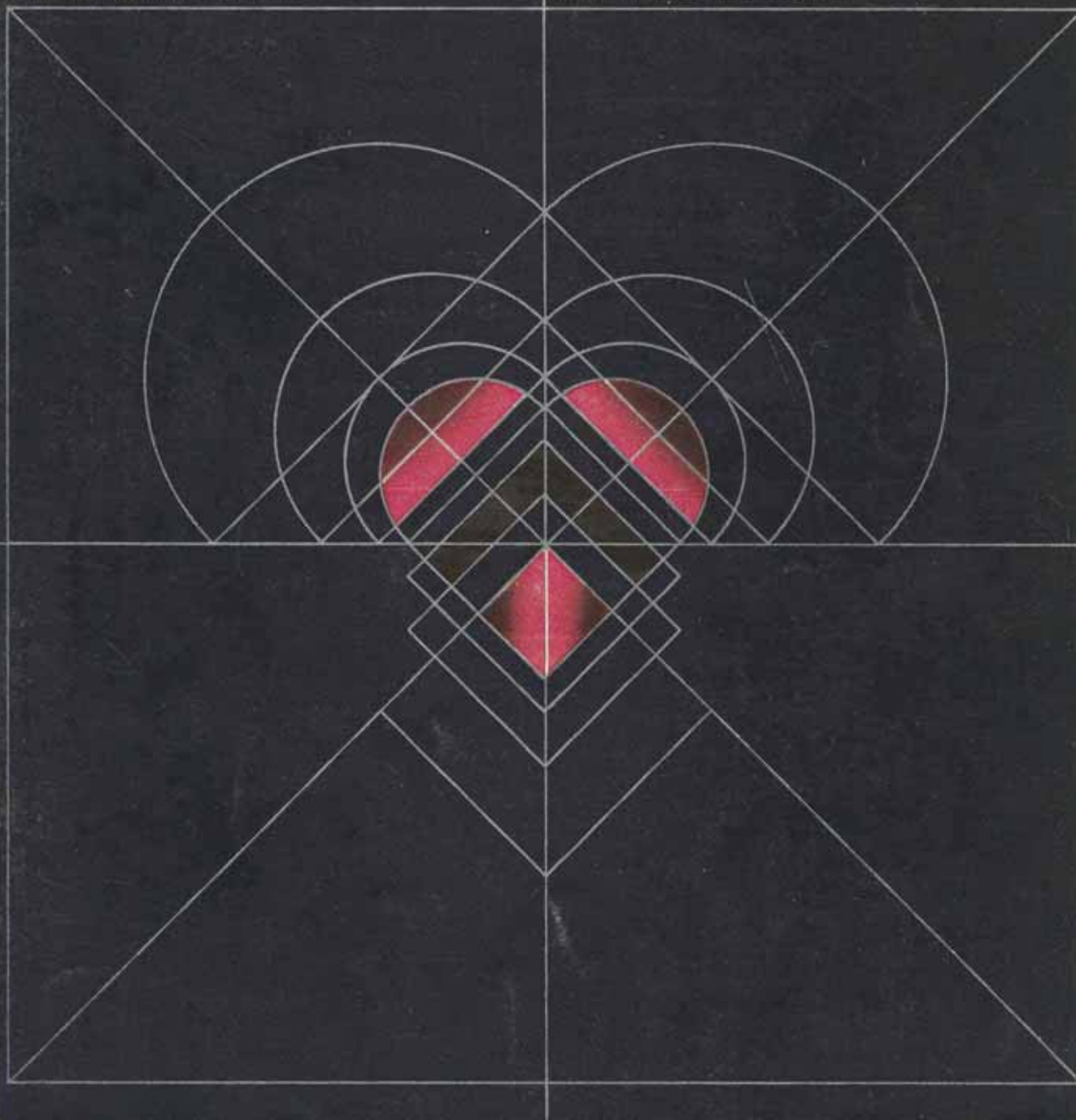
Research 1970

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, including Remembrance Donations, of $2 or more are permissable deductions for income tax purposes.

The Ewen Downie Metabolic 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-FOURTH 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)

FOURTEENTH ANNUAL RESEARCH REPORT

of

THE EWEN DOWNIE METABOLIC UNIT

REPORTS

of

ALFRED HOSPITAL RESEARCH FELLOWS

1970

ALFRED HOSPITAL, PRAHRAN, VICTORIA, 3181, AUSTRALIA

BAKER MEDICAL RESEARCH INSTITUTE

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EDGAR ROUSE, C.B.E., F.C.R.A.(Hon.)

T. H. STEEL, M.D., B.S., F.R.C.P., F.R.A.C.P.

DIRECTOR OF CLINICAL RESEARCH UNIT (ex officio).

ALFRED HOSPITAL RESEARCH FELLOWS, 1970

"Amelia Haigh (Heart)":	S. T. ANDERSON, M.B., B.S., M.R.A.C.P.
"Sol Green": *"R. B. McComas"*:	E. COOPER, M.B., B.S., F.R.A.C.S.
"S. W. Jones":	P. DAILE, B.Sc.
"Victor Y. and Margaret Kimpton": *"Connibere Bequest"*:	A. V. JACKSON, M.D., B.S., F.R.A.C.P., F.C.Path., M.C.P.A.
"James and Elsie Borrowman":	P. A. LOWE, M.B., B.S., F.F.A.R.A.C.S.
"Dr. Henry Laurie": *"Edward Wilson Memorial"*: *"Amelia Haigh (Rheumatoid Arthritis)"*:	T. E. LOWE, D.Sc., M.D., F.R.C.P., F.R.A.C.P.
"Edward Wilson Memorial":	G. T. MARTIN, M.B., B.S., F.R.C.S., M.R.C.P.
"Frederick and Esther Michaelis":	D. J. B. St. JOHN, M.B., B.S., M.R.C.P., M.R.A.C.P.
"Collier":	F. T. McDERMOTT, M.B., B.S., F.R.C.S.
"Edward Wilson Memorial":	R. J. SAWERS, M.B., B.S., F.R.A.C.P., M.C.P.A., M.C.Path.
"S. W. Shields": *"William Buckland"*: *"J. F. Mackeddie"*:	H. P. TAFT, M.D., F.R.A.C.P.
"E. H. Flack":	N. D. YEOMANS, M.B., B.S.
"Ian Gideon McLean": *"Peter Grant Hay"*:	B. G. FIRKIN, B.Sc.(Med.), M.B., B.S., F.R.A.C.P.

APPOINTED TO RESEARCH FELLOWSHIPS FOR 1971

"Edward Wilson Memorial":	E. COOPER.
"Sol Green":	P. DAILE.
"R. B. McComas":	
"William Buckland":	H. DUDLEY.
"A. A. Swallow":	
"Frederick and Esther Michaelis":	A. V. JACKSON.
"James Richardson":	
"Edward Wilson Memorial":	S. KATZ.
"Amelia Haigh (Heart)":	T. E. LOWE.
"Ian Gideon McLean":	
"Sartori":	
"S. W. Jones":	H. A. LUKE and L. M. DUGDALE.
"Victor Y. and Margaret Kimpton":	J. F. McL. OLDHAM.
"J. F. MacKeddie":	
"Connibere Bequest":	D. J. B. St. JOHN and F. T. McDERMOTT.
"George Merriman":	
"Collier":	H. P. TAFT, M.D., F.R.A.C.P.
"S. W. Shields":	
"James and Elsie Borrowman":	
"E. H. Flack":	M. B. Van Der WEYDEN.
"J. R. G. and E. McKenzie":	

TRAVEL GRANT

"J. H. Patterson Travelling Scholarship":	R. D. GLASS.

TABLE OF CONTENTS

Ewen Downie Metabolic Unit

Investigations by Research Fellows of Alfred Hospital in Other Departments

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 it was felt desirable to publish a combined volume entitled "Research". This made its first appearance in 1953, and contained the twenty-seventh annual report of the work of the Baker Institute and the fifth annual report of the work of the Clinical Research Unit and the Alfred Hospital Research Fellows.

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

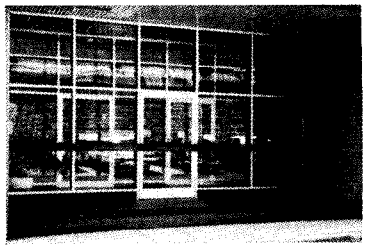
Because of the increasing importance and diversity of the investigational activities conducted in Alfred Hospital, it has been decided to present this report in several sections, indicating the activities of the Baker Institute (including the Clinical Research Unit), The Ewen Downie Metabolic Unit, and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

Administrative Assistant: R. BLAKEMORE, L.L.B.

Graduates:

Mrs. V. CARSON, M.Sc.
E. COOPER, M.B., B.S., F.R.A.C.S.
D. A. COVENTRY, M.B., B.S., M.R.A.C.P. (Acting from 14/5/70).
P. DAILE, B.Sc.
P. FANTL, D.Sc., F.R.A.C.I.
Mrs. H. A. JONAS, M.Sc.
I. E. McINNES, M.B., B.S., F.R.C.S., F.R.A.C.S.
EVA MÄSIAR, M.D. } (from 22/9/70).
P. MÄSIAR, M.D., D.Sc. }
Mrs. D. MILLAR (nee Chipperfield), B.Sc. (to 5/6/70).
C. V. NELSON, Ph.D. (to 6/5/70).
Miss G. M. PICKEN, B.Sc.
Mrs. J. M. RAINEY, M.Sc. (to 5/8/70).
J. STONE, B.Sc. (to 5/6/70).
J. A. STREETON, M.B., B.S., M.R.A.C.P. (to 13/5/70).
G. R. STIRLING, M.B., B.S., F.R.A.C.S.
Miss J. SZETO, B.Sc. (from 1/6/70).
Mrs. J. TAY, B.Sc.
FEDORA R. TRINKER, Ph.D., M.B., B.S.
L. W. WHEELDON, Ph.D.

Technical:

S. HART (Laboratory Supervisor) (to 15/9/70).
R. P. STEELE (Laboratory Supervisor) (from 12/10/70).
A. H. HUCKFIELD (Technical Officer).
Mrs. B. SKYM (Senior Technologist to 25/9/70).
Miss J. DIXON (from 9/2/70).
Mrs. E. MORTENSON (to 2/3/70).
Mrs. R. MUSCUTT.

Librarian: Mrs. M. RAE.

Clerical:

Mrs. C. E. ANGELO (to 26/5/70).
Mrs. C. M. WALKER (from 22/6/70).
Miss J. HAMMOND (from 9/2/70).
Mrs. H. M. CONNOLLY (from 16/11/70).
Miss R. RITCHIE (to 14/8/70).
Mrs. J. E. SALTER (to 5/2/70).
Mrs. P. VINCENT (to 11/9/70).

Laboratory:

Mr. P. BENNETTS (from 13/4/70).
Mr. D. BERRY (from 19/1/70).
Miss H. CHARANIEKA.
Miss K. CLARKSON.
Miss E. DULAK (from 5/1/70 to 29/9/70).
Miss J. DUNCAN (to 20/11/70).
Mrs. C. GALES (to 18/11/70).
Miss A. GREEN.
Mrs. S. HAIN (to 31/7/70).
Mr. K. HARVEY.
Miss F. HIRSCH (to 16/1/70).
Mr. D. LAVENDER (from 31/8/70).
Mr. C. LEWIS.
Miss B. PACE (from 3/2/70).
Mrs. L. PARKER (from 27/1/70 to 20/11/70).
Mrs. E. PAYNE (from 23/3/70).
Miss B. REEVE.
Miss H. SCHRAMM (to 6/6/70).
Miss J. THOMPSON (to 23/3/70).

General: Mrs. M. WRIGHT.

WARD STAFF

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

Resident Medical Officers:

J. R. DOUGLAS, M.B., B.S.
E. T. FAGAN, M.B., B.S.
M. FLAIM, M.B., B.S.
G. L. JOSLIN, M.B., B.S.

Sister: J. HOMEWOOD.

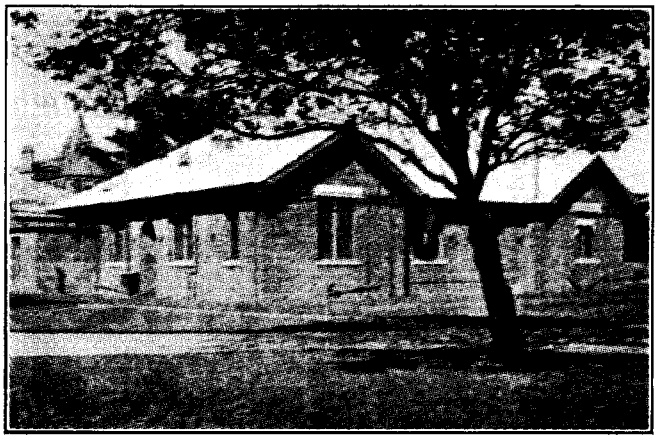
RESEARCH FELLOWSHIPS

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. G. R. STIRLING, M.B., B.S., F.R.A.C.S.
Visiting:	C. V. NELSON, Ph.D.
Anti-Cancer Council of Victoria ("A. A. Thomas"):	(Vacant).
"James and Elsie Borrowman":	Mrs. H. A. JONAS, M.Sc.
"William Buckland":	P. FANTL, D.Sc., F.R.A.C.I.
"The Lang Fellow":	L. W. WHEELDON, Ph.D.

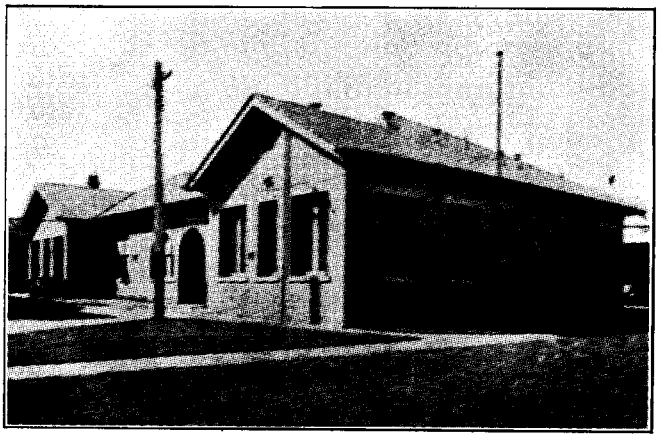
SCHOLARSHIPS

"Laura Nyulasy" (1969):	Mrs. D. MILLAR, B.Sc.
"National Heart Foundation Vacation Scholarship":	K. Y. GAN.



Baker Medical Research Institute (upper) and Alfred Hospital Pathological Block (lower) in 1927.

(Reproduced with permission of Editor, Medical Journal of Australia).



ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

FINANCE

During 1970 one of the most important considerations for the Institute has been a study of ways and means of maintaining its financial stability which, in this context, means a continuing ability to provide funds for the desirable level of expenditure at any particular time. Currently there are problems on both the income and expenditure sides of the ledger.

On the expenditure side the major item is salaries and wages. These are, in most instances, fixed by determinations completely outside our control or influence and they have been continuously rising by small or large jumps for many years. In addition to the growth of this item there needs to be considered the increasing cost of increasingly sophisticated equipment and the cost of the higher grade staff needed for many modern research techniques. This existing economic state of our community makes forward budgetting of expenditure very unreliable.

Unfortunately, the items on the income side are subject to influences which on balance aggravate rather than redress this uncontrolable expenditure. Broadly, funds may come to a research organisation from three sources. Firstly, there is income earned by endowment funds and Institute activities; secondly, there are gifts for general or specified use and, thirdly, there are grants-in-aid, from research-supporting bodies which may be for support of specific projects or block grants for general use.

Of these sources, the value of the income from endowment investment is diminished by the steady fall in purchasing power of the dollar. Not only has the value of individual grants-in-aid for specific projects been falling as the resources of the Funds become less able to cope with the increasing number of applications but also, because such grants rarely cover fully the cost of a project, their acceptance imposes an added load on general funds.

In these circumstances, it is instructive to study the sources of financial support which this Institute (excluding the Clinical Research Unit, which is separately supported) has enjoyed over the past six years. During this time the total running cost has been approximately $1,000,000. Of this, 16 per cent has come from the Institute's own resources of endowment funds and payments for services rendered, 23 per cent from grants-in-aid of specific projects from research-supporting bodies and for the remainder, the Institute has been dependent on generous block grants for general purposes from the Baker Benefaction.

It is recognised that this continuing support from the Baker Benefaction cannot be unlimited and efforts have been made over the past few years to build up the Institute's own resources. However, to build up, adequately and quickly, the endowment fund is difficult for the increase must do two things — offset the depreciation of the capital and provide an increasing working income. Largely, as a result of the very generous response of donors to our appeal launched in 1968, the proportion of income from the Institute's own resources is now approximately 20 per cent in contrast to the five per cent of six years ago. The efforts of many friends have increased the total of the endowment funds considerably but the continuing depreciation of purchasing power of this income indicates that their continuing efforts may be needed for a long time yet before this endowment will reach a size that ensures financial stability. A list of donors for 1970 is included in this report and they are thanked for their generous interest in our work.

The figures quoted refer to ordinary running costs and do not include provision of equipment for new techniques or replacement of obsolescent or unserviceable equipment. The rapid development and improvement of equipment is today responsible for a rate of obsolescence that calls for financial provision on a scale not previously contemplated. Here again I must thank generous donors who have given money specifically for equipment and the "Baker Birds" who have asked that the money they raise should be used for equipment.

That these financial problems are not peculiar to this Institute is emphasised by the public discussions on the support of medical research which have taken place during the year. In addition to the factors just discussed, these have drawn attention to the inequality between the supply and the demand for funds for medical research in the country as a whole.

THE BUILDING

From its inception, the Institute has been closely linked with Hospital activities and, since the formation of the Clinical Research Unit, these links have become even closer in medical research.

This functional association is reflected in the buildings used for research. The original building of the Institute (1927) and the Pathological Block of the Hospital are shown in the photographs on page 12. After the Pathological Block had been converted to a ward and laboratories for the C.R.U., they were physically joined by the C. A. Smith Wing. The new Institute is shown on page 15 as it is seen across the foundations of the new Hospital ward block. It houses the C.R.U. and when the ward block is completed a bridge will connect the Institute building directly with its associated ward.

