

**BAKER INSTITUTE**

**RESEARCH**

**1958**

**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.

BAKER MEDICAL RESEARCH INSTITUTE

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"J. F. MacKeddie" . . . . .	G. R. STIRLING, M.B., B.S., F.R.A.C.S.
"A. A. Swallow" . . . . .	G. WAGNER, M.B., B.S., B.SC. (MED.), M.R.A.C.P.
Anti-Cancer Council of Victoria..	C. W. E. WILSON, M.B., B.S., M.R.C.P., M.R.A.C.P.

## ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

The past year has completed a decade since the Alfred Hospital Clinical Research Unit was formed and its activities integrated with those of the Baker Institute from which at that time all routine duties had been transferred to the hospital Pathology Department. This report is therefore presented in two sections; first, a review of the work of this research group since 1948, and secondly, a review of the work of 1958.

### 1949-1958

The principles upon which the work of the Institute has been directed have been elaborated from time to time in the annual reports, and may be divided into three headings. First, to conduct research in problems of human disease; secondly, to apply the methods of the basic sciences to clinical medicine; and thirdly, to provide facilities for training graduates in the methods of medical science.

Although a large number of different problems have been reported on in this period of ten years, there are a few themes which provide a continuous plan on which the projects have developed. The major theme has been the investigation of the physiology and diseases of the cardiovascular system. This system may be divided into three component parts, the heart, the blood vessels and the fluids which circulate in it. The ability of blood to remain fluid but yet to coagulate when necessary is essential for the integrity of the system as a fluid conductor. Investigations on the heart have, at the clinical level, been concerned with congestive cardiac failure, its treatment and physiology, and the development of cardiac surgery. More basically studies of the heart muscle have been on biochemical, pharmacological, histological and biophysical levels. Occlusive diseases of the peripheral arteries have been investigated in order to improve diagnostic techniques and introduce new therapeutic measures. Study of the biochemistry of blood coagulation has been a major project, and arising from this an extensive study of "bleeding" diseases (haemophilia and others) has led to a considerable increase in knowledge of the clinical management of such conditions. Two long-term studies have considered the cardiovascular system as a whole. One is a long-term assessment of methods of treatment of states of abnormal blood pressure, both raised and lowered, and the other has been the elucidation of the principles governing the control of fluid volume in the human body.

A study of carbohydrate metabolism and its abnormalities has formed a minor theme. This has led naturally to a study of endocrine gland functions, and has been related to the hormones of the pituitary, pancreas, adrenal and thyroid glands. In 1956 these studies were linked with the Diabetic Instructional Unit of the Hospital to form a separate Diabetic and Metabolic Unit.

Another theme, so far minor in the research activities, has been the use of physico-chemical methods to study the proteins of the blood plasma. This technique has been applied to certain neoplastic diseases, to a mapping of plasma protein patterns in Melanesian natives and the disease Kuru.

In addition to these long-term projects, many small investigations into clinical problems related to the gastro-intestinal and urinary tracts, amongst others, have been carried out.

The second principle—to apply the methods of the basic sciences to clinical medicine—is exemplified by the work done on vectorelectrocardiography, plethysmography, cardiac catheterisation and other diagnostic methods for cardiac and pulmonary examinations. These methods were developed to a stage where it was possible to incorporate them into a separate routine service—the Cardiovascular Diagnostic Service.

Thirdly, to enable medical graduates to obtain training and experience in the methods of medical science a series of Fellowships has been established. Two features of these Fellowships are important; they provide opportunities for graduates at all levels of experience from trainees to experienced research workers and, secondly, they provide an opportunity for the two-way exchange of ideas with workers of overseas centres. Travel grants permit graduates from here to go overseas, and the Edward Wilson Memorial Fellowship allows an overseas graduate to work in the Institute each year. A list of the thirty-four Alfred Hospital Research Fellows who have worked in the Institute under this scheme is published in this report (page 14), and includes five from centres in the United Kingdom.

It is of interest to note that four members of the staff and research fellows have received post-graduate degrees for theses on work carried out in the Institute: one M.D., one Ph.D. and two M.Sc. Also the Stawell Memorial Prize was twice awarded for essays written by members of the Institute.

The staff of the Institute contains a limited number of graduates holding permanent appointments. Each of these is skilled in a different scientific discipline, and around these key personnel to form teams working on the various projects are grouped workers supported, full or part time, by grants or fellowships. In this way the scientific disciplines of clinical science, biochemistry, physical chemistry, biophysics and physiology are represented, and provide a broad background against which any research project can be conducted.

During this period there has been a steady increase in the number of workers in the Institute. This has necessitated considerable extension to the buildings that has, however, not completely kept pace with the increase in activity, so that further extensions are planned, and it is hoped will be erected in 1959. The original 5000 square feet of laboratories in 1948 will then have grown to some 11,000 square feet, together with a fifteen-bed ward unit. We have been fortunate that during the growth sufficient financial assistance has been available, and at present the Organisation is supported in varying degree by the Baker Benefactions, Alfred Hospital, Alfred Hospital Research Funds and various national research foundations such as the National Health and Medical Research Council, Life Insurance Medical Research Fund, and the Anti-Cancer Council of Victoria.

The place of the Institute in the medical community is that of a research group directed towards the study of disease and bringing to bear on those problems many diverse disciplines of the basic and clinical sciences. It is,

therefore, complementary to university organisations, and provides an integration of scientific disciplines which, although possible, in universities is difficult because of their sharp division into departments.

## 1958

In general the various research projects under investigation last year have been continued and satisfactory progress made. It is worthy of note that considerable interest from overseas centres is being shown in a number of our projects, in particular the studies concerning the biochemistry of blood coagulation, the control of body fluid volume, the energy production in the myocardium and the serum globulins have been commented upon. It is particularly pleasing to record that Dr. Fantl was invited to be Chairman of the Section of the International Congress of Biochemistry in Vienna dealing with the coagulation of blood. He had a most satisfying visit to Europe as a result of this invitation.

As the number of graduates wishing to pursue research in the Institute continues at the same high level, the space available for personnel and equipment continues to be inadequate. However, during the year negotiations have been started which, it is hoped, will lead to the construction of more laboratory space and so allow for further growth and more efficient working of current projects. Although this will alleviate the overcrowding in laboratories, it will not allow any expansion of clinical activities, which must be restricted until more beds can be made available to the clinical research unit.

A further difficulty encountered this year has been the necessary sharing of a registrar and our inability to obtain a clinical biochemist. This has considerably hampered a number of the clinical projects. However, it is understood that a full-time registrar will be available next year.

The research work in progress in 1958 is detailed in the scientific section of this report, but the following synopsis will give a perspective.

Disturbances in the control of body fluid volume have been further investigated at both clinical and laboratory levels. From an empirical study of data obtained from the observation of patients who had disturbances of fluid volume regulation it has been possible to state that the rate of urine formation is influenced both by some facet of the body fluid volume and by the osmotic pressure of some part of the body fluid. It has been concluded that the fluid of the body is contained in a multi-compartment storage through which there is a continuous flow of fluid controlled on the principles of an "open" system with negative feedback and associated with a complex array of factors determining the partition of fluid between the various compartments. Currently methods of determining details of renal function, plasma volume and plasma content of antidiuretic hormone are being investigated.

The problems associated with the control of bleeding have continued to be a major research project. Emphasis has been placed this year on the part played by platelets in the coagulation mechanism in man and animals, and the conclusion has been reached that platelets do not enter the coagulation process at an early stage. Much detailed work has also been carried out on the control of the coagulation process during and after the use of extracorporeal

pump-oxygenators in cardiac surgery. Clinical studies of various bleeding diseases have also continued.

The long-term clinical trial of drugs for the treatment of severe hypertension continues, and it is noted that the efficacy of newer drugs has improved and also that the prognosis for patients with severe hypertension has been greatly improved by the use of various hypotensive agents.

Investigations into the treatment of occlusive arterial disease continue, and various forms of treatment are being assessed.

As the efficient production of energy by cardiac muscle is essential to the maintenance of the circulation, our studies of cardiac muscle have been expanded during this year. Investigation of the processes associated with the conversion of the energy of glucose into that of moving blood continue to be made using the recycling toad heart preparation. This technique also allows a study of the action of various cardiac drugs. An invariable accompaniment of the activity of cardiac muscle is the production of electrical changes across the cellular membranes, and these are being studied in a preparation akin to that used for the study of energy conversion. Further, as the muscle cell is a very complex structure, as yet incompletely understood, a study of its histology is being made with the electron microscope. This last investigation is preparative to using a histo-chemical approach to the subject, and has been made possible by the help of the Department of Pathology, University of Melbourne.

The immunological investigation of serum proteins previously reported continues, and the electrophoretic study of the serum globulin profiles of Melanesian natives has progressed. This approach is also being used to investigate both serum proteins and haptoglobulins of New Guinea natives suffering from Kuru.

Further developments have been made both in techniques and equipment in the use of extracorporeal pump-oxygenators for open intra-cardiac surgery. Detailed studies have been directed to an assessment of the effect of cardiac surgical procedures and of certain anaesthetics on cardiac function. A study has also been made in animals of the reason for the need for antibiotic cover in cardiac surgery. These studies are being made in co-operation with members of the hospital Thoracic-Surgical Unit and Department of Anaesthesia and Resuscitation.

To encourage younger graduates on the staff of the Institute and Hospital to attempt some research project of their own, however small, a Baker Institute Prize (1958) for a thesis on a research project was instituted this year. This prize results from a gift by the clinical staff of the Institute of clinical fees received by them.

Many of the investigations recorded in this report have been supported by funds provided by the National Health and Medical Research Council, the Life Insurance Medical Research Fund of Australia and New Zealand, the Anti-Cancer Council of Victoria, and Alfred Hospital Medical Research Funds. All of these bodies have allocated larger grants for work in 1959, and the assistance granted is gratefully acknowledged. Acknowledgement is also made of a travel grant from the Rockefeller Foundation towards the overseas visit of Dr. Fantl.

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

Considerable assistance has been given this year by Professors Davies, King, Trikojus and Wright and the staffs of the Departments of Organic Chemistry, Pathology, Biochemistry and Physiology, University of Melbourne, and the staff of the Commonwealth Serum Laboratories, and we thank them and others who have helped for their continuing interest in our work.

It is with regret that we record the death during the year of Dr. A. B. Corkill. He was Director of the Institute from 1938 until he retired owing to ill-health early in 1949. An obituary was published in the Medical Journal of Australia, Volume 2, 1958, p. 512.

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

T. E. LOWE.

31st December, 1958.

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

Adelaide Children's Hospital.  
Auckland Medical Research Foundation.  
Austin Hospital.  
Commonwealth Department of Health.  
Department of Agriculture, Wellington.  
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.  
Instituto de Biología y Medicina Experiment, Buenos Aires.  
International Anaesthesia Research Society.  
Kanematsu Institute.  
Mayo Clinic.  
Medical Research Council, London.  
Middlesex Hospital Medical School.  
National Health and Medical Research Council, Canberra.  
National Institute of Nutrition, Tokyo.  
New York State Department of Health.  
Old People's Welfare Council of Victoria.  
Ophthalmic Research Institute of Australia.  
Organisation for Scientific Research, Indonesia.  
Otago University, New Zealand.  
Queensland Institute of Medical Research.  
Rockefeller Institute, New York.  
Royal Australian Chemical Institute.  
Royal Women's Hospital, Melbourne.  
Strangeways Research Laboratory, Cambridge.  
Staten Seruminstitut, Copenhagen.  
South African Institute of Medical Research.  
University of Melbourne.  
United States Army Medical Library.  
Walter and Eliza Hall Institute.

**ALFRED HOSPITAL RESEARCH FELLOWS IN THE INSTITUTE,  
1949-1958**

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

**OVERSEAS FELLOWS**

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

# REPORT OF SCIENTIFIC INVESTIGATIONS

## BLOOD COAGULATION

### THE THROMBOPLASTIC ACTIVITY OF INTACT BLOOD PLATELETS\*

P. Fantl and H. A. Ward.

Although it is known that reduction of platelets in the circulation may lead to serious haemorrhages, the phase at which platelets enter the coagulation process is not established.

In last year's report it was mentioned that the platelets of horse blood showed apparently less tendency to liberate the clotting factor than those of human blood. A detailed study of the influence of platelets in the early stages of the blood clotting process was therefore carried out. The rate of thrombin formation was determined in normal and thrombocytopenic human blood and in normal horse blood with a platelet count similar to that of normal human blood. It was found that the rate of thrombin formation in horse blood was considerably lower than in normal human blood, and was comparable to that in platelet-deficient human blood. The addition of phospholipid to diluted blood produced a higher rate of thrombin formation.

In order to introduce only minimal changes in the blood composition experiments were carried out with whole blood from normal donors, blood from patients with reduced platelet numbers, and normal blood to which phospholipids were added. Both glass and silicone-coated tubes were used. The clotting times of normal and thrombocytopenic blood were found to be of the same order, and the addition of phospholipid reduced the clotting time in all instances.

