BAKER INSTITUTE

# RESEARCH

1957

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-first Annual Report*

of

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

(Including Alfred Hospital Clinical Research Unit)

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# *First Annual Research Report*

of

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

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# *Reports*

of

ALFRED HOSPITAL RESEARCH FELLOWS

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*1957*

ALFRED HOSPITAL, PRAHRAN,
VICTORIA, AUSTRALIA.

# BAKER MEDICAL RESEARCH INSTITUTE

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Director of Clinical Research Unit (ex officio).

*Appointed from the University of Melbourne.

# 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.

Within the past two years the Board of Management has formed a Diabetic and Metabolic Unit which is engaged in investigation of endocrine and allied disorders. This has also been placed under the supervison 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

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| Pharmacologist | (Vacant) |
| Physiologist | MRS. W. G. NAYLER, M.SC. |
| Research Assistant | M. QUINN-YOUNG, B.SC., B.E.E., M.B., B.S. |
| Ward Staff | SISTER S. A. CITROEN.<br>SISTER M. HENRY (from 8/4/57 to 11/9/57)<br>SISTER R. ARNOLD (from 11/9/57) |
| Registrar | P. J. NESTEL, M.B., B.S., M.R.A.C.P. |
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| "Victor Y. and Margaret Kimpton" | J. K. FRANCIS, M.B., B.S., F.R.C.S. |
| "E. H. Flack" | J. R. E. FRASER, M,D., M.R.C.P., M.R.A.C.P. |
| "Edward Wilson Memorial" | R. J. MARSHALL, M.D. (BELFAST), M.R.C.P, M.R.C.P (IRELAND). |
| "Sol Green" | R. SAWERS, M.B., B.S., M.R.A.C.P. |
| "Alfred Hospital" | M. M. STEVENSON, M.B., B.CH. (BELFAST), B.A.O. |

## ALFRED HOSPITAL RESEARCH FELLOWS

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| "J. F. MacKeddie and Frederick and Esther Michaelis" | E. L. G. BEAVIS, M.B., B.S., D.G.O., M.R.C.O.G., F.R.C.S. |
| "Edward Wilson Memorial" | H. D. BREIDAHL, M.D., M.R.C.P. |
| "E. H. Flack Travelling and E. H. Flack" | I. S. EPSTEIN, M.D., B.S., M.R.A.C.P |
| "Sydney W. Jones Medical Research Foundation" | I. A. L. FERGUSON, M.B., B.S., F.R.A.C.S. |
| "Victor Y. & Margaret Kimpton" | J. K. FRANCIS, M.B., B.S., F.R.C.S. |
| "E. H. Flack" | J. R. E. FRASER, M.D., M.R.C.P., M.R.A.C.P. |
| "Alfred Hospital" | O. M. GARSON, M.B., B.S. |
| "J. H. Patterson Travelling and E. H. Flack Travelling" | W. R. KINGSTON, M.D., M.R.A.C.P. |
| "Edward Wilson Memorial" | R. J. MARSHALL, M.D., (BELFAST), M.R.C.P., M.R.C.P. (IRELAND). |
| "Sol Green" | M. SANDERS, M.B., B.S. |
| "Sol Green" | R. SAWERS, M.B., B.S. M.R.A.C.P. |
| "Alfred Hospital" | M. M. STEVENSON, M.B., B.CH (BELFAST), B.A.O. |
| "Sydney W. Jones Medical Research Foundation" | G. R. STIRLING, M.B., B.S., F.R.A.C.S. |
| "Senior Research" | J. C. Tolhurst, M.SC. |

## APPOINTED TO RESEARCH FELLOWSHIPS FOR 1958

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|---|---|
| E. L. G. BEAVIS, M.B., B.S., D.G.O., M.R.C.O.G., F.R.C.S. | "Frederick and Esther Michaelis" |
| W. C. BOAKE, M.SC., M.B., B.S., B.SC. (OXON.), M.R.C.P. | "Alfred Hospital" |
| H. D. BREIDAHL, M.D., M.R.C.P. | "Victor Y. and Margaret Kimpton" |
| I. S. EPSTEIN, M.D., B.S., M.R.A.C.P. | "E. H. Flack" |
| I. A. L. FERGUSON, M.B., B.S., F.R.A.C.S. | "Sydney W. Jones Medical Research Foundation" |
| W. R. KINGSTON, M.D., M.R.A.C.P. | "R. B. McComas Memorial" |
| V. C. MARSHALL, M.B., B.S. | "Alfred Hospital" |
| F. I. R. MARTIN, M.B., B.S., M.D. | "George Merriman" |
| R. J. SAWERS, M.B., B.S., M.R.A.C.P | "Sol Green" |
| F. O. SIMPSON, M.B., B.CH. (EDIN.), M.R.C.P. (EDIN.) | "Edward Wilson Memorial" |
| G. R. STIRLING, M.B., B.S., F.B.A.C.S. | "J. F. MacKeddie" |
| J. C. TOLHURST, M.SC. | "Senior Research" |
| G. WAGNER, M.B., B.S., B.SC.(MED.), M.R.A.C.P. | "A. A. Swallow" |

# ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

During 1957 all activities of the Institute have continued on a level similar to that of last year. Perhaps the most outstanding event was a three day conference on cardiovascular physiology held in the Institute in August.. This was attended by visitors from overseas and interstate as well as local research workers. The conference provided a forum for a very successful exchange of ideas and information and all who attended appreciate the generosity of the Life Insurance Medical Research Fund who made a grant for this purpose.

The number of graduates wishing to conduct research in the Institute continues at a satisfactory level and this year we have been fortunate in having Drs. R. J. Marshall and Mabel Stevenson from Belfast holding Research Fellowships. It is also pleasing to record that two members of the hospital staff asked for facilities to carry out research projects in their spare time.

The number of research projects being carried out in the Institute has now reached a point where all space available for equipment and personnel is in use. Further, the steady growth in activities which has taken place over the past few years shows no sign of ceasing and in our growing community further expansion must be envisaged. A stage has now been reached when to maintain the present level of activity the building of additional laboratories is desirable but to provide for the anticipated growth further building is essential. It is to be hoped that the tentative plans already discussed to extend the Institute facilities can be carried out in the near future. During the year additional equipment added to the workshop has greatly facilitated the construction of necessary laboratory apparatus.

The research work undertaken during 1957 is detailed later in this report but the following summary gives an overall picture. Many of the projects studied have been in progress for a considerable time and appreciable advances in knowledge in these fields have been made in the year under review.

The studies on the control of body fluid volume have been directed to defining the properties of the control mechanism which exists in the body to regulate its volume. This problem has been approached first as an empirical study of data obtained from the observation of patients suffering from disturbances of fluid volume regulation and secondly, by a comparison of this behaviour with that of known forms of control systems applicable to this type of fluid storage. Many features of the mechanism involved in this physiological control have now been specified and attention is being directed towards a more detailed study of the component parts of the system. At present details of changes in renal function, plasma volume and osmotic pressure in relation to total fluid volume of the body and its partition within various compartments are being considered.

Problems associated with the control of bleeding still provide major projects which may be grouped into the biochemical processes associated with blood coagulation and the importance of platelets and the capillary walls in producing haemostasis. Amongst the biochemical problems studied is a comparison of the

clotting mechanisms in man, horse and marsupials. These studies have been associated with clinical investigations of the various forms of haemophilia, acquired haemorrhagic states and coagulation abnormalities in non-haemotological diseases including coronary atherosclerosis and after haemorrhage or repeated blood transfusions.

Diseases in which the arterial blood pressure is either excessively high or low continues to be a field of investigation. The long-term clinical trial of hypotensive drugs in the treatment of severe forms of hypertension has been continued and the results obtained are good although side effects of the drugs are at times troublesome.

The study of occlusive peripheral arterial disease and intermittent claudication has continued with an assessment of the value of various forms of treatment.

The production of energy to drive the circulation is the essential function of cardiac muscle. With the development of a suitable animal preparation it has been possible to study the effect of various drugs on the production of this energy and to commence a study of the biochemical steps in this process. During the past year the technique of recording intracellular electrical potentials from a beating heart has been developed. This enables the electrical effects accompanying the biochemical changes to be followed in this energy conversion. Arising out of this project has been a study in the toad of the effects of hibernation on cardiac muscle metabolism.

Histochemical, immunological and electrophoretic studies of abnormal proteins occurring in the serum in various diseases have continued with special reference to macroglobulins and myeloma proteins. Electrophoretic studies of the serum proteins of New Guinea natives have also been made.

The development of open intracardiac surgery has been further developed in conjunction with members of the Thoracic-Surgical Unit and many problems associated with the use of an extracorporeal pump-oxygenator both in animals and man have been investigated. Many of these problems appear to be metabolic or associated with blood coagulation disturbances.

The development of investigational techniques of value in clinical diagnosis of cardiovascular disease has continued and equipment to record lung compliance and other lung functions has been constructed. Methods for the estimation of urinary catechols by paper chromatography and of the level of serum transaminase have been studied.

Many of these investigations have been supported by funds provided by the National Health & Medical Research Council, the Life Insurance Medical Research Fund of Australia and New Zealand and Alfred Hospital Medical Research Funds. All of these bodies and the Anti-cancer Council of Victoria have allocated grants for work in 1958. The assistance from the funds is gratefully acknowledged.

The Institute is also the grateful recipient of bequests from the estates of the late Laura S. Nyulasy and the late Catherine A. Smith.

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, Trikojus and Wright and the staffs of the Departments of Organic Chemistry, Biochemistry and Physiology, University of Melbourne and staff of the Commonwealth Serum Laboratories, and we thank them and others who have helped for their continuing interest in our work.

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, and to thank members of the staff and research fellows for their co-operation during the past year.

T. E. LOWE.

31st December, 1957.

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

Adelaide Children's Hospital.
Commonwealth Department of Health.
Hallstrom Institute of Cardiology.
Imperial Chemical Industries of Australia and New Zealand.
*L'Institut Bunge.*
Institute of Dental Research, Sydney.
Institute of Medical and Veterinary Science, Adelaide.
International Anaesthesia Research Society.
Kanematsu Institute.
Mayo Clinic.
Medical Research Council, London.
Middlesex Hospital Medical School.
National Health and Medical Research Council, Canberra.
New York State Department of Health.
Ophthalmic Research Institute of Australia, Inc.
Organisation for Scientific Research, Indonesia.
Queensland Institute of Medical Research.
Royal Women's Hospital.
Staten Seruminstitut, Copenhagen.
South African Institute of Medical Research.
University of Melbourne.
U.S. Army Medical Library.
Walter and Eliza Hall Institute.

# REPORT OF SCIENTIFIC INVESTIGATIONS

## BLOOD COAGULATION

### PHENOLS AS INHIBITORS OF THE HEPARIN CO-FACTOR OF PLASMA**

P. Fantl and A. G. Marr.

Since several biologically active substances are phenolic in nature the influence of phenols on the blood clotting mechanism was investigated.

It was found that a number of phenols antagonise heparin; the prolonged thrombin clotting time of heparinised plasma is markedly reduced by a number of phenols. It was shown that heparin is not affected directly but the phenomenon is due to a partial inactivation of the co-factor which is required for heparin activity.

The most active compounds were phenol, p-cresol, and pyrocatechol but neither the naturally occurring phenols (adrenaline, noradrenaline, tyrosine) oestrogens nor synthetic oestrogens in saturated solutions in the buffer system used had any effect on heparin activity.

### COMPARISON OF BLOOD CLOTTING IN MARSUPIALS AND MAN**

P. Fantl and H. A. Ward.

Several of the blood clotting factors are proteins, and some of them show quantitative differences in activity when homologous and heterologous combinations are compared. Although it is known that clotting factors do not show absolute species specificity it seems likely that the differences of activity which they exhibit are due to characteristic features of molecular structure which are determined by biological relation.

As marsupials are believed to be early forms in the evolution of mammals, the blood coagulation of some Australian marsupials has been investigated and compared with that of man.

From our experiments it is evident that marsupial blood contains all the clotting factors which have been recognised in the blood of mammals. However, a particular factor shows considerable differences in activity in the blood of different species.

It is characteristic for many species that brain thromboplastin gives shorter clotting times with the homologous plasma than with heterologous plasma. This is also true for marsupial components with the limitation that possum brain thromboplastin gave with other marsupial plasmas the same clotting time as with possum plasma. This might indicate a very close biological relationship among the investigated marsupials.

The clotting system of marsupial blood is more efficient that that of human blood. The electrophoretic pattern of marsupial serum differs from that of human serum.

**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 and those marked (*) were supported by grants from the Life Insurance Medical Research Fund.

## THE COAGULATION OF HORSE BLOOD**

P. Fantl and A. G. Marr.

Available information about the coagulation of horse blood is contradictory. Some workers report that horse blood contains clotting factors in concentrations not markedly different from that of human blood, while others conclude that the blood of normal horses of both sexes is deficient in certain coagulation factors.

As naturally occurring deficient blood would be useful for the investigation of the clotting process, it was of interest to investigate the blood coagulation mechanism of the horse.

The experiments showed that all the recognised clotting factors in human blood are active in that of the horse. However marked quantitative differences between the two species are apparent. Thromboplastin generation tests with horse blood components indicated a low activity of the thromboplastin when tested with horse plasma, but an activity equal to that of human plasma was observed when tested on human plasma. This indicated that thromboplastin formation in horse blood proceeded at a similar rate to that in human blood hut that subsequent reactions in horse blood were delayed.

Further the rate of thrombin formation in horse blood was slower than in human blood. This may have been due to inadequate platelet numbers and also to the fact that horse platelets showed less tendency than human platelets to liberate phospho-lipids during clotting, since very rapid clotting could be induced by the addition of brain phospho-lipids.

