Research 1972

BAKER INSTITUTE

ALFRED HOSPITAL

The Baker Medical Research Institute derives its main financial support from the Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefaction. It is also dependent upon donations from private sources. The latter may be allocated to an Endowment Fund. Donations of $2 or more are permissible deductions for income tax purposes.

The Ewen Downie Metabolic Research Unit is a department of the 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.

Forty-sixth Annual Report of

The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute

(Including Alfred Hospital Clinical Research Unit)
(The Institute is affiliated with Monash University)

Sixteenth Annual Research Report of

The Ewen Downie Metabolic Unit

Reports of

Alfred Hospital Research Fellows

1972

Alfred Hospital, Prahran, Victoria, 3181, Australia.

Baker Medical Research Institute

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Alfred Hospital Research Fellows 1972

A. J. Barnett	"Medical Research"
R. C. Buckle	"Sol Green"
E. Cooper	"Sunshine Foundation"
A. V. Jackson	"Peter Grant Hay"
M. W. Johns	"Edward Wilson Memorial"
T. E. Lowe (Clinical Research Unit)	"Amelia Haigh (Heart)" "R. B. McComas" "Ian Gideon McLean" "Sartori" "A. A. Swallow"
H. A. Luke and L. M. Dugdale	"Frederick and Esther Michaelis"
E. Mäsiar	"Victor Y. and Margaret Kimpton" "E. H. Flack"
J. F. McL. Oldham	"Collier" "E. H. Flack"
D. S. Rosengarten	"Connibere" "S. W. Jones" "James Richardson"
H. P. Taft **F. R. Trinker**	"James and Elsie Borrowman" "J. F. MacKeddie" "J. R. G. and E. McKenzie"
M. B. Van Der Weyden	"James and Elsie Borrowman"
I. M. Williams	"Ringland Anderson" "E. H. Flack"

Appointed to Research Fellowships for 1973

E. Cooper	"Edward Wilson Memorial" "James Richardson"
I. A. Ferguson	"William Buckland" "A. A. Swallow"
B. G. Firkin	"E. H. Flack"
B. Ford	"Sol Green" "Ian Gideon McLean"
P. Garcia-Webb	"J. F. MacKeddie"
M. W. Johns	"Edward Wilson Memorial"
T. E. Lowe (Clinical Research Unit)	"H. M. Black" "Paul Henry Christie" "Amelia Haigh (Heart)" "Amelia Haigh (Rheumatoid Arthritis)" "Dr. Henry Laurie" "R. V. Sartori" "Connibere" "Collier"
J. E. Maloney	"R. B. McComas" "S. W. Shields"
A. Pitt	"S. W. Jones"
D. S. Rosengarten	"James and Elsie Borrowman"
J. R. Stockigt	"E. H. Flack"
H. P. Taft	"Frederick and Esther Michaelis" "Victor Y. and Margaret Kimpton" "J. R. G. and E. McKenzie" "George Merriman"

Travel Grants

W. J. Spicer
N. D. Yeomans

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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. The Institute was formally affiliated with Monash University in 1965.

The Clinical Research 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 is 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.

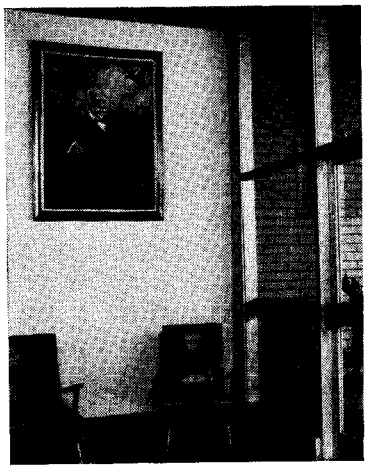
In 1956 the Board of Management formed a Diabetic and Metabolic Unit, which is engaged in investigation of endocrine and allied disorders. In 1969 this unit was renamed The Ewen Downie Metabolic Unit. This has also been placed under the supervision of the Research Advisory Committee.

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 Ewen Downie 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 specialities 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 during the past year illustrating this concept.



Baker Medical Research Institute

Staff

Director	T. E. LOWE, C.B.E., D.Sc., M.D., F.R.C.P., F.R.A.C.P.	
Associate Directors	A. J. BARNETT, M.D., F.R.A.C.P., M.R.C.P. WINIFRED G. NAYLER, D.Sc. (to 31/7/72).	
Administrative Assistant	R. BLAKEMORE, L.L.B.	
Graduates	VALERIE CARSON, M.Sc. ANNABELLA CHANG, B.Sc., Ph.D. E. COOPER, M.B., B.S., F.R.A.C.S. P. DAILE, B.Sc. P. FANTL, D.Sc., F.R.A.C.I. (deceased 30/8/72). G. C. HARD, B.Sc., B.V.Sc., Ph.D. S. KATZ, B.Sc., Ph.D. (to 16/5/72).	T. D. LEWIS, M.B.B.S., M.R.A.C.P. EVA MÄSIAR, M.D. P. MÄSIAR, M.D., D.Sc. DENISE MILLAR, M.Sc. M. SHAW, B.Sc. F. G. SILBERBERG, M.B., B.S., M.R.C.P. FEDORA R. TRINKER, M.B., B.S., Ph.D.
Technical	R. P STEELE (Laboratory Supervisor). Miss J. DIXON (Senior Technologist). A. H. HUCKFIELD (Technical Officer). D. G. BRUCE (from 10/4/72). Mrs. B. M. DOBRASTANSKY (from 14/8/72).	Mrs. R. MUSCUTT. D. G. OAKLEY. Mrs. J. ORTIZ. Mrs. E. RICHARDS (to 1/9/72).
Librarian	Mrs. M. RAE.	
Clerical	Mrs. JUDY MEYER (to 10/3/72). Mrs. FIONA PITHER (from 27/3/72 to 1/9/72). Mrs. CLAIRE McLEAN (from 11/9/72 to 10/11/72).	Miss JANET CLARK (from 4/12/72). Mrs. E. KERN Miss C. MARAZZITA.
Laboratory	P. BENNETTS D. BECKER. D. BERRY. Miss H. CHARANIEKA (to 10/3/72). Miss M. COGDEN. K. HARVEY. Miss E. HEWLETT (to 18/8/72). Mrs. B. HUNTLEY (nee Reeve) (to 1/12/72).	Mrs. K. JONES (to 23/6/72). Miss P. KIRCHNER. R. LOWE (from 21/2/72). Mrs. A. MOORE (nee Green). Mrs. E. PAYNE. Mrs. D. PEARCE (nee Schlunke). Miss A. SCHUTE (from 1/11/72). T. STOBART (from 28/2/72 to 13/10/72).
General	Mrs. M. WRIGHT.	

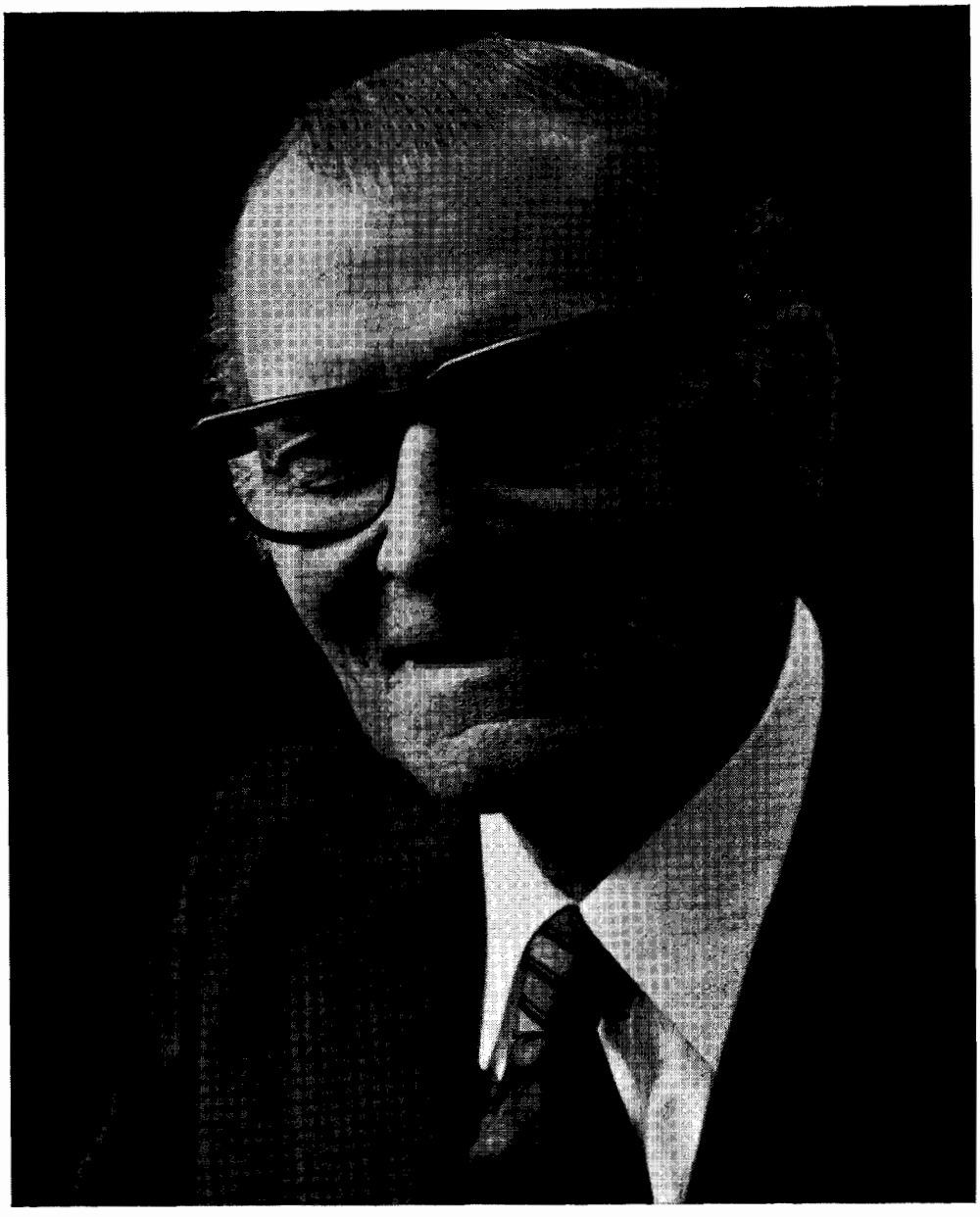
Ward Staff

Registrar	A. HUNTER, M.B., B.S.	
Resident Medical Officers	KAY ANDERSON, M.B., B.S. T. EGGERS, M.B., B.S.	W. MOORE, M.B., B.S. D. CLEEVE, M.B., B.S.
Ward Sister	Mrs. P. NEWELL.	

Research Fellowships

Members of the Institute Staff have held the following Research Fellowships

Honorary	E. COOPER, M.B., B.S., F.R.A.C.S. F. G. SILBERBERG, M.B., B.S., M.R.C.P.
Consulting Fellow	WINIFRED G. NAYLER, D.Sc. (from 1/9/72).
Anti-Cancer Council of Victoria ("A. A. Thomas")	G. C. HARD, B.Sc., B.V.Sc., Ph.D.
"James and Elsie Borrowman"	A. CHANG, B.Sc., Ph.D.
"William Buckland"	P. FANTL, D.Sc.
"The Lang Fellow"	P. MÄSIAR, M.D., D.Sc.



THOMAS E. LOWE, C.B.E., D.Sc., M.D., F.R.C.P., F.R.A.C.P., Director since 1949.

Annual Report of the Director of the Baker Institute

This year is probably the last in which I shall write an annual report for the combined Baker Institute - Clinical Research Unit projects and it therefore seems appropriate to review in brief the research and other activities which have arisen from the successful fusion of these two organisations in order to provide a base for the future evolution of each.

Twenty Four Years

The period 1949 - 1972 has been an eventful one during which a fruitful association was maintained between the Baker Institute and the Clinical Research Unit of Alfred Hospital and in later years a now flourishing affiliation of the Institute was established with Monash University. The period has seen growth in physical size of the Institute from about 5,000 square feet to a new fully air-conditioned laboratory building of approximately 24,000 square feet net usable space. It has also been a period in which the financial support for research from both Hospital and Institute sources has been very greatly increased.

I was appointed to the directorship of both units during 1949 and after considerable discussions it was decided that the two units were to be functionally integrated and the director was asked by the Board of Management of the Hospital and the Trustees of the Institute to carry out Clinical Research and to establish a Cardiovascular Research Centre. These compatible aims were to be achieved by a study of cardio-vascular diseases in sick people, by studies into the physiology of normal individuals and by the application of any appropriate knowledge from any field of human endeavour. In addition it was hoped that training in research methods could be given to suitable persons.
In 1949 advances in scientific techniques were being used successfully to make measurements in investigations in physiology. At this time also these developments were being applied in an expanding manner for the diagnosis of cardiac diseases in man. Further, as surgeons had shown that cardiac surgery had become feasible they were demanding details in pre-operative diagnosis which could only be obtained by use of these new techniques. The most urgent need, therefore, was for this new centre to introduce rapidly into the hospital practice of cardiology the diagnostic techniques which were then evolving, both to bring hospital facilities up to date and to provide techniques for clinical studies of the research groups. Many of these techniques required equipment which, not being commercially available, had to be manufactured by ourselves.

In later years to satisfy another need the research group helped to introduce into hospital practice many newly developed drugs for the treatment of cardio-vascular and other diseases. This role still continues.
The first major project in investigation of human disease was a study of the mechanisms which control the fluid content of the body and later a study of energy production in heart muscle was begun and is still actively engaged in.

Investigations such as these embraced many facets of medical science and it was easy to make opportunities in them for any graduate who wished to obtain some experience of research methods. In the early years of this centre there was little opportunity to obtain such experience elsewhere in the hospital complex and our facilities were much sought after. In recent years the proliferation of hospital departments and of university departments on the campus, each with research facilities, has diffused this demand over them all.

We can identify three distinct types of activity of the group in this quarter of a century and they have continued, albeit at different intensities, throughout the whole time. These activities may be characterised as (a) development of techniques for the diagnosis of human disease; (b) training in research discipline; (c) basic studies of human disease; and they have been dominantly but not exclusively pursued in a cardio-vascular context.

Diagnostic Techniques

The aids to cardio-vascular diagnosis which were available to the clinician in 1949 were X-ray examination of the heart, electrocardiographic recording of the heart's electrical activity and simple measurements of bleeding and clotting times to assess blood coagulability.

The development of radiological methods of examination to their present high pitch was undertaken by H. A. Luke, Director of the Hospital X-ray Department, and forms no direct part of this story except that the combined work led to the publication of a monograph by T. E. Lowe, H. B. Kay and H. A. Luke setting out the practical significance of these and other methods.

With technical developments in the 1950's it became theoretically possible to record the pressure and flow of blood at any point in the circulation, to record the heart's electrical activity in any desired aspect, to record the heart sounds for study at a later time, to record the heart's motion and with biochemical techniques to follow in great detail the processes of blood clotting, whether normal, inadequate or excessive.

All of these techniques were investigated, frequently after first constructing the necessary equipment, and their practical usefulness assessed by clinical application. Because of the demands of the cardiac surgery group in the Hospital, catheterisation of and pressure recording from heart chambers was commenced in July, 1949, and has been continually refined since. At the same time measurement of blood flow in limbs by air plethysmography was commenced by A. J. Barnett.

Development of both the techniques and the theory of electrocardiography also went on from the beginning and included sagittal plane recording, vector-electrocardiography and spatial magnitude electrocardiography. These were all attempts to provide a three-dimensional appreciation of the heart's electrical activity.
In the course of these studies many gaps in our knowledge of the electrical activity of the heart and how it is conducted to the surface of the body became apparent and formed the bases for

further studies. Much of this work was greatly helped by B. McA. Sayers, a physicist who is internationally credited with commencing spatial magnitude electrocardiography. This study he and others still pursue overseas.

Other studies were based on recordings of the heart sounds and the heart's motion and attempts to analyse these both temporally and physically were made. Phonocardiography, vibrocardiography and ballistocardiography were all explored. Although by the end of 1952 these studies had progressed to the stage that a Cardiovascular Diagnostic Service could be formed in the Hospital to embrace the techniques of proved value, others continued to be investigated and if found of value transferred to the Service. The studies of blood flow in limbs were developed in conjunction with the X-ray Department and have formed the basis of the "Vascular Service" now supplied to patients by the Clinical Research Unit in conjunction with the Vascular (Surgical) Service.

In 1955 the use of radioisotopes in clinical medicine was investigated and a small "Radioisotope Centre" established. This was subsequently transferred to the Diabetic and Metabolic Unit and was ultimately to become the Department of Nuclear Medicine.

Prior to the creation of the Clinical Research Unit, P. Fantl had commenced in the Institute the series of investigations into the biochemistry of blood coagulation that were to make for him a world-wide reputation. In these investigations many new tests with which to study aberrations in blood clotting were developed and they made possible the detailed study of haemophilia by R. J. Sawers and also led to the establishment in 1961 of the Hospital Haematology Department which he heads.

In a similar way arising from a research interest in catecholamines, the Institute was able for several years to offer an Australia-wide service for the estimation of these substances in urine samples. This service was much used until simpler methods made an assay generally available

Research Training

The opportunities which the group have provided for graduates in medicine or biological sciences to obtain some research experience have been numerous and the extent to which they have been used is perhaps best illustrated in statistical form. Studies by various workers in the Institute have led to senior university qualifications including Doctorates in Science, Medicine and Philosophy and Masterships in Science. Seventeen past members of staff of this period currently hold high university, or equivalent appointments, in various countries and nineteen others hold senior visiting medical staff appointments at Alfred or other hospitals.

Studies of Human Disease

Most of the many investigations into human disease which have been carried out in these years have had two aspects, one directed to the basic problems of understanding the disturbance and the other directed to the application of new or existing knowledge to the management of the disease.

In planning a research programme in cardio-vascular diseases I took the problem of heart failure as a starting point and defined four major long-term projects concerned with it. At one time or another these have involved every worker and every facility of the Institute but some members of staff have gradually made one or other project their dominant field.

Body Fluid Volume Control

The first project was related to congestive cardiac failure which is associated with inadequate pumping power of the heart. The data accumulated from daily observations on patients (and animals) enabled a mathematical description of the relationship between body fluid volume and daily fluid output to be formulated and disturbances of this relationship in disease states to be noted. As these data were recorded before oral diuretics were introduced into clinical medicine they form a unique set of observations and the mathematical formulation derived with the help of B. McA. Sayers is used as an accepted parameter by workers interested in an appraisal of the behaviour of the circulation in terms of control systems.

Energy Conversion in Cardiac Muscle

The second project considered cardiac muscle as an energy converting device and experimental work was directed to elucidating the mechanisms by which the energy of the carbohydrate and fatty fuels in the blood stream was converted into the energy moving blood around the circulation. Breakdowns in this chain of events could lead to an inadequate supply of energy for moving the blood. With the aid of Sayers and G. A. Bentley we devised and proved a model system consisting of a toad's heart working in a respiratory chamber from which estimates of both the chemical energy input and the mechanical energy output could be measured. This enabled a crude assessment of the efficiency of energy conversions to be made and also provided a means by which aspects of drug action (e.g. digitalis) on the heart could be assessed, as could also the effect of changing the composition of the nutrient fluids. Work with this model pointed the way to many other model preparations using mammalian heart muscle strips, whole hearts (e.g. rat) and large animal hearts with chambers mechanically by-passed and also arterial studies in animals with hearts by-passed with heart-lung machines. All these methods had the common aim of determining how the energy conversions were made and controlled in cardiac muscle. In particular, the role of calcium ions and the action of the digitalis alkaloids has been studied in great detail.

This study of calcium ions and digitalis Winifred Nayler made her particular interest and carried the investigations to intracellular levels with modern techniques of ultracentrifugation and electron microscopy. Early in this investigation it became apparent that blood plasma contained a powerful unknown substance with inotropic action on the heart. Attempts to isolate this substance led to studies on various cardio-vasoactive plasma substances and one fraction isolated was given the name Kinekard.

In the characterisation of the physiological properties of this fraction I was helped by Nayler and for the biochemical properties by several biochemists and recently Paul and Eva Mäsiar have partially identified in the fraction an unusual octapeptide which may be one of its active principles.

Hypertension

The third of the projects was directed towards understanding the mechanism of causation and the treatment of high blood pressure — a condition often associated with some form of cardiac failure. The most outstanding feature of this project has been a clinical trial of drug therapy for hypertension, which A. J. Barnett has conducted for nearly 20 years, in a search for the drug with the best blood pressure control and the fewest side effects. The results of this trial emphasise the great increase in the expectation of life and reduction in morbidity produced by this drug treatment.
A less dramatic feature has been the study of the role of catecholamines in high blood pressure states and in arterial control which was begun by Bentley and recently extended by Fedora Trinker. This forms the basis for the present studies of β-blocking drugs both in the treatment of angina and control of cardiac muscle contractibility.

Peripheral Arterial Disease

The fourth of these projects arose from an interest in the changes in the arteries in atherosclerosis which lead to coronary thrombosis. Early, Barnett concentrated his interest on arteries in the limbs. Changes in these often produced gangrene which is a particular problem for patients with diabetes mellitus. Working closely with vascular surgeons and using techniques for localising the site of arterial blocks, the morbidity from occlusive disease of the peripheral arteries has been greatly reduced by surgical techniques which either unblock arteries or by-pass the block. Also arising out of this interest was a long-term clinical study of Scleroderma. Many of the results of the studies on arteries were embodied in a monograph by Barnett and J. R. E. Fraser and the data of the scleroderma project is in a monograph by Barnett.

