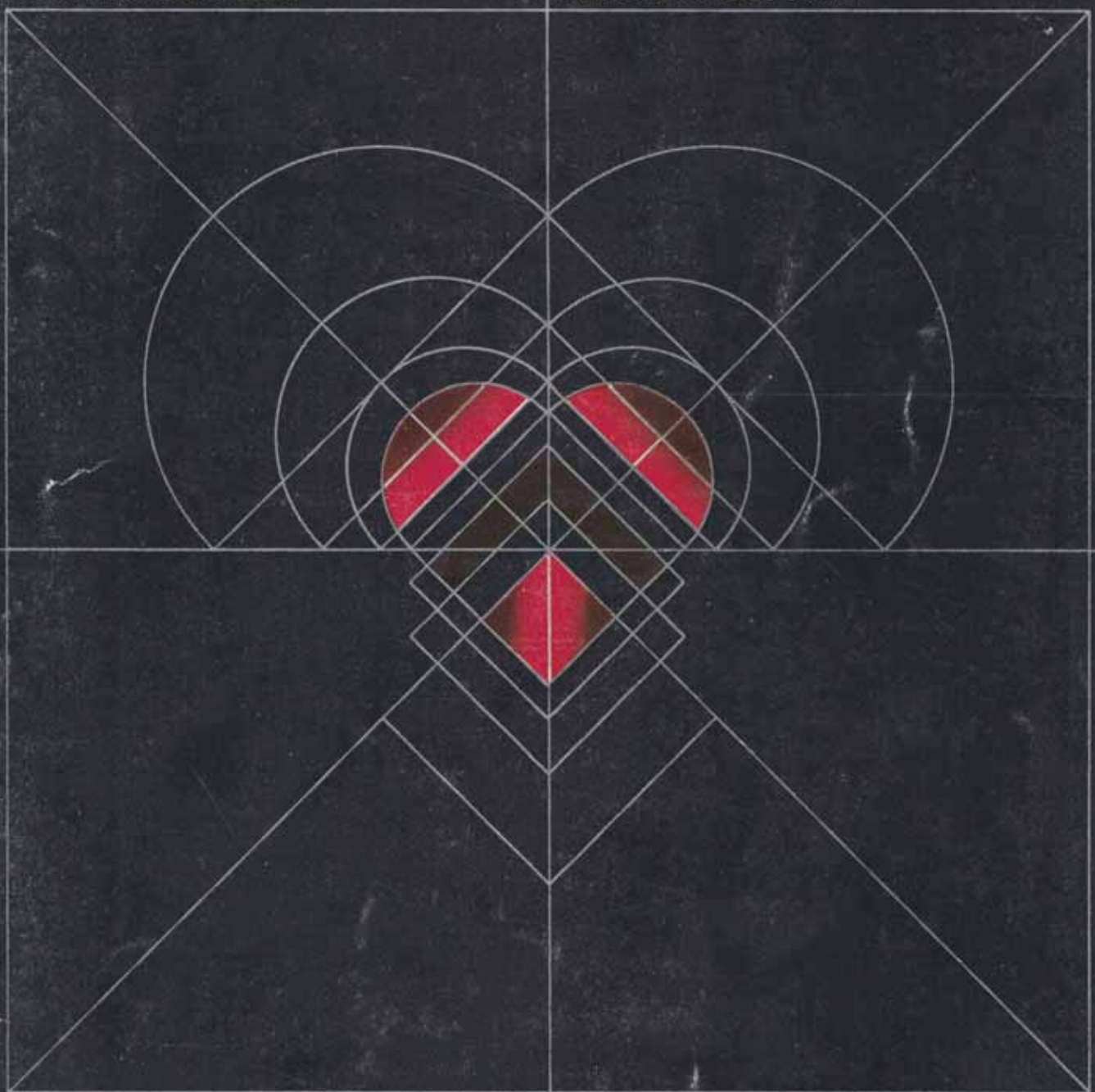
Research 1971

BAKER INSTITUTE

ALFRED HOSPITAL



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

The Ewen Downie Metabolic Research Unit is a department of the Alfred Hospital, part of whose duties is to conduct research in some aspects of endocrinology.

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

FORTY-FIFTH 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)

FIFTEENTH ANNUAL RESEARCH REPORT

of

THE EWEN DOWNIE METABOLIC UNIT

REPORTS

of

ALFRED HOSPITAL RESEARCH FELLOWS

1971

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, 1971

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

APPOINTED TO RESEARCH FELLOWSHIPS FOR 1972

"Medical Research":	A. J. BARNETT.
"Edward Wilson Memorial": *"S. W. Shields"*:	M. BERRY.
"Sol Green":	R. C. BUCKLE.
"Sunshine Foundation":	E. COOPER.
"Peter Grant Hay":	A. V. JACKSON.
"Edward Wilson Memorial":	M. W. JOHNS.
"Amelia Haigh (Heart)": *"R. B. McComas"*: *"Ian Gideon McLean"*: *"Sartori"*: *"A. A. Swallow"*:	T. E. LOWE.
"Frederick and Esther Michaelis":	H. A. LUKE and L. M. DUGDALE.
"Victor Y. and Margaret Kimpton":	
"E. H. Flack":	E. MÄSIAR.
"Collier": *"E. H. Flack"*:	J. F. McL. OLDHAM.
"Connibere": *"S. W. Jones"*: *"James Richardson"*:	D. S. ROSENGARTEN.
"James and Elsie Borrowman":	H. P. TAFT.
"J. F. MacKeddie": *"J. R. G. and E. McKenzie"*:	F. R. TRINKER.
"James and Elsie Borrowman":	M. B. Van Der WEYDEN.
"Ringland Anderson": *"E. H. Flack"*:	I. M. WILLIAMS.

TRAVEL GRANT

R. L. CLANCY.

P. G. HABERSBERGER.

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INTRODUCTION

The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute was founded under the terms of a Deed of Settlement executed in 1926 between the Settlors and the Board of Management of Alfred Hospital. The Institute was established to provide an efficient hospital laboratory service and facilities for medical research. In the course of time it was found more satisfactory for these routine services to be placed under the control of the Hospital staff, and this transfer was completed in 1948. Since then the Institute staff has been entirely concerned with research, with emphasis on the basic medical sciences. This is integrated with projects of the Clinical Research Unit. The Institute was formally affiliated with Monash University in 1965.

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

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

The annual reports of the Baker Institute have been published since 1927, and soon after the formation of the Clinical Research Unit 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.

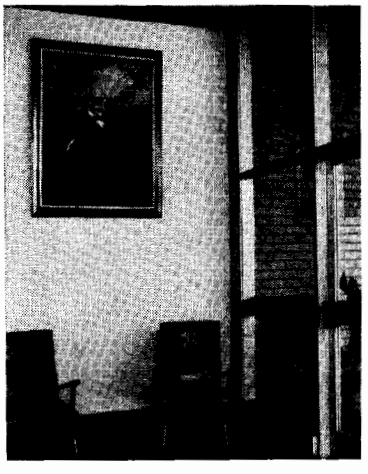
Because of the increasing importance and diversity of the investigational activities conducted in Alfred Hospital, it has been decided to present this report in several sections, indicating the activities of the Baker Institute (including the Clinical Research Unit), The Ewen Downie Metabolic Unit, and 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.
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Graduates:
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Miss A. CHANG, B.Sc.
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P. DAILE, B.Sc.
B. DOWTY, M.B., B.S., M.R.A.C.P.
P. FANTL, D.Sc., F.R.A.C.I.
G. C. HARD, B.V.Sc., Ph.D. (from 10/6/71).
Mrs. H. A. JONAS, M.Sc. (to 13/2/71).
S. KATZ, B.Sc., Ph.D. (from 1/6/71).
I. E. McINNES, M.B., B.S., F.R.C.S., F.R.A.C.S.
P. MÄSIAR, M.D., D.Sc. (from 22/9/70).
EVA MÄSIAR, M.D. (from 22/9/70).
Mrs. D. MILLAR, M.Sc. (from 25/10/71)
W. F. H. M. MOMMAERTS, M.A., Ph.D. (October).
Miss G. M. PICKEN, B.Sc. (to 30/6/71).
M. SHAW, B.Sc.
F. G. SILBERBERG, M.B., B.S., M.R.C.P.
G. R. STIRLING, M.B., B.S., F.R.A.C.S.
Miss J. SZETO, B.Sc. (to 22/10/71).
FEDORA R. TRINKER, Ph.D., M.B., B.S.