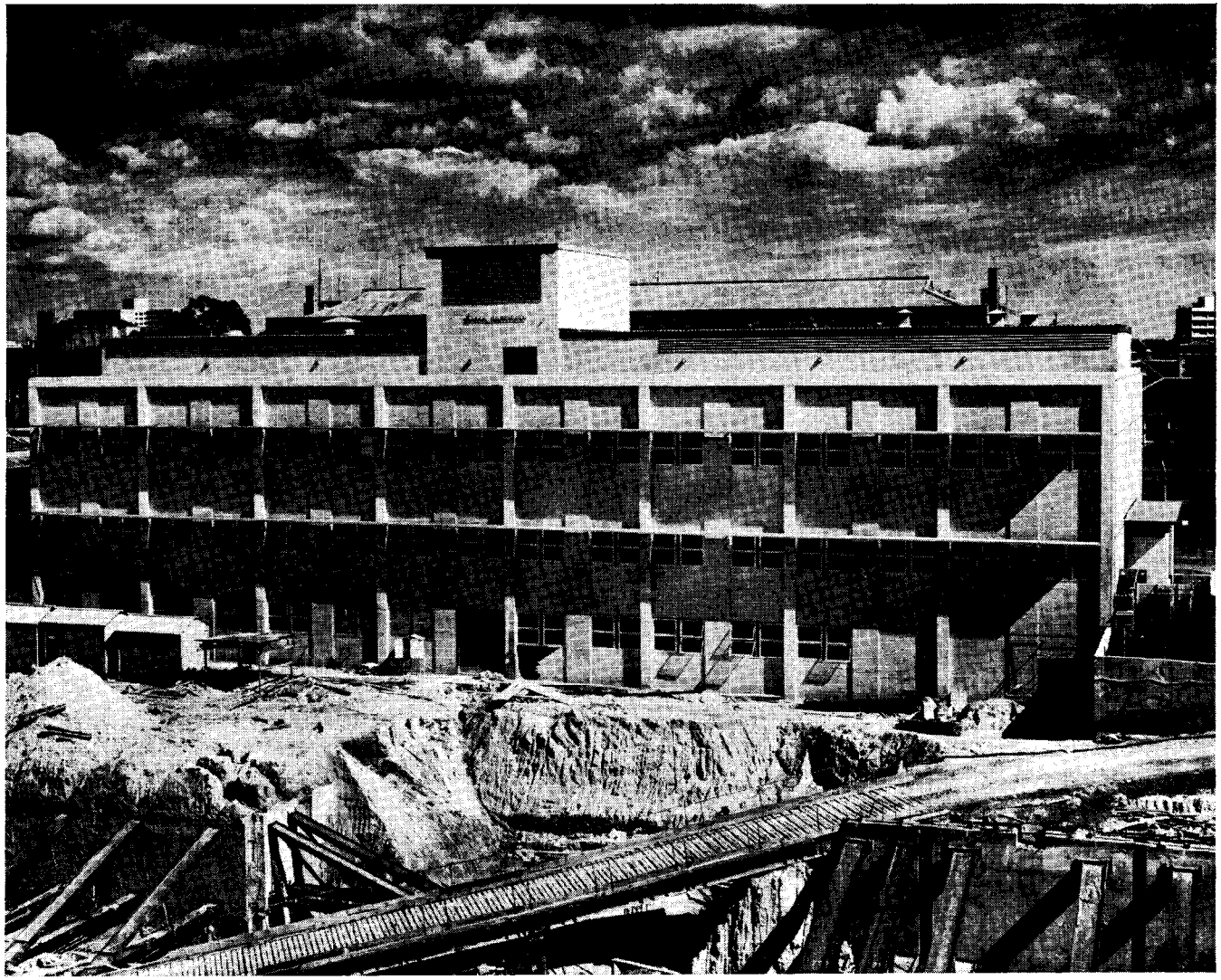
The design of the new building called for an area on the lower ground floor to be left in shell form to provide for future expansion. Already it has become necessary to use some of this space and a suite of rooms for an electron microscope laboratory is being constructed. It is a pleasure to thank the State Government for a capital grant to enable the purchase of equipment and the construction of the laboratory.

This facility is required for many of our projects and will remove our dependence in this regard on the goodwill of others. A joint project is also planned with the Director of the Hospital Morbid Anatomy Department so the prospects are that the instrument will be fully used within its first year of operation.

WORK OF THE INSTITUTE

For a long time, most of the research projects have been directed, immediately or remotely, to the elucidation of problems concerning diseases of the heart and blood vessels. This field of interest is so great that only a few facets of the subject can be studied at any one period. Selection of these has been determined by the desire to use a multi-discipline approach and by the interests and training of available staff and by the investigational techniques available at the time.

However, at all times it is possible to identify projects directed towards — (i) treatment of patients with cardiovascular disease, e.g., the current long-term study of the treatment of high blood pressure, the treatment of obstructive disease of the arteries in limbs; (ii) an understanding of the mechanisms whereby heart muscle develops the power to push blood around the circulation, e.g., the present studies on the fine structure of the muscle cells, the roles of calcium ions and cellular membranes;



The Institute seen across the building site of the new ward block for the Hospital.

(iii) the mechanisms which control the distribution of blood to various regions of the body, typified this year by the studies on the coronary circulation; (iv) the use of chemicals (drugs) to modify the behaviour of the system at either cellular level or the complete circulation either for treatment of disease or as a tool to understand the working of the individual cell. The studies reported here on β-adrenergic blockade and noradrenaline metabolism in cardiac muscle cells illustrate this point; (v) the application of new advances in the biological or other sciences to these general problems. In the past, the introduction of various techniques for the clinical investigation of the circulation which led to the formation of the Hospital Cardiovascular Diagnostic Service and the imminent installation of an electron microscope, which will be used both for laboratory and clinical purposes, illustrate this facet of the work; (vi) in addition, projects in other fields which bring other disciplines and useful techniques into the Institute have been encouraged and are well represented by the small group working in cancer research. This project has been in abeyance during 1970 but will be effective again in 1971. Similarly the studies of drugs on smooth muscle contribute to the cardiovascular projects.

Of these general groupings, the studies related to drug action are worthy of further consideration, especially as the subject of clinical pharmacology has received some recent notice in the general medical press. Clinical pharmacology is the scientific study of the action of drugs in man but it must be based on the action of drugs in all living things and also on a knowledge of their chemical structure and chemical reactions. The functional fusion which exists between the Institute and the Clinical Research Unit provides a unique framework in which to practise clinical pharmacology and opportunities have always been taken to this end, even though the term "clinical pharmacology" has not been applied to the work. Investigations carried out in drug trials and in detailed studies on mammalian cardiac muscle of some new drugs, not yet ready for clinical release, described in the scientific section of the report illustrate this phase of the Institute's work. The relevant seminars conducted indicate the feedback of the results to Hospital circles.

In the scientific section of last year's report an account was given of a study of the effect of excessive thyroid hormone on cardiac muscle cells. Sustained excessive action of the thyroid gland in man produces a disease variously known as Toxic Goitre, Exophthalmic Goitre, Grave's Disease or Hyperthyroidism. In this disease there is excessive heart action and ultimately, if untreated, it leads to a form of heart failure. In the study reported, it has been possible to demonstrate that in the muscle cells of hearts in the presence of hyperthyroidism a tiny structure two millionths of an inch across both accumulates calcium to a greater degree than normal and transfers it at an accelerated rate. Because calcium ions are a most important determinant of muscle action, it is likely that these changes are the basic cause of the effect of hyperthyroidism in heart muscle.

This is perhaps the first time that a clear link between a disease and a change in the working of a specific intracellular structure has been demonstrated. The study was, however, only possible because electron-microscopy enabled the tiny structures to be visualised, an ultracentrifuge enabled them to be separated out and the use of radioactive materials as tracers permitted the minute chemical changes to be followed. All of these techniques are very modern and employ expensive equipment and show how dependent medical research is on the state of development of other sciences and technologies.

A question sometimes asked is how are the details of a discovery such as this communicated to the scientific world? In answer it will be noted in the lists at the end of the report that this discovery has been the subject of written and spoken communications locally, around Australia, and overseas. By these means, together with personal contact and correspondence, the new knowledge becomes widely available. The communications network involved is graphically illustrated on the back cover of this year's report.

STAFF

There have been a considerable number of changes in staff during the year for a variety of reasons. Those who have gone are thanked for their help in the past and wished well in their new ventures.

Mr. S. Hart retired from the position of Laboratory Supervisor after 15 years of service and has been succeeded by Mr. R. P. Steele. Dr. J. A. Streeton left during the year to take up an overseas post-graduate position and Dr. D. A. Coventry has acted as Assistant Physician during the remainder of the year.

Dr. L. W. Wheeldon, after completing a three-year research project, left for another position. Dr. C. V. Nelson, Visiting Fellow, completed his study on Spatial Magnitude Electrocardiography and returned to the U.S.A.

During the year, Dr. P. Mäsiar, M.D., D.Sc., sometime Professor of Biochemistry, Kosice, Czechoslovakia, joined the Institute.

Dr. G. Hard, B.V.Sc., Ph.D., was appointed the A. A. Thomas Fellow of the Anti-Cancer Council of Victoria and is expected to arrive early in 1971.

Dr. S. Katz, Ph.D., has been appointed Edward Wilson Memorial Fellow (1971) and plans to work on a pharmacological project in the Institute.

Named fellowships have been held by the following members of staff:—

The Lang Fellow: L. W. Wheeldon, Ph.D.
William Buckland Fellow: P. Fantl, D.Sc.
James and Elsie Borrowman Fellow: Mrs. H. A. Jonas, M.Sc.

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, the National Heart Foundation of Australia, the Asthma Foundation of Victoria and Alfred Hospital Research Funds, and this continuing assistance is gratefully acknowledged.

It is a pleasure to thank, for generous donations, those whose names are listed in the various financial reports, especially those among our regular contributors who have increased their gifts to help with the rebuilding and those who have so generously helped with the purchase of equipment.

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, 1970.

ALFRED HOSPITAL RESEARCH FELLOWS IN THE INSTITUTE

Anderson, R. Mc.D., 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
Coventry, D. A., 1968
Daile, P., 1970
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
Murfitt, L., 1955
Newman, H. C., 1954
Parsons, P. J., 1951
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Anti-Cancer Council of Victoria.
Auckland Medical Research Foundation.
Australian Medical Association.
Austin Hospital, Melbourne.
Australian and New Zealand Association for the Advancement of Science.
Australian National University.
Baylor University, Texas. Cardiovascular Research Center.
Children's Medical Research Foundation, Sydney.
College of Pathologists, London.
Columbia University, N.Y. College of Physicians and Surgeons.
Commonwealth Bureau of Census and Statistics.
Commonwealth Department of Health.
Commonwealth Department of Labour and National Service.
Commonwealth Serum Laboratories.
Commonwealth X-ray and Radium Laboratories.
Council for Scientific and Industrial Research, South Africa.
Imperial Chemical Industries, London.
Institute of Medical and Veterinary Science, Adelaide.
Instituto de Biologia y Medicina Experimental, Buenos Aires.
International Society of Cardiology, Geneva.
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John Innes Institute, Norwich.
La Trobe University, Melbourne.
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Medical Research Council, London.
Medical Research Council, Wellington, New Zealand.
Melbourne Medical Postgraduate Committee.
Migraine Trust, London.
Monash University, Melbourne.
National Health and Medical Research Council, Canberra.
National Heart Foundation of Australia.
National Institute of Nutrition, Tokyo.
New York State Department of Health.
Ophthalmic Research Institute of Australia.
Otago University, New Zealand.
Papua and New Guinea Department of Health.
Queen Victoria Memorial Hospital, Melbourne.
Queensland Institute of Medical Research.
Refik Saydam Merkez Hifzissihha Enstitusu, Ankara.
Rockefeller Institute, New York.
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Royal Children's Hospital, Melbourne.
Royal Melbourne Hospital.
Royal North Shore Hospital, Sydney.
St. Vincent's Hospital, Melbourne.
St. Vincent's School of Medical Research.
Shionogi Research Laboratories, Osaka.
Societe medicale d'Afrique Noire le Langue Francaise, Dakar.
South African Institute of Medical Research.
South African Medical Research Council.
Statens Seruminstitut, Copenhagen.
Strangeways Research Laboratories, Cambridge.
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Universitas Marie Curie Sklodowska, Poland.
University of Melbourne.
University of Queensland.
Victorian Department of Health.
Walter and Eliza Hall Institute of Medical Research, Melbourne.
Wellington Medical Research Foundation.
World Health Organisation.

REPORT OF SCIENTIFIC INVESTIGATIONS

PHYSIOLOGY AND PHARMACOLOGY OF THE CARDIOVASCULAR SYSTEM

MYOCARDIAL CONTRACTILITY†‡

W. G. Nayler, V. Carson, P. Daile, D. Millar, I. McInnes, G. M. Picken, J. Szeto and T. E. Lowe.

Current concepts of the events involved in excitation-contraction coupling in skeletal and cardiac muscle assign a central role to the mechanisms whereby the intracellular concentration of ionized calcium (Ca^{2+}) is regulated.. It is generally agreed that during excitation depolarization of the plasma membrane facilitates a rise in the intracellular concentration of Ca^{2+}, and that if this intracellular Ca^{2+} concentration exceeds a critical level contraction will ensue. At least two mechanisms are thought to be involved in the process whereby a reduction in the transmembrane resting potential results in a raised intracellular concentration of ionized Ca. These processes are — (1) displacement of Ca^{2+} from the extra- to the intra-cellular phase across the cell membrane; and (ii) displacement of Ca^{2+} from intracellular binding sites, including the sarcoplasmic reticulum.

Contraction in striated muscle involves an interaction between the myofibrillar proteins, actin and myosin. This reaction is triggered by Ca^{2+} and energy for it is derived from the hydrolysis of adenosine triphosphate. However, purified actin and myosin fail to contract in the presence of ATP and Ca^{2+} unless two other proteins are added. These proteins are troponin and tropomyosin, and are both normally present in striated muscle, and apparently form a complex which sensitizes the actomyosin complex to Ca^{2+}. When the Ca^{2+} concentration falls below a critical level, the troponin-tropomyosin complex inhibits the interaction between actin and myosin, and the associated activation of the actomyosin ATPase enzyme. When the Ca^{2+} concentration exceeds a critical level the inhibitory effect of the troponin-tropomyosin complex is abolished. The interaction between actin and myosin and the associated activation of the ATPase enzyme can then occur.

In cardiac muscle a high proportion of the Ca^{2+} needed to raise the intracellular Ca^{2+} concentration above the critical level required to activate contraction is derived from the extracellular space. This is in contrast to skeletal muscle in which a relatively high proportion of the Ca^{2+} which activate contraction is displaced from intracellular binding sites. Relaxation in skeletal and cardiac muscle alike involves the accumulation of Ca^{2+} within specific zones of the sarcoplasmic reticulum.

Calcium Ions

W. G. Nayler

During the past year, studies into the movements of divalent cations associated with excitation-contraction coupling in cardiac muscle have involved detailed investigations into the uptake and release of Ca^{2+} from the sarcoplasmic reticulum and the Ca^{2+}-binding capacity of the cell membrane. Contraction was modified either by the administration of drugs, by the establishment of regional ischaemia, by bilateral adrenalectomy and by varying end-diastolic volume. Lanthanum ions were used to displace Ca^{2+} from membrane-located binding sites.

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

(a) Effect of Ischaemia. In dog left ventricular heart muscle made ischaemic by coronary artery occlusion, cardiac contractility declines immediately. This reduction in contractility was found to be accompanied by reduced concentrations of ATP and CP, and by raised concentrations of cellular Na^+ and IP. The ability of cardiac muscle to accumulate Ca^{2+} at the superficially-located membrane binding sites was found to be significantly reduced after only short (3 - 5 min.) periods of hypoxia and ischaemia. At the same time the microsomal ATPase activity and the Ca^{2+} accumulating activity of the cardiac microsomal fraction (representing sarcoplasmic reticulum isolated by differential centrifugation from homogenized heart muscle) remained unchanged. Prolonged ischaemia resulted in raised cardiac Na^+ and reduced K^+ concentration, in a further decrease in the Ca^{2+}-binding capacity of the membrane-located binding sites and in a reduced Ca^{2+}-binding, but not accumulating, capacity of cardiac microsomes. These results have been interpreted to mean that the altered cardiac contractility found after relatively short periods of either hypoxia or ischaemia may be due to a diminution in the amount of Ca^{2+} stored at membrane-located binding sites. This would result in less Ca^{2+} being available for translocation across the plasma membrane during subsequent membrane depolarizations.

(b) Effect of Drugs with Negative Inotropic Activity. Two drugs—sodium pentobarbitone and verapamil (Isoptin) have been studied in detail. Both have a negative inotropic effect on cardiac muscle, which cannot be explained in terms of β-adrenergic blockade. Sodium-pentobarbitone in high doses was found to diminish uptake of Ca^{2+} by the sarcoplasmic reticulum. In intact cardiac muscle cells, such a diminution in Ca^{2+} uptake by this subcellular fraction probably would result in less Ca^{2+} being available at storage sites within the reticulum for displacement during subsequent excitations. This effect of sodium pentobarbitone could, therefore, contribute to the marked cardiac depressant effect obtained when high doses of the drug are used. Low doses, within the range needed to establish anaesthesia, diminish the Ca^{2+}-binding capacity of the membrane-located but not of the microsomal binding sites. In this respect, therefore, the effect of sodium pentobarbitone on the subcellular distribution of Ca^{2+} in cardiac muscle cells resembles that caused by short periods of ischaemia and hypoxia. By contrast the cardiac glycosides were found to enhance the Ca^{2+}-binding capacity of the membrane-located binding sites.

In contrast to the results obtained for sodium pentobarbitone, the coronary vasodilator drug verapamil fails even at relatively high dose levels to have any effect on the Ca^{2+} accumulating and binding capacity of microsomal fractions prepared from mammalian heart muscle. It does, however, diminish the Ca^{2+}-binding capacity of membrane-located binding sites. This effect of verapamil on the affinity of membrane-located binding sites for Ca^{2+} may be related to the drug's negative inotropic effect, its local anaesthetic and anti-arrhythmic effects, and to its ability to diminish norepinephrine overflow associated with sympathetic nerve stimulation. Other investigations have shown that norepinephrine release in response to sympathetic nerve stimulation depends upon the availability of Ca^{2+}.