With regard to plasma factors required for blood clotting it was observed that horse plasma had similar activity to that of human plasma. It was noted that horse plasma and isolated fibrinogen had a longer thrombin clotting time than human plasma.

It is noteworthy that human fibrinogen gave the shortest thrombin clotting time of all mammals investigated so far. The comparatively long thrombin clotting time is the main reason for an apparent reduced coagulability of horse plasma.

Although the results of some of the tests suggest that the coagulability of normal horse blood is less than that of normal human blood, it should be pointed out that coagulation is only part of the haemostatic process and that there is no evidence that this is impaired in horses.

## CLOTTING FACTORS IN STORED BLOOD

M. M. Stevenson.

It is well known that the initial levels of clotting factors in drawn blood are influenced by the manner of venesection, and that under conditions of storage, blood gradually loses its coagulability.

Analysis of 25 samples taken at random from Red Cross blood transfusion bottles showed that 80% of specimens stored for less than 7 days had satisfactory pro-thromboplastin (anti-haemophilic factor) levels and platelet counts. Samples stored for longer periods showed significant deterioration in the levels of both these constituents.

## A PRE-FIBRONOLYTIC SYNDROME ASSOCIATED WITH EXCESSIVE HAEMORRHAGE

**P. Fantl, R. Sawers and M. M. Stevenson.**

It is a not uncommon observation that patients of both sexes may bleed excessively following psychological upsets, trauma or minor surgical interference such as tonsillectomy or tooth extraction. The haemorrhagic tendency is of temporary nature. Physical examination shows no abnormality and laboratory investigation reveals no defect of any of the known factors required for efficient blood coagulation and haemostasis; anticoagulants are not detectable. The observation to be discussed is connected with the early stages of fibrinolysis, although pronounced fibrinolysis may be absent as judged by in vitro tests.

In the investigated patients, who were of the "worrying type," haemorrhages were out of proportion either to the inflicted trauma or even could not be explained at all. Suspicion that the haemorrhagic condition was due to pathological causes or to deficiency of factors required for normal blood coagulation could in no case be substantiated. However it was noticed that the red cell fall out from the blood clots was so marked that superficially it appeared that fibrinolysis had taken place in the blood specimens incubated at 37°C. Closer inspection indicated the presence of a small clot. Fibrin determinations whether carried out immediately after blood collection and after incubation for 4 hours gave identical results. Other factors which contribute to essential physiological properties of a normal blood clot were not lacking in these patients' blood.

The most likely explanation of the phenomenon is that the precursor of fibrinolysin (plasminogen, profibrinolysin) which is a normal blood constituent becomes activated in the circulation but fibrinolysis is not very marked in shed blood.

It is likely that the fibrinolytic enzyme alters the texture of fibrin to an extent which lessens the ability of the clot to retain the red cells. The red cell fall out is therefore considered as an indicator of fibrinolytic activity.

## HAEMOPHILIA

**R. Sawers.**

The total number of haemophiliacs examined in the present study now numbers 110; twelve of these were seen for the first time during the year. The use of more sensitive techniques has allowed the diagnosis of haemophilia to be established in several patients in whom equivocal results had been obtained previously. The laboratory investigations have shown that 96 patients suffer from alpha-haemophilia (Haemophilia A or "true haemophilia"), and 14 from beta-haemophilia (Christmas Disease). *Approximately two-thirds* of the patients suffer from severe deficiency having less than 3% of the defective clotting factor. The remainder suffer moderate to very mild deficiency and consequently less severe symptoms. It was of interest to discover our first three cases of beta-haemophilia with mild deficiency during the year, for the first eleven cases studied had complete deficiency of beta-prothromboplastin.

Following the successful use of the acid-citrate anticoagulant in preserving the alpha-prothromboplastin activity in plasma, the Red Cross Blood Transfusion Service is now preparing plasma for use in haemophiliacs with "special acid-citrate'

instead of the A.C.D. anticoagulant and preservative. The plasma is collected with special care and is now being stored at the Institute in a deep freeze unit for emergency and routine use. In an occasional bottle of plasma so stored a large amount of insoluble precipitate has formed after storing for several weeks. However, this has not resulted in loss of therapeutic activity.

**Other Haemorrhagic Disorders.**

The great majority of patients referred for investigation on account of bleeding have no coagulation abnormalities but evidence of a vascular abnormality is commonly found. The main feature in these cases is an abnormally prolonged skin bleeding time which is remarkably variable; on some days the bleeding times are grossly prolonged (30 mins.), while on others they may be only slightly prolonged (8 mins.) and on still other days may be quite normal (less than 5 mins.). Capillary fragility is frequently increased, but the severity is usually constant.

Several patients and their relatives with this abnormality were examined during the year. The condition has led to extremely severe post-operative haemorrhage, and in one instance to death. The condition appears to be identical with von Willebrand's disease, but the majority of patients with the abnormality experience less severe bleeding than is usually associated with that disease. No coagulation abnormalities were discovered in any of these cases.

## COAGULATION STUDIES IN PATIENTS WITH GASTRO-INTESTINAL HAEMORRHAGE

**M. M. Stevenson.**

On clinical grounds it has been claimed that direct blood transfusion has a beneficial effect in cases of gastro-intestinal haemorrhage which have not responded to transfusion of stored citrated blood. It was considered that the efficacy of such treatment might lie in the fact that coagulation factors were being supplied to a deficiency state which either was part of the underlying cause of the initial haemorrhage or had resulted from multiple transfusions of citrated blood.

An investigation was therefore carried out to determine whether it was possible to demonstrate by laboratory means the presence of a clotting abnormality in patients admitted to the wards with gastro-intestinal bleeding. Coagulation studies were performed on 58 patients either prior to their first blood transfusion or when a period of at least ten days had elapsed after their last transfusion. In only one patient was a significant coagulation abnormality found. This patient appeared to have a pre-fibrinolytic state. No anatomical lesion was found to account for her haematemesis and subsequent to her admission to hospital, haematuria developed for which again no organic cause was demonstrated.

Subsequent analysis of the cause of bleeding showed that in 13 of the 58 cases no underlying lesion could be demonstrated by radiological or gastroscopic means.

Two patients in the series failed to respond to stored blood transfusion and were treated by direct transfusion. In one case bleeding appeared to stop while in the other it was unaffected by direct transfusion. In both patients coagulation studies done before direct transfusions were normal.

## VASCULAR THROMBOSIS IN ACUTE PANCREATITIS

P. J. Nestel.

Vascular thrombosis appears to play a part in the development of the lesions of experimentally induced acute haemorrhagic pancreatitis. In the experimental animal these lesions can be prevented by the preoperative use of anticoagulants—heparin and tromexan.

Experiments were carried out to determine whether fibrinolytic substances injected into rats after the operative interference would give similar protection.

It was found that using either "Varidase" or human plasmin produced fibrinolysis for one hour only and that thrombosis of pancreatic vessels could not be prevented.

## BLOOD COAGULATION IN CORONARY HEART DISEASE

E. Stock.

Claims have been made that a fatty meal produces in man an increased coagulability of the blood. If true this phenomenon might have a bearing on the development of coronary heart disease.

The blood coagulation time was determined therefore, both fasting and after a fatty meal, in 10 apparently normal individuals and in 15 with proven coronary heart disease.

No difference was observed between the two groups when the fasting clotting time was compared. After a fatty meal the "normal" individuals showed no change in coagulation time but in 7 of the "abnormals" there was a decrease in coagulation time.

## CONTROL OF BODY FLUID VOLUME**

T. E. Lowe, A. J. Barnett, R. J. Marshall and A. G. Marr.

Studies over several years have indicated that flow of fluid through the body and its storage represent a system controlled by a mechanism sensitive to two facets of the stored fluid (volume and osmotic pressure of some part or parts of the stored fluid).

During the past two years attempts have been made to obtain direct evidence of the control of storage and of inflow and outflow by osmotic pressure of the stored fluid. Some evidence of this control has been obtained in conditions where body fluid osmotic pressures are abnormal over long periods (Addison's disease and terminal chronic nephritis). However it seems likely that the limitations of the technique used preclude the obtaining of this evidence in most cases, usually osmotic pressure abnormalities are spontaneously corrected in a time measured in hours.

This year most attention has been paid to the relationship between fluid outflow and body fluid volume which is implied by the hypothesis developed. Both our own studies and those of Adolph (1943) suggest that this relationship produces an S-shaped curve when urine flow is plotted against body weight. However over the central region the curve is essentially linear and this portion has been used to study the effect of disease and drugs upon the relationship. Whilst this linear relationship cannot be demonstrated throughout the whole course of a patient's recovery from a disturbance of body fluid volume, in most instances there are considerable periods in which the relationship holds.

Our observations suggest a fundamental relationship between urine flow and plasma volume, such that these are linearly related. The data also indicate that drugs such as mercurial diuretics and hexamethonium have an effect on the system completely different from diseases such as cardiac failure and nephritis.

Body fluid is stored in many compartments which communicate with one another and the partition of fluid in the compartments has been studied with the aid of a hydraulic model. This has enabled consideration to be given to such factors as differential osmotic pressure across capillary membranes, venous pressure and tissue pressures. The model has emphasised the importance of considering transfer rates of fluid flow across the membranes of the body.

## RENAL CLEARANCE AND PLASMA VOLUME DETERMINATIONS

In order to study renal function and plasma volumes in relation to the regulation of body fluid volume, the work has continued on techniques for the estimation of glomerular filtration rate, renal blood flow and plasma volume. Glomerular filtration is being estimated by the inulin clearance, renal plasma flow by the P.A.H. clearance and plasma volume by the Evans blue space.

In 5 normal subjects the P.A.H. clearance (R.P.F.) varied betwen 726 and 820 ml/minute and the inulin clearance (G.F.R.) between 148 and 168 ml/minute with a filtration fraction (F.F. = G.F.R./R.P.F.) of 18 to 21%.

In a series of 14 hypertensive patients, with one exception the values of the R.P.F. and G.F.R. were reduced compared with those of the normal subjects (R.P.F. 195 to 476 ml/min., G.F.R. 55 to 197 ml per min). Usually the G.F.R. showed a greater proportionate reduction than the R.P.F. giving a F.F. which was usually higher than normal. These findings are in accord with those of previous workers and give us assurance that our technique is a satisfactory one.

# HYPERTENSIVE STATES

## CLINICAL TRIAL OF HYPOTENSIVE DRUGS

**A. J. Barnett, R. J. Marshall and P. J. Nestel.**

The trial of treatment of severe arterial hypertension with ganglionic blocking drugs, commenced in 1950, has continued, the number of patients participating having now reached 102. The overall effect of this treatment in prolonging life, usually useful life, in patients with hypertension in the malignant phase is still demonstrated. The results up to June, 1957, are shown in the following table. 32 patients in the malignant phase had commenced treatment at least 2 years before June, 1957, and are compared with 32 patients in the benign phase observed during the same period.

| Survival time | Malignant phase (32 patients) No. of survivors | Malignant phase (32 patients) Expected No. of survivors without treatment | Benign phase (32 patients) No. of survivors |
|---|---|---|---|
| 1-2 years | 23 | 6 | 27 |
| 2-5 years | 18 | 3 | 25 |
| More than 5 years | 5 | 0 | 4 |

This shows the greatly improved prognosis with treatment for patients in the malignant phase; in fact it approaches that for the benign phase. The expected number of survivors without treatment is based on a study (by Keith, Wagener and Barker) before the ganglionic blocking drugs were available.

The effect of this treatment on the survival of patients in the benign phase is difficult to assess because of the uncertain prognosis and the difficulty in obtaining a suitable series for comparison, but it seems likely that here also life may be prolonged in patients with the more severe forms of the disease. The main indication for treatment in these patients has been the relief of hypertensive symptoms. Side effects have been almost universal but in patients with severe symptoms these are outweighed by the symptomatic relief.

Until this year we were fortunately not troubled with the more serious complications of treatment which have been described by some workers. However in the past year there have been several instances of severe diarrhoea with ulceration of the bowel in patients being treated with ganglionic-blocking drugs administered by mouth, and in one case, this possibly contributed to the patient's death. Care should be exercised in the selection of patients for treatment with ganglion-blocking drugs and probably they are not indicated in the less severe forms of hypertension.

## MECAMYLAMINE

Further experience has been obtained in the use of the new ganglionic blocking agent mecamylamine ("Mevasine," Merck). This drug has been used in 32 patients. In nine the drug was discontinued, mainly on account of complications or side effects. Previously 22 patients had been treated with pyrolidinium ("Ansolysen," M & B), in 12 by the oral route and in 10 by injection, and in comparison with pyrolidinium by injection, mecamylamine did not produce any better blood pressure control, symptomatic relief nor were side effects less, the only advantage being that injections were avoided. Compared with pyrolidinium taken orally mecamylamine produced better blood pressure control in about half the patients but also resulted in more severe side effects in a similar proportion. These findings indicate limitations in the use of ganglionic blocking drugs which are not overcome by the use of this new member.

## CHLOROTHIAZIDE

Chlorothiazide ("Chlotride," Merck) originally introduced as a diuretic agent has antihypertensive effects. This drug was administered over a period of 4 weeks to 12 patients being treated with mecamylamine. It was found that following the administration of chlorothiazide the dose of mecamylamine required for a particular effect on the blood pressure was approximately halved and there was a corresponding reduction in side effects. Arrangements are being made for a more prolonged trial of this treatment.

## PHAEOCHROMOCYTOMA

Assays of urine of patients suspected of harbouring a phaeochromocytoma have been made at a rate of approximately two per week, specimens being sent not only from Melbourne but also from interstate centres. By this method the diagnosis has been confirmed in 4 cases during the year, and from all patients a tumour was removed.