Technical:
R. P. STEELE (Laboratory Supervisor).
Miss J. DIXON (Senior Technologist).
A. H. HUCKFIELD (Technical Officer).
Mrs. R. MUSCUTT.
D. G. OAKLEY.
Mrs. J. ORTIZ (from 1/1/71).
Mrs. E. RICHARDS.

Librarian: Mrs. M. RAE.

Clerical:
Mrs. C. M. WALKER (to 30/11/71).
Mrs. J. MEYER (from 10/12/71).
Mrs. E. KERN (from 29/3/71).
Miss C. MARAZZITA (from 29/9/71).

Laboratory:
Mr. P. BENNETTS.
Mr. C. BECKER (from 2/2/71).
Mr. D. BERRY.
Miss H. CHARANIEKA.
Miss M. COGDEN.
Miss A. GREEN.
Mr. K. HARVEY.
Miss E. HEWLETT.
Mrs. K. JONES (nee Clarkson).
Miss P. KIRCHNER (from 6/9/71).
Mr. D. LAVENDER.
Mr. S. MARK.
Mr. C. LEWIS (to 24/9/71).
Mrs. E. PAYNE.
Miss B. REEVE.
Miss M. ROSIER (from 8/11/71).
Miss D. SCHLUNKE.

General: Mrs. M. WRIGHT.

WARD STAFF

Registrar: G. D. GILL, M.B., B.S.

Resident Medical Officers:
D. A. G. BRACY, M.B., B.S.
J. F. REDMAN, M.B., B.S.
RUTH STEINFELD, M.B., B.S.
J. TOOULI, M.B., B.S.

Ward Sister: Miss J. HOMEWOOD.
Mrs. P. NEWELL.

RESEARCH FELLOWSHIPS

Members of the Institute staff have held the following Research Fellowships:

Honorary:	E. COOPER, M.B., B.S., F.R.A.C.S. F. C. SILBERBERG, M.B., B.S., M.R.C.P. G. R. STIRLING, M.B., B.S., F.R.A.C.S.
Visiting:	W. F. H. M. MOMMAERTS, M.A., Ph.D.
Anti-Cancer Council of Victoria ("A. A. Thomas"):	G. C. HARD, B.V.Sc., Ph.D.
"James and Elsie Borrowman":	A. CHANG, B.Sc.
"William Buckland":	P. FANTL, D.Sc.
"The Lang Fellow":	P. MÄSIAR, D.Sc., M.D.

SCHOLARSHIPS

"Baker Institute Prize:	Mrs. J. TAY, M.Sc.

On December 8 Mr. Edgar Rouse announced his retirement from the position of Chairman of the Trustees of the Institute. His fellow trustees received this with great regret, but recognise its inevitability and with the Director they recorded the following tribute in their minutes.

> "The Trustees desire to place on record their deep appreciation of the outstanding service rendered to the Institute by Mr. Edgar Rouse. As Chairman of Trustees for 27 years he has worked assiduously to place the finances of the Institute on a sound basis. His outstanding efforts in this regard made possible the rebuilding of the Institute in 1968.
>
> The establishment of the Endowment Fund and the continuing support given by the Baker Benefactions are largely attributable to his strong conviction of the importance to the community of medical research. In addition to his dedicated work as a Trustee he has been a most generous benefactor of the Institute.
>
> His warm and sincere interest in the work of the Institute has endeared him to all his fellow Trustees and members of the Staff.
>
> The Trustees desire to record their pleasure at Mr. Rouse's decision to continue as a Trustee of the Institute.
>
> The Director, speaking on behalf of past and present members of the Institute Staff, asked that their warm appreciation of the interest taken in their work by Mr. Rouse be recorded."

EDGAR JOHN ROUSE, C.B.E., F.C.R.A. (Hon.)
Trustee since 1942
Chairman 1944-1971

ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

The cure and prevention of human illness is a major aim of medical research. Although this objective remains constant the milieu in which the research is conducted is continually changing and this may lead to an impression that the broad objectives of medical research change from time to time. The changes in the milieu arise from the effects of causes as diverse as increasing knowledge of disease derived from research developments in many fields of endeavour; the continual development of tools with which to explore the problems; the changing skills needed by research workers and the changing community attitudes which largely determine the support given to any particular field of medical research.

In addition to this utilitarian aspect, medicine has claims to be a science and, as with other sciences, the earliest phase of medical science was the collection and classification of data about human disease by practising doctors. From these data causal correlations were sought and hypotheses created, tested when possible and the results used in attempts to cure or prevent human disease. These aspects of medical research are closely related to individuals and so are often grouped as "Clinical Research" to distinguish them from the more general aspects of the subject which are properly part of "Biological Science." This last embraces a study of all living tissues and often the problems of human disease can be most profitably pursued within its broader field. In this broader context animal tissues can frequently provide models of human disease processes. Since early in the nineteenth century it has been known that living tissues are composed of structural elements called cells. Very large numbers of cells are required to make up a whole body and some form of communication between cells in different regions is essential for co-ordinated activity. This communication is carried out either by electrical impulses conducted and organised in the nervous system or by chemical messengers transported by body fluids from one part to another. Disease may be described as the effects of damage to groups of cells and may be manifest in many ways e.g. structural alterations to tissue as in a tumor or a communication breakdown as in muscle paralysis.

Much of our present knowledge of disease processes has only been acquired within the last few decades because this is the era in which developments in the physical sciences have produced the tools necessary to study the structure and function of individual cells. Examples of these developments are: the electron microscope which permits visualisation of the innermost components of cells and the ultracentrifuge with which to separate these components; the spectrophotometer which, together with radioactive tracer substances, permits cellular chemical reactions to be followed; the electronic devices which permit the electrical activity of tissues to be monitored; tissue culture which is a developing technique with much promise in which cells are grown outside the host and many of their properties can be studied at leisure.

The combined effects of using these tools and techniques have been to remove large areas of medical research away from the sick person to the laboratory and to diminish the clinical proportion of the total effort. The importance of clinical research however has in no way diminished. These trends in medical research have made necessary the use of the skills and assistance of workers trained in various aspects of biology in addition to those medically qualified. These same developments have made research very expensive in manpower and money.

In the scientific sections of this report are accounts of research projects which show how essential these new techniques have become and emphasise the growing dependence on sophisticated equipment and scientific workers who are not medical graduates. The projects described have been carried out in the Baker Medical Research Institute and in Hospital

units and departments, such as the Clinical Research Unit, Ewen Downie Metabolic Unit and the various service departments. A year by year comparison of previous annual reports shows the marked proportional and absolute increase of laboratory studies, as compared with clinical studies, which has taken place; it also shows that the cost of current projects represents in total a very substantial outlay.

All these changes, reflected in the work of the research groups, indicate a healthy growth and evolution of research, but they are taking place at a time when the purchasing power of the dollar is diminishing. It appears therefore, that the most economical use of manpower and money in and between these units must be sought if the standard and amount of our medical research is to be maintained at its present high level. It may be the time has already arrived when some form of federation of the various groups mentioned above is desirable to provide both efficiency, economy and strength to withstand competitive pressures.

RESEARCH PROJECTS

Cardiac Muscle

A substantial proportion of the research projects being carried out is devoted to studies of the individual cardiac muscle cell and its internal structures.

These studies may be classed as cellular biology or even molecular biology for some structures within the cell are so small that their dimensions are only a few large molecules long. The boundaries of cells and of the intracellular structures known as membranes are also only a few molecules thick. These membranes are not inert dividers but functionally active structures which enable different concentrations of molecules, ions and electrons to be maintained in different regions of the cell.