(c) Effect of End-diastolic Volume on the Ca^{2+}-accumulating Activity of the Sarcoplasmic Reticulum. Using dogs on right-sided cardiac bypass to provide a preparation in which left ventricular end-diastolic volume can be regulated, current experiments have provided further evidence in support of the theory that the relationship between left-ventricular end-diastolic volume and left ventricular work involves an effect of end-diastolic volume on the Ca^{2+}-accumulating activity of the sarcoplasmic reticulum. Under conditions of increased left ventricular end-diastolic volume, the Ca^{2+} accumulating-activity of the reticulum was consistently found to be significantly greater than that of sarcoplasmic reticulum extracted from hearts in which left ventricular end-diastolic volume had been maintained at relatively low levels.

(d) Effect of Bilateral Adrenalectomy. Chronically adrenalectomized dogs maintained on saline for ten days exhibited evidence of circulatory collapse. Mean arterial blood pressure was significantly lower than that of sham operated animals and left ventricular contractile force was diminished. This diminution in left ventricular contractile force was not due to an altered Ca^{2+}-accumulating or releasing activity of the sarcoplasmic reticulum. These experiments are being continued to determine if the membrane-binding capacity for Ca^{2+} is altered after adrenalectomy.

Troponin

P. Daile

The interaction between actin and myosin in the presence of ATP represents the essential mechanism of muscular contraction from the molecular standpoint. However, other proteins, troponin and tropomyosin, are essential to make the system sensitive to the physiological action of Ca^{++} ions released during the excitation-contraction coupling process.

During 1969, preparative methods for cardiac troponin were established and calcium binding and binding constant values determined. Ultra-violet scans on a recording spectrophotometer of these troponin preparations revealed a peak of 260 mμ.

This year troponin, tropomyosin and desensitized actomyosin were prepared by standard methods and their interactions studied using the technique of superprecipitation of actomyosin in the presence of ATP. Superprecipitation (measured as an increase in turbidity according to the method of Ebashi) represents a simulated *in vitro* contraction process. In a number of preparations, the sensitization of desensitized cardiac actomyosin by the troponin-tropomyosin complex to the action of Ca^{++} ions was demonstrated. Furthermore, in a limited number of preparations, cardiac troponin and tropomyosin sensitized skeletal actomyosin and *vice versa*. However, more extensive work on this aspect is necessary to elucidate whether the contractile proteins from the two types of muscle are biochemically compatible.

A recent publication by Hartshorne *et al*, has reported that skeletal muscle troponin can be separated into two functionally distinct components, troponin A and troponin B the dissimilarity of the two being evident from amino acid analysis, ultra-violet scans and from their effects on the ATPase activity of desensitized actomyosin. The proportions of the two proteins are quoted as 30% and 70% respectively, troponin A showing an ultra-violet peak at 260 mμ and troponin B a peak at 278 mμ.

Using a method of fractionation with DEAE cellulose, two fractions have been obtained from troponin, one showing a peak at 260 mμ and containing only 2% of the total protein, the other giving a peak of 278 mμ and 98% of the protein. In a limited number of preparations it has been shown that the larger protein fraction binds essentially the same amount of calcium, with the same binding constant, as does the original troponin, whereas the smaller fraction appears to show no binding at all.

Several troponin preparations have been subjected to gel electrophoresis after incubation with 4M urea. The original troponin preparations gave rise to three bands, two heavy and one very faint, whereas the 98% fraction gave rise to only the two heavier bands. The small fraction could not be shown to give rise to a single band since, as prepared, it was too dilute for electrophoresis. A concentrated preparation will, however, be prepared and tested.

In the coming year ATP-ase activity of desensitized actomyosin in the presence and absence of troponin (and fractions) and/or tropomyosin will be investigated using an electrotitrimetric method to follow the liberation of inorganic phosphate during ATP hydrolysis. Also, calcium binding and electrophoretic behaviour of troponin A and troponin B as prepared by Hartshorne will be compared.

Noradrenaline Uptake and Metabolism in Cardiac Muscle

G. M. Picken

(a) Muscle Cell Uptake of Catecholamines. When an action potential travelling down the sympathetic nerves to the heart reaches the

terminal varicosities of these nerves, the neurotransmitter noradrenaline is released and diffuses across the synaptic gap to the muscle cells, where a contraction is initiated through β-receptor stimulation. The noradrenaline may also increase the heart rate, and activate certain metabolic pathways inside the muscle cell. Much of the noradrenaline released from sympathetic nerve terminals is inactivated by re-uptake into the nerve terminals where it is stored in vesicles for subsequent re-use. However, a significant amount is actively taken up into the muscle cells, and is subsequently destroyed chiefly by intra-muscular catechol-O-methyltransferase (C.O.M.T.), and to a lesser extent by monoamine oxidase (M.A.O.). This uptake sequence contrasts with the destruction of acetylcholine — the neurotransmitter in skeletal muscle — by the enzyme cholinesterase on the post-synaptic membrane.

Two explanations have been offered for the noradrenaline uptake by the muscle cell. Firstly, it may serve to convey catecholamine to intracellular receptor sites where it exerts its actions and is subsequently destroyed enzymically. Or, secondly, the receptor sites may be on the surface of the cell, and the uptake process could serve merely to convey catecholamine to intracellular enzymes for destruction. The question towards which the following experiments are directed is, therefore — does the muscle cell uptake process convey noradrenaline to or from receptor sites?

The O-methylated derivatives of the catecholamines, noradrenaline, adrenaline, and isoprenaline, namely normetanephrine, metanephrine and 4-hydroxy, 3-methoxy isoprenaline respectively, are all potent inhibitors of the muscle uptake process. Of these catecholamines, isoprenaline is taken up by the muscle cells but is not accumulated by the nerve terminals and has, therefore, been chosen as the agent in these experiments designed to demonstrate any correlation which may exist between the ability of isoprenaline (i) to be taken up into the myocardium, (ii) to activate myocardial phosphorylase and (iii) to elevate the force of contraction, in isolated rat hearts. The ability of its derivative 4-hydroxy, 3-methoxy isoprenaline to influence each of these three parameters of the isoprenaline response has also been studied. Both the activation of phosphorylase, and the positive inotropic response to isoprenaline are measured, since it has recently been demonstrated that it is very likely that these responses are not mediated via the same receptors.

Muscle uptake of isoprenaline is determined by perfusing rat hearts with tritium labelled isoprenaline, and measuring the amount of radioactive metabolites in the muscle, as a percentage of the total radioactivity of an extract of the heart. Separation of ^{3}H-isoprenaline and its metabolites is achieved by passage of a perchlorate extract of the heart through Amberlite CG-120 cation exchange resin, and elution with differing strengths of hydrochloric acid. This method of separation is 92.7% efficient in our laboratory.

Myocardial phosphorylase activity is determined by the method of Cori and Illingworth[1] and the colour development method for determining the amount of inorganic phosphate produced is as described by Mozersky *et al.*[2]

Force of contraction of isolated hearts is measured by attachment of the heart, by a tie through the apex of the ventricle, to a strain gauge linked to a hot wire recorder.

It has been noted that 4-hydroxy 3-methoxy isoprenaline depresses phosphorylase activity in the isolated rat heart and also inhibits the activation of the enzyme by isoprenaline. Thus it seems that the O-methylated derivative of isoprenaline, which blocks muscle uptake, also inhibits activation of myocardial phosphorylase by this catecholamine.

(b) Catecholamine Tachyphylaxis. In our experiments the O-methylated metabolites of the catecholamine noradrenaline, adrenaline and isoprenaline markedly depress the isolated rat heart when perfused alone. It is

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[1] G. T. Cori and B. Illingworth. **Biochem. Biophys. Acta,** Vol. 2 (1956), p. 105.

[2] S. M. Mozersky *et al.* **Anal. Chem.,** Vol 38 (1966), p. 1182.

thought that this depression arises from C.O.M.T. action on the catecholamine within the muscle cells. The O-methylated derivatives are all potent inhibitors of the muscle uptake process and once formed, limit further entry of catecholamines into the muscle cells. This system may serve to ensure maximum conservation of catecholamines by re-uptake into the nerve terminals. This property of the metabolites may also provide an explanation for the well-known phenomenon of catecholamine tachyphylaxis. Studies are now being conducted to determine whether catecholamine tachyphylaxis in isolated rat hearts can be accentuated by perfusion with these metabolites, and reduced by inhibition of C.O.M.T.

THE CORONARY CIRCULATION‡

W. G. Nayler, V. Carson, I. McInnes, J. Stone, J. Tay, F. R. Trinker and T. E. Lowe

During a series of experiments designed to study the effect that a variety of vasoactive agents had on peripheral vascular resistance our attention has been increasingly directed towards the coronary circulation. Changes in coronary vascular resistance may result from the activation or inhibition of either the α and β-adrenergic or the cholinergic receptors. Alternativly changes in coronary vascular resistance may reflect a changed metabolic demand; or it may be caused by either the activation or inhibition of as yet undefined receptors in coronary vasculature. The reactivity of these various receptors which mediate changes in coronary vascular resistance appeared to be modified if certain drugs are present.

During the past year the reactivity of the coronary vessels has been studied in several ways: (i) the sympathetic nerve supply to these vessels has been stimulated and the changes in blood flow recorded; (ii) the action of catecholamines on isolated segments of coronary arteries has been noted; (iii) the influence of the drugs aminophylline, KO1366 and salbutamol on coronary vasodilator mechanisms has been investigated.

Sympathetic Stimulation

F. R. Trinker

In previous reports a number of preparations has been described in which it has been possible to study perfusion of the coronary circulation of a dog under conditions of either constant flow or constant perfusion pressure. During this year these preparations have been used to assess the influence of sympathetic adrenergic stimulation on coronary blood flow. It had been demonstrated that changes in perfusion pressure alone were accompanied by concomitant changes in coronary blood flow which were sustained so long as the change in perfusion pressure was maintained.

To establish the role of the cardiac sympathetic nerves in the neuronal determinant of coronary blood flow, the left stellate ganglion was electrically stimulated and the coronary blood flow measured directly using a square wave electronic flow meter. Attempts were made to measure variations of the stimulating parameters — voltage and frequency — and to correlate these changes with the changes in coronary blood flow.

These experiments revealed that low frequency (6/sec.) stimulation at low voltage (1-3V.) produced a decrease in coronary blood flow and that this could be abolished by α-receptor blockade.

When the frequency and voltage of stimulation were increased, the vasoconstrictor effect was replaced by vasodilatation reflected in an increase in coronary artery blood flow. The maximum response was obtained by frequencies of 20-25/sec. at voltages of 15-20V.

Since stimulation of the stellate ganglion in this way could involve a variety of types of nerve fibre and the results obtained have varied with frequency of stimulation, the effects on these responses of a number of different drugs were studied.

The ganglion blocking drug, pentolinium, diminished the vasodilator responses at all frequencies of stimulation suggesting that many of the cardiac sympathetic nerve fibres being stimulated are preganglionic.

The adrenergic neurone blocking drugs, guanethidine and bretylium, had differing effects. Guanethidine abolished all responses to both low and higher frequency stimulation whereas bretylium reduced the higher frequency stimulation responses only.

In the human situation it is known that emotional or physical stress produces an increase in sympathetic discharge thereby increasing the demands made on the heart. In the presence of coronary artery disease, the dilatation of coronary vessels possible in response to this stimulation may be inadequate to meet the increased myocardial demands. It is possible, therefore, that drugs such as β-antagonists or adrenergic blocking agents may be used rationally to reduce this sympathetic stimulation of the heart.

Action of Catecholamines

F. R. Trinker

Many investigators have agreed that the changes observed in coronary blood flow following stimulation of cardiac sympathetic nerves arise secondarily from metabolic changes induced in cardiac vessels. This cannot, however, be the complete explanation of the result because the vasoconstriction recorded had no known metabolic counterpart in the muscle. It should also be noted that these arguments are based mainly on data accumulated from experiments using exogenous catecholamines in the non-working heart.

In order to assess the direct action of sympathetic nerves on coronary arteries, an isolated arterial preparation was made. In this the left coronary artery of a dog was isolated, cannulated and perfused with McEwen's solution bubbled with 95% oxygen and 5% carbon dioxide at 37° C. and pH 7.5. The perfusion pressure recorded at constant flow rates gave a measure of the state of constriction or dilatation of the artery segment.

It was found that adrenaline and noradrenaline produced constriction. Isoprenaline and noradrenaline, however, produced dilatation of the vessel provided that its initial resting tone was high, as shown by an initial perfusion pressure of 10-20 mm/Hg. The constrictor action was antagonized by phentolamine and the dilator action by propranolol.

It is apparent, therefore, that there are in the coronary arteries α and β receptors which can elicit constrictor and dilator responses in the absence of any cardiac muscle metabolic changes.

Aminophylline

I. McInnes

The preparation developed last year which allows the coronary circulation of either working or arrested hearts to be perfused under conditions of constant flow or pressure as required while the rest of the circulation is perfused separately under conditions of constant flow, or pressure, has been used to investigate the effect of aminophylline on coronary vasodilator mechanisms. Coronary vasodilator responses to both dipyridamole and adenosine were either abolished or significantly reduced when these drugs were given 10 to 20 minutes after a single i.v. dose of aminophylline. Similarly the coronary vasodilator response to a lowered arterial O_2 saturation was abolished by aminophylline, and an isoprenaline-induced increase in heart rate and force of ventricular contraction was accompanied by only non-significant changes in coronary vascular resistance. These experimental findings have been interpreted to mean that aminophylline interferes with or abolishes the effect of some coronary vasodilator drugs and the coronary vasodilator response to either hypoxia or an increased metabolic demand. These investigations combine to establish under what other conditions these coronary vasodilator responses are abolished or diminished.

KO 1366

J. Tay

KO 1366 (O-2-hydroxy-3-(tert.butylamino) propoxybenzonitril HCl) is a newly developed highly potent β-adrenergic antagonist, which abolishes halothane-adrenaline but not ouabain-induced ventricular arrhythmias. When injected i.v. into intact anaesthetized dogs on

heart-lung bypass KO 1366 causes a reduction in total peripheral and in coronary vascular resistance. KO 1366, therefore, can be used to establish β-adrenergic blockade without increasing coronary vascular resistance, and in this respect it differs markedly from propranolol. The coronary constrictor action of propranolol is well documented.

Salbutamol [2]
W. G. Nayler

Salbutamol is a recently developed bronchodilator agent, the potency of which exceeds that of orciprenaline. Unlike isoprenaline, salbutamol shows selectivity for the β_2 type receptors in the trachea. Hence its bronchodilator action is not accompanied by positive inotropic and chronotropic responses. Studies using dogs on total cardio-pulmonary bypass have shown that doses of salbutamol which are within the range needed to produce effective or significant bronchodilation, reduce the resistance to blood flow in coronary and other vascular fields. The ratio between left ventricular work per minute and O_2 consumption increased suggesting that this β agonist, in contrast to those which stimulate β_1 receptors, enhances left ventricular efficiency.

Effect of Iproveratril (Isoptin, Verapamil)
J. Tay

Investigations carried out during previous years established that the coronary vasodilator drug, Isoptin, reduced peripheral vascular resistance, slowed the instrinsic heart rate and depressed cardiac contractility. Although this drug is known to be clinically useful in the treatment of angina pectoris and for abolishing cardiac arrhythmias its mode of action remains obscure. Isoptin does not block β-adrenergic receptors in the heart and high doses are needed to block β-adrenergic receptors in the peripheral circulation. Investigations carried out this year indicate that this drug may act by reducing the amount of norepinephrine overflow caused by sympathetic nerve stimulation. In this respect isoptin closely resembles the β-adrenergic antagonist propranolol.

[2] See also p. 43.

β-ADRENERGIC RECEPTOR BLOCKADE†

J. M. Rainey and J. Tay

ICI 50172, KO 1366

J. Tay

Because of the potential clinical value of compounds with β-blocking capacities, the studies of ICI 50172 and KO 1366 started last year have been continued.

ICI 50172, KO 1366 and propranolol are specific β antagonists in dogs and rabbits. However, the cardioselective action of ICI 50172 in dogs, but not in rabbits, suggests that the vascular β receptors in dogs and rabbits are different. This conclusion was supported by observations obtained in intact rabbits, and in auto-perfused rabbit hind limb and rabbit isolated thoracic aortic strip preparations where isoprenaline-induced vasodilatation and relaxation were blocked.

(a) Blood Pressure Effects. ICI 50172 has no direct action on mean blood pressure in dogs, rabbits or rats. However, propranolol lowered blood pressure transiently. In contrast, KO 1366 produced a marked fall in blood pressure which persisted for at least an hour. This KO 1366-induced vasodilation may be explained in terms of a direct inhibitory action on the vascular smooth muscle but not by stimulation of β receptors, blockade of α receptors or by a centrally mediated action.

(b) Heart Rate Effects. In intact anaesthetised dogs, rabbits and rats, all three β-antagonists decreased heart rate. ICI 50172 and KO 1366-induced bradycardia may be explained by a reduction in sympathetic activity to the heart by virtue of its β receptor blocking action. Propranolol, however, decreased heart rate by impairing sympathetic activity to the heart (by β-blocking action and by its adrenergic neurone blocking action) as well as by a direct negative dromotropic action.

In experimental preparations in which the heart possessed minimal sympathetic activity, ICI 50172 and KO 1366, unlike propranolol, increased heart rate. This increase in heart

rate could not be explained by β-receptor stimulation since propranolol did not block these responses.

(c) Myocardial Contractility Effects. ICI **50172 and KO 1366** had no significant effect on contractile force in open-chest, anaesthetized dogs whilst propranolol produced a negative inotropic effect. In isolated dog papillary muscles, ICI 50172 had no inotropic action, whilst propranolol produced a negative inotropic effect, which could be associated with its "quinidine-like" action. In contrast, KO 1366 produced a positive inotropic effect. This increase in contractile force was not a result of catecholamine release but probably a result of β receptor stimulation since propranolol abolished this response.