Since it is probable that the platelet factor required for thromboplastin formation is of lipid nature, clotting times of whole blood were determined in the presence of Russell's viper venom (R.V.V.), as it is known that certain lipids increase the accelerating effect of R.V.V. on clotting.

Blood and plasma clotting times in the presence of R.V.V. were not shortened to a degree which would be expected if the platelet factor were freely available. The addition of phospholipid to either platelet-poor or platelet-rich plasma and freezing and thawing of platelet-rich plasma produce a similar reduction in the R.V.V. clotting time. The disruption of platelet structure which occurs in freezing and thawing liberates a component which now becomes active in shortening plasma clotting times in presence of R.V.V. This effect is near optimal, and cannot be appreciably enhanced by addition of phospholipids.

The low rate of thrombin formation in horse blood which had an adequate number of platelets and the increase of this rate by added phospholipid indicate that the thromboplastic component in platelets was not directly available. This

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

is supported by the observation that the addition to normal blood of an amount of phospholipid approximately equal to one-quarter of that found in platelets shortened the clotting time markedly.

On the basis of the above results it can be stated that the component of platelets which is involved in the formation of blood thromboplastin is present in masked form and is set free during the coagulation process or by damaging the platelet structure by freezing and thawing.

The above findings support our previously expressed view that blood platelets do not enter the coagulation process in the initial stages, but come into action by disruption which is most likely induced by catalytic amounts of thrombin.

#### **THROMBIN AND THE THROMBOPLASTIC ACTIVITY OF PLASMA\***

**P. Fantl.**

For the quantitative assay of the anti-haemophilic factor (A.H.F.) it is desirable to have blood available which is free of anti-haemophilic factor but contains all the other clotting components. This obviously is the case in blood of alpha-haemophilic patients with a complete deficiency. However, as such material is not always available, and certainly only in very limited quantities, an investigation of possible artificial systems was carried out.

It is known that the addition of thrombin to human oxalated plasma leads to almost complete loss of anti-haemophilic activity. One would therefore expect that thrombin-treated plasma would be a useful substitute for alpha-haemophilic plasma. Surprisingly, however, it was found that the conversion of prothrombin in this thrombin treated oxalated normal plasma was quite considerable, although the anti-haemophilic activity was destroyed.

The experiments suggested that thrombin reacted with a component of normal plasma, most likely the anti-haemophilic factor, and produced a thromboplastin-like material. In addition, thrombin converted proaccelerin into accelerin, which had greater activity in conversion of prothrombin to thrombin than proaccelerin.

Proaccelerin is required for efficient formation of blood thromboplastin, yet plasma which contained accelerin and was free of anti-haemophilic activity could not be used as a substitute for alpha-haemophilic plasma, because the addition of normal Ba-plasma, as a source of the anti-haemophilic factor, produced longer clotting times in the accelerin-containing plasma than in the alpha-haemophilic plasma. This seems remarkable because accelerin activity as determined in the presence of brain thromboplastin during prothrombin conversion has greater activity than pro-accelerlin.

#### **A HAEMORRHAGIC TENDENCY DUE TO DEFICIENCY OF A CONTACT FACTOR\***

**P. Fantl, R. J. Sawers and H. A. Ward.**

We had the opportunity to investigate a haemorrhagic tendency in a patient with a carcinoma of the lung and multiple metastases.

The laboratory data indicated that the patient's blood took an excessively long time to clot in siliconed tubes, whereas the clotting time in glass tubes was

only slightly raised. In addition, a thrombocytopenia was present. Other clotting factors were present in normal concentrations. The reduction of platelet number only partly explained the pathological results. The data indicated that in the patient's blood a hitherto unrecognised component was missing.

## HAEMORRHAGIC DISEASES

R. J. Sawers.

The combined clinical and laboratory study of patients referred on account of pathological bleeding has continued throughout the year. Thirty-seven new patients were examined on one or more occasions. In addition, the clinical study of management in haemorrhagic disease was continued in conjunction with the Haematology Unit at Alfred Hospital.

### Coagulation Disorders.

#### Haemophilia.

Five new patients with haemophilia were discovered; four have alpha-haemophilia (classical haemophilia), and one has beta-haemophilia (haemophilia B or Christmas Disease).

#### A.H.F. Deficiency.

In one family, with many bleeder members, which was investigated, only the father and two of his sons were found to have a coagulation abnormality due to A.H.F. (alpha-prothromboplastin) deficiency. On the several occasions this family was examined, the skin bleeding time tests were consistently normal in all members. This finding is in contrast to reported cases of von Willebrand's disease in which A.H.F. deficiency has always been found to be associated with a gross prolongation of the skin bleeding time.

#### Vitamin K Deficiency.

An infant of four months developed a severe bleeding condition which was found to be caused by deficiency of many coagulation factors, including complete deficiency of beta-prothromboplastin (Christmas factor). The condition was probably caused by prolonged oral antibiotic administration; all deficiencies disappeared after the administration of Vitamin K<sub>1</sub>.

A second infant, two weeks of age, was examined routinely because his brother has alpha-haemophilia. The infant's plasma was found at first to be completely deficient in beta-prothromboplastin, but this deficiency had completely disappeared when he was re-examined six weeks later.

In this connection it should be recorded that the infant of six weeks of age whose plasma was reported to be deficient in beta-prothromboplastin, in contrast to the deficiency of alpha-prothromboplastin in an older male relative (Fantl, P., and Sawers, R. J. (1956), *Brit. J. Haemat.* 2, 102), has been re-examined recently; he has symptoms typical of severe haemophilia and his plasma is completely deficient in beta-prothromboplastin.

#### Other Coagulation Abnormalities.

Four patients with rare abnormalities were seen, of which two are still undergoing investigation. The fourth patient had deficiency of a contact factor which is referred to above (Fantl, Sawers and Ward).

### Other Haemorrhagic Disorders.

The majority of patients examined had prolonged skin bleeding times and, except for a few with acquired blood platelet abnormalities, were diagnosed as having von Willebrand's disease without associated coagulation defects.

### CONTROL OF BODY FLUID VOLUME\*

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

This study, which has been in progress for several years, has reached a stage where a number of definite conclusions have been made and the nature of the control may be summarised in the following way.

The body must be considered as a compartmented storage of a complex electrolyte solution. Through this storage passes continuously a flow of water and electrolytes and both the partition of fluid in the compartments and its composition are controlled in the manner of self-regulating open systems. The control mechanism appears to be activated by changes in volume and osmotic pressure of some portion of the stored fluid, and it is considered likely from the evidence that this monitored region is part of the plasma volume. It has been demonstrated that the rate of urine flow is linearly related to body weight in a variety of states, and the influence of changes in partition forces on this relationship have been demonstrated.

Further consideration of data has led to the conclusion that control of fluid volume may be upset in three major ways. First, a change in the ability to excrete fluid, secondly, a change in the partition of fluid in the body, and thirdly, a sequestration of fluid outside the influence of the control mechanism.

Although, as previously indicated, the techniques used in this survey have limitations, it has been possible to obtain evidence that changes in plasma osmotic pressure influence the rate of urine flow in the manner assumed from indirect evidence. When values for rate of urine flow are plotted against corresponding body weights (representing body fluid volume) a linear relationship can be seen when all factors concerned in the control are stable. However, in some cases there is a much greater scatter of points about the regression line than in others. It has been assumed that much of this scatter was due to changes in osmotic pressure superimposing variations of urine flow on that due to volume change. Observations of plasma freezing point depression in a number of cases shows this to be true, and a relationship between rate of urine flow (U), body weight (W), and plasma osmotic pressure (P) of the form,

$$U = a + bW + cP \text{ where } a, b, c \text{ are constants,}$$

can be demonstrated. The relationship between osmotic pressure and rate of urine flow appears to be linear to a first approximation, and of similar magnitude to that between urine flow and body weight.

Although these relationships between flow, storage and osmotic pressure describe the behaviour of the system they tell little about the component parts of the control mechanism. One component of the control mechanism is the excretory organ (the kidney), and during the past year efforts have been made to establish techniques for determining glomerular filtration rates (G.F.R.), renal plasma flow (R.P.F.), plasma volume and extra-cellular fluid volume. No

difficulties have been encountered with P.A.H. technique (renal plasma flow) or Evans blue estimation of plasma volume. However, considerable biochemical troubles have attended measurements of G.F.R. with inulin, and at present the use of sucrose as a substitute for inulin is being investigated. A series of normal control subjects have been investigated and also the opportunity to study the renal function in patients with hypertension has been taken. In the patients with hypertension both R.P.F. and G.F.R. were reduced, but the filtration fraction was higher than in normals. Owing to the trouble in establishing the inulin technique for G.F.R. estimation these values have not yet been related to the body fluid volume studies.

The efferent part of the control mechanism may be expected to contain aldosterone and antidiuretic hormone (A.D.H.) as components. Some years ago in this study it was reported that the urinary content of A.D.H. was raised in some patients with oedema. This year a method for the assay of plasma A.D.H. has been sought.

#### ASSAY OF ANTI-DIURETIC HORMONE

A method of assaying anti-diuretic hormone (A.D.H.) by the use of the South American toad, *Bufo marinus*, has been investigated. This work was prompted by the report of a method involving the use of European toads. The basis of these methods is the increase in body weight caused by injections of mammalian A.D.H. (vasopressin) in toads partially submerged in water. This increase in weight is due to increased absorption of water by the renal tubules, by the bladder mucosa and by the skin. A linear log. dose-response curve was obtained with doses varying from 0.003-1.0 milli-units per 10G. of toad body weight. However, the slope of the curve was very flat, and it is thought that the method will not be suitable for routine use.

#### RENAL CLEARANCE STUDIES

Last year a study of renal clearances and estimation of plasma and extracellular volumes in normal and hypertensive persons was commenced. The glomerular filtration was estimated from the clearance of inulin, the renal plasma flow from the estimation of para amino-hippuric acid (P.A.H.), the extracellular space from the distribution space of inulin, and the plasma volume from the distribution space of Evans blue dye.

In the preliminary investigations the values for the clearance of inulin and P.A.H. were similar to those obtained by other workers, both for hypertensive and normotensive persons. These studies were continued until mid 1958, clearance studies being performed on some 49 subjects. At this time routine recovery experiments in association with inulin clearances were commenced, and it was found that the recovery of added inulin from plasma was extremely variable and unpredictable. Uniformly good recovery of inulin could not be obtained in spite of careful attention to detail and modification of technique. The inulin clearance has therefore been abandoned as a measure of glomerular filtration, and the sucrose clearance used instead. It is believed that sucrose is handled by the kidney in the same way as inulin, being freely filtered by glomeruli and neither reabsorbed nor excreted by tubules. Chemical estimation is simple, and there is a uniformly good recovery.

So far sucrose clearances have been determined in twelve hypertensive and fourteen normotensive subjects. Simultaneous P.A.H. clearances and sucrose spaces have been determined.

The results have shown a marked difference between the hypertensive and normotensive subjects. In the hypertensive patients the clearance of sucrose (C.suc.) is usually below 50 ml./sq.metre body surface/min (often much below this figure), and in normotensive persons above this figure. In respect to the clearance of P.A.H. (C.pah), the corresponding values are hypertensive patients below 400 ml./sq.m./min., normotensive patients above 400 ml.sq.m./min. There is a tendency for the filtration fraction (F.F. = C.suc./C.pah) to be higher in hypertensive than in normotensive subjects, with an average of 0.18 in hypertensive subjects, 0.16 in normotensive subjects, but there is much overlap. The filtration fraction C.suc./C.pah is lower both for hypertensive and normotensive persons than the filtration fraction C.in/C.pah.

Concurrently with the renal clearance estimations mentioned, observations are being made on the specific gravity range, ability to excrete a standard water load, and clearances of urea and creatinine, in order to discover whether there is any correlation between the results of tests more readily performed clinically and the sucrose and P.A.H. clearances. The results have not been fully analysed; but there appears to be a fairly good correlation between the clearance of creatine and the clearance of sucrose; but a poor correlation between the clearance of urea and that of sucrose.

## HYPERTENSIVE STATES

### CLINICAL TRIAL OF HYPOTENSIVE DRUGS

A. J. Barnett and F. O. Simpson.

#### Ganglion Blocking Drugs.

In 1950 a clinic was established to investigate the value of ganglion-blocking drugs in the treatment of patients with severe hypertensive disease with the main object to discover the effect of this treatment on the prognosis and long-term benefit to the patient. The number of patients who have commenced the treatment has now increased to 113.

The symptomatic benefit and increased life expectancy of the treated patients previously noted has continued. The following table shows the present position as regards survival in 64 patients with severe hypertension who commenced treatment before June, 1955.

Survival Time	Malignant Phase		Benign phase
	No. of survivors	Expected No. of survivors without treatment	
Over 1 year . . . . .	23	6	27
Over 2 years . . . . .	18	3	25
Over 5 years . . . . .	11	0	7

It is to be noted that with the progress of time the improved prognosis in the patients in the malignant phase receiving treatment, compared with that expected in the pre-treatment era becomes more marked, and also that there is little difference at the 1, 2 and 5 year periods in the numbers of survivors of patients initially with benign hypertension and those initially with malignant hypertension.