The earliest of these cellular studies carried out in the Institute were made in 1956 and concerned the measurement of the electrical forces existing between the inside and the outside of muscle cells and recording the changes in the forces which take place during contraction. As the electrical forces not only have an influence on the behaviour of cell membranes but also indicate the distribution of ions, a record of them provides an excellent way to study the action on cell membranes of various drugs, such as procaine amide, quinidine sulphate, reserpine, fluothane and hormones which act upon cardiac muscle.

In 1959, with the help of colleagues in a number of institutions who gave us access to electron microscope facilities, a study of the internal fine structure of cardiac muscle cells was started. This year our own instrument has been installed and now the project can progress more easily and can become linked with another, commenced in 1968, in which the cell walls are disrupted and the intracellular components are separated by ultracentrifugation. In this way the function of the component parts of cells such as intracellular membranes, microsomes and mitochondria may be explored with exactitude.

These techniques have enabled the action of some drugs and hormones and the role of calcium ions to be related with changes in the subcellular structures seen in cardiac failure, thyroid overactivity and adrenal insufficiency.

Another technique used to explore the working of the intracellular components involved extracting the contractile protein fibres from cells in a manner which left them still able to contract. Commencing in 1963, this project has evolved into the present studies concerning the newly recognised regulatory protein — troponin — which has a controlling action on the contraction of the contractile proteins.

For these significant studies of cardiac muscle function the cells have been obtained

from adult hearts. However, another approach to this cellular study, which we are currently establishing, involves growing cardiac muscle cells in a tissue culture to obtain pure lines of cells. The initial cells are obtained from chick embyros and are grown on glass where they form sheets only one cell thick. These sheets of cells will spontaneously beat and form excellent models for much of the experimental work. It is possible to grow some cells obtained from adult tissues and although to date this has been more the province of cancer research projects, it may be possible in the future to develop such cultures as models for the study of other human disease. With these aims in mind a cancer research project involving the culture of malignant cells is currently being initiated. Tissue culture is becoming an important tool in cellular biology and in medical research.

Clinical Pharmacology

In last year's report mention was made of projects in Clinical Pharmacology which is "the study of what drugs do to the human body and what the body does to drugs, both in health and disease"[1]. The importance of this study is emphasised by mention of the hypotensive drugs used to treat high blood pressure, the tranquilising drugs used in psychiatric practice, the diuretics used in cardiac failure, L-dopa used to control Parkinson's disease and the steroid drugs used in many conditions, all of which have been introduced into medicine in recent years.

For many years workers in Institute projects have been conducting clinical trials of antihypertensive drugs and diuretics and laboratory studies on many drugs which influence cardiac musc'e behaviour. These projects are gradually being broadened and during 1971 some studies were started to investigate interactions between hypotensive and tranquilising drugs when used simultaneously in the treatment of patients.

It is hoped that clinical pharmacology projects will increase and enhance the interaction between clinical and laboratory workers.

[1] B. M. Prichard and P. Turner (1971) Lancet **2**. p. 653.

1971 HIGHLIGHTS

Electron Microscope Laboratory

Through the generosity of the Government of Victoria it has been possible to purchase an electron microscope and to accommodate it and its team of workers in a suite of laboratories built in portion of the uncompleted lower ground floor of the Institute. These laboratories were formally opened by the Minister for Health on October 7, at which ceremony our thanks were conveyed to the Government for providing the whole of the capital cost of this venture.

At present the maintenance finance of the project is being provided jointly by the Institute and the Hospital.

Seminars

In addition to the usual Friday Seminars concerning work of the Institute, several special ones were held during the year.

An all day meeting on the general subject of the Cardiac Muscle Cell brought together research workers from the Institute, Hospital, Monash and Melbourne Universities, and guests from Sydney and Canberra who acted as chairmen of the sessions. This seminar was timed to coincide with the centenary celebrations of the Hospital, and was a successful exchange of information and views that was attended by an overflow audience.

An equally successful one was held on the subject of Beta-adrenergic Blockade. It was restricted to an invited audience and included speakers from other Melbourne research groups and two visitors from England, Dr. J. Hamer and Professor M. Barrett.

During October an extended visit to the Institute was made by Professor W. F. H. M. Mommaerts of the Department of Physiology of the University of California at Los Angeles. He conducted formal seminars in the Institute, at Melbourne and Monash Universities and the

HITACHI ELECTRONMICROSCOPE IN USE
The purchase and installation of this instrument was made possible by the generosity of the Victorian Government.

College of Pharmacy on various aspects of muscle cell function. In addition to these well attended discussions he took an active part in current research projects.

The programmes of these meetings are included in the report and show a successful blending of clinical and laboratory medical research.

Vascular Service

Some 20 years ago a study of clinical methods of measuring the blood flow in the limbs was commenced by our staff. Reliable measurement of these flows was essential for the assessment of treatment of occlusive diseases of arteries by either drugs or surgical intervention.

In 1954 the workers in this project joined with members of the thoracic surgical unit to develop methods by which to unblock arteries or to by-pass blocks. Since then as recounted in the scientific reports very satisfactory techniques have developed which enable many limbs to be saved from gangrene. At various times these techniques have used homograft arteries, synthetic tubes and more recently autogenous vein grafts to by-pass blocks.

This year the importance of this treatment was recognised by the establishment by Alfred Hospital of a Vascular Service to provide the surgical skills necessary. The pre-operative consultation and investigation of these patients however places a considerable service load on the clinical research unit.

STAFF

Apart from the usual changes in junior scientific and technical staff there have been only the changes foreshadowed in last year's report.

Dr. G. Hard, B.V.Sc., Ph.D., arrived in June to take up his appointment as the A. A. Thomas Fellow of the Anti-Cancer Council of Victoria.

Dr. S. Katz, Ph.D., commenced his tenure of the Edward Wilson Memorial Fellowship at the beginning of June.

Miss A. Chang, B.Sc. has joined the Institute staff to supervise the electron-microscopy project.

Dr. F. R. Trinker attended the XXV International Congress of Physiological Sciences held in Munich in July to present a paper dealing with her studies on the influence of the sympathetic nerves on coronary blood flow. She also took part in the International Symposium on cyclic A.M.P. at Milan, and the Symposium on coronary blood flow at Antwerp. In London and in Ann Arbor, U.S.A. she studied developments in the field of clinical pharmacology.

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

The Lang Fellow: P. Mäsiar, M.D., D.Sc.

William Buckland Fellow: P. Fantl, D.Sc.

James and Elsie Borrowman Fellow: Miss A. Chang, B.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 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 and those who have so generously helped with the purchase of equipment, especially The Wellcome Trust who provided a scintillation spectrometer.

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, 1971.

ALFRED HOSPITAL RESEARCH FELLOWS IN THE INSTITUTE
1949 - 1971

Anderson, R. McD., 1953-55
Andrew, R. R., 1949-55
Barnett, A. J., 1949-50
Baumgarten, A, 1962-64
Beavis, E. L. G., 1955-56
Boake, W. C., 1958
Breidahl, H. D., 1952-53
Burnside, K. B., 1951
Cooper, E., 1962-71
Coventry, D. A., 1968
Daile, P., 1970-71
Duffy, D. G., 1952-55
Ferguson, I. A. L., 1957-58
Fowler, R., 1953-54
Francis, J. K., 1956-57
Fraser, J. R. E., 1957
Gardiner, J. M., 1952
Goble, A. J., 1951
Hudson, B., 1952
Jamieson, K., 1954
Kay, H. B., 1959-60
Kincaid-Smith, P., 1959-60
McCutcheon, A. D., 1959, 1965-66
McDonald, W., 1960-61
McNeur, J. C., 1955
McRae, C. J., 1955
Murfitt, L., 1955
Newman, H. C., 1954
Parsons, P. J., 1951
Quinn-Young, M., 1956
Race., D., 1959-63
Sawers, R. J., 1953-60
Silberberg, F. G., 1953
St. Clair, W. A., 1955
Stern, W., 1954-55
Stirling, G. R., 1955, 1969
Swann, J. B., 1967
Wagner, G., 1958