(d) Antiarrhythmic Properties. The antiarrhythmic properties of these three drugs against halothane-adrenaline and ouabain-induced arrhythmias in dogs were also investigated. In the present study, an attempt was made to investigate the mode of action of β antagonists against ouabain-induced tachycardia in dogs.

The antiarrhythmic effect of β antagonists against catecholamine-induced arrhythmia is probably mediated through specific β-receptor blockade. The antiarrhythmic effect against digitalis-induced arrhythmias is thought to be independent of any β-receptor blocking property and probably results from a non-specific "quinidine-like" action or a "neurodepressant" action.

In the present study, all three β antagonists abolished or prevented halothane-adrenaline induced arrhythmias in dogs. However, unlike propranolol, ICI 50172 and KO 1366 failed to abolish ouabain-induced tachycardia, thus supporting the hypothesis that the antiarrhythmic effect of β antagonists against ouabain-induced ventricular tachycardia is not due to β-receptor blockade. The inability of ICI 50172 and KO 1366 to antagonise digitalis-induced arrhythmia can be explained by the absence of any "quinidine-like" actions and by the absence of any neurodepressant action. The latter was shown in a dog splenic nerve stimulation preparation, where ICI 50172 and KO 1366 was found to have no significant effect on noradrenaline overflow. The antiarrhythmic activity of propranolol can be accounted for in terms of its "quinidine-like" action and its ability to decrease noradrenaline overflow in dog splenic-nerve preparation.

Production of Arrhythmias

The mode of action of halothane and adrenaline in producing arrhythmias and fibrillation has also been investigated. It has been suggested that these arrhythmias are elicited only by the simultaneous presence of a sympathomimetic amine with cardiac actions and a critical rise in systemic blood pressure.

The present study shows that, whilst adrenaline is capable of producing fibrillation in halothane-sensitized, bilaterally vagotomised dogs, isoprenaline produces only an increase in heart rate. Similarly, angiotension or phenylephrine alone does not produce fibrillation, but a combination of phenylephrine and isoprenaline consistently resulted in fibrillation. These observations support the hypothesis that fibrillation is produced by a sympathomimetic amine with cardiac action provided that there is a concomitant critical rise in blood pressure. However, the observation that phenylephrine-isoprenaline produced fibrillation more frequently than angiotensin-isoprenaline suggests that although a rise in blood pressure is important, α-receptors which mediate changes in refractory period of the heart may also play an important role.

Antihypertensive Effects of Some β-Adrenergic Antagonists

J. Rainey

Beta-adrenergic receptor blocking drugs, including propranolol and CIBA 39089Ba (Oxprenolol), are useful for the treatment of either angina pectoris or cardiac arrhythmias. The long-term use of these drugs has been found to result in a fall in mean arterial blood pressure, which cannot be accounted for simply in terms of an altered cardiac output and is accompanied by a reduction in peripheral vascular resistance. Because of these findings,

interest has arisen in the possible therapeutic use of various β-adrenergic antagonists as antihypertensive agents. (See p. 41).

The mechanisms whereby these substances cause peripheral vasodilation is obscure. Some investigators have suggested that the antihypertensive action displayed by them reflects their ability to interfere with homeostatic reflex mechanisms. To test this hypothesis, experiments were performed in rats to determine if any of the drugs studied — iproveratril, ICI 50172, propranolol, MJ 1999 or oxprenolol (Ciba 39089Ba) — affected the carotid occlusion baroreceptor response. Drugs were given orally for six to eight weeks, at a dose level of 1 mg/kg/day and the rats were housed in a constant temperature environment.

The results of these experiments showed that drugs which abolish or severely diminish the carotid sinus baroreceptor response do so only after a critical period of time has elapsed. In the case of propranolol, given at a dose level of 1 mg/kg/day, five weeks' contact time was required before this drug had any detectable effect on the carotid occlusion baroreceptor response. It seems unlikely, therefore, that the modified occlusion reflex results from the presence of β-adrenergic blockade, for at the dose levels used, blockade would have been present early during the experiments. This conclusion is supported by the fact that iproveratril mimicked the ability of propranolol to modify the occlusion reflex.

CELLULAR MEMBRANE SYSTEMS

L. W. Wheeldon

It was reported last year that so-called "sarcoplasmic reticulum fragments" in heart muscle microsomes may be resolved into two classes of fragmented membrane material which, because they differ in density, were labelled "light" and "heavy" components. Activities already known to be present in heart muscle microsomes — in particular the ATP-ase activity underlying transport of calcium — were found to be localised in the heavy component. The light component had a complement of enzyme activities which distinguished it functionally from the heavy component. It has not previously been recognised in the microsome fraction of muscle. During the past year, the nature and significance of this new component has been studied.

Light Component

Resolution of the light and heavy components depended upon the inclusion of heparin in the sucrose gradients. This and other polyanions are known to have a dissociating effect on some membrane systems, resulting in inhibition of certain activities in chloroplasts[1] and mitochondria[2]. The possibility that the light component might be a dissociation artefact is emphasised by several differences in properties between the calcium transport ATP-ase in the heavy component from heart muscle and the same enzyme system in skeletal muscle microsomes. These are specificity for ATP in the case of heart muscle, compared with lack of nucleotide specificity in the case of skeletal muscle and a lower affinity for ATP of the transport system in heart muscle. Evidence that the light component is not an artefact is, first, that unfractionated heart muscle microsomes which have not been exposed to heparin exhibit a similar nucleotide specificity with regard to calcium dependence. Non-specific nucleoside phosphatase activity found in the light component was inhibited by azide, a reagent which does not inhibit the calcium-stimulated NTP-ase of skeletal muscle microsomes. This argues against the light component NTP-ase being a degraded calcium transport NTP-ase. Moreover, a similar azide sensitivity of NTP hydrolysis has been reported in liver microsomes[3]. Secondly, when microsomes from skeletal muscle were submitted to the same conditions of density gradient analysis in the presence of heparin, they yielded only one component in which the hydrolysis of both ATP and GTP were stimulated by calcium ions.

It has recently been demonstrated that microsomes from liver[4] and chicken fibroblasts[5] can be resolved into light and heavy fractions, in which the distribution of nucleoside phosphatase and NADH-cytochrome c reductase activities bears a marked resemblance to that found in heart muscle microsomes. In liver, the com-

position of the light component approximates that of hepatocyte plasma membranes prepared by independent methods[6]. Other properties of the light and heavy components lead to the conclusion that these correspond respectively to fragments of plasma membrane and endoplasmic reticulum. In muscle structure corresponding to the endoplasmic reticulum of other cells is the longitudinal component of the sarcoplasmic reticulum, or "L-system", and at least portions of this structure are known to participate in calcium exchanges. This equates the "L-system" with the heavy component of heart muscle microsomes. Another portion of the sarcoplasmic reticulum known as the transverse or "T-system" is continuous with the plasma membrane and this is sufficiently ramified in myocardium[7] to account for the amount of light component found in heart muscle microsomes.

Significance of Plasma Membrane.

Unique roles have been attributed to the plasma membrane for the co-ordination of multicellular processes. For example, surface contact between cultured myocardial cells has marked effects on growth and rhythmic beating. In other tissues, specific antigens are associated with the plasma membrane, as are virus-binding sites and receptor sites involved in hormone and drug action. In molecular terms, this amounts to a view of the plasma membrane as a mosaic of highly specific recognition sites, probably both lipoprotein and glycoprotein in nature. Methods currently being developed to identify such sites are confined to dissociated cells such as erythrocytes and bacteria, where there are few problems in the isolation of the plasma membrane. The extension of these investigations to differentiated tissues such as heart muscle is one of the possibilities presented by the successful isolation of plasma membrane material.

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SPATIAL MAGNITUDE ELECTROCARDIOGRAPHY

C. V. Nelson, T. E. Lowe, J. Stone and K. Harvey

Influence of Electrical Properties of Intracardiac Blood

Since the electrical conductivity of normal blood is about three times that of the myocardium, there has been considerable speculation about the effect of the intracardiac blood on the currents produced in the heart muscle. Brody[1] calculated the potentials which would be produced by a dipole adjacent to a highly conducting sphere. He found that if the dipole were radial, or normal, to the sphere, field potentials would be increased. If, on the other hand, the dipole were tangential to the sphere, potentials would be decreased. These predictions were confirmed experimentally[2]. Therefore, the effects of intracardiac blood should depend on the direction of the excitation wave relative to the heart cavities.

In order to study this problem, isolated dog hearts were set up at the centre of a fluid-filled sphere. Electrodes were mounted on the three major axes (X, Y, Z) of the sphere, plus an additional electrode for ground (earth). The hearts were perfused with oxygenated blood in a modified Langendorff preparation. After allowing time for the hearts to stabilize, voltages from the X, Y, Z electrodes were recorded on an UV galvanometer-type recorder, running at a speed sufficient to give good resolution of the traces. It has been shown that the magnitude and direction of the heart-vector can be determined accurately from the three sets of bipolar voltages across the sphere axes[3].

Two groups of experiments were done: (a) The heart was perfused with normal blood

and the resistivity of the sphere fluid was varied. (b) The sphere fluid was kept constant, and the resistivity of the perfusing fluid was varied.

In group (a), the sphere was filled with different mixtures of isotonic dextrose and Hartmann's solution. As the resistivity of whole blood is about 150 ohm-cm, and the resistivity of myocardium 450 ohm-cm, the sphere fluid resistivity was varied within these limits. In group (b), the sphere fluid resistivity was kept constant at about 450 ohm-cm. The heart was perfused with normal blood. After these measurements were made, the haematocrit of the blood was increased by spinning down the blood in a centrifuge and pouring off the plasma. It has been shown that the electrical resistivity of blood increases with haematocrit content. Method (b) proved to be more successful, since the electrolyte concentration of the perfusate is more nearly physiologically correct. As the blood also goes through the coronary vessels and fills the cardiac chambers, this is an important advantage. The X, Y, and Z voltages were converted to spatial magnitude (M) and two angles, V° and H°, defining the direction of the vector in space calculated[4]. Graphs were made of M. V° and H° as a function of time during QRS.

A considerable amount of difficulty was experienced in obtaining a heart preparation which remained stable long enough to complete the measurements, and a number of unsuccessful experiments resulted. Part of the difficulty was due to the fact that it was necessary to prevent leakage between the perfusion fluid and the sphere fluid.

Results

In the group (a) experiment typical resistivity values were ρ_1 — normal blood = 150, ρ_2 — myocardium = 450, ρ_3 — sphere fluid = 600 ohm-cm, and 140 ohm-cm. In three experiments, the measured dipole moment was greater with the low resistivity fluid in the sphere. With low-ρ fluid, M_{max} was 1.52, 1.37 and 0.77 ma-cm for the three hearts. With high-ρ fluid in the sphere, corresponding values were 0.98, 0.84 and 0.53 ma-cm. Analysis by the method of electrical images[5] showed that the measured values should be greater with the low-resistance fluid. The ratios of dipole moment, M, for the two fluids were not constant throughout the cardiac cycle, indicating that the direction of cardiac excitation influenced the effects observed.

The group (b) experiments provided more information about the effect of the intracardiac blood. In five experiments, M was smaller during the first part of QRS when packed cells were perfused, and greater during the latter part of QRS, corresponding to septal and endocardial-epicardial spread. The excitation is largely tangential during the late portions of QRS, when the basal regions of the ventricles and septum are activated. The results found, therefore, are in accordance with Brody's concepts of the effects of a good conductor on potentials due to radial and tangential dipoles.

A control experiment was done, in which the heart was perfused first with normal blood, then with packed cells and then with normal blood again. The first part of the packed-cell curve was lower than either of the normal-blood curves, in agreement with previous findings.

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CARDIOACTIVE PLASMA SUBSTANCES

T. E. Lowe, H. A. Jonas, E. Mäsiar, P. Mäsiar, and W. G. Nayler.

Blood plasma contains a number of well recognised cardioactive substances which, however, do not account for all of its vasoactive properties. In previous reports it has been

recorded that by gel filtration chromatography on 5% cross-linked polyacrylamide gel two fractions with inotropic activity could be obtained. One fraction would have contained substances with MW greater than 50,000 and the other those with MW between 3,500-10,000. When the fraction with low MW substances was subjected to ion exchange chromatography on DEAE-methacrylate gel a fraction with strong inotropic and vasoconstrictor activity was obtained. This was named Kinekard. The active principle(s) of these fractions with low MW substances is unknown but Nayler and Curtain believed that it could be a polypeptide.

As many of the physiological and clinical properties of this fraction have been examined and recorded recently, further attempts have been made to identify the active principle concerned. In these, plasma has been treated along two separate lines. First, by fractionation in columns containing a variety of gels and, secondly, by ultrafiltration through membranes capable of selective molecular separation. Biological assays are necessary to follow the plasma activity and the response of either a toad heart or dog papillary muscle preparation or the contraction of a strip of rabbit's thoracic aortic muscle has been used as the indicator of inotropic or pressor activity.

Gel Filtration Chromatography

H. A. Jonas.

As supplies of the 5% cross-linked polyacrylamide gel previously used are no longer available Biogel P-10, the closest commercially available equivalent, has been used in this study. As before, two fractions with inotropic activity were obtained when plasma from man and a number of animal species was passed through this gel.

The fraction containing high MW substances was always associated with the bulk of the plasma proteins. The inotropic activity of this fraction usually increased on standing after preparation.

On the basis of gel filtration behaviour on Sephadex G25 and Biogel P-2 gels the MW of substances in the other fraction was considered to be less than 1600. It is also considered possible that the inotropic activity of this fraction might be due to its content of ionized calcium, because when the concentration of calcium ions was lowered by treatment with the chelating agent, disodium EGTA or the strong cation exchange resin, Bio-Rad AG50W-X8, the inotropic response was correspondingly lowered. The magnitude of these inotropic responses could always be duplicated by calcium chloride solutions containing the same concentration of calcium ions. This fraction had no vasoconstrictor activity.

Ultrafiltration

P. Mäsiar and E. Mäsiar.

In the fraction (Kinekard) of low molecular weight separated from heparinized plasma, the amount of active principle thought to be of the order of 60 μg per litre is too small for easy chemical characterisation. Also the relatively high sensitivity of the biological assays to chemicals, ions, pH changes, etc., in the perfusing fluids might arouse suspicion that the biological activity recorded from the fractions is spurious or arises from contaminants introduced in the isolation procedures.

To minimise these difficulties a fresh technique which avoids the addition of any chemicals or water has been used. It is based upon ultrafiltration of blood plasma through membranes which enable selective molecular separation. In "Diaflo" (Amicon Corp.) ultrafiltration cells heparinized blood plasma was placed under a constant pressure 55-60 p.s.i. by compressed nitrogen and passed stepwise through a series of membranes which retained solutes and colloidal material as follows:— (i) MW over 30,000 (PM30), (ii) over 10,000 (PM10), (iii) over 8,000-10,000 (UM10), (iv) over 1,000 (UM2), (v) over 500 (UM05). Nitrogen was used as the pressurizing gas because it was inert and would inhibit any oxidation of plasma components. Individual ultrafiltrates were concentrated by further ultrafiltration through membranes which pass solvent and solutes of lower molecular weight.

One ml fractions of all filtrates were collected and analysed for their content of Ca^{2+} and ninhydrin positive substances. All fractions were found to contain 1.96 ± 0.03 mM Ca^{2+}, which is roughly the amount found in the original plasma. Similarly, the concentration of ninhydrin positive low molecular weight substances as determined by high voltage paper electrophoresis at pH 4.7 was steady.

In concentrated fractions, the protein composition was examined using cellulose acetate electrophoresis in 0.06 M barbitone buffer pH 8.6. The concentration of proteins and large polypeptides was estimated using the biuret reaction. Qualitative analyses of the low molecular weight part of the fractions obtained was performed by two dimensional paper electrophoresis using pyridine-acetate buffer pH 4.7 in one dimension and acetate formiate buffer pH 1.9 in the second dimension.

The inotropic activity of fractions was tested qualitatively on preparations of dog papillary muscle. The fractions obtained from 1.6 l of dog plasma and their partial characterisation is presented in Table 1.

From these results it follows that heparinized dog plasma fractionated by means of ultrafiltration produces fractions containing proteins and peptides of molecular weight which might be considered within the range of 500-30,000. But further concentration of these fractions by ultrafiltration leads to considerable concentration of proteins originally present in plasma. In some of these fractions, all plasma proteins can be found.

See Page 34 for Table 1.

These proteins might be considered according to the results presented in Table 1 as a possible explanation of the appearance of inotropic activity in concentrated fractions obtained by ultrafiltration. However, the presence of inotropic activity in fraction III, which contained only traces of plasma proteins, speaks for the existence of other substances with inotropic activity whose molecular weight can be considered within the range of 1,000-8,000.

It is important to mention here, that in the concentration step plasma loses inotropic activity. A possible explanation of this is that during the filtration procedure a co-factor is lost. The activity can be only partly restored by adding of filtrate to the concentrated plasma.

The chemical nature of this active principle(s) is unknown at present. It is possible only to say that a very large volume of plasma is required to obtain sufficient material for chemical purification. Identification of the cause of inotropic activity of whole plasma and its constitutents appears to be a complex biochemical problem which requires more profound study.

TABLE 1

Fraction	Molecular Weight of Fraction	Concentration of Proteins in Individual Fraction (extrapolated on one litre original plasma)	Proteins Present Detected by Electro-phoresis	Aminoacid and Peptides Present	Concentration Relative to Original Plasma	Inotropic Activity
Plasma Original	—	70 g/l (100%)	All plasma proteins	Not estimated	1	Positive
Concentrated Plasma	Over 30,000	68 g/l (97.15%)	All plasma proteins	Not estimated	6.2 times	Positive
I	Over 10,000	0.172 g/l (0.24%)	Albumins and globulins	All naturally occurring amino-acids and some peptides	414 times	Positive
II	Over 8,000	0.04 g/l (0.057%)	Albumins	All naturally occurring amino-acids and some peptides	484 times	Positive
III	Over 1,000	0.011 g/l (0.016%)	Traces of Albumins	All naturally occurring amino-acids and some peptides	658 times	Positive
IV	Over 500	Traces	—	All naturally occurring aminoacids	220 times	Absent
V	Below 500	None	—	All naturally occurring aminoacids	100 times	Absent

CARDIAC TRANSPLANTATION‡

E. Cooper, G. C. Shardey, D. Davies and G. R. Stirling.

Rejection Phenomenon

Using the techniques described last year, a successful surgically sterile, heterotopic cardiac allograft preparation was established using male dogs as recipients, to obviate the presence of developed antibodies. Cold ischaemia was used to preserve the donated heart while sewing it into position. The rejection phenomenon was studied in four groups of animals.

