

*Thirtieth Annual Report*

of

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

(Including Alfred Hospital Clinical Research Unit)

and

ALFRED HOSPITAL RESEARCH FELLOWS

*1956*

ALFRED HOSPITAL, PRAHRAN,  
VICTORIA, AUSTRALIA.

# BAKER MEDICAL RESEARCH INSTITUTE

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Director of Clinical Research Unit (ex officio).

\*Appointed from the University of Melbourne.

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**Acting-Director:** L. B. COX, M.D., F.R.A.C.P., M.R.C.P. (EDIN.) (28/5/56 to 10/12/56)

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**Pharmacologist** . . . . . (Vacant).

**Research Assistants** . . . . . B. HUDSON, M.D., M.R.C.P., M.R.A.C.P.  
(to 31/3/56).  
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**Biochemists** . . . . . C. C. CURTAIN, PH.D., M.S.C., A.R.A.C.I.  
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**Ward Staff** . . . . . SISTER S. A. CITROEN  
SISTER B. HILLMAN (from 4/4/55 to 5/3/56).  
SISTER E. ARMSTEAD (from 10/4/56 to 5/11/56).  
SISTER J. JEFFREY (from 5/11/56).

**Registrar** . . . . . G. R. WAGNER, M.B., B.S.

**Resident Medical Officers** . . . . . I. E. MCINNES (from 16/1/56 to 8/4/56).  
H. TUNG (from 9/4/56 to 1/8/56).  
D. GEE (from 2/6/56 to 29/9/56).  
C. N. MORGAN (from 1/10/56 to 21/10/56).  
F. D. LANG (from 22/10/56 to 31/12/56).

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S. HART  
MRS. K. GUYOT (to 22/12/56).  
MISS J. PAINTER  
MISS D. VAN ASSCHE (to 3/12/56).

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MISS D. DUDGEON  
MISS J. MOORE

**Animal Husbandry Technician** . . . . . C. GODFREY

**Laboratory Assistants** . . . . . MISS J. BLACK (to 22/12/56).  
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MISS W. DONOVAN (to 24/2/56).  
MISS E. HENDERSON  
MISS J. A. KENT (from 10/12/56).  
MISS M. NEAVE  
MRS. B. STEDMAN

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“Victor Y. & Margaret Kimpton” . . . . .	M. QUINN-YOUNG, B.SC., B.E.E., M.B., B.S.
“R. B. McComas Memorial” . . . . .	R. J. SAWERS, M.B., B.S., M.R.A.C.P.
“Sydney W. Jones Medical Research Foundation” . . . . .	G. R. STIRLING, M.B., B.S., F.R.A.C.S.
Senior Research Fellowship . . . . .	JEAN C. TOLHURST, M.Sc.

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H. D. BREIDAHL, M.D., M.R.C.P. . . . .	“Edward Wilson Memorial”
I. A. L. FERGUSON, M.B., B.S. . . . .	“Sydney W. Jones Medical Research Foundation”
J. K. FRANCIS, M.B., M.S., F.R.C.S. . . . .	“Victor Y. and Margaret Kimpton”
J. R. E. FRASER, M.D., M.R.C.P., M.R.A.C.P. . . . .	“E. H. Flack”
R. J. MARSHALL, M.D. (BELFAST), M.R.C.P., M.R.C.P. (IRELAND) . . . . .	“Edward Wilson Memorial”
M. SANDERS, M.B., B.S. . . . .	“Sol Green”
R. J. SAWERS, M.B., B.S., M.R.A.C.P. . . . .	“Sol Green”

### AWARDED TRAVEL GRANTS FOR 1957

I. S. EPSTEIN . . . . .	Marian and E. H. Flack Travelling Scholarship; E. H. Flack Medical Research Scholarship.
W. R. KINGSTON . . . . .	J. H. Patterson Travelling Scholarship; E. H. Flack Medical Research Scholarship.
G. R. STIRLING . . . . .	Sydney W. Jones Medical Research Scholarship.

## ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

During the thirty years of its existence the Research Organization at the Hospital, first the Baker Institute, then the combined Institute and Clinical Research Unit, has developed investigational techniques and services to a point where they could be separated into routine hospital services. These have then been formed into separate departments or services of the hospital leaving the Research Organization free from the heavy demands of routine services. This year has seen the formation of the Diabetic and Metabolic Unit of the Hospital by fusion of the Diabetic Instructional Clinic and the endocrinological research activities which have been carried out for many years in the Baker and C.R.U.

During the past year both Dr. Fantl and myself made overseas visits. Dr. Fantl was invited to contribute to the International Congress of Haematology in Boston and an International Haemophilia Symposium in New York, and this gave him an opportunity to visit centres of haematological research in the U.S.A., France and Switzerland. The purposes of my visit to the United Kingdom, Scandinavia and New Zealand were threefold. First to study the organization of medical research, secondly to see the growing points of research in cardiovascular disease, and thirdly to make contacts with people who could help in selecting applicants for the Edward Wilson Memorial Fellowship.

To these ends visits of varying duration were paid to medical or other appropriate departments in the following medical centres:—

London—Postgraduate School of Medicine; St. Bartholomew's Hospital; St. George's Hospital; London Hospital; St. Mary's Hospital; Middlesex Hospital; Central Middlesex Hospital; St. Thomas' Hospital; University College Hospital; Institute for Diseases of the Chest; Institute of Cardiology.

Birmingham—Queen Elizabeth Hospital.

Cardiff—M.R.C. Pneumoconiosis Unit, Penarth Hospital.

Edinburgh—Royal Infirmary; Western General Hospital.

Glasgow—Western Infirmary (Gardiner Institute).

Manchester—Royal Infirmary.

Oxford—Nuffield Institute of Medical Research.

Sheffield—University Department of Therapeutics and Pharmacology.

Stockholm—Sodersjukhuset.

Copenhagen—Rigshospitalet.

Auckland—Cardiac Unit, Green Lane Hospital.

Wellington—Dominion Physics Laboratory, D.S.I.R.; Animal Research Station, Dept. of Agriculture.

Dunedin—Medical School.

Perth—Royal Perth Hospital.

Sydney—Department of Physiology, University of Sydney; Department of Pharmacology, University of Sydney; Hallstrom Institute, Royal Prince Alfred Hospital; Clinical Investigation Unit, Royal North Shore Hospital.

Visits and discussions were had with officers of the Royal College of Physicians of London; Royal Society of Medicine, Medical Research Council (London), Ciba Foundation (London), Wellcome Trust (London), and the Hospital Board of Management, Auckland. Also I attended meetings of the Medical Research Society (Oxford), the Physiological Society of Great Britain and Ireland (Cambridge), and the 2nd European Cardiological Congress (Stockholm). I delivered talks, both formal and informal, general and scientific, on the research work, and facilities for research at the Alfred Hospital and Baker Institute at several centres in the United Kingdom and New Zealand.

It is inevitable that, on a tour such as this, one should endeavour to compare our own work and facilities with those seen elsewhere.

As a generalization I feel confident that the quality, and quantity, of the research work carried out at Alfred Hospital is equal to that of any centre visited. In many instances we have started and are pursuing new lines of investigation and not merely following where others have led.

It is however clear, in comparison with other centres, that there are some deficiencies in the facilities of the Baker-C.R.U. Our group is unbalanced between clinical and laboratory facilities. For its size there are an inadequate number of ward beds; even quite small clinical research groups usually had 25 beds under their control. Further, judging by the centre at the Middlesex Hospital, some individual consulting rooms for interviewing and examination of selected out-patients are essential. On the non-clinical side our workshop facilities for the construction and maintenance of apparatus are inadequate by English standards.

I wish to acknowledge the unfailing courtesy, help and advice that I received from the heads and officers of the various departments and institutions visited and in particular to acknowledge the assistance of Professor J. McMichael in planning my itinerary and helping me to make contact with people I desired to meet. It is also a great pleasure to thank Dr. L. B. Cox for his help in assuming the duties of Director in my absence.

The University Department of Surgery has commenced work during the year and we have been able to assist Professor Ewing by making available facilities for experimental surgery, and similar amicable arrangements with the Department of Medicine, when it shortly becomes established in the Hospital, are anticipated.

The research work carried out in 1956 is recorded in detail elsewhere in this report. The major projects have been, as in recent years, concerned with Blood Coagulation, Control of Body Fluid Volume, Hypertensive and Hypotensive States, Peripheral Vascular Disease, The Electrical Activity of the Heart, Energy Production in the Myocardium, Experimental Cardiac Surgery and Abnormal Serum Proteins. In addition to these, many minor problems arising in the course of work have been studied.