OVERSEAS FELLOWS

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

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

Australian Medical Association.
Adelaide Children's Hospital.
Anti-Cancer Council of Victoria.
A.N.Z.A.A.S.
Austin Hospital.
College of Physicians and Surgeons, New York.
Commonwealth Department of Health.
Commonwealth X-Ray and Radium Laboratory.
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Wellington Medical Research Foundation.
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REPORT OF SCIENTIFIC INVESTIGATIONS

PHYSIOLOGY AND PHARMACOLOGY OF THE CARDIOVASCULAR SYSTEM

MYOCARDIAL CONTRACTILITY†‡

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

Role of Calcium Ions

W. G. Nayler

Current concepts of events involved in excitation-contraction coupling in cardiac muscle assign a central role to ionized calcium, Ca^{2+}, some of which probably originates from the sarcoplasmic reticulum and some from superficially-located storage sites, possibly at the level of the sarcolemma and its associated invaginations. Recent investigations have shown that the amount of Ca^{2+} stored at these superficially located binding sites can be estimated by using lanthanum ions (La^{3+}) to displace this Ca^{2+}. Hence it is now possible to correlate changes in contractility with changes in the amount of Ca^{2+} which is stored at these superficially located binding sites, and which presumably is displaced inwards during excitation. The significance of the role played by the sarcoplasmic reticulum in regulating the intracellular availability of Ca^{2+}, and therefore in regulating the amount of Ca^{2+} which is available for interaction with the contractile and regulatory proteins — the actin-myosin and the troponin — tropomyosin complexes respectively, can be estimated either by using the sarcoplasmic reticulum as an isolated subcellular fraction — the microsomal fraction — or alternatively by using whole muscle preparations which have been pretreated in such a way that the selective permeability of the sarcolemma has been destroyed. This latter can be achieved by pretreating the whole muscle preparations with certain chelating agents, including ethylenediamine tetra-acetate (EDTA). The accumulation of Ca^{2+} by the sarcoplasmic reticulum is accompanied by the activation of Ca^{2+}-stimulated ATPase enzymes. Studies in which the rate of accumulation of Ca^{2+} by the sarcoplasmic reticulum has been measured have been parallelled by simultaneous measurements of the activity of this particular ATPase enzyme.

Contraction has been quantitated in terms of the peak tension developed per unit cross sectional area of muscle (gm/mm^2), and the rate at which that tension was developed. Conversely relaxation, which reflects the re-accumulation fo Ca^{2+} by the sarcoplasmic reticulum, has been quantitated in terms of the rate at which the development of tension declined.

(a) Effect of Bilateral Adrenalectomy. As reported last year chronically adrenalectomized dogs maintained on saline for 10 days exhibit circulatory collapse, characterised by a highly significant decline in both the peak tension developed during contraction and the rate at which that tension is developed. At the same time the sensitivity of the heart to the negative inotropic effect of pentobarbital is markedly increased. These changes in contractility and drug sensitivity have been shown to be due neither to an altered Ca^{2+}-accumulating activity of the sarcoplasmic reticulum, nor can they be accounted for in terms of an altered availability of the high energy phosphate stores. Current studies with La^{3+}, however, show that the ability of superficially located binding sites to accumulate Ca^{2+} is grossly impaired. Other studies described elsewhere in this report (p. 27) revealed that the activity of the membrane located Na^+-K^+ activated ATPase enzyme

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

is changed after bilateral adrenalectomy. The superficially located binding sites in cardiac muscle display competitive antagonism for Na^+ and Ca^{2+} and it seems possible that after adrenalectomy this antagonism has been modified in favour of Na^+ binding. Under these conditions the tension developed during contraction would decline.

(b) Negative Inotropic Effect of Quinidine. The negative inotropic effect of quinidine is well documented but poorly understood. Experiments carried out at the Institute some years ago indicated that the magnitude as well as the rate of onset of this negative inotropic effect of quinidine was directly proportional to the extracellular concentrations of Ca^{2+} and inversely proportional to the Na^+ concentration. Studies using isolated sarcoplasmic reticulum preparations have shown that relatively high doses of quinidine (1.0 - 2.0mM) impair the abi'ity of the reticulum to accumulate, bind and to exchange Ca^{2+}. Both the ATP-dependent and the non-ATP dependent binding processes were impaired. Because the ability of cardiac microsomes to bind and to exchange Ca^{2+} is pH sensitive considerable care was taken to ensure that the pH remained constant during these experiments.

In addition to the effect quinidine exerted on the ability of cardiac miscrosomes to both bind and exchange Ca^{2+}, this drug exerted a marked inhibitory effect on the ability of the reticulum to accumulate Ca^{2+}. This effect was highly significant at a dose level of 0.1 mM, and after only two minutes incubation at an even lower dose level (0.01mM) quinidine interferes with the ability of superficially located sites to accumulate Ca^{2+} i.e. the amount of Ca^{2+} which would be displaced by La^{3+} from trabecular muscles which previously had been immersed in Tyrode's solution containing quinidine was significantly ($p < 0.001$) less than that displaced from paired trabecular muscles immersed in quinidine-free Tyrode's solution. In general these experiments with quinidine have been interpreted to mean that quinidine, like pentobarbital, interferes with the binding of Ca^{2+} at the superficially-located binding sites and at the sarcoplasmic reticulum in cardiac muscle cells. The changes caused by quinidine in peak tension developed and in the rate at which that tension is developed therefore can be explained in terms of the interaction of this drug with mechanisms which regulate the availability of Ca^{2+} in these muscle cells.

(c) Effect of β-Adrenoceptor Antagonists on the Availability of Ca^{2+}. Although β-adrenoceptor antagonists improve the efficiency with which the heart performs mechanical work they can, at the same time, impair contractility to a point of seriously intensifying myocardial failure in patients with severe heart disease. The effect that the β-adrenoceptor antagonists exert on cardiac contractility is complex because: (i) the positive inotropic effect of the circulatory catecholamines and of the catecholamines which are released in response to sympathetic nerve stimulation is abolished. (ii) the slowed heart rate will necessarily be accompanied by a negative "staircase" effect, and (iii) some β-adrenoceptor antagonists exert a negative inotropic effect on heart muscle which is not species specific and which cannot be accounted for simply in terms of β-adrenoceptor blockade. This myocardial depressant effect of the β-adrenoceptor antagonists is exerted by all the currently available β-antagonists if sufficiently large doses are used.

Studies carried out during the past year have shown that the rate at which the negative inotropic effect of the β-antagonists develops is directly proportional to the frequency with which excitation occurs and to the extracellular concentration of Ca^{2+}. Studies with isolated microsomal fractions showed that these drugs interfered with the ability of cardiac microsomes to accumulate, bind and exchange Ca^{2+}. In this respect the ability of propranolol > oxprenolol > LB46. The negative inotropic effect of propranolol > oxprenolol > LB46, although the potency of propranolol as a β-adrenoceptor antagonist < oxprenolol < LB46. Studies currently in progress show that these β-adrenoceptor antagonists interfere with the ability of the superficially located binding sites to accumulate Ca^{2+}. This effect is apparent at doses 1/100 of those needed to exert a significant effect on the Ca^{2+} accumulating activity of the sarcoplasmic reticulum.

(d) Effect of Ryanodine. In previous experiments it was shown that ryanodine, which produces sustained contraction in skeletal muscle, causes a progressive decline in the tension produced during contraction in cardiac muscle. The ability of the regulatory protein, troponin, to accumulate Ca^{2+} was not altered by ryanodine but the Ca^{2+} accumulating activity of the microsomal fraction was diminished. Because relaxation in cardiac, as in skeletal muscle, is generally believed to result from the accumulation of Ca^{2+} by the sarcoplasmic reticulum it can be postulated that a drug such as ryanodine should delay the onset of relaxation and should enhance contraction, if any effect this alkaloid may have on the availability of ionized Ca^{2+} is precluded. To test this hypothesis cardiac muscle cells which had been pretreated with EDTA — to destroy the selective permeability of the sarcolemma were used. Electron-micrographs confirmed that the plasma membrane remained intact. Under these conditions ryanodine produced a prolonged and augmented contraction, provided that Ca^{2+} was introduced. Although these are preliminary experiments they indicate that ryanodine does interact with superficially located membranes in cardiac muscle cells, probably the sarcolemma and its invaginations. This hypothesis was strengthened by the results from other experiments which showed that ryanodine reduces the amount of Ca^{2+} displaced by La^{3+} from cardiac muscle cells.