(a) Untreated Group. The first group was untreated and had a mean survival time of eight days. Rejection of the graft was determined by electrocardiographic changes and palpation of the graft. Most grafts were removed while still having a palpable, although irregular and weak, beat. Daily synchronous electrocardiogram tracings from epicardial leads attached to the left ventricle, right ventricle, and right atrium of the donated heart and also bipolar, unipolar and chest leads from the recipient dog were taken and compared on similar time and sensitivity scales. Within this group, a series of dogs were killed beginning at 12 hours post-operatively and daily thereafter up to 10 days. All the donated and recipient hearts were sectioned immediately post-mortem and slides for light microscopy were taken from the areas of the sinus and atrio-ventricular nodes; the bundle of His and distal conduction pathways. Also the free ventricular wall and septal ventricular wall, cardiac valves and sites of anastomosis, were sectioned in most cases.

From these investigations, a significant pattern of rejection became evident. Histological evidence of intense immunocytic infiltration of the sinus node and atrio-ventricular nodes within the first three to four days post-transplant was correlated with the following electrocardiographic arrhythmias; degrees of heart block, sinus arrest, nodal rhythm and complete heart block. All of these findings suggested sinus and/or atrioventricular node damage. Immunocytic cellular infiltration of juxta-nodal autonomic neural ganglia was also found to be as intense and preferential as in the excitatory tissues; however, the main tissue affected was the glia with sparing of the neuronal cell body until the infiltration became so heavy that the latter was finally destroyed.

Destruction of vascular endothelium was seen to occur more heavily in the nodal areas.

Comparison of sections of the excitatory and conduction tissues with the ventricular myocardium showed a lesser degree of infiltration in the latter, which could be graded into less infiltration in the septal myocardium compared to the free wall.

The cardiac allograft thus is rejected, not as an organ *in toto*, but as the result of damage to its component tissues. Preferential infiltration by the effector cells begins in the excitatory and para-nodal neuronal cells, then proceeds to the conduction tissue and finally to the myocytes of the ventricular wall, the septum being the last to be affected. Throughout this process, acute destruction of the endothelium of vessels from capillary size to the nodal arteriolar vessel proceeds, producing occlusion and ischaemia.

(b) Immunosuppressive Therapy. A second group of animals was subject to standard immunosuppressive therapy using azothioprine and hydrocortisone. Survival up to 37 days was obtained. Again the same pattern of rejection was seen, but with a reduced degree of immunocytic response. Also loci of immunocytic cells sub-endocardially around the Purkinje system were seen. Several of these loci resembled lympho-sarcoma histologically.

(c) Antithymocyte Globulin Therapy. A third group was subjected to bovine antithymocyte globulin, using either a single or multiple dose regime and in three dogs, a Sephadex separated IgG fraction was used. There was no prolongation of the graft survival probably because of a thrombogenic property of the non-purified globulin, and a non-immunosuppressive effect of the purified IgG fraction. The use of this anti-thymocyte preparation has been discontinued.

(d) Orthotopic Transplants. In addition to these studies with heterotopic transplants, work has been directed to producing untreated surviving orthotopic preparations, to confirm the above findings, and also by using signs of atrial arrhythmias as an indication of impending rejection, to treat the animal accordingly with increased doses of hydrocortisone. Several survival dogs have been obtained and treatment has been successful up to four weeks post-operatively using the above criteria.

Sections of the heart at this stage showed no infiltration with immunocytes at all. However, death due to superimposed infection following increased immunosuppression has now become a problem. It is obvious that a more specific form of immunosuppressive therapy is required. Further orthotopic preparations are being investigated to elucidate the significance of this line of therapy. Recently, sections of the nodal and conductive tissues of the non-treated and treated rejecting hearts have been subjected to electron-microscopic investigation. Precise location of these tissues has now been achieved and, in one section, there is fusion of an immunocyte with a nodal cell with continuity of the cytoplasm. This is a hitherto unpublished observation.

PERIPHERAL VASCULAR DISEASE

A. J. Barnett, C. Baxter and I. A. Ferguson[1]

ARTERIAL SURGERY

A. J. Barnett and I. A. Ferguson.

Unlike the succession of diagnostic and therapeutic techniques in the past, the procedures today have now become fairly standardised.

Arteriography is performed by puncture of a femoral artery in the groin and contrast material injected, with radiograms taken to show the lower end of the aorta and iliac artery and the arteries of the affected leg(s) from the groin to the ankle.

Aorto-iliac disease is treated by insertion of a Dacron bypass graft, or, if the obstruction is localised, by endoarterectomy. Femoro-popliteal disease is treated by the insertion of a bypass graft of autogenous vein. In patients with obliterative disease of the lower part of the popliteal artery and proximal part of the tibial vessels, a strong effect is made to demonstrate the tibial arteries in the lower part of the calf and if these are of reasonable calibre, a femoro-tibial bypass graft is inserted. Coincident sympathectomy is performed in patients with rest pain or gangrene.

The operations performed for occlusive arterial disease of the lower limbs in 1970 are as follows:

Reconstructive Arterial Operations — 1970

Aorto-iliac "Dacron" graft	13
Aorto-iliac endoarterectomy	7
Femoropopliteal vein graft	41
Femoro-tibial popliteo-tibial vein graft	13
Embolectomy and thrombectomy	10
Miscellaneous and complicated procedures	11
	95

A register is being kept of arterial reconstructive operations and the patients reviewed at regular intervals so that the results of current surgical techniques can be assessed and compared with the extensive series previously studied.

It seems that arterial surgery has reached a stable and fairly satisfactory stage. The main problems in respect to peripheral vascular disease at present seem to be:— (i) Prevention of atherosclerotic arterial disease; (ii) Prevention of rethrombosis after surgery; (iii) Elucidation of causes of, and management of, occlusive disease of small vessels, not amenable to reconstructive surgery.

[1] Honorary Surgeon to Outpatients, Alfred Hospital.

TREATMENT OF HYPERLIPIDAEMIA WITH CH 13437

A. J. Barnett and C. Baxter.

CH 13437[1] is a new drug [(1, 2, 3, 4 tetrahydro-1-naphthyl) phenoxy] proprionic acid reputed to lower plasma liquids. Initial animal experiments showed it to be an orally active hypolipidaemic drug, without toxic effects. Preliminary tests in man showed that it was effective in lowering raised plasma cholesterol and triglyceride levels in atherosclerotic patients.

Subjects and Methods

A trial was set up to compare the effects of treatment with CH 13437 (300 mg/day) with placebo and with clofibrate (1.5 G/day), the order of treatments being varied. In the midst of the trial, information was received that toxic effects had been observed in animals (treated for 1½ years with 50 times the equivalent human dose) and advice was given that, until these were evaluated, further administration of the drug to humans should be discontinued. The original plan had, therefore, to be modified.

In effect, 26 patients were selected for the trial because a raised plasma cholesterol level (> 300 mg/100 ml) had been recorded and/or raised plasma triglyceride level (> 200 mg/100ml), on at least two of an initial series of five observations.

Assessment of the effect on lipid levels was made on the basis of averages of five readings before and during the last month of treatment. Treatment periods were of 14 weeks with an intermission of eight weeks between treatments. The patients were assessed clinically before and after each treatment and a series of urine, biochemical and haematological tests were carried out to detect any toxic effect on kidneys, liver or haematopoietic systems.

Of the 26 patients commencing the trial, nine completed one course of CH 13437, five had a partial course of CH 13437, six completed one course of clofibrate 1.5 G/day, and six completed one course of placebo, when information of possible toxicity from CH 13437 was received and the plan was changed so that no further administration of this drug was given. After an intermission period, the patients originally receiving CH 13437 were treated with clofibrate 2 G/day, those originally treated with clofibrate (1.5 G/day) were treated with a higher dose (3.0 G/day) and those receiving placebo were given a second course of placebo.

Results

The most interesting observation relates to the patients receiving placebo. During the initial treatment period, five of the six patients showed a rise in cholesterol levels (mean change + 49 mg/100 ml) and five of the six patients showed a rise in triglyceride levels (mean change + 64 mg/100 ml).

In the patients who received a second course of placebo, the cholesterol and triglyceride levels which had risen after the first course tended to fall during the intermission and continued to do so during the second course.

During treatment with CH 13437, each of the nine patients showed a fall in cholesterol levels (mean change − 50 mg/100 ml). The change in triglyceride levels was less consistent. In three patients, there was a fall greater than 25 mg/100 ml; in one a rise greater than 25 mg/100 ml), and in the other five, little change (< ± 25 mg/100 ml). It is to be noted that the group contained only three patients with markedly raised triglycerides, in two of whom there was a fall, in the other a rise.

During treatment with clofibrate (1.5 G/day) there was little change in cholesterol levels (three patients showing a rise, three showing a fall, with mean change for the six patients of − 15 mg/100 ml). However, in four of the group with markedly raised triglyceride levels (> 300 mg/100 ml) there was a marked fall, in the other two no change.

In the patients who received the higher levels of clofibrate (2.0 or 3.0 G/day) as their second course, there was usually a marked beneficial effect and the cholesterol and triglycerides eventually achieved normal or near-

[1] Kindly supplied by Hoechst (Australia) Ltd.

normal levels (except in the case of two patients with initially grossly raised triglyceride levels who still had levels > 300 mg/100 ml, which, however, were much lower than their initial levels).

Four patients were lost from the trial during its course; three of these died, one from a coronary occlusion two weeks after starting CH 13437, one from a coronary occlusion following an initial course of CH 13437; the other suddenly (probably coronary occlusion) following an initial course of clofibrate. The third patient ceased to attend after a gastrointestinal haemorrhage. He had completed a first course of CH 13437 and had commenced his second course of clofibrate.

Comment

One of the most interesting results of this trial was the change in lipid values during placebo treatment, tending to rise in autumn-winter and to fall during spring-summer. It is necessary to allow for this seasonal variation in assessing the effect of hypolipidaemic drugs.

CH 13437 gave promise as a hypolipidaemic drug. In a dose of 300 mg/day, it seemed more effective than clofibrate 1.5 G/day in reducing plasma cholesterol levels. The effect on triglyceride levels could not be judged from this study as the group treated with clofibrate contained a higher proportion of patients with gross elevation, probably more sensitive to a hypolipidaemic drug.

HYPERTENSIVE STATES

A. J. Barnett, F. G. Silberberg and F. R. Trinker

Analysis of Therapeutic Trial†

The survey of the results of long-term treatment of hypertension based on the results in the clinic established in 1950 has continued. This has been carried out by the analysis of histories, recording the data on check sheets and subjecting these to computer analysis. In 1969 we outlined the difficulties encountered in a computer study of this type and gave some preliminary figures based on an analysis of 60 cases.

The computer programme has since been somewhat modified and 100 of the 150 case histories on the check sheets have been analysed but there are still defects in the programme to be rectified before the final analysis can be made.

The information required from the computer can be listed under four headings: (i) Patient data on presentation. (ii) General follow-up data. (iii) Information on deceased patients. (iv) Correlations. Some selected results (Tables 1 - 4) demonstrate the type of information which is being obtained. Table 1 shows the age and sex of the patients, the type of hypertension and an analysis of cardiac abnormalities. A similar analysis was carried out in respect of neurological, ophthalmological and renal states.

See Page 39 for Table 1.

TABLE 1

DATA ON PRESENTATION FROM 100 PATIENTS

Age (years)	Under 20	20-29	30-39	40-49	50-59	Over 60	Total
Male	1	2	10	20	18	3	54
Female	—	4	7	14	18	3	46
Total Numbers	1	6	17	34	36	6	100

Type of Hypertension	Benign	Malignant	Renal	Non-Renal
Male	35	19	6	48
Female	32	14	4	42
Total Numbers	67	33	10	90

Blood Pressure

Systolic (mm Hg):	160-179	180-199	200-219	220-239	240-259	260 & over
Numbers	1	11	14	27	25	22
Diastolic (mm Hg):	110-119	120-129	130-139	140-149	150-159	160 & over
Numbers	6	12	26	25	19	12

Main Symptoms

Headache	79
Visual Disturbance	57
Dyspnoea—Exertional	46
Paroxysmal	22
Orthopnoea	4
Nocturia	40
Transient cerebral ischaemia	33
Functional nervous—	
Nervous tension or depression	25
Insomnia	12
Poor memory	3
Cardiac symptoms other than dysponea:	
Palpitation	16
Ischaemic pain	10
C.C.F.	5

[1] One case not assessed.

Cardiac Abnormality:		
Heart clinically normal		19
Heart clinically abnormal		81
Murmur	47	
Cardiomegaly	55	
Triple Rhythm	9	
Heart Size Radiologically:		
Normal		50
Increased		49[1]
Mild	15	
Moderate	12	
Severe	22	
E.C.G.:		
Normal		16
Abnormal		84
Myocardial ischaemia	68	
L.V. hypertrophy	36	

TABLE 2

FOLLOW-UP DATA

General Survival Rate

Number of Survivors Time (years)	Potential	Actual	Lost from Survey
1	100	80	2
2	100	73	2
5	100	55	8
10	100	36	19
15	51	12	4

Incidents

Period (years)	0-1	1-2	2-5	5-10	10-15
Cerebral Vascular Accident	13(9)	4(1)	3(2)	10(1)	1
Coronary Occlusion	3(2)	1	3(2)	1(9)	4(4)
Congestive Cardiac Failure	1	0	5(1)	2	0
Renal Failure	10(8)	2(2)	5(4)	2	1(1)

(The figures in brackets indicate fatal incidents)

TABLE 3

GRADE OF CARDIAC DISEASE

Time (Years)	Initial	1	2	5	10	15
Nil or Mild	60 (60%)	55 (70%)	51 (70%)	39 (72%)	25 (69%)	9 (70%)
Moderate or Severe	40	24	22	15	11	4
Total Numbers	100	79	73	54	36	13
Change in Grade						
Improved		15	5	11	3	2
Unchanged		61	57	37	26	9
Deteriorated		3	11	6	7	2

TABLE 4
CORRELATIONS

Feature Studied	No. of Patients	% Survival at (Years) 1	2	5	10
Systolic B.P.					
160-240 (mm Hg)	51	90	82	59	41
Over 240	49	67	63	51	31
Diastolic B.P.					
110-140 (mm Hg)	44	88	82	56	41
Over 140	56	73	66	54	32
Ocular fundi					
Normal or vessel changes only	37	87	87	65	46
Haemorrhages or exudates (no papilloedema)	30	80	77	57	43
Papilloedema	33	80	60	47	20
Heart Size					
Normal or slightly enlarged (C.T.I. less than 0.53)	65	83	80	62	42
Moderately or severely enlarged (C.T.I. greater than 0.54)	34	62	59	41	26
E.C.G.					
Normal	16	87	68	56	38
Ischaemic	70	77	73	51	33
L.V. hypertrophy	36	72	67	50	30
Fasting blood urea					
below 40 mg/100 ml.	59	83	80	63	41
below 40-60 mg/100 ml.	23	96	83	51	30
above 60 mg/100 ml.	17	47	35	29	12

Table 2 shows that the highest incidence of cerebral vascular accidents and renal failure is in the early period, but cardiac complications reach their peak later; at 2-5 years for coronary occlusion.

In the previous report, a table was shown indicating the improved blood pressure control, symptomatic state and work ability in the later review periods. This is also shown in the recent figures based on a larger number of cases. There was also a tendency to upgrading in respect to severity of vascular disease but it was not clear whether this was due to improvement in individual patients or to elimination by death of the more severely affected persons. Table 3 shows the grading of patients in respect to cardiac disease with time and the change in grade of particular patients. That the improvement in grading at one year is not due to elimination of the unfit is shown by the predominance of improved over deteriorated at this time. A similar but more marked predominance of improved in the early stages occurred in respect to cerebral and ocular disease but not renal disease.

Cause of Death. Analysis of 63 deaths showed that 13 were due to cerebral vascular accident (10 within first two years); 17 were due to coronary occlusion spread over the treatment period, and 15 were due to renal failure (10 within the first two years). Cerebral and renal deaths thus tend to occur early, coronary deaths at any time.

Most deaths occurred in patients aged 40 to 60 years. This age group included 12 of the 13 cerebral deaths, 12 of the 17 coronary deaths and 10 of the 15 renal deaths. Coronary deaths spread into the older age group (five patients over 60) and renal deaths into the younger group (four patients under 40).

In patients dying from a cerebral vascular accident or renal failure, recent blood pressure control was usually poor, but in those dying from coronary occlusion, it was usually good or fair.

Correlations. Correlations between various features on presentation and survival have been studied and some of these are shown in Table 4.

It is noted that in each of the features studied, there is a correlation between the severity of the condition and increased mortality. Heart size is the best prognostic index in these cases. Using the dividing lines given in the tables, systolic and diastolic blood pressure have the same prognostic significance. In these (treated) patients, the severity of the changes in the ocular fundi have not the serious import described in non-treated cases. ECG is less valuable as a prognostic indicator than heart size. Fasting blood urea level does not influence the prognosis until it is over 60 mg/100 ml.

Trial of β-adrenergic Blocking Agents in Hypertension.

Most of the presently used hypotensive drugs act either directly or indirectly on the α-adrenergic component of the sympathetic nervous system. However, recently, there have been reports that in hypertensive patients β-adrenergic drugs have a hypotensive action. (See p. 29).

A small trial along the following lines has therefore been started. A series of patients have been selected in whom (i) there is no evidence of cardiac failure and (ii) the present treatment is not ideal either because blood pressure control is inadequate or there are undesirable side effects. One sub-group is receiving in addition to previous drugs propranolol ("Inderal", ICI), and the other, oxprenolol ("Trasicor", Ciba). Commencing dose is 20 mg. three times daily. The patients are seen at intervals of three weeks and the dose is increased by doubling until either there is a satisfactory response or a dose of 160 mg. three times per day is attained. If a normal blood pressure is achieved, the dose of the original hypotensive drugs is reduced.