Blood Coagulation

Prior to 1949 Fantl had commenced a study of the biochemical reactions concerned in the clotting of blood and he was credited early with the discovery of coagulation Factor V. The availability of his techniques naturally led to a study of various "bleeding" diseases, of which haemophilia is a major member. This study showed that there was more than one type of haemophilia and that there could be quantative differences in each type. A survey of the incidence of haemophilia in Victoria was also conducted. These advanced techniques demonstrated that replacement therapy had a place in the management of haemophilia and that major surgery could be successfully carried out with its aid. These techniques also became essential for patient management in the long-term anticoagulant therapy for intravascular thrombosis. Although the practical application of these methods was taken over by the Hospital's haematology service in the early 60's the fundamental coagulation studies were still being conducted by Fantl up to the time of his death this year.

Endocrinology

The metabolism of carbohydrates and one of its clinical disturbances — diabetes mellitus — had for many years prior to 1949 been an interest of various workers in the Institute. This work continued with attention gradually becoming focussed on the activity of the anterior pituitary gland and the investigations became related to both its diabetogenic and pigmentary functions and are associated with the names of J. Bornstein and Bryan Hudson. In 1956 these endocrine projects were combined with the Diabetic Instructional Clinic of the Hospital to form a new entity — the Diabetic and Metabolic Unit.

Carcinogenesis

The causation and treatment of cancer is a major medical problem and, although at first sight not relevant to a cardiovascular research centre, investigators have been encouraged to work in this field within the Institute with the aid of the Anti-Cancer Council of Victoria. These projects have enabled the skills of molecular biologists and of biochemists interested in chemical carcinogenesis to be made available to workers interested in intracellular processes in cardiac muscle cells. In the same way the biochemical skills needed to study immunoglobulins and haemoglobins in the blood components have been an invaluable help to those interested in the trace peptides of the blood plasma that have a cardio-vaso-activity. In these ways C. C. Curtain, C. Kidson and G. C. Hard amongst others have contributed to the general research theme of the Institute.

Gastro-intestinal and Respiratory Physiology

In a similar co-operative way a continuing interest in gastro-intestinal disease has been maintained by clinical members of the units who have been able to use the scientific skills available. A continuing, although intermittent, interest in respiratory diseases will become a major project in 1973 concerned with the physiology of respiration.

Cardiac Surgery

The cardiac surgery group of Alfred Hospital commenced an association with Institute workers in 1946 and ever since a happy co-operation has led to many joint studies such as the problems of pump-oxygenators for cardiac by-pass surgery, the effects of lowering body temperature in the hypothermia used to permit some forms of cardiac surgery and more recently the development of techniques for transplanting the heart from one animal to another. A study of the rejection phenomena following transplantation continues with the object of finding ways in which to control them. This co-operation has been two-way and these cardiac surgeons have developed for other Institute workers animal models in which to study the physiology and the pharmacology of both the heart and the peripheral circulation independently of each other.

Summary

In summary the activities of the combined units over the quarter century represent a significant contribution to the development of modern medical care in the Hospital, a significant contribution to post-graduate education in the medical and biological fields and an equally significant contribution to the knowledge of basic medical science. The recognised excellence of the scientific work was continually indicated by repeated invitations to various senior members of staff to attend meetings overseas and by the steady stream of research fellows arriving from overseas. An international seal of excellence was placed on the Institute's cardiovascular work by two events. In 1960 I was made President of the 2nd Congress of the Asian-Pacific Society of Cardiology and in 1967, when the American College of Cardiology organised a meeting dedicated to International Cardiology, I was invited to deliver their prestige "Overseas Lecture" on the nominated topic "Kinekard". The data assembled and the ideas generated by these numerous projects have been recorded in some 500 publications in various internationally recognised scientific journals.

The aim of establishing a cardio-vascular research centre of internationally recognised excellence has been achieved. It is, however, composed of several separate entities whose interdependence should be appreciated.

Current Research Projects

Detailed accounts of current research projects are given in the scientific section of this report. Apart from the study of one form of cancer chemically induced in rats and the study related to the gastrointestinal tract, they are all related to some aspect of the cardiovascular system. Three of the cardiovascular projects relate to clinical problems and have been of many years' duration. They are a clinical trial of various drugs used for the treatment of high blood pressure, methods for the diagnosis and treatment of peripheral vascular disease and the disease known as Scleroderma. These three are nearing completion as research projects and the contributions to scientific knowledge and to patient care that have arisen from them are reviewed in the scientific section.

Staff

A few but important changes in the graduate staff occurred during the year.

It is with regret that the death of Dr. Paul Fantl, D.Sc., is recorded and an obituary note is separately placed.

Dr. Winifred Nayler, D.Sc., resigned as Associate Director of the Institute at the end of July because of family reasons and has moved to London where she has accepted a position as Assistant to the Director of the Institute of Cardiology. This post carries with it a Senior Lectureship in the University of London. On her departure she was made a Consulting Fellow of the Institute and, since then and for 1973, a number of cardiovascular research projects jointly supervised by her and me continue to flourish. This is an appropriate place in which to record the indebtedness of the Institute for her services and help since 1955.

Dr. S. Katz, Ph.D., completed his tenure of the Edward Wilson Memorial Fellowship and returned to Canada.

Dr. T. Lewis, M.R.A.C.P., joined the group as Assistant Physician to the Clinical Research Unit and has commenced some research into gastro-intestinal physiology.

Miss A. Chang obtained her Doctorate in Philosophy during the year.

Late in the year Dr. J. Maloney, Ph.D., was appointed as a Senior Physiologist as from January 1, 1973. Together with two assistants he completed moving his laboratary equipment into the Institute before the end of December and next year will be actively prosecuting studies in respiratory physiology in conjunction with members of the Department of Medicine, Monash University.

Overseas Visits

Between February and July I made an extended visit to the U.S.A. and England to study the administration of medical research organisations and the current trends in medical research. A separate detailed report of this visit has been presented, but I take this opportunity to thank the numerous people in so many organisations who helped me so willingly and generously.

In May Dr. Nayler paid a short visit to Denmark and England to deliver papers on β-blocking drugs and the role of calcium in heart function.

Named Fellowships have been held by the following members of staff:
The Lang Fellow: P. Mäsiar, M.D., D.Sc.
William Buckland Fellow: P. Fantl, D.Sc.
James and Elsie Borrowman Fellow:
A. Chang, Ph.D.

Research Assistance

Many of the investigations recorded in this report have been supported wholly or in part by the Life Insurance Medical Research Fund of Australia and New Zealand, the Anti-Cancer Council of Victoria and the National Health and Medical Research Council, the National Heart Foundation of Australia and Alfred Hospital Research Funds, and this continuing assistance is gratefully acknowledged.

It is a pleasure to thank for donations those whose names are listed in the various financial reports and those who have so generously helped with the purchase of equipment. Especially welcome was the grant from the Government of the State of Victoria towards maintenance expenses.

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. The continuing close co-operation between the libraries of the Institute, Hospital and Monash University Medical School is of great benefit to our staff.

Considerable assistance has been given to us through the year by Heads and Staffs of various departments of the University of Melbourne, Monash University and the Australian National University: also by members of the Commonwealth Serum Laboratories, Commonwealth X-ray and Radium Laboratories and C.S.I.R.O., and also by the Honorary Medical Staff and Departmental Staffs of the Hospital. We thank them all for this continuing interest in our projects and their ready help. Such help as we have been able to give in return has been freely availed of, often in the form of lecture and tutorial assistance.

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

T. E. LOWE.
December 31, 1972.

Alfred Hospital Research Fellows in the Institute 1949-1972

Anderson, R. McD., 1953-55.
Andrew, R. R., 1949-55.
Barnett, A. J., 1949-50.
Baumgarten, A., 1962-64.
Beavis, E. L. G., 1955-56.
Boake, W. C., 1958.
Breidahl, H. D., 1952-53.
Burnside, K. B., 1951.
Cooper, E., 1962-72.
Coventry, D. A., 1968.
Daile, P., 1970-71.
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., 1959-60.
Kincaid-Smith, P., 1959-60.
McCutcheon, A. D., 1959, 1965-66.
McDonald, W., 1960-61.
McNeur, J. C., 1955.
McRae, C. J., 1955.
Mäsiar, E., 1972.
Murfitt, L., 1955.
Newman, H. C., 1954.
Parsons, P. J. 1951.
Quinn-Young, M., 1956.
Race, D., 1959-63.
Sawers, R. J., 1953-60.
Silberberg, F. G., 1953.
St. Clair, W. A., 1955.
Stern, W., 1954-55.
Stirling, G. R., 1955, 1969.
Swann, J. B., 1967.
Wagner, G., 1958.

Overseas Fellows

Dawson, J. B., 1961-63 (Oxford).
Emslie-Smith, D., 1955-56 (Dundee).
Hamilton, M., 1954 (London).
Jones, T. G., 1966 (London).
Katz, S., 1971-72 (Montreal).
Lumb, F. H., 1960-61 (London).
Marshall, R. J., 1957 (Belfast).
Moir, T. W., 1968 (Cleveland).
Mommaerts, W. F. H. M., 1971 (Los Angeles).
Nelson, C. V., 1969 (Portland, Maine).
Robertson, P. G. C., 1963-64 (Dundee).
Simpson, F. O., 1958-59 (Edinburgh).
Stevenson, M. M., 1957 (Belfast).
Thomson, J. W. W., 1959 (Edinburgh).

Rouse Library

List of Organisations which have made Gifts to the Library during the Year

Australian Medical Association.
Adelaide Children's Hospital.
Anti-Cancer Council of Victoria.
A.N.Z.A.A.S.
Austin Hospital.
College of Physicians and Surgeons, New York.
Commonwealth Department of Health.
Commonwealth X-Ray and Radium Laboratory.
Department of Health, New Zealand.
Department of Territories, Canberra.
Fox Chase Centre for Cancer and Medical Sciences, Philadelphia.
Halstrom Institute of Cardiology, Sydney.
Instituto de Biologia y Medicina Experimental, Buenos Aires.
Instit Pasteur, Algiers.
Institute of Human Biology, Goroka, TPNG.
Institute of Medicine and Veterinary Science, Adelaide.
Kanematsu Memorial Institute, Sydney.
S. Karger, Basel.
Medical Research Council, London.
Middlesex Hospital Medical School.
National Heart Foundation, Australia.
National Institute of Nutrition, Japan.
New York State Department of Health.
New York University College of Medicine.
New Zealand Medical Research Council.
Ophthalmic Research Institute of Australia.
Queensland Institute of Medical Research.
Rockefeller Institute, New York.
Royal Children's Hospital, Melbourne.
Royal Melbourne Hospital.
Royal Prince Alfred Hospital, Sydney.
Royal Women's Hospital, Melbourne.
St. Vincent's Hospital, Melbourne.
St. Vincent's School of Medical Research, Melbourne.
South African Institute of Medical Research.
Strangeways Research Laboratories, Cambridge.
Staten Seruminstitut, Copenhagen.
Thomas Optical and Scientific Co. Pty. Ltd., Melbourne.
University of Melbourne
University of Otago, New Zealand.
University of Queensland.
University of Sydney.
Universitatis Mariae Curie Sklodowska, Poland.
Walter and Eliza Hall Institute, Melbourne.
Wellington Medical Research Foundation.
World Health Organisation.

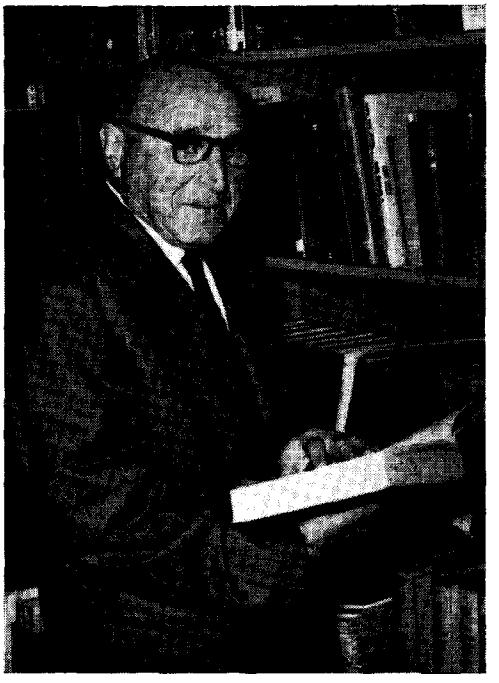
The library has been very busy during 1972 with ever-increasing inter-library loan activity and has, as usual, relied heavily on the resources of the Monash Clinical School and Alfred Hospital libraries.

From early in 1972 the Brownless Medical Library at Melbourne University ceased to make its periodicals available for loan thereby ensuring that its extensive collection was available at all times for reference. The inter-library loan service was replaced with a fast photocopy service which this library has used frequently during the year.
The rising cost of periodical subscriptions is reflected in the financial statements for this year.

Statistics

Monographs on shelves	728	
Journals received regularly	97	
Enquiries received during 1972 —		
from Staff	909	
from Outside Libraries	285	
	—	1,194
Requests to Other Libraries —		
for Loans	666	
for Photocopies	186	
	—	852

Paul Fantl D.Sc.



Paul Fantl, after a period of ill health, died in the Alfred Hospital on August 30, 1972.

He was born at Vienna, August 29, 1900, and became trained in organic chemistry and biochemistry, taking his doctor's degree in technical science (organic chemistry) at the Technische Hochschule zu Wien in 1923.

For a time he worked as a scientific advisor in an industrial laboratory concerned with the preparation of essential oils and natural and artificial perfumes.

From 1931 onwards, however, he worked in laboratories associated with hospitals, either in what would now be called routine biochemistry or in biochemical research projects which had a clinical slant.

Following the annexation of Austria by Germany in 1938, he left Vienna and after a short stopover in Trinidad, obtained an appointment at the Baker Medical Research Institute in Melbourne as a biochemist.

Arriving in Melbourne shortly before the outbreak of war in 1939, he soon found himself involved not only in some routine biochemistry for the Alfred Hospital, but also in a number of wartime projects sponsored by government bodies. These included studies about the feasibility of the manufacturing in Australia, certain drugs in short supply.

Following the cessation of wartime projects and shortly thereafter the transfer of responsibility for the hospital routine biochemistry service to a hospital department, Dr. Fantl was able to devote virtually his full time to research projects, for his duties as Consultant Biochemist to the hospital were not onerous. It was about this period in the late 1940's that he developed a project which was to study the biochemical reactions involved in the "clotting of blood" and to devise methods of controlling them for therapeutic purposes. This became a very successful venture which made him world famous in this sphere and occupied him for the rest of his life. In this connection his work took him on many trips to the Northern Hemisphere and he became a member of "the International Committee for the Standardization of Nomenclature of Blood Clotting Factors".

Apart from his own projects he took a lively interest in the biochemical aspects of other research projects in the Institute and Hospital. This close association that he maintained with others, especially his clinical colleagues, led many to believe, mistakenly, that Dr. Fantl was medically qualified — a tribute to his interest and understanding of their work.

In 1945 he was given some administrative responsibilities as Assistant Director of the Institute and during the illness in 1948 of the then Director, he acted as Deputy Director. Following the appointment of a new director, Fantl was made Associate Director in 1949, a task which he carried until 1966. After a period of leave at that time, he returned to his beloved laboratory on a part-time basis and was active until a very short time before his death.

To those who worked with him, Paul Fantl was much more than a talented colleague, he was always interested in the work of others and even though his comments and criticisms were often extremely frank, these were tempered by a real sense of humour which endeared him to all. His colleagues have lost a friend who was always a joy to be with.

Our sympathy is extended to his wife, his daughter, son-in-law and his grandson, whose loss is so great.

Report of Scientific Investigations

Physiology and Pharmacology of the Cardiovascular System

V. Carson, P. Daile, S. Katz, T. E. Lowe, E. Mäsiar, P. Mäsiar, D. Millar, W. G. Nayler, and F. R. Trinker.

Dog cardiac muscle showing cellular structure. Magnification X-13,500.

Cardiac muscle provides the motive force for the circulation of blood. It is composed of very large numbers of individual units (cells) which contract in unison. The efficacy with which these cells convert the energy of their fuel into mechanical energy determines the ability of the heart to do the work required of it.

Investigations this year have been directed to several aspects of this energy conversion. One study has been directed to the role of calcium ions as regulators of the intracellular processes. Another has been directed to some trace peptides occurring in blood plasma and cardiac muscle which can influence myocardial contraction. Another project has been looking at the control of blood flow to various parts of myocardium. Still another looks at the practical problem of interaction of clinically used drugs that have cardiac actions.

Myocardial Contractility †‡*

W. G. Nayler and T. E. Lowe

Within the muscle cell the contractile unit is the myofibril and within this the unit is the sarcomere. Each sarcomere is bounded by a z-band and contains an orderly but partially overlapping array of thin and thick filaments. The thin filaments consist mainly of actin and the thick filaments of myosin. In addition to actin the thin filaments contain two other proteins — troponin and tropomyosin — that have a modulatory or regulatory function. It is these proteins which make the whole of the contractile process sensitive to calcium ions.

Contraction probably involves the generation of a "sliding force" between the overlapping arrays of actin and myosin filaments. This generation requires the activation of a Mg^{2+}-dependent ATPase enzyme which is associated with a subunit of the myosin molecule. The actin-induced activation of this myosin ATPase enzyme is inhibited by the modulatory proteins if the concentration of ionised calcium falls below 10^{-7}M (approximately). One of the modulatory proteins — troponin — has a relatively high affinity for Ca^{2+} and it is

In this report of scientific investigations those projects marked (†) were supported wholly or in part by grants from Life Insurance Medical Research Fund of Australia and New Zealand; those marked (*) by the National Health and Medical Research Council; those marked (‡) by the National Heart Foundation; those marked (**) by the Anti-Cancer Council of Victoria.

generally agreed that as the intracellular availability of free Ca^{2+} increases an increasing number of binding sites on the troponin moiety of the troponin-tropomyosin complex will be occupied by Ca^{2+}. Under these conditions the inhibitory effect of the troponin-tropomyosin complex is suppressed and the actin-induced activation of the myosin ATPase activity can proceed. If sufficient ATP is available as a substrate for hydrolysis contraction will occur and the magnitude of the mechanical response will be determined by the activity of the myosin ATPase enzyme and by the amount of Ca^{2+} which is available to suppress the inhibitory effect of the modulatory proteins. Within this general scheme the transition from relaxation to contraction and vice versa reflects a regulated phasic change in the amount of Ca^{2+} which is available for interaction with the modulatory proteins.

In cardiac muscle cells regulation of the intracellular availability of Ca^{2+} is a complex process, both morphologically and biochemically. Excitation is known to promote an inwards displacement of Ca^{2+} from the extra to the intracellular phase. In addition it probably causes a release of Ca^{2+} from intracellular storage sites. The distribution of these sites is complex. Some are believed to lie immediately adjacent to the sarcolemma — i.e. the subsarcolemmal cisternae. The plasma membrane itself may act as a reservoir for Ca^{2+} and almost certainly Ca^{2+} can be released from binding sites within the deeper ramifications of the sarcoplasmic reticulum, and possibly from the mitochondria. Although there is convincing evidence from isotope and other studies that the ionised Ca which activates contraction in heart muscle is kinetically homogeneous in origin, morphologically its origin is heterogeneous.

Relaxation reflects the re-sequestration of Ca^{2+} probably by the sarcoplasmic reticulum. Biochemically this process is divided into a "binding" and an "uptake" process depending upon whether or not a precipitating agent is present. The accumulation of Ca^{2+} within the sarcoplasmic reticulum is accompanied by the activation of a Ca^{2+}-activated Mg^{2+}-dependent ATPase enzyme, so indicating that this binding is not merely a physical phenomenon but one which requires energy in the form of high energy phosphate bonds.

During the past year many experiments have been undertaken to gain additional information about the relative importance of the various morphological sites from which Ca^{2+} can be released for participation in the events associated with excitation-contraction coupling. In general, they indicate that the plasma membrane itself exerts a dominant role in this respect. Other studies have been aimed at providing additional information about the biochemical properties of the troponin-tropomyosin complex. Other investigations have concentrated on determining whether the sarcoplasmic reticulum contains sufficient 3'5' AMP to warrant future investigations into its biochemical function within this particular subcellular organelle (the sarcoplasmic reticulum). Morphological studies at the electron microscope level have been aimed at defining the distribution of the "T" invaginations of the sarcolemma and hence their importance in the inwards spread of the excitatory stimulus. It should be emphasised that these studies have been undertaken to provide a background of information against which a study of the various factors which regulate contraction in heart muscle can be continued at the subcellular level.

Positive Intotropic Effect of Cardiac Glycosides

W. G. Nayler

The view of many investigators that the positive inotropic effect of the cardiac glycosides was dependent upon the functional state of the myocardium was probably based on the belief that these drugs exerted an inotropic effect only if the heart had become hypodynamic. Recent experiments have shown, however, that these drugs exert their inotropic effect regardless of the functional state of the myocardium, and that it is characterised by a reduction in the duration of contraction, an increase in the rate of tension development and a decrease in the time to peak tension. These changes resemble those which occur if the peak tension developed during contraction is increased either as a result of an increase in the extracellular concentration of Ca^{2+} or in the frequency of contraction.