## CHROMATOGRAPHIC AND FLUORIMETRIC ASSAY OF URINARY CATECHOLAMINES

C. C. Curtain.

The estimation of urinary, or blood plasma, noradrenaline is now accepted as the most reliable diagnostic test for phaeochromocytoma. The current method used at the Baker Institute is a bio-assay which exploits the relaxing effect of the catecholamines in a urinary extract on the isolated rabbit gut. This method is tedious and subject to interference from substances in the urine and impurities in the reagents. Attempts were made in 1954 to adapt the fluorimetric assay of Lund to the determination of noradrenaline, but were unsuccessful because of the presence of nonspecifically fluorescing substances in the urine.

Because of the heavy demand for estimations the search for a suitable chemical assay has been renewed.

Several methods using chromatographic separation of the noradrenaline have been reported in the literature. Of these paper-partition chromatography in a phenol-aqueous hydrochloric acid system has been used to isolate the noradrenaline from interfering substances in the urine extracts. The noradrenaline was eluted from the paper with dilute hydrochloric acid and estimated fluorimetrically by the method of Weil-Malherbe and Bone. Satisfactory elution proved time consuming and it was felt that much would be gained if the fluorescence could be measured directly on the paper strip. The fluorescence was developed by spraying the strips with a 5% solution of ethylene diamine in 2% aqueous ammonia and heating them at 60°C for thirty minutes. The intensity of the fluorescence was measured by means of an adapted Beckman spectrophotometer. The photocell housing was removed from the body of the instrument and a yellow filter inserted in the photocell aperture. A "Perspex" guide, milled out to take the paper strips, was bolted to the face of the housing and the whole was connected by a light-proof hood to a source of ultraviolet light. Preliminary tests have shown that this technique yields results which are comparable with elution and fluorimetry in solution.

# DISEASES OF THE PERIPHERAL BLOOD VESSELS

## OCCLUSIVE ARTERIAL DISEASE

A. J. Barnett and K. N. Morris.†

Patients with occlusive arterial disease of the legs treated by sympathectomy and by arterial grafting are being examined every 6 months and their symptomatic state and the condition of their limbs assessed.

These reviews confirm the value of sympathectomy in relieving distal ischaemic features (coldness, numbness, rest pain) and emphasise its failure to relieve claudication from muscle ischaemia.

Only 3 arterial grafts have been inserted this year (compared with 21 in 1953-1955 and 14 in 1956) owing to lack of supply of donor material rather than of suitable patients. Prior to 1955 all operations were carried out using an end-to-end technique, but since 1955 in most patients a by-pass technique has been used.

†Thoracic-Surgical Unit, Alfred Hospital.

The following table indicates the results achieved in these patients.

| Result | Type of Graft End-to-end | By-pass | Total |
|---|---|---|---|
| Graft patent | 4 | 14 | 18 |
| „ blocked | 10 | 7 | 17 |
| Patient dead with graft functioning up to death | 2 | 1 | 3 |
| Totals | 16 | 22 | 38 |

The time during which the grafts remained patent (including those still patent) is shown below:

| Survival time of graft | End-to-end technique | | By-pass technique | | Total |
|---|---|---|---|---|---|
| 6 months | 6 | | 6 | | 12 |
| 6-12 months | 3 | | 2 | | 5 |
| 12-18 months | 2 | | 8 | | 10 |
| 18-24 months | 2 | | 3 | | 5 |
| 24-30 months | 1 | 7 | 3 | 14 | 4 |
| 30-36 months | 0 | | — | | 0 |
| Over 36 months | 2 | | — | | 2 |
| Totals | 16 | | 22 | | 38 |

All patients with patent grafts have been virtually relieved of claudication from muscle ischaemia and from distal ischaemic symptoms.

The results indicate that, when feasible, arterial grafting is an efficacious method of relieving symptoms of occlusive arterial disease of the lower limbs. The by-pass technique is proving superior to the end-to-end technique: for whereas 7/16 of the end-to-end grafts have survived one year, 14/22 of the by-pass grafts have survived this period.

## RAYNAUD'S PHENOMENON

A. J. Barnett.

Observations on patients with ichaemic episodes of the hands (Raynaud's phenomenon) continue. In addition to recording clinical features, patients are being investigated by an arterial occlusion reactive hyperaemia test, calorimetry and in some cases by brachial arteriography.

Special interest is being taken in the group of patients with associated sclerodermatous changes in the skin. 20 patients with this condition have now been studied. The classification previously adopted continued to be satisfactory. In this patients are divided into four groups: (a) patients with clinical Raynaud's disease later developing scleroderma localised to the hand; (b) patients with clinical Raynaud's disease later developing widespread scleroderma; (c) patients not suffering from clinical Raynaud's disease and developing scleroderma localised at the hands and (d) patients not suffering from clinical Raynaud's disease and developing widespread scleroderma. There is however

a certain overlap between the groups for features, other than scleroderma and ischaemic episodes which are common to all, present in one group may occur in occasional cases in another group.

## AMNION IMPLANTS

J. K. Francis.

This method of improving the walking distance in patients with occlusive arterial disease was described by Troensegaard-Hansen. He claimed dramatic results and as reported last year a trial of this therapy was commenced.

A series of 21 patients suffering from claudication were assessed prior and subsequent to amnion implantation. Assessment was made by walking distance, and step test performances and by estimation of the limb circulation by clinical and plethysmographic studies.

All the patients were observed for a period of 3 months prior to implantation and the post-implantation assessment was made during the 3 months following treatment.

Results were disappointing in that only one patient was rendered free from claudication (compare Troensegaard-Hansen 75%). This patient subsequently noted return of claudication pain and lessening of his walking distance.

The other 20 patients noted either no improvement or initial improvement of limited extent which later disappeared.

In no case was there any convincing demonstration of an increase in blood flow to the limb.

## SERUM LIPOPROTEINS

P. J. Nestel.

As it has been claimed that the pattern of serum lipoproteins is disturbed in peripheral vascular disease the ratio of beta to alpha lipoproteins has been studied in patients with occlusive peripheral arterial disease and in a control series without this disease.

Paper electrophoresis using Sudan Black B to stain the lipoproteins was used to separate the alpha and beta components which were then measured by photoelectric scanning.

A series of 62 patients admitted to hospital with various diseases but with no evidence of occlusive arterial disease, either peripheral or coronary, and a series of 44 patients with evidence of peripheral vascular disease were studied and the data compared. All patients were receiving a normal diet and were not fasting when the serum sample was obtained. Adequate matching between the two series for age and sex was obtained.

A significant difference in the beta/alpha lipoprotein ratio was observed. The ratio for the control series was 2.68 and 4.47 for the series with peripheral arterial disease.

## ENERGY PRODUCTION IN THE MYOCARDIUM*

W. G. Nayler.

Following on from the previous studies in this series using the modified Warburg type apparatus to record the metabolic and physiological effects of certain drugs on the isolated spontaneously beating toad heart, additional data relating to the mode of action of the cardiac glycosides has been obtained.

The apparatus has been further developed in that terminals suitable for the attachment of E.C.G. leads have been added, enabling a continuous E.C.G. pattern to be recorded throughout the experiment as required. Additional apparatus has been added so that duplicate experiments can be performed simultaneously.

## ACTION OF CARDIAC GLYCOSIDES: METABOLIC INHIBITORS

Since the earlier experiments in this series indicated that the glycosides produced an overall increase in efficiency of the isolated heart associated with increased work output but no significant change in the metabolic pattern, some further experiments were done in an attempt to determine whether or not high energy phosphate bonds played any part in this increased efficiency.

Both 2-4 dinitrophenol (D.N.P.) and sodium salicylate, at suitable concentrations, inhibit oxidative phosphorylation so that either of these drugs can be used to restrict the supply of high energy phosphate bonds in the isolated heart. Thus, in a series of experiments using summer toads the action of strophanthin G ($1 \times 10^{-5}$ final concentration) and Lanatoside C ($2 \times 10^{-5}$ final conc.) on hearts which had been previously perfused for 1½ hours with Ringer enriched with either sodium salicylate (5mM final conc.) or D.N.P. ($10.9 \times 10^{-5}$ M final conc.) were compared with their action on hearts similarly perfused with Ringer solution alone.

In another series the cardiac glycoside and the metabolic inhibitor (either D.N.P. or sodium salicylate) were added simultaneously to hearts perfused in standard Ringer solution and the response compared, as above, with that due to the addition of the glycoside alone.

Whereas the addition of either strophanthin-G ($1 \times 10^{-5}$) or Lanatoside C ($2 \times 10^{-5}$) to hearts perfused with Ringer alone always produced a positive inotropic response associated with augmented oxidative metabolism, the addition of either of these glycosides to hearts pre-treated with the metabolic inhibitors repeatedly failed to show any positive inotropic response or increased oxygen uptake. In those preparations in which the glycoside and the inhibitor were added simultaneously to hearts perfused with Ringer solution as above, a normal positive inotropic response was recorded together with augmented oxidative metabolism.

Apparently, since the inotropic response of the glycosides was abolished by pretreatment of the hearts with D.N.P. or salicylate at concentrations which would interfere with oxidative phosphorylation, it does appear that a supply of high energy phosphate bonds is a necessary part of the positive inotropic response. In those preparations in which the glycoside and inhibitors were added together the phosphocreatine and adenosine triphosphate levels apparently remained sufficiently high to allow the positive inotropic response to be manifested.

## IONIC CHANGES AND QUINIDINE SULPHATE

Since it has been established that the cardiac glycosides increase the rate of loss of potassium from cardiac muscle, it is possible that a changed intracellular ionic pattern is intimately associated with the positive inotrope response. To investigate this possibility a series of experiments was carried out using summer toads, in which the rate of loss of potassium from the cells was restricted by the addition of quinidine sulphate to the perfusate.

Initially the action of quinidine sulphate on the isolated heart was studied.

Without affecting any significant change in the respiratory quotients of the isolated hearts, quinidine sulphate ($1 \times 10^{-5}$ final conc.) produced a marked decline in work output, associated with reduced oxidative metabolism and an overall lowering of the efficiency.

Isolated hearts pretreated with quinidine sulphate at the above concentration for 1½ hours failed to show any positive inotropic response or augmented oxidative metabolism following the administration of strophanthin-G ($1 \times 10^{-5}$) which, as noted above, produces an inotropic response in hearts perfused with standard Ringer solution. In another series the initial dose of quinidine sulphate was reduced to $1 \times 10^{-6}$; which alone produced no cardiac response but which greatly reduced the inotropic effect of the above dose of strophanthin G.

Since these results suggested that the rate of loss of potassium from the cell was involved in the degree of the inotropic response recorded, a further set of experiments was carried out in which the inotropic response of strophanthin G ($1 \times 10^{-5}$) was recorded from hearts perfused with Ringer containing potassium concentration varying from 1.6mM/litre to 4.8mM/litre.

At the lower potassium level, when the rate of loss of potassium from the cell would be maximal, the inotropic response was rapidly apparent but of reduced duration and magnitude. At higher potassium levels the response was greatly delayed and of lesser magnitude when compared with the maximum response recorded at that potassium level normally found in the serum of toad Bufo marinus (3.2mM/litre).

At the lower potassium (1.6mM/litre) levels the toxic dose of strophanthin G was greatly reduced when compared with that at 3.2 or 4.8 mM/litre.

These results suggest that the degree of the response, the time over which the effect is apparent and the toxicity of the glycoside are related, in part at least, to the rate of loss of potassium from the myocardial cells.

## SEASONAL VARIATION IN RESPONSE TO STROPHANTHIN G

### 1. Respiratory Quotients

As noted in earlier experiments hearts from winter hibernating toads showed greatly reduced sensitivity to the glycosides. Since the respiratory quotients of winter hearts fall from the summer level of 0.95 to 0.75, a series of experiments was carried out in which glycogenolytic agents were added together with the glycoside, thereby raising the respiratory quotient of the winter hearts to the summer level. At this raised level strophanthin G ($1 \times 10^{-5}$) failed to elicit any inotropic response from the isolated winter heart.

Similarly, hearts perfused for eighteen hours with Ringer solution enriched with thyroxine failed to respond to strophanthin G at the above concentration.

The results obtained from the experiments using summer hearts in which the inotropic response was limited in part, by the rate of loss of potassium from the myocardium were substantiated from a further set of experiments using winter hearts pre-treated with 9 alpha FF. This cortisone derivative is glycogenolytic and increases the rate of uptake of sodium hy and efflux of potassium from cells. In this series, winter hearts which had heen pre-treated with 9 alpha FF produced a positive inotropic response following the addition of strophanthin G ($1 \times 10^{-5}$). Since other glycogenolytic drugs failed to restore the inotropic response of the winter heart, the action of 9 alpha FF apparently lies in its effect upon ionic exchange. If this is so, then the inotropic response

of the winter heart should be restored hy adjusting the potassium level of the perfusate.

**2. Ionic**

Since ATPase activity is governed in part by the Ca/Mg ratio, the calcium levels in a series of winter hearts was raised from 1.3 mM/litre to 2.6mM/litre and 5.2mM/litre and the response to strophanthin G tested as above. Similarly the response of the winter heart to 9 alpha FF at different calcium levels was compared with the positive inotropic response produced by this drug at the normal calcium concentration in the summer isolated heart (1.3mM/litre).

In this latter series it was apparent that, as with the glycoside, the response of the winter heart to 9 alpha FF ($1 \times 10^{-6}$) was completely suppressed at normal calcium levels. However, when the calcium level of the perfusate used on winter hearts was raised from 1.3mM/litre (used normally) to 2.6mM/litre, the response to both strophanthin G and 9 alpha FF was restored. At the higher calcium concentration (5.2mM/litre) the positive inotropic effect in the winter hearts was again suppressed suggesting that there is a critical calcium level for the contractile system above or below which the glycoside cannot produce an inotropic effect. During hibernation this critical calcium level is higher than that required by summer hearts.

