenosis

The causes of failure in the surgical treatment of aortic stenosis by blind methods led us to design a technique of open aortic valvotomy using left ventricular by-pass and moderate hypothermia. Under these circumstances the aortic valve can be exposed for as long as ten minutes at 28° C. The myocardium is protected by hypothermia, and the use of a left heart by-pass to lessen the load on the left ventricle immediately before and after valvotomy seems a logical addition to the technique. It has been found possible to perform a deliberate valvotomy without the production of incompetence and with a reasonable relief of the stenosis in most cases.

This technique has now been applied to the treatment of thirteen cases of aortic stenosis of both congenital and acquired types. In our clinical experience with these cases ventricular fibrillation which so commonly accompanies interference when no assisted circulation is used, has not occurred. It is possible that the use of halothane and the maintenance of an adequate circulation due to the left heart by-pass are major factors in the freedom from this serious complication.

Surgery of Aortic Incompetence

A study on the surgery of aortic incompetence has commenced. The technique described by Lewis and others of bicuspidisation of the aortic valve has been carried out. It is possible to convert the normally three cusped aortic valve to a competent bicuspid valve, by a plastic procedure on the aortic annulus and the ascending aorta. This technique has been carried out in dogs, and it is confirmed that this may be a promising technique for the treatment of human cases of aortic incompetence in whom the major defect is one of annular dilatation.

SERUM PROTEINS IN KURU*

C. C. Curtain†

Abnormal Serum Globulins in Kuru Patients

The initial findings of disturbed serum globulin patterns in Kuru have been confirmed. A large proportion of patients in the intermediate stages of the disease had high beta globulins, and those in the terminal stages, as a rule, had high gamma globulins. It has been possible to observe six patients over the whole course of their disease (about nine months) and in all cases a rise in beta globulin occurred in the first six months. In the last three months this was replaced in five of the six patients by an elevated gamma globulin.

It is of interest that amyloid-like deposits in the central nervous tissues of kuru victims have been observed by Klatzo, Gajdusek and Zigas, for amyloid deposition in other organs, although rarely in the C.N.S., has been often observed in hyperglobulinaemia.

If the difficulties of obtaining fresh frozen and dried sections in the field can be overcome, it is hoped to carry out fluorescent antibody studies in an attempt to establish the relationship between these deposits and the kuru globulins.

Serum Protein Chromatography in the Study of Kuru

Preliminary studies, carried out on an analytical scale, showed that good separations of the kuru globulins could be obtained on D.E.A.E. cellulose. At present a fully automatic apparatus is being constructed for preparative chromatography.The isolation of the kuru globulins will facilitate more detailed investigations into their structure and relationship to the other serum proteins. This information may help decide whether the distorted serum protein patterns of Kuru are part of the patient's reaction to the disease, or whether the abnormal globulins play some fundamental role in its pathogenesis.

Caeruloplasmins in New Guinea Natives

During the analytical runs on D.E.A.E. cellulose it was noticed that the caeruloplasmin of some Melanesian sera showed marked heterogeneity on the column. It is proposed to investigate this finding further by a combination of preparative chromatography and starch-gel electrophoresis.

Haptoglobins and Kuru

The studies on the association of the haptoglobin type 2 (Hp_2) gene with Kuru have been extended. Close attention has been given to a comparison of the haptoglobin gene frequencies of the Fore and their kuru-free but otherwise similar† neighbours. The largest group studied so far is the Auina, on the north-east boundary of the kuru region. The ratio of Hp_2 to Hp_1 in the Auina is 0.39 : 61, in the normal Fore it is 0.34 : 66, and in the kuru series 0.61 : 39. From these figures, the published data of Bennett

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*In collaboration with Dr. D. C. Gajdusek, National Institute of Neurological Diseases and Blindness, Public Health Service, Bethesda, Maryland, U.S.A., and Dr. V. Zigas, Department of Public Health, Port Moresby.

†On the basis of blood group gene frequencies determined by Mr. R. T. Simmons, of the Commonwealth Serum Laboratories.

and Rhoads, of Adelaide, and mortality data supplied by Zigas and Gajdusek, it has been tentatively calculated that kuru has not expressed itself for more than four or five generations. This calculation is based on the rate of loss of the Hp_2 gene from the Fore population through kuru deaths, assuming that possession of this gene confers no reproductive or survival advantage on those who are not affected by kuru. It was also assumed that the Fore once had the same Hp_2 to Hp_1 ratio as their near-neighbours.

The concept of the relatively recent expression of kuru is an important one. There is abundant evidence that the disease is influenced by genetic factors. Because of its widespread incidence (over 100 cases occur per annum in a population of 11,000), it may be postulated that the genes responsible for kuru had no effect until the appearance of a change in the Fore environment a few generations ago.

If further haptoglobin studies strengthen this hypothesis then a search for such an environmental factor will be an important part of future investigations on kuru.

HETEROGENEITY OF HUMAN HAEMOGLOBIN

C. C. Curtain

Further studies on borate-haemoglobin "complexes" have shown that the interaction is essentially a homogeneous one. It is possible that the heterogeneity observed in the descending limb on electrophoresis in pH 8.6, 0.025M borate buffer is due to microheterogeneity of the haemoglobin itself, and not to the formation of a range of haemoglobin-borate complexes.

Investigations were continued on haemoglobins obtained from patients suffering from various anaemias. It was found that haemoglobins from patients with high reticulocyte counts had less fast material in the descending pattern in borate than normal.

An attractive explanation for this phenomenon is that haemoglobin from young red cells exhibits less microheterogeneity than that from old cells. Possibly ageing of the red cell is accompanied by configurational changes in the protein. A full theoretical treatment of the changes observed in the electrophoresis patterns is being made in an attempt to see if it is possible to devise an electrophoretic test which could be used to obtain the age distribution of a given sample of red cells.

A NEW METHOD OF ESTIMATING HAEMOGLOBIN A_2 IN THE DIAGNOSIS OF THALASSAEMIA MINOR

C. C. Curtain

Estimation of the minor haemoglobin component, A_2, is now regarded as an important diagnostic test for Thalassaemia minor (Thalassaemia trait, microcythaemia). The estimation originally introduced by Kunkel and Wallenius is carried out by electrophoresis of red cell haemolysates in blocks of starch sedimented from pH 8.6 $I = 0.1$ veronal buffer. The slowly migrating haemoglobin A_2 is eluted from the starch and determined photometrically. The new method of estimation uses a starch-gel electrophoresis technique similar to that of Smithies, and the sensitive o-dianisidine hydrogen-peroxide stain of Owen. The haemoglobin A_2 can be estimated by direct photometry of the starch-gels. The method is rapid, and enables the use of very small samples (0.05 ml.) of haemolysate.

ELECTROPHORESIS AND CHROMATOGRAPHY SCANNER

C. C. Curtain

The amount of work involved in the studies on Melanesian sera emphasised the need for a recording scanner for paper electrophoresis patterns.

A double beam scanner was built using cadmium sulphide photo-conductive cells, the output from which was amplified by a direct-coupled transistor amplifier to operate a pen recorder. The paper strips were moved through the scanner by Palmer kymograph, which made possible a wide range of scanning speeds. With an ultraviolet light source and suitable filters, the instrument can be used to scan fluorescent chromatograms.