Arrangements have been completed during the year for a post-graduate Fellow from Belfast to work with us in 1957 during his tenure of the Edward Wilson Memorial Fellowship. These visits by overseas workers are most stimulating to the other research workers and are proving very successful. This success is a tribute to the foresight of the Trustees of the Estate of the late Edward Wilson in founding this Fellowship.

During the year the death occurred of three devoted friends of the Research Group—Mr. J. C. Gates, an Institute Trustee; Sir Wilberforce Newton and Sir Alexander Stewart, who were members of the Research Advisory Committee. We shall miss greatly their advice and help.

The National Health and Medical Research Council and the Life Insurance Medical Research Fund of Australia and New Zealand have continued to support general research projects and have allocated grants for continuance of the projects in 1957. The help of these bodies is gratefully acknowledged.

Many organizations 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 Professor Wright and his staff at the Department of Physiology, University of Melbourne, and we thank them for their continuing interest in our work.

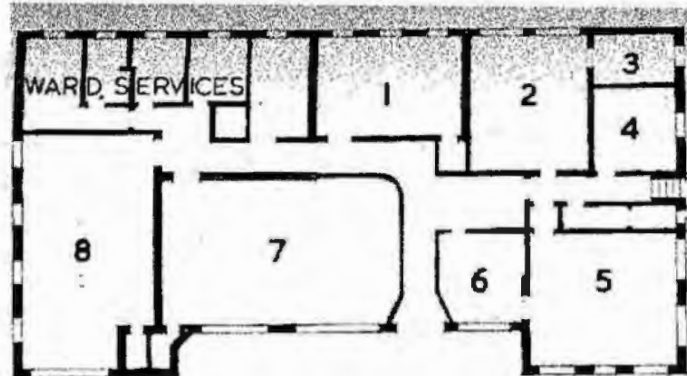
It is a pleasure for me to thank the Trustees of the Institute and the Board of Management of the Hospital for their continued generous support of all our activities, and to thank members of the staff and research fellows for their co-operation during the past year.

T. E. LOWE.

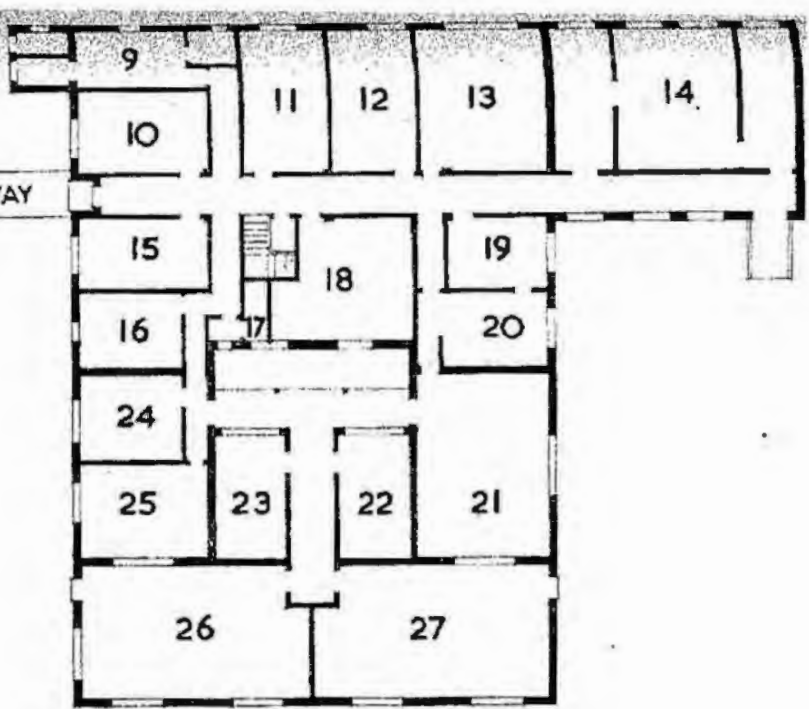
31st December, 1956.

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

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



GROUND FLOOR PLAN



GROUND FLOOR PLAN

LEGEND

- |   |                               |
|---|-------------------------------|
| 1. CLINICAL ROOM                        | 20. RESEARCH FELLOW           |
| 2. CLINICAL BIOPHYSICS                  | 21. WORKSHOP                  |
| 3. DARK ROOM                            | 22. STORE                     |
| 4. CONSULTING ROOM                      | 23. STORE                     |
| 5. CLINICAL ROOM                        | 24. BIOCHEMISTRY LAB          |
| 6. OFFICE                               | 25. ENDOCRINOLOGY LAB         |
| 7. WARD (6 BEDS)                        | 26. CLINICAL BIOCHEMISTRY LAB |
| 8. WARD (6 BEDS)                        | 27. BIOPHYSICS LAB            |
| 9. CHROMATOGRAPHY                       | 28. PHYSIOLOGY LAB            |
| 10. ASSOCIATE DIRECTOR                  | 29. PHYSICAL CHEMISTRY LAB    |
| 11. CENTRIFUGES REFRIG.                 | 30. BALANCE ROOM              |
| 12. BIOCHEMISTRY LAB.                   | 31. PHYSICAL INSTRUMENTS      |
| 13. HAEMATOLOGICAL<br>BIOCHEMISTRY LAB. | 32. RESEARCH FELLOWS          |
| 14. EXPERIMENTAL SURGERY                | 33. LIBRARY & SEMINAR ROOM    |
| 15. ASSOCIATE DIRECTOR                  | 34. GENERAL OFFICE            |
| 16. RESEARCH FELLOW                     | 35. DIRECTOR                  |
| 17. MICROBALANCE                        | 36. STAFF ROOM                |
| 18. ORGANIC CHEMISTRY                   | 37. LABORATORY                |
| 19. RESEARCH FELLOW                     | 38. RESEARCH FELLOW           |



FIRST FLOOR PLAN

RESEARCH CENTRE







# REPORT OF SCIENTIFIC INVESTIGATIONS

## BLOOD COAGULATION

### THE NUCLEOTIDES OF HUMAN BLOOD PLATELETS\*\*

P. Fantl and H. A. Ward.

As certain bleeding tendencies are due to either a qualitative or quantitative deficiency of blood platelets, knowledge of their composition is of interest. During a study concerning the release from isolated human blood platelets of compounds active in vasoconstriction it became necessary to obtain information on the composition of certain nitrogenous substances in platelets.

For this work platelets were isolated from normal human blood in the presence of ethylene diamine tetra-acetate as an anticoagulant and then freed of plasma proteins. An alkaline platelet extract gave an ultraviolet absorption curve which did not resemble that of any known tissue component. On the other hand 80-90% of the platelet nitrogen could be accounted for as proteins. These were isolated and characterised by a quantitative biuret reaction. Ultraviolet absorption measurements of the proteins indicated the presence of tyrosine and tryptophan.

It appeared therefore that the characteristic absorption spectrum of protein was masked by other ultraviolet absorbing compounds in the platelets. These were present in filtrates from precipitated platelet proteins and their ultraviolet absorption spectra suggested that they were purine and pyrimidine derivatives. Paper chromatography and ionophoresis of protein-free platelet extracts indicated the absence of free purine and pyrimidine bases and their ribosides and riboside monophosphates. Adenosine diphosphate, adenosine triphosphate and at least two or three other nucleotides which may be dinucleotides containing adenine, guanine, cytosine and uracil were shown to be present.

The bases of the nucleotides were isolated by paper chromatography after acid hydrolysis and characterised by their specific ultraviolet absorption spectra; adenine and guanine were further identified by counter-current distribution. The sugar of the nucleotides was ribose. Only traces of deoxyribose were detected.

The nucleic acid content of platelets was found to be very low.

The virtual absence of deoxyribose indicates that no nuclear material is present. Platelets are therefore cytoplasmatic particles.

### PRESERVATION OF BLOOD CLOTTING FACTORS\*\*

P. Fantl and A. G. Marr.

It is well known that blood stored for some time loses its coagulability. As the mechanism of the deterioration is not clear, a study of human plasma stored under a variety of experimental conditions was carried out and methods devised to prevent the loss of clotting factors during storage.

\*\*In this report of scientific investigations those projects marked (\*\*) were supported wholly or in part by grants from the National Health and Medical Research Council and those marked (\*) were supported by grants from the Life Insurance Medical Research Fund.

When cell-free plasma is stored at room temperature (19-20°C) in glass containers under sterile conditions and in contact with air, the following changes are noticed: The thrombin and prothrombin clotting times are prolonged, alpha-prothromboplastin concentration (antihæmophilic factor) decreases, prothrombin accelerator activity (Factor V, proaccelerin) disappears, the pH of the plasma rises to between 8 and 9.

