BAKER INSTITUTE

RESEARCH

1960

ALFRED HOSPITAL

The Baker Medical Research Institute derives its main financial support from the Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions. It is also dependent upon donations from private sources. The latter may be allocated to an Endowment Fund.

The Diabetic and Metabolic Unit is a department of Alfred Hospital, part of whose duties is to conduct Research in some aspects of endocrinology.

Research Fellowships are awarded by the Appointors for Research Scholarship Funds of the Hospital in consultation with the Research Advisory Committee of the Board of Management.

THIRTY-FOURTH ANNUAL REPORT

of

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

(Including Alfred Hospital Clinical Research Unit)

FOURTH ANNUAL RESEARCH REPORT

of

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

REPORTS

of

ALFRED HOSPITAL RESEARCH FELLOWS

1960

ALFRED HOSPITAL, PRAHRAN
VICTORIA, AUSTRALIA

BAKER MEDICAL RESEARCH INSTITUTE

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°E. S. J. KING, D.Sc., M.D., M.S., F.R.C.S., F.R.A.C.S., F.R.A.C.P.
Director of Clinical Research Unit (ex officio).

°Appointed from the University of Melbourne.

ALFRED HOSPITAL RESEARCH FELLOWS, 1960

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

APPOINTED TO RESEARCH FELLOWSHIPS FOR 1961

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

INTRODUCTION

The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute was founded under the terms of a Deed of Settlement executed in 1926 between the Settlors and the Board of Management of Alfred Hospital. The Institute was established to provide an efficient hospital laboratory service and facilities for medical research. In the course of time it was found more satisfactory for these routine services to be placed under the control of the Hospital staff, and this transfer was completed in 1948. Since then the Institute staff has been entirely concerned with research, with emphasis on the basic medical sciences. This is integrated with projects of the Clinical Research Unit.

This unit was formed in 1949, and as a result the Board of Management set up a Research Advisory Committee in accordance with suggestions made by the National Health and Medical Research Council at the time of formation of a similar unit in a sister State. The purposes of this Committee were to advise the Board on matters of appointment to the Unit and to accept responsibility that the funds allocated by the Council were expended in accordance with the conditions of the grants.

The appointment of Dr. T. E. Lowe as Director of the Clinical Research Unit in 1948 was followed by his appointment as Director of the Baker Medical Research Institute in 1949, and since that time the Committee has become concerned with an increasing interest and responsibility not only for clinical research conducted within the Clinical Research Unit, but also with Research Fellows who work in various departments of the Hospital, supported from specific research funds bequeathed in trust to Alfred Hospital.

The annual reports of the Baker Institute have been published since 1927, and soon after the formation of the Clinical Research Unit it was felt desirable to publish a combined volume entitled "Research". This made its first appearance in 1953, and contained the twenty-seventh annual report of the work of the Baker Institute and the fifth annual report of the work of the Clinical Research Unit and the Alfred Hospital Research Fellows.

In 1956 the Board of Management formed a Diabetic and Metabolic Unit, which is engaged in investigation of endocrine and allied disorders. This has also been placed under the supervision of the Research Advisory Committee.

Because of the increasing importance and diversity of the investigational activities conducted in Alfred Hospital, it has been decided to present this report in several sections, indicating the activities of the Baker Institute (including the Clinical Research Unit), the Diabetic and Metabolic Unit, and the work of the Research Fellows.

This follows the policy expressed by the Board of Management in the Annual Report of Alfred Hospital in 1950:—

"It is now generally accepted that research into human disease must be conducted predominantly in close relationship with patients undergoing investigation and treatment. Such research is conducted on two levels. The first is concerned with the basic medical sciences (e.g., at Baker Medical Research Institute), and the second is associated with a study of disease as encountered in the sick person, i.e., clinical research. The organisation of Australian

hospitals, which is peculiar to this country, necessitates that the development of the research function of the Hospital be mainly conducted in separate specially equipped units. In addition, many members of the Honorary Medical Staff devote their valuable time to research in their various specialties and the organised research facilities of our Hospital, namely, Baker Institute and Clinical Research Unit, are at all times available to them in this work. Such an arrangement is in conformity with our objects—treatment of the sick, training of doctors and nurses, and provision of facilities for research."

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

BAKER MEDICAL RESEARCH INSTITUTE

STAFF

Director:	T. E. LOWE, D.Sc., M.D., F.R.C.P., F.R.A.C.P.
Associate Directors:	P. FANTL, D.Sc., F.R.A.C.I.
	A. J. BARNETT, M.D., F.R.A.C.P., M.R.C.P.
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	E. C. OSBORNE, Ph.D., B.Sc.
	H. A. WARD, M.Sc.
	Mrs. M. WEISS, Ph.D., M.Sc.
	R. G. WYLLIE, M.B., B.S.
Technical:	S. HART (Laboratory Supervisor).
	Mrs. J. BATHIE (to 29/7/60).
	J. L. BREMNER.
	Miss E. M. REID (to 8/7/60).
	Mrs. R. SABO (from 29/6/60).
	Miss A. STANTKE (from 4/7/60).
Clerical:	Mrs. L. DEMPSTER (to 26/8/60).
	Miss B. DOWDELL (from 7/11/60).
	Mrs. M. HITCHCOCK (from 18/7/60).
	Miss J. MIRAMS (to 2/12/60).
	Mrs. I. ROBINSON (from 29/8/60).
	Miss R. WARD (to 29/7/60).
Laboratory Assistants:	Miss B. BURKE (from 23/5/60).
	D. BREEN.
	Miss P. CLIFTON.
	Miss J. EDYVANE.
	Miss J. HAMMOND.
	Miss J. HARRIS.
	Miss J. HOWELLS.
	Miss W. JENKINS.
	Miss B. LENNIE (to 4/11/60).
	Miss C. A. MILROY.
	Miss A. McARDLE.
	Miss J. NICHOLS (to 20/5/60).

WARD STAFF

Registrar:	I. G. LYALL, M.B., B.S., M.R.A.C.P.
Resident Medical Officers:	K. THEVARAJAH (from 1/2/60 to 22/5/60).
	D. ELLINGHAM (from 23/5/60 to 18/7/60).
	J. CARTLEDGE (from 15/8/60 to 6/11/60).
	A. ZERFAS (from 7/11/60).
Sisters:	P. D. CALLINAN (to 30/10/60).
	J. BOOTHBY (from 7/11/60 to 18/12/60).
Staff Nurses:	M. CROPLY (to 4/4/60).
	C. R. DODD (to 1/2/60).
	A. PHILLIPS (from 15/2/60 to 20/3/60).
	J. BILHAM (from 21/3/60 to 11/9/60).
	D. OWEN (from 4/4/60).
	S. LEIGHTON (from 12/9/60).

RESEARCH FELLOWS

Sydney W. Jones Medical Research Foundation":	P. KINCAID-SMITH, B.Sc., M.B., B.Ch. (W'srand). M.R.C.P., D.C.P.
"Edward Wilson Memorial":	F. H. LUMB, M.B., B.S. (Lond.), M.R.C.P.
"R. B. McConnas Memorial":	W. McDONALD, M.D., B.S., F.R.A.C.S.
"Dr. Henry Laurie"	D. RACE, M.B., B.S.
"Sol Green":	R. J. SAWERS, M.D., B.S., M.R.A.C.P.

ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

Of the many projects in medical research recounted in these reports during past years, the most significant and largest has been referred to by implication only. The development of the Institute in its present form is from the viewpoint of medical research in this country the project of greatest importance. In this we have endeavoured to apply basic science to clinical medicine and, conversely, to present clinical problems to laboratory workers. This co-operation is based on the idea that there is no branch of science whose principles cannot, with advantage, be applied in seeking a solution of clinical problems.

One may ask whether such an organisation achieves something that University departments cannot do equally well or better? In fact it does, because the various branches of science represented in the Institute are not departmentalised, and so much greater interaction between them is achieved than is usually possible between University departments. Such an organisation is not competitive with a University, rather it is complementary.

The Institute has as permanent staff a number of senior established scientists, each carrying out research in a different scientific or medical discipline. With their guidance other projects are carried out by Research Fellows, who may be at any stage of their career from trainees to established research workers. At the present time the fields represented include clinical medicine and surgery, biochemistry, physical chemistry, physiology, biophysics and cellular biology. Continual daily contact between these workers and the clinicians of the hospital enable both groups to be kept informed of each others' problems and the advances in their respective fields. This interplay is of great advantage in keeping the treatment of patients and the training of students abreast with latest developments. Co-operation between these key persons on research projects is a most important feature of this organisation, and a survey of the papers published and the scientific section of the report indicates joint work by clinical and basic science workers as well as by workers in the different laboratory fields. Without this cross linkage the major project must lose its purpose and become a series of perhaps unrelated studies which happen to take place under one roof. The success of this venture shows that the concept is practical and valuable but experience shows that adequate overall direction is essential in much the same way that an orchestra needs a conductor.

Although the permanent staff are skilled in many different disciplines others could, with advantage, be added when appropriate senior workers are available. For example, a physiologist with a good training in mathematics would enable studies in the integration of knowledge of various parts of circulation to be made.

When this project was commenced twelve years ago it was decided to orient studies towards problems of cardiovascular disease. This field, in its broadest meaning, includes the heart, blood vessels and the fluid circulating in the system. At that time the development of instruments for clinical and physiological investigation of the circulation in man was undergoing a great upsurge

everywhere in the world. The Institute played its part in this movement and from this work the Cardiovascular Diagnostic Service of the Hospital grew. Today the instrumentation needed for clinical cardiological studies has become largely stabilised and our research projects are more directed to basic problems which can be studied anew with modern techniques.

Studies at a cellular level are now feasible for electron microscopy has revealed a great deal about cellular structure, and the use of radioactive tracers has made biochemical studies at this level possible.

Recently a group of workers in the Institute have commenced studies of cellular enzyme properties in relation to neoplastic change, and the investigation of bleeding diseases has led to work on genetics. These studies of cellular physiology do not indicate any diversion from the major objective of cardiovascular disease but rather are studies in parallel fields from which results applicable to cardiovascular problems may be obtained, for at the cellular level many processes are common to most cell types.

With the growing complexity of equipment now used for research, the cost in both money and space increases yearly. Although considerable funds are available from both governmental and non-governmental grant-making bodies, these funds are mainly in the form of Grants-in-Aid of specific research projects. Only rarely do these bodies make provision for security of tenure and long-term support for a career investigator or for the buildings in which research is carried out.

This Institute is indeed fortunate in having the assured support of the Baker Benefactions and of the Alfred Hospital with its research endowments which have made possible the maintenance of a research framework within which numerous researchers holding Grants-in-Aid are able to work.

Successful as this project has been, the clinical side still remains disproportionately small and must remain so until a greater number of beds can be made available to the Clinical Research Unit.

Summary of Research Projects

The research projects carried out in 1960 are detailed in the scientific section of this report and are summarised in the following paragraphs.

The concepts derived from the studies on the control of body fluid volume now seem to have been accepted by many overseas workers in this field. At present the component parts of the control system are being investigated in order to obtain details of its mechanism.

The chemical processes involved in the clotting of blood still provide a major project on both clinical and laboratory levels. To further elucidate these problems the clotting mechanism has been studied in a variety of animals. Clinical studies continue in the field of bleeding conditions.

New drugs for the treatment of hypersensitive states are still becoming available and clinical trials of them continue, but the investigation is not being extended further. Studies of peripheral arterial disease have been related to dietary and drug treatment and to arterial grafting to bypass occlusions.

Considerable new data have been accumulated concerning the energy production by heart muscle. The part played in muscle contraction by various electrolytes has formed a focus for these studies this year.

The study of serum proteins and haptoglobins of New Guinea natives continues and a considerable amount of work has been devoted to the development of instruments to assist these investigations.

One group of investigators continues to study the problems of cardiac surgery using total body perfusion techniques. The effect of these procedures on ventricular function has been investigated in detail. Some preliminary investigations of the problem of coronary arterioplasty have been commenced.

The biological studies at a cellular level initiated last year continue with studies on the enzymatic synthesis of components of leucocytes and erythrocytes in inflammatory and neoplastic conditions.

To these large projects must be added a number of shorter and smaller projects which include scleroderma, renal histology, cerebral injury and a study of socio-economic effects of cardiovascular disease.

Overseas Visits

During the year Dr. P. Fantl attended a meeting of the International Committee for the Standardisation of the Nomenclature of Blood Clotting Factors in Princeton, New Jersey, and visited a number of centres in Canada, United States of America, England, Holland and South Africa in connection with his research projects.

Dr. R. J. Sawers attended the 8th Congress of the International Society of Haematology in Tokyo.

Grateful acknowledgment is made to the National Advisory Heart Council, U.S.A., and the Anti-Cancer Council of Victoria for the financial assistance given to them for these visits.

Asian-Pacific Congress of Cardiology

At the end of May, the 2nd Asian-Pacific Congress of Cardiology was held in Melbourne and I had the honour of being President of the Congress. This was a very successful event, and a number of our staff contributed to the scientific programme. The Institute was visited by many of the overseas cardiologists attending the Congress.

Research Grants

Many of the investigations recorded in this report have been supported by funds provided by the National Health and Medical Research Council, the Life Insurance Medical Research Fund of Australia and New Zealand, the Anti-Cancer Council of Victoria, the Trustees of the Patrick Brennan Trust and Alfred Hospital Medical Research Funds, and the assistance granted is gratefully acknowledged.

It is a pleasure to record thanks for generous donations for equipment from the Haemophilia Society of Victoria, Ciba Company Pty. Ltd., and several private donors whose names are listed in detail in the various financial reports.

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

Considerable assistance has been given this year by Professors Davies, King, Trikojus and Wright, and the staffs of the Departments of Organic Chemistry, Pathology, Biochemistry, and Physiology, University of Melbourne,

and the staff of the Commonwealth Serum Laboratories. We thank them and others who have helped for their continuing interest in our work. In reciprocity, clinical and laboratory help has been made available to the Departments of Medicine and Surgery of the University of Melbourne within the hospital.

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

T. E. LOWE.

31st December, 1960.

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

Adelaide Children's Hospital.
Anti-Cancer Council of Victoria.
A.N.Z.A.A.S.
College of Physicians and Surgeons, New York.
Commonwealth Department of Health.
Commonwealth X-ray and Radium Laboratories.
Department of Health, New Zealand.
Instituto de Biologia y Medicina Experimental, Buenos Aires.
Institute Pasteur d'Algerie.
Medical Research Council, London.
Middlesex Hospital Medical School.
National Institute of Nutrition, Tokyo.
New York University College of Medicine.
New Zealand Medical Research Council.
Ophthalmic Research Institute of Australia.
Rockefeller Foundation, New York.
Royal Children's Hospital, Melbourne.
Royal Melbourne Hospital.
Royal Prince Alfred Hospital, Sydney.
Strangeways Research Laboratories, Cambridge.
Staten Seruminstitut, Copenhagen.
University of Melbourne.
University of Otago, New Zealand.
University of Sydney.
Universitatis Mariae Curie Sklodowska, Poland.
Walter & Eliza Hall Institute.