In an additional series the response of the isolated winter heart to additional calcium ions was compared with that of the summer heart under identical perfusion conditions. In all preparations the winter heart showed a markedly increased sensitivity to calcium ions when compared with the non-hibernating counterpart.

## PHOSPHATE ENERGY

Since part of this reduced sensitivity to drugs associated with hibernation may be due to a lowered supply of phosphate bond energy from creatine phosphate stores, creatine phosphate determinations have been carried out regularly throughout the year. Although there has been some variation in phosphate levels throughout the year, the deviations were not sufficiently great to explain the reduced sensitivity. More likely it seems that some ionic disturbance including, amongst others, calcium ions, is responsible for this seasonal pattern of sensitivity.

## INTRACELLULAR CARDIAC MUSCLE POTENTIAL STUDIES*

**M. Quinn-Young and D. McKelvie.**

The aim of this work is firstly to record intracellular potentials from toad (Bufo marinus) heart muscle fibres and to study the effect of various drugs on the resting potential and the amplitude, shape and duration of the action potential records. We have attempted to obtain this data from the intact perfused (recycling) heart with a view to correlating it with the results obtained in other research.

During the year apparatus and recording equipment has been made and adapted to fit the needs of the experiment. A differentiator has been built to measure the maximum rates of rise of action potentials and a multi-channel CRO display unit has been adapted to enable four events (baseline, intracellular potential, maximum rate of rise and a time marker) to be recorded simultaneously on photographic paper. The microelectrodes are inserted into the muscle using a manipulator made from a disused microscope stand.

The main difficulties encountered have been to fill glass microelectrodes with 3M KCl after they are pulled and to prevent their breakage on insertion into the moving heart. Filling by boiling in 3M KCl under reduced pressure has been found fairly successful. Breakage has been reduced by inserting the microelectrodes through a small perspex ring held down on the muscle partially to immobilise it. This is not entirely satisfactory although some long recordings have been made. Much time has been spent investigating other types of electrode which have been described. Two of these which showed promise were fine tungsten wire, electrically pointed and insulated with lacquer and fine tungsten wire tipped with the extreme end of an ordinary KCl-in-glass microelectrode. In the first case it was found impossible to apply the lacquer so that it was a reliable insulator but the second method if care is taken in manufacture shows promise of a lower breakage rate when inserted in the beating heart.

## ABNORMAL SERUM PROTEINS

C. C. Curtain.

### THE HISTOCHEMICAL LOCALIZATION OF MACROGLOBULINS

The term "macroglobulin" was introduced by Waldenstrom in 1948 to describe proteins in human serum having molecular weights in excess of one million. These proteins may be associated with a typical clinical syndrome characterised by lethargy, a bleeding tendency, haemorrhagic purpura and enlargement of the liver, spleen and lymph nodes. The bone marrow is often invaded by so-called lymphocytoid cells, many of which show cytoplasmic loss or irregularity. In smaller amounts macroglobulins may be associated with lymphosarcoma and, very rarely, multiple myeloma.

Because of the variety of pathological cell types found in the tissues of individuals with macroglobulinaemia cellular localisation of macroglobulins by means of the fluorescent antibody technique of Coons has been attempted.

Two cases of macroglobulinaemia were available for study. The first* had a lymphocytic tumour infiltrating the sub-lingual glands and abundant plasma cells in the bone marrow and peripheral blood. The serum contained 1.5 gm per cent. of macroglobulin. Bone marrow smears were treated with highly specific fluorescent anti-macroglobulin and examined in the fluorescence microscope. The cytoplasm of the plasma cells fluoresced brilliantly, indicating the presence of large amounts of macroglobulin. Bright fluorescence was also observed in many large immature cells which with normal staining had a thin rim of basophilic cytoplasm and a large nucleus, and were probably haematogenous stem cells. The similarity between these observations and the description given by Coons of antibody production by the plasma cell and its precursors is striking—suggesting that macroglobulin is synthesised (like antibody) during the process of differentiation and maturation of the (in this case malignant) plasma cell.

The second was a case of multiple myeloma which was producing small amounts of macroglobulin and a myeloma protein of normal molecular weight (M-protein). By serial staining of the marrow smears with specific fluorescent antibody to the macroglobulin and the M-protein it was possible to show that individual plasma cells produced only one kind of protein. No staining of stem cells was observed. Studies of frozen sections of various organs obtained post-mortem from this case revealed no staining by fluorescent anti-globulin or M-protein. The plasma cells of the lymph nodes and spleen, however, stained with fluorescent anti-gamma globulin.

*This patient was studied through the courtesy of Dr. R. Motteram, Peter MacCallum Clinic.

## IMMUNE TOLERANCE AND ABNORMAL SERUM PROTEINS

The pathological serum proteins of myeloma, macroglobulinaemia and cryoglobulinaemia (the so-called paraproteins) have been generally regarded as entities not present in normal serum. Evidence in favour of this hypothesis includes the wide range of physical properties encountered amongst the abnormal serum proteins and that their anti-sera can be made highly specific after absorption with normal human serum to remove cross-reacting antibodies. Recently this view has been challenged by several workers who have succeeded in inhibiting the reactivity of para-protein anti-sera with the aid of various normal human globulin fractions. These workers have concluded that the pathological globulins are gross elevations of proteins occurring in minute amounts in the sera of normal individuals.

In contemplating these hypotheses the question arises as to why sufferers from the paraproteinaemias do not produce antibodies to their abnormal proteins if these are entities not present in normal serum, since in the early stages of these conditions the immune response is unimpaired. Current opinion regarding immune tolerance and "self-markers" suggests that the paraproteins must have been present in the individual from birth for antibody formation not to occur. Recent advances in our knowledge of immune tolerance in laboratory animals provide us with a means of exploring this question experimentally. It is known that rabbits injected with an antigen within a few hours of birth fail to form antibody when challenged with this antigen in adult life, although their ability to respond to other antigens is unaffected. In order to define more precisely the specificity of immune tolerance the following experiment was conducted. A Bence-Jones protein was purified from normal urine and compared with normal gamma globulin immunologically (by double diffusion in agar against the appropriate antisera). The Bence-Jones protein had some, but not all of the antigenic determinants of the gamma globulin. Concentrated solutions of the Bence-Jones protein were injected into rabbits, 0-6 hours old, and at three months these rabbits and uninjected litter mate controls were challenged with the antigen. The controls produced high antibody titres whilst the rabbits injected at birth gave no response. All the rabbits were then injected with gamma globulin. Both groups produced antibody but the immunologically tolerant rabbits produced antibody only to those antigenic determinants which gamma globulin did not have in common with the Bence-Jones protein. This, and a similar experiment with a macroglobulin and gamma globulin, demonstrated that the specificity of immune tolerance is fine enough to distinguish between two proteins differing by relatively few antigenic determinants. New born rabbits were then injected with concentrated solutions of human plasma from either a pool or individual donors. At three months these rabbits were injected with very large amounts of human plasma, with Freund's and other adjuvants. No antibody was detectable in these rabbits, even by the sensitive tanned sheep cell technique. Litter-mate controls rapidly became hyperimmune with such treatment. The unresponsive rabbits were then injected with sera from a case of cryoglobulinaemia, two cases of macroglobulinaemia and four cases of myeloma. Under no conditions was it possible to demonstrate antibody production by these rabbits. It was concluded that the pathological proteins contained no antigenic determinants other than those present in the pooled and individual normal human plasma injected into the rabbits at birth.

This conclusion in no way invalidates the localisation of paraproteins by the fluorescent antibody technique. Orthodox precipitin tests show that the antibodies left after absorption of anti-paraprotein sera with normal globulins are to antigenic determinants occurring to a very small extent in normal plasma—and would presumably be present in very few cells. This is borne out by the negative results obtained with normal bone marrow and fluorescent antibody to paraprotein.

## ELECTROPHORESIS

A recent acquisition has been a Perkin-Elmer Tiselius electrophoresis apparatus. Notwithstanding the proliferation of techniques of electrophoresis on filter paper and other supporting media, electrophoresis in free solution with optical recording remains the method of choice for the precise analysis and characterisation of protein mixtures. The accuracy and sensitivity of the method has been greatly increased by the interferometric optical system with which the Perkin-Elmer instrument is equipped.

As well as playing an important part in the studies on abnormal serum proteins the instrument has made possible a number of new projects.

### (a) THE SERUM PROTEINS OF NEW GUINEA NATIVES†

(i) *The distorted serum protein picture in Kuru.*

An invariably fatal neurological disease, Kuru, peculiar to the Fore people of the Eastern Highlands of New Guinea has been investigated recently by several Australian laboratories. We have used electrophoresis to study the sera of Kuru patients. It was found that many of them had a grossly elevated beta globulin (values of up to 4 gm% were recorded). Some patients had a high alpha$_2$ globulin, whilst a few had patterns with an abnormally low overall globulin concentration. These findings are being correlated with observations made on the disease by workers in the field.

The abnormal electrophoresis pattern is of considerable interest because it is the only positive result from a wide range of biochemical investigations made on the disease.

(ii) *Presence of an additional globulin in the serum of New Guinea Highland Natives.*

In the course of the Kuru study electrophoresis runs were made on the sera of many normal natives. An additional globulin component was found in most of the Eastern Highlands sera examined. This had a mobility slightly greater than that of normal gamma globulin (2.01 $cm^2\ sec^{-1}\ volt^{-1} \times 10^{-5}$ in pH 8.6, I = 0.1 veronal buffer) and is present in concentrations of 6-9% of the total serum proteins. This component could not be detected in the sera of normal Europeans, or of non-Eastern Highland natives on electrophoresis under the same conditions.

It is proposed to study sera from as wide a range of Melanesian peoples as possible to see if this new globulin is unique to the Eastern New Guinea Highlands. Wider investigation may help to decide whether its presence is due to environmental or racial factors. This in turn may help our understanding of the aetiology of Kuru.

†This project is being carried out in conjunction with Dr. D. C. Gajdusek, National Foundation for Infantile Paralysis, U.S.A., and Dr. V. Zigas, Department of Public Health, Port Moresby.

## (b) SICKLE CELL ANAEMIA

The first case of sickle cell anaemia to be recorded in Victoria was diagnosed at the Alfred Hospital early in 1957. The haemoglobins of the patient and his family were examined by moving boundary electrophoresis. The examination showed that the mother was a heterozygous carrier of the sickle cell gene. This information and the fact that the father had an unduly high proportion of foetal haemoglobin suggested that the case was one of sickle cell Thallasaemia.

## (c) FLUORESCEIN-GLOBULIN COMPLEXES

The finding by Louis and Hughes of the Melbourne University Pathology Department that malignant cells do not take up fluorescein conjugated globulin, whereas normal cells do, has focused attention on the phenomenon of non-specific staining by fluorescent antibody. We observed during the fluorescent anti-macroglobulin studies that purification of the conjugated antibody on antigen coupled columns of diazo-benzyl cellulose completely removed non-specific staining. This suggested that the latter was not an intrinsic property of the conjugated antibody but of some fraction of the total fluorescent globulins.

With the availability of the Tiselius electrophoresis apparatus it was decided to investigate the properties of some fluorescein-protein conjugates. The results may be summarised:

1. Conjugation of human or rabbit serum gamma globulin with fluorescein leads to a marked increase in electrophoretic heterogeneity. This is accompanied by the appearance of a sharp negatively charged peak whose size is related to the number of fluorescein groups conjugated.

2. Samples taken from the cell after electrophoresis showed that, in the case of antibody-containing rabbit globulin, most of the activity was in the least negatively charged portion. This also contained the smallest amount of conjugated fluorescein and gave virtually no non-specific staining. The leading peak contained very little antibody, was heavily conjugated and stained normal tissues brilliantly.

3. Conjugation of human serum albumin with fluorescein did not lead to an increase in heterogeneity, but increased the mobility towards the anode by 20%. It was considered that the increased heterogeneity observed with the gamma globulin was due to differential conjugation of the many molecular species known to be present in this serum fraction. Albumin is, relatively, much more homogeneous and uniform conjugation occurs.

These observations also point the way to more effective removal of non-specific staining from fluorescent antibody preparations. The iso-electric points of the antibody and over-conjugated fractions are sufficiently apart to permit separation by electrophoresis-convection. Preliminary experiments along these lines have been most encouraging.

## OPEN INTRA-CARDIAC SURGERY

I. A. LeG. Ferguson and F. Kinross.†

The object of this study has been to investigate and develop methods of conducting intracardiac surgery with safety.

†Thoracic-Surgical Unit, Alfred Hospital.

Initially the effort was concentrated on hypothermia, but this was discontinued about the middle of 1956 owing to the serious intrinsic limitations of the method. These were first, the short time of circulatory arrest allowable, second, the considerable risk of ventricular fibrillation, which is very difficult to revert and precludes ventricular surgery, and last, the time consuming nature and unwieldiness of the whole procedure.

After the cessation of work on hypothermia techniques for the use of a pump oxygenator were investigated. These machines offer two major advantages —the greatly increased time available for intracardiac manoeuvres, and the fact that ventricular fibrillation is hardly a problem. It is important to stress the fact that these machines are not a substitute for the normal heart and lungs, and there is a limit to the time that the circulation can be carried on by them.

There are disadvantages inherent in the use of these machines. Blood has to be circulated through a system of foreign tubes and pumps and may suffer in many ways in consequence. Also, there may be functions of the by-passed organs for which the machine does not substitute, such as deactivation of 5-hydroxytryptamine in the lungs. How important such considerations are is not known with certainty. These may ultimately impose a time limit on the duration of by-pass—even with perfect machines. However it would seem likely that such a time limit will be one of many hours or days, i.e., relevant to medical therapy rather than cardiac surgery. There are some other disadvantages which are characteristic of the particular mechanism used. These will ultimately be eliminated.