#### **Chlorothiazide.**

In a preliminary short term study involving twelve patients it was noted that the administration of chlorothiazide ("Chlotride," Merck) to hypertensive patients reduced the requirement of a ganglionic blocking drug by approximately half. A larger series of patients is now being observed on the basis of long-term treatment. Forty-three of the patients in the severe hypertension clinic (treated with ganglion-blocking drugs) have received chlorothiazide. Twenty-eight patients have received it continuously over a period of six months or more.

The value of this drug indicated in the short term study has been borne out in the more extensive survey. The necessary dose of the ganglion-blocking drug is reduced, and the control of the blood pressure is more even, whilst side-effects from the ganglion-blocking drugs are reduced.

No serious side effects or complications from chlorothiazide have occurred. Serum electrolyte levels have been estimated at intervals, and most of the patients have shown a slight reduction in the level of the serum potassium. When the level has fallen to 3.5 meq./l or below, potassium chloride 1.5 to 2G. per day has been given and has produced a rise in the serum potassium level.

Chlorothiazide has also been used as the initial treatment in some hypertensive patients, usually conjointly with reserpine, in the expectation that if a ganglion-blocking drug is required, it may be given in smaller dosage. In some patients this treatment alone has produced a satisfactory fall in blood pressure.

#### **Clinical Trial of an Analogue of Reserpine.**

As depressive symptoms appear in a proportion of patients receiving Reserpine on account of hypertension, it was decided to carry out a clinical trial of Deserpidine ("Harmony," Abbott). This drug is claimed to equal Reserpine in potency as a hypotensive agent, and to have less tendency to cause mental depression and other side effects. The trial is being conducted on a "double-blind" basis on patients who have had to stop taking Reserpine because of mental depression.

## **DISEASES OF THE PERIPHERAL BLOOD VESSELS**

### **OCCCLUSIVE ARTERIAL DISEASE**

**A. J. Barnett and K. N. Morris.†**

Treatment of suitable cases of occlusive arterial disease by arterial grafting is still being performed, although the supply of grafts does not meet the demand. During 1958, seven grafts were inserted, with a successful result to date in six.

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Since December 1953, 45 grafts have been inserted. Twenty-two of these are still functioning satisfactorily for periods up to four years after operation.

The results continue to indicate the superiority of the by-pass technique over the old end-to-end anastomosis. The following table shows the length of survival of grafts in 35 patients in whom this operation was performed prior to 31st December, 1956.

Period of Functioning of Graft	Technique		Totals
	End to End	By-pass	
Less than 1 year . . . . .	8 (1)	6	14
1-2 years . . . . .	3 (1)	2 (1)	5
More than 2 years . . . . .	4	12	16
	15	20	35

Figures in brackets refer to patients who died with grafts patent up to time of death. The table indicates the superiority of the by-pass technique with twelve of twenty (i.e., approximately 50%) surviving grafts at two years compared with the old (end to end) technique with only four of fifteen (approximately 25%) survivors at two years. The table also indicates that the most common time for a graft to block is in the first year following operation. There were few instances of blockage during the second year after operation. Of those remaining patent until two years after operation, all except two are still patent. One graft is still functioning four years after operation.

## DIET IN ATHEROSCLEROSIS

A. J. Barnett and C. C. Curtain.

Recent evidence indicates that diet may be a factor in the development of atherosclerosis. It is claimed that patients eating much animal fat are more prone to this disease than those eating little animal fat. Abnormalities in the plasma lipids and increase in serum cholesterol level have also been demonstrated in patients with atherosclerosis. It might be expected therefore that a restriction in animal fat and cholesterol intake might retard the development of atherosclerosis, and that in patients with the restricted diet the plasma lipids and cholesterol concentration would be lowered.

In order to determine whether beneficial effects can be obtained in patients with atherosclerosis by the use of a diet low in animal fats and cholesterol, a study has been commenced in which a group of twelve patients treated with a diet low in animal fats and cholesterol is being compared with a control group of twelve patients eating a normal diet. Serial studies are being made of the levels of the serum cholesterol, plasma beta/alpha lipoprotein ratio, fibrinolytic activity of the blood, the patients walking distance and clinical state. It is intended to make a preliminary assessment at the end of two years.

## SMOKING AND OCCLUSIVE ARTERIAL DISEASE

W. C. Boake and A. J. Barnett.

It is widely observed that one type of occlusive arterial disease—thromboangiitis obliterans—is almost entirely confined to heavy smokers, and it has been found that the prognosis can be dramatically improved by total abstinence from tobacco. Also some cardiovascular changes have been thought to arise from smoking tobacco.

From these facts it is commonly extrapolated that smoking is harmful in occlusive arterial disease generally.

It has therefore been considered of interest to determine the effect of smoking on the muscle and skin blood flow in patients with intermittent claudication due to atherosclerosis.

In six patients in whom calf (mainly muscle) and foot (mainly skin) blood flows have been measured at two rates of smoking (one and six inhalations per minute) there has been no significant effect on the blood flow.

These results do not indicate any necessity to forbid or drastically reduce smoking in patients with atherosclerosis because of a supposed vasoconstrictor effect.

## ENERGY PRODUCTION IN THE MYOCARDIUM\*\*

W. G. Nayler.

Throughout the year further investigations into the physiological and metabolic changes associated with drug action on the isolated heart have been made, the drugs studied including reserpine and fluothane as well as further observations relating to the action of the cardiac glycosides and sympathomimetic amines. Preliminary studies on the pressor activity of toad plasma have been made, and additional apparatus constructed so that changes in ventricular volume can be continuously recorded when required.

## RESERPINE

### Pressor Activity of Reserpine.

Although reserpine is used to reduce hypertension several workers have recently noted transient pressor responses immediately following the intravenous administration of this drug. Since reserpine liberates the catechol amines from various tissues, it seemed possible that this pressor response noted during reserpine treatment was due to the pharmacological activity of released amines.

To test this hypothesis experiments were carried out using the technique previously developed for studies of drug action on the isolated spontaneously beating toad heart. Reserpine was added (final conc. 1 microgram/ml.) to a series of hearts and the response compared with that due to the addition of adrenaline (0.01 microgram/ml) and nor-adrenaline (0.1 microgram/ml.) in another series. In a further series Regitine (1 microgram/ml.) was initially added to the perfusate and then reserpine added as above.

At the concentration used reserpine consistently exerted a positive inotropic effect on the isolated heart, and this response was physiologically and metabolically comparable with that of the sympathomimetic amines. Electrocardiograms taken before and after the addition of reserpine to the heart revealed changes typical of those associated with amine activity.

Since the pressor response of reserpine recorded above was completely abolished by regitine at a concentration sufficient to block the adrenergic actions of the amines, and because of the similarity of the pressor responses due to reserpine and the amines it seems probable that the cardiac activity of reserpine is in fact due to the pharmacological action of the released amines.

## CARDIAC GLYCOSIDES

### Cardiac Glycosides and Deaminase Activity.

Rand, Stafford and Thorp (1955) postulated that the positive inotropic activity of the glycosides is primarily due to the accumulation of small concentrations of adenosine-containing compounds due to glycoside inhibition of enzymes responsible for the deamination of adenosine and adenylic containing compounds.

Hence a series of experiments were carried out in which the action of strophanthin-G on the activity of the enzymes responsible for the deamination of adenosine and adenylic acid in freshly shed human blood and freshly excised mouse heart was measured. Deaminase activity was determined by measuring the rate of ammonia liberation (Brown's method). In those experiments in which the enzymes were extracted from the isolated heart substrate concentrations of 1.5 mgm/ml. were used and strophanthin-G added to give a final concentration of 10 microgram/ml. In studies using freshly shed blood, where Conway and Cooke demonstrated that the initial increase in ammonia concentration was due to adenosine or adenylic acid deaminase, strophanthin-G was added to give final concentration 1 microgram/ml. Incubations were made at 37.0° C., in duplicate, and over varying time intervals.

At these concentrations strophanthin-G consistently failed to produce any change in the rate of deamination of adenosine or adenylic acid in either fresh blood or mouse heart extracts, so that if any increased level of adenosine-containing compounds is associated with glycoside activity some mechanism other than changed rates of destruction via deaminase activity must be involved.

### Seasonal Variation in Drug Sensitivity.

In an attempt to find the basis for the greatly reduced sensitivity shown by isolated winter hearts to the glycosides, estimations of phospholipid, phosphocreatine, inorganic phosphate, sodium and potassium concentrations were made regularly throughout the year. Whereas phospholipid, phosphocreatine and inorganic phosphate levels remained relatively constant, a seasonal variation in the sodium/potassium ratio was observed.

The raised potassium levels found in winter hearts, associated with reduced sodium concentrations, may be related to the reduced sensitivity to the glycosides displayed by these winter hearts. Further studies will be directed towards determining the mechanisms whereby this cellular ionic adaptation is accomplished.

### Record of Ventricular Volume.

Data obtained from earlier studies indicated that the cardiac glycosides, 9 alpha fluorohydrocortisone and calcium ions produced a significant increase in the useful work done by the isolated heart. The augmented work output was associated with an increased force of ventricular contraction, as recorded in the maximum aortic pressure records.

Earlier workers interested in cardiac physiology have demonstrated, under certain conditions, a relationship linking the force of ventricular contraction with the end diastolic volume. It is, therefore, necessary to establish whether or not the glycosides act simply by increasing the end ventricular diastolic volume. To do this an apparatus, which is essentially a plethysmograph, has been developed. The ventricle of the isolated perfused heart is completely immersed in ringer solution contained in a bath to which is attached a narrow bore vertical side arm. Any displacement of fluid in the main bath—as occurs during systole and diastole—is reflected in a greater fluid displacement in the side arm. The fluid level in the side arm is followed by means of a waterproofed float which, moving through a light beam, produces variations in the intensity of light falling on a photoelectric cell. In this way changes in ventricular volume can be continuously recorded, together with records of maximum aortic pressure, beat rate and E.C.G. tracings.

Preliminary experiments indicate that the apparatus does give satisfactory records of systolic and diastolic volumes and that it will provide a more accurate estimate of cardiac output per beat than was possible using the drop counting method.

## FLUOTHANE

### Fluothane, Chloroform and Hypothermia.

Fluothane (halothane), because of its hypotensive effect, has been used as an anaesthetic during cardiac surgery where a controlled reduction in cardiac activity is desirable provided that it is not associated with reduced cardiac efficiency. Since anaesthetic agents other than fluothane produce myocardial depression a series of experiments were carried out in which the action of fluothane on the isolated heart was compared with that of chloroform. Also, since hypothermia has been used during cardiac surgery, the changes in myocardial efficiency associated with mild hypothermia were similarly studied.

Fluothane (final conc.  $1 \times 10^{-3}$ ) was added to a series of six hearts after the initial control one hour's perfusion at  $25.0^{\circ}$  C. Changes in beat rate, maximum aortic pressure, drops per beat and rate of oxygen uptake were recorded regularly during the ensuing two hours. In a further series heparin was added initially to the perfusate, and the action of fluothane studied as above.

In a similar series of experiments chloroform (final conc.  $1 \times 10^{-3}$  and  $1 \times 10^{-2}$ ) was added to isolated hearts, after the initial hour's perfusion and observation made as above. In another series, after the usual hour's perfusion at  $25.0^{\circ}$  C., the perfusion temperature was rapidly reduced to  $20.0^{\circ}$  C., and changes in the above indices of cardiac activity recorded as before.

Fluothane, both in the presence and absence of heparin, and chloroform depressed the cardiac work output and rate of oxygen utilisation so that there was, in both cases, a marked fall in cardiac efficiency. Both drugs reduced the aortic pressures, and, at sufficiently high concentrations, both produced bradycardia. There appeared to be no significant difference between the pharmacological actions of these two anaesthetic agents.

In contrast to this reduced cardiac efficiency during either chloroform or fluothane induced anaesthesia, mild hypothermia resulted in a significant and well-sustained increase in cardiac efficiency, associated with a depressed rate of oxygen uptake.

## **PLASMA**

### **Pressor Activity of Plasma.**

Preliminary studies have been made on the pressor activity displayed by heparinised toad plasma when tested against the isolated perfused heart from the donor toad. Positive inotropic activity associated with augmented oxidative metabolism has been consistently recorded. Since the magnitude of the plasma induced inotropic response is related to the calcium concentration of the perfusate the question is raised as to whether calcium binding at the cell membrane is involved? Addition of plasma to perfusates of the standard isolated heart preparations resulted in a marked reduction in sensitivity to the glycosides, suggesting that the factor involved in the plasma response and the cardiac glycosides may have a common site of action.

## **ELECTRICAL ACTIVITY OF THE HEART**

### **MEMBRANE POTENTIALS OF CARDIAC MUSCLE**

#### **D. McKelvie.**

Transmembrane action and resting potential measurements are made from cardiac muscle cells of the toad *Bufo marinus* using glass microelectrodes filled with 3 M potassium chloride solution which are inserted into individual cells. The potential difference across the membrane is amplified and displayed on one trace of an oscilloscope screen for photographing and measurement. The first differential of the action potential profile (a measure of the maximum rate of initial rise of the action potential), an electrocardiogram (if a whole heart is being used), a 1/10 sec. time marker and a reference line for membrane resting potential measurement are displayed on the other oscilloscope traces.