ALFRED HOSPITAL RESEARCH FELLOWS IN THE INSTITUTE 1949-60

Anderson, R. McD., 1953-55
Andrew, R. R., 1949-55
Barnett, A. J., 1949-50
Beavis, E. L. G., 1955-56
Boake, W. C., 1958
Breidahl, H. D., 1952-53
Burnside, K. B., 1951
Duffy, D. G., 1952-55
Ferguson, I. A. L., 1957-58
Fowler, R., 1953-54
Francis, J. K., 1956-57
Fraser, J. R. E., 1957
Gardiner, J. M., 1952
Goble, A. J., 1951
Hudson, B., 1952
Jamieson, K., 1954
Kay, H. B., 1949-53
Kincaid-Smith, P., 1959-60
McCutcheon, A. D., 1959
McDonald, W., 1960
McNeur, J. C., 1955
McRae, C. J., 1955
Murfitt, L., 1955
Newman, H. C., 1954
Parsons, P. J., 1951
Quinn-Young, M., 1956
Race, D., 1959-60
Sawers, R. J., 1953-60
St. Clair, W. A., 1955
Silberberg, F. G., 1953
Stern, W., 1954-55
Stirling, G. R., 1955
Wagner, G., 1958

OVERSEAS FELLOWS

Emslie-Smith, D., 1955-56 (Dundee)
Hamilton, M., 1954 (London)
Lumb, F. H., 1960 (London)
Marshall, R. J., 1957 (Belfast)
Simpson, F. O., 1958-59 (Edinburgh)
Stevenson, M. M., 1957 (Belfast)
Thomson, J. W. W., 1959 (Edinburgh)

NYULASY SCHOLARSHIP

M. McCulloch (1959)

REPORT OF SCIENTIFIC INVESTIGATIONS

BLOOD COAGULATION*

P. Fantl, E. C. Osborne, R. J. Sawers and H. A. Ward

Phospholipids and Blood Coagulation

The fact, that certain lipids influence blood coagulation and may be of clinical importance and that a relation between diet and atherosclerosis and thrombosis has been assumed, is well known. However, the contribution of the factors involved to clinical states has not been established and controversy has been going on regarding the active constituents. As a contribution to this problem we are studying the composition of the phospholipids of egg yolk. Separation has been carried out on columns of silicic acid and by fractional elution with methanol-chloroform mixtures, several components have been isolated. It can be stated that, in contrast to some published reports, phosphatidyl-ethanolamine is inactive in preventing the formation of blood thrombo-plastin and in the conversion of prothrombin to thrombin. We have succeeded in separating an active fraction whose chemical structure is being determined.

Hageman Factor Deficiency

In collaboration with the thoracic-surgical unit the haemostatic mechanism of some fifty patients has been investigated pre-operatively. Three were found to have a deficiency of Factor VII which, however, was not severe enough to produce a haemorrhagic tendency.

One patient showed a severe coagulation abnormality in laboratory tests, and this was found to be due to absence of Hageman factor from his blood. In addition, he had Ehlers-Danlos syndrome of moderate severity. Using an extra-corporeal circulation with heparinised blood in a disc oxygenerator, the patient was operated upon by Mr. K. Morris, who repaired an atrio-ventricular canal. The patient did not have at any time, before or after the operation, excessive bleeding. The half life of Hageman factor in this patient's blood was estimated to be approximately two days. It appears therefore that despite laboratory evidence of a severe blood coagulation defect, no haemorrhagic tendency was associated with the Hageman factor deficiency.

The physiological role of Hageman factor remains obscure. Most laboratory tests are carried out in glass vessels which adsorb this factor, and it is possible that no adsorption of Hageman factor takes place on the endothelial lining of a blood vessel. The activation of blood coagulation by glass or similar surfaces has no physiological counterpart. The patient and one of his brothers had the same degree of deficiency, and their mother was partly deficient in Hageman factor.

Blood Coagulation in Vertebrates

It is to be noted from the above study of Hageman factor deficiency that because of lack of recognisable symptoms the defect can only be detected by chance.

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

The patient being an 11-year-old boy could not be bled too often, and this precluded a study of the significance of Hageman factor in him. Therefore a study of the occurrence of Hageman factor activity among vertebrates was carried out, and the opportunity was taken to study a number of other clotting factors at the same time.

Hageman factor is probably one of the factors in plasma which can be activated by contact with foreign surfaces. Kaolin was used as the foreign surface in this investigation. In addition, the stability of clotting factors after incubation with kaolin was determined from the clotting time and four groups of vertebrates were recognised in order of decreasing activity. In the first group are cane toad, trout, tortoise and echidna, which show very short clotting times after recalcification, but incubation with kaolin results in rapid lengthening of the clotting times. In the second group are possum, dog, guinea-pig and rat, whose plasma, after incubation with kaolin for 1-3 minutes, clots in 15-17.5 seconds. In the third group are man and lizards. In the fourth group are birds and tiger snakes. It was found that incubation with kaolin resulted in shortening of plasma clotting time in all examined plasmata.

Bird plasma clotting time can be shortened by normal human serum but not by serum from patients deficient in Hageman factor or Factor IX deficient patients. It was concluded, therefore, that bird plasma is deficient in Hageman factor or Factor IX. However, bird plasma clotting time can be shortened by kaolin in contrast to the human Hageman factor deficient plasma. It seems therefore that, in the bird, a factor other than Hageman factor can be activated by contact with kaolin. It was observed that the mixture of the two sera, the one deficient in Hageman factor the other in Factor IX (taken from patients with complete deficiency), produces 100% activation only after a time interval. This experiment indicates that there is a reaction between the components of the two different sera, which is time consuming.

Self-Inflicted Haemorrhagic Disorders

The wide-spread use of oral anticoagulants in the treatment of patients and the availability of these drugs to the general public as rodenticides has led, on occasions, to their criminal use and to self-administration with resulting haemorrhages. Especially among nursing staff have cases been reported. Such instances have occurred all over the world, and in each case the patients were treated at first as genuine bleeders and much time and effort has been spent to find a cause for their haemorrhages. Recently we examined the blood of a patient who had a severe haemorrhagic disorder, and a detailed study of the blood clotting factors indicated an almost complete absence of prothrombin, Factor VII and Factor IX from the plasma. Pathologically such a depletion is known only in severe liver disease or malnutrition. No indication for either cause was, however, present in this patient. During the testing procedure a peculiar colour of the plasma, unlike that found in known pathological conditions, was noted. The patient would not admit to the taking of any drug, but the colour of the plasma gave a clue to the presence of indanedione drugs and the plasma was extracted in order to isolate any anticoagulant. An ultraviolet spectrum of the extract indicated beyond doubt the presence of phenylindanedione. From the amount isolated it was calculated that the patient must have taken approximately 500 mg. only a few

hours before her blood was sampled. Confronted with these facts, reluctantly the patient admitted self-administration.

Isolation of Factor VII

The conversion of prothrombin to thrombin in the presence of tissue thromboplastin and calcium ions is accelerated by three blood components, Factors V, VII and X.

While the separation of Factor V from prothrombin and the other two factors presents no difficulty, the separation of Factor VII from Factor X or from prothrombin can only be attempted by modern analytical techniques of zone electrophoresis or column chromatography.

Zone electrophoresis in a supporting medium of agar-gel at a very low concentration was used for this purpose. This procedure minimises contamination of the fractions and enables recovery of the separated proteins.

Electrophoresis in a veronal buffer of pH 8.6 and ionic strength 0.05 resulted in separation of Factor VII from prothrombin. The former was found to migrate as an $alpha_2$ globulin and prothrombin as an $alpha_1$ globulin. Factor X has not as yet been isolated in this system.

HAEMORRHAGIC DISORDERS

R. J. Sawers and P. Fantl

Investigation of new patients with haemorrhagic disorders resulted in the diagnosis of alpha-haemophilia in six patients and beta-haemophilia in one. The severity of haemorrhagic symptoms was, as expected, proportional to the deficiency of the clotting factor. Clinical studies on haemophiliacs have led to the development of a new type of splint which is effective in correcting deformities of the knee and elbow joints, which have been difficult to treat in the past.

Other types of haemorrhagic disorders associated with blood clotting disorders which were investigated, have included a patient with severe fibrinolysis connected with carcinoma of the prostate. The haemorrhagic disorder disappeared when treatment with oestrogens was commenced. A 12-day-old infant, who died from liver atrophy due to a virus infection, had gross deficiency of all clotting factors except for Factor VIII (AHF). This observation is of interest as it gives further support for evidence which suggests that the site of synthesis of this factor is in tissues other than the liver.

A new case wih a congenital intrinsic disorder of blood platelets was investigated. Platelet viscous metamorphosis was absent but clot retraction in whole blood was normal. Isolated platelets when mixed with plasma and clotted with thrombin showed unexpected loss of clot retracting activity.

URIC ACID STUDIES IN LEUKAEMIA

R. J. Sawers

The excretion of uric acid by control subjects and by patients with various blood disorders was estimated on duplicate 24-hour specimens of urine under standard conditions. With improved techniques the daily amount of

uric acid excreted by the control subjects was found to be fairly constant in any one subject and to lie in a narrow range when related to body surface area. Twenty controls excreted between 160 mgm. and 280 mgm. of uric acid/sq. metre/day. The mean was 213 and the SD ±34.

The high amount of uric acid excreted by patients with acute leukaemia was confirmed in nine of 11 patients, the greatest amount being 915 mgm./sq. m./day in one patient and between 465 mgm. and 325 mgm. in the other eight patients. Two gravely ill patients excreted normal amounts of uric acid.

By contrast, three patients with undiagnosed haemotological disorders, but who later developed acute leukaemia, had normal uric acid excretion during the pancytopenic stage of their illness. During the manifest stage of leukaemia one patient had raised uric acid excretion (325 mgm./sq. m./day).

Normal or low amounts of uric acid were excreted by patients with aplastic anaemia, polycythaemia vera, chronic lymphatic leukaemia, and by one patient with malignant lymphoma confined to the bone marrow and one with refractory anaemia associated with hyperplasia of the pronormoblasts.

Abnormally high uric acid excretion occurred in two patients with haemolytic anaemia complicating disseminated lupus erythematosis, in three patients with myelofibrosis and in a 12-year-old boy with severe congenital hypochromic anaemia with hyperplasia of the late normoblasts.

This investigation has failed to reveal that diagnostic assistance can be gained from determination of uric acid excretion in several patients with leukaemia presenting atypical onsets. It does suggest, however, that the rate of turnover of primitive cells in leukaemia and the case of refractory anaemia is not excessively rapid. The increased amount of uric acid excreted by some patients with leukaemia is probably related to the greatly increased mass of primitive cells. The observation of high uric acid excretion associated with rapid red cell formation is an indication that the method can give additional information of the rate of haemopoetic cell turnover in man.

CONTROL OF BODY FLUID VOLUME*

T. E. Lowe, F. H. Lumb, I. G. Lyall, M. Weiss and V. Carson

For many years the changes in body weight, fluid intake and output, and other body functions have been studied with the object of elucidating the control of body fluid volume. Briefly the conclusions reached are that the volume of body fluids is subject to regulation separately from the regulation of the electrolyte dissolved in them and that the volume regulation involves a control system of the feedback type.

Analysis of the data obtained from patients recovering from oedema has shown that the rate of urine flow is usually directly related to the total fluid volume of the body. Further, the data indicate that these patients return to normal fluid volume in a cyclical manner. It seems that the system contains elements which may be called "proportional control" and "antagonists".

These ideas indicate the necessity to identify the component parts of the control mechanism of which it is known that adrenal cortical and posterior pituitary hormones are part, and that the control of renal blood flow is important.

Although such a control system for volume regulation can be postulated it will be related to other controls in the body, and control of osmotic pressure of body fluids is known to be closely associated with volume control.

During the current year, work has been related to two aspects of this problem; the control mechanism as a whole and its component parts.

Subjects with cyclical oedema have been investigated from the viewpoint of overall control of body fluid volume.

Investigation of the components of the control system has been along three lines. First, the electrolyte pattern of the plasma in congestive cardiac failure has been studied. Secondly, the relationship between plasma electrolyte content and the depression of freezing point of that plasma has been investigated as part of the investigation of the relation between osmotic and volume control of body fluids. Thirdly, a method for the assay of aldosterone in urine has been further developed and applied to the study of oedema formation. A report of the existence of a salt excreting factor in the adrenal has also been investigated.

Diuretics and the Low Salt Syndrome

As most diuretics greatly influence the renal excretion of electrolytes it was thought that their use might influence the serum electrolyte concentrations of patients in congestive cardiac failure.

The records of 100 cases of congestive cardiac failure admitted to the Hospital between 1958 and 1960 were therefore studied, with particular reference to the concentration of sodium in their sera and the nature of the therapy they had received. Of 55 patients who had been receiving diuretic therapy, 13 had a serum sodium concentration below 135m.eq/litre at some time in the first three days after admission. Of 45 patients who had not received diuretic therapy eight had a serum sodium concentration below this value.

Although the serum sodium concentration was low in a greater proportion of those on diuretic therapy than in those not having it, the results are not considered to indicate that the diuretic therapy was responsible for this.

Plasma Electrolytes and Freezing Point Depression

As part of a wider investigation of fluid balance and osmotic relationships in patients with congestive cardiac failure, a study is being made of the relationship between the approximate serum osmolarity derived from the sum of the individual components estimated chemically and the actual osmolarity calculated directly from the freezing point depression of the serum. In addition to the usual plasma electrolyte estimations (including total protein), the blood glucose and urea were determined and the sum of all these components taken as one measure of the osmolarity. The freezing point depression of the serum was determined by means of a thermistor in a Wheatstone Bridge circuit using an adaptation of the method previously described.

To date, some 35 dual estimations covering nine patients have been made. For various reasons, it has not been possible to obtain more than three analyses at closely spaced intervals except in two cases. Case A had 14 observations and Case B nine.

A regressional analysis was made of both the data on these two patients individually and of the combined data from all patients.

The line fitted to data from Case A showed a significant regression at the 1% level, as did the line from data covering all patients, whereas the fit of the line in Case B was barely significant at the 5% level. However, in the three cases there was a wide variation in the slope of the individual lines. Case A approached most closely to the ideal 45° line; B was almost horizontal, whilst the line for the combined data was intermediate.

These preliminary observations suggest that it is not possible to predict the serum osmolarity (and hence the osmotic pressure) from the serum chemistry with any degree of certainty, as the relationship is subject to a high degree of individual variation. It is proposed to investigate individual behaviour in this regard with further observations.

Aldosterone Assay

A suitable method for the estimation of aldosterone in ¼ or less of a 24-hour urine specimen was sought and a number of hydrolytic procedures and solvents for extraction were tried. The continuous extraction of the acidified urine using dichloromethane was found the most suitable. Most of the extracted pigments were eliminated by differential elution on silica-gel columns. Isolation and further purification of aldosterone was achieved by acetylation and chromatography in various systems, after three chromatographic separations. The quantitative evaluation was done using the NaOH fluorescence reaction. Recoveries of added aldosterone ranged from 70 to 78 per cent.

Urinary Excretion of Aldosterone

Serial estimations of aldosterone excretion were commenced in a series of patients with different cardiac disorders. Fluctuation in excretion ranged from low normal to higher than normal in some cases. The aim of this investigation is to correlate aldosterone excretion with various physical and laboratory findings.

Cyclical Oedema

Observations have been continued on the water and electrolyte balances of patients suffering from oedema of various aetiology and, more recently, these studies have been correlated with the urinary excretion of aldosterone.