If the positive inotropic effect of the cardiac glycosides results from an increase in the intracellular availability of Ca^{2+} then the identification of the site from which this Ca^{2+} is derived is important. In previous experiments we have ruled out the possibility that this Ca^{2+} is derived from either the sarcoplasmic reticulum or from the mitochondria. Experiments were therefore undertaken to test the possibility that the additional Ca^{2+} was being derived from storage sites closely associated with the plasma membrane itself. In one experiment the plasma membrance was rendered freely permeable to Ca^{2+} and under these conditions strophanthin-G no longer exerted a positive inotropic effect. In another experiment Ca^{2+}, labelled with $^{45}Ca^{2+}$, was displaced from the plasma membrane by the trivalent cation La^{3+}. The amount of $^{45}Ca^{2+}$ which was displaceable by La^{3+} was increased by doses of strophanthin-G within the range 10^{-8} - 10^{-6}M.

These experiments led to the conclusion that doses of strophanthin-G, which exerted a positive inotropic effect on human and dog heart muscle, increase the capacity for Ca^{2+} of binding sites within the plasma membrane. As it is generally agreed that Ca^{2+} is displaced inwards from these sites during the rising phase of the cardiac action potential, it is not impossible that the positive inotropic effect of the cardiac glycosides results from their interaction with the plasma membrane in such a way that its ability to release Ca^{2+} for inwards displacement during the rising phase of the action potential is increased. Indeed it may be this same interaction with Ca^{2+}-receptor sites within the plasma membrane which results in the well-documented glycoside-induced inhibition of the plasma membrane located Na^+ - K^+ activated ATPase enzyme.

Negative Inotropic Effect of Ryanodine

W. G. Nayler

Ryanodine is an insecticide which has a marked negative inotropic effect on heart muscle, despite the fact that it causes contracture in skeletal muscle. Previous investigations have failed to show conclusively the mechanism responsible for this negative inotropic effect.

During the past year experiments with preparations in which the plasma membrane of cardiac muscle cells had been rendered freely permeable to Ca^{2+} and to which ryanodine was added were carried out. Under these conditions ryanodine failed to exert any inotropic effect irrespective of the dose used. Action potential studies have shown that the process of excitation is not changed by doses of ryanodine which abolish contraction. It seems probable, therefore, that the drug acts at the level of the plasma membrane in such a way that it weakens the link between excitation and contraction.

Effect of Beta-Adrenoceptor Antagonists

W. G. Nayler

The β-adrenoceptor antagonist drugs have a complex effect on cardiac contractility. Some of them, e.g. oxprenolol and prindolol, have weak sympathomimetic activity. Others, e.g. practolol and MK950, are practically devoid of intrinsic sympathomimetic activity, and some, e.g. propranolol, are without any intrinsic sympathomimetic activity and instead exert only a negative inotropic effect. In part this negative inotropic effect is due to the removal of sympathetic support, but experiments in many laboratories have shown that relatively large doses of β-adrenoceptor antagonists exert a negative inotropic effect quite apart from that which can be explained in terms of β-adrenoceptor blockade.

Experiments carried out during this past year have consistently shown that those β-adrenoceptor antagonists, which have a marked non-specific negative inotropic effect, interfere with the ability of the sarcoplasmic reticulum to bind Ca^{2+} and secondly, that this effect is accompanied by an inhibition of the Ca^{2+}-activated ATPase enzyme located in this reticulum. However, because there is a marked difference between the amount of β-adrenoceptor antagonist needed to depress cardiac contractility and that which is needed to inhibit the Ca^{2+}-accumulating activity of the sarcoplasmic reticulum, some other site of action may be involved in the mediation of their non-specific negative inotropic activity. The Na^+-K^+ activated ATPase enzyme was found to be unaffected by these drugs, but the ability of the plasmalemma to bind Ca^{2+} was found to be significantly impaired in much the same way as was previously observed in experiments in which verapamil was used. These experiments have helped to substantiate our conclusion that the plasma membrane, as well as the sarcoplasmic reticulum, represents a major site of drug action. Previously we have shown that relatively short periods of ischaemia in the dog exerted a marked deleterious effect on the ability of the plasma membrane prepared from cardiac muscle to accumulate and exchange Ca^{2+}. These observations show that it is necessary to determine whether the plasma membrane surrounding the myocardial cells of patients who have developed cardiac failure is able to accumulate and exchange Ca^{2+} in a manner which is comparable with that exhibited by the plasma membranes of non-failing cardiac muscle cells. Because changes in the extracellular concentrations of Na^+ occur early in some forms of cardiac failure in man experiments were undertaken to determine whether a changed intracellular concentration of Na^+ alters the ability of the plasma membranes to either accumulate or exchange Ca^{2+}. The intracellular concentration of Na^+ was enhanced (a) by a period of rapid stimulation; and (b) hypothermia. Generally either a raised intracellular or a reduced extracellular concentration of Na^+ enhanced the ability of the plasma membrane to both accumulate and exchange Ca^{2+}, indicating an increase in the size of the readily exchangeable pool of Ca^{2+} which is located either within or immediately adjacent to the plasma membrane.

Localisation of Adenyl Cyclase in Purified Microsomal Preparation of Cat Myocardium

D. Millar and S. Katz

The possible role of the accumulation of cyclic 3',5'-AMP (cAMP) in cardiac tiisue has been in contention since the initial discovery of adenyl cyclase in nuclear fractions of heart homogenates[1]. In 1965 evidence was put forward indicating that cAMP, already implicated as a second messenger in a variety of hormonal responses, was also involved in the positive inotropic response to adrenaline. These studies[2] indicated that adrenaline, and similar agents, acted first at the plasma membrane to stimulate adenyl cyclase activity thereby increasing the level of cAMP and ultimately leading to an increased strength of contraction.

In 1969 Entman **et al**[3] found adenyl cyclase activity associated with a microsomal fraction of canine myocardium thought to represent sarcoplasmic reticulum. This fraction of adenyl cyclase was stimulated by adrenaline and glucagon with the β-blocking agent propranolol abolishing the adrenaline stimulated activation. It was concluded from these studies that adenyl cyclase may play a role in the augmentation of sarcotubular calcium stores, and ultimately, in the increase in inotropy.

Recently other workers[4] have reported adenyl cyclase activity associated with "sarcoplasmic

reticular fragments" prepared from heart muscle. Analysis of the preparation procedures employed and the lack of proper indicators for mitochondrial and, specifically, plasma membrane contamination in these studies suggest that the activity found might not be native to the Ca^{2+} accumulating system of the heart.

The localisation of native adenyl cyclase in close proximity to the Ca^{2+} accumulating system of heart muscle and different from the plasma membrane bound enzyme would be of definite interest.

The aim of our work was therefore —

1. to develop an accurate, sensitive and readily performed assay for the measurement of cAMP which would be capable of detecting levels as low as 10^{-12}M;
2. to obtain a purified preparation of sarcoplasmic reticular fragments, employing the techniques of systematic differential centrifugation and discontinuous sucrose density gradient centrifugation;
3. to characterise the adenyl cyclase in this fraction of purified sarcoplasmic reticulum fragments and determine its relationship to Ca^{2+} accumulation and ultimately, its role in myocardial contractility.

The first objective, to set up a sensitive assay for the measurement of cAMP, was accomplished as described last year with a modification of the Gilman assay[5].

The second part of the study involves the isolation of a purified preparation of sarcoplasmic reticulum fragments. A standard microsomal preparation was prepared and various methods were employed to purify this fraction. Nucleotidase (5'-AMPase) was employed as a marker for plasma membrane fragments[6], and cytochrome c oxidase to determine mitochondrial contamination. Oxalate-supported calcium uptake, using ^{45}Ca as an indicator, was found to be the most suitable marker for intrinsic sarcoplasmic reticular fragment activity. Ca^{2+}-ATPase studies were also informative.

The standard microsomal preparation routinely used and described as sarcoplasmic reticulum fragments was found to be highly contaminated with mitochondria and plasma membrane fragments. An adenyl cyclase was found in this preparation which responded to adrenaline and NaF with an increase in cAMP accumulation. When this fraction was further purified by separation on a discontinuous sucrose density gradient two major components were found; one major component collected at the bottom of the gradient was designated as the heavy fraction (H) and the other was recovered at the interphase of the 20% and 35% sucrose and was designated the light fraction (L). Both major fractions were contaminated with mitochondrial fragments. The L fraction exhibited considerably more plasma membrane contamination than the H fraction. The ATPase present in the H fraction was also found to be considerably more sensitive to activation by calcium than the L fraction and exhibited a profile quite different from the latter. Ca^{2+} uptake studies were performed on both these fractions and it was found that the H fraction exhibited significantly greater ability to accumulate calcium, either in the presence or absence of oxalate. It was concluded from these studies that the H fraction with its higher Ca^{2+} accumulating characteristics and Ca^{2+}-ATPase activity and lower plasma membrane contamination represented a more purified sarcoplasmic reticular preparation than the L.

When the adenyl cyclase activity of these fractions was studied and compared to the untreated microsomal preparation and plasma membrane preparations, it was shown that whereas the L component possessed an adenyl cyclase similar to that found in untreated microsomal preparations and in plasma membrane the H component possessed adenyl cyclase activity quite unlike this. Neither adrenaline nor NaF, which markedly stimulated these other preparations, increased cAMP accumulation in the H fraction; adrenaline, under certain conditions, signficantly inhibited cAMP accumulation in the H fraction.

It was concluded that the H component prepared represented a purification of sarcoplasmic reticular elements and that the adenyl cyclase found in this fraction was that native to the Ca^{2+} accumulating system of the cardiac muscle cell. The inhibition of this enzyme by adrenaline implies that decreased cAMP levels might alter Ca^{2+} accumulation by this system and lead to higher levels of free calcium and increased contractility.

Effect of Beta-Blocking and Other Drugs on the Adenyl Cyclase Activity of Cat Myocardium

V. Carson

Using an assay for adenyl cyclase developed from the method of Krishna, Weiss and Brodie[7] a significant increase in the production of cAMP can be demonstrated when noradrenaline, adrenaline or isoprenaline are present in a coarse homogenate of cat heart.

This system has been used to study the effects of the β-blocking drug oxprenolol (Trasicor) and prindolol (LB46) and verapamil (Isoptin) both in the presence and absence of adrenaline. In every case, an appropriate concentration of the drug was added to the homogenate or homogenate plus adrenaline immediately before the homogenate was added to the incubating medium. Controls containing no drug and tests containing adrenaline alone were always included. All tests were done in duplicate. All tests contained 10^{-2}M aminophylline but no sodium fluoride.

After pilot runs to determine the limiting concentrations of the drug with the final concentrations of adrenaline always 10^{-4}M, each drug was then tested in six experiments at the selected concentrations.

Oxprenolol. In the case of oxprenolol the drug was tested at final concentrations of from 10^{-5} to 10^{-8}M. The control level of adenyl cyclase activity was 25.6 ± 2.1 (S.E.) p moles of cAMP per mg. protein per min. Whilst adrenaline caused a mean rise of 78%, this effect was completely blocked by 10^{-5}M oxprenolol, and almost completely by 10^{-6}M oxprenolol. At 10^{-7} and 10^{-8}M, there was no effect on the stimulation caused by adrenaline. The drugs by themselves had no effect on the control levels of cAMP.

Prindolol. Similar experiments were carried out using prindolol This drug is insoluble in water and must be dissolved in acid and carefully neutralised before being added to the homogenate. The upper final concentration of prindolol which can be tested is therefore limited to between 10^{-3} and 10^{-4}M. After pilot studies, prindolol was tested in six experiments at 10^{-6} and 10^{-7}M. In this case, although blocking activity was evident, the results were not so clear-cut.

The control level of adenyl cyclase activity was 22.3 ± 1.8 (S.E.) p moles cAMP per mg protein per min. Prindolol on its own did not significantly alter this figure. However, in the presence of adrenaline at 10^{-6}M the cAMP level was 27.7 ± 3.3 ($p < 0.02$ compared with controls), and at 10^{-7}M, the cAMP level was 34.5 ± 4.4 ($p < 0.01$ compared with controls), whilst with adrenaline alone it was 39.7 ± 4.4 ($p < 0.005$ compared with controls). Further concentrations (10^{-5} and 10^{-8}) are now being tested.

Verapamil. This was tested for its β-blocking activity. This drug was originally thought to belong to the β-blocking class but our experiments confirm that this is not so.

We were unable to demonstrate any effect, either alone or in the presence of adrenaline, by verapamil on the production of cAMP at concentrations of 10^{-4} and 10^{-5}M.

It appears that the **in vivo** effects of these three drugs, with respect to their antagonism of the effects of catecholamines are mirrored in the **in vitro** assay designed to demonstrate the stimulatory effect of catecholamines on cAMP production.

It is intended to show whether or not this blocking effect is specific for β-blockers, by testing an α-blocker (phenoxybenzamine) in the same system.

A Protein-binding Assay for the Determination of 3'5' Cyclic AMP in Rat Myocardium

V. Carson

A protein kinase which binds cAMP has been prepared from rabbit muscle according to the method of Myamoto **et al**[8] and used to assay cAMP according to the method of Gilman[5].

The assay has been used on frozen myocardium in the first place taken from freshly killed rats and secondly from rat hearts perfused with Krebs-bicarbonate medium.

In early experiments, no purification procedures were used before assaying the heart for cAMP. However, it was found that anomalies occurred in the levels of cAMP obtained from undiluted extracts and those diluted 1:2 and 1:4. The undiluted extracts gave results which were too high compared with those of the diluted extracts, indicating that other substances were competing for the protein-binding sites in addition to cAMP. The most likely of these was ATP.

The purification procedure of Walton and Garren[9] using zinc sulphate-barium hydroxide precipitation followed by chromatography on Dowex 50 was therefore used and the recovery monitored by the addition of a small amount of ^{3}H-cAMP to the extract before the purification procedure was started.

Recoveries of about 40% were obtained, and dilutions of the final extract gave the same levels of cAMP as the undiluted extract.

The mean content of cAMP in unperfused rat heart (5 experiments) was 10.1 ± 1.0 (S.E.) p moles per mg P under the conditions of test, i.e. duplicate analyses of three dilutions.

The assay is extremely senitive (1-2 p moles) but the precision is not high so that it is necessary to do several replicates to reduce the standard error. Testing of three dilutions is desirable also to ensure that the values obtained lie on the standard curve (which is linear over a limited range on a log-log plot).

The purpose of this study is to follow up the work of Paddle and Haugaard[10] who stated that in the presence of a high magnesium concentration (20mM), adrenaline stimulated the contraction of the heart but did not cause an increase in lactate production or glycogen breakdown, but did cause increases in phosphorylase **a** activity to the same extent as that produced in hearts perfused with the control (low Mg^{2+}) medium.

Phosphorylase activity and changes in cAMP levels under these conditions of high Mg^{2+} concentration with and without stimulation by adrenaline are being studied.

The protein-binding assay would seem to be the best tool available for this kind of experiment provided it is sufficiently reproducible and the variation in cAMP levels from one heart to another is not too great. By rapid freezing of the heart **in situ** with liquid nitrogen after perfusion, it is hoped to minimise one of the sources of variation, that is enzymic degradation of cAMP.

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Troponin *

P. Mäsiar and P. Daile

The isolation and purification of cardiac troponin from cardiac muscle of greyhounds has been carried out. The purification procedure was based on electrofocusing studies and it was found that electrofocusing of cardiac troponin produced a pattern different to that of rabbit skeletal troponin.

On the basis of the results of electrofocusing studies, a new 0.5M sodium phosphate buffer pH 6 for cellulose acetate strip electrophoresis was introduced and found to be satisfactory. When this electrophoresis of greyhound cardiac troponin was carried out several minor bands and one major band were found.

The purification performed by gel-filtration on sephadex G-200 column (2.5 x 50 cm) in 0.5M KCl, 0.5mM dithiothreitol, 0.05M sodium phosphate buffer, pH 7.0 produced four fractions, only one of which was found to contain protein possessing calcium binding properties. Electrophoretic examination of this protein showed that a small degree of contamination was still present. Further purification by ion exchange chromatography on DEAE cellulose yielded one electrophoretically homogeneous component when examined in the presence of dithiothreitol. This protein component was shown to exhibit calcium binding properties. The amino acid composition of the calcium binding component has been determined and it was found to be different to that of a rabbit skeletal component determined by several authors.

Beta-Adrenoceptor Antagonists ‡

W. G. Nayler and T. E. Lowe

During the past year experiments were undertaken to compare the intrinsic properties of two newly developed β-adrenoceptor antagonists with those exhibited by the currently available compounds. The two newly developed drugs were MK 950 and HOE 893, both of which were available in the laevo form. HOE 893 is 1-tert.butylamino-3-(2-cyclopentylphenoxy)-2-propanol sulphate. MK 950 has the following formula $C_{17}H_{28}N_4O_7S$. Propranolol and oxprenolol were used as the reference drugs.

To establish whether either HOE 893 or MK 950 has any intrinsic sympathomimetic activity doses of between 5 and 150 μg/kg were added to isolated segments of human and dog trabecular muscle immersed in aerated Tyrode's solution. Isometric conditions were maintained throughout. Neither of these compounds exerted any direct sympathomimetic effect, nor did they display non-specific negative inotropic activity. This lack of intrinsic sympathomimetic activity was confirmed in intact animal studies, during which doses of between 5 and 300 μg/kg were injected intravenously into anaesthetised dogs, and changes in heart rate and force of left ventricular contraction were recorded.

In terms of potency the compound HOE 893 is between 6 and 10 times more potent in blocking β-adrenoceptor mediated changes in rate and force of contraction than is **dl** propranolol. Isoprenaline infusions (0.25 μg/kg/min) were used to evoke changes in heart rate and force of contraction. MK 950 is between 10 and 15 times more potent than **dl** propranolol.

In 14 experiments performed on intact anaesthetised dogs doses of HOE 893 ranging between 5 and 400 μg/kg caused significant dose-dependent decreases in the rate at which the myocardium utilised oxygen. Experiments using dogs on right-sided cardiac bypass showed that cardiac efficiency was enhanced under these conditions, and that the myocardial stores of high energy phosphates actually increased. Coronary blood flow was reduced. MK 950, in doses ranging between 5 and 100 μg/kg caused significant dose-dependent changes in myocardial oxygen consumption similar to those which were caused by either HOE 893, propranolol or oxprenolol. Myocardial efficiency was increased and the high energy phosphate stores maintained. The fall in coronary blood flow was significantly less than that caused by an equipotent blocking doses of **dl** propranolol.

Some additional experiments were undertaken to study the effect of the compound BAY 1040. This drug increases coronary blood flow and decreases cardiac contractility without establishing β-adrenoceptor blockade. It reduced the myocardial demand for oxygen and improved cardiac efficiency, presumably because it diminished the rate at which tension developed during contraction.

Cardioactive Peptides

P. Mäsiar, E. Mäsiar and T. E. Lowe

Blood Plasma

A number of inotropically active fractions can be obtained from heparinised blood plasma by means of selective membrane filtration. Using a method of membrane ultrafiltration reported last year an intermediate molecular weight (1,000-10,000 daltons) fraction was isolated. From this fraction several peptides have been isolated by sephadex gel filtration (G-25 and G-10). The study of inotropic activity has shown that this was mainly present in the fractions eluted from sephadex columns in the region of low molecular weight compounds, however it was also observed that these fractions displayed a very high degree of adsorption on the sephadex column, so that the molecular weight would not necessarily correspond to the one expected according to the theory of gel filtration.

The inotropically active peptides were purified and their amino acid composition was estimated with an amino acid analyser. An attempt to elucidate the primary structure of one of the peptides with particularly striking inotropic activity has been made. It was found that this particular peptide contained some other organic molecule(s) attached to its peptide chain. The amino acid sequence of its peptide compound has been shown to be as follows: X.iLeu.iLeu.iLeu.iLeu.Gly.iLeu.iLeu.iLeu. The chemical nature of compound X remains as yet unknown.

Cardiac Muscle

Last year its was remarked that cardioactive substances can be isolated from the myocardium. This year an attempt has been made to fractionate an extract of heart muscle homogenate by membrane ultrafiltration.

Using 0.1M N-Ethylmorpholine pH 7.5 buffer extracts from 8 hearts of normal healthy dogs and 5 rejected neck transplants have been fractionated by membrane ultrafiltration using XM-100, XM-50, PM-30, UM-2, UM-0.5 in succession. Each fraction was lyophilised, the aliquots of those with high molecular weights were studied by using the DEAE cellulose column fractionation and electrofocusing.

Those with low molecular weights were studied by sephadex gel filtration. Comparison of the patterns of individual fractions obtained by either of the above mentioned methods revealed that those obtained from healthy dogs were identical in each case but that the fractions obtained from the extracts of rejected hearts displayed considerable differences in their patterns.

Distribution of Myocardial Blood Flow in the Dog

F. R. Trinker

During this year the technique of using isotopically labelled carbonised microspheres (15 μ diam.) to determine total and regional blood flow through the myocardium of a dog has been established. Microspheres labelled with the isotope ^{85}Sr were injected as 0.2 ml (20 μCi) of a 20% dextran suspension through a catheter situated in the left atrium and this was flushed in by 10 ml of saline.