CELLULAR ENZYMES

ENZYMES IN LEUCOCYTES***

R. G. Wyllie

Neutrophil leucocytes in myeloid leukaemia have been shown to have abnormalities in their enzyme systems, and the defect in alkaline phosphatase, in particular, is being investigated both in myeloid leukaemia and other diseases.

Wackstein (1946) histochemically demonstrated alkaline phosphatase in neutrophils by the method of Gomori and Takamatsu (1939). Films of human blood showed neutrophils containing various proportions of alkaline phosphatase, and this proportion was found to be highest in infected patients and lowest in patients suffering from myeloid leukaemia. Subsequent workers have confirmed these results, extended the range of disease types studied to include all major pathological processes, developed a quantitative histochemical method, and found that the neutrophil alkaline phosphatase content is independent of the leucocyte count and the level of serum alkaline phosphatase.

A survey of 500 patients has shown that in 96 per cent of people neutrophil alkaline phosphatase content is a sensitive index of the severity and progress of acute inflammation, infarction and necrosis of tissue, and neoplastic proliferation of bone marrow cells, myeloid leukaemia excepted. The diagnostic value of this estimation in equivocal cases has been demonstrated on many occasions. The remaining 4 per cent of people are weak reactors, and at present the test is of no diagnostic value in subjects of this group.

Specimens of bone marrow, peripheral blood and sections of acute inflammatory lesions made at the same time show that neutrophils at the inflammatory site contain many times more alkaline phosphatase than bone marrow or peripheral blood cells. This observation has been confirmed by making minimal lesions which show high concentrations of enzyme in neutrophils of the inflammatory exudate but no change in level of peripheral blood. An attempt to develop an in-vitro method of inducing increased amounts of alkaline phosphatase in neutrophils is currently being sought.

With this tool it is proposed to explore the nature of the alkaline phosphatase defect in myeloid leukaemia neutrophils and those of the weak reactors.

ENZYMES IN ERYTHROCYTES

A. D. McCutcheon

Drug-induced Haemolytic Anaemia

Recent work has shown that the erythrocyte enzyme, glucose-6-phosphate dehydrogenase is deficient in some patients with a haemolytic anaemia due to various drugs (primaquine, phenacetin, acetanilid, some sulphonamides, nitrofurantoin) or due to eating broad beans (favism). Two techniques have been established for the investigation of such cases. First, the glutathione stability test, in which the concentration of reduced glutathione (G.S.H. in erythrocytes is measured before and after incubation with hydroxylamine or acetyl phenylhydrazine. G.S.H. is a reducing substance capable of protecting cells to some extent from the action of various oxidising agents.

Normal cells maintain G.S.H. in a reduced state by means of the enzyme glutathione reductase, which is dependent on the presence of reduced triphosphopyridine nucleotide (T.P.N.H.). T.P.N.H. is generated primarily from the oxidation of glucose-6-phosphate by glucose-6-phosphate dehydrogenase (G6P.D.). Cells deficient in G6P.D. are therefore unable to maintain G.S.H. in a reduced state, because of a secondary inability to produce T.P.N.H.

Secondly, the in-vitro Heinz body incubation test, in which blood is incubated with hydroxylamine or acetyl-phenylhydrazine for four hours, stained with two per cent crystal violet, and the percentage of red cells with five or more Heinz bodies counted. This test gives an indication of the susceptibility of erythrocytes to damage by various agents, and in patients with deficiency of G6P.D., more than 45 per cent of red cells contain five or more Heinz bodies. These tests have been applied to a group of patients with drug-induced haemolytic anaemia (mostly due to phenacetin), all of whom gave normal results. Some other mechanism must be sought to explain the occurrence of haemolysis in these cases.

In contrast, one patient of Italian origin who was thought to have had an acute haemolytic anaemia after eating broad beans, was found to have abnormal Heinz body and G.S.H. stability tests—confirming the presence of G6P.D. deficiency.

Sulphaemoglobinaemia

A high level of reduced glutathione (G.S.H.) was found in the blood of a patient with sulphaemoglobinaemia, and this prompted the investigation of similar cases. Nine cases were studied, four of whom had considerably elevated erythrocyte G.S.H. values.

The present view that sulphaemoglobinaemia results from the action of drugs (phenacetin), and hydrogen sulphide absorbed from the bowel in constipated people, is considered unsatisfactory for several reasons.

It is postulated that an elevated erythrocyte G.S.H. concentration increases the susceptibility of a subject to sulphaemoglobin formation, and that the source of hydrogen sulphide is probably within the erythrocyte rather than the bowel, being liberated during the breakdown of oxidised glutathione (G.S.S.G.).

CYTOTOXIC ACTION OF NITROMIN

J. W. W. Thomson

The control of neoplasia by chemotherapy is an attractive idea, and various cytotoxic agents have been used for this purpose. In the present investigation the effects of nitromin administration on normal rabbits has been studied.

"Nitromin" has a chemical structure—methyl-bis-(beta-chloroethyl) amine-N-oxide hydrochloride—and was discovered during a study of growth-retarding substances by Ishidate and Yoshida in Tokyo. It has been used clinically for some time in cases of leukaemia and Hodgkin's disease, and given in a dose of 0.5-2 mg/Kg.

It is recognised as a dangerous substance, being a strong marrow depressant, producing leucopenia and other side effects such as nausea, vomiting and anorexia.

The results of prolonged high dosage on rabbits have been investigated. Two groups, each of five rabbits, received Nitromin. One group were given 4mg/Kgm for six doses over seventeen days, the other group eight mg/Kgm for twelve doses over 56 days. As this cytotoxic substance has its maximum effect on the cell in active mitoses scattered, but high doses, were administered rather than daily exhibition.

In the first group (4mg/Kg body weight) the average fall in the white cell count was 11,430, and was shown between the sixth and ninth days of treatment. The average fall in haemoglobin was 30%. The condition of the rahbits at this time was poor, but a five-day break without Nitromin allowed an immediate rise in white count and improvement in the amount of food eaten. All counts had returned to normal limits twelve days after therapy had finished. Two rabbits were killed, and no histological or macroscopic abnormality noted. The others have subsequently been used for breeding with success.

In the second group (8 mg/Kg body weight), one rabbit was found dead seven days after commencement of therapy. This rabbit had a haemoglobin 65% and a W.B.C. of 15,450 in the day prior to death. Post mortem revealed no cause for death.

This group had a bigger dosage, but over a much longer period, and the average fall in white cell count was 5900. The cell count recovered slowly, but had returned to normal fifteen days after the course ended. The animals appeared to be in fair condition during treatment. The average fall in haemoglobin was 12%, and this too recovered quickly.

From these results it is concluded that the laboratory rabhit will tolerate fairly high doses of "nitromin", and if a method of transplanting human tumours with a reasonable number of positive results could be found then the anti-cancer effect of "nitromin" could be easily tested.

CHOLESTEROL AND BILIARY CALCULI

J. W. W. Thomson

Although much work has been recorded on the feeding of animals with cholesterol to produce biliary calculi, the dosage level used has usually been grossly in excess of normal physiological intake.

A series of rabbits was therefore fed with 0.24 gm. of 3 alpha cholesterol every second day. Rabbits were killed at varying times, and the biliary tract and liver examined histologically.