One case of particular interest was that of a 31-year-old married woman who, for 14 years, had been troubled by recurrent oedema of increasing severity. The attacks occurred at approximately monthly intervals, with a weight increase of up to 6 kgm. There was no definite association with menstruation. No other obvious cause for her recurrent oedema was found, and she was thus similar to the cases of cyclical oedema previously described by Thorn (Amer. J. Med. 1957. Vol. 23, p. 507).

During the detailed observation of one of her episodes, it was found that the period of fluid retention and positive water balance coincided with sodium retention, and a negative potassium balance. Her daily aldosterone output at this stage was raised to 15 micrograms, and the findings were reversed in the subsequent diuretic phase, the aldosterone excretion falling to 4.0 microgram. It thus seemed that aldosterone was possibly concerned with the production of her cyclical oedema, though there was no evidence that it was the primary factor.

Salt Excreting Factor

Recently a new compound (SEF) has been isolated from hog adrenals and the urine of patients with adrenogenital syndromes of a salt-losing type. There is some evidence that this compound, which has been synthesised, produces salt loss in the urine when injected into rats under certain conditions.

The salt excreting activity of synthetic SEF* has been investigated in both intact and adrenalectomised rats. It is concluded that under the conditions used SEF did not cause an increased sodium excretion and that it had no inhibitory action on the biological effect of aldosterone.

HYPERTENSIVE STATES

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

Clinical Trial of Hypotensive Drugs

The value of the more potent hypotensive drugs in the treatment of severe arterial hypertension is now well established. A trial of the ganglion blocking drugs was commenced some 10 years ago, and over this period the drugs used have changed as the more potent ones became available. These have been augmented with reserpine and chlorothiazide. Our study confirmed that with effective treatment the prognosis of severe hypertension is greatly improved and, in particular, that hypertension diagnosed in the "malignant" phase no longer has the sinister outlook of earlier times.

Until recently the ganglion-blocking drugs have had an unwanted action on the parasympathetic system which has hampered the treatment of hypertension. New agents becoming available have been developed which are ganglion-blocking but without undesirable side effects.

(a) Ismelin.

Last year we described our early experience with guanethidine ("Ismelin" Ciba), which we found to have sympatholytic effect in man without parasympathetic blocking activity. During this year we have given the drug a more extended clinical trial. Thirty-two patients commenced treatment prior to 1st June, 1960. Of these, seven have been treated over 12 months, three from 9 to 12 months, 15 from 6 to 9 months, and seven have stopped treatment in less than 6 months. In general, blood pressure control with guanethidine has been comparable to that obtained with recent ganglion blocking drugs. Side effects due to parasympathetic blockade have been absent, but diarrhoea and muscular weakness have been present. Most patients are happier on treatment with the new drug. No toxic effects on the liver or blood-forming tissues have been observed. A rise of blood urea concentration of at least 15 mg. per 100 ml. has occurred during treatment in nine patients, and albuminuria has occurred in three. These changes suggest that the drug may sometimes have a deleterious effect on renal function. The reasons for stopping treatment in seven cases within 6 months of commencement were excessive

* Synthetic SEF was kindly supplied by Ciba.

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response in one, lack of control of blood pressure with a dose of 100 mg./day in three, side effects in three. The drug is cumulative, and its full effect on the blood pressure is not apparent for two or more weeks. The average early maintenance dose (43 mg./day) is almost the same as that after treatment for 6 months or more (50 mg./day). Tolerance therefore is not a prominent feature, although there has been an increase in the maintenance dose of 50 per cent. or more in four cases.

In conclusion, guanethidine represents an important advance in hypotensive therapy, for, in most cases, it has an efficacy comparable to that of the recent ganglion-blocking drugs but fewer side effects, but it is not effective in in all cases and there is a suspicion that its administration may sometimes be associated with impairment of renal function.

(b) Darenthin

A clinical trial of Darenthin was commenced but, as has been reported by others, patients with severe hypertension quickly developed tolerance to it and their clinical state sometimes deteriorated. The trial was therefore not proceeded with.

In a number of these patients the acute effect of Darenthin on the renal clearance of sucrose, creatinine and PAH was studied. The results confirm that, 2½-3 hours after an intravenous dose of the drug, renal function has returned to or risen above control level although the arterial blood pressure is still reduced.

Renal Causes of Hypertension

In order to investigate and treat cases of hypertension due to renal disease, particularly those due to unilaterial renal disorder or a renal vascular abnormality, a "renal hypertension panel" has been constituted. Cases of interest seen by the members, or referred by other physicians and surgeons, are discussed. Special investigations such as divided renal clearance studies, water-load pyelography and aortography, were carried out when considered necessary. To date some 16 cases have been studied. There have as yet been no cases with a demonstrable vascular abnormality or indications for removal of a diseased kidney.

DISEASES OF THE PERIPHERAL BLOOD VESSELS

A. J. Barnett, V. Carson, I. G. Lyall, K. N. Morris[1] and G. R. Stirling[1]

Arterial Grafting

The treatment of certain cases of occlusive arterial disease commenced six years ago continues. It has given dramatic results in some cases with block in a major artery, but gives only a partial answer to the problem of occlusive arterial disease, for it is applicable to only a small proportion of these patients. The success rate of the operation is much less than desired.

Review of our past experience shows that the commonest disease for which patients were referred to us was atherosclerosis obliterans, and males and females were in the ratio 6 : 1. With one exception they were all aged more

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than 40 years, with the major group between 60 and 70 years. Fifty-two patients (less than 10% of those referred) were considered suitable for operation, and on them 62 arterial grafting procedures were carried out.

Four types of operative procedures were adopted: homograft end-to-end (16), homograft shunt (30), teflon end-to-end (2) and teflon shunting (13). The immediate success rate was 53 of 62 operations (85%) and did not seem to vary with the type of operation. Immediate operative mortality was nil, but one patient died 3 months after operation from chronic wound sepsis. There were four cases of haemorrhage from graft sites. Only half the grafts inserted over the whole period were still patent at the time of this review. Only 14 of 35 grafts inserted prior to 31st December, 1956, survived for three years or more. If the early end-to-end grafts were excluded this figure was improved to 12 of 23. These figures do not include "Teflon" grafts which have been inserted more recently. Although the results justify continuing to use the operation, they do not warrant extending its use until it is possible to obtain a better prognosis of graft survival.

In 1960, a further 20 grafts, all "Teflon", have been inserted in 18 patients. We have now used a total of 36 "Teflon" grafts over a period of 1½ years (compared with a previous 46 homografts over a period of five years). Seventeen of these "Teflon" grafts have blocked (including one in a patient who died from chronic infection), one remained patent until the patient's death, leaving 18 (50%) still patent. In all instances of occlusion of the graft, this occurred within 12 months of operation. There have been three cases of severe haemorrhage at the site of grafting several months after operation. Marked narrowing of the graft through development of a thick false intima was found in the patient who died with the graft still patent, and narrowing of the lumen of the graft has been demonstrated radiographically in two patients. These results indicate that "Teflon" has no advantage in respect to graft survival and complications over homografts and that a search should be made for a better grafting material.

Diet and Atherosclerosis

(a) Diet

The ineffectiveness of methods of treatment of established occlusive arterial disease due to atherosclerosis stresses the need for a more basic approach by prevention or retardation of the development of the condition. It has been claimed that atherosclerosis is associated with an abnormality of the blood lipids, due in part to excessive intake of animal fat. In the previous report we outlined a study being conducted to determine whether a long-term effect on the serum cholesterol concentration of patients could be produced by a diet, low in animal fat and cholesterol, containing a supplement of unsaturated fats in the form of maize oil. There were originally 12 patients in the control group and 12 in the diet group. In the control group one patient absconded, in the diet group one patient died from cardiac infarction soon after starting the trial, and one left the country. The results in the remaining patients are as follows:—

Symptomatic Assessment

Symptom Group	Treatment	Number of Patients — Worse	Unchanged	Improved	Totals
Cardiac	Control	1	9	1	11
	Diet	0	10	0	10
Cerebral	Control	0	11	0	11
	Diet	0	10	0	10
Peripheral Vascular	Control	1	8	2	11
	Diet	1	3	6	10

Serum Cholesterol Concentrations

Treatment	Number of Patients Showing Rise	Fall	Total
Control	10	1	11
Diet	4	6	10
	14	7	21

Probability = 0.063.

When patients were considered individually only one man (in "diet" group) showed a statistically significant ($p < 0.05$) fall in his serum cholesterol. He was the only man in the group under 50 years of age; he adhered rigidly to his diet.

In conclusion, although the results tend to support the thesis that serum cholesterol level can be lowered by a diet low in animal fat and with a supplement of unsaturated fat, they do not reach statistical significance (at the 0.05 level). The success rate is so low and the treatment so arduous that it is doubtful whether it is warranted in patients with the type of disease and in the age group studied (men suffering predominantly from atherosclerosis of the lower limbs and over 50 years of age).

(b) MER-29 (Treparanol).

The difficulty of lowering serum cholesterol by dietetic measures indicates the need for a more practical and more effective method. The new drug MER-29 has been shown to interfere with synthesis of cholesterol in the body. This drug is being given to a group of 36 patients with clinical atherosclerosis (24 predominantly peripheral and 12 predominantly coronary) to determine the effect on the serum cholesterol level and the cause of the disease. Owing to difficulties associated with frequent estimations of serum cholesterol concentrations in a large group of patients it has been necessary to "stagger" the dates of commencement of treatment and only half the subjects have as yet commenced taking the drug. With a dose of 250mg./day there have been only moderate reductions in the serum cholesterol concentration, and the effect of 500mg./day is being investigated.

MER-29 is believed to inhibit cholesterol synthesis by interfering with the final step in the process—the transformation of desmosterol to cholesterol, and the former compound is present in increased amount. Unfortunately, the currently used method for determination of cholesterol gives a reaction with both cholesterol and desmosterol, and the values found for cholesterol are only apparent because of the contribution of desmosterol to the result. Attempts are being made by modified techniques to obtain values for the concentrations of cholesterol and desmosterol individually.

Clinical Trial of Persantin in Angina Pectoris

European research workers have claimed favourable results for a coronary dilator drug known as "persantin"* in the treatment of angina pectoris. A pilot study of oral and intravenous "persantin" was undertaken with patients proven to be suffering from angina pectoris.

Six patients had an electrocardiogram taken prior to, and after, a modified Master's step test. This was repeated ten minutes later after 10 mgms. of "persantin" had been given by intravenous injection. In this small series it was apparent that "persantin" did not prevent angina pectoris, nor did it cause favourable changes in the electrocardiograms.

Twelve patients underwent a blind trial with two placebo tablets three times a day for two weeks, and then two "persantin" tablets (25 mgm. dose) three times a day for four weeks. No beneficial effect of "persantin" in the prevention of angina was observed in these cases.

ENERGY PRODUCTION IN THE MYOCARDIUM†

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

In this study of the mechanisms by which the chemical energy supplied to the heart is converted into useful mechanical work many different approaches have been used. Whilst in some techniques it has been necessary to use toad hearts which contain no coronary circulation, in others the guinea pig, rat, fish, tortoise and snake have been the experimental animal. This report summarises current work proceeding on whole hearts, strips of cardiac muscle, muscle cells, cellular components and plasma.

Effect of Glycosides on Oxidative Metabolism

Data recorded in previous experiments indicated that the positive inotropic response following the addition of glycosides to isolated spontaneously beating hypodynamic hearts was associated with raised levels of oxidative metabolism, of efficiency and of useful work output. The augmented rate of oxygen uptake could be due to a basic metabolic action of the glycosides and not causally related to the positive inotropic response. Alternatively it could be directly linked with the increased mechanical activity recorded during the positive inotropic response.

To investigate these alternatives, the effect of the glycosides, strophanthin-G and lanatoside C, on the rate of oxygen uptake by actively beating hearts was determined and compared with that recorded following the similar

* The "persantin" was kindly made available by C. H. Boehringer, Sohn.

addition of these drugs to hearts arrested in either diastole or systole. The concentrations of glycosides used were sufficiently high to evoke well defined positive inotropic responses without any associated toxic effects and the chlorides of potassium and of calcium employed to induce diastolic and systolic arrest as required.

Both strophanthin-G and lanatoside C stimulated oxidative metabolism in the actively beating hearts but failed to do so in hearts arrested in diastole or in systole. It seems probable therefore that the augmented rate of oxygen uptake is not due simply to a metabolic action of these drugs but that it is related to the increased work output recorded during the inotropic response.

These findings may help to clarify the confusion which has arisen regarding the action of the glycosides on myocardial oxidative metabolism, since results obtained by workers using actively beating preparations have been compared and contrasted with results published by other workers using tissue homogenates and breis. The oxygen consumption of hearts arrested in diastole (1.59ml./min./100gm. wet weight) was significantly less than that recorded from those hearts arrested in systole (4.233ml./min./100gm. wet weight) indicating that energy is utilised in maintaining the steady state of contraction.

Cardiac Contractility

(a) "Staircase" in Ventricular Muscle

Although the classical phenomenon of step-like augmentation of cardiac contractile force following a rest period was described as early as 1871, the fundamental physiological and metabolic events associated with it have not yet been elucidated. Three different theories to explain the staircase are current—one invokes the progressive loss of potassium ions, another the surface accumulation of calcium ions and a third the accumulation of catechol amines during successive beats.

Tension-duration curves of stimulated isometrically contracting toad ventricular strips were recorded during successive beats of the staircase and compared with those noted during conditions of enhanced cardiac contractility evoked by the addition of calcium, of strophanthin-G, of various amines and by the reduction in the potassium concentration of the perfusate. These curves separated into two distinct classes, those recorded during the staircase and following the addition of calcium ions and of strophanthin-G belonging to one class and those recorded during amine activity and as the result of a decrease in the potassium ion concentration to the other. The first class was characterised by an increased rate of tension development and a reduced twitch duration throughout the period of enhanced contractility.

These results have been interpreted by assuming that the accumulation of calcium at specific sites on or about the myocardial membranes is the fundamental event involved in the conditions of enhanced contractility recorded both during the staircase and throughout the positive inotropic response recorded following the addition of the glycosides. In other experiments performed under isotonic conditions it has been shown that a critical potassium concentration is required for these accumulated calcium ions to exert their positive inotropic effect.

(b) Post stimulation potentiation.

A series of experiments were carried out to determine whether or not post stimulation potentiation as well as the classical "staircase" could be explained in terms of calcium ion accumulation or redistribution associated with rapid repetitive stimulation (post stimulation potentiation being the transient increase in cardiac contractility which follows a period of rapid stimulation).

Experiments were performed under isotonic conditions using a photo-electric method to record the amplitude of contractions of stimulated isolated toad ventricles. Whereas the presence of either catechol amines or of the specific amine antagonist, dichlor-isoproteronal, did not influence the percentage potentiation recorded the presence of the glycosides and a raised perfusate calcium concentration abolished it. Similarly, deletion of magnesium ions from the perfusate and a reduction in the sodium ion concentration depressed the potentiation. Potentiation was enhanced following the use of a calcium poor perfusate or when the number of stimuli delivered during the rapid stimulation phase was increased.

From these results it seems possible that the potentiation recorded following a period of rapid repetitive stimulation is due to the accumulation of calcium at specific sites in the myocardium, the rate of this accumulation being linked in some way with the stimulation frequency and with the perfusate calcium sodium ion ratio.

(c) Cardiac contracture.