Troponin

P. Daile.

The interaction of actin and myosin in the presence of Mg-ATP represents the essential mechanism of muscle contraction. The final mediator in this process is a minute amount of Ca^{2+} released in the vicinity of the myofibrils during the excitation-contraction coupling process. The two regulatory proteins troponin and tropomyosin are required to render the actin-myosin interaction sensitive to the calcium ions.

Because others have shown that skeletal troponin binds tightly large amounts of calcium, it was considered of importance to attempt to purify and characterise cardiac troponin.

Purification

DEAE cellulose, cellulose acetate strip low voltage electrophoresis, electrofocusing and Sephadex column chromatography have been used to purify troponin preparations. DEAE cellulose purification of greyhound cardiac troponin yielded two fractions, as judged by ultra-violet scans of absorbence. Troponin and fraction 2, eluted at 1.OM KCl concentration, had a peak at, or very near to 260nm, whereas fraction 1, eluted at 0.3M KCl concentration, had a peak at 278nm. Fraction 1 contained approx. 95% and fraction 2 approx. 5% of the total protein respectively. Using low voltage electrophoresis with cellulose acetate strips, in 0.1M phosphate buffer, pH 6.0, a clear separation of unpurified rabbit skeletal troponin into 3 and 4 bands was achieved. As yet these constituents have not been characterised. Electrofocusing techniques and Sephadex column chromatography of a crude rabbit skeletal troponin preparation yielded a number of fractions consisting of protein-like material. The characterisation of these fractions and the investigation of their physiological activities is in progress.

Physiological Activity. (i) Calcium binding parameters. Calcium binding and binding constant values of unpurified cardiac troponin and fractions were determined by a radiometric method based on the partition of Ca^{2+} between a soluble phase containing the calcium binding protein and the calcium chelating resin Chelex-100, using $^{45}Ca^{2+}$ as the radioactive tracer isotope. In 10 preparations unpurified troponin was found to bind 13.9 ± 3.59 μM Ca^{2+} per gm. protein (mean $\pm$ 1 S.D.) with a binding constant of $0.64 \pm 0.23 \times 10^6$ M^{-1}. Fraction 1 obtained by DEAE cellulose purification of the same preparations, bound significantly less calcium ($p < 0.05$), 10.8 ± 4.32 μM Ca^{2+} per gm. protein, but the binding constant was significantly higher ($p < 0.01$), $0.95 \pm 0.27 \times 10^6$ M^{-1}. Fraction 2 from these preparations showed no calcium binding property and its nature has not been resolved.

As a preliminary investigation to purification work by electrofocusing and Sephadex column chromatography, two cardiac troponin preparations were chromatographed on a

Sephadex column. One preparation led to two major protein peaks and one minor and at least one of the major peaks was shown to possess calcium binding properties. The other preparation yielded two major and two minor peaks and both of the major peaks bound significant amounts of calcium.

(ii) Superprecipitation and ATPase activity of actomyosin. The interaction of actin and myosin is associated with a change in the light scattering properties which can be monitored by recording the turbidity at 660 nm. This phenomenon is termed superprecipitation and is due to shrinkage and dehydration of the actomyosin gel in the presence of low concentrations of ATP.

The ATPase activity of actomyosin can be readily followed using pH-stat equipment since upon hydrolysis of ATP to ADP phosphoric acid is liberated. Depending on the pH, a varying amount of acid is liberated per molecule of ATP split. If this ratio is known then the amount of alkali necessary to maintain constant pH in an unbuffered system is a measure of inorganic phosphate (Pi) formed. To record such changes the cell compartment of a Unicam SP 700 recording spectrophotometer was modified so as to incorporate a 15ml. reaction vessel (glass optical cell) in the light path, situated over a small magnetic stirrer, with pH electrodes, cell holder and alkali delivery tube suitably located in the compartment lid.

This apparatus made possible simultaneous recording of turbidity (superprecipitation) and volume of alkali delivered for constant pH (ATPase activity) and served as a very useful tool in the study of the interactions of the contractile proteins.

For this work it was necessary to prepare actomyosin and tropomyosin as well as troponin. For the calcium binding investigations troponin had been prepared from greyhound cardiac muscle. Difficulties in preparing tropomyosin from this muscle were not encountered, but it was found difficult to isolate satisfactory myofibrils and extract actomyosin from cardiac muscle. Consequently, to establish investigational procedures, all the muscle proteins were prepared from rabbit white skeletal muscle (using hind leg and back muscle).

The troponin from this preparation was fractionated with DEAE cellulose as described before, and it was found that both unpurified troponin and fraction 1 brought about the Ca^{2+} sensitization of desensitized actomyosin with respect to superprecipitation and ATPase activity, whereas fraction 2 was ineffective, and not necessary, in this respect. The Ca^{2+} binding parameters of this preparation were not investigated. However, more preparations will be necessary to establish the above observations conclusively.

Myocardial Uptake of Isoprenaline

G. Picken

Catecholamines are transported into cardiac muscle cells and then metabolised by the enzymes COMT and MAO. The metabolites of catecholamines — normetanephrine and metanephrine inhibit the uptake process. Studies undertaken during the year were aimed at determining the physiological role of the uptake process in cardiac muscle cells. Normentanephrine was found to potentiate the isoprenaline-induced activation of the myocardial phosphorylase enzyme, but metanephrine and 4-hydroxy, 3 methyl-isoprenaline produced little significant potentiation. The accumulation of tritiated isoprenaline by isolated rat heart was inhibited by both normentanephrine and metanephrine, and more strongly by the O-methylated metabolite of isoprenaline. It was concluded that the transport of catecholamines into cardiac muscle cells is independent of, but competitive with, receptor activation by these substances. Muscle uptake may, therefore be a mechanism working in conjunction with the neural uptake process, to clear the synaptic gap of catecholamines.

LEFT VENTRICULAR FUNCTION

I. McInnes

Bilateral Adrenalectomy. During the course of other concurrent studies (see p. 23) it was noted that dogs after bilateral adrenalectomy became hypersensitive to pentobarbitone, both

with respect to the amount needed to induce anaesthesia and the amount needed to produce cardiotoxic effects. Left-ventricular work studies in dogs carried out 7-10 days after removal of both adrenals showed a flattened "Frank-Starling" work function curve. Myocardial oxygen consumption was within normal limits, the cardiac stores of energy-rich phosphates were well maintained and the concentration of calcium within heart muscle cells was normal. Perfusion-fixation techniques were employed to provide cardiac muscle suitable for electron microscopy. These sections showed that the fine structure of the heart muscle, including that of the sarcolemma, T-system and reticulum was within normal limits apart from some depletion of glycogen stores.

Partial Nephrectomy. Left ventricular work studies in partially nephrectomized dogs showed that up to 80% of the kidney volume could be destroyed without producing any significant change in left ventricular function, high energy phosphate stores or calcium content, at 2-3 days after removal of the kidney tissue.

Bilateral Adrenalectomy and Myocardial ATPase.

J. Szeto

Previously it had been demonstrated that chronically adrenalectomized dogs maintained on saline for 7-10 days had a decrease in plasma Na^+ and an increase in plasma K^+ concentrations as well as a decrease in ventricular contractile force. It was therefore decided to investigate the effect of bilateral adrenalectomy on Na^+, K^+ dependent ATPase and basic Mg^{2+} dependent ATPase activities in the myocardium. Using the method of Matsui and Schwartz[1] membrane particles of heart tissue were obtained by differential centrifugation and washed with deoxycholate to bring a large proportion of the ATPase activity into solution. The preparation resulting from bilateral adrenalectomized animals was compared with a similar preparation from control animals.