The glass electrode filled with potassium chloride has been used—

(a) as produced, with a rigid 3 mm. glass tube shank, or

(b) as a semi-flexible type. This is made by breaking the last centimetre from an electrode of type (a) to give a potassium chloride filled tube of glass tapering from about 0.5 mm. outside diameter to less than 0.001 mm. outside diameter at the tip. This tube is then slipped on a 5 cm. length of 50 gauge

nylon-coated silver wire suitably bent to give a flexible coupling to the micro-manipulator. Use of the flexible electrode allows recordings to be made with fair success from complete recycling heart preparations.

During the early part of the year recordings were made with the rigid electrode from portions of spontaneously beating toad heart pinned out in Ringer's solution. A series of recordings made to determine the range of variation of the various parameters gave the following values for cellular membrane activity:

Resting potential,  $86 \pm 6$  mV.

Action potential,  $115 \pm 15$  mV.

Maximum rate of rise,  $27 \pm 10$  volt sec.<sup>-1</sup>.

Drugs added to the bathing solution enabled changes in the magnitude of the resting and action potentials, and the maximum rate of rise and shape of the action potential to be studied. Procaine amide ( $1 \times 10^{-4}$  final conc.) reduced the maximum rate of rise of the action potential to one-third of the normal value with little other change. Quinidine sulphate ( $5 \times 10^{-6}$  final conc.) acted similarly. No effect could be shown when insulin (1.5u/1000 ml. final conc.) or reserpine ( $1 \times 10^{-5}$  final conc.) was added. Solutions containing the anaesthetic fluothane ( $5 \times 10^{-4}$  to  $2 \times 10^{-3}$  final conc.) caused the maximum rate of rise of the action potential to be increased by up to 50 volts sec.<sup>-1</sup>.

Using the recycling heart method and flexibly mounted electrodes the recorded value of the action potential is slightly higher ( $119 \pm 9$  mV). Complete immersion of the heart in Ringer's solution has been abandoned as the surface film takes control of the electrode as it is lowered through it and results in electrode breakage. So far difficulty has been experienced in recording the effects on the outer cells of the myocardium of drugs added to the perfusate, although changes in the electrocardiograms can be noted.

## THE ELECTROCARDIOGRAM OF THE TOAD (BUFO MARINUS)

F. O. Simpson.

Electrocardiograms are being recorded from a series of anaesthetised toads in order to determine the normal range of the voltage of the ventricular complex. This value will be compared with that obtained from another series of toads in whom ventricular hypertrophy is being induced.

It was noted in this study that an electrical wave due to activation of the bulbus cordis can be readily identified and a study to investigate the function and mode of activation of this region has commenced.

## HISTOLOGY OF CARDIAC MUSCLE

F. O. Simpson.

Preliminary work on a study of the structure of the cardiac muscle cell by electron microscopy is being carried out. This study will form the basis for a project on the histochemistry of these cells.

## CARDIAC SURGERY

G. R. Stirling, I. A. L. Ferguson and W. C. Boake,

in conjunction with

K. N. Morris†, F. Kinross† R. Orton,‡ and W. Crosby.‡

For several years now methods of conducting intracardiac surgery with safety have been under investigation. During 1958 three aspects have been studied in detail. First, the pharmacological action of the anaesthetic fluothane; secondly, developments of extracorporeal pump-oxygenators, and thirdly, the reasons behind the need for pre-operative antibiotic cover to be given to animals to enable them to survive cardiac by-pass. The general work on the study of physiological, biochemical and haematological problems associated with cardiopulmonary by-pass in both man and animals has also been continued.

### STUDIES ON FLUOTHANE (HALOTHANE)

(See also p. 25, FLUOTHANE, by W. G. Naylor.)

#### Fluothane and Circulatory Occlusion.

Methods of depressing body metabolism and of thus prolonging the period for which the circulation may be interrupted safely, have an obvious application in intracardiac surgery. In 1955 hypothermia was studied in this regard, and it was established that the dog tolerated eight to ten minutes of circulatory occlusion if its body temperature was reduced to 30° C. The method proved to be somewhat cumbersome and time-consuming, and ventricular fibrillation was frequently experienced.

An alternative approach, using drugs to lower the rate of metabolism, is at present being studied. Commencing in 1957, a series of experiments was aimed at determining the value of fluothane for this purpose. Fluothane (Halothane) is a volatile, non-inflammable and rapidly acting anaesthetic agent which is administered by inhalation. Others have shown that its inhalation is associated with a significant reduction in total body oxygen consumption.

A series of dogs submitted to complete circulatory occlusion whilst deeply anaesthetised with fluothane tolerated well occlusion for periods of six minutes. However, when the circulation was occluded for eight to ten minutes, although the majority of the dogs survived, cerebral damage was evident in a number of the survivors. It was found that fluothane reduced the incidence of ventricular fibrillation after circulatory occlusion when compared with hypothermia.

#### Heparin and Circulatory Occlusion.

The alleged protective effect of high concentrations of heparin in dogs undergoing circulatory occlusion was investigated. It was found that heparin in a concentration of 10 mg. per Kg. body weight did not materially improve

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the results when compared with a series of dogs who had been submitted to circulatory occlusion after the administration of heparin in a dose of 2 mg. per Kg. body weight.

#### **Clinical Experience.**

With some appreciation of the limitations of the method, the technique of deliberate circulatory occlusion together with fluothane and heparin has been applied to clinical cases. The cases chosen have been those in which a short time of circulatory occlusion (less than five minutes) is necessary for the completion of the intra-cardiac procedure. To date twenty cases have been operated on and include ten cases of aortic stenosis in which open trans-aortic valvotomy was carried out, seven cases of congenital pulmonary valvular stenosis, and three cases of simple atrial septal defect. The results have been very encouraging.

#### **The Hypotension of Fluothane Anaesthesia.**

The mechanism of the hypotension which is a marked feature of deep fluothane anaesthesia has been analysed. A series of experiments was carried out in which the cardiac output of the dog was kept constant by controlling the rate of venous inflow. When fluothane was administered a fall in blood pressure was always recorded, suggesting a fall in peripheral vascular resistance. When fluothane was administered to a dog with complete cardio-pulmonary by-pass at a constant rate of perfusion a similar fall in blood pressure was noted. From these experiments it was concluded that fluothane exhibited marked vaso-dilator activity.

The effects of fluothane on cardiac performance have also been investigated. By varying the volume load on the right ventricle and measuring the pressure responses in the pulmonary artery and right atrium it is possible to derive curves relating the minute work of the right ventricle to the mean right atrial pressure. Such curves have been called "right ventricular function curves." The right ventricular function curve of a dog after the administration of fluothane shows depression of function when compared with the control curve obtained in the same animal. The degree of disturbance of ventricular function is directly related to the concentration of fluothane which is used.

#### **Extra-Corporeal Pump Oxygenator.**

During the year the major components necessary to assemble a rotating disc-type oxygenator appropriate to the perfusion of all sizes of human patients were received. The construction of this new oxygenator was carried out and an extensive evaluation of its performance made with animals.

Preparation of the machine involved the study of three major aspects of the machine. These aspects were:

1. The physical arrangement of the machine with additional reservoirs, tubing, etc., and the development of a technique of running it.
2. Investigation of the effect on arterial pH of varying CO<sub>2</sub> tension in the ventilating gas.
3. Investigation of the effect on platelet survival of silicone coating on the discs.

The final arrangement of the components of the machine was arrived at after considerable trial and error. It consists in using venous reservoirs into which blood is gravitated from the patient, and from which the blood is gravitated into the oxygenator itself. These reservoirs also act as the cardiomy return reservoirs and as the path for priming the machine. An hydraulic system for raising and lowering the reservoirs has been constructed by the workshops in order that the level of blood in the oxygenator can be kept constant at all times. This latter has been found to be particularly important since the oxygenating capacity of the oxygenator is seriously affected by any fall in the level of blood in it.

Arterial blood is pumped directly from the oxygenator to the patient via a stainless steel mesh filter.

This arrangement appears to be quite satisfactory, and to be about as simple as can conveniently be devised.

We have to thank Mr. Kohler and the hospital workshop staff for the very considerable amount of work they did in modifying the machine and making stainless steel connections, all in a relatively short period of time.

It was necessary to investigate the effect of the CO<sub>2</sub> tension in the ventilating gas on pH of arterial blood emerging from the oxygenator. A series of estimations using pure oxygen were followed by a series with 5% CO<sub>2</sub>, and the latter concentration was adopted. This gave mean pH and CO<sub>2</sub> tension figures of

pH	CO <sub>2</sub> Tension
7.44	23.5
7.52	21.5
7.453	22.5

The mixture containing 5% CO<sub>2</sub> is convenient since it can easily be obtained as "carbogen," and small variations in the percentage of CO<sub>2</sub> do not seem to be critical.

Dog's heparinised blood was circulated over siliconed and non-siliconed discs and it was found that the number of platelets was less severely depressed by the siliconed discs. It was also noted that there was greater platelet damage in the de Wall apparatus than with the rotating discs.

Similar experiments, at present being carried out with human blood, indicate that human platelets are more stable to mechanical trauma than those of the dog.

It is considered therefore that the improved platelet stability obtained by silicone coating of the apparatus does not compensate for the difficulties of using such apparatus.

#### STUDIES ON ACHROMOBACTER BACTERAEMIA IN DOGS SUBJECTED TO CARDIAC BY-PASS PROCEDURES

Dogs which have not been given penicillin and streptomycin pre-operatively invariably die after cardiac by-pass procedures in a state of peripheral circulatory failure. From the blood of these dogs a gram-negative organism, identified as a member of the genus *Achromobacter*, has been repeatedly isolated. This

organism is not present in the peripheral blood of normal dogs, but is found in the heart blood immediately after thoracotomy. Evidence suggests that these organisms reach the blood stream from the dog's gut, and are normally removed from the blood during passage through the lungs.

The relation of *Achromobacter* bacteraemia to the subsequent fate of the dog is uncertain. Dogs immunised with killed *Achromobacter* vaccine, and others immunised with gram-negative endotoxin, did not survive by-pass.

The problem is complicated by the observation that the *Achromobacter* strains isolated are not sensitive to streptomycin and penicillin *in vitro*, and that the organisms are sometimes found in the blood of dogs which have been given penicillin and streptomycin pre-operatively.

#### **EFFECT OF PARASYMPATHOMIMETIC DRUGS ON THE PULMONARY CIRCULATION**

The effect of a parasympathomimetic amine, compound 45/50 (Burroughs Wellcome, New Jersey) has been investigated in one patient with pulmonary hypertension (secondary to ventricular septal defect with reversed shunt). No alteration in pulmonary artery pressure, or of systemic arterial pressure, was observed when the pulmonary artery was infused with this drug.

The drug has no effect on the heart rate, pulmonary artery pressure, or femoral artery pressure of normal sheep.

#### **BLOOD DYE-DILUTION CURVES IN THE DIAGNOSIS OF CONGENITAL HEART DISEASE**

**G. R. Wagner.**

Investigations involving the injection of indicator substances into the circulation and the analysis of their time-concentration relationship in the blood at various sites in the circulatory pathway date back to the nineteenth century. However, only in the last 30 years has this technique been applied clinically. The object of the present study has been to develop a technique of recording indicator-dilution curves, using Evans Blue as indicator, in patients with congenital heart disease and to attempt to assess their value as a clinical test. A dye-dilution curve is obtained from the heat-flushed ear through an oximeter ear-piece or from an oximeter cuvette through which the blood from an in-dwelling arterial needle is allowed to flow, the output of ear-piece or cuvette being amplified by a D.C. amplifier and recorded by a direct-writing recorder.

Studies of patients with congenital heart disease were undertaken as they came for special investigation of their cardiac lesion, but satisfactory dye curves were obtained in a proportion of patients only. Difficulties arose in getting the apparatus sufficiently stable at the sensitivity required to obtain a curve of reasonable amplitude and also in obtaining a satisfactory blood flow from an artery in a child, most of the patients with congenital heart disease being less than twelve years of age. The curves were usually obtained at the time of cardiac catheterisation, the dye being injected through the cardiac catheter. This has the advantage that the site of injection can be anywhere on the right side of the heart.

In the case of a lesion without a shunt the dye curve has the normal configuration, showing a primary peak and a recirculation peak. In cases with L to R shunts the recirculation peak is absent and the down slope from the primary peak is flattened due to the slow liberation of dye from the pulmonary circuit. In contrast R to L shunting lesions give rise to an early peak before the primary curve with dye injections upstream to the site of shunt and a normal dye curve with injections downstream to the site of shunt. The early peak is due to some dye short-circuiting the lungs by way of the defect and appearing early in the systemic circulation.

Using certain approximations, the different types of dye curves can be evaluated to measure proportionately the size of a shunt.

During the course of this work it became obvious that the usefulness of dye studies in the investigation of patients with congenital heart disease is limited largely by the complexity of the test. Only infrequently does a dye-dilution curve provide information of fundamental importance which cannot be obtained from cardiac catheterisation or angiocardiology. However, if the test could be simplified and could be carried out reliably it would provide some useful information more quickly and easily than otherwise obtainable.