An investigation of the mechanism of the prolonged thrombin clotting time has shown that antithrombins are not increased during storage. The prolonged thrombin clotting time can be partly overcome by the addition of pyrocatechol. The phenomenon is best explained by a change in the physico-chemical properties of the surface of the fibrinogen molecule. No major chemical alteration takes place because the yield of fibrin is not diminished after storage for four weeks at room temperature. It was found that acidification of oxalated or citrated plasma to pH 6.4 resulted in preservation. The thrombin clotting time is not altered in such specimens. Further the antihæmophilic factor is preserved. Prothrombin accelerator losses are also greatly reduced. Usually under these conditions 50% of prothrombin accelerator activity is still present. The plasmas can be stored both under aerobic as well as under anaerobic conditions with equally good results. It is suggested that for transfusion purposes plasma should be stored under carbon dioxide. In this case refrigeration is not necessary.

#### IDENTIFICATION OF ANTI-BETA-PROTHROMBOPLASTIN\*\*

P. Fantl, A. G. Marr and R. J. Sawers.

This investigation concerned the hæmorrhagic tendency of a seven year old boy, who was stated to have suffered hæmorrhages from the age of two months. He had received nine blood transfusions and was seen by us for the first time six months after the most recent blood transfusion. Laboratory examination indicated the presence of a powerful coagulation inhibitor in his blood. In addition it was found that his blood was completely deficient in beta-prothromboplastin (PTC, Christmas factor). Since no laboratory data are available prior to this investigation, we have assumed that the patient was born with a beta-prothromboplastin deficiency and following several blood transfusions he developed a coagulation inhibitor; the assumption being that beta-prothromboplastin of the donor's blood stimulated antibody formation. However, serological evidence for the presence of antibodies against components of normal human plasma was absent.

The inhibitor was isolated from the patient's blood and showed the following properties. It was not adsorbed by barium sulphate; it was salted out between 25-46% ammonium sulphate concentration; it was absent from the cuglobulin and albumin fractions of his plasma; it was stable on storage below 65°C. Since serological evidence of the antibody nature of the inhibitor could not be obtained, biochemical tests were applied. As antibodies are usually species specific experiments with homologous and heterologous blood mixtures were carried out. These experiments indicated that the inhibitor, although very active in preventing formation of thromboplastin in human blood, is without effect on thromboplastin formation of rabbit's and guinea-pig's blood.

The following technique was devised for identification and to study the mode of action of the inhibitor. Beta-prothromboplastin was adsorbed on barium sulphate and the adsorbate was treated with the inhibitor isolated from the patient's blood. It could be established that the inhibitor reacted only with adsorbates which contain beta-prothromboplastin of normal human plasma and serum and that this is not due to a non-specific combination of the barium adsorbate with the inhibitor because animal beta-prothromboplastins showed no affinity to the inhibitor. It was thus possible to identify the inhibitor as an anti-human beta-prothromboplastin. The barium sulphate adsorbate technique was also applied to plasmas of other congenital beta-prothromboplastin deficiencies. It was observed in some cases that the barium adsorbates did not remove inhibitory activity, which is to be expected since the plasmas were free of beta-prothromboplastin. In other cases, however, it was found that appreciable inhibitory activity which was not very much lower than that obtained from normal plasma, was removed by barium adsorbate. These experiments allow the following conclusions to be drawn. Beta-prothromboplastin deficiencies are instances of genetically determined errors in protein synthesis. It would appear that we have to divide patients with a complete beta-prothromboplastin deficiency into two groups. In the one the patient can produce a protein which although inactive as thromboplastin precursor, is still active as antigen. In the other group the patient cannot produce even a protein with antigenic properties. This would explain the observation that approximately 6% of all haemophilic patients who have received a number of blood transfusions become refractory to further transfusion therapy. It is very likely that these patients are unable to synthesise even defective beta-prothromboplastins with antigenic properties, and they therefore will produce antibodies following transfusion of the particular factor they lack.

## HAEMOPHILIA

R. J. Sawers.

Accumulation of data in the combined clinical and laboratory study has continued throughout the year. Case finding has revealed several new pedigrees with complete prothromboplastin deficiencies and a greater number with milder grades of deficiency. It has now been shown that the group of patients with greater than 3% of alpha-prothromboplastin in their plasma suffer symptoms much less serious than those occurring in patients with less than that amount. In spite of the much higher average age of the former group, only 50% have experienced haemarthroses, and then usually on only one occasion. Also spontaneous haemorrhages are rare and traumatic haemorrhage is relatively slight. Many of these patients have never required a blood transfusion. On clinical grounds the diagnosis is difficult to make especially when, as sometimes happens, there is no prior family history of the disease. The laboratory diagnosis is also difficult and in the very mild cases a number of different tests may be required before the clinical suspicion can be confirmed.

During this year, the studies on the social aspects and on clinical management have received greater attention than previously. The assistance of a social worker trained in psychology research was obtained for five months, and a detailed study of 31 haemophilic men was made and analysed. Also thirteen mothers of haemophilic children were interviewed to determine the requirements for the home care and education of the children. The results of this work will be published as no similar study has been reported.

Study of the clinical management of haemophilic haemorrhage and its complications has continued with the assistance of the Haematology Clinic in the Alfred Hospital. Principles for management are being defined and it is evident that there is a considerable role for surgery which can now be carried out with reasonable safety. The problem of having adequate supplies of plasma available has been in the main part solved with the assistance of the Red Cross Blood Transfusion Service. By using an acid citrate anticoagulant developed by Dr. Fantl it is now possible to hold plasma for several days, without any loss of its alpha-prothromboplastin content. The use of this plasma immediately prior to and at intervals following major surgical procedures has produced good results.

These genetic studies have revealed two haemophiliacs in different pedigrees who also suffer from colour blindness. In each case the patient has had no haemophilic relatives. A combined statistical and genetic study made in conjunction with members of the Department of Statistics, University of Melbourne, has been forwarded for publication.

#### **Other Haemorrhagic Diseases**

Three Victorian families with members suffering from thrombasthenia (a thrombocyte abnormality causing haemorrhage) have been studied. Unlike previously reported cases, the condition in each case appears to have been inherited as a somatic recessive character. No linkage with other genetic characters could be established.

An acquired haemorrhagic disease affecting both men and women and causing easy bruising and bleeding from bronchial and gastrointestinal mucosa has been studied in part. No coagulation abnormality can be demonstrated. Vascular fragility and prolonged skin bleeding time occurs and there is an apparent disturbance of protein and amino-acid metabolism.

#### **CONTROL OF BODY FLUID VOLUME\*\***

T. E. Lowe, A. J. Barnett and G. R. Wagner.

It was reported last year that from clinical observations, study of a mechanical model and animal studies a hypothesis concerning the regulation of body fluid volume had been propounded. This hypothesis considers the body fluid to be represented by an "open" storage, with a transport system through which a continuous flow occurs and with input and output controlled by at least two facets of the stored fluid. One of these is thought to be the volume and the other the osmotic pressure of some portion of the stored fluid.

In previous reports an account has been given of the evidence indicating the role of volume in the control of this system. Recently we have been determining the plasma osmotic pressure of oedematous patients and have obtained some evidence showing a direct relationship between plasma osmotic pressure and urine flow, which is in accord with the hypothesis.

Further observations have also been made on the quantitative relationship between urine flow and body volume and these indicate that, whilst over considerable ranges this relationship is linear, the overall relationship is probably represented by a sigmoid curve with an almost linear central portion. In general, urine flow rates increase with increasing body volume. In a variety of pathological states it has been observed that the slope of the curve representing this relationship is much less steep than in the normal. During recovery from oedema states the slope of the curve usually increases, often in a stepwise fashion.

With a view to extending clinical observations on fluid volume control to a wider variety of conditions a bed-weighting machine has been installed. This should make it possible to study the changes in fluid volume occurring post-operatively. During the year much time has been spent in devising suitable weighing techniques to overcome errors due to variable weights of bed clothes and other objects attached to the bed. It has been determined that reproducible readings can be obtained from the machine and this facet of the project should now proceed.

In an endeavour to investigate the nature of the stepwise changes in outflow-volume relationships reported during recovery from various oedema states, techniques are being established to study renal function in detail with particular reference to glomerular filtration rates and tubular reabsorption.