No biliary calculi or sludge was seen in rabbits killed up to the thirtieth day of ingestion of cholesterol, but a number killed after the fortieth day had biliary calculi.

RENAL BIOPSY

P. Kincaid-Smith

This procedure is now being performed regularly, and fourteen successful biopsies have been done in the past five months. There has been one failure. Histological examination of this material has been particularly useful in determining the underlying lesion in anuric patients admitted for haemodialysis to determine whether a potentially recoverable lesion is present. This may save many futile dialyses in an unrecoverable condition which cause distress to patient and relatives.

Valuable information about prognosis and treatment has also been obtained in certain generalised glomerular lesions such as nephritis, lupus erythematosus, Henoch Schonlein pupura and Goodpasture's syndrome.

CONN'S SYNDROME

A. J. Barnett and F. O. Simpson

A case of primary aldosteronism (Conn's syndrome), the second to be discovered in this unit, was studied. A metabolic balance study was conducted, and the effect observed of a high potassium intake and of the administration of a reputed aldosterone antagonist, a spirolactone, "Aldactone" (Searle). It was found possible to induce a positive potassium balance and negative sodium balance by the administration of a high potassium supplement and thus the biochemical abnormalities were largely corrected before operation. Administration of Aldactone in a dose of 1G per day had little effect, whereas a dose of 2.5G per day produced increased loss of sodium. The administration of the drug after operative removal of the tumour produced approximately half the effect before operation. There was no appreciable effect on the potassium balance.

A peculiar feature noted in this case was a paradoxical response to the injection of pitressin, following which there was an actual diuresis, instead of the normal anti-diuresis.

SCLERODERMA

A. J. Barnett

Over the past ten years a number of patients with scleroderma and Raynaud's phenomena have been studied, and the clinical histories of 27 patients with these two features analysed and the vascular disturbance studied. Clinically the patients can be divided into four groups on the basis of whether or not the onset was typical of Raynaud's disease and whether or not the

scleroderma was localised to the hands. In general, in patients with scleroderma localised to the hands visceral disturbance was not prominent, whereas in patients with scleroderma of other parts as well (diffuse scleroderma) visceral lesions of various types were common.

The vascular abnormality in the various groups were investigated by calorimetry, a cold immersion test, an arterial occlusion reactive hyperaemia test, and by arteriography. In all groups there was evidence of structural arterial disease.

The treatment of these patients is still unsatisfactory, but adrenal cortical hormones appear to produce subjective benefit and amelioration of skin changes in some cases.

SOCIAL ASPECTS OF CARDIOVASCULAR DISEASES

B. B. Thomas†, G. I. Howard‡ and T. E. Lowe

During the latter part of this year a pilot study was conducted to ascertain the effects produced on the economic and social life of patients and their families by cardiovascular diseases. A series of 100 consecutive admissions to the hospital for these conditions was taken as a sample, and included 92 adults and eight children. In each case the patient and/or his relatives were interviewed by an almoner.

This small survey indicates that many problems, about which little factual information is available, exist in this community. These relate to the degree of physical disability, financial embarrassment, rehabilitation and re-employment of adults. The number of children in the survey is too small for any valid conclusions to be drawn.

As a result of this pilot study, a more detailed and prolonged investigation has been planned for 1960.

†Senior Almoner—Alfred Hospital.

‡Medical Superintendent—Alfred Hospital.

PAPERS PUBLISHED DURING 1959

BARNETT, A. J.—"Experience with Use of Ganglion-blocking Agents in Hypertension". *Post-grad. Comm. Med. Bull., Univ. Sydney*, Vol. 14 (1958), p. 199.

BARNETT, A. J., and M. C. DAVIS.—"Phaeochromocytoma: Diagnosis and Management". *M.J.A.*, Vol. 2 (1959), p. 396.

CURTAIN, C. C.—"Starch-gel Electrophoresis of Melanesian Sera". *Aust. J. Sc.*, Vol. 21 (1959), p. 195.

CURTAIN, C. C.—"Possible Sites of Macroglobulin Synthesis: A Study made with Fluorescent Antibody." *Aust. Ann. Med.*, Vol. 8 (1959), p. 143.

CURTAIN, C. C.—"The Use of Acquired Immunological Tolerance and Immunological "Paralysis" in the Study of the Antigenic Relationships of Normal and Abnormal Serum Globulins." *Brit. J. exp. Path.*, Vol. 15 (1959), p. 255.

EMSLIE-SMITH, D., G. E. SLADDEN and G. R. STIRLING.—"The Significance of Changes in the E.C.G. in Hypothermia". *Brit. Heart J.*, Vol. 21 (1959), p. 343.

FANTL, P.—"Thrombin and the Thromboplastic Activity of Plasma". *Aust. J. exp. Biol. & Med. Sc.*, Vol. 36 (1958), p. 581.

FANTL, P.—"The Influence of Certain Metal Salts on Blood Coagulation." *Aust. J. exp. Biol. & Med. Sc.*, Vol. 37 (1959), p. 309.

FANTL, P.—"Nomenclature of Blood Clotting Factors. Report of International Committee for the Standardisation of the Nomenclature of Blood Clotting Factors".

FANTL, P., and H. A. WARD.—"Influence of Organ Extracts on Blood Clotting Factors". *Thromb. et Diath. Haemorr.*, Vol. 3 (1959), p. 491.

LOWE, T. E.—"The Nature of the Control of Body Fluid Volume in Man." *Physics in Med. & Biol.*, Vol. 4 (1959), p. 126.

LOWE, T. E.—(Technical assistance of June Sheath).—"The Role of Body Fluid Volume and Plasma 'Active' Osmotic Pressure in the Control of Urine Flow and Fluid Intake in Man." *Aust. Ann. Med.*, Vol. 8 (1959), p. 113.

MARSHALL, R. J., A. E. DOYLE and J. R. E. FRASER.—"Reactivity of Forearm Vessels to Vasoconstrictor Substances in Hypertensive and Normotensive Subjects". *Clin. Sci.*, Vol. 18 (1959), p. 441.

NAYLER, W. G.—"The Action of Fluothane, Chloroform and Hypothermia on the Heart." *Aust. J. exp. Biol. & Med. Sc.* 37 (1959), p. 279.

NAYLER, W. G.—"Positive Inotropic Activity of Cytidine Triphosphate". *Nature*, Vol. 184 (1959), p. 67.

STIRLING, G. R., R. de WALL, H. E. WARDEN, R. L. VARCO, C. WALTON LILLEHEI. "Open Heart Surgery Using Total Body Perfusion". *Post Grad. Med.*, Vol. 25 (1959), p. 156.