Results have not yet been analysed but we have found that in some patients the addition of the β-blocking agent is very useful while in others it has had no effect in the dose used.

A remarkable feature has been the absence of side effects from these additional drugs.

W. G. Nayler, I. McInnes and J. Tay

Salbutamol*

Sympathomimetic amines are widely used in the treatment of reversible airways obstructive disease but their value is often limited by undesirable other effects. Of them isoprenaline has been extensively used, but its value is limited because of its rapid destruction by the catechol-o-methyl transferase enzyme, its uptake into tissues and its positive inotropic and chronotropic effect on the heart. Likewise another of these amines, orciprenaline, is a potent bronchodilator but its usefulness is limited by the fact that it causes significant increases in heart rate, force of ventricular contraction and myocardial oxygen demand.

During the past year laboratory investigations have concentrated upon determining the properties of a newly developed bronchodilator drug, Salbutamol. Particular attention has been given to its effect on the cardiovascular system.

Preliminary investigations confirmed that salbutamol (2-t-butylamino-1-(4-hydroxy-3-hydroxymethyl) phenylethanol) differed significantly in action from either orciprenaline or isoprenaline. It displays marked selectivity for β_2 receptors, which mediate a dilator response to bronchiolar smooth muscle, and at the same time stimulates glucose metabolism. High doses of salbutamol have a small positive inotropic and chronotropic effect on mammalian heart muscle.

In guinea pig tracheal chain preparations, which had been equilibrated in aerated Tyrode's solution, salbutamol produced relaxation slightly in excess of that produced by equimolar doses of isoprenaline. The bronchodilator effect of salbutamol differed from that of isoprenaline in that it was maintained for significantly ($p < 0.001$) longer periods of time even when comparatively low doses were used. The bronchodilatation caused by salbutamol, like that produced by isoprenaline, involves activation of β-adrenergic receptors, because it is abolished by propranolol.

The effect of salbutamol on β-adrenergic receptors in heart muscle was investigated, using dog and human heart muscle. The results of these experiments indicated that significantly higher doses ($p < 0.001$) of salbutamol than isoprenaline are needed to stimulate β-adrenergic receptors in the heart. Doses of salbutamol which reduced resting tension in tracheal smooth muscle by 50% failed to cause any significant change in myocardial oxygen demand.

Dogs on right-sided cardiac bypass were used to investigate the effect of salbutamol on peripheral vascular resistance. Small doses of salbutamol produced small non-significant changes in peripheral vascular resistance but large doses caused vasodilatation. Coronary vascular resistance was decreased, an effect which involved stimulation of β-adrenergic receptors. In contrast to the vasodilator response to either hypoxia, adenosine, or dipyridamole that caused by salbutamol persisted in the presence of aminophylline.

Left ventricular work-function studies indicated that salbutamol may improve the efficiency with which the left ventricle performs mechanical work, because the ratio between left ventricular work performed and oxygen extracted by the heart from the coronary circulation increased after the administration of doses of salbutamol which produced effective bronchodilatation but which failed to have any positive inotropic or chronotropic effect. Analyses of biopsies from left ventricular muscle showed that effective bronchodilator doses of salbutamol did not cause any significant change in the high energy phosphate stores (adenosine triphosphate and creatine phosphate) of cardiac muscle.

P. Fantl

Protection from Mercury Inactivation of Factor XIII by Mercaptalbumin.

During studies which required the measurement of factor XIII (fibrin stabilising factor) activity difficulties in the assay were encountered. It was found that when reagents which had "Merthiolate" (ethylmercurithiosalicylate) as a preservative and which had been stored for months were used, factor XIII was inactive. In contrast, freshly prepared reagents containing merthiolate in similar amounts did not inactivate factor XIII. Apparently during storage at 4°C. merthiolate decomposed to one or more active mercury compounds. It was found that free mercuric ions could inactivate factor XIII and that this inactivation could be reversed by mercaptalbumin.

Clinically in chronic liver disease with reduced plasma albumin concentration factor XIII activity in the blood plasma is diminished and it is believed that the plasma factor XIII activity is related mainly to plasma mercaptalbumin concentration.

A detailed analysis of normal plasma indicated that it contained three groups of thiol compounds: non-protein thiols (approx. 5%), mercaptalbumin (75%) and in other proteins (20%). When the thiol groups of the mercaptalbumin were blocked these derivatives did not reactivate mercury inactivated factor XIII.

For the assessment of factor XIII activity it is therefore necessary to determine both the factor XIII as well as the thiol concentration, as the lowest concentration of either will decide the activity.

Since mercaptalbumins are the major source of thiol compounds in plasma, conditions where reduced albumin concentration occurs will, with tests which depend on dilution of plasma for factor XIII activity, give results which are too low. This was shown to be so in a case of nephrotic syndrome and in another patient with a kidney transplant who had markedly reduced albumin concentration but normal factor XIII activity. Plasma albumin may be reduced in pregnancy and indeed apparent low factor XIII values have been reported.

Thiol Distribution and Mercaptalbumin Reactivity in the Blood Plasma of Different Vertebrates.

The observation that mercaptalbumins protect factor XIII from inactivation by mercuric ions had made it desirable to determine mercaptalbumin concentration in mammalian plasma. This has been done with a disulphide-thiol exchange reaction. Treatment of plasma and the isolated albumins with di-(5-carboxy-4-nitrophenyl)-disulphide indicated a marked difference in the reactivity of mercaptalbumins of different species.

Human plasma, and its isolated albumin fraction, like that of guinea-pig showed a low reactivity in the thiol-disulphide exchange reaction. Rat's plasma on the other hand reacted almost as fast as non-protein thiol compounds. These observations indicate that the thiol groups in human and guinea-pig mercaptalbumin are located in less reactive sites than in the rat's mercaptalbumin.

Although albumins are species specific in immunological reactions, it is not common to observe marked differences in chemical reactivity of homologous proteins.

Grouping of animals according to mercaptalbumin reactivity with di-(5-carboxy-4-nitrophenyl)-disulphide gave the order starting with the slowest reaction rate: guinea-pig, man, rabbit, mouse, rat. It is interesting to speculate on the significance of the marked difference of the thiol reactivity in vertebrate mercaptalbumins. Differences between human and rodent mercaptalbumins may not be surprising but the difference in reactivity between rat and mouse mercaptalbumin is unexepected. It is probably justified to assume that mercaptalbumins which comprise over half of the total albumin have similar biological properties in the circulation of all animals. There are therefore from a Darwinian point of view no obvious advantages of mutation but it seems probable that the differences in mercaptalbumin structure are largely due to non-Darwinian evolution.

PUBLICATIONS IN 1970

PHYSIOLOGY AND PHARMACOLOGY OF CARDIOVASCULAR SYSTEM

Role of Calcium†‡

NAYLER, W. G. — "Ion Movements in Heart Muscle" in "Membranes and Ion Transport" Ed. E. E. Bittar. Vol. 2 (1970).

NAYLER, W. G., P. DAILE, DENISE CHIPPERFIELD and K. GAN. — "The Effect of Ryanodine on Calcium in Cardiac Muscle." *Amer. J. Physiol.* Vol. 219 (1970) P. 1620.

NAYLER, W. G., I. McINNES, DENISE CHIPPERFIELD, VALERIE CARSON and P. DAILE. — "The Effect of Glucagon on Calcium Exchangeability, Coronary Blood Flow, Myocardial Function and High Energy Phosphate Stores." *J. Pharmacol. exp. Therap.* Vol. 171 (1970) P. 265.

NAYLER, W. G., I. McINNES, D. CHIPPERFIELD, V. CARSON and J. B. KURTZ. — "Ventricular Function and the Calcium-Accumulating Activity of the Sarcoplasmic Reticulum." *J. Molec. Cell. Cardiology*, Vol. 1 (1970) P. 307.

NAYLER, W. G. and N. C. R. MERRILLEES. — "Cellular Exchange of Calcium." *J. Molec. Cell. Cardiol.* In Press.

NAYLER, W. G., N. C. R. MERRILLEES, DENISE CHIPPERFIELD and J. B. KURTZ. — "Influence of Hyperthyroidism on the Uptake and Binding of Calcium by Cardiac Microsomal Fractions and on Mitochondrial Structure." *J. Cardiovasc. Res.* In Press.

NAYLER, W. G., J. STONE, VALERIE CARSON and DENISE CHIPPERFIELD. — "Effect of Ischaemia on Cardiac Contractility and Calcium Exchangeability." *J. Molec. Cell. Cardiol.* In Press.

Pharmacology‡

NAYLER, W. G. — "Cellular Function and Beta-adrenergic Blockade." "New Horizons in Medicine." Vol. 1 (1970) P. 25.

NAYLER, W. G. — "Some Observations on the Cardiovascular Effects of Salbutamol, with Particular Reference to the Cardiovascular System." *Postgrad. Med.* In Press.

NAYLER, W. G., I. McINNES, J. STONE, V. CARSON and T. E. LOWE. — "Catapres (ST155) — induced changes in Coronary Vascular Resistance." *Cardiovasc. Res.* Vol. 10 (1970) P. 457.

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NAYLER, W. G. and JANINE TAY. — "Effect of 0-2-hydroxy-3-(tert. butylamino) propoxy-benzonitrile HCl (KO1366) on Beta-adrenergic Receptors in the Cardiovascular System." *J. Pharmacol. exp. Therap.* Submitted.

NAYLER, W. G. and JANINE TAY. — "Effect of Antiarrhythmic Drugs on the Release of Norepinephrine in Response to Splenic Nerve Stimulation. *J. Pharmacol. exp. Therap.* Submitted.

TRINKER, F. R. — "The Significance of the Relative Potencies of Noradrenaline and α-methylnoradrenaline in Relation to the Mode of Action of α-methyldopa". *J. Pharm. Pharmacol.* In Press.

TRINKER, F. R. and A. J. BARNETT. — "Trial of a New Hypotensive Drug — Clonidine ('Catapres')". *Med. J. Aust.* Vol. 2 (1970) P. 675.

TRINKER, F. R. and V. CARSON. — "The Pharmacological Effects of H56/28 — A new β-antagonist on the Cardiovascular System". *Cardiovasc. Res.* In Press.

Myocardial Function‡

NAYLER, W. G. — "The Effect of Beta-adrenergic Receptor Blocking Drugs on Myocardial Function" in "Beta-Adrenergic Receptor Blocking Drugs." Ed. F. O. Simpson. (1970) P. 1.

NAYLER, W. G. — "The Effect of Beta-adrenergic Receptor Blocking Drugs on Myocardial Function: An Explanation at the Subcellular Level." *Postgrad. Med. J.* Vol. 46 (1970) Supp. Nov. P. 90.

NAYLER, W. G., I. McINNES, J. STONE and V. CARSON. — "Effect of Dopamine on Coronary Vascular Resistance, Ventricular Efficiency and High-Energy Phosphate Stores." *Cardiovasc. Res.* In Press.

WHEELDON, L. W. and K. GAN. — "Resolution of Fragments of Plasma and Sarcotubular Membranes in Heart Muscle Microsomes." *Biochem. Biophys. Acta.* In Press.

Plasma Vasoactivity

MOIR, T. W. and W. G. NAYLER. — "The Coronary Vascular Effects of Glucagon." *Circulation Res.* Vol. 26 (1970) P. 1.

CARDIAC SURGERY‡

SHARDEY, G. C., E. COOPER and W. G. R. M. DE BOER. — "Differential Rejection of Neurons and Neuroglia in Canine Cardiac Allografts." *Nature.* Vol. 228 (1970) P. 69.

COOPER, E., G. C. SHARDEY and W. G. R. M. DE BOER. — "The Differential Rejection of Excitatory and Conduction Tissues of Untreated Canine Cardiac Allografts with Electrocardiographic Correlation." *Thorac. Cardiovas. Surg.* Submitted.

DISEASE OF BLOOD VESSELS

BARNETT, A. J., I. A. FERGUSON, K. N. MORRIS, K. E. STUCHBERY, A. SHANNAHAN and E. G. STAFFORD. — "The Results of Surgical Treatment of Occlusive Arterial Disease Affecting the Lower Limbs." *Med. J. Aust.* Vol. 1 (1970) P. 745.

BLOOD COAGULATION

FANTL., P. — "Protection from Hg-Inactivation of factor XIII by Mercaptalbumin." *Thrombosis et Diathesis Haemorrhagica.* In Press.

FANTL, P. — "Thiol Distribution and Mercaptalbumin Reactivity in the Blood Plasma of Different Vertebrates." *Aust. J. exp. Biol. med. Sci.* In Press.

CARCINOGENESIS**

HUGHES, P. E. — "Mitotic Responses to Partial Hepatectomy in Pre-neoplastic Rat Liver." *Chemico-Biol. Interactions.* Vol. 1. (1970) P. 315.

HUGHES, P. E. — "Liver Cell Responses to the Carcinogen 3'-Methyl-4-dimethylaminoazobenzene." *Chemico-Biol. Interactions.* Vol. 1 (1970) P. 301.

HUGHES, P. E. and R. PILCZYK. — "The *in vivo* Binding of Metabolites of 2-napthylamine to Mouse Liver DNA, RNA and Protein." *Chemico-Biol. Interactions.* Vol. 1 (1970) P. 307.

MISCELLANEOUS

ST. JOHN, D. J. B. and F. T. McDERMOTT. — "Influence of Achlorhydria on Aspirin-induced Occult Gastrointestinal Blood Loss." *Brit. Med. J.,* Vol. 2 (1970) P. 450.

LECTURES DELIVERED DURING 1970

"Atrial Septal Defect: a review of 216 cases and four methods of closure." — (i) *Paediatric Surgical Congress, Cardiac Surgery, Melbourne.* (ii) *University of Malaysia, Kuala Lumpur.*	E. COOPER
"The Use of Fascia Lata Prosthetic Heart Valves." (i) *Mayo Clinic, Minnesota.* (ii) *University of Edinburgh.*	E. COOPER
"Correlation Between Observed ECG and Histological Changes in Rejecting Cardiac Allografts." —*University of Edinburgh.*	E. COOPER
"Troponin from Cardiac Muscle." — *Muscle Biology Conference — C.S.I.R.O., Brisbane.*	P. DAILE
"Effect of Thyroxine on Ca^{++} — accumulating Activity of Cardiac Sarcotubules." *Muscle Biology Conference, C.S.I.R.O., Brisbane.*	W. G. NAYLER
"Cellular Function and Beta-adrenergic Blockade." — *Symposium on Beta Adrenergic Blockade, Sydney.*	W. G. NAYLER
"β-Adrenergic Blockade: An Explanation at the Subcellular Level". *Symposium on β-Adrenergic Blockade, Auckland.*	W. G. NAYLER
"β-Adrenergic Blockade and its Effect on Myocardial Function". *University of Leeds.*	W. G. NAYLER
"β-Adrenergic Blockade: A Subcellular Action". *Symposium on β-Adrenergic Blockade, Oxford University.*	W. G. NAYLER
"The Action of Salbutamol." — *National Study Workshop on Asthma, Sydney.*	W. G. NAYLER
"Calcium, Exchange in Heart Muscle." *National Heart Institute and University of London.*	W. G. NAYLER
"Effect of Thyroxine on Heart Muscle." — *Women's Post-Graduate Medical Society, Melbourne.*	W. G. NAYLER
"Some Observations on the Pharmacology of Salbutamol, with special reference to its Effect on the Cardiovascular System." — *Symposium on Salbutamol, London.*	W. G. NAYLER
"Clinical Experiences with Clonidine ('Catapres')". — *Alfred Hospital Clinical Society.*	F. R. TRINKER
"Meaning of Nucleoside Triphosphate Hydrolysis in Cardiac Microsomes." — *Muscle Biology Conference, C.S.I.R.O., Brisbane.*	L. WHEELDON

SEMINARS HELD DURING 1970

Immunological Aspects of Scleroderma.	A. J. BARNETT
Rejection of Heart Transplants.	E. COOPER
Cardiac Troponin.	P. DAILE
Inactivation and Reactivation of Factor XIII.	P. FANTL
Role of Mercaptalbumin and Some Genetic Implications.	P. FANTL
Kinekard.	H. A. JONAS
Changes in Structure of some Proteins during Phylogeny and their Possible Reflections in Ontogeny.	P. MÄSIAR
Fine Structure of Cardiac Muscle.	N. C. R. MERRILLEES
Cardiac Muscle Structure in Hyperthyroidism.	N. C. R. MERRILLEES
Effects of Thyroxin on Sarcoplasmic Reticulum in Cardiac Muscle.	D. MILLAR
Spatial Magnitude Electrocardiography.	C. V. NELSON
Noradrenaline Metabolism in Cardiac Muscle.	G. M. PICKEN
Blockade of Carotid Sinus Reflex.	J. M. RAINEY
Storage of Canine Hearts.	J. STONE
Salbutamol.	J. A. STREETON
β-receptor Blockade.	J. TAY
Control of Coronary Circulation.	F. R. TRINKER
Characterisation of Microsomal ATPase in Heart Muscle.	L. W. WHEELDON

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

Revenue Account for the Year Ended December 31, 1970

EXPENDITURE		INCOME		
Salaries and Wages	$154,837	Donations —		
Laboratory Supplies and Isotopes	18,882	Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions		$140,890
Library Maintenance	6,316	Grants-in-Aid of Research —		
Postage and Telephone	1,618	Asthma Foundation	$4,493	
Printing and Stationery	2,259	National Heart Foundation of Australia	20,027	
Light and Power	15,645	Life Insurance Medical Research Fund of Australia and New Zealand	8,500	
Insurance	4,892	The James and Elsie Borrowman Research Trust	4,000	
Repairs and Renewals	3,735	The William Buckland Research Fund	1,000	
Animal House Contribution	4,000			38,020
Sundries	3,835	Interest from Investments —		
Travelling Expenses	1,000	Held by Trustees of The Baker Institute Grant Trust	$1,700	
Public Relations	402	Other Income	23,966	
Surplus for Year	24			$25,666
		Sundry Sales, Recoveries and Refunds		12,869
	$217,445			$217,445

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

Balance Sheet as at December 31, 1970

FUNDS AND LIABILITIES			ASSETS		
Funds —			Fixed Assets (Note 1)		
Accumulated Revenue brought forward	$365		Investments —		
Add, Surplus for year	24		Held by the Trustees of the Institute:		
Accumulated Revenue	389		Commonwealth Inscribed Stock	$2,937	
Restricted Fund	148,292		M.M.B.W. Stock	7,202	
Endowment Fund	373,528		S.E.C. Stock	6,556	
Development Fund	70,675		Treasury Bonds	5,000	
The William Buckland Research Fund	20,870		Argo Investments Co. Ltd. Shares	29,806	
Laura Nyulasy Research Scholarship Fund	3,940		Softwood Products Treatment Co. Pty. Ltd.	100	
Lang Research Scholarship Fund	4,246		Short Term Deposits	70,976	
		$621,940		$122,577	
Current Liabilities —			Held by the Trustees, Executors & Agency Co. Ltd.:		
Sundry Creditors and Accrued Expenses		4,786	Laura Nyulasy Research Scholarship Fund	3,940	
			The William Buckland Research Fund	20,870	
			Endowment Fund	316,180	
			Restricted Fund	145,681	
					$609,248
			Current Assets —		
			Cash at Bank	$15,988	
			Sundry Debtors	1,490	
					$17,478
		$626,726			$626,726

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, 1970, other than the building totalled $265,867. The cost of the present building to December 31, 1970 totalled $1,300,000.
2. Retention monies paid to December 31, 1970 in respect of the contract for a new building amount to $45,800 and have been deposited in a bank account. The release of these funds to the builders or sub-contractors is dependent on the satisfactory completion of the contracts.
3. 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

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, 1970.