## HYPERTENSIVE STATES

### CLINICAL TRIAL OF HYPOTENSIVE DRUGS

A. J. Barnett and G. R. Wagner.

The clinical trial of the use of ganglionic blocking agents commenced over 6 years ago has continued and the number of patients participating in the trial has now reached 91. The efficacy, described in the previous report, of these drugs in the more severe forms of hypertension, particularly the malignant phase, and the advantageous combination of treatment with various ganglionic blocking agents has been confirmed. Disadvantages of the type of treatment available until recently have been that a certain proportion of patients can only be controlled by injections and that even in patients controlled by oral treatment the effect is variable due to irregularity in absorption of the drug. A new ganglionic blocking drug "Inversine" (Merck), which has been claimed to be completely absorbed orally, has recently become available in small quantities and it is hoped that with the use of this drug the particular disadvantages mentioned will be avoided. Enough of the new drug to treat twelve patients for six months has been made available to us. It is being administered to subjects in whom treatment by injection of pentolinium has been required, or in whom treatment with pentolinium by mouth is producing an inadequate blood pressure control. Results to date indicate that treatment with "Inversine" is giving better control than the previous treatment with pentolinium. It is however too early to assess results satisfactorily.

## ALDOSTERONISM

B. Hudson, J. Bornstein and A. J. Barnett.

A patient, participating in a trial of reserpine for benign hypertension and whose symptoms and hypertension were not relieved by the treatments used, was admitted to hospital for further investigation. As an electrocardiogram showed the changes associated with a low serum potassium concentration the serum electrolytes were investigated and showed that not only was there a marked lowering of the serum potassium but also a slight increase in the serum sodium concentration and an alkalosis. These were features described in Conn's syndrome of primary aldosteronism.

Further investigations showed excessive amounts of aldosterone in the patient's plasma and urine and X-ray examination following perirenal air insufflation showed a small suprarenal tumour. This was removed surgically, following which the patient's symptoms were relieved, her hypertension decreased and the abnormalities in the serum electrolyte pattern abolished.

This case indicates the advisability of looking for aldosteronism in patients with unexplained hypertension, particularly if they are not responding to treatment with anti-hypertensive drugs.

## HYPOTENSIVE STATES

A. J. Barnett.

Previously studies made on three patients with orthostatic hypotension have been recorded. During this year another patient with severe orthostatic hypotension due to disease of the sympathetic nervous system has been investigated. This patient differed from the others in that he was young and the lesion developed suddenly following an acute illness. Tests of sympathetic nervous reflexes indicated that the loss of sympathetic nervous function was almost complete.

During investigation of this patient the mode of action of sympathicomimetic amines was able to be studied. Noradrenaline, which is generally believed to act directly on blood vessels, produced an excessive pressor response. Neosynephrine and methoxamine also produced a marked effect indicating that these substances also acted directly on vessels. Other pressor amines—ephedrine, methedrine, dl-amphetamine, mephentermine—had no such effect indicating that the pressor action which they have in normal people is mediated by another mechanism and probably depends on the presence of some sympathetic nervous activity.

## DISEASES OF THE PERIPHERAL BLOOD VESSELS

### INTERMITTENT CLAUDICATION

J. K. Francis and A. J. Barnett.

The study of the natural history of intermittent claudication has been continued by two main methods: first, by following the patient's performance with a standard step test (23 patients), and secondly by analysis of clinical notes and replies to a questionnaire relating to patients examined more than two years ago (51 patients).

Although there was a marked range in the step test performance, the variability as assessed by the standard deviation was reasonably small when calculated on 12 tests within four weeks after "training" for three months. None of the various drug treatments used was superior to a deliberate placebo treatment. The course of the symptoms in the patients observed can therefore be taken as following its natural history (plus placebo). For over twelve months when studied by step tests claudication remained the same or decreased.

The clinical study (over an observation period of two to five years) showed that in about half the patients the severity of claudication remained unchanged; of the remainder, in about half it decreased and in half it became worse. Only two of the patients studied required major amputation.

The incidence of clinical atherosclerotic disease in heart and brain was high; about 50% of the patients were affected at the end of a period of observation of two to five years.

The survival period from the onset of claudication was over five years in 50% of the patients and over two years in 80% (many of the patients are still living).

It is concluded that the treatment of intermittent claudication is not an urgent matter, its main importance being the interference with the patient's way of life. The incidence of development of ischaemic features elsewhere, although high, did not preclude operation.

## ARTERIAL GRAFTING

A. J. Barnett and K. N. Morris†.

Last year we reported on the use of arterial grafting for the relief of claudication. In addition to the cases in which an arterial graft is used for symptomatic relief, this operation is also being performed in some patients with incipient gangrene although it is not expected that the results will be as satisfactory in these patients. To date 36 grafts have been inserted in 30 patients. Two patients have since died. Twelve grafts in 9 patients have blocked. Three grafts were unsuccessful for other reasons (haemorrhage, infection, treatment difficulty). At present there are 19 patients with grafts. In two of these the grafts have survived for more than 2 years and in three more than one year. In the others less than one year has passed since operation was performed. (In two of the cases in which the graft blocked and in one of the patients who died the graft had functioned for more than one year.) In patients with patent grafts, claudication has been completely relieved and several ischaemic limbs have been saved from amputation by this method. The results indicate that, where feasible, arterial grafting is a worthwhile procedure in the treatment of occlusive arterial disease of the lower limbs.

## AMNION IMPLANTS

J. K. Francis and A. J. Barnett.

Following the dramatic results quoted by Troensegaard-Hansen (B.M.J. 2, 262 (1956)) those patients unsuitable for arterial grafting, (either suffering from generalised disease or an anatomically unsuitable local lesion) had amnion implants made in the thigh under local anaesthesia. It is too early to assess the results of this technique as only 4 cases have been operated on and that very recently.

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## VARICOSE OR STASIS ULCERATION OF THE LEGS

J. K. Francis.

Stasis ulceration has been studied, first, by the investigation and treatment of the established ulcer and secondly by a follow-up of a series of 98 patients who had suffered from phlebitis or thrombosis 5 years prior to their assessment by means of a questionnaire and clinical examination.

*Treatment of the established ulcer* was along several lines. A simple compression bandage until the ulcer is healed and then the wearing of an elastic stocking is often the only one possible owing to the condition of the patient, allied with the shortage of hospital beds. In the main most ulcers heal by this means in a matter of a few weeks to a few months.

In larger ulcers, which after repeated compression bandages show clean granulation tissue but slow healing because of their size, a split skin graft has been employed as an out-patient procedure under local anaesthesia. The patient rests at home for 2-3 days and is then ambulatory with a compression bandage. These grafts all took exceedingly well but despite a continued compression regime they were found to re-ulcerate in most cases.

Recurrent or resistant ulcers were investigated by venography. The technique used varied but that most suitable for demonstrating the patency and valve status of the deep veins was ascending venography. In all cases the deep veins were patent and showed that they were still returning blood from the lower limb but in most cases the valves were absent and the lumen irregular and tortuous indicating past thrombosis and recanalisation. Attempts at the same time to show, by venography, "blowouts" beneath the ulcer bed were unsatisfactory, owing to the multiplicity and overlap of the veins. Probably three dimensional radiograms would improve the localisation of these "blowouts" making surgical attack thereon more simple and certain.

The surgical approach to those showing "blowouts" near the ulcer (either clinically or by radiography) was to excise the ulcer bed and the underlying "blowout" and to repair the defect by a split skin graft. Initial healing of the ulcer was employed in one case by merely skin grafting over the granulations as a first stage and then excising the "blowouts" by incision through this graft. This is difficult as the underlying fibrous tissue is still left and if undercut (as it was in this case) then the original graft is jeopardized and will necrose. It seems preferable to excise the granulations (along with the blowouts) without prior grafting. The ulcers so treated did not become infected, provided the granulations were clean and firm beforehand.

*Follow-up of sequelae of thrombophlebitis of the legs* was retrospective as the sequelae following recanalisation require several years to produce symptoms. Those patients reviewed showed a high percentage of sequelae, mainly oedema and varicose veins, but the number of ulcers present was much lower than figures quoted by other workers. For this reason a further project is being undertaken to follow-up those cases seen personally who have suffered thrombosis and to record the subsequent development of complications.

## THE HEART'S ELECTRICAL ACTIVITY RESTING AND ACTION POTENTIAL OF CARDIAC MUSCLE CELLS

D. McKelvie and M. Quinn-Young.

During the year a project designed to enable the recording and measurement of membrane potentials with a micro-electrode inserted into a single muscle fibre of cardiac muscle has been started. Ultimately it is intended to study variation in membrane resting and action potentials under the influence of various drugs.

Work carried out so far has been mainly construction of apparatus. A mechanical electrode puller for the manufacture of micro-electrodes from 3mm Pyrex glass has been made. The electrodes, being rather fragile, are used in fairly large quantities and it is important to keep their physical characteristics as uniform as possible. The machine is entirely automatic and makes electrodes with a tip diameter of 0.5 to 1.0 micron. They have a resistance of 10-50 megohm when filled with 3M potassium chloride solution by boiling under reduced pressure.