Contractures were induced in stimulated isotonic toad ventricular preparations by the introduction of a hypertonic Ringer-sucrose solution, the onset of contracture being preceded first by contractions of reduced amplitude and then by a transient tetanic state. The amplitude of the contracture varied with the stimulation frequency used as well as with the ionic composition of the perfusate, high calcium concentrations and rapid stimulation rates favouring the development of contracture whilst low calcium concentrations and slow stimulation rates impaired it.

Following replacement of the hypertonic Ringer-sucrose with standard Ringer's solution cardiac contractility was transiently enhanced, the percentage increase in contractile force being influenced by the calcium concentration of the perfusate.

In these experiments the relationship between stimulation frequency, perfusate calcium concentration and the cardiac contractility which was noted during the post-stimulation experiments was again evident. Isotonic rather than isometric preparations were used since additional experiments have shown that the distribution of fluids throughout the intercellular cardiac spaces is aided by the movements associated with isotonic contraction and relaxation. Thus events which depend upon the surface accumulation of certain ions will be more pronounced under isotonic than under isometric conditions. Experiments confirmed this viewpoint.

The results of these investigations into some of the factors which regulate cardiac contractility indicate, therefore, that the surface accumulation of calcium plays at least some part in determining the force of contraction.

Potassium is necessary for calcium to exert its positive inotropic effect, and the rate at which calcium is stored at the specific sites is related to the stimulation frequency used. The glycosides appear to limit the rate at which calcium diffuses away from these specific sites.

Plasma Pressor Activity

Previous mention has been made to the observation that heparinised toad plasma has a pressor effect on the isolated perfused heart of the donor toad. This phenomenon has now been observed in several species, including guinea pig, rat, fish, tortoise and snake.

Experiments recorded last year indicated that this plasma factor might be under hormonal control. The experiments have therefore been repeated in a series of 12 previously adrenalectomised rats and similar results obtained. It seems unlikely therefore that the plasma factor originated in or is controlled by either adrenal cortex or medulla.

Further observations show that the factor is heat stable and dialysable and is still present when plasma is prepared in contact with silicone instead of glass.

Membrane Potentials of Cardiac Muscle Cells

During this year the form of cellular action potentials of cardiac muscle has been examined. It has been noted that the portion of the action potential most sensitive to alterations in cellular activity is the initial phase of reversal of polarisation. This phase has been displayed on a C.R.O. screen of long persistence at a trace speed of 2000 cm./sec. and photographed on fast film (300 ASA). Three distinct phases can be seen in the trace from a "normal" cell. In the first 3 m.sec. the potential falls from the resting value of about 70 mV (negative to the bath solution) at a rate of approximately 3 V /sec. Then follows a rapid transition to a rate of approximately 50 V/sec. for 1 m.sec., by which time the potential of the membrane is zero. This phase is then succeeded by a fall in rate to a steady potential of 30mV positive in 1.5 m.sec. In "injured" cells these phases are not seen and the potential changes at an essentially uniform rate and the time for reversal of potential is markedly increased.

The effect of various cardioactive drugs on this phase of the action potential is at present being investigated.

The electrical resistance to current flow between a a microelectrode within a cell and the solution in the bath is also being studied in the presence of these drugs. This resistance is of the order of 12 megohms at a current of 10^{-9} amp.

Actomyosin Studies

Some reports suggest that cardiac failure may be associated with a change in the physical or chemical properties of the contractile proteins actin and myosin. Studies are therefore being made on actomyosin extracted under carefully controlled conditions from mammalian hearts following certain operative procedures. Preliminary results indicate that under some conditions the extractability of actomyosin varies.

Phosphorylase Determination

Cardiac muscle contains the enzyme which catalyses the conversion of glycogen into glucose-1-phosphate. Experiments indicated that this enzyme was involved in the action of the amines on the myocardium but that it was not involved in the positive inotropic activity of either the glycosides or of calcium ions.

The endogenous cardiac catechol amines may partly regulate cardiac contractility via this enzyme system since phosphorylase activity was low in hearts depleted of their normal amine stores by reserpine.

Toxic Effects of "Latex" Tubing

It was found in the course of the various perfusion experiments that the use of "Latex" tubing in the apparatus accelerated the onset of a hypodynamic state in the perfused heart. With laboratory rubber tubing these preparations were fully active for about three hours, but with "Latex" tubing their activity had fallen to half within 90 minutes.

Experimental Congestive Cardiac Failure

The production of congestive cardiac failure in an experimental animal would be of great value in the study of myocardial failure. Attempts have therefore been made to produce this condition in rats by applying a constricting band to the ascending aorta.

The ascending aorta was partially occluded by a silver clip to reduce its cross-sectional area to one-third of normal. Seven rats survived for three months without developing any clinical signs of cardiac failure and were destroyed. Autopsy showed no cardiac abnormality. Eleven rats died suddenly, about the seventh post-operative day, without having developed any evidence of congestive cardiac failure. Autopsy revealed in each case rupture of the aorta at the site of the clip. It would seem that the technique used does not permit accurate enough adjustment of the clip on the aorta and has been abandoned.

CARDIAC SURGERY†

G. R. Stirling[1] and K. N. Morris[1]

Total Body Perfusion

Our total human experience with this technique for open heart surgery now exceeds 1000 cases. The main advances in the last year have included the frequent use of mild hypothermia, which is induced and controlled by the use of a blood heat exchanger unit which has been developed and constructed in our laboratories.

The techniques of direct perfusion of the coronary arteries during cardiac by-pass in which the aortic valve is exposed, have been studied. A satisfactory cannulation technique has been developed which enables prolonged operations on the aortic valve to be undertaken without allowing myocardial anoxia to occur. Two cases of aortic stenosis with heavy calcification of the cusps have been successfully submitted to operation in which, in addition to division of

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fused commissures, the calcified deposits have been excised to mobilise the cusps. It is our plan to use this technique for all future cases of aortic stenosis.

Mitral incompetence has also been subject to successful surgical attack. The field of surgical replacement of the mitral valve is one of our current laboratory studies.

Studies on Ventricular Function

The studies commenced in 1957 on the effects of various methods of producing asystole on ventricular performance have now been completed.

(a) Potassium Citrate Asystole.

The Melrose technique invariably results in some impairment of function which persisted for at least 1 to 2 hours after the injection of the potassium citrate. Although the degree of impairment of function was proportional to the time for which asystole was induced, significant impairment was obvious even after asystole periods of five minutes. Our clinical experience with the Melrose technique seemed to confirm these findings so this technique has been abandoned in clinical practice. Other workers have drawn attention to myocardial necrosis following the use of potassium citrate asystole. We have been able to confirm this finding in at least one of our clinical cases. Further histological studies on this problem are in progress.

(b) Simple Anoxic Arrest.

Anoxic arrest of the heart has proven to be less harmful than that produced by potassium. Five-minute periods of myocardial ischaemia are tolerated without a detectable effect on ventricular function. As the duration of ischaemia is prolonged the impairment of myocardial performance becomes more severe. After 20 minutes of myocardial ischaemia there is always a significant deterioration in ventricular function, although not to the degree seen in those dogs in which potassium citrate was also used.

Repeated short periods of myocardial ischaemia interrupted by periods in which coronary circulation was allowed for one minute were well tolerated. In fact, a total period of 20 minutes of ischaemia consisting of four five-minute periods was as well tolerated as a single five-minute period of ischaemia. These experiments suggest that the damage resulting from simple myocardial ischaemia was more closely related to the longest period of uninterrupted ischaemia than to the total time for which the myocardium was ischaemic.

This technique of using short periods of aortic occlusion to produce myocardial ischaemia, and thus cardiac asystole, has been adopted clinically and at normal temperatures. The time for which the myocardial blood supply is prevented does not exceed five minutes. At 10° C. reduction in body temperature seems to provide about 50% safety factor so that the impairment of ventricular function after 10 minutes of circulatory occlusion at 28° C. seems to be negligible. This practice has been also adopted clinically.

(c) Chloroform.

In 1958 and 1959 an analysis was made of the hypotensive effect of halothane. It was concluded that halothane has two effects; it impairs ventricular function and it reduces the total systemic vascular resistance. Both effects

are reversible with removal of the drug from the anaesthetic circuit. A similar study was made during this year using the anaesthetic agent chloroform. The qualitative effects of the two drugs were found to be the same, but the speed of production and recovery from the effects of chloroform were found to be less than when halothane was used.

Coronary Arterioplasty

Experience with autopsy material and with one clinical case, in which endarterectomy of the coronary vessels was carried out, suggested that a limitation inherent in the technique was the encroachment on the lumen of the vessel brought about by suture of the arteriotomy. To overcome this difficulty a technique has been developed whereby a gusset or patch of autogenous material is sutured into the incision, thus allowing an arteriotomy to be closed without loss of diameter. The materials used have been arterial wall and parietal pericardium. The technique of the insertion of the patch into the arteriotomy involves the use of a temporary shunt from a convenient artery to the distal end of the coronary artery which is to be grafted. The surviving animals from these experiments will later be studied by coronary arteriography and post-mortem injection and dissection. Long-term experiments are planned to study the fate of the autogenous materials.

Autogenous Pericardium as a Material for Vascular Replacement

The physical properties and the availability of autogenous pericardium during intra-thoracic procedures have suggested its use as a material well-suited for tissue replacement during intra-cardiac surgery. A series of dogs is being studied in which tear-drop shaped patches of pericardium have been incorporated in the repair of a longitudinal ventriculotomy in the right ventricle. The clinical counterpart would be in the plastic reconstruction of the out-flow tract of the right ventricle in Fallot's Tetralogy. This technique has been used clinically by Gross and Sauvage. These dogs will be studied by selective angiography and will later be sacrificed for histological study of the autografts.

Air Embolism

The opportunities for air embolism during open intra-cardiac surgery are obvious. While the cerebral effects of left heart air embolism are well known, little attention has been paid to the cardiac effects which may follow the introduction of the air into the left heart. Preliminary studies show the heart to be exquisitely sensitive to even small amounts of air thus introduced. Air embolism of the major coronary arteries results in acute ischaemia with a rapid fall in cardiac work-output; the distribution of the ischaemic myocardium seems to be largely determined by position. Acute arrhythmias such as ventricular fibrillation have also been observed. When air is deliberately injected into the right heart in small amounts, the major effects are related to pulmonary arterial obstruction. In dogs with a stabilised rate of cardiac inflow the injection of 5 ml. of air into the pulmonary artery may result in a prompt rise in pulmonary arterial pressure from 40 to 140 mm. Hg., indicating very gross obstruction of the pulmonary arterial tree. When air is injected into the right atrium or right ventricle a second factor becomes apparent. This is sequestration of air in the right ventricle with progressive distension of the right ventricle—if the flow load be maintained.

CARDIAC VENTRICULAR FIBRILLATION

D. Race and P. J. Armstrong

Ventricular fibrillation can be produced by weak electrical currents applied to the heart and it can be stopped by the application of large electrical currents. This effect of weak currents on the dog's heart can be measured in terms of the threshold current necessary to produce fibrillation. This threshold is remarkably constant for the same dog both before and after the exhibition of various volatile anaesthetic agents and in the same dog from day to day.

Across the ventricles of a dog with a standard surgical thoracotomy, anaesthetised with pentothal, nitrous-oxide and oxygen, gradually increasing electrical currents are applied until ventricular fibrillation appears. The threshold voltage, current and stimulus duration are recorded and the heart then defibrillated with a very high current.

The dog acts as his own control, since the various volatile anaesthetic agents are rapidly excreted and the thresholds can be quickly determined before and after the use of such agents. In all cases the threshold was shown to be the same before the exhibition and after the excretion of the anaesthetic agent being tested.

The Effect of Halothane

This anaesthetic agent has been used during operations on the aortic valve. At such operations, requiring circulatory occlusion for a few minutes, the risk of ventricular fibrillation is considerable, but since the use of halothane the incidence of ventricular fibrillation has been much less.

Halothane in low concentration (0.5%) raises the threshold current for ventricular fibrillation considerably. Higher concentration does not raise this proportionately. In very high concentrations 3-4% the cardiac action is weak, the tone of the heart poor, the volume of the ventricles increases and there is considerable difficulty in effecting defibrillation.

The Effect of Chloroform

This agent has similar cardiac actions to halothane but they are not as well marked. High concentrations (2%) produce cardiac arrest, but at no time did spontaneous ventricular fibrillation appear.

The Effect of Hypothermia

Seventy dogs were cooled by surface means to temperatures of 15-18° C. At no time did spontaneous ventricular fibrillation appear.

The ventricular fibrillation threshold current was raised as the temperature decreased, but defibrillation could at all temperatures be brought about by electrical means.

As these results conflict with those of other investigators the effect of low blood CO_2 and normal blood CO_2 was studied but no differences could be detected.

Conclusions

Halothane seems to have a general depressant effect on cardiac irritability and this can be used to reduce the incidence of ventricular fibrillation in cardiac operations.

Ventricular defibrillation can always be effected as long as the heart is not irreversibly poisoned by anoxia, or drugs, if a sufficiently high current is used and if the wave form of the stimulus is such that following the defibrillation pulse a low voltage pulse is not then introduced. The effect of the latter is to cause fibrillation again.

BLOOD PROTEINS*

C. C. Curtain

Studies of the proteins occurring in blood involve many problems of technique and instrumentation, the solution of which greatly speeds and simplifies the investigations. This report is therefore divided into three sections covering instrumentation, techniques and investigations.

(a) Instrumentation

Gradient Generator for Chromatography

To overcome the difficulties of producing accurately controlled reproducible pH and salt gradients for protein chromatography a photo-electrically programmed electrolytic gradient generator was designed and built. This device uses differential electrolytic gas generation to pump buffers from two bottles, each representing a gradient limit, to the top of the column. The electrolysis current is produced by a pair of complementary transistor amplifiers which are controlled by a pair of photo-cells scanning a photographically prepared mask of the gradient function. The mask is reproduced on a strip of 35 mm. film, the "sound track" edge of which carries cue marks which activate a photo-transistor controlled selector switch. This allows the current from the amplifiers to be automatically applied to any pre-arranged sequence of electrolysis and buffer vessels. The instrument provides an extremely wide choice of conditions.

The gradient generator is installed in a cold room as part of a protein chromatography apparatus which includes a double-beam ultra-violet column effluent scanner and a fraction collector operated by an integrating photo-electric drop counter, which automatically compensates for the variations in drop size that occur during gradient elution owing to changes in the viscosity of the solvent. This is achieved by measuring the size of each drop photo-electrically and firing a trigger which operates the collector at any pre-determined sum of drop sizes.

Photo-electric Scanning Microscope

The motor-driven stage of the electrophoresis Rayleigh fringe counter and comparator has been modified to provide a traverse (in steps adjustable from 5-15 mm.) at right angles to the continuous drive. An area of 5mm. by 500mm. can be scanned in a series of overlapping tracks. It is intended to use this instrument to carry out differential counts on blood smears stained for foetal haemoglobin. The light source is "chopped" by a 200 cycle disc, enabling a high gain drift-free A.C. amplifier to be employed ahead of the scanning cell (931A photo-multiplier). Alternating complementary light filters may be inserted in the chopper disc. Each alternate hole of the disc has a smaller hole directly below it on the radius. In line with this hole is a photo-

transistor which electronically switches the output of the amplifier to alternate "decatron" scalers. In a modification of the foetal haemoglobin method, due to Mrs. Tepper, of the Victorian Red Cross Blood Bank, foetal cells stain red whilst adult cells stain blue. Differential counts are performed on such slides by placing alternating red and blue filters in the disc. The blue scaler is set to stop the scan when it has counted 1000, and the number of foetal cells is read from the red scaler. It is expected that this instrument may find application to other histochemical problems.