It was found that there is a non-uniform regional distribution of myocardial blood flow. This can be illustrated by the following ratios which summarise the flow data obtained in twelve control experiments:

$$\frac{LA}{RA} = 1.7 \pm 0.1$$

$$\frac{LVend.}{LVepi} = 1.2 \pm 0.06$$

$$\frac{LVepi}{RV} = 1.3 \pm 0.1$$

$$\frac{LV}{RV} = 1.5 \pm 0.1$$

$$\frac{LVsept}{RVsept} = 1.2 \pm 0.05$$

[where LA, RA are left and right atria; LV, RV are left and right ventricles; end, epi, sept are endocardial, epicardial and septal regions.]

Analysis by paired "t" test established that the differences between blood flow in different regions of the myocardium were significant at the following levels:

$LA > RA$	$p < 0.001$
$LVend > LVepi$	$p < 0.001$
$LV > RV$	$p < 0.005$
$LVend > RV$	$p < 0.001$
$LVsept > RVsept$	$p < 0.001$

This normal distribution of regional blood flow through the myocardium will be used as a base from which to investigate possible changes in the distribution pattern that may occur under various pathological conditions, such as coronary occlusion or excessive cardiac sympathetic stimulation, and to study the effects of "coronary dilator" drugs on myocardial blood flow. To this end it will be necessary to make a second injection of spheres labellel with ^{46}Sc to give a before and after, or control and experiment, picture.

Cardiac Surgery

Eric Cooper and G. R. Stirling

Cardiac Transplantation

During previous experiments on cardiac transplantation, we had noted isorhythmic atrioventricular synchronisation in the transplanted heart during rejection. This finding could not be explained by any extrinsic reflex pathway and the following alternative hypotheses were suggested: (i) electrical or mechanical interaction between adjacent separate sections of cardiac muscle, (ii) paired coupled oscillations between atrial and ventricular pacemakers, (iii) synchronisation of the sinus node with pulsations in the sinus node artery, (iv) the release of catcholamines from the isolated heart, (v) a combination of two or more of the above.

An acute heterotopic cardiac transplant was used to investigate these hypotheses. Perfusion of the donated heart by arterial blood of the recipient was either direct or through a calibrated, non-pulsatile pump. Its aortic arch was cannulated for pressure measurements and its pulmonary artery was anastomosed to the recipient's venous system for return of coronary sinus blood. The donated heart was placed in either the abdominal or thoracic cavity to maintain normal temperature. An electromagnetic flow probe placed around the root of its aorta measured net coronary blood flow. Its atrial and ventricular electrocardiograms were recorded simultaneously with aortic root pressure, net coronary flow and the electrocardiogram of the recipient.

The effects on the sinus node rate and rhythm of the following manoeuvres were studied: (i) a rise in the aortic root pressure with or without a concomitant flow increase, with normal sinus rhythm, (ii) induced ventricular fibrillation whilst maintaining a regular atrial rhythm and varying the aortic root pressure, (iii) division of the Bundle of His, (iv) varying aortic root pressure in the unpaced blocked heart, (v) induction of ventricular fibrillation in the blocked heart, and subsequent changes in aortic root pressure, (vi) ventricular pacing of the blocked ventricle.

Paired blood samples from the aortic root and coronary sinus of the donated heart were taken before and after each sequence change, and the content of serum catecholamines estimated.

Significant changes in sinus node rate and rhythm have been noted following an increase in the aortic root pressure, after inducing ventricular fibrillation, and in pacing the blocked ventricle at or near the rate of the beating atria. The last two changes also change the aortic root pressure, and therefore are probably acting as secondary factors. Samples of blood estimated for catecholamine levels show that the transplanted heart releases significant quantities of these substances, particularly after each procedure. We are continuing this year to elucidate these problems.

Autologous Tissue in Valve Surgery

A second project concerns the changes that occur in autologous de-vascularised, denervated tissue used to replace diseased cardiac valves.

The clinical results of the use of autologous fascia lata to fashion tricuspid heart valves, suggest that most of the transplanted tissue becomes, and remains, non-viable. The consequences are rupture and infection of the cusps.

The fact that the fascia lata is transplanted from its normal site into the cardio-vascular system, may in itself lead to changes. To examine this possibility we have removed the anterior leaflet of the dog's mitral valve, on cardio-pulmonary bypass in a sterile manner, and resutured it into place. As this is normal tissue for the valve site, we would expect any changes that may occur after an arbitrary time of six to twelve months to be associated with the results of the surgical procedures.

At the present surviving dogs have been obtained and are awaiting the arbitrary period before examination.

Hypertensive States 1950-1972

A. J. Barnett

Introduction

In 1950 the aetiology and pathogenesis of essential hypertension were unknown and, apart from some rather drastic measures by pioneers, treatment was generally ineffective. Since then there has been a great increase in knowledge concerning aetiological factors, pathogenesis and physiological disturbances, but much remains unsolved. The problem has proved more complex than anticipated and it is now generally accepted that the aetiology of essential hypertension is multifactorial.

In 1950 many prominent workers believed that the raised blood pressure was due to an increased peripheral resistance rather than to an increased cardiac output and, since structural changes in arteries could not be demonstrated in early cases, this increased resistance must be "functional" and due to some undetermined vasoconstrictor agent. Following Goldblatt's historic discovery that hypertension could be produced in the dog by clipping one renal artery, the renal pressor agent renin was the prime suspect.

Also at that time because the haemodynamic effects of adrenaline, previously considered to be the sympathetic transmitter, differed greatly

from the findings in essential hypertension, adrenaline excess and sympathetic over-activity were generally not favoured as pathogenic agents. However, the role of the sympathetic system needed reassessment following the demonstration that the sympathetic transmitter was noradrenaline. Because of my previous participation in a study on the effect of noradrenaline in man[1] and on the mechanism of hypertension, a project to study the causes and treatment of hypertension in man was commenced. Some aspects of this study have been completed as research projects and are now reviewed. Other aspects such as the role of sodium ions in the pathogenesis of hypertension will continue.

The Diagnosis and Treatment of Secondary Hypertension

Phaeochromocytoma

Hypertension is one of the symptoms associated with phaeochromocytomata and for the diagnosis of these tumours two types of tests were investigated — one involved the estimation of the amount of catecholamines excreted in the urine and the other the determination of the effect on the blood pressure of a specific blocking agent for adrenaline and noradrenaline.

Using a rabbit ileum preparation a biological assay for adrenaline and noradrenaline in urine was established by G. A. Bentley and was used for investigating patients not only of the Alfred Hospital but also of other centres throughout Australia.

In 1950 no adrenergic blocking agent suitable as a reliable test for phaeochromocytoma was available. Benzodioxane had been suggested but was found sometimes to produce an alarming rise in blood pressure in some cases of essential hypertension during an infusion of adrenaline. Also when injected during an infusion of noradrenaline benzodioxane produced only a slight fall in blood pressure[2]. Dibenamine proved more effective in reducing or reversing the pressor effect of infused noradrenaline or adrenaline, but toxic effects and the two hours required for an infusion militated against its usefulness as a diagnostic test[3]. Phentolamine ("Regitine") however blocked the rise of systolic blood pressure from adrenaline and both systolic and diastolic blood pressure rises from noradrenaline infusions. It was rapidly effective and non-toxic and suitable for a diagnostic test for phaeochromocytoma[4].

Although urine samples were being received from all over Australia for catecholamine assay and phaeochromocytomata were being diagnosed in other centres as a result, it was not until 1959 that the value of the tests was demonstrated in cases at the Alfred Hospital[5].

Conn's Disease

Another cause of secondary hypertension is an aldosterone secreting tumour of the adrenal cortex described by Conn in 1955. Such a tumour was diagnosed following from the observation by D. Emslie-Smith of ECG findings suggestive of a low serum potassium concentration. The patient was then fully investigated and shown to have the characteristic biochemical abnormality, an increased urinary secretion of aldosterone, and a radiologically demonstrated adrenal tumour which was successfully removed[6]. This was the first reported case of this condition in Australia. In 1960 F. O. Simpson and A. J. Barnett[7] reported another case showing the effect of the newly introduced aldosterone antagonist — spironolactone — on promoting sodium excretion and potassium retention. It also showed an anomalous (reversed) effect of the anti-diuretic hormone vasopressin which produced increased diuresis[8].

Mechanism of Essential Hypertension

As already mentioned there was a widespread belief that the increased peripheral vascular resistance in essential hypertension was largely due to a functional arteriolar constriction. The cause of this functional vasoconstriction was not clear. The three main theories were that it was due to (a) sympathetic nervous over-activity; (b) the action of some humoral vasoconstrictor agent; (c) to increased vascular responsiveness to normal vasconstrictor agents.

Barnett and Fraser (1954)[9] showed that there was no correlation between the fall in blood pressure in a "Seconal" sedation test and that following an injection of hexamethonium bromide and concluded that any conclusions about the mechanism of hypertension which were based on findings of the "Seconal" test should be regarded with suspicion. They[10] then studied the effect of an injection of relatively large doses (2 mg per kg) of the ganglion blocking agent hexamethonium bromide in a group of 40 hypertensive and 18 normotensive subjects. They observed that a greater relative fall in both systolic and diastolic blood pressures in the hypertensive subjects was associated commonly with a rise in pulse rate in the normotensive subjects but usually no change or a slight fall in the pulse rate in the hypertensive subjects.

The probable significance of these findings was that the blood pressure changes indicated an increased sympathetic effect in hypertensive compared with normotensive subjects, that the pulse rate changes indicated a decrease in vagal effect in hypertensive subjects (the ganglion-blocking agents block both sympathetic and parasympathetic activity) and that both excessive sympathetic and reduced vagal activity might arise from decreased responsiveness of the cardiac sinus mechanism. The decreased vagal activity in essential hypertension was overlooked by subsequent workers and has only recently been rediscovered by others elsewhere.

Action and Effectiveness of Hypotensive Drugs

The development of the ganglion-blocking drugs introduced a new era in the treatment of hypertension and a clinical trial of their use was commenced in 1951. The short-term beneficial effects of symptomatic relief, regression of papilloedema and retinopathy and apparent retardation of vascular disease in patients with severe hypertension were soon apparent [11, 12].

Other hypotensive drugs introduced a little later included reserpine and hydralazine. Reserpine was found to lower blood pressure in patients with benign hypertension[13] and was later added with benefit to the ganglion-blocking agent pentolinium in the treatment of severe hypertension[14].

Side effects were common with ganglion-blocking agents. Bowel effects were particularly troublesome and there were several instances of paralytic ileus and pseudo-obstructions and acute enterocolitis, a previously unrecognised complication, was noted in some patients being treated with ganglion-blocking drugs[15].

The introduction of the thiazide diuretics was a major advance in the treatment of hypertension. Barnett and Marshall (1958)[16] confirmed in a short-term study that the addition of chlorothiazide in a dose of 1 gm per day potentiated the effect of the ganglion-blocking drug, mecamylamine, so that the dose of the latter could be reduced by 50 per cent. without loss of blood pressure control but with considerable diminution of undesirable side effects due to parasympathetic blockade. Barnett and Simpson in 1960[17] confirmed the beneficial hypotensive effect of the addition of chlorothiazide over longer periods but drew attention to the fact that in half of the patients the serum potassium level had fallen to below 3.5 m eq/1 after six months.

The next major advance in hypotensive treatment was the introduction of guanethidine which has a selective action on the post ganglionic sympathetic-neurone and therefore avoids the side effects due to the cholinergic blockade experienced with the ganglion blocking drugs. The effect of this drug on reduction of sympathetic activity without significant effect on parasympathetic activity was confirmed[18] and later (1962) favourable results of a clinical trial were reported[19]. Although side effects from parasympathetic blockade were absent with guanethidine other side effects such as postural faintness, diarrhoea, tiredness and weakness were troublesome. Other sympathetic blocking drugs, bethanidine and debrisoquin, have since been introduced and have the advantage of a more rapid onset and offset of their actions.

Other drugs acting on the sympathetic nervous system more recently introduced have been included in this trial on our hypertensive patients. They include methyldopa, which causes a decrease in the formation of the neuro-transmitter — noradrenaline — by the nerve endings by substituting a false transmitter, and clonidine which decreases sympathetic activity by a central action. Studies of the action of clonidone in man[20] showed that it reduced blood pressure without serious impairment of sympathetic reflexes. A satisfactory hypotensive effect was obtained in 17 of 19 patients with benign hypertension and in the single patient with malignant hypertension[21]. Side effects were moderate but acceptable.

Long-term Study of the Effect of Treatment on Severe Hypertension

This long-range project was to determine the effect of strenuous medical treatment on the course of severe hypertension. Patients with severe hypertension were admitted to a special clinic and, after assessment and stabilisation on treatment, were seen at frequent intervals for blood pressure measurements and adjustment of drug dosage. They were reviewed with the aid of a battery of tests to assess progress of the hypertensive disease, particularly in the ocular, cardiac and renal fields. At first only patients with malignant hypertension were accepted, but later patients with Grade III retinopathy and more recently patients with severe complicated hypertension irrespective of the ocular fundus grading were accepted into the project.

Treatment has been with the most effective drugs available at the time. Recently the data have been analysed and it has been possible to describe the course of severe hypertension with treatment and to compare the results with those previously obtained in patients who were either untreated or only mildly treated and with the results of two other studies which have been published while our analysis was in progress.

It is difficult to summarise briefly the extensive material surveyed but the following tables and figures indicate some of the main findings.

Table I

Severity of Hypertension

A. Blood Pressure

	Systolic (mm Hg)			Diastolic (mm Hg)		
	160-199	200-239	240 +	110-119	120-139	140 +
Number of Patients	24	92	75	16	81	94
		88%			93%	

B. Ocular Fundus Grade

	0-1	II	III	IV
Per cent. Patients	21	23	35	21

Table I summarises the subject material and shows that 21 per cent. were in the malignant phase and all had severe diastolic hypertension. Figures 1 - 3 show survival curves with subdivision into age and sex groups, and according to severity of certain initial features which were believed to affect prognoses.

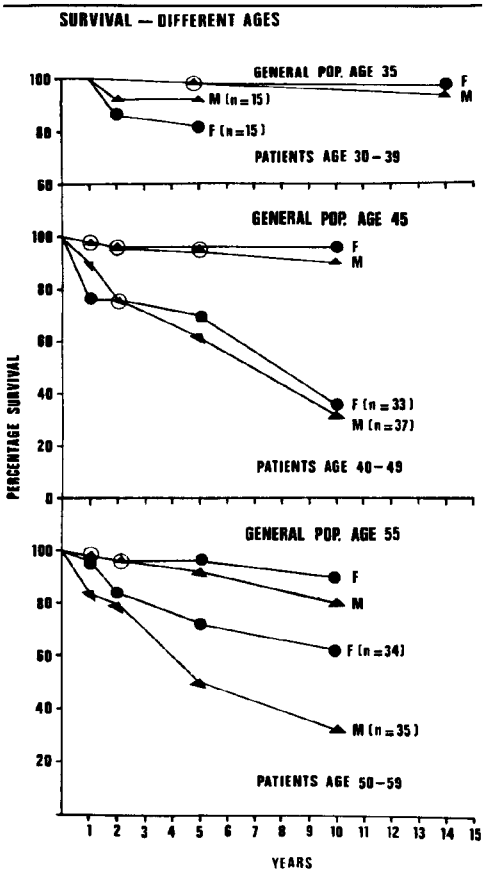

Figure 1

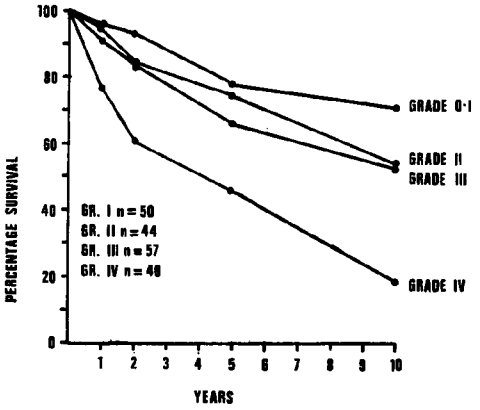

Figure 2

Although comparison with other reported studies indicates that our treated hypertensives did better than untreated or mildly treated hypertensives of similar severity they still do much worse than the general population (Figure 1). Factors influencing prognosis in untreated hypertension operate similarly in treated hypertension. Ocular fundus grade and degree of renal impairment are more important than the degree of cardiac impairment. Surprisingly the height of the systolic blood pressure proved more significant than that of the diastolic pressure.

Table II

Causes of Death in Treated Hyptertension

	0-5 Years	Over 5 Years	Total
Cardiac	9	18	27
Cerebral	18	3	21
Renal	19	2	21
Other and Unknown	8	9	17

Table II shows the causes and times of the 86 deaths. It is seen that cerebral, cardiac and renal causes contributed about equally, but whereas deaths from cerebral and renal causes occurred early (under 5 years), those from myocardial infarction occurred late (after 5 years).

It would seem that the best methods left to improve the prognosis of hypertensive persons is early diagnosis — before vascular damage has occurred — and avoidance of the risk factors for myocardial infarction.

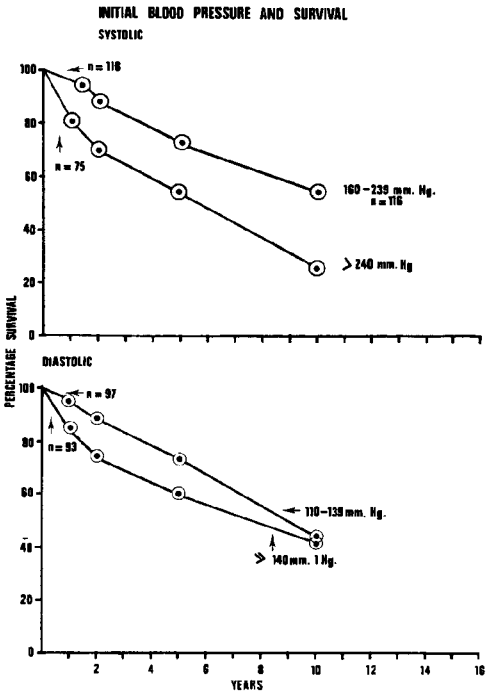

Figure 3

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Clinical Pharmacology

Plasma Concentrations and Urinary Excretion of Prindolol

F. R. Trinker

In order to understand the use of β-blocking drugs in the treatment of hypertensive patients it is essential to know in what way plasma concentration and urinary excretion of the drug relate to the injection of the drug and to each other. Such measurements have been made in a number of healthy volunteers to provide a base line for observations to be made in hypertensive patients. The drug used is prindolol (LB46, Visken).

Modification of a fluorimetric method for determining plasma and urinary concentration of prindolol has established a range of plasma levels with a minimum of 5 ng/ml. Standard curves for plasma and urinary extraction procedures closely approximated to external standard concentrations of prindolol, exhibiting linearity and recoveries of greater than 80%.

Three healthy volunteers taking 5 mg tablets of prindolol thrice daily have shown the following plasma levels of prindolol:

	FT	PB	DP
Day 1			
2½ hrs. after first 5 mg dose	21 ng/ml	10.5 ng/ml	27 ng/ml
Day 2	18 ng/ml	10.0 ng/ml	39 ng/ml
Day 3	23 ng/ml	10.0 ng/ml	26 ng/ml

The urinary levels of prindolol which were too high to be measured until diluted to 1:10 dilution of a 1 ml sample, were as follows:

	PB	DP
Day 1 2½ hours after first 5mg dose	Too high, further dilution necessary	2500 ng/ml
Day 2	2800 ng/ml	3700 ng/ml
Day 3 12 hours after last dose	3000 ng/ml	5000 ng/ml

Interaction between Amitriptyline and Guanethidine in Rat Heart

F. R. Trinker

Following the **in vivo** experiments reported last year on the interaction between tricyclic anti-depressant and adrenergic neurone blocking drugs a study to help determine the mechanism(s) that may be involved in this interaction was undertaken.

In perfused rat hearts the noradrenaline content and the active noradrenaline uptake mechanism were investigated simultaneously. Tissue noradrenaline content was measured using a modification of the Bio-Rad catecholamine column test. This method gave consistent, reproducible results and recoveries were generally greater than 75%.

A tracer dose of titriated (^{3}HNA) noradrenaline permitted the uptake system in adrenergically innervated tissue of the rat heart to be studied.

The following table gives a summary of the results obtained:

	Control	Guanethidine (30 mg/kg)		Amitriptyline (30 mg/kg)
		Acute	Chronic (7 days)	
NA content μg/gm tissue	1.10	0.60	0.15	0.89
Na uptake μCi/gm tissue	4.1	1.9	2.5	1.4

Guanethidine is a potent drug which depletes, even after a single dose 18 hours prior to experiment, the heart muscle of noradrenaline and reduces its ability to take up noradrenaline. Amitriptyline does not significantly alter the noradrenaline level in cardiac tissue, but the noradrenaline uptake system is markedly inhibited. Potentially, therefore, a competition between drugs exists for the active transport of noradrenaline. Possibly this may reduce the efficacy of either drug.

Chronic treatment (up to 11 days) with amitriptyline and guanethidine reduced the NA content 0.08 μgm/gm and the NA uptake 1.12 μCi/gm.

It would appear (although more experiments are necessary) that the noradrenaline depleting action of guanethidine was not antagonised despite inhibition of the uptake mechanism.

Furthermore, if the two drugs are given acutely and within ½ hour of each other, the noradrenaline depleting action is substantially reduced, i.e. the noradrenaline content in rat heart is minimally reduced (to 0.90 μg/gm).