Various electronic devices necessary for recording and measuring the potentials have been constructed. First a cathode follower input stage mounted on a disused microscope stand carried the micro-electrode mounted directly on the grid of a 954 pentode. The output is direct coupled to a DC main amplifier and thence to a DC cathode ray oscilloscope. A calibrating voltage can be applied to the electrode tip to give a comparison voltage for measurement and also to enable the electrode resistance to be calculated.

The duration of action potentials is measured by comparison with time-making pulses obtained from an electronic circuit which converts a sine wave input into sharp pulses. This is driven from an audio frequency oscillator and is adjustable to provide timing marks from  $\frac{1}{2}$  sec. to 200 micro-seconds apart.

A stimulator locked to the time base sweep has been built to provide a controllable stimulus of up to 80 volts in the form of a flat-topped pulse whose length can be varied over the range of 20 milliseconds to 20 microsecs. The time of occurrence of the stimulus in relation to the start of the time base sweep is also adjustable. The duration of the time base sweep is adjustable from one sweep in 10 seconds to one sweep in 1 millisecond.

In the initial stages until the technique of insertion of electrodes and obtaining reproducible and accurate results has been perfected, the sartorius muscle of the toad *bufo marinus* is being used as the values for this preparation have been well established by other workers.

## THE EFFECT OF TEMPERATURE ON THE ELECTROGRAM OF ISOLATED TOAD ATRIUM PREPARATIONS

D. Emslie-Smith.

Previous work on hypothermia has shown that cooling both man and dog to temperatures of about 30°C produces similar and consistent changes in the ST segment and T wave of the electrocardiogram. The effect of temperature upon the electrogram of the isolated toad atrium was investigated to determine whether the changes seen in man and dog might be interpreted in terms of some intrinsic property of cardiac muscle.

Cooling the preparation produces little alteration in the contour of the polyphasic electrogram but produces progressive lengthening in the various measured intervals. Over the temperature range tolerated by the preparation, from about 30°C to 9°C, the duration of the spread of depolarization and the duration of the depolarized state both appear to lengthen with cooling in a manner consistent with the Arrhenius equation which describes the velocity of simple chemical reactions as a function of temperature. The rate of repolarization is depressed proportionally more than the rate of depolarization.

Frequently the effect of cooling is characterized by an abrupt change in energy of activation at a critical temperature (about 18°C). This sudden alteration may be an artefact, but the factor which is responsible appears to exert opposite effects on the two processes of depolarization and repolarization.

The study therefore raises some interesting theoretical problems about the nature of the accession and regression phases of the atrial electrogram and their relationship to the excitation process in cardiac muscle.

## **VECTORELECTROCARDIOGRAPHY\*\***

**T. E. Lowe and J. M. Gardiner.‡**

The routine collection of data towards an assessment of the clinical value of this technique is still being carried on, and it is proposed to link these findings with data obtained from the spatial magnitude electrocardiograph which has been previously described.

## **VIBROCARDIOGRAPHY—STUDY OF CARDIAC VIBRATIONS\***

**Margaret Down.**

Continuing on from the study of vibrations in the normal heart the cardiac vibrations in patients with hypertension or mitral stenosis have been recorded. The vibrocardiograph, described previously, was used. Tracings were taken at the mitral, tricuspid, pulmonary and aortic areas. In most of the cases of mitral stenosis studied, a recording medial to the apex beat was also made. Detailed study of vibrations in terms of time of onset after the Q deflection of the electrocardiogram, duration and qualitative energy content were made, and statistical analyses performed.

### **Hypertension**

These cases were selected at the Clinical Research Unit Clinic for hypertensive patients. The number of satisfactory tracings was 34; of these 17 were male and 17 female patients. The age ranged from 34 to 65 years. In all cases the systolic blood pressure exceeded 140 mms Hg. and the diastolic blood pressure 100 mms Hg.

Clinical findings were variable. The heart was not necessarily clinically enlarged. At auscultation there was a consistently accentuated second sound principally at the aortic area but also heard well over the entire praecordium. All cases exhibited a systolic murmur at the apex which varied from case to case in quality and duration, being either soft or loud and blowing; or short or long.

‡Cardiovascular Diagnostic Service, Alfred Hospital.

As in the normal cases previously described (1955), there were two Main Vibration Complexes. Within each of these was seen the Principal Vibration, the Fundamental Vibration, frequently associated with harmonic vibrations in higher frequency channels, and occasionally Chest Waves were distinguished. As part of the First Vibration Complex, Preliminary Waves occurred at times between the P and S deflections of the electrocardiogram.

The vibrocardiogram of hypertensives displayed certain characteristic features—

The Preliminary Waves were more frequently seen and were of greater amplitude and intensity than in normals; both the Main Vibration Complexes, more particularly the first, were of greater than normal amplitude and intensity. Aortic tracings were more readily recordable than normal. Harmonics, in higher frequency channels, associated with the second Main Vibration Complex were very frequent and occurred at all areas in almost every case.

Statistically, when expressed as a percentage of cycle length, the time of onset of vibrations, after the Q deflection of the electrocardiogram of the mitral First Vibration Complex in channel 1\*, showed a significant difference in the means of normals and hypertensives ( $P < .001$ ). This difference is due to the common occurrence of Preliminary Waves in the hypertensives. When expressed as a percentage of cycle length the duration of the two main Vibration Complexes in channel 1 at the mitral areas and the first Vibration Complex (channel 1) at the aortic and pulmonary areas were all significantly prolonged in cases of hypertension ( $P < .001$ ).

### Mitral Stenosis

Satisfactory recordings numbered 16, of which 4 were male, and 12 female patients. The age ranged from 22 to 57 years. All cases were normotensive—the systolic blood pressure not exceeding 140 mms Hg., nor the diastolic blood pressure 90 mms Hg.

Clinically the heart was not necessarily enlarged. In 15 of the 16 cases, the first sound at the mitral area was markedly accentuated, and there was a mid-diastolic murmur with or without a presystolic accentuation. The 16th case had no diastolic bruits clinically, but showed mitral valve calcification at radiography.

Although the recordings showed similar vibration complexes to the normal cases, certain dissimilarities were noted. Although Preliminary Waves were present in only 4 of the 16 cases, their qualitative amplitude was increased. In 11 of the 16 cases there was a marked increase in harmonic vibrations in higher frequencies associated with the first Main Vibration Complex.

In 5 of the 16 cases, a low amplitude vibration of approximately 100 c.p.s., was recorded at the apex and/or tricuspid areas. The occurrence varied from 0.04 to 0.07 seconds after the second Main Vibration Complex. The duration ranged from 0.02 to 0.03 seconds, and harmonics were demonstrable in higher frequency channels in decreasing intensity and duration.

\*Channel 1 records vibrations with a frequency between 0.5 and 50 c.p.s.

In 7 of the 15 cases where there was a mid-diastolic murmur detected clinically, there were noted vibrations of 30 to 40 c.p.s. following the second Main Vibration Complex. The vibrations were variable in duration, either extending throughout the interval between the second and first Main Vibration Complexes, or occupying only a segment of this interval. It is to be noted that these vibrations which might reasonably be considered to correspond to the clinical diastolic murmur are not easily recorded on our machine.

Statistically the number of cases of mitral stenosis studied is too small to be of any significance, and tests applied showed no significant difference from normal cases in time of onset or duration as percentage of cycle length.

## ENERGY PRODUCTION IN THE MYOCARDIUM\*

W. G. Nayler.

The modified Warburg type apparatus previously developed to study the relationship between the work capacity and metabolic activity of the isolated toad heart has been used throughout a series of experiments without any further modification.

In brief the preparation used consists of a Straub recycling heart placed in the modified Warburg respiratory chamber from which outlets are provided for the measurement of the maximum aortic pressure, number of drops per beat and beats per minute. Changes in oxygen uptake and carbon dioxide output were observed simultaneously with the above.

This preparation has proved to be reliable and stable during the initial four-hour perfusion period and all observations have been made within this time interval.

### Action of the Cardiac Glycosides

In a series of preparations the action of strophanthin-C (final conc.  $1 \times 10^{-5}$  and  $1 \times 10^{-6}$ ), digoxin (final conc.  $1 \times 10^{-6}$ ) and lanatoside C (final conc.  $2 \times 10^{-5}$ ) has been observed and measured. These three glycosides displayed a uniform pattern of response, reflecting a marked increase in efficiency and work capacity of the heart associated with increased oxidative metabolism. The glycosides failed to produce any significant change in the respiratory quotient values.