Automatic Computation Programme

The burden of calculating and analysing an increasing amount of serum protein data led us to consider, with the assistance of Mr. N. Holmes, of IBM (Australia), the advantages of automatic computation. In our studies two types of serum electrophoresis pattern must be analysed: napthaline-black-stained paper electrophoresis strips, and interferometric recordings from the Perkin-Elmer and Tiselius apparatus.

The varying density of dye due to the distribution of the proteins along the paper strip is recorded on a chart by a densitometer. This chart is transferred to a plotting table, where each component peak is integrated by a curve follower which controls the frequency of a pulse generator. The pulses are counted by a "decatron" scaler. The integrator contains dividing and multiplying circuits which enable the operator to correct each component for its characteristic dye binding capacity and, in the case of β and δ_2 regions of the patterns of sera from haemolysed blood samples, for haemoglobin. The integrals are at present read from the scaler and converted into percentage concentrations with the aid of an electric desk calculator, although the scaler is designed to drive a calculating punch if the amount of work justifies its use. These figures are transferred to an IBM punching detail, where they are combined with geographical, anthropological, clinical and serum protein genetic data. From this detail punched cards are produced by the IBM service bureau. The punched cards can be machine-sorted into desired groupings. Statistical processing of the data in these groups is carried as far as obtaining sums of values, and of their squares on the IBM electric punch card accounting machine. Tests for statistical significance are then carried out with the aid of the desk calculator.

The interference fringe patterns recorded on the chart of the Rayleigh fringe comparator are also analysed on the plotting table. Fringe spacings are converted into reciprocal distances every 0.22 mobility units. Since the reciprocal of the fringe spacing is equal to the height of the electrophoresis curve at any given point the ordinates obtained by this procedure give a complete description of the pattern. The output of the reciprocal computer is read on a "decatron" display and the information transferred to punched cards and processed in the same manner as the paper electrophoresis data.

This system, combining analog and digital techniques appeared to provide the most economical method of computation for the study in hand—the analysis by paper electrophoresis of some 2000 Melanesian sera, with more detailed analysis of 300 randomly selected samples by moving boundary electrophoresis.

If it were desired to survey a larger number of sera the process could be made fully automatic, and for more elaborate statistical analyses the figures

produced by the punch-card accounting machine can be processed by a digital computer.

(b) Techniques.

Electrophoresis in the Presence of Free Haemoglobin

The presence of haemoglobin in a large number of the samples of Melanesian sera received has made quantitative electrophoretic studies of the serum proteins difficult, since it is considered that haemolysis may be due to increased red cell fragility in certain individuals, these samples cannot be discarded without making the collection of sera unrepresentative of the population. A method has been devised in which the absorption due to haemoglobin and haptoglobin-bound haemoglobin, and therefore the serum haptoglobin content in the Tiselius cell, is measured densitometrically at 545 mμ.

Analysis of Electrophoretic Patterns

Two methods are used for the resolution of complex electrophoresis patterns such as serum. In one, due to Kabat and Tiselius, ordinates are dropped from the minima between the components in the pattern. In the other, due to Svedberg and Pedersen, Gaussian curves are drawn under each component so that the sum of the ordinates at any one place is equal to the observed value of the electrophoresis curve. The two methods give slightly different results, the component values being dependent on the degree of separation in the first. Analysis of a large number of electrophoresis patterns with the aid of the photo-electric comparator showed that this dependence upon the degree of separation practically disappeared after electrophoresis under such conditions that the pattern extended for nearly the full height (41 mm.) of the Perkin-Elmer 2 cc. cell.

Protein Chromatography on Ion Exchange Paper

The recent availability of experimental batches of diethylamino-ethyl-cellulose paper prompted an investigation into its suitability for the chromatography of proteins on an analytical and micropreparative scale. As in the case of ion exchange columns, gradient elution is necessary for satisfactory resolution and gives rise to two difficulties. The first is that the complete gradient system necessary to separate components of a mixture must be developed within the length of the paper strip; the second is that the gradient generating device must be capable of delivering flow rates as low as 1 ml. per hour. It was with these requirements in mind that the prototype of the electrolytic gradient generator was built.

Using the electrolytic generator, successful chromatograms of gamma-globulins and caeruloplasmins have been prepared and it is expected to apply the technique to the study of individual variation in these proteins.

(c) Investigations.

Chromatographic Purification of Fluorescein-antibody

The most serious difficulty encountered in the use of fluorescein-conjugated antibody is the presence of non-specifically staining fluorescent material in the crude conjugater. Part of this material, consisting of low molecular weight fluorescein derivatives can be removed by exhaustive dialysis but the remainder

can only be removed by absorption with acetone-dried rat liver powder. In our previous investigations we found that this non-dialysable material was more heavily conjugated than the specifically staining antibody and was more negatively charged. It was therefore possible to purify specifically staining antibody by electrophoresis convection.

As electrophoresis convection is a cumbersome method, chromatographic purification has been investigated. Low molecular weight fluorescein derivatives can be removed completely by passing the crude conjugate through a column of "Sephadex" G 25 cross linked dextran gel. The filtrate which contains fluorescent antibody is then chromatographed on a column of diethylamino-ethyl-cellulose. Conditions are selected such that the non-specifically staining material is retained on the column whilst the purified antibody passes through.

Structure of Haptoglobins and Transferrins

The haptoglobins and the transferrins are examples of genetically controlled polymorphism of proteins with a specific biological function. The products of the two haptoglobin genes Hp_1 and Hp_2 differ from each other in electrical charge and molecular size. The two transferrins TfC and TfD appear to differ in charge only. It seems desirable to determine the chemical basis of these differences in the interest of elucidating the relationship between gene action and chemical structure.

As a preliminary approach we decided to investigate the effect of treatment with highly purified crystalline neuraminidase* on the starch gel electrophoresis pattern of human serum. It was found that neuraminidase markedly reduced the mobility of the Hp_1 and Hp_2 haptoglobin bands in homozygous and heterozygous sera. The slow haptoglobins seemed less affected than the fast ones. Both C and D transferrins were reduced in mobility to the same extent. The reduction in mobility is caused by the splitting off of neuraminic acid, and the experiments suggest that in the case of the transferrins the difference in charge between the two types is not due to different neuraminic acid content.

Electrophoretic Analysis of Serum Proteins of Melanesians‡

(a) Regional variations in the serum globulin profile throughout Melanesia.

Moving boundary interference fringe patterns of the sera of some 300 Melanesian natives have been studied by the method outlined under "Instrumentation". It was found that the serum globulin profile varied significantly from region to region, confirming an impression gained from a preliminary survey in 1957-58. The most marked variation in the profile is the hypergammaglobulinaemia found in the coastal regions and in New Britain. That this appears to be due to environmental factors is illustrated by the marked rise in the gamma-globulin level of 47 Menyamya Kukukuku (Eastern Highlands) labourers after they had spent six months in New Britain.

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* Prepared and kindly supplied by Dr. G. L. Ada, of the Walter & Eliza Hall Institute, Melbourne.

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

(b) Abnormal globulins in Kuru.

Studies on the 1959-60 collection of kuru sera confirmed our earlier findings of variable abnormalities in the serum globulins of kuru patients. Our present hypothesis is that the changes are reactive in nature and not fundamentally related to the pathogenesis of the disease. This is supported by our finding that patients in the early stages of the disease have normal electrophoresis patterns and approximately 100 subjects related to kuru victims had normal electrophoresis patterns.

Serum Protein Genetics

(a) Regional variations in haptoglobin gene frequencies in Melanesia and its relation to malaria.

Haptoglobin typing carried out by starch gel electrophoresis on over 1000 Melanesian sera has shown a significantly higher Hp_1 frequency (0.80-0.87) in the coastal regions of New Guinea and in New Britain compared with the Highlands (Hp_1 = 0.67-0.71). Studies of the haemoglobin-binding capacity of Melanesian sera revealed that type 1-1 and type 1-2 Highland individuals could complex an average of 110 mg. of haemoglobin per 100 ml. with a standard deviation of 18. Type 2-2 individuals could only complex 87 mg. per 100 ml. with a standard deviation of 20. A similar difference was found in coastal individuals where the figures were found to be 40 (S.D. 16) and 27 (S.D. 9). This has considerable significance in relation to malaria. Individuals suffering from malaria are subject to intravascular haemolysis and since it has been demonstrated that, unlike free haemoglobin, haptoglobin-bound haemoglobin is not lost through the kidney, more efficient haptoglobin binding would be an advantageous trait from the point of view of iron conservation. This could be of great importance in a typical environment where there are other factors favouring anaemia, such as the intestinal iron loss from hookworm. In this situation one could expect selection in favour of Hp_1, whose product binds haemoglobin more efficiently.

If this hypothesis can be sustained it may explain the higher Hp_1 frequency found amongst many tropical populations, particularly in Africa.

(b) Transferrins in normal Melanesians.

A survey carried out on the recent sera collection has confirmed our earlier finding of a high frequency of the D transferrin (10%) in Melanesia. This also agrees with the figures of Kirk (personal communication) and the recently published data of Barnicott and Kariks. None of the series studied have been sufficiently large to draw any conclusions as to regional variation in transferrins.

(c) Haptoglobins in Kuru.

Independent observations by Kirk and our own survey of the 1959-60 collection of kuru sera have failed to confirm our earlier finding of an excess of Hp_2 in kuru patients. A recheck of the 1957-58 collection, which was kindly carried out by Dr. Kirk, of the Department of Zoology, University of Western Australia, showed that this was due to our earlier starch gel electrophoresis technique having insufficient resolving power to differentiate between type 1-2 and type 2-2 in partly denatured sera, which formed a significant part of the 1957-58 collection. We have confirmed Kirk's finding with the aid

of Poulik's tris-borate discontinuous buffer system which we have found to give satisfactory results, even with sera which have been incubated at 37° C. for 72 hours.

(d) Transferrins in Kuru.

No differences in the frequencies of the C and D transferrins could be found between the kuru and the control sample.

CELLULAR ENZYMES**

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

These studies, commenced last year, concern enzyme systems in leucocytes and erythrocytes and, in particular, facets related to lipids, zinc and alkaline phosphatase in leucocytes in normal, inflammatory and leukaemic situations, and of glucose-6-phosphate dehydrogenase in erythrocytes.

Leucocytes

(a) Lipid synthesis in human leucocytes.

Studies carried out in collaboration with Drs. Marks and Gellhorn at Columbia University, New York, in 1959, on lipid synthesis in normal erythrocytes, leucocytes and platelets, and in the cells of chronic myeloid and chronic lymphatic leukaemia, have been extended this year to blood from patients with acute leukaemia. In acute myeloid leukaemia marked acceleration of total lipid synthesis by the leucocyte, when compared to the normal granulocyte or to the cell of chronic myeloid leukaemia, has been demonstrated. In acute lymphatic leukaemia the pattern observed is similar to that of chronic lymphatic leukaemia, synthesis being diminished below that of the normal lymphocyte. The leucocytes of all types of leukaemia exhibit a higher percentage synthesis of phospholipid than do normal cells.

Other conditions involving leucocyte aberrations studied are:

1. Polycythaemia vera, in which leucocytes may exhibit markedly depressed lipid synthesis rates despite the presence of a large percentage of young cells.

2. Myelosclerosis and myeloid metaplasia, where lipid synthesis is increased.

3. Infective leucocytosis, where the rate of lipid synthesis appears to be in the lower normal range.

These results suggest that lipid turnover and de novo synthesis provide a valuable parameter for further understanding of metabolic derangements of the leukaemic cell. The interpretation of these results has been considered in terms of cell age, of rate-limiting steps, and of more specific enzyme abnormalities. An examination is being made of the mechanisms governing quantitative and qualitative aspects of lipid synthesis in the normal and abnormal leucocyte, and in vitro phagocytosis is being employed as a system for studying changes under conditions of increased energy requirement by the cell.

Some evidence has been obtained suggesting that a considerable proportion of newly synthesised lipid in the leucocyte is transferred to or near to the cell surface. The finding of increased phospholipid production by leukaemic cells has initiated an investigation of the surface phospholipids and lipoproteins, utilising immunochemical and cell-fractionation techniques.

These lipid studies employ acetate-C^{14} as substrate, and a Packard tricarb. liquid scintillation spectrometer for counting purposes, and have been made possible by the co-operation of the Department of Physiology, University of Melbourne.

(b) Lipoidoses.

In conjunction with Dr. L. Taft, of the Royal Children's Hospital, a metabolic study is in progress on a small group of patients with lipoidoses. Thus far two patients with Nieman-Pick's disease and one patient with Gaucher's disease have been employed. The study aims to evaluate quantitative and qualitative abnormalities of lipid metabolism in the reticuloendothelial cell, with a view to gaining some evidence concerning the enzymatic defects involved.

(c) Leucocyte enzyme genetics.

A detailed study has commenced of qualitative aspects of certain enzymes known to be quantitatively altered in leukaemic cells. The stimulus for this work derives from recent evidence that quantitative gradations in enzymic activity possibly represent multiple mutational effects. While most previous studies in leukaemia have used mixed normal leucocyte populations for comparison with leukaemic cells, all these studies employ separated granulocytes and lymphocytes as normal prototypes.

The first single enzyme chosen for this study is leucocyte catalase; the activity of normal lymphocyte catalase is considerably less than that of the normal granulocyte enzyme. There is greatly augmented activity, as high as 400%, in myeloid leukaemia leucocytes compared to normal granulocytes; in acute and chronic lymphatic leukaemic lymphocytes, the activity is of the same order or slightly less than that of the normal lymphocyte.

The rate-behaviour and properties of the enzyme in crude and in purified form are being studied, an attempt is being made to elucidate the normal function of catalase in the leucocyte. Preliminary studies have been done on normal and leukaemic leucocyte phosphorylase.

(d) Neutrophil Alkaline Phosphatase.

Further studies on neutrophil alkaline phosphatase have shown this parameter to be a suitable tool for analysis of acute inflammatory lesions in complex situations.

In cardiac surgery the skin incision is commonly far removed from the site of the defect. Secondary infection at or near the cardiac site, should it then occur, has no direct line of egress for inflammatory exudate. By using serial neutrophil alkaline phosphatase estimations it has proved possible to estimate the frequency of this event. While the study is not complete it appears that in some cases the persistence of acute inflammation over many weeks is related to foreign bodies rather than bacteria, introduced at the time of the operation.

The choice of antibiotics in cases where the causal organism cannot be recovered, continues to be a problem in clinical medicine. A preliminary survey of such cases has shown that when the appropriate antibiotic is used the neutrophil alkaline phosphatase titre returns to normal, an event which

does not occur when the wrong antibiotic is used. This approach has been found especially useful in cases of sub-acute bacterial endocarditis when no organism has been recovered.

Serial studies in the malignant blood dyscrasias have continued. It is apparent that, with the exception of myeloid leukaemia, in all these conditions neutrophils respond to an acute inflammatory response in the normal way. In acute and chronic myeloid leukaemia some neutrophils show the normal response acute inflammation. With progress of the disease the percentage of neutrophils behaving in this way decreases until a stage is reached when the neutrophils do not respond to acute inflammation by generating increased amounts of alkaline phosphatase. A comparison of the functional characteristics of these two populations of cells is being undertaken.

(e) Zinc Metabolism.