It was observed that the ratio of Na^+, K^+ dependent ATPase activity to basic Mg^{2+} dependent ATPase activity was significantly reduced in the adrenalectomized animals compared to the controls ($P < 0.05$). This was due, in part, to an increase in the basic Mg^{2+} dependent ATPase activity of these preparations. No change was observed in the optimal concentrations of Na^+ and K^+ required for maximal ATPase stimulation.

It was concluded that bilateral adrenalectomy produces a slight decrease in Na^+, K^+ dependent ATPase activity as well as a slight increase in basic Mg^{2+} dependent ATPase activity. Whether these changes in myocardial ATPase are associated with the alterations in plasma Na^+ and K^+ concentrations noted in adrenalectomized animals is uncertain.

Biochemical Techniques (a).

V. Carson

Plasma Catecholamines. A method has been developed for the determination of plasma catecholamines expressed as noradrenaline equivalents. The method however is not sensitive enough to enable a differential assay for adrenaline and noradrenaline in dog plasma because of the very low level of adrenaline present there.

Approximately 100 ml of heparinized blood is collected in a vessel containing 50 mg of sodium metabisulphite in an ice-bath. The blood is centrifuged in the cold for 15 min. at 2000 x g and the plasma removed to a stoppered measuring cylinder.

A 50 ml aliquot is brought to 0.4 M with respect to perchloric acid, by the addition of concentrated perchloric acid dropwise with shaking. The cylinder is stoppered and shaken vigorously for 5 min. The contents are transferred to nylon centrifuge tubes, the cylinder washed out with 2 x 5 ml. volumes of 0.4M perchloric acid and the washings added to the centrifuge tubes. They are then centrifuged at 30,000 x g for 10 mins. The supernatant

[1] H. Matsui and A. Schwartz, Biochem. Biophys. Acta Vol. **128** (1968) p. 380.

is transferred to a beaker; 0.5 ml of 0.2M Na_2EDTA is added for every 20 ml of supernatant and the pH adjusted to 8.4 with 5N and 0.5N NaOH.

The supernatant is chromatographed on a column of alumina prepared as follows: Woelm alumina is sieved so as to retain particles which will pass through a 150 mesh but be retained by a 200 mesh sieve and activated according to the method of Crout[1]. 0.3g of alumina is washed into a column consisting of a thistle funnel with a 30 cm stem and approximately 6 mm external diameter. This is drawn out at the tip and plugged with glass wool.

The supernatant, kept in ice, is added to the column and allowed to run through. The column is washed with iced distilled water, using a head of pressure, until the pH is below 7.

The catecholamines are eluted with 5 ml of ice-cold 0.2M acetic acid after first adding 0.5 ml acetic acid and discarding the first 0.5 ml of eluate.

Development of Fluorescence. Fluorescence is developed using the trihydroxyindole method at pH 6.5.

One ml of 1M sodium acetate buffer pH 6.5 is added to an appropriate number of 6 x ½" test tubes. 0.5 ml of sample eluate is added to three of the tubes (sample, "faded" blank and internal standard) and water added to bring the volume to 2.9 ml.

External standards consisting of 10, 20 and 40 μl of L-noradrenaline (B.D.H.), 1 μg/ml., in 0.01 N HCl are likewise prepared, and 20 μl of L-noradrenaline are added to the internal standard tube.

0.1 ml of 0.25% K_3FeCN_6 is added to the "faded" blank and mixed and after 3 min., 1.8 ml of 5N NaOH is added and mixed. After 30 min., 0.2 ml of 2% ascorbic acid (freshly prepared) is added and one drop of ethylene diamine (EDA). The fluorescence is read after five mins.

Alkaline ascorbate containing EDA is prepared just before use as follows: 2% ascorbic acid is added to 5N NaOH in the proportion of 1:9 (v/v). EDA is added to the alkaline ascorbate in the proportion of 1:50.

To the external and internal standards, sample and reagent blank (containing water instead of sample) 0.1 ml of 0.25% K_3FeCN_6 is added and the contents mixed. After exactly 3 mins., 2 ml of alakine ascorbate are added with thorough mixing. Timing should be arranged so that all tubes can be read together against the reagent blank.

Fluorescence is read after 5 mins. in a Turner Model 110 fluorometer using the x30 sensitivity setting and the 360nm — 525 nm filter combination (Turner 7-60 and 58).

Fluorescence is stable for 30 minutes.

Values. The mean value of the catecholamine level in dog plasma was found to be 1.08 ± 0.36 (S.E.) μg/l.

The recovery of added noradrenaline varies between 50 and 70%.

Efforts are currently being made to improve the recovery by the combined use of an ion exchange resin and alumina adsorption which if successful would permit the use of a smaller sample volume of plasma.

Tissue Calcium. The method of Sparrow Johnstone[2] has been modified to permit estimation of calcium in dog cardiac muscle.

Immediately after killing the animal, the heart is excised and rinsed quickly in two changes of de-ionised water and gently blotted dry with filter paper. Approximately three grams of tissue are taken, minced finely with scissors and passed through a tissue press with 1 mm holes. One gram of minced tissue is extracted (in duplicate) with 2 ml of a mixture of equal volumes of glacial acetic acid and 3M trichloracetic acid in plastic disposable 10 ml centrifuge tubes, together with a solvent blank. The tubes are heated in a boiling water-bath

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1 J. R. Crout Standard Methods of Clinical Chemistry, Vol. 3 (1961) p. 62. Academic Press, New York.

for 5 mins.; 3 ml of de-ionised water are added and the tubes boiled for a further 5 mins. The tubes are allowed to cool and stand for one hour at room temperature, with occasional mixing using a Vortex mixer. One ml. of a solution of 2% La_2O_3 dissolved with the aid of concentrated perchloric acid and neutralised with 2N NaOH, is added, followed by 2 ml of 200 meq/1 NaCl. The volume is then brought to 10 ml. with de-ionised water.

The tubes containing the precipitate are spun at 3500xg for 10 minutes and the supernatant removed to plastic tubes.

The calcium content of samples and solvent blanks are determined by atomic absorption spectrophotometry and compared with standard solutions containing the same proportion of acetic acid-trichloracetic acid, La_2O_3 solution and 40 meq/1 added Na^+. The acetic acid-trichloracetic acid mixture has a strong enhancing effect on the atomic absorption of Ca^{2+}, whilst the normal sodium content of the tissue has a small enhancing effect. The enhancing effect of Na^+ increases with the concentration of Na^+, reaching a plateau between 20 and 40 meq/1 of Na^+.

As the amount of Na^+ contributed by the tissue to the extract is approximately 8 meq/1, the addition of 40 meq/1 Na^+ to both extracts and standards compensates for any small enhancing effect of Na^+.

The enhancing effect of the acetic acid-trichloracetic acid mixture is approximately 25%; hence the necessity to include the same concentration of the latter in the calcium standards.

The presence of lanthanum counteracts the interference caused by phosphate ions.

Recovery of added Ca^{2+} to the initial extract was 100%. The normal level of Ca^{2+} in dog cardiac muscle was found to be 0.688 $\pm$0.025 (Mean $\pm$ S.E.) μM/g wet weight (N = 9).

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[2] M. P. Sparrow and B. M. Johnstone, Biochim. Biophys. Acta Vol. **90** (1964), p. 425.

Adenyl Cyclase System. In order to study the effect of catecholamines and β-blocking agents on the adenyl cyclase system in cardiac muscle, a reliable and reproducible assay of cardiac muscle adenyl cyclase which exhibits the characteristic catecholamine sensitivity reported in the literature is needed and the radioactive method Krishna, Weiss and Brodie[3] was adopted with some modifications. Aminophylline was substituted for theophylline for the purpose of inhibiting phosphodiesterase activity because of its greater potency in this regard in our hands. An ATP-regenerating system (phosphoenol pyruvate plus pyruvate kinase) was included in the assay medium, and we have shown that its inclusion maintains the ATP concentration constant throughout the incubation period of 10 mins. and also results in higher yields of 3'5' cyclic AMP than were obtained without such a system.