PAPERS ACCEPTED FOR PUBLICATION

BARNETT, A. J.—"Scleroderma and Raynaud's Phenomenon." *Alfred Hospital Clinical Reports.*

BARNETT, A. J., and W. C. BOAKE.—"Cigarette Smoking and Occlusive Arterial Disease of the Legs." *M.J.A.*

BARNETT, A. J., and I. K. FRANCIS.—"Amnion Implantation in Peripheral Arterial Disease." *Alfred Hospital Clinical Reports.*

BARNETT, A. J., and F. O. SIMPSON.—"Treatment of Hypertension with Combination of Ganglion Blocking Drugs and Chlorothiazide." *M.J.A.*

LOWE, T. E.—"The Concept of a Volume Receptor." *Aust. Ann. Med.*

McCUTCHEON, A. D.—"Sulphaemoglobinemia and Glutathione." *Lancet.*

STIRLING, G. R.—"The Surgery of Atrial Septal Defect." *Alfred Hospital Clinical Reports.*

PAPERS SUBMITTED FOR PUBLICATION

CURTAIN, C. C.—"A Study of the Serum Proteins in Natives from the Kuru Region of New Guinea." *J. Clin. Invest.*

CURTAIN, C. C.—"A Photoelectric Comparator for Rayleigh Fringe Electrophoresis Patterns". *J. Sc. Instr.*

FANTL, P. and H. WARD.—"Clotting Activity of Maternal and Foetal Sheep Blood". *J. Physiol.*

NAYLER, W. G., and M. McCULLOCH.—"The Positive Inotropic Activity of Plasma". *Aust. J. exp. Biol. & Med. Sc.*

NAYLER, W. G., and M. McCULLOCH.—"The Actions of Anions on Cardiac Muscle". *Aust. J. exp. Biol. & Med. Sc.*

SIMPSON, F. O.—"The Assay of Anti-diurctic Hormone by the Use of Toads." *Aust. J. exp. Biol. & Med. Sc.*

STIRLING, G. R., K. N. MORRIS, F. KINROSS.—"Total Body Perfusion." *Aust. & N.Z. J. Surg.*

STIRLING, G. R., K. N. MORRIS, R. H. ORTON, W. C. BOAKE, D. R. RACE, FAY KINROSS, J. W. THOMSON, W. CROSBY.—"Halothane and Circulatory Occlusion". *Brit. J. Anaesth.*

LECTURES DELIVERED DURING 1959

"Cardiac Abnormalities in Scleroderma"—*Victorian Cardiac Group.*	A. J. BARNETT
"Serum Proteins and Kuru"—*Victorian Society of Pathology & Experimental Medicine.*	C. C. CURTAIN
"Coagulation in Open Heart Surgery"—*Alfred Hospital Clinical Society.*	P. FANTL
"Blood Coagulation"—*Biochemistry Department, University of Melbourne.*	P. FANTL
"Influence of Organ Extracts on Blood Clotting Factors"—*Victorian Society of Pathology & Experimental Medicine.*	P. FANTL
"The Cellular Basis of the Electrocardiogram"—*Cardiac Society of Australia & New Zealand.*	T. E. LOWE
"Simulation of Biological Systems by Mechanical Devices"—*Seminar on Computors in Biology, Baker Institute.*	T. E. LOWE
"Your Heart and You"—*Constitutional Club.*	T. E. LOWE
"Cardiac Research"—*Rotary Club, Prahran.*	T. E. LOWE
"Drug-induced Haemolytic Anaemia"—*Alfred Hospital Clinical Society.*	A. D. McCUTCHEON
"Electron Microscopy of Cardiac Muscle"—*Victorian Cardiac Group (in collaboration with S. Oertelis, Commonwealth Serum Laboratories).*	F. O. SIMPSON
"Electron Microscopy of Cardiac Muscle"—*Alfred Hospital Clinical Society.*	F. O. SIMPSON
"Microstructure and Function of Cardiac Muscle"—*Victorian Society of Pathology & Experimental Medicine.*	F. O. SIMPSON
"The Pathology, Physiology and Selection of Patients for Surgery of Aortic Stenosis"—*Royal Australasian College of Surgeons, Sydney.*	G. R. STIRLING
"The Effects of Various Surgical Procedures on Ventricular Performance"—*Royal Australasian College of Surgeons, Sydney.*	G. R. STIRLING
"The Surgical Treatment of Atrial Septal Defect"—*Royal Australasian College of Surgeons, Melbourne.*	G. R. STIRLING

"A New Laboratory Technique for the Diagnosis of Suppuration"—*Alfred Hospital Clinical Society.* R. G. WYLLIE

"Neutrophil Alkaline Phosphatase in the Diagnosis of Acute Inflammation and Myocardial Infarction"—*British Medical Association, Clinical Pathology Section.* R. G. WYLLIE

"Neutrophil Alkaline Phosphatase in the Diagnosis of Acute Inflammation"—*Victorian Society for Pathology & Experimental Medicine.* R. G. WYLLIE

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

Revenue Account for the Year Ended 31st December, 1959.

EXPENDITURE.				INCOME.					
Drugs, Chemicals, Provisions, etc.	£1,405	0	8	Donations—					
Fuel and Lighting	130	18	2	Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions	£15,800	0	0		
Instruments and Glassware	1,606	15	5	Marian and E. H. Flack Trust	350	0	0		
Insurance	461	15	11	George F. Little Trust	148	6	6		
Library Maintenance	670	18	5	Mr. and Mrs. Edgar Rouse	105	5	0		
Printing and Stationery	510	9	3	Kodak (Australasia) Pty. Ltd.	50	0	0		
Repairs and Renewals	216	5	7	Eagle Star Insurance Company Ltd.	10	10	0		
Salaries and Wages	20,710	14	7	Dr. and Mrs. John Rouse	5	5	0		
Telephone	142	19	4	Mr. J. C. Habersberger	5	5	0		
Travelling Expenses	70	3	0	Mrs. Lawrence Simpson	5	5	0		
Sundries	1,075	18	7	Mr. Elvin Teasdale	5	0	0		
Workshop Equipment	208	16	8	Mr. Ernest Page	2	2	0		
				Miss N. E. Cameron	2	2	0		
				In Memory of Harry D. Giddy	5	0	0		
				In Memory of Dr. Merrill C. Sosman	5	0	0		
				In Memory of Myra Wiley	5	0	0		
				In Memory of E. T. Wilkinson	2	2	0		
								£16,506	2 6
				Grants-in-aid of Research—					
				National Health and Medical Research Council	3,943	19	4		
				Anti-Cancer Council of Victoria	2,231	5	0		
				Life Insurance Medical Research Fund of Australia and New Zealand	2,400	0	0		
								8,575	4 4
				Interest from Investments—					
				Held by the Trustees of the Estate of the late Thomas Baker	719	14	0		
				Endowment Fund	738	13	2		
								1,458	7 2
				Interest from Commercial Bank of Australia Ltd.				180	3 3
				Sundry Sales				208	10 4
				Deficit for Year				282	8 0
	£27,210	15	7					£27,210	15 7

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

Balance Sheet at 31st December, 1959.

LIABILITIES.					ASSETS.				
Current Liabilities—					Current Assets—				
Sundry Creditors			£1,500	9 11	Cash at Bank	£2,182	16 8		
Endowment Fund—					Prepayments	601	7 6		
Balance at 31/12/58	£8,500	0 0						£2,784	4 2
Add Estate of C. A. Smith	14,642	8 4			Endowment Investments—				
			23,142	8 4	Inscribed Stock—				
Capital Grants and Gifts			1,015	7 5	Commonwealth Government	£11,300	0 0		
Accumulated Revenue			3,524	6 1	Grain Elevators Board	2,500	0 0		
						13,800	0 0		
					Treasury Bonds—				
					Commonwealth Government	3,500	0 0		
					Shares in Companies	5,265	1 4		
					Cash at Bank	77	7 0		
					Mortgage Loan	500	0 0		
								23,142	8 4
					Restricted Funds (represented by Cash at Bank)—				
					Capital Grants and Gifts			1,015	7 5
					Fixed Assets—				
					Furniture and Fixtures			2,240	11 10
			£29,182	11 9				£29,182	11 9

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

In our opinion the annexed Balance Sheet is properly drawn up to show a true and fair view of the state of the Institute's affairs at 31st December, 1959, according to the best of our information and the explanations given to us and as shown by the books of the Institute.