Thus, with a respiratory quotient value of 0.95, the addition of strophanthin C ( $1 \times 10^{-5}$ ) to the heart preparation resulted in 70% increase in efficiency, this increase being apparent over a prolonged time interval. Likewise digoxin and lanatoside C produced a 60% increase in efficiency without any alteration in respiratory quotient values.

It is interesting to compare this marked and well sustained increased efficiency following the administration of the glycosides with the small and poorly sustained increased efficiency previously noted after the addition of adrenaline in a similar series of experiments. Like the cardiac glycosides, the sympathomimetic amines adrenaline and nor-adrenalin both produced a marked increase in oxidative metabolism.

Throughout this series observations have been made at  $25.0 \pm 0.05^\circ\text{C}$ , using glucose as the sole exogenous substrate.

### **Ionic Changes**

In a further series of experiments the inotropic response resulting from the addition of 9-a-fluorohydrocortisone to the heart preparation has been investigated. This response appears to be identical with that noted above in the case of the glycosides, i.e., a marked and sustained increase in efficiency, work output and oxidative metabolism associated with a lack of any apparent change in the respiratory quotient values.

These results are particularly interesting since it is established that 9-a-fluorohydrocortisone enhances the rate of uptake of sodium from the perfusion fluid.

The inotropic response recorded following the addition of calcium to the hypodynamic heart appears to be identical with that noted in the case of the cardiac glycosides.

These results appear to indicate that the inotropic response due to the glycosides, calcium ions and 9-a-fluorohydrocortisone is not due to any changed metabolic substrate but rather due to an increased efficiency in energy utilization. The increased oxidative metabolism associated with the change in mechanical activity may be due to a change in high energy phosphate level.

### **Seasonal Variations in Cardiac Metabolism and Response to Strophanthin G**

Respiratory quotient values of hearts perfused in the standard apparatus at  $25.0 \pm 0.05^\circ\text{C}$  and using glucose Ringer Solution displayed marked seasonal variations.

From October to April values 0.90-0.95 were recorded; in May the average value fell to 0.85, a minimum of 0.70 being recorded throughout July and August. During October the respiratory quotient value returned to the summer level.

Similarly the rate of glucose utilization and glycogen content of the ventricle (estimated by the Anthrone method) showed comparable seasonal changes. Thus the glycogen content and rate of glucose utilization was minimal during the period of natural hibernation.

During hibernation, an inotropic response was not recorded following the addition of strophanthin G ( $1 \times 10^{-5}$ ) to the preparation. Substrates other than glucose-pyruvate, lactate, succinate, propionate, glycerophosphate, glycerol and glycine—failed to restore the normal inotropic response in the hibernating heart. Insulin (5U/litre) and the intramuscular injection of anterior pituitary extract (2-5 days before use) both restored the response of the isolated heart to the glycoside without restoring the respiratory quotient value to the summer level.

These results suggest that some change in permeability of the cardiac cell membrane occurs during hibernation, thereby slowing the rate of entry of the glycoside into the cell. Since exogenous substrates other than glucose did not restore the inotropic response it seems unlikely that the lack of a suitable substrate is involved in the seasonal changed response to the glycoside.

## OPEN INTRACARDIAC SURGERY

D. R. Stirling, J. M. Collison† and D. Harrison.‡

The last twelve months or more have seen the exit of hypothermia from the field of Cardiac Surgery in most centres, and the entrance on to the stage of the pump oxygenator and its successful use in clinical cases. The American School at Minneapolis under the leadership of Lillehei and Varco have produced the most stimulating work and have applied their findings to some 200 human patients with cardiac defects.

In keeping with general opinion, we too have forsaken hypothermia and turned our attention to by-pass of the heart by mechanical means.

Our work during the year has covered various fields, namely: the pathology of hypothermia, the use of various plastics as arterial grafts, the development of a means of by-passing the heart and lungs for periods of up to one hour.

### Hypothermia

Our interest in the pathology of hypothermia developed as a result of routine pathological examination of the hearts taken at post-mortem from animals subjected to hypothermia in 1955. The hearts of many of these animals were subjected to defibrillation, cardiac massage and intracardiac injections, which complicated the interpretation of the findings.

A control series of 4 animals was cooled to terminus at 18-20°C over a period of 4-5 hours. Using special staining methods, the histology of the myocardium of all hearts was investigated in sections from upper and lower regions of both ventricles and the septum. Two changes were consistently recorded. A loss of striation of isolated twigs, or small foci, of muscle fibres was seen in all hypothermic hearts. The other change was a bizarre pattern of intensely staining transverse bands interrupting the normal fibre structure. This was commonly an extensive sub-endocardial or sub-epicardial strip. These bands were hyaline and frequently had a larger diameter than the adjacent normal fibre. The length of the band varied from just larger than striation size, to several times the fibre diameter.

These two changes appear to be seen more frequently in the animals subjected to defibrillation and massage than in the control animals with pure hypothermia to terminus.

Our results partly confirm the worth of Samuli Saraja in Stockholm, who described hyaline necrotic muscle fibres with frequent instances of moderate cellular reaction. Little evidence of cellular infiltration has been recorded throughout our investigations. No other reports of the disruption of fibres by hyaline bands have been found.

The significance of the changes seen will be difficult to assess until a series of hearts from normal dogs have been investigated with similar procedure as the hypothermia hearts. There is evidence that these changes described are not peculiar to hypothermia, but have been seen occasionally in hearts from cross-circulation experiment animals.

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‡Department of Pathology, Alfred Hospital.

## Arterial Grafting

In the second field, under general anaesthesia, a portion of the abdominal aorta below the origin of the renal vessels was resected and was replaced by Ivalon, which has some degree of elasticity.

During these experiments notice was taken of graft thickness, the need for repair in such grafts, the use of gel foam around the grafts to prevent oozing and the relation of these various factors to patency of the vessel post-operatively and to pathological changes.

Animals on which this procedure was carried out were sacrificed at various intervals and the grafts examined macroscopically and microscopically.

From these observations it is concluded that clamping of the aorta below the renal vessels, for periods up to one hour, produced no ill effects.

The majority of the grafts were patent clinically post-operatively and thrombosis was seen at post-mortem only once in seven specimens.

Correct thickness for the grafts was difficult to obtain for too thick a graft was hard to manage surgically, and too thin a graft tended to leak blood, after reconstitution of the circulation.

Microscopic examination showed that a new intima was formed in the graft and this was separated from the layer of Ivalon by a thin (in some cases thicker) layer of organised blood clot which was only loosely attached to the Ivalon itself. Tissue reaction to Ivalon was not marked.

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The above projects are but the tempting by-ways explored as we have followed the main road toward open cardiac surgery. We are not attempting to pioneer the way but are following the road already traversed by the workers in Minneapolis, U.S.A., who have been so successful.

The problem in open heart surgery is to provide an adequate time (up to one hour in some cases) and adequate exposure of a dry heart in which to carry out repair of defects.

The best means of obtaining these requirements is to occlude the heart from the circulation and to provide a mechanical pump as the heart and have a means of obtaining oxygenation of venous blood removed from the body.

During the year we have tried three means of providing oxygenation of venous blood. First by using the heart and lungs of another animal, i.e., cross circulation; secondly, by having a reservoir of arterialized or oxygenated blood and pumping this into the arterial side whilst a similar quantity is removed from the venous side of the animal. This is excellent but the volumes of blood required for anything but the smallest animal are prohibitive; thirdly, the use of an oxygenator as the lungs, and a pump as the heart, to remove blood from the body and return it again.

Cross circulation was found to be much more satisfactory than hypothermia, but this was passed over in favour of using a pump oxygenator because of difficulties on moral and ethical grounds when dealing with patients.



The oxygenator we are using is simply made of plastic tubing and disposable after use. It is a copy of the one designed by the Minneapolis group. Venous blood is taken from the patient and the fine bubbles of oxygen are mixed with the blood in a long vertical plastic tube. The blood then passes into a defoaming chamber where by means of an antifoam silicone the bubbles are broken and excess oxygen and carbon dioxide are allowed to escape. The blood then passes into a helix tube which acts as a reservoir of blood and a place where further oxygen and carbon dioxide may escape. Arterialized blood is then pumped from this helix tubing and returned to the patient.

Blood passing to the oxygenator is removed from the I.V.C. and S.V.C. and is returned to the subclavian artery.

Our work has been hampered by the lack of a satisfactory type of pump and it is only since the recent arrival of the Sigmamotor pumps from the U.S.A. that we have been able to make much progress towards our ultimate goal. We have accumulated, however, valuable information about haemolysis and the effect of the apparatus on red and white blood cells, platelets, etc. We have studied pH changes and serum chemistry changes which occur due to the passage of blood through the apparatus and due to the reduction of cardiac output.