Leucocytes of the granular series normally contain a large amount of zinc, 80% of which is attached to a soluble protein. In myeloid leukaemia, however, the zinc concentration of leucocytes is markedly reduced to 10% of the normal value.

A specific histochemical stain for zinc which could be applied to blood cells was described by McNary in 1957.

Using this stain it has been found that eosinophils are the only granulocytes in which zinc can be demonstrated. The zinc-protein complex is probably extracted from neutrophilic granulocytes by the watery zinc-specific stain, as counter-staining with Leishman shows a loss of neutrophilic granules and vacuolisation of the cytoplasm.

With certain modifications in staining technique, it has been possible to demonstrate metal which is almost certainly zinc, in dog and human neutrophil leucocytes. Cells of the lymphocytic series do not take up zinc chloride added to the surrounding medium, whereas cells of the granular series, including myeloblasts (in one case of acute myeloblastic leukaemia), do take up zinc chloride. This may prove a useful test with which to distinguish acute myeloid from acute lymphatic leukaemia.

(f) Physico-chemical differences.

While using a dithizone stain said to be specific for zinc, it was noted that neutrophil and eosinophil granulocytes, but not lymphocytes, showed a brownish, discrete granulation easily seen with a high dry objective in the microscope. When viewed under oil, or after mounting, the granulation is no longer visible, and for this reason is regarded as a surface effect due to scattering of light at irregularities in the cell surface. Accentuation of this effect is obtained by using phase contrast and a dry objective when the neutrophils are seen to be dotted with black granules which again disappear when viewed through oil.

The dithizone stain contains diphenylthiocarbazone (dithizone) dissolved in absolute acetone, water, 1N acetic acid, sodium potassium tartrate and a complex-forming buffer containing sodium thiosulphate, sodium acetate, potassium cyanide and glacial acetic acid, at a pH of 5.5.

Freshly prepared air-dried blood smears were tested against the separate components of this stain, and it was shown that dithizone was not necessary for production of the surface granulation, which cannot therefore be regarded as a staining effect. Acetone, or acetone and water alone, do not produce "surface" granulation either. Sodium thiosulphate in the presence of acetone and water is effective in producing neutrophilic surface granulation, but the change is dependent on pH as well.

If blood smears are fixed first in formalin vapour or osmium tetroxide vapour, neutrophils are no longer capable of showing surface granulation.

The different results in lymphocytes and neutrophils must reflect biochemical differences in cell proteins. These differences are being investigated in lymphocytic and granulocytic cells at various stages of maturation.

(g) Lipid transport in blood.

Using in vitro systems, the percentage transfer of newly formed lipids from leucocytes and platelets to plasma was shown to depend on the plasma/cell ratio, probably due to the availability of protein sites for lipid binding. Albumin concentration does not appear to be a very significant factor. Electrophoresis combined with auto-radiography and paper strip scanning is being used to identify globulin fractions binding C^{14}-labelled lipids.

The plasma/cell ratio has some governing effect on the rate of cellular lipid synthesis. There appears to be a preferential re-utilisation by the cell of phospholipids compared to glycerides from the plasma. Dialysis of plasma prior to incubation results in depressed synthesis and transport of lipids in the system: studies are in progress to determine the nature of the dialysable factor or factors concerned.

Lipid kinetics are being studied in relation to hormonal effects, drug effects, temperature, etc., using acetate-C^{14}, glucose-C^{14} and pyruvate-C^{14} as substrates.

Erythrocytes

(a) Thyroid Disease.

Erythrocytes from patients with thyrotoxicosis have been reported by others to have markedly increased activity of glucose-6-phosphate dehydrogenase. Experiments here with erythrocytes from both thyrotoxic and myxodematous patients have thrown some doubt on these findings: in myxoedema the enzyme activity is within the normal range, while in thyrotoxicosis it is normal or only slightly elevated. The opportunity to study some 50 patients from New Guinea with endemic goitre or cretinism showed that in the extreme state of thyroid hormone deprivation the enzyme still retains normal activity. Control experiments are being done employing in vitro incubation with thyroxine and triiodothyronine prior to estimation of the enzyme.

(b) Erythrocyte glucose-6-phosphate dehydrogenase deficiency in New Guinea and New Britain.

Screening of 689 persons from New Guinea and New Britain revealed for the first time the existence of erythrocyte glucose-6-phosphate dehydrogenase deficiency in that area. This trait was found to be present in 16 out of 155 persons (10%) in the Gazelle Peninsula, New Britain, but in only 3 out of 534

persons ($<$1%) in the Eastern Highlands, New Guinea. This marked difference in incidence is thought to be related to the incidence of malaria, which is high in the Gazelle Peninsula and low or absent in the Eastern Highlands. The findings add weight to Motulsky's conclusions from studies in the Congo, that the enzyme deficiency protects against the malarial parasite, since the depleted dehydrogenase diminishes grossly the amount of reduced TPN available in the erythrocyte, TPNH being an essential requirement for the parasite's existence.

This is the first population in which the trait has been reported which does not also possess the gene for sickle-cell haemoglobin.

Further studies are proceeding on the properties of the enzyme in deficient persons, for comparison with those of affected negroes and caucasians, with the aim of elucidating further the nature of the gene enzyme interaction concerned.

RENAL HISTOLOGY

P. Kincaid-Smith

Renal Biopsy

The application of this technique to the study of renal disease has been continued and extended during 1960.

Experience in 150 biopsies done by the modified Vim-Silverman technique has shown that this method is safe in experienced hands when certain precautions are taken. No case has required a transfusion or had any serious complication. Macroscopic haematuria occurred in only seven patients after biopsy, and two patients passed blood clots accompanied by some ureteric colic. No other complications have occurred. Only five patients experienced pain at the time of the biopsy, and four complained of subsequent pain. Pain was severe enough to require analgesics in one patient, but there was no clinical evidence of bleeding or any other abnormality associated with it. Fewer complications were encountered in this series than in 50 lung biopsies done by the same method.

Compared with a series of biopsies done with a serrated cutting needle by a number of different persons, these complications are very few and make it likely that the Vim-Silverman technique is much safer.

Very valuable information about the nature of various renal diseases has been obtained in this series. Repeat biopsies have been done in several patients to assess the results of the exhibition of steroids and other treatment on the histological lesions. It is becoming increasingly apparent that renal biopsy is an essential investigation in the diagnosis and evaluation of treatment in renal disease. The results will be published shortly.

Renal Disease and Hypertension in the Bantu

Renal hypertension appears to be very common in the Bantu. At the request of the South African Institute of Medical Research, a study of a series of kidney sections (biopsy and autopsy) has been commenced in an attempt to assess the frequency and aetiology of renal hypertension in the Bantu. Three hundred slides have already arrived and this work has commenced.

SCLERODERMA

A. J. Barnett and F. H. Lumb

Following the clinical evaluation of 27 patients with scleroderma and Raynaud's phenomena, further studies are being performed on this group of patients. Three main lines of investigation are at present bing pursued:—

(i) Investigations regarding the nature and extent of visceral involvement.

All patients are being assessed with respect to cardiac pulmonary, renal and especially gastro-intestinal involvement. The latter appears to be a not infrequent occurrence in diffuse sclerosis, and patients are being examined regarding oesophageal, gastric and intestinal function.

(ii) Basic information concerning the nature of the disorder.

This is being studied first, by the examination of biopsy material, initially by light and subsequently by electron microscopy. Secondly, tests for disturbance of protein electrophoretic patterns have been carried out. Thirdly, an investigation of the various factors which might indicate an auto-immune mechanism for scleroderma has been undertaken; the results of the latter so far being negative.

(iii) The effect of new treatment on scleroderma.

Following the report of Zarafonetis (Ann. Int. Med. 1959, Vol. 50, p. 343) regarding the efficacy of potassium para-amino-benzoate in scleroderma, a controlled trial of this therapy has been undertaken. Results are being assessed by clinical observations, the estimation of the objective change in skin elasticity using an electrical strain gauge, and changes in limb blood flows, measured by strain gauge plethysmography performed at rest and following reactive hyperaemia under standard conditions.

HEAD INJURIES

W. M. McDonald

A progressive study of all patients with head injuries admitted to the Alfred Hospital was commenced in March, 1960. The nature of the accident, time of accident in relation to hour, day and month, type of injury and the duration of hospital stay and convalescence were noted. Presence of neurological signs at the time of admission and during the later stages, and injuries coincident to the head injury were also recorded. Blood alcohol estimations at the time of admission were not able to be performed, as no laboratory would agree to undertake these because of medico-legal difficulties. With the co-operation of the Coroner's Office, post-mortem examinations have been carried out on fatal cases.

Hypothermia

Three very severe, eventually fatal, head injury cases were managed by hypothermia at a temperature of 30-32° C. for periods of five to 21 days. Although beneficial control of basic physiological functions was easily obtained considerable trouble was experienced in their maintenance. Daily bronchoscopy was needed to deal with exudative blocking of main bronchi. The exhibition of cortisone was found to be helpful. Initially larger doses of

chlorpromazine, pethidine and phenergan were needed to establish and maintain the hypothermic state, although daily requirements gradually lessened. No trouble with urine excretion or intestinal absorption occurred. On re-warming one case the serum sodium concentration rose to 175m.eq/l., but this rapidly returned to normal with the re-establishment of the hypothermic state.

Hypothermia has been found to be an expensive, prolonged procedure from which little practical benefit can be obtained for these patients. However, maintenance of controlled normo-thermic states by using hypothermic methods has been carried out with advantage in many cases.

Hypertonic Infusions

An intravenous infusion of 30% urea solution has become a routine procedure for all cases undergoing cerebral surgery in which a raised intracranial pressure is evident, or where ease of access to areas of the brain surface is required. The solution has also been used with success on six cases of acutely raised C.S.F. pressure following head injury. Improvement of conscious state or of basic physiological functions was evident in all cases.

In one terminal case, the urea was combined with large molecular size dextran solution. This was rapidly followed by improvement in respiration rate, pulse, blood pressure, reaction to painful stimulus and return of the light reflex to the fixed dilated pupils.

An endeavour to gain a broad picture of the action of intravenous urea solution was attempted by serial tracking of changes in serum chemistry patterns. At present evidence of large expansion of the extra-cellular fluid space has been obtained, and it appears that a considerable shift of sodium ions must take place from the intracellular to the extracellular compartment.

SOCIAL ASPECTS OF CARDIOVASCULAR DISEASE‡

B. B. Thomas[1], G. I. Howard[2], and T. E. Lowe

The pilot study to ascertain the effects produced on the economic and social life of patients and their families by cardiovascular diseases has been continued on a series of patients. Experiences during the year seem to indicate that many patients need considerably more help than they have been getting to understand the amount and kind of activity in which they may engage and the type of employment they may follow. This last problem is related to physical impairment, patients' motivation, employers' attitudes to the employment of such persons and the state of the employment market. All of these aspects require a considerable amount of fact-finding study before any generalisations will be possible.

[1] Senior Almoner—Alfred Hospital.

[2] Medical Superintendent—Alfred Hospital.

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BARNETT, A. J., and F. O. SIMPSON—"Primary Aldosteronism—Conn's Syndrome". *Med. J. Aust.*

CURTAIN, C. C., D. C. GAJDUSEK, and V. ZIGAS—"Studies on Kuru: II Serum Proteins in Natives from the Kuru Region of New Guinea". *Amer. J. Trop. Med.*

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NAYLER, W. G.—"The Importance of Calcium in Post-stimulation Potentiation". *J. Gen. Physiol.*

SIMPSON, F. O., and S. J. OERTELIS—"Relationship of the Sarcoplasmic Reticulum to Sarcolemma in Sheep Cardiac Muscle". *Nature.*

STIRLING, G. R.—"The Preservation of Myocardial Function During Open Heart Surgery". *Amer. Heart J.*

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

WYLLIE, R. G.—"Control of Body Fluid Volume". *Amer. Heart J.*

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CURTAIN, C. C.—"The Quantitative Electrophoresis of Serum Proteins in the Presence of Haemoglobin". *Aust. J. Exp. Biol. & Med. Sci.*

CURTAIN, C. C.—"The Analysis of Electrophoresis Patterns—A Comparison of Two Methods with the Aid of Rayleigh Interference Optics". *Aust. J. exp. Biol. & Med. Sci.*

FANTL., P., and H. A. WARD—"Blood Coagulation Problems in Open Heart Surgery". *Thorax.*

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McCUTCHEON, A. D.—"Surface Granulation in Myeloid Leukocytes". *Nature.*

NAYLER, W. G.—"The Action of Reserpine, Tyramine and Cocaine on the Phosphorylase 'A' Activity of the Heart". *Aust. J. exp. Biol. & Med. Sci.*

NAYLER, W. G.—"Surface Phenomena in Cardiac Muscle under Isometric and Isotonic Conditions". *J. Gen. Physiol.*

NAYLER, W. G.—"The Influence of Hypertonic Perfusion Fluids on the Contractile Activity of Isolated Toad Ventricular Muscle". *J. Physiol.*

NAYLER, W. G.—"Modification of the Inotropic Activity of Calcium by Potassium". *Nature.*

STIRLING, G. R., K. N. MORRIS, and D. RACE—"The Effect of Induced Asystole on Ventricular Function". *Aust. & New Zealand J. Surg.*

LECTURES DELIVERED DURING 1960

"Treatment of Hypertension with Guanethidine"—*Alfred Hospital Clinical Society.*	A. J. BARNETT
"Arterial Grafting in Occlusive Arterial Disease of the Lower Limbs" *Second Asian-Pacific Congress of Cardiology.*	A. J. BARNETT
"Recent Developments in the Field of Medical and Biological Electronics"—*Institute of Radio Engineers.*	C. C. CURTAIN
"Anticoagulant Drugs and Thrombolytic Agents"—*Royal Australasian College of Surgeons, Melbourne.*	P. FANTL
"Coagulation Problems in Open Heart Surgery"—*Second Asian-Pacific Congress of Cardiology.*	P. FANTL
"Certain Aspects of Blood Coagulation and Fibrinolysis"—*Banting and Best Institute, Toronto, Canada.*	P. FANTL
"Current Views on Blood Coagulation"—*Hoffman-La Roche, New Jersey, U.S.A.*	P. FANTL
"Blood Coagulation"—*University of Melbourne.*	P. FANTL
"The Auscultatory Findings in Hypertension"—*Victorian Cardiac Group.*	P. KINCAID-SMITH
"Medical Diseases of the Kidney"—*College of Radiologists of Australia.*	P. KINCAID-SMITH
"Presidential Address"—*Second Asian-Pacific Congress of Cardiology.*	T. E. LOWE
"Oedema in Cardiac Failure"—*Second Asian-Pacific Congress of Cardiology.*	T. E. LOWE
"Cell Membrane Permeability as a Basis of Glycoside Activity"—*Second Asian-Pacific Congress of Cardiology.*	W. G. NAYLER
"The Effect of Induced Asystole on Ventricular Function"—*Second Asian-Pacific Congress of Cardiology.*	G. R. STIRLING
"The Effect of Local Intra-arterial Injections of Sodium Salts on Corticosteroid Secretion by the Autotransplanted Adrenal Gland of a Conscious Sodium Depleted Sheep"—*Endocrine Society of Australia.*	M. WEISS
"Leucocyte Alkaline Phosphatase in Leukaemia"—*Victorian Cancer Congress.*	R. G. WYLLIE
"Leucocyte Alkaline Phosphatase Studies"—*Royal Children's Hospital.*	R. G. WYLLIE

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

Revenue Account for the Year Ended 31st December, 1960.