FLACK & FLACK,
Chartered Accountants (Australia),
Honorary Auditors.

Melbourne,
29th April, 1960.

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, 1959

CAPITAL GRANTS AND GIFTS.

Balance at 31st December, 1958		£1,558	12 6
Add			
Donations—			
Drs. T. E. Lowe, A. J. Barnett and R. J. Sawers		44	13 0
For Building Extensions—			
Alfred Hospital Medical Research Fund	£7,297 10 0		
Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions	7,297 10 0		
		14,595	0 0
		£16,198	5 6
Deduct			
Cost of Building Extensions—			
C. A. Smith Wing	14,595 0 0		
Baker Institute Prize—			
R. G. Wyllie	100 0 0		
Laboratory Equipment—			
C. A. Smith Wing	341 13 1		
Architect Fees for cool room	146 5 0		
		15,182	18 1
Balance at 31st December, 1959		£1,015	7 5

ACCUMULATED REVENUE.

Surplus at 31st December, 1958	£3,806	14 1
Deduct		
Deficit for Year	282	8 0
Surplus at 31st December, 1959	£3,524	6 1

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

1959

STAFF

Honorary Physician:	EWEN DOWNIE, M.D., F.R.C.P., F.R.A.C.P.
Assistant Physician Scientific Studies:	JOSEPH BORNSTEIN, D.Sc., M.D., F.R.A.C.P.
Assistant Physician Clinical Studies:	BRYAN HUDSON, M.D., Ph.D., M.R.C.P., F.R.A.C.P. (on leave)
Honorary Assistant Physician:	HARALD BREIDAHL, M.D., M.R.C.P., M.R.A.C.P.
Registrar:	B. C. RITCHIE, M.B., B.S.
Biochemists:	DORA WINIKOFF, M.Sc.
	JUNE SHEATH, M.Sc.
	DEIRDRE HYDE, B.Sc.
	DILYS SARGEANT, B.Sc.
Technical Staff:	Mr. W. HUDSON.
	Miss A. EKKEL.
	Miss L. GIBSON.
	Miss B. ANDERSON.
	Mrs. P. KEEN.
	Miss M. ZWART.
	Miss S. SEMMEL.
	Miss P. HORE.
Secretary:	Miss J. SHARP.

DIABETIC CLINIC

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

RESEARCH FELLOWS

"Victor Y. and Margaret Kimpton":	HARALD BREIDAHL, M.D., M.R.C.P., M.R.A.C.P.
Roussel Fellow:	KEVIN CATT, M.B., B.S.
Wellcome Fellow:	PETER DAVOREN, Ph.D., B.Sc.

HONORARY ALFRED HOSPITAL RESEARCH FELLOWS

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

The past year has seen the completion of the constructional work of the Unit. The Laboratories, which were completed in April, 1957, formed the first part of this work. Thanks to a generous gift from the Trustees of the Baker Charitable Trust, and later to a substantial contribution from the Trustees of the Estate of the late Marie Paser, it became possible to proceed with the completion of the physical structure of the Unit. Early this year this work was completed. A single bed ward and two two-bed wards now provide accommodation which permits the treatment of acute metabolic emergencies and the investigation of patients presenting problems requiring intensive investigation. In addition, two small laboratories, a Seminar Room, an examination room, and a small workshop were included in this phase of development.

On 15th April, 1959, exactly two years after the opening of the laboratories, the Honourable Ewen Cameron, M.L.C., performed the opening ceremony in the presence of a representative gathering which included the Trustees and relatives of the late Marie Paser. The ward bears a plate commemorating the name of the late Carl Paser, whose memory is perpetuated by his sister's bequest. This has completed a Unit for the study of a variety of endocrine disorders, which although small in size is unique in this country. Thanks to the generosity of many individuals and organisations, it is well equipped to provide the appropriate facilities for the investigation and treatment of patients and to pursue research into a variety of metabolic problems.

The clinical activities of the Unit have been presented in the Annual Report of the Alfred Hospital, and will not be repeated in detail here. These embrace three out-patient sessions per week, concerned with the treatment of patients suffering from diabetes mellitus, thyroid disorders, adrenal disease and a variety of other endocrine disorders. In addition to five beds within the hospital, the Unit controls 24 beds in the Caulfield Convalescent Hospital, and in addition provides a consultative service in Endocrinology to Alfred Hospital.

The research activities were set out in detail in my last report, and have continued during the past year. Detailed description of these investigations are given later in this report.

As mentioned last year, Dr. Bryan Hudson spent this year in the Department of Biological Chemistry in the University of Utah, U.S.A., under the direction of Doctor Leo Samuels. His work was concerned with advanced studies of steroid chemistry, and the experience gained by him will be of much value in stimulating interest and improving the techniques of investigation and treatment of patients suffering with adrenal diseases.

Dr. Ian Martin, who left the Unit in 1957, has pursued his studies under a Cleveland Fellowship in the Medical School of Western Reserve University, Cleveland, U.S.A. He was engaged in fundamental studies on Diabetes Mellitus under the direction of Dr. Max Miller. He has recently been granted a Leverhulme Fellowship of the Royal College of Physicians of London, and is proceeding to the Middlesex Hospital to work under the direction of Dr. John Nabarro.

Early this year Dr. Joseph Bornstein was invited to participate in an Insulin Conference organised by the Novo Terapeutisk Laboratories in Copenhagen. He paid brief visits to appropriate centres in U.S.A., Great Britain

and Germany during his time away and gained important information on current trends of research work into various aspects of diabetes mellitus.

In May last the Annual Meeting of the Endocrine Society was held in Adelaide and attended by several members of the Staff of the Unit, four of whom presented papers to the Scientific Session of the Society. The Unit was also represented at the Annual Meeting of the Royal Australasian College of Physicians in Adelaide and at a General Meeting in Canberra in October last.

The Unit continues to attract attention both in Australia and abroad. During the year a number of overseas visitors have visited us, including Doctor Knoppers and Mr. Ekaireb, of Merck, Sharp and Dohme, U.S.A.; Doctor Upjohn, of Upjohn Inc., U.S.A.; Doctor E. Sayers, Dean of the Faculty of Medicine, Otago, New Zealand; Professor R. Farquharson, Department of Medicine, Toronto; Professor Kark, Department of Medicine, University of Illinois, Chicago; Doctor Merkel, Hoescht, Frankfurt; Professor Leo Samuels, University of Utah, U.S.A.; Doctor Franklin B. Peck, Sen., Eli Lilly and Co., U.S.A.; Doctor Adamson, Burroughs Wellcome Pty. Ltd., London; Doctor John Nabarro, Middlesex Hospital, London. In addition, numerous visitors from within the Commonwealth have been received throughout the year.

The interest displayed by our visitors has proved stimulating, and has been a source of encouragement to the members of the Staff. Much valuable advice and suggestions have been received; these are gratefully acknowledged.