There is a wide field from which to pluck information and we have touched on the outskirts only as regards these basic factors.

Our year's work has been very satisfactory because now we can exclude the heart for fifteen minutes from the circulation and we have had a series of surviving animals after this procedure. This places the field of human surgery within our grasp.

## ABNORMAL SERUM PROTEINS

C. C. Curtain.

The previous work on abnormal serum proteins is being continued by the application of the immunochemical techniques of Coons et al. to the investigation of the paraproteins of multiple myeloma. This technique involves the preparation of rabbit antibodies to the paraproteins, conjugating the antibodies with a fluorescent label, applying the conjugate to sections and smears of tissues from myeloma patients and observing the disposition of the label under the fluorescence microscope. There are several serious difficulties in the application of this technique. The first is the tendency of the conjugates to stain non-specifically. This cannot be eliminated in human bone marrow by absorption of the conjugate with rat liver or other animal organ powder as suggested by Coons. It has been found, however, that non-specificity can be minimised by rigorous purification of the antibodies followed by absorption with human spleen powder after labelling. A second difficulty arises from uncertainty regarding the antigenic structure and "uniqueness" of the paraproteins. An extreme view is represented by those who claim that they contain no antigenic determinants other than those normally present in the plasma proteins—which would condemn to failure any attempt to demonstrate the presence of myeloma protein in tissues by an immunochemical technique. On the other hand there is much in the literature to suggest that many cases of myeloma do produce proteins which are antigenically distinct from any found in the serum of normal individuals.

Consequent to this controversy, it has been necessary to start a collateral investigation into the antigenic relationships between the proteins obtained from the cases of myeloma under study and various fractions of human serum. The established techniques of supernatant analysis and double diffusion in agar are being used. To supplement these an attempt is being made to exploit the phenomenon of immunological tolerance in rabbits. It has been shown by a number of workers that exposure of the rabbit to a particular antigen from birth results in an inability to produce antibody to this antigen, although, usually the animal will react normally to the injection of another, unrelated protein. The rabbits used in the present study are being made tolerant to normal human serum.

The initial results of these studies have shown that it is possible to obtain highly specific antibodies to a number of myeloma serum proteins and to a few Bence-Jones proteins. Limited microscopic studies with the fluorescent conjugates of these suggest that the serum and the Bence-Jones proteins are produced in the cytoplasm of the marrow plasma cells and are found in negligible amounts in other cells and tissues.

## ASSAY OF PITUITARY GONADOTROPHIC HORMONES

E. L. G. Beavis.

During the past twelve months work on the development of an assay method for Pituitary Gonadotrophic Hormones has been carried out.

In order to reduce the variation in the response of different mice to the same dose of extract, four selected strains of mice were inbred for several generations. The progeny of each strain were then tested by giving a given dose of an extract to those animals which fulfilled the requirements regarding age and weight. The mice of the strain which showed the least variation were then selected for further breeding. By this means a highly inbred strain has been developed, and it appears to have diminished the degree of variation.

Using a standard preparation of pituitary gonadotrophin\* it was shown that the response of the mice used in the assays, in this laboratory, was the same for a given dose as that described in the literature.

Further tests using this standard powder demonstrated that with a small increase in the dose, there was a marked increase in the response, which is an important property of the assay method.

Further experiments were performed to determine the efficiency of the method of extraction, by re-extracting known amounts of the standard powder and estimating the percentage recovery.

The results of these latter experiments were extremely disappointing and reference to the literature indicates that similar problems have been experienced by others in this field.

\*Standard was supplied by courtesy of Organon Laboratories Ltd.

## STUDIES ON MYCOBACTERIA

### MYCOBACTERIUM BALNEI

J. C. Tolhurst.

*Mycobacterium balnei* was first described in 1951 by Norden and Linell.† It produces granulomatous lesions of the skin in man. It resembles *Mycobacterium ulcerans*, which also causes skin lesions in man, in that lesions are produced at low temperature sites in susceptible animals, as for example, in the scrotum and in the skin and subcutaneous tissues of the limbs and tails of mice. Both organisms grow on the egg yolk media used for the cultivation of the tubercle bacillus when the temperature of incubation is 30°C, but not when it is 37°C.

It is well known that some bacteria are susceptible to the toxic effects of minute amounts of unsaturated fatty acids, such as oleic acid, which are contained in various media, and that these effects can be neutralized by the addition of bovine serum albumin or of activated charcoal.

Experiments with *Mycobacterium balnei* have shown that this organism is more susceptible to the action of oleic acid at 37°C than at 30°C. Certain media containing oleic acid fail to support growth at 37°C unless very large inocula are used; yet small inocula grow readily at 30°C. When either serum albumin or activated charcoal is incorporated in these media, small inocula are enabled to grow at 37°C.

These observations are in line with our knowledge of the enhancing effect of temperature on the action of soaps on bacteria. They do not explain the apparent preference of *Mycobacterium balnei* for growth in low-temperature sites in the tissues, but are important because of their general implications. Thus cultural methods of diagnosis sometimes fail, as for example, in some cases of subacute bacterial endocarditis and in brucellosis, when the size of the inoculum available may be small. It is suggested that incubation at temperatures below 37°C and attempts to neutralize the toxic effects of fatty acids in media should be considered in any infection where difficulty in cultivating bacteria is experienced.

### MYCOBACTERIUM ULCERANS

#### 1. Laboratory diagnosis of infection

J. C. Tolhurst and G. Buckle.

A child from Bairnsdale was recently admitted to hospital suffering from an ulcer of the leg caused by *Mycobacterium ulcerans*. The ulcer was about the size of a shilling and, although induration was present, there was no indication of the true extent of the infection. Surgical incision of tissue was required from above the knee to the ankle and the child was in hospital for five months. The great importance of early diagnosis was thus made evident and available methods were compared.

†See Linell, F. and Norden, A., *Mycobacterium balnei* and *Acta Tuberc. Scand.*, Suppl. 33, 1954.

(1) Microscopic examination of ulcers of the skin for acid-fast bacilli should always be made in spite of the presence of pyogenic organisms. If the ulcer is dry, saline washings should be collected with a pipette or syringe. A presumptive diagnosis can be made on the clinical picture and the demonstration of acid-fast bacilli.

(2) After treatment to destroy contaminants, culture on egg yolk media incubated at 30-33°C yields characteristic growth in from 6 to 8 weeks. Growth fails at 37°C and so excludes the tubercle bacillus.

(3) Inoculation of the foot pad of the mouse produces progressive swelling and ulceration (Fenner). This may commence within a week or two if the inoculum is large or may be delayed for 3 or 4 months if the inoculum is small. Since certain other acid-fast bacilli produce swelling of the foot, culture is still necessary to prove the identity of the organism.

(4) Inoculation of male rats intraperitoneally produces characteristic epididymal lesions and ascites in from 4 to 10 months.

It is concluded that the most rapid and satisfactory method of proving the diagnosis is by direct culture of material from the human ulcer.

## 2. Epidemiology

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

Studies on the epidemiology of infections with *Mycobacterium ulcerans* have been continued. Since this infection was first described in 1948 from Bairnsdale and Colac in Victoria, other cases have occurred in Bairnsdale, Yallourn, Sydney, and also in the Belgian Congo and in Mexico.

Thirty-one opossums in the Bairnsdale district were examined but no lesions were found.

A mouse trapped at Springvale showed skin lesions caused by an acid-fast bacillus not *Mycobacterium ulcerans*.

Skin lesions recently discovered in a herd of cattle at Oakleigh (N. A. M. Wellington) were caused by an acid-fast bacillus. They are under investigation.

Several calves were inoculated with cultures of *Mycobacterium ulcerans*. The animals became sensitised to tuberculin. They developed minor skin and subcutaneous lesions which regressed.

## STUDIES ON CHEMOTHERAPY

J. C. Tolhurst and G. Buckle.

Studies are in progress on the management of penicillin-resistant staphylococcal infections occurring in man and experimentally produced in animals.

†Pathology Department, Alfred Hospital.

‡Department of Agriculture, Victoria.