PRICE WATERHOUSE & CO.,
Chartered Accountants.

Melbourne. March 29, 1971.

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

Year Ended December 31, 1970

DEVELOPMENT FUND

Balance at December 31, 1969		$69,151
Add —		
Transfer from Endowment Fund	$7,499	
Income from Investments	3,844	
		$11,343
		$80,494
Deduct —		
Building Costs	$2,206	
Furnishing and Equipment Costs	7,613	
		$9,819
Balance at December 31, 1970		$70,675

RESTRICTED FUND

Balance at December 31, 1969		$57,086
Add —		
Transfer from Estate of Thomas Baker	$276,350	
Income from Investments	222	
Income from Laura Nyulasy Research Scholarship Fund	200	
Donations	31,852	
Transfer from The Ethel Mary Baillieu Fund	10,000	
		$318,624
		$375,710
Deduct —		
Transfer to Revenue Account	$136,890	
Transfer to The Ethel Mary Baillieu Fund Savings Account	51,073	
Loss on Redemption of Investments	7,778	
Equipment Costs	26,422	
Transfer to The William Buckland Research Fund	4,670	
Payment of Laura Nyulasy Research Scholarship	200	
Sundry Payments	385	
		$227,418
Balance at December 31, 1970		$148,292

ENDOWMENT FUND

Balance at December 31, 1969		$356,004
Add —		
Donations	$18,125	
Transfer from Appeal	13,200	
Interest Received	167	
		$31,492
		$387,496
Deduct —		
Transfer to Lang Research Scholarship Fund	$386	
Transfers to Development Fund	7,499	
Equipment Costs	1,888	
Transfer to Revenue Account	4,000	
Sundry Payments	195	
		$13,968
Balance at December 31, 1970		$373,528

DONATIONS

The following gifts towards the funds of the Institute were received during the year:—

Mrs. M. J. Wallace	$5,700
William Buckland Foundation (Trustees Executors and Agency Co. Ltd.)	4,670
J. &. E. Borrowman Research Fund (Trustees Executors and Agency Co. Ltd.)	4,000
Estate of H. & L. Hecht	2,000
Boehringer Ingelheim Pty. Ltd.	1,500
Mr. and Mrs. F. Crane	1,500
Edward Wilson Estate	1,450
Appel Family Trust (Trustees Executors and Agency Co. Ltd.)	1,200
Estate of Truby and Florence Williams (Trustees Executors and Agency Co. Ltd.)	1,200
Edgar Rouse	1,100
Merck Sharp and Dohme (Australia) Pty. Ltd.	1,000
William Angliss (Victoria) Charitable Fund	900
Estate of Alfred Edments (Trustees Executors and Agency Co. Ltd.)	750
Marion & E. H. Flack Trust	700
Sandoz Australia Pty. Ltd.	500
George F. Little Trust	475
J. C. Habersberger	250
Laura Nyulasy Estate	200
Siegfried Meyer	100
Pethard Tarax Charitable Trust	100
Stuart Sanderson	75
J. C. Miller, Painting	50
Norris and Partners Pty. Ltd.	50
Specialty Press Pty. Ltd.	50
Miss N. E. Cameron	10
N. Payne	10
Geo. Turner Pty. Ltd.	10
Mrs. Glover	5
Mrs. Helmore	5

Further contributions were received from:—

J. W. Archer, Associated Broadcasting Services Pty. Ltd., W. A. J. Baker, R. Blakemore, Miss N. E. Cameron, Eagle Star Insurance Co. Ltd., Goulburn Murray Television Ltd., J. C. Habersberger, Miss J. Hall, W. E. Helmore, A. Helper, S. J. Kemp, G. Knox, Kodak (Australasia) Pty. Ltd., F. Manning, N. Payne, B. K. Phelan, Edgar Rouse, E. Teasdale, Tasmania T.V. Ltd., A. G. Walton.

In memory of:

Miss B. D. Bretherton, Sir Giles Chippendall, Flora Colquhoun, Mrs. M. E. Dixon, Gregson Davis, Mrs. Alice Glover, Justin Hancock, Roy Heath, Mrs. J. Holman, Bruce Jackson, C. V. Jackson, Henry Jacobson, Dick James, W. C. Jones, Mervyn King, Betty Kitchen, Frank Lang, Mrs. A. Linn, R. Mair, Bruce Mead, E. Middenway, Mrs. H. Middenway, John Minter, Grantley Morgan, Lionel Newton, Constance Oliver, L. O'Meara, A. Rochlin, Mary Rouse, Mrs. M. Smith, Archdeacon L. A. Wade, Mrs. M. Ward, Mrs. S. Wright, Yip In Tsoi.

Totalling $491.40

In addition to the abovementioned, the activities of the "Baker Birds" produced a further	$6,995.67
AND	
those of "B Group"	$1,400.00
making a grand total of	$38,447.07

Further contributions to the Appeal included in the financial statements have been acknowledged elsewhere.

The Trustees record with deep appreciation the efforts of the two women's groups — the "Baker Birds", under the chairmanship of Mrs. Eric Rogers, and "Group B", under the chairmanship of Mrs. John Officer, who have been responsible for contributing $17,873.81 since the commencement of their activities.

THE EWEN DOWNIE METABOLIC UNIT — 1970

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Chiropodist:	J. HEFFERNAN, F.Ch.A.V., M.Ch.I.A.
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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

In the report by Dr. Zimmet and his co-workers the comment is made that it is hoped to test a synthetically prepared polypeptide in human diabetics. There are valid reasons for attempting such a therapeutic endeavour, theoretical considerations making it possible that a beneficial effect might be observed. However much preparatory work will be necessary prior to such tests in order to determine the safety of administration to humans. Preliminary toxicologic studies are well recognised precautions with the use of new drugs, and have become more comprehensive since the disaster associated with the use of thalidomide a few years ago.

Untoward side effects of widely used drugs, regarded after preliminary tests and quite extensive clinical use as safe, are being increasingly reported. For example the renal complications of a variety of analgesic drugs are now well known, and the extended and extensive use of oral contraceptives has engendered furious differences of opinion regarding their safety. This aspect of drug safety has now moved into the field of diabetes therapy. The first reports of a long term trial in which a study of the effect of control of diabetes on the development of the long term complications have suggested that the oral hypoglycaemic agent tolbutamide, a sulphonylurea compound, has a deleterious effect. In patients treated with this drug the death rate from cardiovascular disease was reported to be higher than in groups of similar patients treated with placebo tablets, or with insulin and diet. The release of this report has caused considerable anxiety in the minds of doctor and patient alike. It is a cause of major concern, since if the results of the study are accepted — currently there is controversy — the number of patients currently under treatment who will need to undergo a change of therapy is considerable. Two percent of the population is diabetic. Some 25-30 per cent of these patients are treated with oral hypoglycaemic agents for whom insulin has now been recommended as the treatment of choice by the American Diabetes Association. An interesting year or two lies ahead as this major problem in the therapy of diabetes becomes resolved.

No annual report of this department would be complete without reference to happy relationships with, and welcomed assistances from, the medical staff of the Alfred Hospital, the clinical departments of Monash University, and with the biochemistry departments at Alfred and Monash. We are appreciative too of collaboration with the University Department of Anatomy. The generosity of donors of grants in aid and kind is again gratefully acknowledged.

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THE ROLE OF InG — A GROWTH HORMONE DERIVED PEPTIDE — IN THE PATHOGENESIS OF DIABETES MELLITUS

P. Zimmet[1], P. Taft, J. Chan, J. Bornstein[2], F. Ng[2], and J. McD. Armstrong[2]

In the report last year, it was mentioned that a polypeptide fragment of growth hormone code named AcG (acceleratory fragment) had been administered to normal and diabetic subjects. In the former a potentiation of the hypoglycaemic action of insulin was noted and in the latter, fasting blood glucose levels fell, presumably due to the action of endogenous insulin released now from inhibition by a further growth hormone fragment — InG.

A biochemical model for diabetes, based on the action of this inhibitory fragment of growth hormone InG, which simulates the known diabetogenic effects of growth hormone, has been proposed. InG and AcG can be obtained by extraction from the pituitary gland by means of specific enzyme hydrolysis of human growth hormone, and have recently been synthesised in small quantities by the Merrifield technique.

In vitro, InG inhibits the action of glyceraldehyde phosphate dehydrogenase, α-glycerophosphate dehydrogenase, and acetyl CoA carboxylase. The overall effect of this inhibition causes impaired glucose utilisation, decreased fat synthesis, and accelerated lipolysis — the known diabetogenic effects of growth hormone. These effects are reversed by AcG. Considering these *in vitro* effects of InG and the *in vivo* actions of AcG, it seemed important to establish whether InG existed in human plasma.

The project has revealed the following:—

(i) That ultrafiltrates (M.W. < 6000) of human plasma contain a polypeptide which inhibits the action of glyceraldehyde phosphate dehydrogenase. That this inhibition is due to InG can be inferred by its partial reversal with ovine AcG, and by its absence from the plasma of hypophysectomised or hypopituitary subjects. InG caused a mean inhibition of the action of glyceraldehyde phosphate dehydrogenase to the extent of 23% in fasting normal subjects, 52% in juvenile onset, and 45% in maturity onset diabetics.

(ii) The chromatographic behaviour of the peptide isolated from human blood conforms to that of InG produced from ovine pituitary extracts on Dowex-50 and CG-50 columns.

(iii) The plasma derived peptide causes inhibition of the same enzymes as the pituitary derived InG, and doesn't inhibit other glycolytic enzymes such as hexokinase, glucose-6-phosphate dehydrogenase, lactate and malate dehydrogenases.

(iv) The amino-terminal amino acid of the plasma peptide InG is arginine, and the carboxyl-terminal is phenyl-alanine. These correspond to the known sequences of human InG derived from human growth hormone, and the carboxyl-terminal of the human growth hormone molecule as proposed by Li and Dixon.

(v) The inhibitory effects of plasma InG are reversed by synthetic AcG.

It is suggested that InG and AcG exist *in vivo,* and are derived from growth hormone by specific enzyme hydrolysis either in the pituitary or plasma. Whereas insulin plays a role in promoting glucose transport into the cell, it is suggested that the relative balance of InG and AcG exert a modulatory effect on intracellular glucose utilisation, and thus on insulin sensitivity.

The concept of diabetes mellitus as an absolute insulin deficiency syndrome is no longer tenable, as 70-80% of diabetics have insulin in the plasma in varying amounts. An excess of InG, or deficiency of AcG in the plasma, could be an important factor in the pathogenesis of human diabetes.

We intend to study the role of InG in the carbohydrate intolerance of renal failure, pregnancy, oral contraceptive therapy and prediabetes. It is hoped that synthetic AcG will be available in quantity, and preliminary tests will be carried out to assess its role in the therapy of diabetes mellitus.

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[1] P. Zimmet is the recipient of an N.H. & M.R.C. Medical Postgraduate Research Scholarship.

[2] Department of Biochemistry, Monash University.

THYROID INVESTIGATIONS

Dora Winikoff and Associates[1]

Studies of total binding capacity of thyroxine binding globulin (TBG):

The direct assay of TBG introduced last year has been applied to various categories of patients in whom the "thyroid profile" suggested possible binding abnormalities of TBG. The thyroid parameters we use, namely protein bound iodine (PBI) and/or serum thyroxine (T_4), triiodothyronine resin uptake (RU), electrophoretic index (EI), and free thyroxine index (FTI = PBI x RU), follow a distinct pattern in the face of high and low binding of hormone by TBG.

TBG	PBI	T_4	RU	EI	FTI
High	↑	↑	↓	↑	Normal
Low	↓	↓	↑	↓	Normal

(i) In adults: A TBG assay helps to explain discordant tests and greatly aids diagnosis.

(ii) In children: The TBG binding is high in infancy but gradually falls to adult levels between one and two years of age. In a group of 164 children in whom thyroid function tests were carried out we found the following distribution of TBG values:

		TBG — μg T_4 per 100 ml.		
No. of subjects	Age in years	< 30 (low)	30-40 (normal range)	> 40 (high)
75	less than one	2	16	57
89	one to fifteen	12	39	38

In adults thyrotoxicosis was associated with low normal or normal TBG level. Low PBI values associated with a high TBG on the other hand indicated overt hypothyroidism. However if a low TBG is found in the face of a low PBI, the diagnosis in most cases has to be confirmed by a TSH stimulation test. (TSH has no effect on the binding capacity of TBG).

(iii) In pregnancy: TBG rises at the end of the first trimester and gradually attains very

[1] The technical assistance of Rosalind Witchell and and Janis Upton for part of the year is acknowledged.

high values (up to 70 μg%). A plateau is reached which is sustained up to the time of parturition. In abnormal thyroid states, a TBG of less than 45 μg T_4% was encountered in hyperthyroidism co-existing with pregnancy. In hypothyroidism however both low and high TBG levels could be found.

(iv) Women who were prone to habitual abortions had, when pregnant, a bizarre thyroid profile when PBI and FTI were at normal pregnancy levels, while other parameters resembled those of a non-pregnant state. TBG was usually below 45 μg T_4% or rising very slowly. (Preliminary observations).

(v) In malignancy: low values of TBG were often encountered resulting in thyroid parameters difficult to interpret. Odd protein patterns add to inconsistent RU and EI values.

The Thyroid and the Pill:

Our trial commenced in 1964 and an investigation the effect of oral contraceptives on thyroid function tests continues.

At present we are studying the long term effect of the new type of low oestrogen preparations. The value of TBG which is usually over 40 μg T_4 100 ml is characteristic for each individual, the response from pre-treatment level however is oestrogen dependent. A plateau is reached eventually, the height of which is again individual.

Another interesting observation is that PBI values are higher than when measured as thyroxine-iodine by competitive binding. The borderline toxic patient may be hard to diagnose when this technique is used as RU can still be in the normal range of values. EI seems to be the most reliable index when the low-oestrogen type of contraceptives are used.

Binding Ratio:

The value of a new empirical parameter termed the "Binding ratio" (BR) was assessed. It is expressed by a formula $\frac{RU}{EI}$ and its range extends from 1.0 - 2.0, providing a normal binding of TBG is present. The degree of T_3 and T_4 binding by TBG is involved in the RU and EI tests respectively. Their ratio differentiates between thyrotoxicosis ($BR > 2.0$) and high binding of TBG ($BR < 1.1$) on one hand and hypothyroidism ($BR < 1.0$) and low binding of TBG ($BR > 1.8$).

We found this index is of a better discriminatory value than the Free Thyroxine Index in a large number of instances when drugs, hormones or an illness upset the TBG.

TBG Assay By Dextran-Coated Charcoal: Worker — Marie Snook

We commenced a trial of a TBG method using a dextran-coated charcoal system (Roberts and Nicolai 1969). It was attempted as an alternative method to our TBG assay by electrophoresis, because of its simplicity.

The preliminary investigation is encouraging. However, contrary to the author's claim, we found that the percentage of radioactivity remaining unabsorbed by the TBG and the charcoal is not constant but rises with a lower TBG binding capacity. With a high TBG its value could be under-estimated by approximately 5 μg T_4 per 100 ml. serum.

COMPUTER ANALYSIS OF RESULTS OF GLUCOSE TOLERANCE TESTS

D. Feiglin, H. D. Breidahl and L. Dugdale

A mathematical model for the glucose tolerance test has been established. It is based upon the effect of glucose-insulin double feedback which allows the solution of a second degree constant coefficient differential equation. This permits the development of specific constants for each individual glucose tolerance tests.

It has been found by previous workers that there is a good separation between normal and diabetic responses to oral glucose when examined in this fashion, and that as a consequence the determination of these constants is of diagnostic value. Theoretically similar constants for values of plasma insulin should be obtainable. It is planned to attempt correlation of these with the constants found for glucose.

ANTIDIURETIC HORMONE SECRETION IN DIABETIC KETOACIDOSIS

D. Feiglin and P. Taft[1]

With the high plasma osmolality seen in diabetic acidosis it would be expected that stimulation of antidiuretic hormone secretion occurs. This is not normally observed as a diminished urine flow, because of the substantial diuresis induced by the excretion of the large quantities of urine sugar seen in this clinical situation.

An examination of antidiuretic hormone action has been made by the calculation of free water clearance over a half hour period early after the admission of patients with marked hyperglycaemia and symptoms of diabetes.

In nine patients studied where a range of plasma osmolality from 273-350 milliosmoles per kilogram of water was noted, the urine osmolality ranged from 380-970 milliosmoles per kilogram. There was a positive free water clearance in each patient studied indicating antidiuretic hormone action. There was a negative correlation between urine osmolality and urine sugar excreted, indicated that despite adequate antidiuretic hormone secretion as indicated by a positive free water clearance, the diuretic effect of excreted glucose and electrolyte was sufficient to produce diuresis at a rate ranging from 3.5 - 26.5 litres per day.