## BLOOD SERUM PROTEINS

C. C. Curtain.

### THE ANTIGENIC RELATIONSHIPS BETWEEN ABNORMAL AND NORMAL SERUM GLOBULINS

The immunological tolerance studies, reported last year, have been supplemented by experiments with the phenomenon of immunological "paralysis." Normal adult rabbits were given large amounts (10 ml./Kg./day) of normal human plasma by combined subcutaneous and intravenous routes. After four weeks of such injections the sera of the rabbits contained no detectable antibodies to most of the major components of human plasma, although they reacted weakly with isolated gamma globulin and whole plasma, indicating that the rabbits had produced antibodies to some of the minor components of plasma. However, the same sera reacted quite strongly with four out of five myeloma globulins, two macroglobulins and a cryoglobulin. It was concluded that some of the antigenic determinants of the abnormal globulins could be found in the minor components of normal human plasma.

It has been reported previously that rabbits with an acquired immunological tolerance to normal human plasma will not form antibodies to abnormal serum globulins, and it was concluded that all the antigenic determinants of the abnormal globulins are to be found in normal plasma. This and the related conclusion drawn from the immunological paralysis experiments do not necessarily mean that the abnormal globulins are merely gross elevations of minor, normally occurring globulin components. It is possible that they could be hybrid proteins derived from the pool of sub-units found in the population of normal globulin molecules. This hypothesis could reconcile the supposed antigenic identity of the normal and abnormal globulins with the great physical and chemical diversity of the latter.

## THE SERUM GLOBULIN PROFILES OF MELANESIAN NATIVES

### (a) Moving Boundary Electrophoresis.

Further studies have been made on variations in the serum protein pattern between different groups of Melanesian natives. Considerable inter-group variation is observed, the most unusual variation being found in a group of eighteen Bougainville natives, where a split beta, or a well-separated gamma<sub>2</sub> component was observed.

### (b) Starch-Gel Electrophoresis.

Melanesian sera were examined by the starch-gel electrophoretic technique of Smithies. With the exception of the Bougainville group, whose patterns showed a split beta-C component, little regional variation was observed. The principal significance of starch-gel electrophoresis lies in the fact that it can be used to study the distribution of the genetically determined haptoglobin groups (genes Hp<sub>1</sub> and Hp<sub>2</sub>). Haptoglobin typing has been carried out on over 200 sera. The frequencies of the two genes in the Melanesian population differ markedly from the frequencies reported for Caucasians. In Melanesians the ratio Hp<sub>1</sub> : Hp<sub>2</sub> is 0.73 : 0.27, whilst in Caucasians it is stated to be 0.42 : 0.58. The frequency of Hp<sub>1</sub> appears to be somewhat lower in the more elevated regions of the territory, although many more sera must be examined before this difference can be regarded as significant.

## SERUM PROTEINS AND KURU†

### (a) Moving Boundary Electrophoresis.

Fresh studies have confirmed the existence of variable abnormalities in the sera of natives of the Eastern Highlands of New Guinea suffering from the neurological disease Kuru. The results obtained with Kuru sera are being correlated with stage-of-disease, sex and kinship data, and a search is being made for possible environmental and genetic factors which may influence the serum protein pattern. This study so far indicates that high values for beta globulin (exceeding 25% of the total protein of 7-8 gm.%) are found in many patients in the middle stages of the disease, and are virtually absent in the terminal stages. The majority of the terminal patients, however, have elevated levels of gamma globulin (>30%).

### (b) Haptoglobin-Typing and Kuru.

Kuru sera also show abnormalities in the starch-gel patterns obtained by the method of Smithies. These may be correlated with the abnormalities observed in patterns obtained by conventional methods. In spite of the abnormalities it is still possible to determine the haptoglobin types of the Kuru patients from the patterns. It has been found that the frequency of the Hp<sub>2</sub> gene (0.62) is considerably higher amongst the Kuru sample (64 sera) than amongst the normal Fore population. As far as can be determined in such a small sample the frequency of the gene does not vary significantly in the two sexes or in three age-groups (0-10, 10-20, 20-30 years).

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

Although only a preliminary result, this increase in the frequency of the  $Hp^2$  gene is of great interest. Any genetic theories of Kuru must explain the increased susceptibility of carriers of the gene, and it is the first correlation reported between a haptoglobin group and a disease.

#### **ELECTROPHORESIS OF HUMAN HAEMOGLOBIN IN BORATE BUFFERS**

It was found that human haemoglobin showed considerable spreading when submitted to electrophoresis by the starch-gel technique of Smithies. This was in marked contrast to the behaviour of serum albumin which, at comparable concentrations, migrated as a sharp band. It was decided to investigate the phenomenon by the moving boundary technique with the pH 8.6, ionic strength 0.023 borate buffer which had been used to make the gels. In these experiments it was found that normal adult haemoglobin gave a diffuse asymmetric peak in the descending limb, although the ascending boundary remained sharp. Samples of the trailing edge of the descending pattern were removed from the cell and were run again in the borate buffer. This material gave a symmetrical fairly sharp peak suggesting that conditions in the descending limb had given rise to a true fractionation of the haemoglobin. The most probable explanation is that normal haemoglobin consists of a population of molecules heterogeneous in their ability to react with the borate ions. This heterogeneity is emphasised by the conductivity and pH gradients which occur in the descending limb of the electrophoresis cell.

Most previously reported cases of protein-borate interaction have involved proteins of significant carbohydrate content. Haemoglobin is not considered to contain carbohydrate, and it is difficult to understand the nature of the reaction.

The experiments were repeated with a sample of sickle-cell haemoglobin. The preparation used gave a symmetrical diffuse peak, quite different in appearance to normal haemoglobin. Other experiments were made with haemoglobins of apparently normal mobility under conventional conditions and from cases of megaloblastic anaemia, and it was found that a significant amount of the fast material observed in the normal descending patterns was missing.

Further work is being carried out to confirm these results and to investigate the effect of borate on as many normal and abnormal haemoglobins as possible.

#### **PHOTOELECTRIC FRINGE COUNTER**

In recent years the precision and sensitivity of the technique of moving boundary electrophoresis has been improved considerably by the introduction of interferometric recording of the refractive index changes in the electrophoresis cell. Commercial electrophoresis apparatus which can detect the refractive index change due to 0.004 gm. of protein per 100 ml. is readily available. It is usually claimed that the single operation of counting fringes is less laborious than tracing and planimetry of the gradient pattern obtained with the conventional Philpot-Svensson optical system. However, with complex patterns, such as given by serum, fringe counting does impose a considerable strain on the operator. In such patterns with asymmetric mobility envelopes, reflecting

multiple components, the conventional gradient curve is most desirable for detailed analysis. In the case of interferometric patterns a very accurate gradient curve can be calculated from the spacing between the fringes.

If a photographic plate of an interference electrophoresis pattern is magnified until the finest fringes appear quite separate, each appears as an irregular band of silver grains, with no clearly demarcated point from which to measure the spacing. It was decided that the simplest method of measuring spacing was to scan the fringes photo-electrically and measure the distances between a set point on each photometric curve.

In its final form the fringe-counter measure consists of a binocular microscope with a mechanical stage operated by a micrometer drive adapted from a Bendix azimuth indicator. The image from one eyepiece (at x650 magnification) is focused on to a 0.5 x 2 mm. slit in a mask on the surface of cadmium sulphide photoconductive cell. The other eyepiece is used to check the focus visually. The current from the cell is amplified by three stages of D.C. amplification with an overall gain of 3000 and used to operate either a Schmitt trigger, coupled to a counter and indicator light meter, or a chart recorder. The trigger is set to fire at a voltage corresponding to the "shoulder" of the photometric curve of each fringe. To measure fringe spacing it is merely necessary to record the readings of the micrometer between pulses of the trigger as the plate is slowly moved along the stage. For the most accurate measurements the micrometer is driven by a constant speed motor and the photometer output fed into a chart recorder. Fringe spacing is then measured directly on the recorder chart.

This instrument will be of considerable value in analysing the complex electrophoresis patterns obtained from the sera of New Guinea natives.

### CARCINOMA OF THE LUNG\*\*\*

C. W. E. Wilson.

An analysis of over 500 cases of carcinoma of the lung admitted to Alfred Hospital in the period 1946-1958 is being carried out. The survey is as yet incomplete, but the following tentative observations may be made.

There have been five male cases to each female case, and a similar proportion of each sex in whom surgical removal was attempted. The mean age of the group is 59 years, but the age of those operated upon is slightly lower than those in whom operation was not advised. The occupation of the patients was not recorded frequently enough to enable any generalisations to be made, but one patient had been employed refining arsenic for fourteen years. This probably was an occupational hazard. Although a number of patients were heavy smokers, smoking habits had been recorded in less than half of the cases, and no statement can be made about the incidence of smokers in the series.

As there have been suggestions that some diseases occur more frequently in persons with various blood groups, a comparison was made of the incidence of different blood groups, when it was recorded, with that of the general population, as surveyed by the Red Cross blood transfusion service. This showed no difference between patients with carcinoma of the lung and the control series.

It is of interest to note that one-third of the patients sought advice because they had a chronic cough, and in 5% the diagnosis was suspected following attendance at a mass chest X-ray survey.

Although a variety of neurological lesions has been recorded in association with lung carcinoma, in this group only three cases of myasthenia gravis were recorded.

An unusual finding in these cases is that carcinoma of the lung was commonly in the left lung in those cases operated upon. This is in contrast to the more common occurrence in the right lung as was seen in those cases not treated surgically.

In only about half of the 40% of the cases upon whom thoracotomy was performed could the neoplasm be removed. The expectation for survival of these cases has been not more than twelve months, unless removal of the carcinoma was possible. After removal of the carcinoma the operative mortality was 8.5% and the five year survival 25%.

In conclusion, it may be noted that one-quarter of the patients from whom the neoplasm was removed had a history of symptoms extending for more than twelve months.

#### PUBLICATIONS DURING 1958

- T. E. Lowe: "Further Observations on the Relation Between Body Fluid Volume and Urine Flow in Man." *A/Asian. Ann. Med.*, Vol. 7 (1958), p. 235.
- P. Fantl and A. G. Marr: "The Coagulation of Horse Blood." *J. Physiol.*, Vol. 142 (1958), p. 197.
- P. Fantl and H. A. Ward: "The Thromboplastic Component of Intact Blood Platelets is Present in Masked Form." *Aust. J. Exp. Biol & Med. Sci.*, Vol. 36 (1958), p. 499.
- P. Fantl, R. J. Sawers and A. G. Marr: "Coagulation Inhibitors in the Blood of Patients with the Hemophilic Syndrome." *Proc. Int. Soc. Haematology* (1958), p. 403.
- A. J. Barnett and G. Wagner: "Calorimetry. II. Clinical Application in Peripheral Vascular Disease." *Med. J. Aust.*, Vol. 1 (1958), p. 391.
- A. J. Barnett and G. Wagner: "Severe Orthostatic Hypotension." *Amer. Heart J.*, Vol. 56 (1958), p. 412.
- A. J. Barnett: "Colitis in Patients Treated with Ganglion-blocking Agents." *Med. J. Aust.*, Vol. 2 (1958), p. 525.
- A. J. Barnett and R. J. Marshall: "Treatment of Hypertension with a Combination of Chlorothiazide and Mecamylamine." *Med. J. Aust.*, Vol. 2 (1958), p. 521.
- W. G. Naylor: "Further Observations on the Seasonal Variation in Drug Sensitivity of the Isolated Toad Heart." *Aust. J. Exp. Biol. & Med. Sci.*, Vol. 36 (1958), p. 133.
- W. G. Naylor: "The Effect of Potassium on the Response of the Isolated Heart to Strophanthin G." *Aust. J. Exp. Biol. & Med. Sci.*, Vol. 36 (1958), p. 323.
- C. C. Curtain: "Electrophoresis of Fluorescent Antibody." *Nature*, Vol. 182 (1958), p. 1305.
- F. Binet, R. J. Sawers and G. S. Watson: "Heredity Counselling for Sex-linked Recessive Deficiency Diseases." *Ann. Human Genetics*, Vol. 22 (1958), p. 144.
- G. R. Stirling: "The Effects of Cardiac By-pass and Ventriculotomy upon Right Ventricular Function." *Surg. Forum*, Vol. 8 (1957), p. 433.
- G. R. Stirling: "The Surgical Anatomy of the Heart." *Alfred Hospital Clin. Repts.*, Vol. 8 (1958), p. 93.
- D. Elmslie-Smith: "The Spatial Vectercardiogram in Hypothermia." *Brit. Heart J.*, Vol. 20 (1958), p. 175.
- R. J. Marshall, "Chlorothiazide: A New Diuretic," *Med. J. Aust.*, Vol. 2 (1958), p. 517.
- R. J. Sawers: "Haemorrhagic Disorders Due to Coagulation Abnormalities" in *Clinical Haematology*, by G. C. de Gruchy, Blakewell, Oxford.