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- T. E. Lowe: "CONTROL OF BODY FLUID VOLUME IN MAN: FURTHER OBSERVATIONS ON INTAKE, OUTFLOW AND VOLUME OF BODY FLUID," *A/sian Ann. Med.*, Vol. 5 (1956), p. 42.
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- W. G. Nayler and D. McKelvie: "A METHOD FOR THE STUDY OF CARDIAC METABOLISM AND EFFICIENCY IN THE TOAD, BUFO MARINUS," *Aust. J. Exp. Biol. & Med. Sci.*, Vol. 34 (1956), p. 239.
- W. G. Nayler: "THE ACTION OF ADRENALINE AND NOR-ADRENALINE ON THE ISOLATED TOAD HEART," *Aust. J. Exp. Biol. & Med. Sci.*, Vol. 34 (1956), p. 247.
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- D. Emslie-Smith: "CHANGES IN THE ELECTROCARDIOGRAM DURING PREOPERATIVE HYPOTHERMIA IN MAN," *A/sian Ann. Med.*, Vol. 5 (1956), p. 62.
- Margaret Down: "VIBROCARDIOGRAPHY—A STUDY OF VIBRATIONS IN THE NORMAL HEART," *A/sian Ann. Med.*, Vol. 5 (1956), p. 54.
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- P. Fantl and H. A. Ward: "NUCLEOTIDES OF HUMAN BLOOD PLATELETS," *Biochemical Journal.*
- P. Fantl and A. G. Marr: "THE PRESERVATION OF COAGULATION FACTORS IN HUMAN PLASMA," *Aust. J. Exp. Biol. & Med. Sci.*
- Bryan Hudson and G. A. Bentley: "THE BIOLOGICAL ASSAY OF MELANOCYTE STIMULATING HORMONE," *Aust. J. Exp. Biol. & Med. Sci.*
- Bryan Hudson and G. A. Bentley: "THE NATURE OF THE PIGMENTARY DISTURBANCE IN ADDISON'S DISEASE," *A/sian Ann. Med.*
- J. C. Tolhurst: "THE ROLE OF THE HOSPITAL ADMINISTRATOR IN THE PREVENTION OF HOSPITAL INFECTION," *National Hospital.*

#### PAPERS SUBMITTED FOR PUBLICATION

- W. G. Nayler: "SEASONAL VARIATION IN CARBOHYDRATE METABOLISM AND DRUG SENSITIVITY OF THE ISOLATED TOAD HEART (BUFO MARINUS)."
- G. A. Bentley: "STUDY ON INHIBITORY ACTION OF ADRENALIN. PART I."
- G. A. Bentley and Bryan Hudson: "A COMPARISON OF METHODS FOR THE ISOLATION OF MELANOCYTE STIMULATING HORMONE FROM BLOOD."
- Bryan Hudson and F. E. Binet: "THE ACCURACY OF EOSINOPHIL COUNTS."
- B. McA. Sayers and F. G. Silberberg: "FURTHER STUDIES OF THE ELECTROCARDIOGRAPHIC SPATIAL MAGNITUDE CURVE (in the rabbit)."

## LECTURES DELIVERED DURING 1956

- "MANAGEMENT OF CONGESTIVE CARDIAC FAILURE" . . . . T. E. Lowe  
Postgraduate School of Medicine, London.
- "CONTROL OF BODY FLUID VOLUME" . . . . . T. E. Lowe  
Postgraduate Lectures, Middlesex Hospital, London, and Central Middlesex Hospital, London.
- "CONTROL OF BODY FLUID VOLUME" . . . . . T. E. Lowe  
Postgraduate Lecture, Auckland, N.Z.
- "PHYSIOLOGY AND PATHOLOGY OF BLOOD COAGULATION," P. Fantl  
Royal Australasian College of Physicians, Melbourne.
- "LECTURES ON BLOOD COAGULATION" . . . . . P. Fantl  
Universities of Detroit and Chapel Hill, U.S.A.
- "PARAHAEMOPHILIA (HYPO-PROACCELERINEMIA)" . . . . . P. Fantl  
International Haemophilia Symposium, New York.
- "COAGULATION INHIBITORS IN THE BLOOD OF PATIENTS WITH THE HAEMOPHILIC SYNDROME" . . . . . P. Fantl  
International Congress of Hematology, Boston.
- "RAYNAUD'S PHENOMENON AND SCLERODERMA" . . . . A. J. Barnett  
Royal Australasian College of Physicians, Melbourne.
- "USE OF GANGLIONIC BLOCKING AGENTS IN THE TREATMENT OF HYPERTENSION: A LONG TERM STUDY" . . . . . A. J. Barnett  
Alfred Hospital Clinical Society.
- "A METHOD FOR THE STUDY OF CARDIAC METABOLISM," W. G. Nayler  
Victorian Society for Pathology and Experimental Medicine.
- "SEASONAL VARIATIONS IN CARBOHYDRATE METABOLISM AND DRUG SENSITIVITY IN THE ISOLATED HEART" . . . . W. G. Nayler  
Royal Australian Chemical Institute.
- "THE CLINICAL SIGNIFICANCE OF VARIATIONS IN THE PHYSICAL CHEMISTRY OF PLASMA PROTEINS" . . . . . C. C. Curtain  
Royal Australian Chemical Institute.
- "THE SIGNIFICANCE OF CHANGES IN THE PLASMA PROTEINS" . . . . . C. C. Curtain  
Alfred Hospital Clinical Society.
- "THE GENETICS OF SOME HAEMORRHAGIC DISEASES" . . R. J. Sawers  
Royal Australasian College of Physicians, Melbourne.
- "THE GENETIC BACKGROUND OF HAEMORRHAGIC DISEASES" . . . . . R. J. Sawers  
Alfred Hospital Clinical Society.

- "VIBROCARDIOGRAPHY" . . . . . Margaret Down  
Victorian Society for Pathology and Experimental Medicine.
- "THE ROLE OF THE HOSPITAL ADMINISTRATOR IN THE PREVEN-  
TION OF HOSPITAL INFECTION" . . . . . J. C. Tolhurst  
School in Hospital Administration, University of Melbourne.
- "GROWTH STUDIES ON MYCOBACTERIUM BALNEI" . . J. C. Tolhurst  
Victorian Society of Pathology and Experimental Medicine.

**MONOGRAPH SERIES**

- No. 1. "PRACTICAL ANAESTHESIA."  
1932. (Asian Med. Pub.)
- No. 2. "SPREAD OF TUMOURS IN THE HUMAN BODY" . . . . R. A. Willis  
1934. (Churchill.)
- No. 3. "BLOOD CULTURES AND THEIR SIGNIFICANCE," Hildred M. Butler  
1937. (Churchill.)
- No. 4. "HUMAN TORULOSIS" . . . . . L. B. Cox & J. Tolhurst  
1946. (M.U.P.)
- No. 5. "THE PRACTICAL SIGNIFICANCE OF MODERN CARDIOLOGICAL  
INVESTIGATIONS" . . . . . T. E. Lowe, H. B. Kay & H. A. Luke  
1951. (M.U.P.)
- No. 6. "PERIPHERAL VASCULAR DISEASE" . . A. J. Barnett & J. R. E. Fraser  
1955. (M.U.P.)
- No. 7. "CHEMOTHERAPY WITH ANTIBIOTICS AND ALLIED DRUGS" .  
Jean C. Tolhurst, G. Buckle & S. W. Williams  
1955. (N.H. and M.R.C.)





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

Revenue Account for the Year Ended 31st December, 1956

EXPENDITURE.			INCOME.		
Drugs, Chemicals, Provisions, etc. . . . .	£930	19 5	Donations—		
Fuel and Lighting . . . . .	360	9 1	Thomas Baker (Kodak), Alice Baker and		
Instruments and Glassware . . . . .	1,060	6 0	Eleanor Shaw Benefactions . . . . .	£15,732	1 2
Insurance . . . . .	375	9 7	Mr. Edgar Rouse . . . . .	100	0 0
Library Maintenance . . . . .	722	17 7	Marian and E. H. Flack Trust . . . . .	400	0 0
Printing and Stationery . . . . .	187	2 4	Sir William Angliss . . . . .	100	0 0
Repairs and Renewals . . . . .	346	8 7	George F. Little Trust . . . . .	148	3 6
Salaries and Wages . . . . .	14,818	7 9	"Strathcona" Baptist Girls' Grammar School . .	1	1 0
Telephone . . . . .	119	9 9			
Sundries . . . . .	531	4 4		£16,481	5 8
Travelling Expenses . . . . .	2,797	0 9	Appropriation of Grant—Life Assurance		
			Medical Research Fund of Australia and		
			New Zealand . . . . .	1,970	15 5
					18,452 1 1
			Government Grant—		
			National Health and Medical Research Council . . . . .	2,681	13 7
			Interest from Investments—		
			Held by the Trustees of the Estate of the late		
			Thomas Baker—		
			Commonwealth Government Inscribed Stock	551	10 0
			Endowed to the Institute—		
			Commonwealth Government Inscribed Stock	178	2 6
			Australian Consolidated Treasury Bonds . .	16	5 0
			Grain Elevators Board Inscribed Stock . .	103	2 6
					849 0 0
			Sundry Sales . . . . .	287	0 6
					£22,249 15 2
					£22,249 15 2