When it was decided to take up this work here, there were two tried and proven machines in use. These were the de Wall-Lillehei bubble oxygenator at Minneapolis and the Gibbon filming type at the Mayo clinic. At that time there was no great disparity in the results obtained with each machine. The Gibbon machine is very expensive and complex, while the de Wall-Lillehei oxygenator is relatively cheap and maintenance problems hardly exist, hence we decided to begin work with the latter.

Our aim since beginning work with this machine has been first to learn to use it safely, and secondly to investigate the physiological changes associated with its use. We began by duplicating so far as was possible the technique used at Minneapolis and initially met with complete lack of success. All the experimental animals died within 24 hours of a period of thirty minutes' by-pass on the machine. All further information we obtained from Minneapolis failed to help with the problem.

In an attempt to isolate the important factor in the failure of our technique we conducted a series of experiments. With thirty minutes of pumping from the external jugular vein to the carotid artery at standard outputs without a thoracotomy, we found that the animals survived. Pumping for thirty minutes from the venae cavae to the left subclavian artery, without excluding the heart and lungs from the circulation, resulted in the survival of all animals. Fifteen minutes of pumping from the venae cavae to the left subclavian artery with the exclusion of the heart and lungs—in other words, fifteen minutes of full by-pass also gave successful results. However, with thirty minutes of by-pass the animals all died.

During the period of by-pass we found by investigation that the arterial oxygenation was usually in the region of 100%, and the arterial blood pressure

remained at normal values. The biochemical changes produced were not such that they were necessarily fatal. In general we found that the bicarbonate values fell during the by-pass and rose afterwards quite rapidly. At the end of by-pass the level would often be as low at 10 mEq/litre, rising to 20 mEq/litre after one to two hours following the by-pass. The pH readings showed a rise from 7.35 before by-pass to 7.43 at the end of by-pass, with a progressive fall thereafter, the lowest value reached being 7.3. One might interpret these changes as representing a partly compensated respiratory alkalosis arising during the by-pass changing to a metabolic acidosis following the by-pass.

From the investigations we concluded that the causes of death did not lie among the following:

(a) Toxins produced by or added during circulation of blood through the machine.

(b) Haematological changes due to use of the machine.

(c) Emboli from the machine.

(d) Cerebral anoxia.

(e) Biochemical changes induced by complete by-pass.

(f) Magnitude of the operation, type of thoracotomy used, or in fact any technical aspect of the operation other than the period of by-pass.

In considering the possibilities remaining, the question of bacterial infection arose. The operations were done without using an aseptic technique, and also dogs have a well-known susceptibility to anaerobic septicaemia following circulatory disturbances, particularly those affecting the liver. Bacterial infection had not previously been seriously considered owing to the rapidity with which death followed the operation—always within 24 hours.

We found that providing the animals were given a large dose of penicillin before operation, the great majority survived thirty or even forty-five minutes' by-pass.

This could be the explanation of the failure of a number of groups to duplicate the work done at Minneapolis, since the lack of preoperative chemotherapy is unlikely to be considered to be of importance in the prevention of early post operative deaths.

## MECHANICAL ASPECTS OF LUNG FUNCTION

J. R. E. Fraser.

This project was commenced with the object of establishing techniques for the study of various mechanical aspects of lung function. The theoretical basis of these studies was clearly analysed over 30 years ago, particularly by Rohrer in Basle, but was not regularly applied in human studies until recent years, probably due in part to the belief held earlier that intra-oesophageal pressure was not an adequate index of intrathoracic pressure, which is an essential measurement in this work. Several studies have shown that this objection is not of great consequence, and a considerable amount of physiological information has been obtained in the last few years.

As with most new techniques, the method has not yet been standardised, and indeed several of the important specifications of the equipment have not

been clearly stated, though no doubt recognised. (This applies more particularly to the performance of spirometers, which were chosen for this particular work.) Our attention has therefore been directed to establishing our equipment with particular reference to the performance of the spirometer, and the electro-mechanical transducer which was designed to translate the spirometric movements to the photographic record.

Studies of patients with pulmonary or cardiac disease were undertaken as they were referred for other lung function tests, and in the course of developing the method, but specific studies have been deferred during this period.

In general, our observations follow the same pattern as those reported elsewhere.

During the course of this work, several reports appeared describing a tendency for the "effective" lung compliance to fall with increasing respiratory rates in patients with pulmonary disease. Theoretical analysis and studies on models indicated that this phenomenon results from regional inequalities in both the lung elasticity and non-elastic resistances. This observation has been confirmed here, and has required extensive records. Whether it will afford a clinically helpful test remains to be seen. Other observations concerning the effects of pulmonary vascular congestion have been confirmed, and it is possible that variation of venous return to the heart by various means will serve to reveal functional disturbances in cardiac patients which are not easily apparent under resting conditions. It is hoped that useful information will be accumulated in the course of the routine performance of these tests, as well as by planned study.

## TRANSAMINASE AND MYOCARDIAL INFARCTION

C. C. Curtain.

In 1954 Karmen, Wroblewski and LaDue observed that the activity of the enzyme serum glutamic-oxaloacetic transaminase (S.G.O.T.) was greatly increased immediately after myocardial infarction. Since then the American literature has contained numerous reports on the diagnostic value of S.G.O.T. determinations. The method of choice in the U.S.A. employs a coupled dehydrogenation reaction involving a co-enzyme the oxidation of which can be followed by ultraviolet spectrophotometry.

S.G.O.T. can, however, be estimated by quantitative paper chromatography of the glutamic acid formed on incubation of a mixture of serum, aspartate and alpha-ketoglutarate. The method is slow, but simple, inexpensive and foolproof. It was used to estimate the S.G.O.T. of 500 subjects, comprising normal donors and hospital patients with various conditions. The results were substantially the same as reported by other workers. High S.G.O.T. activities were found in cases of proven myocardial infarction, in a number of doubtful cases of infarction and in cases with hepatic necrosis. Patients with other conditions and all the normals had low activities. Towards the conclusion of the study colorimetric estimations by the method of Reitman and Fraenkel (Am. J. Clin. Path., 28, 1, 1957) were run in parallel with the chromatographic technique.

## THE DIURETIC PROPERTIES OF CHLOROTHIAZIDE

R. J. Marshall.

Chlorothiazide ("Chlotride")* has recently been introduced as an orally effective diuretic compound. In experimental animals it induces a marked increase in the output of water, sodium and chloride, and, unlike other carbonic anhydrase inhibitors, does not induce a metabolic acidosis. A trial has been undertaken to study its diuretic properties in non-oedematous subjects and in patients with abnormal fluid retention. Studies of its anti-hypertensive effects are referred to elsewhere in this report.

In non-oedematous subjects a diuretic effect is noted within 2 hours of administration, and persists for 6 to 10 hours. Surplus sodium and chloride are excreted in approximately equivalent amounts; there is a slight increase in potassium output and little change in bicarbonate output. The drug is more effective when given in divided doses. Continuous administration over periods of 4 to 6 weeks is well tolerated; there is a fall in body weight averaging 1.9 kg (12 subjects), a moderate fall in plasma chloride and slight and inconstant falls in plasma sodium and potassium. These changes revert to normal on cessation of therapy.

Chlorothiazide usually induces a prompt diuresis in patients with congestive cardiac failure and retains its effectiveness on prolonged administration. It is generally more effective than acetazolamide and often comparable with the parenteral organic mercury compounds. Little or no response occurred in 3 patients with intractable cardiac failure and impaired renal function, who were also resistant to all other diuretics employed. Encouraging results were obtained in a small group of patients with nephrotic oedema.

No toxic effects were encountered during the trial which could be attributed to the drug. However great care will be required to ensure that patients, especially those with restricted intakes of sodium, do not develop serious electrolyte disturbances during prolonged spells of treatment.

## PTERYGIUM

J. K. Galbraith and R. Fowler.†

In 1954 studies on the causation of pterygium were carried out in the Institute and some evidence was obtained that exposure of the cornea to hot air might be a contributory factor. This year the investigation has been resumed.

In a series of rabbits one eye was exposed daily to a current of warm air for period of one half to one hour for many weeks. In some animals drying of the cornea was prevented by the repeated instillation of artificial tears.

In almost all cases a change was produced in the cornea which had the macroscopic appearance of a pterygium and many microscopic similarities to that lesion.

When drying of the cornea was prevented the change still developed and seemed dependent on the temperature of the air jet—at all times this was kept below the burning temperature.

*Merck & Co. Inc., West Point, Pa.

†Royal Children's Hospital.

## PUBLICATIONS DURING 1957

T. E. Lowe: "Control of Body Fluid Volume: Some Observations and a Hypothesis." *Amer. Heart J.*, Vol. 53 (1957), p. 265.

P. Fantl: "Parahaemophilia (Proaccelerin Deficiency) Occurrence and Biochemistry." *Proc. Internat. Soc. Haematology*, p. 79.

P. Fantl and A. G. Marr: "The Preservation of Coagulation Factors in Human Plasma." *Aust. J. Exp. Biol. & Med. Sci.*, Vol. 34 (1956), p. 433.

P. Fantl and A. G. Marr: "Prothromboplastin Activities of Some Mammalian Plasmas and Sera." *Aust. J. Biol. Sci.*, Vol. 10 (1957), p. 351.

P. Fantl and A. G. Marr: "Phenols as Inhibitors of the Herapin Co-Factor of Plasma." *Nature*, Vol. 180 (1957), p. 990.

P. Fantl and H. A. Ward: "Nucleotides of Human Blood Platelets." *Biochem. J.*, Vol. 64 (1956), p. 747.

P. Fantl and H. A. Ward: "Comparison of Blood Clotting in Marsupials and Man." *Aust. J. Exp. Biol. & Med. Sci.*, Vol. 35 (1957), p. 209.

W. G. Nayler: "Seasonal Variation in Carbohydrate Metabolism and Drug Sensitivity of the Isolated Toad Heart (Bufo Marinus)." *Aust. J. Exp. Biol. & Med. Sci.*, Vol. 35 (1957), p. 131.

W. G. Nayler: "Cardiac Metabolism: Ionic Changes. Influence of Calcium Ions, (9.a, Fluorohydrocortisone and Cardiac Glycosides on the Isolated Toad Heart)." *Aust. J. Exp. Biol. & Med. Sci.*, 35 (1957), p. 241.

W. G. Nayler: "The Action of Salicylate, Dinitrophenol and Cardiac Glycosides on the Isolated Toad Heart." *Aust. J. Exp. Biol. & Med. Sci.*, Vol. 35 (1957), p. 491.

G. A. Bentley: "Study of the Inhibitory Action of Adrenaline: 1. Effects on the Carbohydrate Metabolism of Isolated Rabbit Intestine." *Aust. J. Exp. Biol. & Med. Sci.*, Vol. 34 (1956), p. 485.

G. A. Bentley and Bryan Hudson: "A Comparison of Methods for the Isolation of Melanocyte Stimulating Hormone from Blood." *Aust. J. Exp. Biol. & Med. Sci.*, Vol. 35 (1957), p. 157.

G. A. Bentley and Bryan Hudson: "The Nature of the Pigmentary Disturbance in Addison's Disease." *A/sian Ann. Med.* Vol. 6 (1957), p. 98.

G. A. Bentley and Bryan Hudson: "The Biological Assay of Melanocyte Stimulating Hormone." *Aust. J. Exp. Biol. & Med. Sci.*, Vol. 35 (1957), p. 45.

Bryan Hudson and Alison Doig: "Observations on the Nature of Hormone Induced Eosinopenia." *A/sian. Ann. Med.*, Vol. 6 (1957), p. 228.

Bryan Hudson, A. J. Barnett and J. Bornstein: "Primary Aldosteronism." *A/sian Ann. Med.*, Vol. 6 (1957), p. 250.

Bryan Hudson and F. E. Binet: "The Accuracy of Eosinophil Counts." *Aust. J Exp. Biol. & Med. Sci.*, Vol. 34 (1956), p. 479.

B. McA. Sayers and F. G. Silberberg: "Further Studies of the Electrocardiographic Spatial Magnitude Curve (in the Rabbit)." *Am. Heart J.*, Vol. 53 (1957), p. 558.

## PAPERS ACCEPTED FOR PUBLICATION

T. E. Lowe: "Further Observations on the Relation Between Body Fluid Volume and Urine Flow." *A/sian Ann. Med.*

A. J. Barnett and G. Wagner: "Calorimetry. 11. Clinical Application in Peripheral Vascular Disease." *Med. J. Aust.*

W. G. Nayler: "Further Observations in Seasonal Variations in Drug Sensitivity of the Isolated Toad Heart." *Aust. J. Exp. Biol. & Med. Sci.*

D. Emslie Smith: "The Spatial Vectocardiogram in Hypothermia." *Brit. Heart J.*

## PAPERS SUBMITTED FOR PUBLICATION

A. J. Barnett and G. Wagner: "Severe Orthostatic Hypotension."

D. Emslie Smith, G. E. Sladden and G. R. Stirling: "The Significance of Changes in the Eloctrocardiogram in Hypothermia."

P. Fantl and A. G. Marr: "Coagulation of Horse Blood."

## LECTURES DELIVERED DURING 1957

"The Value of Mathematics in Biological Problems" . . . . . T. E. Lowe
Mathematics Society, University of Melbourne.

"Coagulation Problems in Open Heart Surgery" . . . . . P. Fantl
Royal Australasian College of Surgeons.

"Biochemistry of Blood Clotting" . . . . . P. Fantl
National University, Canberra.

"Medical Aspects of Blood Coagulation" . . . . . P. Fantl
University of Melbourne.