[1] The assistance of the Department of Biochemistry is gratefully acknowledged.

GLUCOSE AND GLYCEROL METABOLISM IN DIABETES

J. Herington, S. Menahem, P. Taft and J. Bornstein

As reported last year, the aim of this study is to determine the rate of metabolism of glucose, the rate of metabolism of glycerol, and the rate and extent of conversion of glycerol to glucose in fasting normal and diabetic subjects. (The methodology has been developed through 1968 and 1969 by the first two workers who also undertook the preliminary animal studies).

It was proposed to make these measurements by administering a single dose of radioactive (^{14}C labelled) glucose and glycerol (in separate experiments) to human volunteers and to obtain the desired information by following the level of radioactivity in the blood over a period of time.

To date five normal and three diabetic subjects have been tested.

The decay curves of administered radioactivity are mathematically complex to solve, and it has been necessary to use computer analysis. The programme being used is Berman and Weiss' SAAM. This is a general purpose computer programme developed for the analysis of data in terms of models. It permits simulation and data fitting by adjusting the parameter values of a model until a least-squares best fit is obtained.

As with most computer analyses, this is proving very time consuming, the problem being to postulate a model for the biochemical process under study which is not only mathematically correct but which is physiologically feasible.

A three-compartment model which appears to fit these requirements has been established for the glucose data, and from the flow-rates between compartments the desired parameters have been calculated for the normal patients. The average total turnover of glucose is 0.278 gm/kg/hr of which 56.5% is recycled back to the glucose pool and the remaining 43.5% (0.121 gm/kg/hr) is irreversibly metabolised, mostly to CO_2.

Analysis of the glucose data for diabetic subjects is at present incomplete, but it appears that in the diabetic the total glucose turnover is larger than in the normal person, the excess being recycled back to glucose and a smaller proportion being irreversibly metabolised.

Analysis of the glycerol data has not yet been possible, and work in this area is continuing. It also appears necessary to study more subjects, both normal and diabetic, in order to obtain statistical significance in the differences between their parameter values.

CARBOHYDRATE METABOLISM IN URAEMIA

J. S. C. Chan, P. Taft, J. Bornstein and R. Dargaville

Although there is a general acceptance that glucose intolerance exists in uraemic patients there are also contrary evidences. It has been shown after an oral glucose load that uraemic patients display a delayed clearance of insulin and glucose, and after an intravenous injection of insulin there is a delayed and prolonged fall in serum glucose levels which do not return to the fasting level within two hours as is usually observed.

Other groups of workers on the other hand have found no difference in responses between normals and uraemics to an oral glucose load. They did find however an enhanced insulin response to administered glucose. This has prompted us to look again at the pattern of plasma insulin levels after glucose in uraemic patients.

Patients and controls under test were given an intravenous dose of 25 grams of glucose, followed by an intravenous injection of insulin (0.1 unit per kilogram) two hours later. Plasma insulin and blood glucose values were measured. The logarithmic disappearance rate constant for glucose was calculated and shown to be normal in the uraemic patient. Insulin responses were greatly enhanced, and plasma insulin — blood glucose ratios were increased above the normal level. It was interesting to observe a much delayed clearance of insulin in the uraemic patients.

The increased insulin secretion in uraemic patients seemed to indicate the presence of an insulin antagonist in the plasma. It may also be that there is secretion of an immunologically active but biologically inactive insulin. The delayed clearance of insulin may be due to an increased half life of insulin which has been suggested by some reported studies.

Extension of last year's work utilising *in vitro* conditions has revealed little, if any, effect of urea upon insulin action on ^{14}C glucose incorporation into muscle glycogen. There seemed therefore to exist no peripheral resistance to insulin action under these conditions.

^{14}C glucose incorporation into lipid and fatty acid of rat fat pads was inhibited in the presence of urea. This observation may infer a block of entry of glucose into fat cells, a reduction in fatty acid synthesis, or an inhibition in the process of esterification, or all three processes. It must be borne in mind however that urea in the incubation medium is only a simulator of the total uraemic plasma.

It is hoped in the forthcoming year to employ tissues from rats rendered uraemic for *in vitro* experiments, and to use plasma from uraemic patients as an incubation medium.

ENDOCRINE CHANGES IN LIVER CIRRHOSIS PITUITARY-TESTICULAR RELATIONSHIPS

P. Taft, C. S. Seah, B. Hudson, H. Burger and A. Mirovics

In the report of 1968 an account of the combined Singapore-Melbourne study of endocrine changes in liver cirrhosis was given. Further data regarding pituitary gonadal function is now available.

It was earlier reported that urinary "general" gonadotrophin (G.G.H.) was present in all patients, and that in the three borderline high values noted there was no correlation with testicular atrophy.

Plasma luteinising hormone (L.H.) levels were in the normal range save three, where levels were high. Again there was no correlation with clinical endocrine abnormality, and only one high L.H. corresponded with a high G.G.H.

Plasma testosterone levels were low in general and responded poorly to stimulation with HCG. Plasma oestradiol initially at high levels paradoxically fell with this stimulation.

These results are presently being studied and will form the basis of a later report.

LECTURES GIVEN DURING 1970

"Calcium Metabolism." "Adrenal Glands." — *Royal Australasian College of Surgeons Post-Graduate Course.*	H. D. BREIDAHL
"Treatment with Glibenclamide." — *International Meeting on Oral Hypoglycaemic Therapy, Alfred Hospital.*	H. D. BREIDAHL
"Hypogonadotrophic Hypogonadism: The Effects of Gonadotrophin Replacement Therapy" — with H. G. Burger (Senior Author), B. Hudson, D. M. de Kretser and A. Mirovics. — *Endocrine Society of Australia.*	I. EKKEL & P. TAFT
Presidential Address. — *Endocrine Society of Australia.*	P. TAFT
"The Aetiology and Treatment of Diabetic Ketoacidosis." "On The Aetiology of Diabetes." "Endocrine Disease in Pregnancy." "Endocrine Changes in Cirrhosis of the Liver." — *Pfizer Lectures, Adelaide.*	P. TAFT
"The 'Thyroid Profile' in Abnormal Pregnancy." — *Annual Meeting of the Endocrine Society of Australia.*	D. WINIKOFF
"The Importance of Thyroid Function Tests during Pregnancy in Health and Disease." — *Queen Victoria Hospital.*	D. WINIKOFF
"Modern Thyroid Function Tests Based on Radioisotopes, Theory and Practice." — *Royal Melbourne Institute of Technology.*	D. WINIKOFF
"Clinic Interpretation of Thyroidal Parameters." — *Royal Melbourne Institute of Technology.*	D. WINIKOFF

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

ULTRA-THIN SECTIONS FOR HISTOLOGY

A. V. Jackson[1]

The original general objective of this study was to determine whether ultra-thin (0.5μ) araldite-embedded sections could assist "routine" diagnostic histopathology. More specifically, the tissues that were to be specially studied included lymph nodes, spleen, jejunal mucosa and problem soft tissue tumours. Such ultra-thin sections, stained by polychrome dyes, did in fact reveal a beautiful precision of detail such as is never seen in paraffin-embedded material. Photo-micrographs of these preparations made an obvious impression during teaching sessions. Early changes in the micro-villi of the jejunum can be recognised and in lymph nodes nuclear differences between various members of the lymphocyte and histiocyte series are quite evident and permanently demonstrable in photos. However, the more one studied cells and tissues in the greater depth made available by this technique, the more one felt the need to go further and to use full electron microscopy, even if only in a limited way, for routine diagnosis.

[1]Morbid Anatomy Department.

CEREBRO-SPINAL FLUID STUDIES

Graham Martin[1]

Ten years ago Lundberg observed that when the pressure of the cerebrospinal fluid in the ventricles was raised it fluctuated widely in waves lasting 30 to 120 seconds and doubling or trebling the basal pressure. These had not been observed before because the crude pressure measuring apparatus used had introduced a very large damping factor. Continuous isovolumetric pressure recording is needed to demonstrate them.

An apparatus to continually record these waves in patients in the ward has been partially assembled on a trolley which can be wheeled up to the bedside but has not yet been in use for this purpose because of the failure of a suitable pressure transducer to arrive. It has been used with a borrowed transducer for measuring pressures during carotid ligation.

A technique for observing the speed of transit of blood through the skull using colloidal sulphur labelled with technecium has been evolved with the aid of Dr. Dugdale of the Department of Nuclear Medicine. The intention has been to use these transit curves to investigate the hypothesis that these cerebrospinal fluid pressure waves are generated by intermittent obstruction of the deep cerebral veins.

As a subsidiary project, an examination of the effect of hyperventilation on the cerebrospinal fluid partial pressure of oxygen and the blood-brain barrier was undertaken. A single clinical experience that hyperventilation lowered the pO_2 of the cerebro-spinal fluid suggested that hyperventilation, known to cause cerebral vasospasm might also deprive the brain of oxygen and that this would be reflected in the cerebro-spinal fluid oxygen content. Moreover, this might damage the blood-brain barrier and so account in part for some cases of cerebral oedema. Damage to the blood-brain barrier could be detected by the passage of ^{131}I labelled serum albumin across it and into the cerebro-spinal fluid.

In a series of dogs after three hours of hyperventilation, ^{131}I albumin was detected in excess in the cerebro-spinal fluid. The partial pressure of oxygen in the cerebro-spinal fluid appeared to be lowered by hyperventilation but the

[1]Assistant Neurosurgeon

interpretation of this was difficult as the previous investigations on dogs had been made under anaesthesia and there was no control over the degree of hyper- or hypoventilation. In our dogs, indwelling catheters were placed in the cisterna magna and cerebrospinal fluid drawn off when the dog was recovered from the anaesthetic.

VENOUS THROMBOSIS FOLLOWING ACUTE MYOCARDIAL INFARCTION

P. G. Habersberger, A. Pitt and S. T. Anderson[1]

Using isotope labelled human fibrinogen 65 patients have been studied to assess (i) the incidence of leg vein thrombosis following myocardial infarction and (ii) the value of prophylactic anticoagulation in prevention. Although the present series is small, the initial results would suggest that prophylactic anticoagulation is of little benefit in preventing leg vein thrombosis following acute myocardial infarction.

[1] Cardiovascular Diagnostic Service.

ASSAY OF CARDIAC GLYCOSIDE LEVELS IN HUMAN PLASMA

S. T. Anderson, A. Pitt[1] and L. Dugdale[2]

A method for the estimation of the concentration of cardiac glycosides in the plasma of patients was investigated. The method chosen was the inhibition *in vitro* of the uptake of ^{86}Rubidium by human red cells. The procedure proved time consuming and the results were not reproducible. It is hoped in 1971 to investigate the immuno assay technique.

[1] Cardiovascular Diagnostic Service.

HAEMODYNAMIC AND ANGIOGRAPHIC CONSEQUENCES OF MITRAL AND AORTIC VALVE REPLACEMENT

R. Zimmett, A. Pitt and S. T. Anderson[1]

The data on mitral valve replacement has now been collected and the results analysed. During 1970, all patients with aortic valve prosthesis were reviewed and haemodynamic assessments commenced.

The use of autologous tissue valves by the cardiac surgeons has provided an additional comparative group for assessment.

[1] Cardiovascular Diagnostic Service.
[2] Department of Nuclear Medicine.

PROPHYLACTIC USE OF LIGNOCAINE IN ACUTE MYOCARDIAL INFARCTION

A. Pitt, H. Lipp and S. T. Anderson[1]

Two hundred and twenty-two patients entered the trial up until June 30, 1970. The results show a statistically significant reduction in the incidence of ventricular tachyarrhythmias in the overall group, Class I myocardial infarction, Class II myocardial infarction and anterior myocardial infarction. Of interest is that the reduction in incidence of tachyarrhythmias in inferior myocardial infarction was not statistically significant and the trial is continuing to elucidate this point.

[1] Cardiovascular Diagnostic Service.

EXPERIMENTAL GASTRIC ULCERATION

D. J. B. St. John[1] and F. T. McDermott[2]

The response of the gastric mucosa to continued injury by acetylsalicylic acid (aspirin) is being investigated by histology and autoradiography with tritiated thymidine in the Sprague-Dawley rat. The principal aims of the project are to determine the mechanism by which aspirin damages the gastric mucosa and to study the mucosal response to continued dosage with aspirin. Our earlier studies indicated that mucosal adaptation occurred with continued aspirin. Single doses (120 mg/kg body weight) given by oesophageal intubation, regularly produced multiple linear erosions in the fundic mucosa of the stomach but, with repeated daily doses, the erosions were shown to have healed.

[1] Department of Medicine, Monash University, Alfred Hospital.
[2] Department of Surgery, Monash University, Alfred Hospital.

Further studies have been performed on groups of rats given aspirin each day for periods up to 16 weeks and the previous observations confirmed. The effect of interruption of aspirin dosing for three days before a final single dose of aspirin has been examined. In addition, the responses to carbenoxolone and carbenoxolone combined with aspirin have been investigated. Evidence of healing is present after three daily doses of aspirin, but no further morphological changes occur with longer periods of dosing. Mucosal adaptation is lost when dosage is discontinued for three days. The autoradiographic studies have shown an increase in incorporation of tritiated thymidine in the adapted mucosa. Quantitation of the autoradiographic changes is in progress.

VITAMIN B_{12} ABSORPTION IN THE SPRAGUE-DAWLEY RAT

N. D. Yeomans and D. J. B. St. John[1]

Studies are being undertaken to investigate the mechanism of absorption of Vitamin B_{12} by the epithelial cells of the small intestinal mucosa.

The technique of electron microscopic autoradiography has now been established and will be used to study the intra-cellular localisation of labelled Vitamin B_{12} in the absorptive cell.

The absorption of ^{57}Co-Vitamin B_{12} by the Sprague-Dawley rat was estimated using whole body counting and daily stool counting. Findings confirmed previous observations on the rate and site of absorption and on the tissue distribution of B_{12}. The rat was shown to have a high rate of continuing B_{12} loss, compared to man. These data indicate that the rat can relatively quickly develop experimental B_{12} deficiency. These techniques are suitable for investigating the influence of a variety of agents upon B_{12} absorption and studies have been performed using alcohol and cytotoxic drugs. An immuno-assay for intrinsic factor (I.F.) in rat gastric juice was established, using modifications of the methods of Gottlieb and of Ungar. This has proved to be sensitive and reproducible.

Homogenates of small intestinal mucosa were used for *in vitro* studies of the uptake of the intrinsic factor-B_{12} complex, according to the methods of Carmel. Homogenates from the distal three-quarters of rat small intestine bound three times more B_{12} than homogenates from the proximal one-quarter. The difference was dependent on the presence of intrinsic factor. Dose-response curves for I.F.-mediated binding were established using the same technique and have enabled the physiologic range of B_{12} absorption to be established for the system.

[1] Department of Medicine, Monash University, Alfred Hospital.

Studies have been commenced with suspensions of small intestinal epithelial cells isolated by mechanical vibration or incubation in EDTA. This approach will facilitate investigation of the effects of metabolic inhibitors on B_{12} uptake. Initial studies are in progress using ^{57}Co-B_{12} and ^{3}H-B_{12} for light autoradiography. Modifications of autoradiographic techniques are being introduced to minimise losses of labelled B_{12} during fixation and embedding.

ACUTE LEUKAEMIA IN ADULTS

M. B. Van Der Weyden[1]

(a) Ferrokinetic Studies. These were performed in seven patients, two with erythroleukaemia; the remaining five patients included one with subacute leukaemia. The pattern of iron metabolism in the two patients with erythroleukaemia was similar to that reported in the literature. The remaining patients showed varying patterns of iron metabolism, ranging from normal to increased or decreased erythropoiesis. There was no correlation between bone marrow morphology and the ferrokinetic patterns.

(b) Red Cell Survivals. This was determined in 14 patients with acute leukaemia. Three patients had normal red cell survivals. The remainder had shortened $T_{\frac{1}{2}}{}^{51}Cr$, ranging from 10 to 20 days. No distinct correlation can be found between decreased red cell survival and the presence of positive autohaemolysis, or enzymatic changes, either membrane or intra-corpuscular in nature.

(c) Gastrointestinal Blood Loss. This was measured in five patients. Significant blood loss was found in four patients and was related to the presence of thrombocytopenia.

(d) Erythropoietin Assays[2]**.** In general, erythropoietin levels were parallel with the degree of anaemia present, although a number of patients exhibited inappropriate erythropoietin levels in relation to the degree of anaemia.

Platelet Function[3]

Fourteen patients were studied either in relapse or remission. Significant defects in platelet aggregation were disclosed in a number of these patients. Platelet survivals were studied on six occasions and there appears to be a correlation between the presence of platelet abnormalities and decreased platelet survival.

Pyrimidine Biosynthesis

As reflected by red cell orotidylic decarboxylase (O.D.C.) and pyrophosphorylase (O.A.P.) levels, this was studied in — (i) patients with megaloblastic anaemia; and (ii) patients with acute leukaemia. In the former group, two distinct patterns have emerged. In one, the changes in O.D.C. and O.A.P. activities paralleled the maturity of the red cell population studied, as reflected by glucose-6-phosphate dehydrogenase activity. In the other, however, there is reduction in O.A.P. and O.D.C. activity in conflict with the maturity of the red cell population.

In the patients with acute leukaemia studied, in general the changes in O.D.C. and O.A.P. activity paralleled the maturity of the red cell population. However, again, anomalies between maturity of the red cell population and changes in O.A.P. and O.D.C. activity have been disclosed.

^{3}H-Thymidine Incorporation

The incorporation of thymidine into peripheral blood and marrow cell population was studied. In particular, sequential bone marrow cell population D.N.A. synthesis capacity has been followed. Interesting patterns have emerged and this will be further studied.

Chemotherapeutic Trial

Of twenty-six patients with acute leukaemia studied this year, 23 entered into the chemotherapeutic trial using combination cytosine arabinoside and rubidomycin therapy. Of those, nine have achieved either complete or partial remission. This chemotherapeutic regime is, at present, under scrutiny, and changes are envisaged in future.

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[1] Department of Medicine, Monash University, Alfred Hospital.
[2] In conjunction with H. Greville.
[3] In conjunction with M. Howard and R. Clancy.

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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.

- Members of the Institute staff have visited it in recent years to deliver lectures or seminars or to exchange data on current research projects.

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

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