Apart from this personal stimulus, generous assistance has continued to be provided by many individuals and organisations who have provided financial support and gifts in kind. This has come not only from Australia, but from Great Britain, the United States of America, and from several of the countries on the Continent of Europe. It is gratifying to those who initiated the formation of this Unit to realise that in the short period of its existence it has attracted such interest and support.

With the Unit now developed fully along the lines envisaged in 1956, it is realised that it would have been impossible for this to have been achieved in such a short time had it not been for the generous help and assistance which it has received from many sources. Together with this, the loyal support of all members of the Staff, with advice and suggestions both from the Honorary Medical Staff, from University colleagues and others have materially contributed to the success of this development.

As I said last year, our future activities will, I trust, justify the confidence and support which have been given to us.

EWEN DOWNIE.

31st December, 1959.

Grateful acknowledgment is made of financial assistance provided by—

Alfred Hospital Research Fund.
Burroughs Wellcome & Co. (Australia) Ltd.
Dr. Margaret Clark.
Estate of the late E. H. Flack.
Estate of the late Marie Paser.
Eli Lilly & Co., Indianapolis, U.S.A.
Life Assurance Medical Research Fund, Australasia.
National Health and Medical Research Fund, Australia.
Pfizer Pty. Limited, Australia.
Roussel Laboratories Ltd., London.
Upjohn Co., Kalamazoo, U.S.A.
Boots Pure Drug Co. (Aust.) Pty. Ltd.
Schering Corporation (Australia).
Mr. R. Willmoth.

Acknowledgment of gifts in kind is acknowledged from—

Ames Co. (Lindon) Ltd.
Burroughs Wellcome & Co. (Australia) Ltd.
Ciba Medical Department, Melbourne.
Fawns & McAllan Pty. Ltd.
Eli Lilly & Co., Indianapolis, U.S.A.
Merck, Sharp & Dohme (Australia) Pty. Ltd.
Novo Terapeutisk Laboratories, Denmark.
Pfizer Pty. Limited, Australia.
Roussel Laboratories Ltd., London.
Sigma Chemical Co., Missouri, U.S.A.
Upjohn Co., Kalamazoo, U.S.A.

Sincere appreciation is expressed for the unfailing assistance given by Professor Trikojus and members of the staff of the Department of Biochemistry, University of Melbourne, and for assistance received from the Directors and Heads of the Hospital Departments of Alfred Hospital, from the Director and Members of the Staff of the Baker Institute, the Clinical Research Unit, and from the University Departments of Medicine and Surgery, from Messrs. Dryden (Electronic Industries), Kirton (Selby & Co.) and McGee (Austronics Aust.).

CLINICAL STUDIES IN DIABETES MELLITUS

Ewen Downie, Harald Breidahl and Blair Ritchie

Sulphonylurea Compounds in the Treatment of Diabetes Mellitus

Long term studies of patients under treatment with tolbutamide have so far shown no deleterious side effects. A small proportion have shown secondary failure of response. In some instances this has been associated with infections or emotional disturbances. In others no obvious reason has been determined.

Clinical trials have been continued with chlorpropamide. This substance seems to be more effective in some instances than tolbutamide. The dosage is appreciably smaller, the maximum of 500 mgs. per day has been thought desirable to determine response. Based on an experience of 200 patients, 64 were well controlled on 250 mgs. daily, while nineteen required 125 mgs. It is of interest that 23 patients failed to respond to Rastinon either primarily or secondarily, and were treated with chlorpropamide. Of these nineteen showed a satisfactory response, which has continued for more than one year in six, and for more than six months in ten. No significant side effects have been observed in this. However, personality changes of appreciable degree have been noted in three, and in three others antabuse-like effects have been observed. The studies are continuing.

CLINICAL STUDIES

H. D. Breidahl.

During 1959, clinical studies on hyperparathyroidism and hypercalcaemic states, abnormalities of sexual development, and thyroid diseases were continued. Some of the work will be collected and presented during 1960.

INVESTIGATION INTO DIABETES MELLITUS

(a) Pituitary and Plasma Insulin Antagonists

J. Bornstein and Deidre Hyde

The pituitary fraction mentioned previously has been purified and identified as a polypeptide of molecular weight 4400, and its biological activity further investigated. It has been shown to depress the utilisation of glucose by muscle and liver, the synthesis of protein by muscle, and the synthesis of fatty acids by liver, while apparently accelerating the synthesis of cholesterol by that organ.

Initial results from injection of the material into rabbits show a rise in blood ketones, a rise in blood cholesterol, and a rise in plasma amino acids.

The possible clinical significance of this material is being investigated at present.

(b) Synthesis of Insulin by the Perfused Pancreas of the Cat

P. R. Davoren and J. Bornstein

The development of techniques for the isolation of pure insulin from a single cat's pancreas has been continued. This project has assumed a major part of the programme of investigation of the factors which control the incorporation of labelled amino acids into the perfused isolated cat's pancreas.

A number of methods of protein purification have been applied to the above problem in order that their relative values could be assessed with regard to the purification achieved, and more particularly the retention of the biological activity of the very small quantities of insulin involved.

A new technique of insulin purification which provides a small protein fraction containing the insulin in high yield has been developed.

Work is proceeding on the development of the terminal stage of the purification which will provide insulin able to satisfy rigid criteria of isotopic and biological purity.

(c) (i) A clinical study of the comparative side-effects of prednisolone, 6-methyl-prednisolone and dexamethasone was carried out.

(ii) An investigation of the presence and absence of the plasma insulin antagonist in non-diabetic arteriosclerotics was commenced with, so far, inconclusive results.

THYROID HORMONE STUDIES

Dora Winikoff

Microiodine Assays. An ashing modification for protein-bound iodine determination has been worked out. The basic stages of precipitation, ashing, leading of the ash with acid solution and colorimetry have been suitably adjusted in order to bring the results derived by this technique in close agreement with the ones obtained by Barker's distillation method. The new modification permits P.B.I. determinations to be carried out in batches, and is suitable for diagnostic purposes.

In order to determine the normal range, one hundred normal individuals have been investigated. Their P.B.I. ranged from 2.8-8.7 μg%; mean 4.7 μg%; standard deviation $\pm$ 0.8, which is lower than for Barker's method (5.2 μg%). However, the difference in concurrent estimations by both methods does not usually exceed 1-2 μg%.

Column Electrophoresis. Preliminary work is being carried out in applying zone electrophoresis in vertical columns using ethanolized cellulose as the supporting medium. Suitable conditions are being sought in order to achieve the best separation between serum albumins and alpha1-globulins. Both of these fractions are involved in the transport of thyroid hormone by blood proteins.

The ultimate aim of this investigation is is the localisation of thyroid hormone bound to these fractions in various states of thyroid function.

THE ESTIMATION OF CORTICOSTEROIDS IN BIOLOGICAL FLUIDS

J. B. Sheath K. Catt and A. Ekkel

During 1959 techniques for the estimation of plasma 17-hydroxycorticosteroids have been investigated, and a technique selected and brought into routine use.

A number of techniques for the estimation of plasma 17-ketosteroids have been investigated.

Dr. Catt has developed apparatus for automatically scanning corticosteroids on chromatography paper and the applications of this technique are now being examined.