"Structural Requirements for Phospholipid Activity in the Activation of Prothrombin by Russell Viper Venom" . . . . . P. Fantl, A. G. Marr, H. Ward
Australian Biochemical Society.

"Smooth Muscle Contracting Compounds of Human Blood Platelets" . . P. Fantl, H. Ward
Victorian Society Pathology and Experimental Medicine.

"Experience with the use of Ganglionic Blocking Agents in Hypertension" . . A. J. Barnett
Hypertension Colloquia, Sydney.

"Seasonal Variation in Drug Sensitivity of the Isolated Heart" . W. G. Nayler
Victorian Society for Pathology and Experimental Medicine.

"Serum Transaminase—Its Diagnostic Value in Myocardial Infarction" . . C. C. Curtain
Alfred Hospital Clinical Society.

"Bleeding in the Surgical Patient" . . . . . R. J. Sawers
St. Vincent's Hospital Clinico-Pathological Meeting, August.

"Vascular Effects in Man of 5-Hydroxytryptamine and its Antagonists" . . R. J. Marshall
Alfred Hospital Clinical Society.

"Bleeding Diseases in Northern Ireland" . . . . . M. M. Stevenson
Alfred Hospital Clinical Society.

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

**Balance Sheet as at 31st December, 1957**

| LIABILITIES. | | | | ASSETS. | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Current Liabilities— | | | | Current Assets— | | | | | |
| Sundry Creditors | £837 | 3 | 6 | Cash at Bank | | | £2,774 | 16 | 2 |
| Endowment Fund (per Contra) | 8,500 | 0 | 0 | Endowment Investments (per Contra) | | | | | |
| Capital Grants and Gifts (per Contra) | 1,473 | 13 | 1 | Inscribed Stock— | | | | | |
| Accumulated Revenue | 4,037 | 12 | 8 | Grain Elevators Board— | | | | | |
| | | | | £2,500 4½% due 1/5/1964 | £2,500 | 0 0 | | | |
| | | | | Commonwealth Government— | | | | | |
| | | | | £5,000 3½% due 15/10/1960 | 5,000 | 0 0 | | | |
| | | | | £500 3½% due 15/10/1963 | 500 | 0 0 | | | |
| | | | | Bonds— | | | | | |
| | | | | Commonwealth Government Treasury— | | | | | |
| | | | | £500 3¼ % due 15/9/1961 | 500 | 0 0 | | | |
| | | | | | | | 8,500 | 0 | 0 |
| | | | | Restricted Funds (represented by Cash at Bank)— | | | | | |
| | | | | Capital Grants and Gifts (per Contra) | | | 1,473 | 13 | 1 |
| | | | | Fixed Assets— | | | | | |
| | | | | Furniture and Fixtures | | | 2,100 | 0 | 0 |
| | | | | NOTE.—In addition to receiving interest from the Investments as shown on the Balance Sheet, the Institute receives the income from 3½% 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. | | | | | |
| | £14,848 | 9 | 3 | | | | £14,848 | 9 | 3 |

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

We have examined the annexed Balance Sheet with the books of the Institute and have obtained all the information and explanations we have required. In our opinion the Balance Sheet presents a true and fair view of the state of the affairs of the Institute at 31st December, 1957, according to the best of our information and the explanations given to us and as shown by the books of the Institute.

Melbourne,
11th February, 1958.

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

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

**Revenue Account for the Year Ended 31st December, 1957**

| EXPENDITURE. | | | | INCOME. | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Drugs, Chemicals, Provisions, etc. | £1,386 | 5 | 0 | Donations— | | | | | |
| Fuel and Lighting | 182 | 17 | 4 | Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions | £14,467 | 18 | 10 | | |
| Instruments and Glassware | 727 | 5 | 3 | Marian and E. H. Flack Trust | 400 | 0 | 0 | | |
| Insurance | 261 | 9 | 0 | Kodak (Australasia) Pty. Ltd. | 50 | 0 | 0 | | |
| Library Maintenance | 884 | 11 | 5 | George F. Little Trust | 148 | 6 | 6 | | |
| Printing and Stationery | 282 | 6 | 0 | Eagle Star Insurance Co. Ltd. | 50 | 0 | 0 | | |
| Repairs and Renewals | 368 | 1 | 1 | Dr. Ewen Downie | 5 | 5 | 0 | | |
| Salaries and Wages | 14,898 | 11 | 4 | Mr. E. Teasdale | 5 | 5 | 0 | | |
| Telephone | 116 | 12 | 1 | Sir George Coles | 5 | 0 | 0 | | |
| Sundries | 556 | 19 | 8 | Sir Archie Michaelis | 5 | 0 | 0 | | |
| Travelling Expenses | 21 | 5 | 0 | Miss N. Cameron | 2 | 0 | 0 | | |
| Workshop Equipment | 241 | 4 | 7 | | | | | 15,138 | 15 | 4 |
| *Total Expenses* | 19,927 | 7 | 9 | Appropriation of Grant—Life Assurance Medical Research Fund of Australia and New Zealand | | | | 1,846 | 14 | 3 |
| Surplus for Year | 1,262 | 2 | 4 | Government Grant— | | | | | | |
| | | | | National Health and Medical Research Council | | | | 3,251 | 3 | 0 |
| | | | | Interest from Investments— | | | | | | |
| | | | | Held by the Trustees of the Estate of the late Thomas Baker for the benefit of the Institute— | | | | | | |
| | | | | Commonwealth Government Inscribed Stock | | | | 551 | 10 | 0 |
| | | | | Endowed to the Institute— | | | | | | |
| | | | | Commonwealth Government Inscribed Stock | 178 | 2 | 6 | | | |
| | | | | Commonwealth Government Treasury Bonds | 16 | 5 | 0 | | | |
| | | | | Grain Elevators Board Inscribed Stock | 103 | 2 | 0 | | | |
| | | | | | | | | 297 | 9 | 6 |
| | | | | Sundry Sales | | | | 103 | 18 | 0 |
| | £21,189 | 10 | 1 | | | | | £21,189 | 10 | 1 |

# ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

# STAFF

| | |
|---|---|
| Honorary Physician | Ewen Downie, M.D., F.R.C.P., F.R.A.C.P. |
| Assistant Physician—<br>Scientific Studies | Joseph Bornstein, D.Sc., M.D., M.R.A.C.P. |
| Assistant Physician—<br>Clinical Studies | Bryan Hudson, M.D., M.R.C.P., M.R.A.C.P. |
| Biochemists | Peter Davoren, Ph.D., B.Sc.<br>Dora Winikoff, M.Sc.<br>June Sheath, B.Sc. |
| Registrar | Ian Martin, M.D., B.S. |
| Chiropodist | Maida O'Connor, F.Ch.A.V., M.Ch.I.A. |
| Technical Staff | Mr. W. Hudson<br>Miss A. Ekkel<br>Miss L. Gibson<br>Mrs. P. Keen |
| Secretary | Miss J. Sharp |

## RESEARCH FELLOWS

| | |
|---|---|
| J. F. Mackeddie and Frederick and Esther Michaelis | E. L. G. Beavis, M.B., B.S., D.G.O., M.R.C.O.G., F.R.C.S. |
| Edward Wilson Memorial | H. D. Breidahl, M.D., B.S., M.R.C.P. |
| Sol Green | Margaret Sanders, M.B., B.S. |

In November, 1955, on the recommendation of the Honorary Medical Staff, the Board of Management of Alfred Hospital decided to reorganise the Diabetic Instructional Clinic which was founded in 1929. It was felt that concentration on a single aspect of metabolic disorders was no longer in the best interests of patients. Accordingly the Diabetic and Metabolic Unit was founded for the purpose of studying general problems in the whole field of Endocrinology. The functions of the Unit were defined as the treatment of patients, the prosecution of research into Diabetic and Metabolic disorders and the teaching of undergraduate and postgraduate students.

The initial step in the formation of the Unit was the appointment of Dr. Ewen Downie as Honorary Physician in Charge, with Dr. Bryan Hudson as Assistant Physician (Clinical) and Miss June Sheath as Biochemist. In addition Dr. J. Bornstein was appointed as part-time Assistant Physician (Scientific).

Early in 1956, plans were drawn up for the construction of a laboratory for the purpose of investigation of patients. Money for this development was made available from the Capital Construction Grant to Alfred Hospital and a magnificent donation of £10,000 was given by the Alfred Hospital Auxiliary to provide equipment for the Laboratory. Further equipment to the value of £8000 was subsequently added by Dr. Bornstein, from grants made to him personally by Eli Lilly & Co., of the United States of America.

The embryo unit was enabled to function in 1956 thanks to temporary accommodation provided by the Director of the Baker Medical Research Institute, Dr. T. E. Lowe, and to facilities made available by Professor V. Trikojus of the Department of Biochemistry, University of Melbourne. Four beds for the investigation and treatment of patients were allocated from the general medical beds in Alfred Hospital in addition to twenty beds available in Ward 1 at Caulfield Convalescent Hospital.

During 1956, the first task of the Unit was to undertake an investigation into the value of the recently developed sulphonylurea compounds in the treatment of diabetes. This work was carried out in the closest co-operation with research centres in the United States of America, Canada and Great Britain. In June, 1956, a preliminary account of the value of these substances was published and a subsequent report in October of that year made some contributions to the possible mode of action of the drugs. During the year studies were also undertaken on the humoral factors in patients suffering from insulin resistance, and in other fields of endocrinology a start was made on fundamental work concerned with the investigation of pituitary, adrenal and thyroid disorders. By mutual arrangement, the routine investigation of patients suffering from thyroid diseases by means of radioactive isotopes was transferred from the Baker Research Institute to the control of the Unit.

Early in 1957, Dr. Joseph Bornstein assumed whole time duty with the Unit and during the year paid a short visit to the United States of America and to Canada at the invitation of the organising committee of the Insulin Symposium. In addition he attended the Annual Meetings of the Endocrine Society and of the American Diabetes Association, and visited the following research centres:—

| | |
|---|---|
| The Department of Hormone Chemistry | University of California |
| The Department of Metabolism . . . . | University of California |
| The Department of Biological Chemistry | Washington University, St. Louis |
| The Department of Physiology . . . . . | University of Chicago |

| | |
|---|---|
| The Departments of Biochemistry, Pharmacology and Physiology | Western Reserve University, Cleveland |
| The Department of Experimental Medicine | University of Michigan |
| The Department of Physiology | Vanderbilt University, Nashville |
| The Charles Best Institute | University of Toronto |

In October, 1957, Dr. Ewen Downie and Dr. Bryan Hudson visited Sydney and were present at the Inaugural Meeting of the Diabetes Federation of Australia. Dr. Downie spoke at the opening ceremony and Dr. Hudson read a paper to the Medical Section of this Meeting. Together they participated in preliminary discussions on the formation of an Endocrine Society of Australasia.

Work on the Unit Laboratory was commenced in October, 1956, and this was completed in April, 1957 by which time the Unit had increased its staff and activities. During the past year the staff has undertaken a number of important studies on various aspects of endocrinology, the details of which are set out in a later part of this report. Plans have been approved which will permit an extension of the laboratories and will provide five beds alongside the laboratories together with an appropriately equipped examination room for patients. When completed this project will make the Unit self-contained and therefore unique in Australia.

During 1957, work has proceeded on the differentiation of the types of human diabetes and on the investigation of insulin antagonists present in human blood. Studies have been conducted into the state of pre-diabetes and into the physical condition of children born of diabetic mothers. Much assistance has been given by the Honorary Medical Staffs of the Royal Women's and Queen Victoria Hospitals in this investigation. A research is to be undertaken into some aspects of the problem of arterial degeneration in diabetes and, along with this, an enquiry is to be made into the clinical state of juvenile diabetics of long duration. An important investigation has been commenced in an attempt to determine the factors responsible for the production of insulin by the pancreas.

In the field of thyroid diseases, techniques for the study of thyroid function have been further developed particularly in regard to the use of radioactive isotopes and investigations of protein and globulin bound iodine. Some fundamental work has been commenced on adrenal function in determining the pattern of certain steroids produced by the adrenal gland. An investigation is proceeding into certain disorders of bone structure with particular reference to diseases of the parathyroid gland. Work is also proceeding on the mode of action of gonadotrophic hormones of the pituitary gland.

The formation of the Unit has attracted attention not only in Victoria but in Australia and abroad. Much generous assistance has been afforded by many organisations and individuals by gifts in kind and by financial support. In addition valuable advice has been given and the greatest help and co-operation has been received from many interested people and organisations both within and outside Alfred Hospital. To all of these donors and well-wishers grateful thanks is extended. The present status of the Unit is largely a tribute to the help and assistance it has received. Its future activities will, I trust, justify the confidence and support afforded in these early days of development.

EWEN DOWNIE.

31st December, 1957.

Grateful acknowledgment is made of financial assistance provided by:—

The Alfred Hospital Auxiliary.
Baker Charitable Trust.
British Drug Houses.
Burroughs Wellcome & Co. (Australia) Ltd.
Dunklings Pty. Ltd., Melbourne.
Eli Lilly and Co., Indianapolis.
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Dr. Margaret Sanders.
Miss D. Stirling.
The Upjohn Company, U.S.A.

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Miss Janet Downie.
The Colonial Sugar Refining Co. Ltd.
Fawns and McAllan.
Eli Lilly and Co., Indianapolis.
Merck, Sharp & Dohme (Australia) Pty. Ltd.
Roussel Pharmaceuticals Pty. Ltd.
The Upjohn Company (Australia) Pty. Ltd.
Mr. J. L. William, Melbourne.

and in addition for advice received from Professor Trikojus, and members of the staff of the Department of Biochemistry, University of Melbourne, from the Directors and Heads of the Hospital Departments of Alfred Hospital, and from the Director and Members of the Staff of the Baker Institute and Clinical Research Unit, from Mr. Dryden of Electronic Industries and Mr. McGee of Austronics Australasia. Books have been generously donated to the Unit by Lange Publishing Company of the U.S.A. and Geigy (Australasia) Pty. Ltd.