EXPENDITURE.				INCOME.					
Drugs, Chemicals, Provisions, etc.	£1,543	19	0	Donations—					
Fuel and Lighting	367	3	10	Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions	£26,000	0	0		
Instruments and Glassware	2,385	1	0	Other Donations as per attached schedule	914	5	6		
Insurance	543	0	8					£26,914	5 6
Library Maintenance	1,410	13	7	Grants in aid of Research—					
Printing and Stationery	634	0	5	National Health and Medical Research Council	5,356	8	1		
Repairs and Renewals	1,066	8	4	Anti-Cancer Council of Victoria	8,132	15	0		
Salaries and Wages	33,720	9	8	Life Insurance Medical Research Fund of Australia and New Zealand	2,372	15	6		
Telephone	557	6	11					15,861	18 7
Travelling Expenses	630	9	11	Interest From Investments—					
Sundries	933	0	0	Held by the Trustees of the Estate of the late Thomas Baker	849	0	0		
Portion Cost Animal House	992	9	0	Endowment Fund	1,072	17	2		
Surplus for Year	135	18	11					1,921	17 2
				Interest From Commercial Bank of Australia Ltd.				168	0 0
				Sundry Sales				54	0 0
	£44,920	1	3					£44,920	1 3

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

Balance Sheet at 31st December, 1960.

LIABILITIES.				ASSETS.					
Current Liabilities—				Current Assets—					
Sundry Creditors	£6,789	12	7	Cash at Bank	£7,559	11	11		
Endowment Fund	24,039	6	10	Prepayments	270	11	9		
Capital Grants and Gifts	1,780	15	9	Sundry Debtors	303	0	0		
Accumulated Revenue	3,660	5	0					£8,133	3 8
				Endowment Investments—					
				Inscribed Stock—					
				Commonwealth Government	£11,380	0	0		
				Grain Elevators Board	2,500	0	0		
					13,880	0	0		
				Treasury Bonds—					
				Commonwealth Government	3,510	0	0		
				Shares in Companies	6,032	2	1		
				Mortgage Loan	500	0	0		
				Cash at Bank	117	4	9		
								24,039	6 10
				Restricted Funds (represented by Cash at Bank)—					
				Capital Grants and Gifts				1,780	15 9
				Fixed Assets—					
				Furniture and Fixtures				2,316	13 11
	£36,270	0	2					£36,270	0 2

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

In our opinion the annexed Balance Sheet is properly drawn up to show a true and fair view of the state of the Institute's affairs at 31st December, 1960, according to the best of our information and the explanations given to us and as shown by the books of the Institute.

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

Melbourne,
April, 1961.

NOTE: In addition to receiving interest from the Investments as shown on the Balance Sheet, the Institute receives the income from 5% Commonwealth Government Inscribed Stock face value of £17,000, which is inscribed in the name of the Trustees of the Estate of the late Thomas Baker for the benefit of the Institute.

THE THOMAS BAKER, ALICE BAKER AND ELEANOR SHAW MEDICAL RESEARCH INSTITUTE
YEAR ENDED 31st DECEMBER, 1960

CAPITAL GRANTS AND GIFTS.

Balance at 31st December, 1959		£1,015 7 5
Add		
Donations—		
Drs. T. E. Lowe, A. J. Barnett and R. J. Sawers		8 9 7
For Building Extensions—		
Alfred Hospital	£618 19 0	
Estate Thomas Baker	618 19 0	
		1,237 18 0
Anti-Cancer Council of Victoria		105 0 0
Estate of Thomas Baker		500 0 0
Estate of R. C. MacDonald		100 0 0
Life Insurance Medical Research Fund of Australia and New Zealand		500 0 0
National Health and Medical Research Council		450 0 0
Anonymous		260 0 0
		£4,176 15 0
Deduct		
Cost of Building Extensions—		
New Wing	£1,237 18 0	
Laboratory Equipment	1,158 1 3	
		2,395 19 3
Balance at 31st December, 1960		£1,780 15 9

ACCUMULATED REVENUE.

Surplus at 31st December, 1959	£3,524 6 1
Add	
Surplus for Year	135 18 11
Surplus at 31st December, 1960	£3,660 5 0

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

OTHER DONATIONS RECEIVED DURING YEAR TO 31st DECEMBER, 1960

Marian and E. H. Flack Trust	£350	0	0
Geo. F. Little Trust	148	6	6
Mr. and Mrs. Edgar Rouse	105	5	0
Mr. S. Meyer	100	0	0
Kodak (Australasia) Pty. Ltd.	50	0	0
Eagle Star Insurance Co. Ltd.	15	15	0
In Memory of May Mendelsohn	15	5	0
Mrs. Lawrence Simpson	10	0	0
Mr. J. C. Habersberger	7	7	0
Mr. Elvin Teasdale	5	5	0
Dr. and Mrs. John Rouse	5	0	0
In Memory of Sir William Angliss	5	0	0
„ „ „ Michael J. Canny	5	0	0
„ „ „ Percy Clarey	5	0	0
„ „ „ Deric Deane	5	0	0
„ „ „ Lady Gullett	5	0	0
„ „ „ Sam Horden	5	0	0
„ „ „ Ken Lord	5	0	0
„ „ „ Ida Florence Luke	5	0	0
„ „ „ Cecil Gordon McAdam	5	0	0
„ „ „ Dr. Kenneth Mees	5	0	0
„ „ „ Gretel Packer	5	0	0
„ „ „ Mrs. William S. Philip	5	0	0
„ „ „ Lieut.-General Sir Horace Robertson	5	0	0
„ „ „ Dr. David Rosenthal	5	0	0
„ „ „ Dorothy Julia Smith	5	0	0
„ „ „ Lady Stewart	5	0	0
„ „ „ Reta Thomas	5	0	0
„ „ „ "Tosh" White	5	0	0
„ „ „ Dr. Thomas Wood	5	0	0
Antony Rouse	3	0	0
Miss N. E. Cameron	2	2	0
Juliet Rouse	2	0	0
	£914	5	6

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

1900

STAFF

Honorary Physician:	EWEN DOWNIE, M.D., F.R.C.P., F.R.A.C.P.
Assistant Physician Scientific Studies:	JOSEPH BORNSTEIN, D.Sc., M.D., F.R.A.C.P.
Assistant Physician Clinical Studies:	BRYAN HUDSON, M.D., Ph.D., M.R.C.P., F.R.A.C.P.
Honorary Assistant Physician:	HARALD BREIDAHL, M.D., M.R.C.P., M.R.A.C.P.
Registrar:	J. H. EVANS, M.B., B.S.
Biochemists:	DORA WINIKOFF, M.Sc. JUNE SHEATH, M.Sc. DEIRDRE HYDE, B.Sc. FRANCES WALKER, B.Sc. AUSMA DULMANIS, B.Sc.
Technical Staff:	Mr. W. HUDSON. Miss I. EKKEL. Miss L. GIBSON. Miss B. ANDERSON. Miss M. ZWART. Miss S. SEMMEL. Miss P. HORE. Miss MARIANNE DALHOFF.
Secretary:	Miss J. SHARP.

DIABETIC CLINIC

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

RESEARCH FELLOWS

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

HONORARY ALFRED HOSPITAL RESEARCH FELLOWS

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

The past year has seen further consolidation of the Unit. For the first time since its formation in 1957 it has functioned for a year as a corporate body. The association of Laboratory and Ward with the close proximity of patients to an adequately equipped investigational service has proved to be advantageous both to patients and investigational research.

The clinical activities of the Unit for the year have already been described in the Annual Report of Alfred Hospital, 1960, and will not be repeated in detail here. Suffice it to say that the routine services in the investigation of Thyroid disorders and of abnormalities of steroid and carbohydrate metabolism and other endocrine problems has been continued.

The research activities of the various workers are detailed later in this report.

During the year Dr. Joseph Bornstein was invited to participate in an Insulin Conference organised by Eli Lilly Research Laboratories, U.S.A. While abroad he paid brief visits to a number of centres in U.S.A., Great Britain and Germany, and studied current trends of Research Work into various aspects of Diabetes Mellitus.

Dr. Bryan Hudson returned from overseas in December, 1959, after sabbatical leave spent in Professor Samuels' Unit in Salt Lake City, U.S.A. Since his return he has been engaged in developing techniques in advanced study of adrenal disorders. As a result, elaborate methods of investigation of patients suffering from adrenal disease are soon to be introduced as routine procedures by the Department of Biochemistry. In addition, he has been engaged in research into the complex problems of steroid bio-synthesis and metabolism.

Dr. Peter Davoren, who was awarded a Fulbright Scholarship, left during the year to spend 12 months working as a Research Fellow with Professor Earl Sutherland in Cleveland, U.S.A. He will proceed to England at the completion of his Fellowship for further experience, and will return to the Unit in 1962.

An important function of the Unit is that of providing opportunities for the training of young graduates in various aspects of Endocrinology. Dr. Ian Martin, a graduate of the Royal Melbourne Hospital Medical School, spent 18 months in the Unit and was then awarded a Fulbright Scholarship and a Fellowship which permitted him to work with Dr. Max Miller in Cleveland during 1959. He was then awarded a Leverhulme Fellowship by the Royal College of Physicians, London, and worked with Dr. John Nabarro at the Middlesex Hospital. He returned at the end of this year and has taken up duties at the Royal Melbourne Hospital with Dr. Pincus Taft.

During this year, at the request of the late Professor John Hayden, Dr. Henry Burger, a graduate of St. Vincent's Hospital Clinical School, joined the Unit as a Research Fellow and has been engaged in studies of carbohydrate and protein metabolism. He has recently been awarded a Nuffield Fellowship, and late in 1961 will go to London to work with Dr. John Nabarro. He will later visit the United States and return to Australia in 1963, when it is anticipated he will resume his activities at St. Vincent's.

Dr. James Evans, a graduate of Alfred Hospital Clinical School, has returned to the Unit after post-graduate training at the Royal Women's Hospital. He is acting as Registrar at present. His interests are primarily in the field of

gynaecological endocrinology, and he will leave for Edinburgh in 1961 for further studies. It is anticipated that on his return he will rejoin the Royal Women's Hospital to continue Endocrine work there.

From this it is apparent that in the short space of four years the influence of the Unit has extended beyond the confines of Alfred Hospital. It has provided opportunities for training in Endocrinology to graduates from other Clinical Schools who, on their return to their parent hospitals, will be capable of stimulating interest in the study of endocrine disorders.

During the year, Dr. Henry Burger obtained his M.D. Degree in the University of Melbourne and was awarded the David Grant Prize. He also obtained his M.R.A.C.P. Dr. Kevin Catt, a former Roussel Fellow, also obtained his M.D. and M.R.A.C.P. Dr. Blair Ritchie, a former Registrar, obtained his M.R.A.C.P.

The Annual Meeting of the Endocrine Society of Australia was held in Melbourne in May last, and the Society later met with the Royal Australasian College of Physicians in Plenary Session during the Annual Meeting of the College. At both these meetings papers were presented by members of the Unit.

The Unit continues to attract attention by visitors both from Australia and abroad. During the year a number of visitors have been welcomed, including: Professors Frank Young, Department of Biochemistry, Cambridge; John McMichael, Post-graduate Medical School, London; Leon Israel, Department of Obstetrics, University of Pennsylvania; Rupert Willis, Emeritus Professor of Pathology, University of Leeds; John Goligher, Department of Surgery, University of Leeds; J. A. Dauphine, University of Toronto; Graham M. Wilson, Department of Medicine, University of Sheffield; Chester Jones, Department of Zoology, University of Sheffield; Bob Morton, Department of Agricultural Chemistry, University of Adelaide, and Drs. Richard Havel, Cardiovascular Research Unit, University of California; David Long, Wellcome Foundation, London; Max Gahwyler, Pfizer International, New York; I. R. Falconer, University of Adelaide; Francis J. Thomas, University of Queensland; Mansel Thomas, Brisbane General Hospital, and K. R. Gollan, Royal Prince Alfred Hospital, Sydney. These visits have proved stimulating and are a source of encouragement. Much valuable advice and helpful suggestions have been given, and these are gratefully acknowledged.

In addition, generous assistance has been provided by a number of individuals and organisations, who have given substantial financial support and gifts in kind. It is stimulating to realise that in the short time since its formation the Unit has attracted such interest and support not only from Australia but from the United States, Great Britain, and several countries on the continent of Europe.

Generous assistance and advice has been given by the Honorary Medical Staff, by University colleagues and many others, and their help is gratefully acknowledged.

In conclusion, I desire to pay tribute to the loyalty and support of all members of the staff of the Unit.

EWEN DOWNIE.

31st December, 1960.

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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National Health and Medical Research Fund, Australia.
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Schering Corporation (Australia).
Mr. R. Wilmoth.

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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.

THE EFFECT OF STEROIDS ON PROTEIN SYNTHESIS BY BONE MARROW

June Sheath and J. Bornstein

It has been demonstrated that the biologically active steroids, hydrocortisone and cortisone are capable of inhibiting incorporation of amino acids into the protein of bone marrow. The effect of variations of structure has been studied.

It has been shown that if the marrow cells are undisturbed in their matrix, then the full structure of the steroid must be intact. However, if the cells have been freed and thus, presumably, their surfaces altered, the presence of the oxygen function at carbon 11 is not necessary. It is thus concluded that the oxygen function at 11 is necessary for the steroid to enter the cell.

INCORPORATION OF C^{14} AMINO ACIDS INTO INSULIN BY THE ISOLATED PERFUSED CAT PANCREAS

P. R. Davoren

The technique of isolation of insulin from a single cat pancreas having been developed, a study of the incorporation of C^{14} valine into insulin was then carried out. It was shown that valine incorporates into insulin and that the amount incorporated is proportional to the radioactivity of the added valine. The influence of tolbutamide was then studied and it was found that tolbutamide appears to depress the rate of incorporation.

METABOLICALLY ACTIVE PITUITARY POLYPEPTIDES

J. Bornstein, Deirdre Hyde, Frances Walker

The directly active pituitary polypeptides have been purified and extensively investigated. Three peptides have been isolated in a substantially pure form. The first of these has growth activity in all assays and is capable of stimulating glucose use by muscle, and the incorporation of radioactive amino acids into the protein of muscle and peripheral tissues, but no effect has been demonstrable in liver.

The second inhibits the utilisation of glucose by muscle, the oxidation of glucose by peripheral fat and liver, the incorporation of amino acids into muscle protein and the incorporation of acetate into fatty acids and cholesterol. The third, which is extremely unstable under present conditions of investigation, stimulates the incorporation of amino acids into protein of muscle and also the incorporation of acetate into the fatty acids and sterols by liver. In so far as structure has been determined there is no great difference between peptides obtained from human or animal pituitaries.

By a variation of the technique similar polypeptides have been isolated from the pituitary growth hormones of various species.

STUDIES ON STEROID METABOLISM

Bryan Hudson, Ausma Dulmanis, Ida Ekkel

(a) **Androgen Metabolism:** The clinical problem of excessive androgenic activity in the female has been intensively investigated in a number of different

laboratories. A variety of techniques have been used, but common to most has been the determination of neutral 17-ketosteroid *excretion* combined with the fractionation of individual C_{19} 17-ketosteroids. These techniques have been used to compare the amounts of each metabolite excreted in normal and abnormal subjects. Some claims have been made that the ratio of 11-desoxy-17-ketosteroids/11-oxy-17 ketosteroids is abnormal, and others that excessive amounts of etiocholanolone and/or androsterone are excreted. In general, these studies have not been particularly revealing with respect to a common pattern or patterns of abnormal steroid hormone production. As a result of these studies it is not possible to be certain that the fault in one particular patient is primarily adrenal or in another primarily ovarian.