#### PAPERS ACCEPTED FOR PUBLICATION

- T. E. Lowe: "The Role of Body Fluid Volume and Plasma 'Active' Osmotic Pressure in the Control of Urine Flow and Fluid Intake in Man." *A/Asian. Ann. Med.*
- P. Fantl: "Thrombin and the Thromboplastic Activity of Plasma." *Aust. J. Exp. Biol. & Med. Sci.*
- W. G. Nayler: "The Action of Reserpine on the Isolated Heart." *Aust. J. Exp. Biol. & Med. Sci.*
- W. G. Nayler: "Effect of Strophanthin-G on the Rate of Deamination of Adenyl Compounds." *Aust. J. Exp. Biol. & Med. Sci.*
- C. C. Curtain: "Starch-gel Electrophoresis of Melanesian Sera." *Aust. J. Sci.*
- C. C. Curtain: "Possible Sites of Macroglobulin Synthesis: A Study Made with Fluorescent Antibody." *A/Asian. Ann. Med.*
- M. C. Davis and A. J. Barnett: "Phaeochromocytoma. Case Reports Illustrating Diagnosis and Management." *Med. J. Aust.*
- D. Elmslie-Smith, G. E. Sladden and G. R. Stirling: "The Significance of Changes in the Electrocardiogram in Hypothermia." *Brit. Heart J.*

#### PAPERS SUBMITTED FOR PUBLICATION

- C. C. Curtain: "The Use of Acquired Immunological Tolerance and Immunological 'Paralysis' in the Study of the Antigenic Relationships of Normal and Abnormal Serum Globulins." *Brit. J. Exp. Path.*

#### LECTURES DELIVERED DURING 1958

- "Control of Body Fluid Volume" . . . . . T. E. Lowe  
Series on Scientific Basis of Medicine.
- "Blood Coagulation Factors" . . . . . P. Fantl  
Chairman's Address—International Congress of Biochemistry, Vienna.
- "Medical Aspects of Blood Coagulation" . . . . . P. Fantl  
University of Melbourne.
- "The Clinical and Laboratory Diagnosis of Phaeochromocytoma" A. J. Barnett  
College of Pathologists of Australia.
- "A New Globulin Component Present in Certain Melanesian Sera" C. C. Curtain  
Australian Biochemical Society, Adelaide.
- "Immunological Tolerance and the Antigenic Structure of the Abnormal Serum Globulins of Myeloma, Macroglobulinaemia and Cryoglobulinaemia" . . . .  
C. C. Curtain  
A.N.Z.A.A.S., Adelaide.
- "The Use of Fluorescent Antibody in the Study of the Sites of Synthesis of Abnormal Serum Globulins" . . . . . C. C. Curtain  
A.N.Z.A.A.S., Adelaide.
- "Sickle Cell Thalassaemia" . . . . . C. C. Curtain and R. A. Hayes  
Alfred Hospital Clinical Society.

- "Halothane and the Heart" . . . . . W. G. Nayler  
 Alfred Hospital Clinical Society.
- "Cardiac Physiology and Drug Action" . . . . . W. G. Nayler  
 A.N.Z.A.A.S., Adelaide.
- "Tobacco Smoking and the Incidence and Symptomatology of Common  
 Respiratory Diseases" . . . . . W. C. Boake  
 Victorian Society for Pathology and Experimental Medicine.
- "Recent Advances in Cardiac Surgery" . . . . . G. R. Stirling  
 Cardiac Society of Australia and New Zealand (Victorian Group).
- "Cardiac Surgery" . . . . . G. R. Stirling  
 Austin Hospital Clinical Society.
- "Halothane and Ventricular Performance" . . . . . G. R. Stirling  
 Alfred Hospital Clinical Society.
- "Bleeding Patients: A Review of Diagnostic and Therapeutic Problems in  
 Haemorrhagic Disorders" . . . . . R. J. Sawers  
 Repatriation General Hospital.
- "Prognosis in Hypertensive Vascular Disease" . . . . . F. O. Simpson  
 Alfred Hospital Clinical Society.

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

Revenue Account for the Year ended 31st December, 1958

EXPENDITURE.		INCOME.	
Drugs, Chemicals, Provisions, etc. . . . .	£1,278 7 4	Donations—	
Fuel and Lighting . . . . .	202 10 10	Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions . . . . .	£16,800 0 0
Instruments and Glassware . . . . .	685 9 1	Marian and E. H. Flack Trust . . . . .	350 0 0
Insurance . . . . .	520 12 9	George F. Little Trust . . . . .	148 6 6
Library Maintenance . . . . .	729 19 8	Rockefeller Foundation . . . . .	443 15 1
Printing and Stationery . . . . .	275 2 10	Mr. and Mrs. Edgar Rouse . . . . .	105 0 0
Repairs and Renewals . . . . .	1,768 14 8	Kodak (Australasia) Pty. Ltd. . . . .	30 0 0
Salaries and Wages . . . . .	18,755 16 0	Mr. J. C. Habersberger . . . . .	10 10 0
Telephone . . . . .	114 17 6	Mr. Henry Foote . . . . .	5 0 0
Travelling Expenses . . . . .	1,613 11 8	Miss N. E. Cameron . . . . .	2 2 0
Sundries . . . . .	529 7 9	Mr. W. R. Clarkeson . . . . .	2 2 0
Workshop Equipment . . . . .	5 19 0	Mr. Ernest Page . . . . .	2 2 0
		Mr. Elvin Teasdale . . . . .	5 0 0
<i>Total Expenses</i> . . . . .	26,480 9 1	Eagle Star Insurance Co. Ltd. . . . .	10 0 0
		Dr. John Rouse . . . . .	2 0 0
		Mrs. Lawrence Simpson . . . . .	2 0 0
		Dr. Ewen Downie . . . . .	5 0 0
		Life Assurance Medical Research Council of Australia and New Zealand . . . . .	2,400 0 0
			20,322 17 7
		Government Grant—	
		National Health and Medical Research Council . . . . .	4,280 19 11
		Anti-Cancer Council of Victoria . . . . .	615 0 0
		Interest from Investments—	
		Held by the Trustees of the Estate of the late Thomas Baker—	
		Commonwealth Government Inscribed Stock . . . . .	£551 10 0
		Endowed to the Institute—	
		Commonwealth Government Inscribed Stock . . . . .	178 2 6
		Australian Consolidated Treasury Bonds . . . . .	16 5 0
		Grain Elevators Board Inscribed Stock . . . . .	103 2 6
			849 0 0
		Sundry Sales . . . . .	181 13 0
		Deficit for Year . . . . .	230 18 7
			£26,480 9 1
			£26,480 9 1



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

YEAR ENDED 31st DECEMBER, 1958

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CAPITAL GRANTS AND GIFTS.

Balance at 31st December, 1957 . . . . .	£1,473 13 1
<i>Add,</i>	
Donation—Drs. T. E. Lowe and A. J. Barnett . . . . .	84 19 5
Balance at 31st December, 1958 . . . . .	<u>£1,558 12 6</u>

ACCUMULATED REVENUE.

Surplus at 31st December, 1957 . . . . .	£4,037 12 8
<i>Deduct,</i>	
Deficit for Year . . . . .	230 18 7
Surplus at 31st December, 1958 . . . . .	<u>£3,806 14 1</u>

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ALFRED HOSPITAL DIABETIC AND METABOLIC  
UNIT

1958

## STAFF

Honorary Physician . . . . .	EWEN DOWNIE, M.D., F.R.C.P., F.R.A.C.P.
Assistant Physician— Scientific Studies . . . . .	JOSEPH BORNSTEIN, D.S.C., M.D., F.R.A.C.P.
Assistant Physician— Clinical Studies . . . . .	BRYAN HUDSON, M.D., PH.D., M.R.C.P., M.R.A.C.P. (on leave).
Honorary Assistant Physician . . .	HARALD BREIDAHL, M.D., M.R.C.P.
Biochemists . . . . .	PETER DAVOREN, PH.D., B.SC. (Wellcome Fellow). DORA WINIKOFF, M.SC. JUNE SHEATH, M.SC. DEIRDRE HYDE, B.SC.
Registrars . . . . .	B. C. RITCHIE (from 3/2/58 to 25/5/58). W. KIMBER (from 26/5/58 to 17/8/58). W. HUNTER (from 18/8/58 to 9/11/58). J. G. HOWARD (from 10/11/58 to 1/2/59).
Technical Staff . . . . .	MR. W. HUDSON. MISS A. EKKEL. MISS L. GIBSON. MRS. P. KEEN. MISS B. ANDERSON. MISS J. HUGHES.
Secretary . . . . .	MISS J. SHARP.

## ALFRED HOSPITAL RESEARCH FELLOWS

“Frederick and Esther Michaelis”	E. L. G. BEAVIS, M.B., B.S., D.G.O., M.R.C.O.G., F.R.C.S.
“Victor Y. and Margaret Kimpton”	HARALD BREIDAHL, M.D., M.R.C.P.
“George Merriman” . . . . .	IAN MARTIN, M.D., M.R.A.C.P.

## DIABETIC CLINIC

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

The past year has been one of consolidation and development. The closest co-operation has been maintained with the Baker Research Institute, the Department of Biochemistry and the Professorial Units of the University of Melbourne within Alfred Hospital. The Standing Committee on Co-ordination of Laboratory services, at its regular meetings, provides a satisfactory means of integrating the activities of the several investigating groups within the Hospital, and has proved valuable in saving unnecessary duplication of effort and of equipment. During the year the Hospital Animal House has been reorganised to the mutual benefit of all concerned.

Broadly, the work of the Unit can be divided into clinical activities and research activities, although no sharp demarcation can be drawn between the two, as much of the work is complementary, and many research projects are concerned with problems of human endocrinology.

The clinical activities of the Unit are presented in the Annual Report of Alfred Hospital, and details of these are not relevant to a research report. Suffice to say that three out-patient sessions are held each week, and are devoted to the management of diabetes mellitus, thyroid disease, adrenal disease and other endocrine disorders in human patients. In addition the Unit controls 24 beds in Ward 1 of Caulfield Convalescent Hospital, provides a consultant service in Endocrinology to Alfred Hospital, and has the use of four beds within the Hospital for investigation of special problems.

The research activities are well defined and consist of a number of projects under the direction of a senior member of the staff, each with a team engaged on different aspects of the work. The vast amount of this activity is supported by specific grants from various research funds and organisations, and the personnel and their work conform to the terms of the grants applied. Fundamental studies on the problems of insulin resistance and antagonism have continued throughout the year. A study of aspects of diabetic vascular disease is proceeding, further observations have been made on the effects and the place of sulphonylurea drugs in the management of diabetes, and work has continued on the prediction of diabetes and on the physical state of the progeny of diabetic mothers. Three important surveys have been concluded on the various aspects of treatment of patients suffering from diabetic coma, Addison's disease and hyperthyroidism. Further work has been undertaken on the behaviour of various steroids, and improved techniques have been developed to facilitate their study and clinical trials have been undertaken to study the effect of several new compounds on human patients. Studies have proceeded on the variations of activity of the thyroid gland in human beings under differing circumstances, and improved techniques are being developed to facilitate these investigations. A study has been made of pituitary dysfunction, and a satisfactory technique for determining one aspect of this has been developed. In addition, a significant contribution has been made to the familial incidence of diabetes insipidus. Improved techniques have been developed which have resulted in determining more exactly evidence of over activity of the parathyroid glands. Details of these investigations are given later in this report.

Early in 1957, thanks to a generous gift from the Trustees of the Baker Charitable Trust, and later by a substantial contribution from the Trustees of the Estate of the late Marie Paser, it became possible to proceed with plans

for the completion of the construction of the Unit. In October last the student lecture theatre was moved from the fifth floor, adjoining the Unit laboratories, and construction of one single-bed ward and two two-bed wards, two small laboratories, an examination room, a seminar room and a small workshop, was commenced with the approval of the Hospitals and Charities Commission. This work will be completed early in 1959, and will greatly facilitate the investigational and research activities of the Unit.

During the past year Dr. Bryan Hudson obtained the Ph.D. degree in the Melbourne University for original work related to adrenal and pituitary functions. Miss June Sheath was awarded the degree of M.Sc. for original work concerned with methods of estimation of urinary steroids. In addition, Dr. Ian Martin was successful in attaining Membership of the Royal Australasian College of Physicians.

Dr. Bryan Hudson received a United States Public Health Service Fellowship, tenable in the Department of Biological Medicine in the University of Utah, U.S.A., and was granted leave of absence for one year to pursue advanced studies in steroid chemistry. Dr. Ian Martin received a scholarship tenable in the Medical School of Western Reserve University, Cleveland, U.S.A., and has left to study under the direction of Dr. Max Miller. Following the award of these Fellowships, both Dr. Hudson and Dr. Martin were granted Fulbright Scholarships to enable them to proceed abroad.

In September Dr. Ewen Downie paid a short visit to the United States of America and Canada in order to attend, by invitation, a symposium conducted by the New York Academy of Sciences and Chas. Pfizer Ltd. relating to the place of sulphonylurea drugs in the treatment of diabetes mellitus with particular reference to the value of chlorpropamide.