It may be postulated that the interaction between guanethidine and amitriptyline is time and probably dose-dependent. It appears that inhibition of the active noradrenaline transport by amitriptyline is a short lived phenomenon and provided guanethidine is administered at a reasonable time after (perhaps 2-3 hours) the degree of interaction is minimal. However, more experiments are required to confirm these preliminary findings.

Peripheral Vascular Disease 1950-1972

A. J. Barnett

In 1950 the treatment of peripheral vascular disease was very inadequate and sympathectomy was the only treatment occasionally used to improve blood flow in the limb. Consequently the ischaemia produced by the arterial obstruction often led to amputation and this was usually delayed until demanded because of intolerable pain being suffered by the patient who was in addition wasted and debilitated by prolonged anorexia and toxaemia.

A study of the pathogenesis and optimum treatment of peripheral vascular disease appeared at that time to be a desirable clinical research project. It was commenced in 1950 and now may be considered to have passed from being a research project to a service-to-patients commitment. It is therefore appropriate to review the evolution of adequate methods for managing this distressing condition.

There have been many facets to this investigation — adequate diagnosis of the site and extent of arterial blockage and its nature; a detailed clinical study of the condition; investigation of methods of treatment; rehabilitation of patients; possible prophylactic measures.

Diagnostic Procedures

It seemed at that time that in order to investigate methods of improving blood flow, one needed a clinical method of measurement of flow.

Venous occlusion plethysmography using an air filled plethysmograph was developed[1, 2] and although not an instrument of precision it was shown to have sufficient accuracy for clinical measurement of limb blood flow. Using this instrument it was found possible to distinguish between structural arterial occlusion and spasm and to study the effectiveness of vasodilator drugs.

A calorimeter for measurement of hand blood flow was constructed later (1953)[3] and was found valuable in the study of the circulation of the hands, particularly in distinguishing between cases with predominantly vasospastic disturbance and those with obstruction of arteries[4]. These methods were time-consuming but from experience with them it became possible to predict the results in most cases from the clinical features. They are therefore not now used clinically.

With the necessity for visual demonstration of the state of the arteries preparative to arterial surgery, arteriography, introduced into this Hospital by the late Berwyn Deans and developed by H. A. Luke and his colleagues at Department of Diagnostic Radiology, has become the main diagnostic tool used.

Clinical Studies

In 1955 Barnett and J. R. E. Fraser prepared a monograph[5] which presented a practical approach to the problems of peripheral vascular disease of the extremities and the treatment available at that time (before the era of arterial grafting). This was based on clinical observations and physiological measurements using the methods described above.

Two particular aspects of occlusive arterial disease were subject to special study — occlusive arterial disease of the hands and intermittent claudication. In 1955 a study[6] of 30 cases of occlusive arterial disease of the hands, using special tests including calorimetry, revealed that primary Raynaud's disease accounted for only 12, the others being secondary to other conditions. It is of interest that among the latter were included five cases of scleroderma.

In 1956, a study of 125 patients (185 ischaemic limbs enabled Barnett and St. Clair[24] and Barnett and Francis[25] to report on the clinical aspects and natural history of intermittent claudication. Important findings were that of 51

patients followed over a period from two to five years in half the patients the severity remained unchanged, in about a quarter it decreased and in about a quarter it became worse; only two patients required major amputation. This study, conducted before the era of arterial surgery, has influenced our thinking on indications for operation where the only symptom is claudication. It is advised only for the relief of unacceptable pain and not for the preservation of limbs.

Atherosclerosis is by far the main cause of peripheral vascular disturbance. However, the less common conditions are of great interest and have been the basis of various reports from the unit. These include —

(a) A case of subclavian aneurysm due to compression at the thoracic outlet[7].
(b) Thrombophlebitis associated with hidden malignancy[8] (in one case cured by removal of the tumour).
(c) Ischaemic episodes in cardiac failure, some apparently due to critical closure of arteries[9].
(d) Cystic myxomatous degeneration of the popliteal artery[10][11].
(e) cryoglobulinaemia producing purpuric skin infarcts[12] or Raynaud's phenomenon[13].

Treatment of Occlusive Arterial Disease

Lumbar Sympathectomy

This was an established treatment for ischaemia of the lower limbs and many surgeons claimed that the operation produced relief both of distal ischaemic symptoms and claudication. However, in a small series[14] of cases it was found that although there was improvement in the skin blood flow and the patients sometimes claimed improvement in walking, the latter was not substantiated by careful observation using a step test before and after operation and was probably a placebo effect. Using venous occlusion plethysmography, it was possible to demonstrate a high blood flow in the feet of patients[15] who had been sympathectomised up to 20 years previously. A detailed follow-up study was made of the results in 245 limbs of 147 patients in whom sympathectomy had been performed over the past eight years[16]. The findings confirmed that there was a marked immediate relief of symptoms of distal ischaemia in about 50 per cent. of patients and long-term relief in a substantial proportion, but that claudication was not relieved.

Arterial Grafting for Claudication

The treatment of intermittent claudication remained unsatisfactory. It was shown[17] that intermittent intramuscular injection of heparin, advocated at that time, was no better than a placebo and that amnion implantation[18] for which extravagant claims had been made, was also useless.

Reports of the use of preserved arterial homografts for occlusive arterial disease appeared in overseas literature in the early 1950's and the first such operation in the Alfred Hospital was performed by C. J. Officer Brown (1953). Patients selected for operation were few because of rigid selection criteria that required a patient with severe symptoms but a good artery distal to the block. Also there was difficulty in obtaining suitable grafts from young donors. Later, teflon grafts were used and at first seemed satisfactory and an answer to supply problem. In 1960[19] it was possible to report on a series of 62 grafts in 52 patients with an immediate success rate of 85 per cent. and a three-year patency rate of 40 per cent.

Since then there has been a growing interest in vascular surgery and co-operation between physicians of the C.R.U. and surgeons, first, of the Cardiothoracic Surgical Unit and more recently of the surgical Vascular Service has led to many advances in the treatment of occlusive arterial disease. New techniques and grafting materials, including dacron and autogenous vein have been used. Thombectomy is also used in suitable cases.

A report in 1970 on the results of reconstructive arterial surgical porcedures in 560 limbs of 490 patients[20] showed an immediate success rate between 80 to 90 per cent. In the case of aorto-iliac disease there was an overall patency at three years of 63 per cent. with endarterectomies and 44 per cent. in the case of grafts (mainly dacron). Patency in survivors (a more meaningful figure as late deaths were generally not due to the occlusive disease affecting the lower limbs or its treatment) was 77 per cent.

In the case of operations for femoro-popliteal disease best results were obtained with vein grafts with an overall patency rate at three years of 58 per cent. and a patency in survivors of 68 per cent. The prosthetic grafts (dacron and teflon) did particularly badly. Operative mortality for aorto-iliac disease was 6 per cent. and for femoro-popliteal disease 1.3 per cent. Since this study vein grafts for femoro-popliteal occlusions have been used almost exclusively.

The amount of arterial surgery has increased greatly and patients are accepted with a disease severity which would previously have been considered inoperable. More complicated procedures are being performed and include combined grafting and endarterectomy and continuation of grafts if necessary down to the tibial vessels just above the ankle. In 1971, arterial surgery (including also surgery for carotid artery occlusions) had reached such proportions that a special surgical Vascular Service, was established by the Hospital.

Amputation

Prior to reconstructive arterial surgery, amputation was a common treatment for ischaemia following arterial occlusion, and even now in spite of restorative surgical endeavours, some patients still require amputation. In the early part of the period under review amputees were often neglected and few walked again. A survey[21] of amputations performed in the Alfred Hospital in the year 1964 showed that of

the 39 patients 26 had died, 21 had been supplied with prostheses but only 13 were using them. There were great inadequacies in the psychological preparation of patients for amputation and in their rehabilitation. Since then an amputee clinic has been set up, new methods of limb fitting and training have been used and it is believed that because of this more active approach better results are being achieved, although a new follow-up study has yet to be made.

Anti-Lipidaemic Treatment

The work outlined above involves treatment of the developed disease. Although the risk factors in peripheral arterial disease are not well defined, there is circumstantial evidence that a high intake of saturated fats and hyperlipidaemia are causative in the development of atherosclerosis generally. Reduction in plasma lipid levels should therefore be beneficial in tending to prevent or slow down the development of atherosclerosis.

Barnett and Carson[22] found that it was possible to produce a moderate lowering of serum cholesterol levels by a low fat, low cholesterol diet with the addition of corn oil; by oral triparanol (Mer 29)* in doses of 0.25 to 0.5 g per day and by oral nicotinic acid, 3 g per day and a more marked lowering by a combination of nicotinic acid and triparanol.

Later, falls in the levels of cholesterol, triglyceride and total lipids following treatment[23] with chlorphenisate (Atromid) were noted. Although some of the patients claimed clinical benefit it was not possible to demonstrate any objective improvement from the short courses of treatment used. A more logical approach would be to eliminate hyperlipidaemia and any other risk factors before the development of atherosclerosis.

A survey is currently being conducted to determine the risk factors in occlusive arterial disease.

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* This drug has since been withdrawn by the makers because of the possibility of toxic effects.

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Carcinogenesis**

G. C. Hard and D. M. Shaw

The principal aim of this research is to define the processes which are involved in the induction of cancer by chemicals, with particular emphasis on renal neoplasia. The studies are planned to investigate these aspects at the cellular level with a view to providing a basis for the interpretation of studies at a molecular level. Definition of the mechanisms involved in the inductive period, which precedes the uncontrolled proliferation of malignant cells into a clinically recognisable tumour, may facilitate the determination of the strategy required for the control and prevention of cancer.

DMN Renal Carcinogenesis

The studies are based on an experimental model of carcinogenesis utilising N-nitroso compounds in the rat, and in particular dimethylnitrosamine (DMN). In recent years it has become evident that N-nitroso compounds might offer a serious carcinogenic hazard to man. It has been shown that they are able to induce cancer of various organs in all laboratory animals tested, including the monkey. Not only have some of these substances been found in various food items, but they can be formed endogenously by simple interactions between secondary or tertiary amines and nitrite in an acid medium. Furthermore, man metabolises DMN in liver tissue in the same fashion as does the cancer-susceptible rat.

A single, intraperitoneal injection of DMN administered to rats that have been pre-conditioned by a diet lacking in protein but high in carbohydrate, suppresses microsomal enzyme activity in the liver which results in the kidney metabolising larger amounts of DMN than would normally occur. All rats surviving the acute cytotoxic action of DMN develop renal tumours. Such a model, involving a single pulse of carcinogen to produce an invariable effect, facilitates interpretation of interim lesions with respect to the acute phase of injury and the final stage of tumour manifestation.

Investigations utilising this DMN model were commenced whilst working at the Toxicology Unit, Medical Research Council Laboratories in Carshalton, U.K. The completed studies have been used to analyse the morphological structure of the tumours induced, to identify the target cells injured by DMN and to trace the sequential tissue changes that occur in the kidney during tumour development following a single dose of DMN. They provide the basis for the current project. An essential adjunct to the system is the method of perfusion fixation of the kidney and a preparative technique involving the embedding of whole organ slices in resin so that infrequent and small lesions can be identified first with the light microscope and the relevant area of fixed tissue then selected for examination in the electronmicroscope.

The sequential events relating to the development of the renal mesenchymal tumour commence with the identification of focal intracellular degenerative changes in periglomerular, fibroblastic cells at 24 hours after carcinogen administration. From two days there is an increase in mitotic figures within the cortical intertubular space, again located in the vicinity of glomeruli. Then follows a generalised inflammatory response throughout the cortex by seven days which is associated with evidence of cell damage mainly in the first segment of the proximal tubules. With resolution of the tubular change the diffuse interstitial inflammatory response subsides within 2-3 weeks. However, sparsely distributed periglomerular aggregates of intertubular cells persist beyond the acute phase. For the first 8-10 weeks these lesions consist predominantly of lymphocytes, lymphoblasts, plasma cells and some macrophages, together with occasional abnormal, fibroblast-like cells. Later than 12 weeks the lesions present can be identified ultrastructurally as very small tumour foci of fibroblast-like cells with little evidence of immunological reactivity. By 16 weeks tumour cell foci are recognisable at the light microscope level and by 20-25 weeks rapid proliferation results in macroscopic, mesenchymal neoplasms.

The experiments in progress are designed to explore the various points of inquiry which these findings raise.

Immunological Aspects

A long-term study has been commenced to determine the modifying effect of immunosuppression on the incidence and the mode of development of renal tumours following a single dose of DMN. Kidneys are being examined by light and electron-microscopy at the significant stages of induction in neonatally thymectomised rats that have received supplementary doses of antithymocyte globulin, and in sham-operated rats receiving normal immunoglobulin.

In conjunction with this study, the activity of DMN as an immunosuppressive agent is being tested and the results again will be assessed in the light of the known developmental events leading to tumour growth in the kidney. Immunological function in rats following the administration of a single carcinogenic dose of DMN is being determined by each of three parameters. Thymus-dependent lymphocyte function is measured by tritiated thymidine uptake in cultures of splenic lymphocytes following phytohaemagglutinin stimulation. Bone marrow-dependent lymphocyte function is indicated by a measure of the agglutinin response to immunisation with **Brucella abortus** antigen. The third assay measures the plaque-forming cell response to immunisation with sheep erythrocytes.

Radioautographic Studies

In the mesenchymal tumour model outlined, radioautography, combined with electronmicroscopy, is being employed to investigate the relationships which might exist between the cells exhibiting toxic injury in the acute phase, the cells stimulated to divide from two days, the abnormal fibroblast-like cells found in persisting lesions between two and ten weeks and the ultimate, malignant cells of the proliferating neoplasm. Injection of tritiated thymidine at sequential stages after DMN administration will identify the cell types which are stimulated to synthesise DNA in the acute phase of injury. The same technique will also indicate whether the large, abnormal cells characteristic of the "latent" phase are capable of division.

In Vitro Application of the Model

In correlation with the sequential, **in vivo** stages of renal tumour development, cortical cells are being studied in culture to determine the stage at which transformed cells can be isolated from the kidneys of carcinogen-treated rats. Preliminary results suggest that within one week following a single **in vivo** dose of DMN, clones of renal fibroblasts develop **in vitro** which differ in morphology and behaviour from normal. The success of this aspect of the research will depend on the establishment of the system in an inbred strain of rat, as the ultimate test of malignant transformation in cells is their transplantability to produce tumours in recipient hosts. Consequently, the susceptibility of various strains of inbred rats to the renal carcinogenic action of DMN is being determined.

Recognition of altered clones of cells will also be dependent upon a knowledge of the **in vitro** characteristics of the tumour cells themselves. Thus, a number of renal mesenchymal tumours have been seeded into culture and their behaviour, morphology and cytogenetics are being followed through successive subcultures.

The aim of the tissue culture studies is twofold; to set up a system which will enable the subcellular analysis of relatively pure populations of precursor cells, and to provide a system for the **in vitro** investigation of the role of immunological factors in chemical carcinogenesis. Biochemical characterisation of carcinogen-induced, macromolecular alterations usually rely on the analysis of preparations from whole or parts of organs. However, it is likely that the macromolecular alterations pertinent to ultimate neoplastic transformation may occur in only a very small sample of the susceptible cell population within that organ. Thus, the development of an **in vitro** model employing critical selection of susceptible cells would facilitate the analysis of the significant changes that occur as these cells progress through the premalignant to the malignant state.

The Nature of Renal Neoplasms

In order to identify likely target cells of origin in chemical carcinogenesis and to interpret the significance of interim lesions during the latent phase of development, it is necessary to understand fully the nature of the neoplasm under investigation. The complexity of the renal mesenchymal tumour induced in rats by DMN and related compounds, is well illustrated by the fact that it has been described by no less than seven different classifications including such apposite terms as anaplastic carcinoma, nephroblastoma and fibrosarcoma. Histologically this complex neoplasm closely resembles the mesenchymal component of Wilm's tumour in children.

Over 150 of these tumours have been carefully examined and it has been established that there is a consistent histological spectrum in all specimens, thus indicating histogenetic uniformity. The consistent features include sarcomatous areas of spindle-shaped fibroblasts, areas of embryonic mesenchyme, tracts of smooth muscle of vascular type, vasoformative organisation by groups of tumour cells, a matrix of reticulin and collagen and an assortment of epithelial structures such as cysts, tubules and nests of transitional epithelium. These latter epithelial elements represent sequestered, pre-existing components of the renal parenchyma. A proportion of the tumours also contains mature striated muscle, rhabdomyoblasts, pericytes and areas of haemangiomatous nature. The histological spectrum suggests therefore that the tumour may be a vascular neoplasm. Histochemical methods will be used to explore further the vascular properties of these neoplasms. Enzymes studied will include $NADH_2$ — tetrazolium reductase, ATP-ase, 5′ nucleotidase, lactic dehydrogenase, alkaline phosphatase and nucleotide diphosphatase.

Tissue culture of mesenchymal tumours is also helping to establish their histogenetic uniformity. So far, those that have been seeded into culture display a consistent, uniform pattern of behaviour and morphology.

Comparative aspects of renal tumour morphology are under investigation in species other than the rat. The nephroblastoma of the pig resembles Wilm's tumour of children in that both malignant epithelial and mesenchymal elements are present. In the rat however these two components appear to exist as separate entities and the two have not been described in mixed form. Comparative morphological studies may enable a better understanding of the extent to which animal cancer models may be extrapolated to the carcinogenic process in man.

Studies with Alternative Renal Carcinogens

Preliminary tests are being conducted to explore the possibility of setting up a model of rat nephroblastoma by means of dimethylbenzanthracene administration to the ovariectomised female.

The sequential stages in the development of tumours induced by alternative renal carcinogens will be compared with the process induced by DMN. Ethyl methanesulphonate which is associated with the production of mesenchymal neoplasms and diethylnitrosamine or the diabetogenic antibiotic streptozotocin which induces only epithelial neoplasms are several of the carcinogens that will be studied.

Scleroderma 1950-1972

A. J. Barnett

Introduction

Although there were numerous reports of scleroderma in the late nineteenth and early twentieth centuries, in 1950 it was still considered as a rare disease. Early workers had regarded it as a dermatological condition with associated vascular disturbances and it was not until near the mid-century that it was generally recognised as a systemic disturbance and the term "progressive systemic sclerosis" was introduced. About this time there were reports of involvement of particular internal organs: gastro-intestinal tract, heart, lungs, kidney. Classification of types of scleroderma was confused and the name was applied to two conditions which are now generally considered separate entities. One is a purely dermatological form (morphoea) and the other is a form with associated vascular phenomena and in some cases visceral disturbance. It was not clear whether the latter comprised a single disease or several for at one extreme was the form with marked vascular phenomena and sclerosis limited to the fingers (Raynaud's disease with sclerodactylia) and at the other the form with widespread skin changes, frequently less prominent vascular features but often with marked visceral disturbance (diffuse scleroderma).

Clinical Description

My interest in scleroderma arose primarly from a study of vascular disease. In a group of 30 patients with ischaemic symptoms in the hands there were five cases of the supposedly rare disease — scleroderma[1].

Awareness of this association led to the recognition of further cases, and by 1959 twenty-seven cases had been seen[2]. These were grouped according to whether the onset seemed typical of Raynaud's disease and whether the sclerosis was confined to the hands or also involved other areas. (This classification is no longer used as "typical" Raynaud's disease requires a female sex and early age of onset and scleroderma of similar severity may occur in either sex or at any age.)

In this series visceral disturbance and a fatal outcome was more common in patients with widespread skin changes and there was uncertainty as to whether the occasional visceral disturbances in cases with sclerosis confined to the hands were a part of the disease or were incidental.

A special study was made of the vascular disturbance in 30 cases. Immersion in cold water produced cyanosis or pallor of the digits; in most cases there was a delay in flushing of one or more digits in response to an arterial occlusion reactive hyperaemia test; heat elimination during a reflex hyperaemia test was subnormal. In the seven cases studied by arteriography, including examples from each of the three groups according to the classification used (see below), there was structural narrowing or blockage of arteries. It was concluded that the vascular features were mainly due to structural arterial disease.

Classification and Course

In 1969[3] sixty-one were reviewed and three types were described. Type I in which the skin changes remained confined to the digits; type 2 in which the skin changes extended beyond the digits but spread slowly and type 3 in which the skin changes were diffused from an early stage. This grouping was adopted to avoid confusion with descriptive terms used by other workers which did not seem to always mean the same thing to different people. This study showed a predominance of females (approximately 3 to 1), onset mainly in middle age, visceral involvement in a high proportion of patients from all three "types" and a general tendency to progression. Four of the 20 type 1 cases, four of the 32 type 2 cases and six of the nine type 3 cases had died. However, only one of the deaths in the type 1 cases was related to the scleroderma whereas all 10 of the deaths in the other two types were definitely or probably related to the disease. No treatment was of great value but long-administration of adrenal steroids produced softening of the skin and sympathectomy (cervical or lumbar) produced improved skin circulation in about half of the cases in which it was used. A detailed study[4] of the frequency and nature of systemic involvement of various regions — skin, peripheral vessels, alimentary canal, heart, lungs, kidneys, bones and joints — was made in 31 patients available for study. Abnormalities in the various systems occurred in all three types of scleroderma and were of similar type to those described by other workers.

During the past year a monograph[5] which embodies our data on scleroderma has been prepared. It includes a follow-up study of 78 patients and the data are summarised in the following tables.

The data in these tables show that Types 1 and 2 scleroderma are compatible with many years of life but the prognosis is particularly bad in Type 3 for no patient in the series has survived more than 10 years.