Because of this, attempts are being made to estimate *secretion* rates of at least two steroid hormones with androgenic properties. Experimentally, this consists of the administration of radioactive dehydroepiandrosterone (labelled with tritium) and radioactive testosterone (labelled with carbon-14).

Secretion rate can be determined by the isolation of a known metabolite in the urine and the determination of the specific activity (cpm/μMol) of this metabolite. The ratio: $\frac{\text{c.p.m. tracer administered}}{\text{specific activity of metabolite}}$ enables the secretion rate to be calculated.

These studies have been in progress for the past three or four months and a number of technical difficulties have yet to be overcome. Perhaps the most important difficulty is that the metabolites are excreted in the urine almost exclusively in the form of conjugates—sulphates and glucuronides. As such, they are thought to represent two distinct metabolic pools with different fractional rates of turnover. Thus, sampling from each pool has been found to give different estimates of secretion rates. These problems are being further investigated.

In parallel with these studies changes in the levels of C_{19} steroids in plasma in response to the administration of human chorionic gonadotrophin and adrenocorticotrophic hormone are being investigated. The purpose of such studies is to determine whether any differences exist in the response to normal and abnormal subjects to these trophic hormones.

An important and outstanding problem in the overall investigation of androgen function is a method for the measurement of testosterone in plasma. Since there is good reason to believe that the normal level is less than 0.5μg/100 ml. it is essential that some highly sensitive procedure be developed for the detection of the small amounts that are present in 10 ml. of plasma or less. Conventional colour reactions make detection impossible; therefore, the use of a technique of "double labelling" is being investigated. A known amount of 4-C^{14}-testosterone is added to plasma, this and the testosterone present in plasma extracted, isolated chromatographically and the acetate formed by using tritium labelled acetic anhydride. A difficulty in this procedure that is yet to be overcome is to achieve radiochemical purity by freeing the acetylated extract of excess tritiated material.

(b) General Steroid Studies: These involve the application of what are now standardised techniques in steroid estimation to the study of clinical problems—

C^{14}-cortisol for the determination of cortisol secretion rates, SU4885 for the study of pituitary function, plasma 17-hydroxysteroids for the diagnosis of adrenal hypo- or hyperfunction, also with the estimation of 17-ketogenic and 17-ketosteroids for the routine screening of patients suspected of having adrenocortical or other disorders of steroid producing tissues.

STUDIES OF PROTEIN SYNTHESIS

H. G. Burger and J. Bornstein

The object of these studies was to investigate the metabolism of protein in kidney tissue and perhaps to throw some light on the disturbances of renal structure and function which occur in diabetes mellitus and in arterial disease.

Initially, a perfusion system was devised for the simultaneous perfusion of the two isolated kidneys of the rabbit, but, after a number of experiments, it was found that the presence of a variable arterio-venous shunt in the rabbit kidney rendered that organ unsuitable for this type of study. The perfusion experiments have therefore been suspended for the present.

Investigations are now being carried out on rat kidney slices, and it has been found that in the presence of glucose, the incorporation of lysine C^{14} into rat kidney protein is accelerated, whereas insulin has no effect. Differences exist between the incorporation rate in fed and fasted animals. These studies are continuing, and it is proposed to investigate the effects of other hormones, including various pituitary peptides.

THYROID HORMONE STUDIES

Dora Winikoff

Mixtures of proteins, peptides and amino acids can be fractionated by filtration through a bed of "Sephadex" (dextran gel containing only small amounts of carboxylic acid).

Mixtures of tyrosine, thyroxine and phenylalanine were run on Sephadex column in 0.1M pyridine, pH 9 as solvent. By the use of a fraction cutter three distinct peaks could be observed. To detect the amino acids a colour reaction with diazotised sulphanilic acid (a modification of Moser's method) has been used.

A mixture of blood plasma and thyroxine up to 20 mgs. per cent. filtered through a "Sephadex" bed under similar conditions was separated, proteins forming the first peak, followed by the thyroxine peak.

In order to study binding capacity of proteins in various thyroid states, radiothyroxine I^{131} in quantities up to 1μg per ml. was added to plasma. Separation has been achieved, but a certain amount of trailing could not be avoided.

It is intended to continue this work with radiothyroxine of higher specific activity, as the concentrations used previously are far above the physiological levels, exceeding perhaps the binding capacity of the thyroxine-binding proteins.

DISTRIBUTION OF THYROID HORMONE BETWEEN PROTEIN FRACTIONS

Dora Winikoff and C. C. Curtain

The distribution of protein bound iodine is being studied in fractions of normal and thyrotoxic sera obtained by chromatography on diethylamino-ethyl cellulose (DEAE).

At present two fractions are being investigated, the combined (α_1 and α_2) globulin and albumin.

The results are very similar to the ones obtained with salt fractionation with half saturated ammonium sulphate by one of us (D.W.). The bulk of protein bound iodine up to 75% are albumin bound, the remaining 25% being in the combined globulin fraction. There is a small but distinct shift in thyrotoxic patients from the albumin-bound to globulin-bound fraction. In both types of patient the albumin plus globulin-bound iodine (ABI and GBI) add up to total protein-bound iodine (PBI) within the limits of experimental error.

It is intended to isolate more fractions by the use of DEAE column and obtain more detailed information on thyroxine iodine binding capacity of various proteins.

THE RESULTS OF TREATMENT OF THYROTOXICOSIS WITH RADIOACTIVE IODINE

H. D. Breidahl

The results of I^{131} treatment of 105 cases of thyrotoxicosis have been assessed, and the figures presented to the October meeting of the Alfred Hospital Clinical Society. The full report will be published in the Alfred Hospital Clinical Reports.

CLINICAL STUDIES IN DIABETES MELLITUS

H. D. Breidahl

The studies commenced some years ago on the sulphonylurea compounds in the treatment of diabetes mellitus have been continued, and have been extended with the newer hypoglycaemic tablets available. We have now treated over 20 cases of diabetes with DBI, or phenformin, a biguanide derivative. The cases here have been mainly those of brittle or unstable diabetics in the younger age group, and the response has been fairly good. However, side-effects are fairly frequent, and often preclude further treatment with this substance. D.B.I. is now available in Australia, and it should have a place in the control of brittle, young diabetics. Recently, further sulphonylurea compounds have been introduced, and clinical trials are proceeding with these. At present, they do not offer any advantage over the currently existing compounds.

CLINICAL STUDIES

H. D. Breidahl

Studies of the clinical aspects of hyperparathyroidism, metabolic bone disease and abnormalities of sexual development have continued during 1960, with more cases being encountered. When sufficient numbers of cases have accumulated, the results will be collected.

PUBLICATIONS DURING 1960

HUDSON, BRYAN, DORA WINIKOFF, P. TAFT and F. I. R. MARTIN—"Thyroid Stimulating Hormone and Triiodothyronine as Aids in the Diagnosis of Thyroid Disorders". *Aust. Ann. Med.* 9: 1960: p. 194.

HUDSON, BRYAN, MARJORIE BICK and F. I. R. MARTIN—"Observations on the Treatment of Severe Diabetic Ketosis". *Aust. Ann. Med.* 9: 1960: p. 34.

BORNSTEIN, J., and DEIRDRE HYDE—"Polypeptides of the Human Pituitary with Direct Action on Metabolism". *Nature*, 187: 1960: p. 125.

HUDSON, BRYAN—"Melanocyte Stimulating Hormone", in *Clinical Endocrinology* I: 1960: p. 649. Grune & Stratton, Inc., New York.

PAPERS ACCEPTED FOR PUBLICATION

HUDSON, BRYAN, and GEORG W. OERTEL—"Determination of Dehydroepiandrosterone and Total Neutral 17-Ketosteroids in Human Plasma". *Analytical Biochemistry.*

SANDERS, MARGARET J.—"The Effect of Prednisolone on Glucose Tolerance in Respect to Age and Family History of Diabetes Mellitus". *Diabetes* 1961.

PAPERS IN PREPARATION

HUDSON, BRYAN, JUNE SHEATH and AUSMA DULMANIS—"β-glucuronidase from the Female Rat Preputial Gland in the Hydrolysis of Steroid Glucuronides".

BORNSTEIN, J., and DEIRDRE HYDE—"The Preparation of Pituitary Polypeptides with Direct Action on Metabolism".

BORNSTEIN, J., and FRANCES WALKER—"A Pituitary Polypeptide with Growth Activity".

BORNSTEIN, J., DEIRDRE HYDE and FRANCES WALKER—"An Insulin Inhibitory Pituitary Polypeptide".

SHEATH, JUNE, and J. BORNSTEIN—"The Action of Steroids on Protein Synthesis by Bone Marrow".

BREIDAHL, H. D.—"Hyperparathyroidism"—A Review of Cases.

BREIDAHL, H. D.—"Hypercalcaemia due to Malignant Ovarian Tumour"—A Case Report.

BREIDAHL, H. D.—"Phenformin (D.B.I.) in the Treatment of Diabetes"—A Preliminary Report.

BREIDAHL, H. D.—"Results of Treatment of Thyrotoxicosis with I^{131}".

LECTURES DELIVERED DURING 1960

"Insulin Antagonists"—*Harvard University Medical School.*	J. BORNSTEIN
"Pituitary Polypeptides with Direct Action on Metabolism"—*Western Reserve University Medical School.*	J. BORNSTEIN
"Pituitary Polypeptides with Direct Action on Metabolism"—*International Insulin Symposium, Indianapolis.*	J. BORNSTEIN
"Theories of Insulin Action"—*University of California Medical School.*	J. BORNSTEIN
Pituitary Polypeptides with Direct Action on Metabolism"—*Royal Australasian College of Physicians.*	J. BORNSTEIN
"Body Weight"—*University of Melbourne,* November, 1960.	BRYAN HUDSON
"Cushing's Syndrome"—*Royal Australasian College of Physicians,* May, 1960.	BRYAN HUDSON
"Results of I^{131} Treatment of Thyrotoxicosis"—*Alfred Hospital Clinical Society.*	H. D. BREIDAHL
"Diabetes Detection Drives"—*Diabetes Federation of Australia.*	H. D. BREIDAHL
"Medical Emergencies"—*Post-graduate Committee, Bendigo.*	H. D. BREIDAHL

MEETINGS ATTENDED DURING 1960

Endocrine Society of Australia, Melbourne, May, 1960.	EWEN DOWNIE BRYAN HUDSON J. BORNSTEIN H. D. BREIDAHL H. G. BURGER D. WINIKOFF J. SHEATH
Royal Australasian College of Physicians, Melbourne, May, 1960.	EWEN DOWNIE BRYAN HUDSON J. BORNSTEIN H. D. BREIDAHL H. G. BURGER D. WINIKOFF J. SHEATH
Royal Australasian College of Physicians, Sydney, October, 1960.	BRYAN HUDSON
Royal Prince Alfred Hospital Post-graduate Week (Participant in two Panel Discussions).	BRYAN HUDSON
Diabetes Federation of Australia, Melbourne, May, 1960.	H. D. BREIDAHL

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

STUDIES OF MYCOBACTERIA

J. C. Tolhurst[1], G. Buckle[1], and N. A. M. Wellington[1]

Owing to the absence of Mr. G. Buckle overseas, little field work was done on Traum's disease of cattle. Cultures already obtained from cattle with Traum's disease were inoculated into uninfected cattle and lesions resembling those of Traum's disease were produced. It has now to be determined whether the lesions will progress or regress. Much interesting information has been accumulated, but this disease, like most mycobacterial diseases, advances slowly.

STUDIES ON CHEMOTHERAPY

J. C. Tolhurst[1], A. Perceval[1], and M. Dorr[1]

A new edition of the monograph on Chemotherapy published in 1955 has been in preparation and is more than half completed.

Considerable time has been devoted to the study of the significance of tests of the bacterial action of antibiotics.

The results of most sensitivity tests refer to bacteriostatic tests indicating merely the inhibition of bacterial growth over a period of 18-24 hours by the antibiotic, and in infections in which the patient's defence mechanisms can give him some help these are a fairly reliable indication of which antibiotic it is best to use. However, in some types of infection the patient can do very little to assist himself, and then it is desirable to employ an antibiotic or a combination of antibiotics which will kill every one of the infecting organisms if that is possible. The tests which serve this purpose are, unfortunately, intricate and slow, but may be well worthwhile. There have been a number of patients this year for whom such tests have been done because they failed to respond to treatment with bacteriostatic drugs, and it has been gratifying to see cures follow the use of a bactericidal combination of drugs.

Allied to this is another test to check the efficacy of treatment. Patients vary in the efficiency with which they absorb antibiotics, the degree in which they alter or destroy them within their bodies and the speed with which they excrete them, so that in these cases where the outcome depends a great deal on the effect of an antibiotic it is necessary to make sure that the patient has in his blood stream sufficient concentration of antibiotic to kill the infecting bacteria. These tests also are laborious and slow but, as with those mentioned above, there have been instances of dramatic improvement following alteration of therapy in accordance with the result of the test.

It is evident that no general rules will emerge in respect to the use of antibiotics in this way; the effective combinations and concentrations are unpredictable. Therefore, attention must be directed to facilitating the performance of the test, to simplifying and hastening its execution so that it can become routine work.

[1] Department of Pathology, Alfred Hospital.

SURVEY OF ROAD ACCIDENTS

I. McNicol Smith[1]

The survey of accident cases admitted to the Alfred Hospital begun in 1959 has continued. In April, 1960, the collection of material was completed, and the second year has been devoted to a follow up, by interview, of as many cases as possible, in conjunction with Miss B. F. Letheren, of the Department of Social Studies, as outlined in last year's report. This follow up was terminated at the end of the year, and during the remaining three months the information obtained will be studied and a final analysis prepared.

RENAL DIALYSIS

B. W. Fox[2]

The existence at the Hospital of an artificial kidney unit has continued to attract very many patients with renal failure. Of these, a relatively small number has come to dialysis, but during the early part of 1960 the unit was in action on an average of once weekly.

This form of treatment, which is undertaken in consultation with the members of the Staff of the University Department of Medicine, can now be regarded almost as a routine adjunct to the well-established conservative management of acute renal failure.

There is, however, continuing difficulty in diagnosis and many fruitless dialyses must necessarily be carried out before it is apparent that the renal lesion is, by its nature, an irrecoverable one.

Useful experience is being accumulated in the management of renal failure in surgical patients, but the outlook for such patients continues to be very unsatisfactory.

It is apparent that infection is often an important factor in determining both morbidity and mortality in acute renal failure, and it is often not clear whether this is the primary cause of the renal damage or is a preventable complication of treatment.

URINARY CALCULI IN SHEEP

B. W. Fox[2]

An attempt has been made to induce urinary calculi in sheep by adding potassium acid phosphate to a standard diet, but at the end of an initial period of three months none of a group of 12 sheep had any stones.

The diet varied a little from that described by the original workers in this field, and it was intended to repeat the trial with a further modification of the diet.

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[2] Department of Surgery, University of Melbourne, Alfred Hospital.