In June last, several members of the Unit attended the Inaugural Meeting of the Endocrine Society in Sydney, and presented papers both to this gathering and to the Annual Meeting of the Royal Australasian College of Physicians. In addition, Mrs. D. Winikoff attended the A.N.Z.A.A.S. Meeting in Adelaide in August and presented papers relating to her recent work on thyroid disorders.

This Unit, the first to be established in Australia for the study of Clinical Endocrinology, has attracted attention both at home and abroad. The initial action by the Board of Management of Alfred Hospital in creating this opportunity has been followed by generous assistance provided by many individuals and organisations both by financial support and gifts in kind. It is indeed encouraging to realise that this assistance has come not only from Australia, but also from Great Britain and the United States of America. In addition, valuable advice, technical help and co-operation has been received from many sources within Alfred Hospital, the University of Melbourne and elsewhere. To all these donors and well-wishers grateful thanks are extended.

Within the coming year the Unit will be completed according to the pattern envisaged in 1956. This development could never have been achieved in this time had it not been for the generous help and assistance it has received from many sources and the loyal support of all members of its staff. As stated last year, it is confidently anticipated that its future achievements will justify the confidence and support which have been afforded it in these early years of development.

EWEN DOWNIE.

31st December, 1958.

Grateful acknowledgment is made of financial assistance provided by—

Alfred Hospital Research Fund.  
Burroughs Wellcome & Co. (Australia) Ltd.  
Dr. Margaret Clark.  
Estate of the late E. H. Flack.  
Estate of the late Marie Paser.  
Eli Lilly & Co., Indianapolis, U.S.A.  
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Roussel Laboratories Ltd., London.  
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Eli Lilly & Co., Indianapolis, U.S.A.  
Merck, Sharp & Dohme (Australia) Pty. Ltd.  
Novo Terapeutisk Laboratories, Denmark.  
Pfizer Pty. Limited, Australia.  
Roussel Laboratories Ltd., London.  
Sigma Chemical Co., Missouri, U.S.A.  
Upjohn Co., Kalamazoo, U.S.A.

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

## CLINICAL STUDIES IN DIABETES MELLITUS

Ewen Downie.

Observations on the clinical pattern of insulin resistance have been continued, and further evidence has been obtained on the differentiation of types of human resistance and the response to treatment.

### Vascular Changes in Juvenile Patients of Long Duration.

In association with Dr. Ian Martin, a clinical review was undertaken of 47 patients, all of whom had developed diabetes in childhood or adolescence. All patients in the series had lived for a minimum of twenty years, and some for over thirty years. The examination was primarily aimed at determining the presence or absence of vascular complications, and included ophthalmological, electrocardiographic, oscillometric examinations and urine microscopy. In addition, determinations of serum lipids by electrophoresis and in some cases biochemical estimation were carried out. Dr. J. Bornstein assayed the presence or absence of insulin inhibitors in 24 cases. The data collected was analysed for correlation between the presence of vascular complications and factors such as "control," insulin dose, presence of inhibitors. No conclusion was reached as to the etiology of vascular complications in this group.

It is proposed to continue these observations on a series of patients in similar age groups who are suffering from arterial disease of non-diabetic origin. It is also hoped to study certain aspects of vascular change in older diabetics and in a similar control group.

### Sulphonylurea Compounds in the Treatment of Diabetes Mellitus.

In conjunction with Dr. J. Bornstein and Dr. H. D. Breidahl, observations have been continued in an endeavour to define more clearly the place of these compounds in treatment. Long term observation of patients under treatment with tolbutamide seem to indicate that there is a secondary failure of response in a small percentage. The reason for this is not apparent. No serious side effects have been observed in patients who have been under treatment with this drug for periods of one or two years.

Clinical trials have been undertaken with another compound, chlorpropamide. This substance seems equally satisfactory in the control of many middle-aged diabetic patients. It is apparently effective in smaller dosage than with tolbutamide, and within the dosage limits recommended no serious side effects have been noted, and in a few instances patients who have failed to respond to tolbutamide seem to have shown satisfactory response. These studies are continuing.

## INVESTIGATION OF INSULIN ANTAGONISTS IN DIABETES MELLITUS

J. Bornstein and Deirdre Hyde.

The previously reported work on insulin antagonists in diabetes mellitus has been extended, and the compound previously detected has been partially purified from the plasma of suitable patients. It has been shown to be an ultrafiltrable polypeptide, but the small amounts available from plasma have halted further studies on its purification.

Accordingly, an investigation of human pituitary material was undertaken and a similar ultrafiltrable polypeptide was isolated, purified by column chromatography, and its properties are now being investigated.

#### **Insulin Antagonists in the Plasma of Long Standing Juvenile Diabetic Patients.**

As part of a study on the incidence of complications in juvenile diabetes an assay of insulin antagonists was carried out on the plasma of 24 such patients, thirteen of whom had evidence of vascular disease, and eleven were free of complications.

It was found that the fraction referred to above could be demonstrated in all complicated patients, and in only three of the eleven who were free of vascular disease. A report of this investigation has been accepted for publication by "Diabetes."

### **PERFUSION OF THE RAT AORTA AND KIDNEY**

**J. Bornstein, Margaret Sanders and Delys Sargeant.**

Extensive preliminary studies have been performed on the problem of the study of the metabolism of aorta and kidney. It has been shown that it is possible to successfully perfuse the dorsal and abdominal aorta and the left kidney of the rat, and a perfusion pump capable of handling six such preparations simultaneously is now being constructed. It is proposed to study the influence of hormones on the cholesterol metabolism of these organs.

### **ISOLATION OF INSULIN FROM SMALL QUANTITIES OF PANCREAS**

**J. Bornstein and P. R. Davoren.**

As a part of the project which follows, it became necessary to develop a technique for the isolation of insulin from small quantities of pancreas, as orthodox methods were too cumbersome for the purpose. A number of techniques have been tried, and a completely new technique developed for the early stages of extraction.

### **INCORPORATION OF C<sup>14</sup> LABELLED AMINO ACIDS INTO INSULIN BY THE PERFUSED PANCREAS**

**P. R. Davoren.**

A suitable perfusion pump capable of maintaining a cat pancreas for many hours has been developed. It has been shown by use of C<sup>14</sup> glucose that respiration remains constant for up to four hours. Preliminary experiments on transamination, osmolarity, buffering capacity of the medium have been carried out, and the most suitable conditions for the experiment selected.

## AN ENQUIRY INTO THE GROWTH AND DEVELOPMENT OF CHILDREN BORN TO MOTHERS WITH DIABETES

H. D. Breidahl.

This project has proceeded further during 1958. Now, 100 children, born to 65 mothers, have been examined, and of this hundred 27 have shown significant congenital abnormalities. A detailed analysis will be prepared for publication at a later date. At present, one can say that most of these abnormalities are ones that handicap the child physically or mentally or require surgical operation for correction. There has, as yet, been no instance of diabetes in these children, although some have shown abnormal glucose tolerance curves when stimulated with cortisone, and they have been included in Dr. Margaret Sanders' series of cases.

The type of congenital defect one sees is of a variable pattern, and of the major ones one can list an absent hand, severe spastic paraplegia, a duodenal atresia requiring surgery at an early age, an imperforate anus, and epilepsy. Of the minor abnormalities, strabismus, requiring surgical correction, is the most common; next in order of frequency is an inguinal hernia. Other minor abnormalities are knock knees, undescended testicles, coloboma of the iris, and tracheocele, to mention some.

This survey is going to be extended to include as many children as we can contact, and to include a review of these children at three to five year intervals to see, firstly, if any other abnormalities appear, and secondly, to attempt to determine the incidence of diabetes in this group.

## METABOLIC BONE DISEASE

H. D. Breidahl.

During 1958 further estimations of urinary calcium were carried out on cases of recurrent renal calculi. Another 100 estimations were made, and five more calcium infusions were performed. From the cases on which urinary calcium estimations were performed, a further six cases of parathyroid tumour have been found and successfully operated. One case with hypercalcuria was found to have Cushing's syndrome by subsequent steroid analyses, and this will be dealt with fairly soon. However, we are still left with the frequent problem of the case of idiopathic hypercalcuria, with an occasional high serum calcium. As yet we have not been keen to advise operation in this case, and we are going to try the effects of sodium phytate in an attempt to reduce the urine calcium excretion, and hence the tendency to form renal calculi.

An extension of the work on urinary calcium excretion has been to determine the effect of hormones on the excretion of calcium in the urine in patients with metastatic malignancy in bone. Most of these patients are women with carcinoma of the breast, and with bony secondaries the urinary calcium excretion is usually high. We then see the effect of a course of cortisone, then a course of oestrogen, then a course of testosterone on the level

of the urine calcium. If it can be shown that one of these hormones increases the excretion of calcium, an attempt is made to ablate the organ producing that hormone in the body, and to maintain the patient on a hormone with the opposite action. So far, three or four cases have been screened in this way, and appropriate surgical therapy undertaken to date, with considerable success.

The intravenous calcium tolerance test has been done on about twelve patients now. It is not proving of any value in the diagnosis of metabolic bone disease of obscure origin.

There are plans for extension of the work on the diagnosis of metabolic bone disease to be carried out in 1959.

## PROTEIN-BOUND AND GLOBULIN-BOUND IODINE INVESTIGATIONS

*Dora Winikoff.*

(a) Recent studies with radiothyroxine added to blood serum in vitro revealed an increased binding capacity for this amino-acid by the alpha 1-alpha 2 globulin fraction in normal pregnancy. In cases of habitual abortion, however, no increase above the normal non-pregnant level was observed.

In this project (carried out jointly with the Professorial Unit of the Royal Women's Hospital, who kindly supplied the clinical material) a series of cases of normal and abnormal pregnancy was investigated. Total protein-bound and globulin-bound iodine assays were carried out. The globulin-bound iodine levels have proved, in our hands, to be a very sensitive index of borderline thyroid hyperactivity. It was thought, therefore, that it could detect minor thyroid deficiencies or altered mode of transport of the thyroid hormone by blood proteins. The mean "total" protein-bound iodine and globulin-iodine values in a series of normal pregnant women were well above the normal non-pregnant levels; while in a series of cases of habitual and first spontaneous abortions these were well below the control cases. The difference was statistically significant and more pronounced for globulin-bound iodine values.

It is felt that although in its present form an isolated globulin-bound iodine estimation cannot be used in individual cases to predict the outcome of pregnancy due to overlap of normal and abnormal values, nevertheless, certain assumptions can be made. The mean globulin-bound iodine values for non-pregnant and pregnant controls being 1.2 microgram% and 2.1 microgram% respectively, a value below 1 microgram% could be regarded as a warning sign, while above 2.3 microgram% would almost exclude the possibility of abortion. It would seem desirable to correlate these assays with pregnandiol excretion studies.

(b) An attempt is being made to introduce a simplified ashing method for iodine determination in order to be able to handle more thyroid cases for diagnostic purposes.

## STEROID RESEARCH

June Sheath and Aleida Ekkel.

During the past year several problems in the steroid field have been investigated.

### (a) Plasma Corticosteroids.

Techniques for the estimation of plasma corticosteroids have been investigated, and it is expected that in the near future the estimation will be available for research and routine use. Chromatography of corticosteroids has been investigated by means of the Bush and Zaffaroni systems.

### (b) Hydrolysis of 4-C<sup>14</sup> Cortisone Acetate to 4 C<sup>14</sup> Cortisone.

Various methods of hydrolysis of cortisone acetate were investigated, the most satisfactory one of which was employed for the hydrolysis of 20 mg. of the radioactive material. A yield greater than 80% of pure cortisone was obtained, as shown by colorimetry and chromatography. The product is a suitable reference compound to assist in steroid estimation and identification.

### (c) Enzymatic Hydrolysis of Steroid Conjugates.

(i) It is frequently necessary to extract large volumes of urine to obtain sufficient amounts of steroids for their identification. Investigations into a preliminary extraction of urine in the presence of ammonium sulphate show that the steroid conjugates can be extracted from the urine. These can then be dissolved in a small volume of water, for hydrolysis and subsequent extraction. Thus, the handling of large volumes of urine throughout the procedure is eliminated.

### (ii) The preparation, purification and assay of Molluscan beta-glucuronidase.

Hydrolysis of steroid conjugates necessitates the use of the enzyme beta-glucuronidase. Owing to the high cost and limited availability of the bacterial preparation, beta-glucuronidase has been satisfactorily prepared from molluscs. These were collected from the Victorian coast, and the enzyme was extracted and purified from the viscera of the animals. A product of high activity satisfactory for the enzymatic hydrolysis of steroid conjugates was obtained.

### (d) The 2,4-Dinitrophenylhydrazine Method for Steroid Identification.

This method has been investigated, and may be employed satisfactorily in the identification of steroids, with particular reference to the  $\Delta^4$ -3-ketosteroids.

### (e) Techniques for the Estimation of 17-Ketogenic Steroids.

Two methods were investigated, either one of which could be used for the estimation of 17-ketogenic steroids, when required.

(f) As part of an investigation by Drs. Downie and Martin into the incidence of complications in long standing juvenile diabetics, techniques for the estimation of serum lipids were set up, and 47 patients were investigated.