Scleroderma is responsible for only one quarter of the deaths in Type 1 cases, one half of the deaths in Type 2 cases and all the deaths in Type 3 cases. Renal failure occurred predominantly in Type 3 cases.

Approximately half of the survivors regard their current health as good.

Table I

Present Status of Seventy-eight Patients

Type	Patients			
	Alive	Deceased	Lost	Total
1	19	12	1	32
2	14	15	6	35
3	1	8	2	11
Total	34	35	9	78

Table II

Information on Thirty-four Living Patients

Type	Number	Known Duration of Illness (years)			Present Health*		
		0-9	10-19	> 20	Good	Fair	Poor
1	19	6	9	4	9	8	2
2	14	2	7	5	7	4	2
3	1	1	0	0	0	1	0
Total	34	9	16	9	16	13	4

* One patient not assessed

Table III

Information on Thirty-five Deceased Patients

Type	Total Deaths	Relation to Scleroderma		Age at Death (Years)			Duration of Illness (Years)		
		Probable	Unrelated or Unknown	0-39	40-59	60+	0-9	10-19	20+
1	12	3	9	1	6	5	7	3	2
2	15	6	9	0	7	8	6	5	4
3	8	8	0	2	5	1	8	0	0
Totals	35	17	18	3	18	14	21	8	6

Table IV

Nature of Deaths Related to Sclerdoerma in Seventeen Cases

Type	Gastro-intestinal	Cardiac	Pulmonary	Renal	Total
1	1	—	2	—	3
2	1	1	3	1	6*
3	—	3	1	4	8
Total	2	4	6	5	17

* In two additional cases, scleroderma probably contributed.

Pulmonary Function

In conjunction with B. C. Ritchie[6] and M. Smith[7] two separate investigations of pulmonary function were carried out in many of these patients. In almost all there was evidence of diffusion and restrictive defects.

Immunological Studies

In 14 out of 33 of these patients examined, autoantibodies were present[8] (rheumatoid factor 14, antinuclear factors 12). In kidneys obtained at autopsy from two patients accumulations of immunoglobulins and complement were demonstrated[9] in the glomeruli, small arteries and arterioles. It was thought possible therefore that circulating immune complexes might be an important factor in the development of renal disease in scleroderma subjects.

Another interesting observation[10] is the finding that chromosome abnormalities occur more frequently in scleroderma patients than in controls.

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Gastrointestinal Diseases

T. D. Lewis

Gastrointestinal Protein Loss

This form of protein loss has become increasingly recognised as an important, although often clinically unsuspected, cause of hypoalbuminaemia. Patients typically present with oedema and gastrointensinal disease is often inapparent so that cardiac, renal or hepatic diesease is suspected. Although the technique of measuring such loss is well established it has not been available within the Alfred Hospital and the initial work in this project concerned development of the technique.

The method used required the intravenous injection of a dose of radioactive chromic chloride ($^{51}CrCl_3$) — a small dose in the range of 50-100 micrograms is used — and the collection of faeces for the succeeding five days. The radioactivity in the stools is then counted and is compared with that in an aliquot of the original sample and so from this the percentage faecal excretion, compared with the injected amount of radioactivity, can be calculated. The normal level of excretion is up to 1 per cent., while definitely abnormal results are in excess of 2 per cent.

The rationale of the investigation is that the chromic chloride becomes bound to circulating plasma proteins and passes into the gastrointestinal lumen with these proteins. However, when the protein molecules become digested and reabsorbed, the chromic ion which is released remains within the lumen as it is unable to cross the gut mucosa in ionic form, and so is excreted in the faeces. Thus increased radioactivity in the stools is indicative of increased exudation of plasma protein into the gut lumen (the only precaution required is to prevent urinary contamination, as this gives falsely high results).

Though more definitive information can be gained from more detailed investigation, this imposes more hardship on the patient and the technique instituted here provides the required information for diagnostic purposes.

Gastro-oesophageal Reflux

At the present time, the medical management of clinically significant gastro-oesophageal reflux is far from effective and so any therapy which relieves the reflux would be a great aid in treatment. As a means of investigating the effects of some newer forms of treatment, a pH electrode (Titron) has been acquired. This is a small unit which is not inconvenient to swallow and will remain in position in the lower oesophagus, while a lead runs from the electrode to the exterior through the patient's mouth. This lead is attached to a pH meter, and to a multi-channel recorder to obtain a permanent record. Any acid reflux into the oesophagus will significantly lower the pH (a value less than 4 is indicative of reflux) and bile reflux will elevate the pH. This technique gives a means of defining the extent of reflux.

Up to the present time we have been gaining experience in the use of instrument with a view of establishing this as an available diagnostic technique for the elucidation of atypical chest pain and also as a valuable tool in the assessment of the efficacy of drugs in preventing gastro-oesophageal reflux.

Also used in the assessment of gastro-oesophageal reflux are oesophagoscopy (to note the degree of oesophagitis) and oesophageal manometry (to localise and assess the state of tone of the lower oesophageal sphincter).

Electron Microscopy

A. Chang

The work of the electron microscopy laboratory during this year has been collaborative in nature and has provided ultrastructural studies in several projects.

An ultrastructural study of dog cardiac muscle was carried out in collaboration with W. G. Nayler (see page 20). Current concepts of events involved in excitation-contraction coupling in cardiac muscle assign a central role to ionised calcium. The intracellular distribution of Ca^{2+} in cells has been shown to occur in association with many subcellular structures, but it is bound in such a way that it is available for excitation-contraction coupling only in the presence of extracellular Ca^{2+}. The sarcolemma, its tubular invaginations and the sarcoplasmic reticulum may therefore play a significant role in regulating the intracellular availability of Ca^{2+}, and in regulating the amount of Ca^{2+} which is available for interaction with the contractile and regulatory proteins. By treating whole cardiac muscle preparation with certain chelating agents including ethylenediamine tetra-acetate (EDTA), the selective permeability of the sarcolemma is destroyed. Such preparations were examined to determine what effect, if any, this had at the ultrastructural level.

An ultrastructural study of human breast cancer was carried out by A. V. Jackson and J. Frisch from the Alfred Hospital Morbid Anatomy Department (see page 62). In view of the observation that scirrhous breast cancer and salivary tumours have a high content of elastin, and the belief that this elastic tissue is a pre-malignant indicator, specimens submitted for frozen section diagnosis were examined under light and electron microscopy to study the elastic tissue in these specimens.

This interest in elastic tissue is shared by D. Challis (Alfred Hospital Morbid Anatomy Department), who has, towards the end of 1972, initiated an electron microscopy study of endocardial fibro-elastosis.

Tumours induced in rat kidneys by the chemical carcinogen dimethylnitrosoamine (DMN) are being studied by G. C. Hard and D. M. Shaw (see page 36). The sequence of histopathological processes which precede and lead to the appearance of macroscopic tumours is defined by light and electron microscopy.

The E.M. facilities have also been utilised in conjunction with D. Millar to determine the purity of microsomal fractions prepared from rat myocardium and used in the study of adenyl cyclase activity (see page 22).

Publications in 1972

Physiology and Pharmacology of Cardiovascular System

Role of Calcium†‡

Nayler, W. G. "Effect of Inotropic Agents on Cardiac Muscle Rendered Highly Permeable to Calcium". **Amer. J. Physiol.** In Press.

Nayler, W. G. "An Effect of Ouabain on the Superficially-located Stores of Calcium in Cardiac Muscle Cells". **J. Molec. Cell. Cardiol.** In Press.

Nayler, W. G. "Calcium and Ventricular Function". **CIBA Symposium.** In Press.

Nayler, W. G. "Influence of Electrolyte Disturbances on Electro-mechanical Coupling". **Cardiologia.** In Press.

Nayler, W. G. and J. Szeto "Effect of Sodium Pentobarbital on Calcium in Mammalian Heart Muscle". **Amer. J. Physiol.** Vol. 222 (1972) p. 339.

Nayler, W. G. and J. Szeto "Effect of Verapamil on Contractility, Oxygen Utilisation and Calcium Exchangeability in Mammalian Heart Muscle". **J. Cardiovasc. Res.** Vol. 6 (1972) p. 120.

Pharmacology‡

Nayler, W. G. "β-Adrenoceptor Agonists and Antagonists'. **Med. J. Aust.** Vol. 2 (1972). Supp., Sept., p. 43.

Nayler, W. G. "Comparative Partial Agonist Activity of β-Adrenoceptor Antagonists". **Brit. J. Pharmac.** Vol. 45 (1972), p. 382.

Nayler, W. G. "Cellular Pharmacology of Beta-blocking Drugs" in **New Perspectives in β-blockade.** In Press.

Nayler, W. G. and J. Tay "Effect of 0-2-hydroxy-3-(tert. butylamino) propoxybenzonitrile HCl (KO1366) on beta-adrenergic Receptors in the Cardiovascular System". **J. Pharm. Therap.** Vol. 180 (1972), p. 302.

Trinker, F. R. "The Effects of Catecholamines on Isolated Perfused Coronary Arteries in the Dog". **Brit. J. Pharmac.** Submitted.

Myocardial Function‡*

Mäsiar, P. and P. Daile "A Study of Cardiac Troponin: Its Comparison with Skeletal Troponin". **Biochem. Biophys. Res. Com.** Submitted.

Nayler, W. G. "Regulation of Myocardial Function". **J. Molec. Cell. Cardiol.** In Press.

Nayler, W. G. "Salbutamol, Isoprenaline et Orciprenaline — Etude Comparative de leur Action sur la Fonction Cardiaque". Symposium. Salbutamol. In Press.

Nayler, W. G. and V. Carson "Effect of Phentolamine on Myocardial Function, Efficiency and Noradrenaline Levels in Blood Plasma". **Cardiovasc. Res.** Vol. 6 (1972), p. 500.

Nayler, W. G. and V. Carson "Effect of Stellate Ganglion Stimulation on Myocardial Blood Flow, Oxygen Consumption and Cardiac Efficiency during Beta-adrenoceptor Blockade". **J. Cardiovasc. Res.** In Press.

Nayler, W. G. and I. McInnes "Salbutamol and Orciprenaline-induced Changes in Myocardial Function". **Cardiovasc. Res.** Vol. 6 (1972), p. 725.

Plasma Vasoactivity

Mäsiar, P., E. Mäsiar and D. G. Oakley "An Uncommon Inotropically Active Peptide from Dog Blood Plasma". **Biochem., Biophys. Res. Com.** In Press.

Blood Coagulation

Fantl, P. "Evolutionary Trends in Plasma Mercaptalbumin Composition". **Comp. Biochem. & Physiol.** Vol. 42B (1972), p. 403.

Fantl, P. "Thiol Distribution in the Plasmas of Native Australian Mammalia". **Aust. J. exp. Biol. med. Sci.** Vol. 49 (1971), p. 521.

Fantl, P. "The Relationship In Man Between Age And Plasma Concentration of Mercaptalbumin". **J. Geront.** Submitted.

Cardiac Surgery

Cooper, E. "Aortic and Mitral Valve Replacement with Autologous Fascia Lata Valves". **Med. J. Aust.** Vol. 2 (1972), Spec. Supp., p. 47.

Miscellaneous

Barnett, A. J. "Scleroderma". Monograph. In Press.

Chang, A. and S. Faine "Effect of Anti-cell and Anti-axial Filament Sera on **Leptospira". Aust J. exp. Biol. med. Sci.** Submitted.

Chang, A. and S. Faine "Association Between the 'Incomplete Subserotypes and the Cryptic Axial Filament Antigen of **Leptospira". Aust. J. exp. Bio. med. Sci.** Submitted.

Chang, A. and S. Faine "Relative Specificity of Immunoglobulins Induced by Leptospiral Axial Filament Antigens". **Aust. J. exp. Biol. med. Sci.** Submitted.

Chang, A., S. Faine and W. T. Williams	"Cross-reactivity of the Axial Filament Antigen as a Criterion for Classification of **Leptospires**". **Aust. J. exp. Biol. med. Sci.** Submitted.
McGiven, A. R., W. G. R. M. de Boer and A. J. Barnett	"Renal Immune Deposits in Scleroderma" . **Pathology** Vol. 3 (1971), p. 145.
Mäsiar, P. and D. C. Shaw	"Two Isoenzymes of Arginine Kinase from Panuliras longipes". **Biochem. Biophys. Acta.** In Press.

Lectures Given During 1972

A. Chang *et al*	"Leptospiral Axial Filament Antigen: Computer Analysis of Its Relationship Among 36 Strains"	Australian Society for Microbiology, University of Sydney.
A. Chang *et al*	"Antigens of Leptospires"	Australian Society for Microbiology, University of Sydney.
E. Cooper	"The Role of Surgery in Myocardial Infarction"	Melbourne Medical Post-graduate Committee.
G. C. Hard	"Aspects of Experimental Renal Tumours"	Monash University.
G. C. Hard	"Morphogenesis of a Renal Neoplasm"	Australian Society of Experimental Pathology, Sydney.
D. Millar	"Properties of the Cardiac Microsomal Fraction"	Australasian Society of Clinical and Experimental Pharmacologists, Sydney.
W. G. Nayler	"β-adrenoceptor Agonists and Antagonists"	Symposium on "Migraine and Hypertension", Post-graduate Committee in Medical Education, University of New South Wales.
W. G. Nayler	"Coronary Blood Flow in Relation to Cardiac Work — Some Drug-induced Changes"	Alfred Hospital Clinical Society
W. G. Nayler	"Cellular Pharmacology of β-blocking Drugs"	Symposium on "New Perspectives in β-blockade", Aahus, Denmark.
W. G. Nayler	"Pharmacology of beta Stimulants"	Symposium on Cardiac Stimulants, Melbourne.
W. G. Nayler	"Calcium and Ventricular Function"	Symposium on "Prospects on the Management of Ischaemic Heart Disease", Cardiff.

Seminars Held During 1972

	Adrenergic Receptors (May 4).
G. Kroneberg	Cerebral α-adrenergic Receptor Stimulation and Hypotensive Activity.
H. J. Schümann	Influence of Temperature Changes and Inhibition of the Metabolic State on the Sensitivity of Adrenergic α and β-recptors of Isolated Organs.
G. A. Bentley	Drug-induced Cardiovascular Reflexes.
	Friday Seminars.
V. Carson	Cardiac Cycle 3'5' AMP.
J. F. Chalmers	Brain Amines and Neurogenic Hypertension.
A. Chang	Immunological Response to Leptospiral AF Antigen.
E. Cooper	Control Mechanism of the Sinus Node.
P. Daile	The Calcium-Binding Component of Cardiac Troponin.
G. C. Hard	Morphology of Experimental Renal Tumours in Rat.
G. C. Hard	Morphological Development of a Renal Mesenchymal Tumour in Rat.
G. C. Hard	Morphological Development of a Renal Adenocarcinoma in Rat.
S. Katz and D. Millar	Adenyl Cyclase Activity.
T. D. Lewis	Pancreatitis in Some Clinical Complications.
T. E. Lowe	Visits to Some Research Institutes.
E. and P. Mäsiar	The Significance of Proteins and Peptides in Relation to Heart Failure II and III.
F. Silberberg and A. J. Barnett	Study of Long-Term Treatment of Hypertension.
F. R. Trinker	Mechanism of Drug Interactions.
I. M. Williams	Micro-emboli in Cardiac By-pass Surgery.

The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute

Revenue Account for the Year Ended 31st December 1972

EXPENDITURE		INCOME		
Salaries and wages	$199,876	**Donations from Baker Benefactions**		
Laboratory supplies and isotopes	39,664	Transfers from Restricted Fund		$153,399
Library maintenance	7,032	**Grants in aid of Research Projects**		
Postage and telephone	1,564	Anti-Cancer Council	$13,748	
Printing and stationery	2,258	National Heart Foundation of Australia	16,730	
Light and power	18,860	Life Insurance Medical Research Fund of Australia and New Zealand	13,287	
Insurance	6,304	National Health and Medical Research Council	6,302	
Repairs and renewals	7,589	**Other Grants**		
Animal house contribution	4,000	The James and Elsie Borrowman Research Trust	5,000	
Sundries	4,645	The William Buckland Research Fund	680	
Travelling expenses	2,578	Victorian State Government	20,000	
Public relations	1,133			75,747
		Interest from Investments		
		Held by Trustees of The Baker Institute Grant Trust	$1,700	
		Other Income	45,651	
				47,351
		Sundry Sales, Recoveries and Refunds		18,616
		Deficit for Year		390
	$295.503			$295,503

The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute

Balance Sheet as at 31st December 1972

FUNDS AND LIABILITIES		
Funds		
Accumulated (deficit) brought forward	($1,906)	
Add (deficit) for year	(390)	
Accumulated (deficit)	(2,296)	
Restricted Fund	39,078	
Endowment Fund	784,740	
William Buckland Research Fund	20,870	
Laura Nyulasy Research Scholarship Fund	3,940	
Lang Research Scholarship	4,246	
		$850,578
Curent Liabilities		
Sundry creditors and accrued expenses		14,930
		$865,508

ASSETS		
FIXED ASSETS (Note 1)		
INVESTMENTS		
Held by Trustees of the Institute:		
Commonwealth Inscribed Stock	$2,937	
M.M.B.W. Stock	7,202	
S.E.C. Stock	5,062	
Treasury Bonds	5,000	
Argo Investments Co. Ltd. shares	29,806	
Softwood Products Treatment Co. Pty. Ltd. shares	100	
Short term deposits	22,651	
Mortgage loans	217,000	
North Broken Hill Ltd shares	1,224	
	$290,982	
Held by Trustees, Executors & Agency Co. Ltd.		
Laura Nyulasy Research Scholarship Fund	3,940	
William Buckland Scholarship Fund	20,870	
Endowment Fund	489,864	
		$805,656
CURRENT ASSETS		
Cash on Hand	$100	
Cash at Bank	53,470	
Sundry debtors	6,282	
		59,852
		$865,508

Notes to the Balance Sheet

1. Expenditure included in present or past periods on fixed assets including laboratory equipment, motor vehicles, buildings, improvements and furniture and fittings, have been charged against appropriate funds, grants or revenue accounts.
   The insured value of all assets at December 31, 1972, including the building, totalled $1,785,000.
2. In addition to receiving income from investments shown above, the Institute receives interest on $34,000 5% Commonwealth Inscribed Stock which is held by the Trustees of The Baker Institute Grant Trust for the benefit of the Institute.

Auditor's Report to the Trustees of the Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute

As an audit procedure it was not practicable to extend our examination of contributions and donations beyond the accounting for the amounts received as shown by the books and records of the Institute.

Subject to the above reservation, in our opinion, the above balance sheet together with the notes thereto is properly drawn up to show a true and fair view of the state of the Institute's affairs at December 31, 1972.

PRICE, WATERHOUSE & Co.

Melbourne. Chartered Accountants.
February 5, 1973.

The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute

Year Ended 31st December 1972

DEVELOPMENT FUND		
Balance at December 31, 1971		$15,616
Deduct:		
Transfer to Restricted Fund		15,616
Balance at December 31, 1972		NIL
RESTRICTED FUND		
Balance at December 31, 1971		$88,613
Add:		
Transfer from Estate of Thomas Baker	$268,113	
Transfer from Development Fund	15,616	
Transfer from Revenue Account for equipment ordered but not delivered at balance date	16,000	
Further donations from Victorian Government relating to electron microscope	1,238	
Donations — Other	4,968	
Sundry Receipts	375	
		306,310
		$394,923
Deduct:		
Transfer to Endowment Fund	$187,556	
Transfer to Revenue Account	153,399	
Equipment Costs	14,890	
		355,845
Balance at December 31, 1972		$39,078
ENDOWMENT FUND		
Balance at December 31, 1971		$564,664
Add:		
Donations	$22,457	
Transfer from Restricted Fund	187,556	
Accretion of Trustees, Executors & Agency Co. Ltd. investments	801	
Interest — Blake & Riggall short-term	9,095	
Sundry Receipts	167	
		220,076
Balance at December 31, 1972		$784,740

Donations

The following gifts to the Institute were received during the year.