The technique developed makes use of the fact that biologically active corticosteroids fluoresce when treated with alkali. The steroids are separated by paper chromatography. The strips are then suitably treated with alkali and mechanically run under an ultraviolet source, the fluorescence being detected by a photomultiplier, the output of which is amplified and recorded automatically. The technique appears particularly valuable in the measurement of corticosterone.

The return of Dr. Bryan Hudson from study leave in the United States will lead to considerable expansion in these activities.

PUBLICATIONS DURING 1959

DOWNIE, EWEN, and F. I. R. MARTIN—"Vascular disease in juvenile diabetic patients of long duration". *Diabetes* 8: 383: 1959.

DOWNIE, EWEN, JOSEPH BORNSTEIN and HARALD BREIDAHL—"Preliminary clinical and experimental studies with chlorpropamide in diabetes mellitus". *Bull. N.Y. Acad. Sci.* 74: Art. 3: 810: 1959.

BORNSTEIN, J., and DEIRDRE HYDE—"Insulin antagonists in blood of juvenile diabetics with arterial disease". *Diabetes*, 8: 92: 1959.

BORNSTEIN, J., C. W. BAIRD—"Assay of insulin-like activity in the plasma of normal and diabetic human subjects". *J. Endocrinol.* 19: 74: 1959.

DAVOREN, P. R., and J. BORNSTEIN—"Effect of glucagon on metabolism of glucose and acetate by isolated rat liver". *Amer. P. Physiol.* 197: 887: 1959.

HUDSON, BRYAN—"The diagnosis and treatment of Addison's disease". *Aust. Ann. Med.* 8: 182: 1959.

HUDSON, BRYAN—"Fluoxymesterone ("Halotestin")—A new androgen". *Med. J. Aust.*, Oct., 1959: p. 468.

HUDSON, BRYAN, and F. I. R. MARTIN—"The use of radioactive iodine (1131) in the diagnosis of thyroid disorders". *Med. J. Aust.*, May, 1959: p. 731.

SHEATH, JUNE—"Factors in the colorimetric estimation of 17-ketosteroids in urine". *Aust. J. Expt. Biol. & Med. Sci.* 37: 133: 1959.

SHEATH, JUNE—"Chromatography of urinary ketosteroids". *Aust. J. Expt. Biol. & Med. Sci.* 37: 147: 1959.

SHEATH, JUNE—"The specificity of laboratory tests for occult blood in Faeces"—*M.J.A.* 46: 836: 1959.

PAPERS ACCEPTED FOR PUBLICATION

HUDSON, BRYAN, F. I. R. MARTIN and MARJORIE BICK—"Observations on the treatment of severe diabetic ketosis". *Aust. Ann. Med.*

HUDSON, BRYAN, DORA WINIKOFF, P. TAFT and F. I. R. MARTIN—"Thyroid stimulating hormone and triiodothyronine as aids in the diagnosis of thyroid disorders". *Aust. Ann. Med.*

HUDSON, BRYAN—"Melanocyte stimulating hormone", in progress in clinical endocrinology (1960). *Ed., E. B. Astwood.*

BORNSTEIN, J. and DEIRDRE HYDE—"Polypeptides of the human pituitary with direct action on metabolism". *Nature.*

WINIKOFF, D., K. H. CLARKE and S. F. McCULLAGH—"The use of intramuscular depot of iodised oil as a lasting source of iodine". *Med. J. Aust.*

WINIKOFF, D., R. D. DICKINSON and C. WADE—"Globulin-bound iodine, levels in normal and abnormal pregnancy". *Brit. J. Obst. & Gynecol.*

SUBMITTED FOR PUBLICATION

HUDSON, BRYAN, and GEORGE W. OERTEL—"The determination of dehydroepiandrosterone and total neutral 17-ketosteroids in plasma".

PAPERS IN PREPARATION

DOWNIE, EWEN, HARALD BREIDAHL and BLAIR RITCHIE—"Clinical experiences with chlorpropamide".

BREIDAHL, H. D.—Hypogonadism at puberty".

LECTURES DELIVERED DURING 1959

"Natural History of Diabetes Mellitus—*University of Melbourne.*	EWEN DOWNIE
"Obesity"—*University of Melbourne.*	EWEN DOWNIE
"Presidential Address"—*Endocrine Society of Australia, Adelaide.*	EWEN DOWNIE
"Living with Diabetes"—*Tasmanian Diabetes Association, Hobart.*	EWEN DOWNIE
"The Changing Pattern of Diabetes Mellitus"—*The Actuarial Society of Australasia, Victorian Branch.*	EWEN DOWNIE
"Lipid Metabolism and Arterial Disease"—*Royal Australasian College of Physicians.*	J. BORNSTEIN
"Concepts of Insulin Action"—*Department of Hormone Chemistry, University of California, U.S.A.*	J. BORNSTEIN
"Insulin Antagonists"—*Lilly Research Laboratories, Indianapolis, U.S.A.*	J. BORNSTEIN
"A Pituitary Polypeptide with Anti-Insulin Activity"—*Department of Pharmacology, Western Reserve University, Cleveland, U.S.A.*	J. BORNSTEIN
"Complications of Diabetes Mellitus"—*Upjohn Research Laboratories, Kalamazoo, U.S.A.*	J. BORNSTEIN
"A Pituitary Polypeptide with Anti-Insulin Activity"—*Best Institute for Medical Research, Toronto, Canada.*	J. BORNSTEIN
"A Pituitary Polypeptide with Anti Insulin Activity"—*Sir William Dunn School of Biochemistry, Cambridge University, England.*	J. BORNSTEIN
"A Pituitary Polypeptide with Anti-Insulin Activity"—*Novo Terapeutisk Laboratories, Copenhagen, Denmark.*	J. BORNSTEIN
"Complications of Diabetes Mellitus"—*Novo Terapeutisk Laboratories, Copenhagen, Denmark.*	J. BORNSTEIN
"Pituitary Polypeptides with Direct Action on Metabolism"—*Waite Institute of Agricultural Chemistry, Adelaide.*	J. BORNSTEIN
"Biological and Biochemical Aspects of Melanocyte Stimulating Hormone"—*Department of Medicine, University of Utah,* U.S.A.	BRYAN HUDSON
"Familial Diabetes Insipidus"—*Department of Medicine, University of Utah, U.S.A.*	BRYAN HUDSON
"Androgens in Plasma"—*Department of Obstetrics, University of Wisconsin, U.S.A.*	BRYAN HUDSON
"Melanocyte Stimulating Hormone"—*Department of Oncology, Chicago Medical School, U.S.A.*	BRYAN HUDSON
"Hormone Induced Eosinopenia"—*Department of Anatomy, University of Utah, U.S.A.*	BRYAN HUDSON
"Methods for the Estimation of D.H.E.A. and Androgens in Peripheral Plasma"—*Department of Biochemistry, University of Utah, U.S.A.*	BRYAN HUDSON
"Diabetes Insipidus"—*Department of Clinical Investigation, New York University, Syracuse, U.S.A.*	BRYAN HUDSON
"Androgens in Plasma"—*Metabolic Unit, University of California, U.S.A.*	BRYAN HUDSON
"Hyperparathyroidism"—*Junior Physicians' Club.*	H. D. BREIDAHL
"Diabetes"—*Postgraduate Course for Anaesthetists, Royal Australasian College of Surgeons.*	H. D. BREIDAHL
"New Work in the Field of Diabetes"—*Victorian Diabetes Association.*	H. D. BREIDAHL
"Protein Anabolic Hormones"—Victorian Dieticians' Association.	H. D. BREIDAHL
"Paediatric Problems of the Diabetic"—*Australian Institution of Chiropody.*	H. D. BREIDAHL
"Thyroid Hormone Binding Proteins"—*The Endocrine Society of Australia, Adelaide*	D. WINIKOFF