## CLINICAL STUDIES IN DIABETES MELLITUS

**Ewen Downie.**

Transient insulin resistance occurring in patients has been studied in relation to the clinical pattern of the resistance and is being correlated with temporary appearance of inhibitors in the plasma. This work is continuing.

It appears that diabetic patients of long duration develop vascular change in some instances and in such a way that it cannot be related to diabetic control. Preliminary studies suggest that it may be related to insulin inhibitor diabetes as compared with pure insulin deficient diabetes. It is proposed to investigate this hypothesis by careful study of a group of juvenile diabetics of long duration, that is, of 20 years or more.

## SULPHONYLUREA COMPOUNDS IN THE TREATMENT OF DIABETES MELLITUS

**E. Downie, J. Bornstein, B. Hudson, H. Breidahl.**

Observations have been continued in an attempt to define more clearly the place of these drugs in treatment. 43 patients have been treated with Rastinon. Of these, it was expected that 3 would fail to respond. This proved to be the case. Of the remaining 40, there were 11 failures and 29 successes. 4 patients were under the age of 50, and there were 13 cases in each decade from 50 to 80. 6 cases had shown ketonuria at some stage prior to treatment with Rastinon.

Ten cases had bad diabetes for 10 years or more, 10 for between 5 and 10 years, 11 for between 2 and 5 years, 3 for less than 2 years, and there were 9 new cases. Of those previously needing insulin, 6 had required over 40 units per day, 6 between 20 and 40 units per day, and 3 less than 20 units daily.

Rastinon has been used for less than 3 months on 24 cases, for 3-12 months on 7 cases, for 12-18 months on 6 cases, and for longer on 5 cases. 11 showed a relapse when Rastinon was stopped, and subsequent return of control on reintroduction of the drug.

There were 7 complete failures of response, and 7 partial successes—by this is meant imperfect control of diabetes, but of necessity adequate under the circumstances (failing vision, tremor of hands, etc.). Side effects noted were severe mental depression in 4 cases, drug eruption in 2 cases, headache and dyspepsia in 2 cases, and hepatitis in 2 cases. It is of interest to note that 3 cases underwent surgical procedures with general anaesthesia. All showed increased glycosuria, but none needed supplementary insulin.

The usual plan of treatment has been to give an initial loading dose of 6 gm, the first day, and a maintenance dose of ½ gm. t.d.s. This is adequate for most cases, but some have found 1 gm. t.d.s. necessary to control their glycosuria. 3 cases are adequately controlled on ½ gm. daily. None of the patients treated has shown a weight gain of more than a few pounds.

## INSULIN ANTAGONISTS AND PLASMA INSULIN IN DIABETES MELLITUS

**J. Bornstein.**

Throughout 1957 work has continued on the long standing project of determining the respective roles of failure of insulin secretion and the excess production of insulin antagonists in the aetiology of diabetes mellitus.

The application of fractionation methods during the past few years has greatly simplified this problem, and these methods have proved sufficiently reliable to begin a systematic study of diabetic patients.

The present investigation aims to determine the relationship between the plasma insulin level and the presence of antagonists in the plasma and the stabilisation characteristics of the patient. During a later follow-up of such patients it is proposed to relate these factors to the subsequent development of complications.

During the year 17 patients newly admitted to the Instructional Clinic of the Unit have been studied. These have consisted of 3 juvenile diabetics under the age of 18 at onset, 3 diabetics between 23 and 40, and 11 diabetics over the age of 40.

In all three cases under the age of 18 at onset, neither insulin nor antagonist has been demonstrated in the plasma and these must be regarded as cases of pure insulin deficiency. In each case the patient was easily stabilised on less than 36 units of insulin per day.

In the cases over 18, insulin in varying amounts was demonstrated in all but one, a male of 25. However, in all these cases insulin antagonists were demonstrable in varying quantities. This series is at present too short to warrant any conclusion but it appears that patients with insulin in the plasma and minimal amounts of inhibitor will readily stabilise on diet alone whereas with increasing amounts of antagonists insulin is required.

In addition two patients with moderate insulin resistance were investigated and in each case large amounts of antagonist were demonstrable.

Work is at present proceeding with the aim of purifying and characterising the inhibitory fraction or fractions.

During the year at the invitation of the organising committee of the Insulin Symposium a visit was paid to the United States of America and Canada. Two papers were read at the Symposium, and a number of research centres were visited.

## SYNTHESIS OF INSULIN BY THE PERFUSED PANCREAS

P. R. Davoren† and W. H. Hudson.

During the past year an investigation into the control of the synthesis of insulin by the isolated perfused cat's pancreas has been commenced. An apparatus has been designed and constructed which allows the replacement of the normal blood supply to the pancreas by an artificial supply of fluid of controlled pulsatile pressure. The subsequent transfer of the organ from the cat into a constant temperature enclosure is effected without interruption to the supply of oxygen and nutrients to the tissue. The artificial heart-lung machine provides for the recirculation of a small volume of fluid through the organ and is of a form which facilitates the use of radioactive isotopes in the fluid.

The major part of the project carried out to the present time has been the development of the perfusion apparatus and the accessory equipment required for the dissection of the cat and the transfer of the preparation to the apparatus with a minimum of trauma. Preliminary observations on the carbon dioxide produced in the apparatus have shown that the preparation respires actively in its artificial environment.

†Wellcome Fellow.

## STUDIES OF THYROID FUNCTION

**Bryan Hudson and Mrs. D. Winikoff.**

During the past twelve months methods for the study of thyroid function have been more fully developed. More than 100 patients with suspected thyroid disorders have been studied by the use of tracer doses of radioactive iodine ($I^{131}$) during this period. These studies clearly indicate that the most satisfactory parameters of thyroid function are the measurement of the 4-hour uptake of $I^{131}$ and the determination of plasma protein bound $I^{131}$ at 48 hours. These findings are of some practical significance since most other workers have used the 24-hour uptake of $I^{131}$ as being the best index of thyroid function. In our hands this latter parameter is of little value in the separation of patients with hyper- and hypothyroidism from euthyroid persons.

The combined use of the 4-hour retention value and the level of the plasma $PBI^{131}$ at 48 hours misclassifies between 5 and 10% of patients with thyroid disorders. The usual direction of misclassification is to label hyperthyroid patients as euthyroid. The chemical determination of the non-radioactive protein bound iodine value further reduces the degree of misclassification of patients with thyroid disease, so that the overall misclassification by these laboratory tests is of the order of 4%.

Investigations are still proceeding into methods for improving the laboratory diagnosis of thyroid function. These have taken three forms:—

(1) *Suppression of thyroid function with tri-iodothyronine*

The administration of thyroid hormone to a person with normal thyroid function will suppress the uptake of $I^{131}$ by the gland, whereas no such suppression occurs in persons with hyperthyroidism. This fact has been used in some patients with questionable hyperthyroidism in whom other investigations have yielded equivocal results. The uptake of $I^{131}$ is measured before and two weeks after the administration of tri-iodothyronine. Further studies of this procedure are being made.

(2) *The use of thyroid stimulating hormone (TSH) in the diagnosis of hypothyroidism.*

This procedure is being applied to two groups of patients. The first group consists of patients who are manifestly hypothyroid but in whom there is some doubt whether the cause of the hypothyroidism is primarily in the thyroid or is due to failure of pituitary function. Patients with failure of pituitary function show a good response to TSH, the response being measured by an increase in $I^{131}$ retention in the gland and rise in plasma protein bound iodine value. Patients with primary hypothyroidism show no such responses.

The second group of patients are those manifesting many of the clinical features of hypothyroidism but in whom there is no evidence of impaired thyroid function by the parameters that have been described. The hypothesis has been advanced that such patients show a diminished thyroid reserve and that they are unable to produce additional thyroid hormone in response to the demands made by the environment. The responses ($I^{131}$ uptake and PBI) of such patients to TSH is being studied.

(3) *The determination of globulin bound iodine (GBI).*

(See next project.)

## GLOBULIN BOUND IODINE ($GBI^{127}$)

Mrs. D. Winikoff.

Recent studies on the nature of thyroid hormone using paper electrophoresis technique have suggested the association between this hormone and alpha-1/alpha-2 globulins.

Using a salting-out method, it has been established in a previous project* that the level of the globulin bound iodine ($GBI^{127}$) of plasma can be used as an index of thyroid activity.

Further investigations are being conducted into the value of this determination for:—

(1) The differential diagnosis of thyroid disease—carried out on patients diagnosed by alternative tests and clinical assessment.

(2) Establishing a pattern of thyroid function during normal pregnancy and in cases of habitual abortion. (Material for this project is being kindly supplied by the Professorial Unit of the Royal Women's Hospital.)

It is hoped that this fractional PBI test will give some information on the transport of thyroid hormone by the blood proteins in health and disease.

*Carried out at the Biochemistry Department, University of Melbourne.

## STUDIES IN ADRENAL FUNCTION

Bryan Hudson and Ada Ekkel.

The development of techniques of steroid analysis for the study of adrenal function has been outlined in previous reports. These techniques lend themselves quite readily to the diagnosis of primary and secondary adrenocortical hypofunction.

A pressing problem in the field of adrenocortical physiology is the diagnosis of hyperfunction of the adrenal cortex. There are a number of patients in whom many of the features of the excessive production of adrenal steroids appear to be present, but when investigated by conventional laboratory techniques, sufficient for the demonstration of adrenocortical hypofunction, there is no obvious disorder of adrenocortical secretion. Methods are being studied for the detection of qualitative defects of steroid production. These include chromatographic analysis of steroid excretion. This year the technique of paper partition chromatography has been applied to urinary fractions from normal persons and to those patients with suspected secretory disorders of the adrenal cortex.

Another approach to this problem has been to measure the urinary output of adrenocortical metabolites in response to a standardised stimulus with corticotrophin. These studies are continuing.

## AN INVESTIGATION INTO THE DETERMINATION OF URINARY 17-KETOSTEROIDS

June Sheath.

During 1957 an investigation has been made into aspects of urinary 17-ketosteroid determination as a means of assisting the assessment of adrenocortical function.

The work has been approached along three lines:—

(a) *An investigation of existing methods for the routine determination of 17-ketosteroids.*

(b) *The compiling of a reliable method for this routine determination.*

(c) *Chromatographic research into the aspects of enabling these ketosteroids to be estimated and identified individually to assist in diagnosis.*

(a) This investigation has proved that existing methods are somewhat unreliable due to the necessity to adopt an empirical formula for calculation. When ketosteroids are extracted from urine, certain extraneous chromogenic materials are also extracted which cause serious error in the final colorimetric estimation. In order to overcome this many workers suggest the use of colour correction formulae and extensive work has been done in this direction. A general correction to suit the conditions of estimation employed in this laboratory has been formulated.

(b) From the work carried out as above, the climination of a colour correction was desirahle. A technique to enable this has been produced, whereby ketosteroids may be extracted from urine, whilst chromogenic materials are rendered inextractable. Thus a direct calculation of total 17-ketosteroids is made possible by a speedy method which can be applied to routine analyses.

(c) By means of paper chromatography it has been shown that the use of acid hydrolysis destroys certain labile steroids and that combined enzymatic and acid hydrolysis must be used.

A simple chromatographic system for the separation of certain ketosteroids is in use. This is being applied to the study of the secretion patterns in normal controls and to the investigation of patients suffering from adrenal disorders. Difficulties have been encountered in that slowly moving compounds tend to tail into each other. Under such circumstances these may be eluted and run for a longer period. Both 19-C-ketosteroids and 21-C-ketosteroids are extracted by the procedure in use, and hence it is desirable to separate these prior to paper chromatography. This has been accomplished by adsorption chromatography on florisil.

Identification of individual steroids presents problems. When pure steroids are availahle, reasonable identification has been obtained by showing that the pure compound and the unknown have similar Rf values. When certain of these pure steroids have been unobtainable the technique of sulphuric acid spectrophotometry has been used, which has enabled identification purely by this technique. Amongst other methods being investigated for this purpose are the use of 2,4-dinitrophenylhydrazine spectra, alkaline fluorescence and a number of other colour reactions.

## METABOLIC BONE DISEASE

H. D. Breidahl.

During the year an attempt has been made to elucidate some of the problems involved in the diagnosis of metabolic diseases of bone, as compared with other conditions causing hypercalcaemia, hypercalcuria and replacement of bone with neoplastic tissue.

The first aspect studied was the urinary excretion of calcium in various conditions. The method used involved ashing the urine at 600-700°C. The method although reliable and accurate, is time consuming, and a comparison is being

made between the results obtained by this method and those obtained by flame photometry. In all, 102 estimations of urinary calcium were carried out. The range of excretion of 10 normal people on normal diet was 60-280 mgm./calcium per 24 hours. Two cases of osteomalacia had excretions below 30 mgm. per day, and two proven cases of hyperparathyroidism had excretions over 400 mgm./day. These figures dropped to less than 150 mgm./day after removal of the parathyroid tumours. Sixteen cases of recurrent renal calculi had urinary calcium excretions of 100-700 mgm./day, except one inexplicable case which had an excretion of 42 mgm./day. A case of Cushing's syndrome excreted 330 mgm./day, and a case of thyrotoxicosis 500 mgm./day.

Another aspect of this investigation has been concerned with the differentiation of cases with recurrent renal calculi with hypercalcuria from cases of hyperparathyroidism. The effect of a low calcium diet on the urinary output of calcium has been determined. After 3 days on a 50 mgm. calcium intake, the urinary calcium will drop to less than 150 mgm./day in normal persons, but in the presence of hyperparathyroidism the hypercalcuria persists.