## DIABETES INSIPIDUS

Ian Martin.

A study of familial diabetes insipidus commenced in 1957 was completed. Two families in Victoria, including some 90 members, were studied to determine the mode of inheritance, clinical features and physiological defect of the condition.

The inheritance appeared to be due to a simple dominant gene in both families. Hypertonic saline infusion tests were performed on nine individuals, and the anti-diuretic effect of intravenous nicotine was measured in three. The results obtained indicated that nicotine produced an anti-diuresis, but that the osmotic stimulus of hypertonic saline did not; further, all cases were normally sensitive to pitressin. The genetic, clinical and physiological aspects are discussed in a paper submitted for publication.

## REVIEWS OF TREATMENT

Ian Martin.

In association with Dr. Bryan Hudson, two reviews were carried out—one on the course and management of severe diabetic acidosis in the Alfred Hospital in the years 1952-1957, and the other on the efficacy of the radio-isotope tests used in the diagnosis of thyroid disease in the D.M.U. laboratories over the preceding two years. Both these reviews have been submitted for publication.

A further review of the use of thyrotrophic hormone and thyroid suppression tests in the diagnosis of thyroid disease was commenced with Dr. Hudson, Mrs. Winikoff and Dr. H. P. Taft.

Also, in association with Dr. Hudson, the section "The Role of Adrenocortical Hormones in Infections" in N.H.M.R.C. handbook on "Chemotherapy with Anti-biotics and Allied Drugs" was revised, and the recent literature reviewed.

## ASSAY OF PITUITARY GONADOTROPHIC HORMONES

E. L. G. Beavis.

During 1958 a further series of assays were conducted to test several variable techniques used in preparing the extract, of which freeze drying was found to be the most useful. Although this added to the time taken to prepare each extract, it was entirely justified by the low toxicity and stability of the final preparation.

Difficulties were experienced in the breeding of mice, probably due to the degree of in-breeding and to the presence of other animals in the same room.

It was found that, in women, during the reproductive phase of life a titre of between three and five units for 24 hours could be detected, using an amount of powder equivalent to between ten and twelve hours for each animal. When there was clinical evidence that a high titre of pituitary gonadotrophin could be expected it was found more appropriate to use an amount of powder

equivalent to four hours, which would detect titres of approximately between three and twelve units. No claims are made that it is possible to use this technique to detect qualitative differences between the pituitary gonadotrophins, should indeed such differences exist. Quantitatively, it is a moderately positive assay, and during the year was employed to confirm the diagnosis of several cases of Klinefelter's syndrome, in which the titre is high, and of pituitary failure, when none could be detected below a level of approximately one unit for 24 hours.

Finally, a laboratory technician has been trained in all aspects of the assay, and is capable of carrying it out through all the stages.

#### PAPERS ACCEPTED FOR PUBLICATION

- Ewen Downie, Joseph Bornstein and Harald Breidahl: "Preliminary Clinical and Experimental Studies with Chlorpropamide in Diabetes Mellitus." *Bull. N.Y. Acad. Sci.*
- J. Bornstein and Deirdre Hyde: "Insulin Antagonists in Arterial Disease of Juvenile Diabetics." *Diabetes.*
- Bryan Hudson and F. I. R. Martin: "The Use of Radioactive Iodine ( $I^{131}$ ) in the Diagnosis of Thyroid Disorders." *Med. J. Aust.*
- Dora Winikoff, K. H. Clarke and S. F. McCullagh: "The use of Intramuscular Depot of Iodised Oil as a Lasting Source of Iodine." *Med. J. Aust.*
- June Sheath: "The Specificity of Laboratory Tests for Occult Blood in Faeces." *Med J. Aust.*

#### PAPERS SUBMITTED FOR PUBLICATION

- Ewen Downie and F. I. R. Martin: "Vascular Disease in Juvenile Patients of Long Duration."
- J. Bornstein and C. W. Baird: "Assay of Insulin Like Activity in the Plasma of Normal and Diabetic Humans."
- B. Hudson: "The Diagnosis and Treatment of Addison's Disease."
- B. Hudson: "Fluoxymesterone (Halotestin): A New Androgen."
- F. I. R. Martin: "Familial Diabetes Insipidus."
- P. R. Davoren and J. Bornstein: "The Effect of Glucagon on the Metabolism of Glucose and Acetate by the Isolated Rat's Liver."
- Margaret Sanders: "The Effect of Prednisolone on Glucose Tolerance in Respect to Age and Family History of Diabetes Mellitus."
- June Sheath: "Factors in the Colorimetric Estimation of 17-Ketosteroids in Urine."
- June Sheath: "Chromatography of Urinary Ketosteroids."

#### PAPERS IN PREPARATION

- J. Bornstein, C. W. Baird and Deirdre Hyde: "Insulin Resistance and Insulin Antagonists."
- J. Bornstein and Deirdre Hyde: "Preparation of an Insulin Antagonist from Human Pituitary."
- B. Hudson: "Observations on the Treatment of Severe Diabetic Ketosis."
- B. Hudson, D. Winikoff, F. I. R. Martin and P. Taft: "Thyroid Stimulating Hormone and Triiodothyronine as Aids in the Diagnosis of Thyroid Disorders."
- H. D. Breidahl: "Clinical Aspects of Hyperparathyroidism."
- H. D. Breidahl: "Results of an Enquiry into the Growth and Development of Children Born to Mothers with Diabetes."
- P. R. Davoren: "An Apparatus for the Controlled Pulsatile Perfusion of Isolated Organs."
- D. Winikoff, R. D. Dickinson and G. Wade: "Globulin-Bound Iodine Levels in Normal and Abnormal Pregnancy."

### LECTURES DELIVERED DURING 1958

"Natural History of Diabetes Mellitus" . . . . .	Ewen Downie
University of Melbourne.	
"Obesity" . . . . .	Ewen Downie
University of Melbourne.	
"Vascular Disease in Diabetes Mellitus" . . . . .	Ewen Downie
North-Western University, Chicago.	
"Clinical Uses of Chlorpropamide" . . . . .	Ewen Downie
New York Academy of Sciences.	
Diabetes Abroad . . . . .	Ewen Downie
Victorian Diabetes Association.	
"Presidential Address" . . . . .	Ewen Downie
Endocrine Society of Australia, Sydney.	
"Vascular Disease in Juvenile Diabetics of Long Duration" . . . . .	Ewen Downie
Toronto Diabetes Association.	
"Insulin Antagonists" . . . . .	J. Bornstein
Baker Institute.	
"Influence of Hormones on Metabolism" . . . . .	J. Bornstein
University of Melbourne.	
"Structure and Action of Hormones" . . . . .	J. Bornstein
University of Melbourne.	
"The Diagnosis and Treatment of Addison's Disease" . . . . .	B. Hudson
The Royal Australasian College of Physicians, Sydney.	
"A Case of Cushing's Syndrome" . . . . .	B. Hudson
Alfred Hospital Clinical Society.	
"Pituitary and Adrenal" . . . . .	B. Hudson
The Royal Australasian College of Surgeons, Melbourne.	
"Pituitary Disorders" . . . . .	H. D. Breidahl
Postgraduate Medical Course, Prince Henry's Hospital.	
"Hypoparathyroidism" . . . . .	H. D. Breidahl
Alfred Hospital Clinical Society.	
"Familial Diabetes Insipidus" . . . . .	F. I. R. Martin
Alfred Hospital Clinical Society.	
"Thyroid Disease and its Diagnosis" . . . . .	D. Winikoff
Society of Clinical Scientists.	
"Iodine Metabolism" . . . . .	D. Winikoff
Baker Institute.	
"Thyroid Gland in Pregnancy" . . . . .	D. Winikoff
A.N.Z.A.A.S., Adelaide.	
"The Estimation of Urinary 17-Ketosteroids: An Appraisal of Current Methods" . . . . .	J. Sheath
The Endocrine Society of Australia, Sydney. Association of Hospital Scientists in Victoria.	

**MEETINGS ATTENDED DURING 1958**

Inaugural Endocrine Society of Australia Meeting, Sydney. Ewen Downie  
J. Bornstein  
B. Hudson  
F. I. R. Martin  
P. R. Davoren  
J. Sheath

Annual Meeting, Royal Australasian College of Physicians, Ewen Downie  
Sydney . . . . . B. Hudson  
J. Bornstein

Symposium on Chlorpropamide, New York . . . . . Ewen Downie

Annual Meeting Biochemical Society, Adelaide . . . . . D. Winikoff

A.N.Z.A.A.S. Meeting, Adelaide . . . . . D. Winikoff



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

## STUDIES ON MYCOBACTERIA

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

Studies on unusual acid-fast bacilli have been continued.

### *Mycobacterium springvale*

*Mycobacterium springvale* is a new species of mycobacterium with unusual characters which was isolated from lesions in a wild mouse. Subcultures have been made and inoculated into experimental mice and have produced typical lesions. Thus Koch's postulates have been satisfied.

This work has given us a wider knowledge of the bacteriology of the genus and is also of value in serving as a new diagnostic and research "tool."

In 1916, Traum, in the United States of America, described lesions of the skin and subcutaneous tissue in cattle. These lesions contained acid-fast bacilli which could not be identified, although the animals sometimes showed a positive reaction to tuberculin. Since 1916 there have been further reports from U.S.A. as well as from England and Sweden, but the disease appears not to have been recognised in Australia until 1956-57, when one of us (N.A.M.W.) discovered typical lesions in cattle in a number of different herds in Victoria. The results of bacteriological studies on a number of these lesions conform with descriptions of Traum's disease.

## STUDIES ON TORULOSIS

A. Perceval† and J. C. Tolhurst.

Early in 1958 a patient with torulosis of subcutaneous tissue and bone was admitted to hospital. In spite of our long-standing interest in this disease, this was the first time that we had seen a case with lesions of the skeletal system.

The infecting organism was unusual. It was small and appeared non-capsulated in the patient's tissues, and in culture it produced matt colonies instead of the common mucoid type. It was shown to be pathogenic by the intraperitoneal route, for the mouse in which it produced the characteristic capsulated forms which made its identity certain.

The possibility that the type of lesion in the patient was related to the character of the organism was considered, although a similar strain had been isolated by one of us in 1942 from cerebrospinal fluid. This strain was preserved and is available for comparison. Studies are proceeding.

## STUDIES ON CHEMOTHERAPY

G. Buckle† and M. Dorr.†

The problem of organisms acquiring resistance to antibiotics has continued, as in previous years, to be a major interest. Several new antibiotics and combinations of antibiotics are being investigated for their efficacy in the therapy of these resistant infections.

†Department of Pathology, Alfred Hospital.

‡‡Department of Agriculture, Victoria.

†Department of Pathology, Alfred Hospital.

**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.)



## STUDIES ON HOSPITAL INFECTIONS

G. Buckle,† A. Perceval,† M. Shallard† and M. Dorr.†

Since 1949 the problem of infections acquired in hospital has been studied with the object of determining and eliminating the sources of infection.

In the past three years a Register has been kept of these infections and much bacteriological data accumulated to serve as a base line for the projected investigations to be carried out in conjunction with the hospital Wounds Infection Committee.

## THE ARTIFICIAL KIDNEY

V. C. Marshall.††

Experiments have been performed with the Kolff twin-coil dialyser. The technique of dialysis and heparinisation was studied on sheep and the efficiency of the dialysis determined by loading doses of urea.

It was found that the Kolff machine compared favourably with other types, and it has since been used clinically on twelve patients.

There have been no serious complications of dialysis to date, and improvement in a proportion of those treated has been gratifying.

## RADIO-ISOTOPE TECHNIQUES IN HAEMATOLOGY

Ivan S. Epstein.

During the past year the establishment of isotope techniques in diagnostic haematology has been developed to the stage of routine use, and some investigations of problems in haematology have been carried out with this aid.

Radio-active chromium is being used for the determination of red cell mass as an aid to the diagnosis of polycythaemia and for measurement of red cell survival time in the investigation of patients suspected of having haemolytic anaemia.

Vitamin B<sub>12</sub> labelled with radio-active cobalt is being employed to aid the diagnosis of megaloblastic anaemias. Both urinary and faecal excretion of the labelled vitamin are being measured to estimate the absorption of an oral dose of labelled vitamin.

Labelled vitamin B<sub>12</sub> is being used to study the absorption of the vitamin, in both man and animals, in the presence of carcinoma of the colon and after small bowel resection.

Using radio-active chromium to tag red cells it has been possible to show that chronic blood loss from many ulcerations in the alimentary canal is intermittent.

†Department of Pathology, Alfred Hospital.

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

**PUBLICATIONS DURING 1958**

J. C. Tolhurst: "The Effect of the Medium on the Cultivation of *Mycobacterium Balnei* at 37° C." *J. Gen. Microbiol.*, Vol. 18 (1958), p. 295.

**PAPER ACCEPTED FOR PUBLICATION**

J. C. Tolhurst, G. Buckle and N. A. M. Wellington: "The Experimental Infection of Calves with *Mycobacterium Ulcerans*." *J. Hygiene*.