The source of the enzyme is a homogenate of the particulate fraction of cardiac muscle. The preparation of this requires considerable care. After killing the animal with anaesthesia, the heart is excised immediately and placed in ice-cold 0.25M sucrose. After rinsing in 0.25M sucrose and blotting dry, 1-2g of tissue are minced finely with scissors and homogenized in approximately 15 ml of 0.002M glycyl-glycine buffer, pH 7.5, containing 0.001, $MgSO_4$, using a loosely-fitting teflon motor-driven pestle-glass homogenizer, for 10-15 sec. The homogenate is then centrifuged in the cold at 600xg and the pellet resuspended and washed twice with glycyl-glycine buffer and finally made up to 15% (w/v) with buffer.

The incubation medium consists of tris-HCl 50 mM, pH 7.4; $MgCl_2$ 4.6 mM; aminophylline 10^{-2}M; bovine serum albumin 0.014%; ATP-^{3}H 1.4-1.6 mM, 16.7 μCi/μM; phosphoenol pyruvate 2.4 mM; pyruvate kinase 50 μg; together with NaF 10^{-2}M or catecholamines 10^{-4}M, depending on the experiment, in a final volume of 0.6 ml.

When catecholamines were included the bitartrate salt was dissolved in water and added to the homogenate immediately before the reaction was initiated by the addition of the homogenate (containing approximately 0.5 — 1 mg protein) to the reaction mixture.

Incubation was carried out at 30°C for 10 mins. in a shaking water-bath. The reaction was stopped by the addition of 0.1 ml of ATP 40 mM, containing 15 mM carrier 3'5' cyclic AMP and plunging the tubes into a boiling water-bath for 3 mins.

After centrifuging in the cold at 2000xg for 20 mins., the supernatant was transferred to a column of Dowex AG 50 ion-exchange resin, 200-400 mesh (5 x 0.5 cm) and eluted with water. The third 2 ml fraction was then precipitated twice with 0.25M $ZnSO_4$ and $Ba(OH)_2$. 0.5 ml of the supernatant was added to phosphor and counted in a Packard Liquid Scintillation Counter, and another aliquot was diluted and the optical density at 260 nm determined and hence the recovery of 3'5' cyclic AMP.

The protein precipitates were dissolved in 1N NaOH and the protein determined by the Lowry method.

Blanks in which boiled homogenate was substituted were carried through the whole procedure. All estimations were done in duplicate or triplicate.

Early work was carried out using dog myocardium as the enzyme source. The mean level of adenyl cyclase activity, expressed as picomoles 3'5'AMP/mgP/min was 32.7 ± 3.26 (S.E.), in the absence of fluoride. Fluoride caused a 3-4 fold rise in this value.

However, repeated exhaustive attempts to demonstrate a stimulation of adenyl cyclase activity by L-isoprenaline, L-noradrenaline and L-adrenaline gave generally unimpressive results.

Whilst considered over a large group of experiments, a small stimulation by these three hormones was observed (25-38%; $p < 0.05$ - $p < 0.01$), in many individual experiments, no significant stimulation by a particular hormone was obtained.

The reason for the failure is not known, but is believed to be related to the extreme lability of the membrane-bound specific receptor responsible for hormone sensitivity to harsh methods of cell disruption. This has been noted for some other tissues by other workers.

The mildest methods of homogenization compatible with obtaining a workable homogenate were used but in spite of this the results were variable and disappointing.

As the majority of published work on hormone stimulation of cardiac adenyl cyclase has been performed on cats, with guinea pigs and rats next in preference, the remainder of this study has been carried out on cat heart homogenate.

Using this preparation, it has been possible to demonstrate routinely a 50-100% stimulation of 3'5' AMP production in the presence of L-isoprenaline, L-noradrenaline and L-adrenaline.

In one set of seven experiments, the control level of adenyl cyclase activity was 27.2±1.12 (S.E.) picomoles 3'5' AMP/mgP/min. In the presence of 10^{-4}M L-isoprenaline it was 50.5 ±2.20 ($p < 0.001$); with L-noradrenaline 48.7±1.97 ($p < 0.001$) and with L-adrenaline 50.9±2.04 ($p < 0.001$).

We are presently investigating the effect of oxprenolol directly on the adenyl cyclase system and also its ability to block the stimulation caused by L-adrenaline.

Biochemical Techniques (b)

S. Katz

Adenyl Cyclase Activity in Sarcoplasmic Reticulum. Adenyl cyclase activity has recently been reported to be associated with "sarcoplasmic reticulum fragments" prepared from heart muscle[1] [2] but the preparation procedures used did not exclude the possibility of contamination of the "fragments" by some other cellular component such as low density plasma membrane fragments. The methods used to determine adenyl cyclase activity ap-

[3] G. Krishna, B. Weiss and B. Brodie, J. Pharmacol. Exp. Ther. Vol. **163** (1968) p. 379.

peared to be inaccurate, and too insensitive. As a study of the adenyl cyclase activity of sarcoplasmic reticulum fragments was important to our studies of cardiac muscle an accurate, readily perfomed and sensitive technique for the assay of adenosine 3', 5'-cyclic monophosphate (cAMP) capable of detecting picomole quantities of this nucleotide, and a preparation of sarcoplasmic reticulum devoid of significant plasma membrane and mitochondrial contamination have been sought. The methods developed were then used to characterise the adenyl cyclase activity of this preparation.

Assay of Cyclic AMP. A modification of the method of Gilman[3] was developed for the measurement of cAMP. This method is based on the competition for protein binding of the nucleotide to a cAMP dependent protein kinase. A cyclic AMP dependent protein kinase was prepared from homogenized rabbit skeletal muscle by acid precipitation of the supernatant solution resulting from centrifugation at 27,000 x g at 4°C followed by ammonium sulphate precipitation and DEAE cellulose chromatography. Inhibitor protein was prepared from homogenized dog skeletal muscle by precipitation with 50% TCA followed by dialysis and centrifugation. The above-mentioned protein kinase preparation in the presence of the heat stable inhibitor, was shown to bind 0.05 pM AMP/μg protein. Both the kinase and its inhibitor were stable at -18°C and sufficient quantities for more than 1000 assays can be prepared from less than 1 kg muscle.

The assay is based upon the isotopic dilution of tritiated cyclic AMP by the cyclic AMP being measured. Separation of free ligand from that bound to the protein is effected by passage of the mixture through a cellulose ester filter. At the levels of cyclic AMP which this assay permits, a high specificity was observed even in the presence of similar concentrations of other nucleotides. This method has been successfully utilised in deproteinized tissue extracts from various whole cell and lysed preparations and is accurate at concentrations below 2 pM cyclic AMP. Less than mg quantities of tissue are therefore sufficient for this assay.

Preparation of Sarcoplasmic Reticulum. Various enzyme assays were employed to determine the extent and type of combination of each of the sarcoplasmic reticulum preparations employed. Nucleotidase activity and Na^+, K^+ dependent ATPase activity were used to identify plasma membrane contamination and cytochrome oxidase and succinic dehydrogenase activity for mitochondrial contamination. For each preparation the ratio of Ca^{2+} dependent ATPase activity to basic Mg^{2+} dependent ATPase activity was determined as a measure of the specific activity of the Ca^{2+} accumulating system present.

With these techniques, a sarcoplasmic reticulum preparation from cat heart, prepared by differential centrifugation and sucrose density gradient separation, followed by LiCl treatment, was shown to contain a component that was relatively low in nucleotidase and Na^+, K^+ dependent ATPase activity, contained slight mitochondrial contamination and exhibited a high Ca^{2+} stimulated ATPase activity. This component also contained adenyl cyclase activity that was stimulated by adrenaline as well as NaF. This component is being further purified.