Victorian Government	$21,258.00
H. and L. Hecht Estate (Perpetual Executors & Trustee Co.)	5,000.00
"Group B"	3,617.76
Edgar Rouse	2,475.00
Edward Wilson Estate (Trustees Executors and Agency Co. Ltd.)	2,000.00
"Baker Birds"	1,512.06
William Angliss (Victoria) Charitable Trust	1,500.00
Appel Family Bequest (Trustees Executors and Agency Co. Ltd.)	1,400.00
Bushell Trust	1,000.00
J. B. Were & Son	1,000.00
Alfred Edments Estate (Trustees Executors and Agency Co. Ltd.)	800.00
Truby and Florence Williams Estate (Trustees Executors and Agency Co. Ltd.)	800.00
M. and E. H. Flack Estate	700.00
Carlton & United Breweries Ltd.	500.00
Mrs. Laura Hannan	500.00
B. J. Jones	500.00
Lauri Newton	500.00
Vulcan Australia Ltd.	500.00
George F. Little Trust (The Equity Trustees Co. Ltd)	475.00
Darren Baillieu	375.00
Mayne Nickless Ltd.	250.00
The Danks Trust	250.00
Roche Products Pty. Ltd.	200.00
General Motors-Holden Pty. Ltd.	150.00
W. J. Baker	100.00
C. A. Gordon	100.00
Mrs. R. Hewgill	100.00
Klamis Investments Pty. Ltd.	100.00
Mrs. L. Jones	100.00
Siegfried Meyer	100.00
W. J. L. Ould	100.00
Pethard Tarax Charitable Trust	100.00
Dr. James Syme	100.00
Stuart Sanderson	75.00
Bayer Pharma Pty. Ltd.	50.00
Berry Currie Pty. Ltd.	50.00
Mr. Eric Cooper	50.00
Alan Drayton	50.00
The Specialty Press Ltd. (4th Instalment)	50.00
Mrs. N. G. Baker	40.00
A. Huckfield	33.40
Sir William Howarth	25.00
Reginald Blakemore	19.96
Mrs. J. Ortiz	15.30
Miss N. E. Cameron	10.00
J. C. Habersberger	10.00
K. Harvey	5.00
	$51,376.49

Further donations were received from—

J. W. Archer, Mrs. B. J. Barnett, Reginald Blakemore, Mrs. N. E. Cameron, M. A. Cuming, Mrs. M. A. Cuming, Eagle Star Insurance Company Ltd., Mrs. J. Grecian, J. C. Habersberger, L. Harmer, George F. Knox, Kodak (Australasia) Pty. Ltd., Kodak Senior Staff Social Club, A. Lazer, F. Manning, Melbourne University Rugby Football Club, N. H. Payne, Ringwood Elderly Citizens' Club, Edgar Rouse, Mrs. M. Rumble, H. E. Tobin, H. C. Trinnick, West Brighton Club.

In memory of—

M. Clancy, Mary Clarkson, Frank Crane, Arthur Leith, Margaret Reilly, Charles Sims, L. S. Andrew, Dr. A. Cooper, Roy Grave, L. R. Stillman, N. W. Swann, Mrs. A. Canham, Mrs. A. Vowles, Mrs. Lillian Maxwell, Prof. W. Rawlinson, Raymond Allsop, Mrs. N. M. Wollaston, D. J. Roney, Charles Chitts, Maj-Gen. E. J. Milford, C. C. Blaikie, Dr. W. H. Ward. Mrs. Ruth Barrett, C. H. Barrett, Charles Barnett, L. A. Boardman, Mrs. Elsa Carver, T. a'B. Cutbush, Norman E. Jones, Dr. Paul Fantl, Sam Fripp, Joan Grecian, T. Griffiths, Dr. W. G. Holman, A. McColl, Mrs. M. Jones, Mrs Ethel MacGowan, Dale Teasdale, James Baillieu, John Williams, Mr. and Mrs. C. Campbell, W. R. Thompson, G. Mitchelmore.

Total: $415.10

The Trustees record their appreciation of the assistance rendered by Berry Currie Pty. Ltd. in the presentation of this report.

The Ewen Downie Metabolic Unit

Staff

Honorary Consulting Physicians:
EWEN DOWNIE, M.D., F.R.C.P., F.R.A.C.P.
BRYAN HUDSON, M.D., Ph.D., F.R.C.P., F.R.A.C.P.

Honorary Consulting Biochemist:
JOSEPH BORNSTEIN, D.Sc., M.D., F.R.A.C.P.

Physician-in-Charge:
PINCUS TAFT, M.D., F.R.A.C.P.

Honorary Physician:
HARALD BREIDAHL, M.D., M.R.C.P., F.R.A.C.P.

Assistant Physician:
R. M. DARGAVILLE, M.B., B.S., M.R.A.C.P. (Resigned June, 1972).

Registrar:
C. VARLEY, M.B., B.S.

Biochemists:
J. S. C. CHAN, B.Sc.
M. MALINEK, M.Sc.
JUNE SHEATH, M.Sc., F.A.A.C.B. (Deceased June, 1972).
DORA WINIKOFF, M.Sc., F.A.A.C.B.

Technical Staff:
Miss I. EKKEL.
Mr. W. HUDSON.
Mrs. F. RABOLD.
Miss J. ROSS (Resigned October, 1972).
Mrs. F. FELLER (From October, 1972).

Secretary:
Miss J. EWENCE.

Diabetic Clinic

Clinical Assistants:
MARGARET SANDERS, M.B., B.S.
G. T. SEILER, M.B., B.S.

Chiropodist:
V. DeVERE, M.A., Ch.A.

Honorary Consulting Chiropodist:
M. IMPEY, F.Ch.A.V., M.Ch.I.A.

Research Fellows

Burroughs Wellcome Research Fellow:
H. MOSMAN, B.Sc.

N.H. & M.R.C. Research Scholar:
PAUL ZIMMET, M.B., B.S., Ph.D., M.R.A.C.P. (Until April, 1972).
G. T. SEILER, M.B., B.S. (From April, 1972).

Honorary 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.

Annual Report

The past ten years have seen remarkable changes in the practice of endocrinology both clinically and in the laboratory. Important contributions to these changes have been made by the development of a variety of techniques for the measurement of a wide spectrum of hormones, peptides and steroids, present in very low concentration in blood. Such measurements have enabled studies of endocrine function and interrelationship to be made such that control mechanisms and behaviour under physiological conditions of various endocrine systems have become more clearly defined and better understood. They have facilitated the recognition of endocrine disease and provided the means of following the course of illness and the influence of treatment. Notable advances in diabetes, various pituitary diseases, reproductive disorders and abnormalities of thyroid function have been made.

It is essential in a unit such as this that these techniques are available for the investigation of patients under its care. The introduction of these methods although not novel work, requires considerable care and attention to detail, quality control of experimental techniques and the definition of a "normal range" and a "disease range" for local laboratory conditions. This has been and will continue to be a responsibility of the unit which, along with the application of such methods and more specific investigation procedures, has occupied much of the time of its staff.

With the return in 1973 of Dr. J. R. Stockigt, Registrar in 1964, to the Unit as Deputy Director, new ground is again to be broken. His studies of endocrine aspects of hypertension during his work in the United States of America and the United Kingdom will enable him to add to the scope of the Unit both in clinical expertise in this area and in investigative studies involving renal and adrenal aspects. New methods will be introduced, a new dimension of investigation will become available, and the facilities for diagnosis and management of yet another group of patients will be upgraded.

The role of the Unit in more basic research is reflected in the recent award to Dr. Paul Zimmet of the degree of Ph.D. (Monash) for his studies in collaboration with Dr. Frank Ng, Department of Biochemistry, Monash University, on the influence of growth hormone derived peptides on carbohydrate metabolism. A progress account of this work is included in this year's research report.

It is with sadness that the death in 1972 of Miss June Sheath, M.Sc., F.A.A.C.B., a foundation member of the Unit, is reported. A meticulous worker, Miss Sheath was concerned with the establishment of a wide variety of biochemical methods which had diagnostic and investigative applications and had published a number of novel methods. She was also associated with many publications by members of the Unit with whom she collaborated by means of her methodological skills. Her final paper, published after her death, was typical of her work involving as it did the use of micro-enzymatic techniques in studies of intra-uterine foetal metabolism undertaken in collaboration with the staff of Professor Carl Wood, Department of Obstetrics and Gynaecology, Monash University.

As has always been our happy lot, this past year has seen continued valued collaboration with and assistance from colleagues in both clinical and laboratory departments at Alfred Hospital and Monash University. Assistance by grants-in-aid and in kind have enabled the acquisition of the facilities necessary to maintain the laboratory back-up in support of the technologic advances referred to earlier. Of special mention is the generous grant made this year in the name of Dr. Ewen Downie, founder of the Unit now bearing his name, by Eli Lilly and Company Australia in recognition of his initiation of, and service as chairman of the Australian Committee responsible for the selection of the incumbent of the yearly Lilly Fellowship. And too of the long continued support — initiated in collaboration with Dr. Downie when he was Honorary Physician in charge of the then Diabetic and Metabolic Unit — by Burroughs Wellcome and Co. (Australia) Ltd. This yearly grant has supported a distinguished list of Research Fellows now represented in academic and clinical positions here and abroad.

PINCUS TAFT,
Physician-in-Charge.

Grateful Acknowledgment is made of financial assistance and gifts in kind from—

Estate of the late Vincenza E. M. Acton.
Alfred Hospital Research Funds.
Burroughs Wellcome & Co. (Australia) Ltd.
Eli. Lilly (Australia) & Co.
Fawns & McAllan Pty. Ltd.
Mr. B. Gishen.
Hoechst Australia Ltd.
Monash University.
Miles Laboratories Aust. Pty. Ltd.
D. M. Rollason.
Mr. C. Rotstein.
William R. Warner & Co. Pty. Ltd.

Studies on the Role of Pituitary Growth Hormone Fragments in the Regulation of Glucose Metabolism in Man

P. Zimmet(1), G. T. Seiler(1), H. Mosman, P. Taft, F. Ng(2) and J. Bornstein(2).

That growth hormone under both clinical and experimental conditions can produce diabetes in man and animals has long been known. It has been demonstrated recently that a small fragment of the hormone, termed somantin, can produce diabetic-like effects in experiments on isolated tissues and cells. A second fragment cataglykin, a distant part of the molecule, reverses these actions. This project is concerned with an examination of the possible role of excess action of the first fragment in the cause of human diabetes and of the second in its treatment. During this year Dr. Paul Zimmet, previously Medical Post-Graduate Research Scholar, was awarded the degree of Ph.D. (Monash) for his work on this project. He has taken a post at Guy's Hospital, London, in the Department of Medicine, and his position as Research Fellow for 1972 has been filled by Dr. Gwen Seiler.

The evidence for the presence of somantin and cataglykin in blood and urine respectively referred to in last year's report has been extended. In particular plasma somantin has been shown to cause lipolysis in intact rabbits, in rat epididymal fat pads and in rat hemidiaphragm. Urinary cataglykin reverses lipolysis in the **in vitro** experimental situation.

Small amounts of synthetic cataglykin have been available for the necessary toxicity studies preliminary to its use in a clinical trial in the treatment of human diabetes. Twelve rats of similar weight and age were used, six serving as controls, the test animals being given daily injections of cataglykin. Two animals from each group were sacrificed at one, two and four weeks. The parameters monitored included growth, activity and general health, weekly haematological and serum electrolyte profile, terminal urea and creatinine levels and histological examination of a wide variety of tissues. No abnormality was observed in the treated animals. While there was no difference in the glucose response in the two groups to administered glucose or insulin, there was a significantly increased level of liver glycogen in the treated group indicating a biological effect on carbohydrate metabolism. We are indebted to Dr. Alan Jackson, Dr. R. Sawers and Dr. P. Garcia-Webb for their assistance and that of members of staff of their departments in undertaking the morbid anatomical, haematological and biochemical studies in this aspect of the programme.

The absence of change in glucose levels **de novo** or following insulin or glucose in these animals was reflected to some extent in extensive studies of insulin sensitivity and glucose tolerance in rats given single doses of synthetic cataglykin at various dose levels. Inconsistent responses indicative of facilitation of insulin action were noted.

Rabbits have also been used as animal models in an examination of the effects of cataglykin, synthetic and urinary. In contrast to the rat experiments it has been shown that the rate and extent of glucose removal is increased after glucose administration in cataglykin primed animals. Considerable interest is aroused by the observation in these animals that for up to six days following cataglykin priming, despite unchanged or improved glucose tolerance, insulin secretion is significantly less than pre-treatment levels again implying an increase in insulin sensitivity generated by cataglykin. An increased degree and duration of hypoglycaemia in response to a small (0.025 μ/kg) intravenous injection of insulin has also been observed in rabbits primed with urinary cataglykin. This parallels the observations previously made in normal man primed with ovine growth hormone derived cataglykin. The study has been extended in the animal experiments in that it has been observed that heightened insulin sensitivity persists for up to six days, the effect being apparently dose dependent. This observation is similar to the persistence of the effect of cataglykin in improving glucose tolerance with a paradoxically lowered insulin secretion for a similar period after cataglykin priming. This protracted effect could have clinical implications.

In previous limited studies it has been shown that blood drawn from normal people during glucose assimilation, when appropriately extracted, produces a fall in the degree of activity of the enzyme glyceraldehyde-3-phosphate dehydrogenase — a marker of the presence of somantin. With the completion of glucose assimilation and storage this effect disappears and the degree of inhibition returns to a baseline level. In "genetic" diabetics the baseline inhibition is greater and is not diminished by a glucose load. These preliminary studies are being extended and 23 patients suffering from various forms of diabetes have been examined. These include patients with diabetes due to excessive growth hormone production (acromegaly), with lack of insulin due to known disease of the pancreas (pancreatitis and haemochromatosis) as well as "genetic" diabetes both of the juvenile and maturity onset types. Blood has been drawn prior to and during glucose assimilation after oral glucose loading. Examination of glucose, insulin and growth hormone levels has been made and the plasma samples have been ultra-filtered, freeze dried and vacuum sealed prior to mass assay in an examination of the somantin responses.

Thus far cataglykin has been found in urine only. Further examination of blood in a search for cataglykin is being made. In addition the effects of food and of insulin on cataglykin and on somantin levels are being studied in order more clearly to delineate the role of these peptides in health and disease in a comparative study of a number of normal and diabetic patients.

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(2) Department of Biochemistry, Monash University.

Thyroid Investigations

Dora Winikoff and Malvina Malinek (with the technical assistance of Jenny Ross and Vreni Feller)

In the course of the last year a change has been made in the routine diagnostic regimen employed for screening patients suspected of thyroid disease.

Although we are still of the opinion that to evaluate patients whose tests are in the borderline range of values, or receiving interfering medication, a full thyroid profile of tests is essential, in the majority of cases an abbreviated procedure can suffice.

This consists of the determination of serum thyroxine (ST4) plus a Diagnostic Ratio (D.T.R.) introduced recently and both carried out with the aid of "Tetralute" Kits (Ames)*.

1. Diagnostic Thyroxine Ratio (DTR)

[Med. J. Aust. Vol. 2 (1972), p. 1035]

This parameter developed by us is based on the principle employed in the "Mallincrodt" Kit method, the "Effective Thyroxine Ratio" (ETR), based on the "Res-O-Mat-T_4", and which the makers claim provides an appraisal of the thyroid state by a single assay procedure.

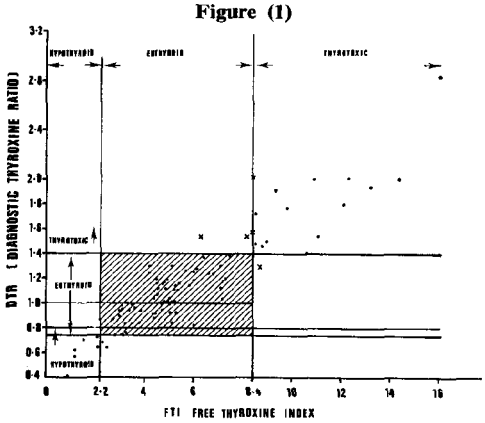
The Principle of DTR*: Into the "Tetralute" Competitive binding system, at the stage when serum thyroxine and tracer, detached from its binding carrier protein, are trapped on the sephadex column, a 10 ul sample of the original whole serum is introduced. Following this, the addition of the standard solution of thyroxine binding globulin (TBG), which elutes some of the trapped thyroxine, results in a competition between the TBG of the added serum, the TBG of the eluting fluid, and the sephadex gel. A certain amount of radioactivity is retained on the column, proportional to the amount of thyroxine as well as TBG present in the original sample.

By relating this to the percentage of radioactivity remaining on the column of a standard reference serum treated similarly, a "Ratio" is obtained which correlates both with ST_4 and TBG of the tested serum.

The advantage of DTR as an additional parameter to ST_4 (estimated concurrently) is obvious, due to the simplicity and speed of the procedure. the elimination of the alcohol extraction necessary for other techniques, and the results expressed as serum thyroxine level plus "DTR" simultaneously. This is far more convincing than an "ETR" assay which provides the result only in terms of an abstract "Ratio". Normal range under conditions in our laboratory is from 0.75 - 1.35, mean 1.00 when "Metrix" Normal Clinical Chemistry Control Serum (Armour Pharmaceuticals) is used as the reference standard. The original "Tetralute" serum is then used as a quality control, or vice versa.

Fig. (1) illustrates the Correlation between Free Thyroxine Index (FTI) and DTR in 100 consecutive samples received for diagnostic assay.

Figure (1)



x — refers to patients on thyroxine medication.

A comparison of diagnostic accuracy of "ETR" and "DTR" based on a full thyroid profile was carried out on 50 patients who were euthyroid, hypo-hyperthyroid and including some on interfering medication (thyroxine, corticosteroids, oestrogens), children and patients following ^{131}I therapy. (See Table.)

With the development of DTR the streamlining of our diagnostic service became possible. We still have recourse to other tests when indicated.

* "DTR" has been also effectively estimated using "Tertasorb" (Abbott Lab.) kits and it can be adjusted to any procedure of thyroxine assay by the method of competitive binding.

Table

	Correct %	Incorrect %	Uncertain %
ETR	74	14*	12
DTR	86	6**	8**

* These were misleading as normal results obtained in the face of abnormalities.
** If ST_4 levels estimated concurrently with DTR were considered, a correct diagnosis of all patients was feasible.

2. Oral Contraceptives and Thyroid Function Tests

This study initiated in 1964 is drawing to an end.

The object this year was to ascertain whether the "changing scene" due to the almost universal use of oral contraceptives containing low levels of oestrogen had altered the validity of our Diagnostic Criteria [D. Winikoff, Med. J. Aust. Vol. 1 (1971), p. 1059] for defining thyroid disease during the intake of the "Pill".

We found as a general rule that the serum thyroxine iodine levels (ST_4I) measured with

"Tetralute" Kits were appreciably lower when compared to those encountered in the previous years, particularly when compared to the PBI values then employed. Other thyroid parameters also showed less dramatic departure from normal levels of untreated patients. This, of course, makes the detection of borderline states much harder. However, in cases with proved hypo- or hyperthyroidism, the same criteria were still valid. For patients suffering from subclinical hypothryroidism while on the "Pill", DTR is often much more helpful than FTI which is almost invariably in the (low) normal range, due to an increase in ST_4I values while T_3-RU is only slightly lowered.

3. Further Investigations into the Problem of Habitual Abortion

As previously stated, a distinct thyroid profile of a rise in ST_4, TBG and EI and a fall in T_3RU is characteristic in normal pregnancy after the first trimester.
The EI is almost invariably over 80%, FTI remains in the normal range of values and so does the DTR.

We have undertaken a systematic study of women who previously had two or more spontaneous abortions. A group of these when pregnant again, and followed at monthly intervals, exhibited the characteristic "normal" pattern when pregnancy was sustained.

Three women whose parameters were abnormal before miscarriage, in subsequent pregnancies when maintained on small doses of thyroxine (0.1 mg/day), reverted to the normal pregnancy pattern. In contrast, a group of women tested at the time of actual miscarriage showed a high percentage of an altered thyroid profile, namely normal (non-pregnant) ST_4 and T_3RU, and EI under 80%. TBG was normal or only slightly elevated in sharp contrast to the usual high pregnancy values.

4. Thyroid Function Tests after the administration of Thyrotrophin Releasing Factor (TRH)

The diagnosis of marginal hypothyroidism, and differentiation of thyroid from pituitary or hypothalamic insufficiency, can be facilitated by the thyrotrophin (TSH) assay following the administration of the synthetic tripeptide (TRH).

High levels of TSH before, and a substantial rise after the administration of TRH, indicate hypothyroidism of primary origin.

We have investigated a series of patients suffering from frank thyrotoxicosis and myxoedema as well as euthyroid patients with high and low levels of TBG, after removal of pituitary gland, after I^{131} therapy and while on oral contraceptives.

Specimens were taken at −10, +60 and +120 minutes and after 24 and 48 hours following the intravenous administration of TRH.

Although minor variations in all thyroid parameters were observed, they did not follow any clearly defined pattern and none were significant. It is concluded that the use of thyroid parameters to follow the changes produced in TSH levels could possibly be justified only if very high doses of TRH were administered.

5. Thyroid Function Tests during Sleep

A series of tests was carried out to observe the thyroid profile in normal volunteers during sleeping hours.

The results were manifested as small but significant changes. They are reported elsewhere by Dr. M. Johns, of the Department of Surgery of the University of Monash.

Diabetes Eye Study

F. W. Billson, H. D. Breidahl, P. Cowen, J. B. Foster, P. Taft, and R. H. West.

Work has continued in the Special Diabetes Eye Clinic during this year. One hundred and seven patients have been studied and are at various stages of follow-up. Careful retinal colour photographs concentrated on standard views of the optic disc and of the macula area in each eye are used as the permanent record of progress of the retinal disease. The simultaneous projection of two or three photographs of comparable areas taken at intervals, enable detailed examination of any changes in lesions of blood vessels and retina occurring over the period covered by the photographs.

Fifty-nine of these patients have had no special treatment prescribed and are being examined serially to follow the course of any retinal changes noted. An attempt is made to correlate these with the clinical history of the patient. A feature of these examinations has been the fact that the pattern of lesions can alter quite markedly between photographs with, not infrequently, the appearance of improvement of the retinopathy. Alterations in vascular patterns in a number of patients suggest pre-retinal contraction of the hyaloid face of the vitreous producing distortion of the blood vessels.

Thirty-two patients have been treated by retinal photocoagulation. One eye only has been treated — the other serving as a control. It is too soon to make any assessment of results of treatment. It can be said, however, that the basis of selection and type of treatment undertaken has not been associated with deleterious response.