MEETINGS ATTENDED DURING 1959

Endrocrine Society of Australia, Adelaide.	EWEN DOWNIE H. D. BREIDAHL K. CATT P. R. DAVOREN D. WINIKOFF J. SHEATH
Annual Meeting, Royal Australasian College of Physicians, Adelaide.	EWEN DOWNIE
General Meeting, Royal Australasian College of Physicians, Canberra.	EWEN DOWNIE
Ordinary Meetings, Tasmanian Diabetes Association, Hobart and Launceston.	EWEN DOWNIE
41st Annual Meeting, The U.S. Endocrine Society, Atlantic City, New Jersey, U.S.A.	BRYAN HUDSON
Annual Meeting, American Diabetes Association, Atlantic City, New Jersey, U.S.A.	BRYAN HUDSON

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

STUDIES ON MYCOBACTERIA

J. C. Tolhurst, G. Buckle* and A. V. Jackson*

The new mycobacterium isolated from a wild mouse and mentioned in previous reports has proved to be of considerable biological interest. Not only is it unique in requiring a low temperature of incubation and an increased concentration of CO_2 in the atmosphere for growth, but the histopathology of the lesions in animals is distinctive. Section of the swollen foot of a mouse inoculated in the footpad shows a remarkable degree of bacillary multiplication with a minimal amount of cellular inflammatory response and virtual absence of any necrosis of collagen or of other parts of the tissue. The subcutaneous space between the bones and muscle centrally and the skin externally is greatly widened by a honeycomb of thin-walled cavities distended by a coiled and tangled mass of acid fast bacilli.

In mice and rats the infection shows the characteristic chronicity of a mycobacterial disease, and some animals have been observed for periods of from one to two years. Guinea pigs are not susceptible to infection by subcutaneous injection, but after intraperitoneal inoculation minor peritoneal lesions develop.

Studies on other unusual mycobacteria isolated from man and from animals are still proceeding. Frogs and toads inoculated with a culture of M. ulcerans developed destructive lesions of the liver and kidneys. This observtion may be important in elucidating the epidemiology of this infection in man.

TORULOSIS

A. Perceval*

Using a strain of torula (Cryptococcus neoformans) isolated from bone lesions in a patient, experiments on the active immunisation of mice have been performed. Animals have resisted challenge with living cultures of both the immunising strain and of other strains of the organism.

SEROLOGICAL STUDIES IN THE DIAGNOSIS OF RHEUMATOID ARTHRITIS

M. Shallard*

During the past two years serological tests for the diagnosis of rheumatoid arthritis have been studied. Using the method of Alexander and de Forest, which is a modification of Rose's sheep cell agglutination test, 206 sera were tested from patients suffering from rheumatoid arthritis, other types of arthritis, rheumatic fever, disseminated lupus erythematosus or from various unrelated diseases. 59.1% of rheumatoid arthritis sera and 4.5% of other sera gave positive results by this method.

Ninety-three of the 206 sera were also examined by a method resembling that described by Singer and Plotz, using latex particles coated with gamma globulin set up as drop tests on glass slides. Using this method, 68.4% of rheumatoid arthritis sera and 4.7% of other sera gave positive results.

*Department of Pathology, Alfred Hospital.

Of twelve doubtful cases diagnosed clinically as "probable rheumatoid arthritis" none were positive by the sheep cell agglutination test, but five reacted in the latex particle test.

Further studies are projected.

POST-OPERATIVE URINARY TRACT INFECTIONS

A. Perceval*, M. Shallard*, M. Dorr*

Urological infections, especially those following trans-urethral resection, have been investigated, particular note being taken of infections by "hospital type" antibiotic-resistant organisms which are sometimes fatal. Many of the sources of these infections and possible measures to combat them have been studied. The ward pan-room equipment appears to be the main reservoir of antibiotic-resistant organisms causing the majority of post-operative infections.

SURVEY OF ROAD ACCIDENTS

I. McNicol Smith†

In conjunction with Miss B. F. Letheren, of the Department of Social Studies, a personal survey has been made of all persons admitted to the Alfred Hospital as a result of accidents during 1959. A follow-up in 1960 will be carried out to determine some of the more widespread effects of the accident to the individual, the family and the community, in terms of the cost in money, loss of man hours, domestic and personal readjustment. All types of accidents are included, but an intensive study is being made of traffic accidents. In addition, the opportunity is being taken to analyse specific injuries which occur in sufficient numbers during the twelve months to make a significant series.

ARTIFICIAL KIDNEY

V. C. Marshall†

Further experience in the management of patients with renal failure has been gained, and the artificial kidney has now been used on 39 occasions on 34 patients, with no serious complications. Chronic renal failure does not appear to be benefited by dialysis, but diagnosis of an irreversible renal lesion is difficult to predict before an autopsy, and dialysis is probably the most useful diagnostic aid. Dialysis is of most benefit in acute renal failure if used at a comparatively early stage, before irreversible changes occur. It has been in several instances undoubtedly life-saving, and may need to be repeated if a diuresis does not occur within two weeks.

Clinical indications that dialysis is necessary are more important than biochemical ones, and symptoms attributable to renal failure which are progressing despite conservative management will be improved, often dramatically, by a timely dialysis. Biochemical indications are a dangerous elevation of the potassium or urea levels, or a lowering of the bicarbonate level in the plasma.

*Department of Pathology, Alfred Hospital.

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

The presence of a diuresis should not alter the decision to dialyse. There have been twenty patients with acute renal failure who have been dialysed, and eleven are surviving.

The mortality in post-operative and post-traumatic renal failure is very high, and could possibly be improved by earlier transfer of patients to a dialysing centre.

In conjunction with Dr. George Berci, a colour film of ten minutes' length depicting the artificial kidney in action, with a discussion of the indications for its use, has been completed.

CHRONIC RETENTION OF URINE

V. C. Marshall†

Observation of a small series of cases shows undoubtedly that sodium diuresis can occur after relief of the obstruction by a catheter, and fluid and electrolyte loss may be striking.

POTENTIATION OF CLOSTRIDIUM WELCHII BY ADRENALINE-IN-OIL

V. C. Marshall†

In view of two recent cases of gas gangrene developing after the injection of adrenaline-in-oil, experiments were performed using the guinea pig, which showed that—

a. aqueous adrenaline increases the infectivity of Clostridium welchii 1000 fold.

b. adrenaline-in-oil has a similar effect.

c. oil alone has no effect.

The implications of these observations has been discussed, and strict care in sterilisation of syringe, needle and injection site is urged when using adrenaline.

PUBLICATIONS DURING 1959

TOLHURST, J. C., G. BUCKLE and N. A. M. WELLINGTON—"The experimental infection of calves with mycobacterium ulcerans". *J. Hygiene*, 57 (1959), p. 47.

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

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