Three cases of recurrent renal calculi with urinary calcium excretion of over 300 mgm./day showed a drop to less than 200 mgm./day on the diet, but another three cases failed to show a drop. In the absence of other findings of hyperparathyroidism, these last cases were considered not to have this condition, and on a maintenance low calcium diet the high excretion has gradually diminished.

The third aspect of the investigation has been to study the urinary calcium excretion following an intravenous infusion of calcium gluconate. Five cases have been studied, but no conclusions can be drawn from the results at this juncture.

## ASSAY OF PITUITARY GONADOTROPHIC HORMONES

**E. L. G. Beavis.**

The first experiments in 1957 consisted in investigating the reproducibility of the whole assay. The results were good at first, but subsequently they became very poor. The cause was ultimately found and corrected. The possibility of using aluminium hydroxide was investigated, but the potential advantages were less than the immediate practical problems, and this was not pursued. A fortuitously discussed alteration in timing during extraction was of great advantage and was adopted.

Since it had been shown that the assay was effective in specimens containing a high concentration of hormone, the possibility of demonstrating the presence of low concentrations was investigated.

So far, the experiments have been extremely satisfactory. The levels of the hormonal concentrations during active reproductive life are being established, and since it appears that relatively very small amounts can be detected, the significance of negative results becomes very much greater.

The responses elicited in each assay have been capable of critical statistical analysis, which has also improved the standard obtained. These improvements have considerably increased the scope of clinical application. The most interesting case investigated during the year was a young girl suffering from precocious puberty. This was associated with demonstrable pituitary gonadotrophin.

## PUBLICATIONS DURING 1957

E. Downie: "Diabetes and Clinical Research—A Study of Insulin Resistance." (Lilly Lecture.) *Ann. Int. Med.* Vol. 46 (1957), p. 126.

E. Downie: "The Management of Diabetes Mellitus." *Aust. J. Pharm.*, Vol. 38 (1957), pp. 453, 1050.

E. Downie: "Oral Treatment of Diabetes." *Aust. Med. Digest*, Vol. 1 (1957), p. 13.

E. Downie, J. Bornstein, B. Hudson: "Hypoglycaemic Sulphonamides in the Management of Diabetes Mellitus." *A/sian. Ann. Med.*, Vol. 6 (1957), p. 105.

E. Downie and M. O'Connor: "Infection and Gangrene in the Foot of Diabetic Patient: The Virtues of Conservatism." *A.N.Z. Jl. Surg.*, Vol. 26 (1957), p. 195.

J. Bornstein: "Inhibition of Alanine Transaminase by the Hypoglycaemic Sulphylurea Derivatives." *Nature*, Vol. 179 (1957), p. 534.

J. Bornstein and C. W. Baird: "Plasma Insulin and Insulin Resistance." *Lancet*, Vol. 1 (1957), p. 1111.

B. Hudson and G. A. Bentley: "The Biological Assay of Melanocyte Stimulating Hormone." *Aust. J. Exp. Biol. & Med. Sci.*, Vol. 35 (1957), p. 45.

B. Hudson and G. A. Bentley: "The Nature of the Pigmentary Disturbance in Addison's Disease." *A/sian Ann. Med.*, Vol. 6 (1957), p. 98.

B. Hudson and Alison Doig: "Observations on the Nature of Hormone-Induced Eosinopenia." *A/sian Ann. Med.*, Vol. 6 (1957), p. 228.

B. Hudson, A. J. Barnett, and J. Bornstein: "Primary Aldosteronism." *A/sian Ann. Med.*, Vol. 6 (1957), p. 250.

Dora Winikoff: "The Value of Globulin-bound Iodine Determination in the Differential Diagnosis of Thyroid Disease." *Acta Endocrinologica*, Vol. 26 (1957), p. 243.

## PAPERS IN PREPARATION

E. Downie: "The Employment of Diabetic Patients."

J. Bornstein and P. R. Davoren: "The Effect of Glucagon on the Oxidation of Glucose by the Perfused Rat's Liver."

B. Hudson: "The Diagnosis and Treatment of Addison's Disease."

H. D. Breidahl: "Clinical Aspects of Hyperparathyroidism."

H. D. Breidahl: "Recent Advances in Endocrinology."

Margaret Sanders: "The Effect of Prednisolone on the Glucose Tolerance of the Relatives of Diabetics."

Dora Winikoff: "Neohydriol Depot as a Continuous Source of Iodine. Its Application in Goitre Areas."

June Sheath: "The Specificity of Laboratory Tests for Occult Blood in Faeces."

June Sheath: "Chromatography of Urinary Ketosteroids."

June Sheath: "The Colorimetric Estimation of 17-ketosteroids in Urine."

## LECTURES DELIVERED DURING 1957

"Natural History of Diabetes Mellitus" . . . . . E. Downie
University of Melbourne.

"Obesity" . . . . . E. Downie
University of Melbourne.

"The Course of Diabetes Mellitus" . . . . . E. Downie
University of Sydney.

"The Employment of Diabetic Patients" . . . . . E. Downie
Inaugural Meeting, Diabetics Federation of Australia, Sydney.

"The Management of Diabetes Mellitus" . . . . . E. Downie
Pharmaceutical Society of Victoria.

"The Role of Glucagon in Metabolism" . . . . . J. Bornstein
Department of Hormone Chemistry, San Francisco.

"Plasma Insulin and Insulin Resistance" . . . . . J. Bornstein
Department of Metabolism, San Francisco.

"Glucagon and Liver Metabolism" . . . . . J. Bornstein
Insulin Symposium, Indianapolis.

"The Assay of Insulin in Plasma" . . . . . J. Bornstein
Insulin Symposium, Indianapolis.

"Plasma Insulin and Insulin Resistance in Diabetes Mellitus" . . . J. Bornstein
Department of Pharmacology, Physiology and Biochemistry, Cleveland.

"The Assay of Insulin in Plasma" . . . . . J. Bornstein
Charles Best Institute, Toronto.

"An Effect of Glucagon on Liver Metabolism" . . . . . J. Bornstein
Charles Best Institute, Toronto.

"The Assay of Insulin in Plasma" . . . . . J. Bornstein
Department of Experimental Medicine, Ann Arbor—University of Michigan.

"The Aetiology of Diabetes Mellitus" . . . . . J. Bornstein
Veterans' Administration Hospital, Ann Arbor—University of Michigan.

"The Action of Antidiabetic Sulphonamides" . . . . . B. Hudson
Alfred Hospital Clinical Society.

"Radioactive Iodine in the Diagnosis and Treatment of Thyroid Disorders" . .
Alfred Hospital. B. Hudson

"The Diagnosis of Adrenal Hypersecretion" . . . . . B. Hudson
The Royal Australasian College of Surgeons.

"The Management of Diabetes" . . . . . B. Hudson
Swan Hill.

"Recent Advances in Endocrinology" . . . . . H. D. Breidahl
Postgraduate Meeting, Dietetic Association of Victoria.

"Pituitary Syndromes" . . . . . H D. Breidahl
Postgraduate Course in Medicine, Alfred Hospital.

"Protein Bound Iodine as an Index of Thyroid Activity" . . . . . Dora Winikoff
Alfred Hospital Clinical Society.

## MEETINGS ATTENDED DURING 1957

Inaugural Diabetes Federation of Australia Meeting, Sydney .. .. E. Downie
The Insulin Symposium, Indianapolis .. .. .. .. .. .. .. .. .. .. J. Bornstein
The American Endocrine Society, New York .. .. .. .. .. .. .. J. Bornstein
The American Goitre Association, New York .. .. .. .. .. .. .. J. Bornstein
The American Diabetes Association, New York .. .. .. .. .. .. J. Bornstein
Annual Meeting R.A.C.P., Brisbane .. .. .. .. .. .. .. .. .. .. B. Hudson
Inaugural D.F.A. Meeting, Sydney .. .. .. .. .. .. .. .. .. .. .. B. Hudson
A.N.Z.A.A.S. Meeting, Dunedin .. .. .. .. .. .. .. .. .. .. .. Dora Winikoff

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

## STUDIES ON MYCOBACTERIA

J. C. Tolhurst, G. Buckle† and N. A. M. Wellington‡.

Of recent years world-wide interest has been shown in acid-fast bacilli other than the various tubercle bacilli which may cause, or may be found in, pathological lesions in man. Some of these organisms have been isolated from sputum or from infected lymph nodes and it is important to assess their true significance in relation to human disease. A number of strains of such organisms isolated in Australia has been examined in this laboratory and a study of their cultural characters and pathogenicity for laboratory animals is being made. In general these acid-fast bacilli are easy to grow at 37°C. This is in contrast to the organisms *Mycobacterium ulcerans* and *Mycobacterium balnei* which cause ulceration of the skin in man and either fail to grow at 37°C or do so only under special conditions. Recently a new mycobacterium which also shows a preference for a low temperature of incubation has been isolated by us from subcutaneous lesions in a wild mouse. It is not yet known whether this organism is pathogenic for man but it is felt that the study of members of the group which cause "natural" infections in animals may well throw light on similar infections in human beings.

Investigations on skin lesions and lymphangitis in cattle caused by an acid-fast bacillus are therefore being pursued. Attempts to cultivate this organism have so far failed which suggests that it is not one of the well-known mycobacteria.

All these studies are as yet incomplete and do not justify a detailed report.

## STUDIES ON CHEMOTHERAPY

G. Buckle†.

The most important defect in chemotherapy with antibiotics has been the ability of one particular organism, *Staphylococcus aureus*, to develop resistance to them. *Staphylococcus aureus* was originally sensitive to all the major antibiotics with the exception of a few strains which have an inherent ability to destroy Penicillin. As each new antibiotic has been introduced the same pattern of events has occurred; at first all staphylococci are sensitive to it, then a few resistant strains appear. These strains become disseminated among the population and soon are found causing infections or being carried by people who have never received the antibiotic.

Of 1200 strains of *Staph. aureus* recently tested, 71% were resistant to Penicillin, 57% to Sulphonamides, and 40% to Streptomycin, 39% to Tetracycline, 38% to Terramycin, 37% to Aureomycin and 6% to Chloramphenicol. In the past,

†Department of Pathology, Alfred Hospital.
‡Department of Agriculture, Victoria.
†Department of Pathology, Alfred Hospital.

this phenomenon has been minimised by the introduction of new drugs but recently most of the new drugs introduced have proved to be relatives of the old ones. The manufacturers have attempted to avoid the difficulty of the resistance of *Staph. aureus* by marketing combinations of drugs, assuming from theoretical reasoning that if one staphylococcus in a million is resistant to either of two drugs, then only one in a million million will be resistant to both. In experiments here it has been found that if it takes 5 subcultures to "train" a staphylococcus to a small degree of resistance to Oleandomycin and 7 subcultures to "train" it to a similar degree of resistance to Tetracycline, then it will take 32 (approximately $35 = 5 \times 7$) subcultures to induce that degree of resistance to the mixture of drugs, so that the theory is borne out in fact. However, it is unlikely that such combinations are the answers to the staphylococcus problem. Firstly, these incredibly large numbers are not enormous for bacteria; secondly, a significant proportion of stapyhlococci is already resistant to one or other member of the combinations.

A further point which must be elucidated is in connection with the claim by the manufacturers that such combinations are synergistic; that is, that the effect of the combination of drugs is greater than the total of the effects of the separate drugs. This has not been found to be so in our tests. If synergism does occur it is uncommon.

These studies are being continued.

## RADIO-ISOTOPE TECHNIQUES IN HAEMATOLOGY

**Ivan S. Epstein.**

During the latter part of the year equipment was obtained to enable radioactive chromium ($Cr^{51}$) to be used to label red blood cells. Injection into patients of these labelled cells has enabled studies on blood volume, red cell survival time and estimations of the size of alimentary blood loss to be carried out.

Although some patients have been investigated most of the work carried out has been devoted to standardisation of the techniques and obtaining normal values.

## PUBLICATIONS DURING 1957

J. C. Tolhurst: "The Role of the Hospital Administrator in the Prevention of Hospital Infection," *National Hospital*, Vol. 1, No. 4 (1957), p. 9.

G. Buckle: "Wound Infections: A Study of Wound Cultures and Sensitivity Tests at the Alfred Hospital," *Alfred Hospital Clinical Reports*, Vol. 7 (1957), p. 47.

## PAPERS ACCEPTED FOR PUBLICATION

J. C. Tolhurst: "The Effect of the Medium on the Cultivation of Mycobacterium Balnei at 37°C," *J. Gen. Microbiol.*

## MONOGRAPH SERIES

No. 1. "Practical Anaesthesia."
1932. (A/sian Med. Pub.)

No. 2. "Spread of Tumours in the Human Body" . . . . . R. A. Willis
1934. (Churchill.)

No. 3. "Blood Cultures and Their Significance" . . . . . Hildred M. Butler
1937. (Churchill.)

No. 4. "Human Torulosis" . . . . . L. B. Cox and J. Tolhurst
1946. (M.U.P.)

No. 5. "The Practical Significance of Modern Cardiological Investigations" . .
T. E. Lowe, H. B. Kay and H. A. Luke
1951. (M.U.P.)

No. 6. "Peripheral Vascular Disease" . . . A. J. Barnett and J. R. E. Fraser
1955. (M.U.P.)

No. 7. "Chemotherapy with Antibiotics and Allied Drugs" . . . . . .
Jean C. Tolhurst, G. Buckle and S. W. Williams
1955. (N.H. and M.R.C.)

No. 8. "The Collected Papers of Hugh Trumble" . . . . . .
L. B. Cox, R. S. Lawson and T. E. Lowe (Ed.)
1957. (M.U.P.)