Nine patients have been treated with medroxyprogesterone acetate or chlorpromazine with the intention of suppressing pituitary function, so as to produce a "medical

hypophysectomy". The results of this programme have so far been very disappointing in that tests of pituitary function have not indicated that suppression has been achieved. It has not been surprising then that there have been no dramatic retinal responses to this therapeutic approach. In seven patients where the typical hard exudate has been the dominant feature, clofibrate has been used. In some patients there has been a diminution in the amount of exudate with improvement in vision. The numbers as yet are small and the period of follow-up too short to draw valid conclusions from our experiences with this form of therapy.

Continuing Studies of the Aetiology of Diabetic Ketoacidosis

P. Taft, C. Varley and H. D. Breidahl.

Between 30 and 45 patients are admitted each year suffering from diabetic ketoacidosis. It has been our experience over a number of years of study of the cause of diabetic coma among patients admitted to our care, that neglect of treatment by the patient or improper medical advice in the period prior to admission to hospital was a much more common cause of acidosis than intercurrent infection.

More than half of our patients have had no previous contact with the Diabetes Clinic, being referred directly to Alfred Hospital with this acute complication. During 1972, prompted by the treatment of a patient in whom the development of ketoacidosis followed medical advice to withhold insulin because of vomiting, an examination was made of the records of patients suffering episodes of ketoacidosis where they had been under the care of a doctor prior to admission. In all, 21 patients fell into this category.

It was striking that in 12 patients there was a delay of from 12 hours up to one week in referring the patient to hospital after the presence of symptoms and signs which clearly pointed to the diagnosis. In another patient the referral was immediate but the coma had been misdiagnosed as hypoglycaemia and the patient given glucose prior to admission. Three of these patients died.

Eight other patients were recognised immediately to be in severe metabolic disarray and were referred to hospital promptly for treatment. None of these patients died. In this group the fluid, electrolyte and insulin requirement to restore the deficit was significantly less than in the "neglected" group.

This clinical study highlights the contribution made by ignorance on the part of patient and all too often of his doctor, of the type of event which will determine the onset of coma, of its early recognition and effective emergency treatment.

Carbohydrate Tolerance in Uraemia

J. Fung, P. Taft, J. Bornstein, and R. Dargaville.

Studies of the carbohydrate intolerance observed in uraemia have continued. Artificial "uraemic" media with high urea concentrations have been prepared and used to study glucose utilisation by muscle, liver and fat. It has been found that glucose uptake by muscle — measured both by examination of its disappearance from the medium and appearance in glycogen — is unaffected. Glucose conversion to liver glycogen studied in liver slices is likewise unaffected by urea in the medium. The incorporation of ^{14}C glucose into lipid or into fatty acids or rat epididymal fat pads is not altered by urea nor is the rate of insulin secretion from pancreas slices.

On the other hand serum collected from uraemic patients does have an inhibitory effect on glucose utilisation by rat soleus muscle. There is also a slight inhibition of ^{14}C glucose conversion to lipid and fatty acid by such serum.

These studies indicate that urea alone is not the agent responsbile for the frequently observed carbohydrate intolerance in uraemia. It is hoped to fractionate uraemic serum in an effort to identify the physical properties of the inhibitory agent.

The Treatment of Acromegaly by Transphenoidal Hypophysectomy

R. Dargaville, H. D. Breidahl, P. Taft, H. Millar, H. G. Burger(1) and G. W. Baker(1).

A review has been made of the course of acromegalic patients treated by trans-sphenoidal hypophysectomy. This has been undertaken in conjunction with the Endocrine Unit at Prince Henry's Hospital, 14 patients from both hospitals having been studied. The clinical result has been judged as fair to good in 10 and poor in the remaining four. Although assessment of clinical results is subject to observer error, it appeared that the changes in growth hormone levels following surgery correlated to some extent with clinical response, falling most and being suppressed best in those patients with good responses.

Despite the attempt to remove all pituitary tissue at operation this was not achieved in that three patients with persisting disease and elevated growth hormone required post-operative radiotherapy to procure remission and three others require no hormone replacement therapy.

There was no mortality and little morbidity and the clinical results achieved appear to compare favourably with other forms of therapy.

(1) Prince Henry's Hospital.

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De Kretser, D. M., D. Fortune, B. Hudson, A. R. Long, C. A. Paulsen and H. P. Taft— "Hormonal, Histological, and Chromosomal Studies in Adult Males with Testicular Disorders". **J. Clin. Endocrinal. Metab.** Vol. 35 (1972), p. 392.

Lectures Given During 1972

H. D. Breidahl	"Obesity and Its Treatment"	*H.M.A.S. "Cerberus" (R.A.N. Medical Service).*
H. D. Breidahl	"Recent Advances in Diabetes and Endocrinology"	*R.A.A.F. Medical Services, Point Cook.*
H. D. Breidahl	Five Lectures at City and Country Centres on Diabetes and Endocrinology	*Royal Australian College of General Practitioners.*
P. Taft	"Selection of Patients for Gonadotrophic Treatment" "Oral Treatment of Diabetes"	*Post-Graduate Committee in Medical Education, University of New South Wales.*
P. Taft	"Steroid Therapy—Indications and Control" "The Assessment of Thyroid Function" "The Treatment of Female Infertility"	*Annual Meeting, Gold Coast Medical Society, Queensland.*
P. Taft	"Obesity — Aetiology and Management" "Diabetes in Pregnancy" "Investigation and Treatment of Amenorrhoea"	*Melbourne Medical Post-graduate Committee, Wangaratta.*
P. Taft	"Further Evidence for the Existence of Growth Hormone-derived Peptides in Man"	*Endrocrine Society of Australia, Sydney.*
P. Taft with E. J. Keogh *et alii*	"Pituitary Apoplexy"	*Royal Australasian College of Physicians, Sydney.*
P. Taft *et alii*	"The Biologic Activity of Growth Hormone-derived Polypeptide Fragments — Physiological and Clinical Implications"	*International Union of Physiological Sciences Regional Meeting, Sydney.*
D. Winikoff	"Use of Radioisotopes of Iodine in Diagnosis and Treatment of Thyroid Disease" (Series of three lectures)	*Medical Nucleography, Royal Melbourne Institute of Technology.*

Report of Investigations By Research Fellows of Alfred Hospital in other Departments

Attempted Suicide and the General Hospital

Roger Buckle[1]

Following on from two studies of patients who attempted suicide and were interviewed at the Casualty Department of the Alfred Hospital (1963 and 1967) a third similar study was undertaken in 1971.

Comparison has been made between these groups of patients and it is anticipated that this will provide valuable information for planning facilities of the hospitals in the future.

During 1972 a follow-up study of the patients has been carried out with the help of an experienced Social Worker. The broad aims were to determine the effect and nature of hospital intervention in attempted suicide and to identify predictors of repeated attempted suicide.

The follow-up of the patients from the 1971 group has been carried out, and it is anticipated that during the early part of 1973 the follow-up of the 1963 group will be completed.

It has been decided to abandon the follow-up study of the patients in the 1967 group, since it has proved difficult to trace these patients because of their extraordinary social mobility.

(1) Assistant Psychiatrist.

Breast Cancer

A. V. Jackson[1]

The orginal breast tumour project was to repeat and, if confirmed, to expand the claim by Murad[1] that medullary and scirrhous carcinoma could be precisely identified and distinguished by a combination of histochemistry and electronmicroscopy. The bases of the claim were (i) the probability that scirrhous carcinoma arises from myoepithelial cells; medullary carcinoma from ductal epithelial cells, and (ii) the demonstration that myoepithelial cells retained the membrane-associated ATPase after neoplastic change; so that scirrhous carcinoma cells have in E-M, a membranous layer of electron dense material after lead staining of thick frozen sections incubated appropriately in ATP. The histochemical aspect of the project was the technical responsibility of Mr. Fred Jackson (technologist on loan, for training, from Gippsland Base Hospital, and who has now returned to Sale), and from June, of the Morbid Anatomy Department's new laboratory manager, Mr. John Young. Excellent and consistently thick (0.5μ) "Paragon"-stained and araldite-embedded sections are routinely prepared by Judy Frisch. These show, much better than paraffin sections, precise distinction between abnormal elastic fibres and collagen (see later). However, the rest of the project has been held up temporarily because, with the glass knives available, it has been impossible to cut satisfactory E-M. sections of the araldite-embedded, 40μ thick and histochemically manipulated sections. Aided by a grant from the Whole Time Medical Officers Private Practice Fund, two diamond knives have been ordered from Rondikn Corporation in Honolulu and when they arrive (in about, we hope, April) we may make further attempts.

In the meantime our attention has been redirected to the elastic tissue content of scirrhous carcinomas. Although the yellow flecks and streaks which are of practical importance to the surgeon and to the surgical pathologist in the macroscopic diagnosis of breast cancer are commonly believed to be due to necrosis, numerous and widely scattered reports over 40 years or more have pointed out that these are, in fact, areas of excess elastic tissue (or possibly "elastotically degenerated collagen"). This was reported as long ago as 1931 by Cheatle and Cutler[2]. The origin of elastic tissue is not clear, but it has been suggested by Jackson and Orr[3] that it may be a pre-malignant indicator. A similar suggestion has been made with reference to skin cancer by Gillman[4]. Very recently (July, 1972) Azzopardi and Zayed[5] have shown that "mixed" salivary tumours which also (like scirrhous breast cancer) arise, we believe, from myoepithelial cells, have a very high content of elastin. Other E-M studies such as those by Kadar, Gardner and Bush[6] have shown a very close association between smooth muscle cells (and ? myoepithelial cells) and elastinogenesis. These various, and hopefully related, threads have stimulated us to make routinely in all breast cancers submitted for frozen section diagnosis, a light and electronmicroscope study of elastic tissue in these specimens. About 30 breast tumours have been studied in this way. The paraffin sections have been stained by a battery of elastic tissue stains, the most satisfactory of which proved to be Weigert's resorcin-fuchsin stain. These confirmed previous reports of the intense peri-ductal accumulation of elastin. Araldite-embedded sections stained by "Paragon" (a proprietary preparation of polychrome alkaline methylene blue) showed very precise localisation and precise polychrome distinction from collagen of the elastic tissue. So far, because the scirrhous tumours proved to be stubbornly and almost impossibly tough, glass knife E-M sections have often been unsatisfactory and further progress of this aspect (along with the continuation of the ATPase studies) has been deferred until the diamond knives are available.

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(1) Morbid Anatomy Department.

Endocardial Fibroelastosis

A. V. Jackson and D. Challis[1]

The hospital Morbid Anatomy Department has another project in hand which was initiated towards the end of 1972 and which will be continued in 1973. Dr. David Challis — Senior Morbid Anatomy Registrar — is making cardiopathology his special interest and is hoping to undertake an E-M study of endocardial fibroelastosis. This will link up with our current interest in elastic tissue. Though a severe degree of endocardial fibroelastosis is seen most strikingly in congenital heart disease and in tropical cardiomyopathy, a lesser degree is not uncommon in other heart conditions and is, we feel, an important topic to study. So far Dr. Challis is busy acquiring technical expertise but we hope that he will be able to spend up to half time on this project in the next two years.

(1) Morbid Anatomy Department.

Post-operative Deep Vein Thrombosis

D. S. Rosengarten[1]

Venous thrombosis often occurs without clinical indications and may be followed both by life threatening pulmonary embolism and the potentially incapacitating post-phlebitic syndrome. Ideally both these sequelae can be prevented by a prophylactic regime. In the past, study of this problem has been hampered by the difficulties in establishing the diagnosis of deep vein thrombosis and so of obtaining a background of incidence against which both prophylactic and therapeutic measures can be compared.

Recently, using ^{125}I fibrinogen with surface scanning as a sensitive criterion for the diagnosis of venous thrombosis it has been found that approximately one-third of all patients after operation develop venous thrombosis in the calf. Furthermore, 50% of these thromboses occur on the operating table, and only those thromboses which extend to involve a major axial vein of the leg produce pulmonary embolism. This technique provides a tool to study the surgical population to assess prophylactic measures.

Using this technique, the first part of the year was spent completing a study from 1971 which showed that post-operative electrical calf stimulation reduced the incidence of post-operative venous thrombosis in patients over the age of 60 years who did not have malignant disease. It was ineffective in patients with malignant disease. The difference in the incidence of thrombosis between the control and treated group was significant at $p = 0.001$. It has been found that perioperative, low dose heparin also reduces but does not abolish thrombosis. As these regimens have different modes of action, the former by decreasing venous stasis and increasing fibrinolysis and the latter by anticoagulation, a study was designed to determine whether a combination of the above was more effective than either alone.

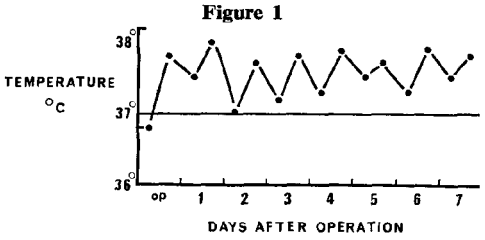
Patients undergoing elective general surgical procedures, under general anaesthesia were allocated at random into one of three groups, viz. electrical calf stimulation, low dose heparin or a combination of these regimens.

Of the 255 patients studied the number who developed thrombosis was nine of 86 having low dose heparin and 12 of 84 having calf stimulation. None of 85 having a combination regimen developed thrombosis. These groups were comparable for age, sex, height, weight, type of operation, length of anaesthesia, presence of cancer and varicose veins as shown by standard statistical tests of X^2 for frequency and Student's "t" test for parametric data. It may be concluded that each technique alone was equally effective in the reduction of thrombosis, but the combination abolished thrombosis and was more effective in prophylaxis than each technique alone (exact $p = 0.002$).

Finally, a retrospective temperature chart analysis was performed on 55 consecutive post-operative patients in whom radioactive fibrinogen studies had been undertaken. These were examined by eye to see if a pattern could be established which was presumptively typical of the disorder. On the accepted criteria, 20 of this group had deep vein thrombosis and 35 did not. The features which emerged were —

1. Almost all patients had some fever in the first 48 hours after operation.
2. The temperature lay in the range 37.4 - 38° C. in those patients who, apart from increased local counts, were making an uneventful convalescence.
3. A single spike of fever for one day was never associated with increased radioactive fibrinogen uptake.
4. High and continuous fever beyond the second post-operative day did not reveal any consistent pattern which would permit recognition of deep vein thrombosis or other conditions by simple inspection.

Figure 1



These criteria were then used to divide the same group into those who should have, or should not have, deep vein thrombosis. The model case is shown in Fig. 1. The first two days post-operative fever must be neglected but,

(1) Department of Surgery, Monash University, Alfred Hospital.

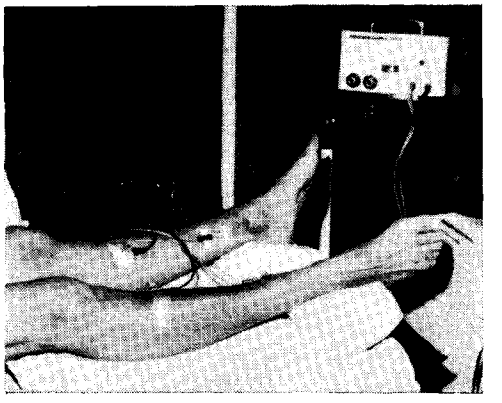
provided that the patient is making a normal recovery, the recrudescence of fever for two consecutive days in the range 37.4 - 38° C. suggests deep vein thrombosis. All the criteria were applied to each patient and weighting was not used. Half the patients were correctly assigned and the incidence of false positive diagnoses is one in five.

A further 89 patients were prospectively studied and similar results found. If clinical signs were added to temperature chart analysis a 60% diagnostic rate was obtained.

Detection of half the patients who sustained deep vein thrombosis in the post-operative period is not particularly satisfactory unless reasons can be found for failure in the other half and it can be shown that it is the detected group who are of clinical importance in the sense that they are likely to suffer harm from their thrombotic process. Our data do not permit us to answer these questions unequivocally. Nevertheless, comparison of the detected group with all those not detected shows that the clot was of longer duration in those with fever as compared with those without. If undetected afebrile patients only are considered, the clot was transient except in one instance where silent extension occurred. The undetected febrile patients either had an atypical fever or obscuring causes; their thrombi were of longer duration. Therefore, the clinician can detect half of those who are developing clots in the leg and can be confident that with some exceptions at least half of the remainder will not have a thrombus of consequence. He may feel reassured that, if a patient is afebrile, any leg clot present is more likely to be of short duration and therefore less likely to extend into the axial vessels. If the temperature is high or atypical he must be anxious about the presence of thrombi which neither clinical signs nor fever will necessarily detect.

Thus his strategy of action could be based on applying radioactive fibrinogen studies to patients who have typical post-operative fever patterns and those in whom other complications are occurring so that the temperature chart cannot be interpreted. Further, as the incidence of thrombosis falls under the influence of prophylactic measures for use of routine detection methods which involve specialised measurement becomes less cost-effective. Under such circumstances the development and refinement of pattern recognition techniques on data that is already being collected for other purposes may well prove an effective screening method. To this end we have transferred our pattern recognition system to the computer and will now produce a daily list of patients who, according to the temperature chart analysis, are in three probable classes — with thrombosis, without thrombosis, and indeterminate. The clinician can use this preliminary analysis to decide what he should do in the way of further study.

Figure 2



Electrical Calf Stimulator on Patient

Microcirculatory Events During Open-Heart Surgery *

Isla M. Williams(1)

Whilst outstanding technical advances in cardiac surgery each year are increasing the life-span of approximately 1,400 Australians with heart disease (such as malfunctioning valves or coronary ischaemia) many operated upon develop neurological complications. Tufo, in a prospective study of 100 patients in the United States of America, showed that half the sample on waking from anaesthesia had neurological abnormalities. The majority of neurological abnormalities appear to be transient; permanent changes in higher cortical function, such as impaired concentration, being extremely difficult to assess, are sometimes overlooked.

Open-heart surgery imposes complex intravascular changes. The retina offers a unique opportunity to document microcirculatory events. The aim of this project is to gain an understanding of microcirculatory changes during open-heart surgery by studying the retinal circulation, to relate the changes to the neurological complications, and to apply this knowledge to the development of a regimen which will prevent the complications.

Observations in this study have revealed that, in one-third of patients undergoing open-heart surgery, white emboli (some forming **in situ**) traversed the small vessels of the retina, usually occluding capillaries. The patients in whom white emboli occurred were principally those who developed neurological complications and those who failed to survive. Refractile emboli were also observed; whilst more common, their occurrence appeared unrelated to post-operative complications. The opportunity for pathological examination of the retina arose in three patients. It was shown that the white emboli comprised blood constituents.

Multiple capillary occlusions were demonstrated by trypsin digest preparations of the retinas.

In open-heart surgery, microemboli are known to be an important cause of tissue damage; their numbers have been decreased by the introduction of blood filters. Despite the use of the Swank filter and, in the last seven cases the Pall filter as well, white retinal emboli were observed in 10 of the 31 patients studied and refractile emboli in 20 patients.

The syndrome has been reproduced experimentally by placing a dog on total cardiopulmonary bypass. To encourage the formation of retinal emboli, filters were omitted and bypass time was prolonged. Many white emboli were sectioned for electron microscopic examination. They comprised blood constituents demonstrating every degree of degenerate change. Homogeneous black material, the nature of which is being investigated, was present in each plug. It was also found in a second series of experiments that small doses of dipyridamole (a substance which prevents platelet aggregation and adhesion) added to the perfusate prevented the formation of white emboli.

Trypsin digestion of the non-vascular components of the retina permitted visualisation of capillary occlusions too small to be detected with the ophthalmoscope or dissection microscope. Both in the patients studied and in the dogs, this technique revealed many capillary occlusions. Some comprised blood constituents, others components of Antifoam A (the defoaming agent used in the oxygenator for patients undergoing open-heart surgery) or of silicone grease which is used in the oxygenator for the experimental animals). Both Antifoam A and silicone grease injected into the internal carotid artery experimentally produced retinal emboli very similar to those observed in patients and in dogs after cardiopulmonary bypass.

Currently, attempts are being made to identify the components of the plugs and of the changes induced by dipyridamole, to study the effects of the plugs on vessel walls with fluorescein angiography and the effects of the addition of dipyridamole which may prevent this damage, and to study the effects of ischaemia induced by these plugs on nerve cells. It is hoped to conduct a trial of prophylactic dipyridamole in patients undergoing open-heart surgery. The patients already studied are being re-examined.

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Publications in 1972

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Lectures Given During 1972

R. C. Buckle	"Attempted Suicide and the Student"	*Australian and New Zealand Triennial Student Health Conference, Canberra.*
D. S. Rosengarten	"Temperature Chart Anaylsis in the Diagnosis of Post-operative Deep Vein Thrombosis"	*Royal Australasian College of Surgeons, Hobart.*
D. S. Rosengarten	"Prevention of Post-operative Deep Vein Thrombosis by Calf Stimulation"	*Royal Australasian College of Surgeons, Hobart.*
D. S. Rosengarten	"Prevention of Post-operative Deep Vein Thrombosis by Calf Stimulation"	*Alfred Hospital Cinical Society.*
D. S. Rosengarten	"Prophylaxis and Treatment of Venous Thrombosis"	*Royal Melbourne Hospital.*

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Each spot on this map of the world indicates a research centre in communication with the Institute in one or more of the following ways.

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- **There have been visitors from there to the Institute for similar purposes.**

- **There has been an exchange of correspondence concerning research projects.**