ADRENOCEPTOR AGONISTS AND ANTAGONISTS

W. G. Nayler

α-Receptors

Phentolamine. The infusion of phentolamine in doses smaller than those required to produce significant α-adrenoceptor blockade has recently been shown to cause an increase in myocardial blood flow and heat production. Because it has been suggested that this increase in myocardial blood flow is secondary to an increase in cardiac work (and the resultant change in metabolism and oxygen demand) ex-

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1 N. S. Dhalla, P. V. Sulakhe, R. L. Khandelwal and I. R. Hamilton. Life Sciences Vol. **9** (1970) p. 625.
2 M. L. Entman, G. S. Levey and S. E. Epstein. Circ. Research. Vol. **25** (1969) p. 429.
3 A. G. Gilman. Proc. Nat. Acad. Sci. Vol. **67** (1970) p. 305.

periments were planned to determine if the increase in cardiac contractility caused by infusing relatively small doses of phentolamine into animals is accompanied by significant changes in myocardial oxygen consumption, efficiency and high energy phosphate stores, and in the plasma levels of noradrenaline. The results obtained showed that relatively small doses of phentolamine cause a significant increase in heart rate, ventricular contraction, myocardial oxygen consumption, coronary blood flow and plasma noradrenaline levels. At dose levels > 10mg/kg/min phentolamine caused a reduction in cardiac efficiency, irrespective of whether the resistance in the outflow tract was maintained constant or allowed to increase.

Probably the increase in heart rate and force of contraction caused by phentolamine reflects the presence of a raised plasma noradrenaline level. The phentolamine-induced increase in myocardial oxygen consumption therefore involves a component which can be linked with the known calorigenic effect of the catecholamines. When relatively large doses of phentolamine were used the cardiac stores of high energy phosphates were significantly reduced, whilst the levels of inorganic phosphates increased, suggesting that at high doses (> 20mg/kg/min) phentolamine does cause an imbalance between rates of energy release and energy utilization. At low doses (5mg/kg/min) however, this effect was absent although heart rate and peak tension developed by the left ventricle were increased.

Although considerable care is needed when extrapolating laboratory findings to clinical situations the results obtained during these particular studies indicate that the proposed use of large doses of phentolamine in the treatment of shock, irrespective of whether this be either of cardiogenic or haemorrhagic origin, may be accompanied by the depletion of the cardiac high energy phosphate stores, by a raised plasma noradrenaline level and by a reduction in cardiac efficiency.

β-Receptors.

Many β-adrenoceptor antagonists display agonist activity, particularly at relatively low dose levels. Dose-response curves which express the agonist activity in terms of an increase in heart rate are available, but similar dose-response curves expressing this agonist activity in terms of positive inotropic activity are not available. Therefore experiments were undertaken to establish the relative agonist potencies of a series of β-adrenoceptor antagonists using inotropic responses of isolated trabecular muscles as an index of agonist activity. These experiments showed that the agonist activity of K01366 > LB46 > oxprenolol > practolol. Propranolol did not display any agonist activity. The dose-response curves obtained for the inotropic activity of LB46, K01366, practolol and oxprenolol were flatter than those obtained for isoprenaline. They were displaced to the right by 0.5mg/kg propranolol, indicating that this positive inotropic activity reflected β-receptor stimulation. Pretreatment of the trabecular muscles with phenoxybenzamine did not significantly change the shape of the dose-response curves, indicating that the unusual shape of these curves, and in particular their flatness, cannot be explained in terms of tissue uptake of the drug.

Effect of Stellate Ganglion Stimulation. Provided that β-adrenoceptor antagonists are absent stellate ganglion stimulation increases the amount of noradrenaline present in the coronary circulation and increases coronary blood flow. The plasma concentration of noradrenaline increased from a control value of 1.08± 0.36ng/ml to a level of 2.06±0.24ng/ml after 30 seconds of stimulation. This increase was significant ($p < 0.001$). LB46, oxprenolol, K01366 and practolol all resembled propranolol in that their addition in equipotent β-blocking doses all resulted in a slight reduction in the rate at which the myocardium utilized oxygen. With the exception of practolol these β-antagonists all evoked a significant fall in coronary blood flow. Each of the β-antagonists used in this study, including practolol, enhanced the calculated index of cardiac efficiency, irrespective of whether or not the particular antagonist used possessed agonist activity.

The effect of relatively high doses of either propranolol, LB46 or oxprenolol on the increased levels of noradrenaline found in coronary venous blood after 30 seconds of stellate

ganglion stimulation was determined, using dogs on right-sided cardiac bypass in which coronary blood flow was artificially maintained at a constant level. The results showed that relatively large doses of either propranolol, LB46 or oxprenolol all significantly reduced the effect that stellate ganglion stimulation had on the noradrenaline content of coronary venous blood. On a weight basis propranolol appeared to be more active in inhibiting noradrena'ine release than did LB46 or oxprenolol; practolol produced only minor changes at the highest dose levels (3mg/kg) used. This inhibitory effect that the β-adrenoceptor antagonists exert on noradrenaline release in response to stellate ganglion stimulation possibly may be involved in the mechanisms which underly the successful use of these drugs in the treatment of angina pectoris.

Salbutamol and Orciprenaline

In recent years there has been an unexpected increase in the incidence of sudden death in asthmatic patients and it has been suggested that this may be correlated with the use of pressure-packed aerosols containing metered doses of various bronchodilator substances, including isoprenaline, orciprenaline and adrenaline. Now another bronchodilator substance has recently been introduced — salbutamol (2 - t - butylamino - 1 - [4 hydroxy - 3 - hydroxymethyl] phenylethanol) (Ventolin). Experiments were carried out therefore, to study the effects of this new drug, and orciprenaline as a representative of the older group, on ventricular function.

The results obtained showed that orciprenaline had a significantly greater effect ($p < 0.001$) on heart rate and left ventricular contractile force than did salbutamol. These effects of orciprenaline were accompanied by a marked increase in the rate at which the myocardium extracted oxygen from the coronary circulation, so that the efficiency with which the left ventricle performed mechanical work was reduced. After salbutamol, however, ventricular efficiency was slightly increased. Both drugs reduced peripheral vascular resistance. The decline in left ventricular efficiency caused by orciprenaline was accompanied by a reduction in the ventricular high energy phosphate stores so that the levels of adenosine tri — and creatine phosphate fell, whilst the levels of inorganic phosphate increased.

After equipotent bronchodilator doses of salbutamol had been given the myocardial high energy phosphate stores were not depleted, a finding which is compatible with the fact that this bronchodilator drug has little effect on heart rate or force of contraction, and that it does not lower ventricular efficiency.

These results indicate that the salbutamol-induced changes in cardiac function differ significantly from those caused by orciprenaline. The reduction in efficiency caused by orciprenaline may indicate that the orciprenaline-induced increase in heart rate and left ventricular force of contraction involves an energy wasting process. The failure of salbutamol to depress cardiac efficiency possibly reflects the absence of this "oxygen-wasting" effect.

In general these results confirm an earlier conclusion that salbutamol selectively stimulates the β_2 receptors. In contrast orciprenaline lacks this marked selectivity and therefore stimulates the β receptors mediating an in crease in heart rate and force of contraction, as well as the β_2 receptors — mediating broncho and vasodilation. Probably the order of potency for selective β_2 receptor stimulation follows the sequence: salbutamol > orciprenaline > isoprenaline.

CARDIOACTIVE PEPTIDES

T. E. Lowe, E. Mäsiar, P. Mäsiar and W. G. Nayler

A number of inotropically active fractions can be obtained from heparinised blood plasma by means of selective membrane ultrafiltration. These active principles are found in fractions containing substances of molecular weight from 2,000-10,000.

Studies this year have been directed towards purification of these principles and their chemical characterisation.

Purification

The inotropically active fractions obtained by membrane ultrafiltration were combined and then subjected to Sephadex gel filtration using distilled water, Ringer-phosphate buffer (pH7) and 0.1N ethyl morphaline HCl buffer. This enabled four inotropically active fractions to be prepared and these were further purified with high voltage paper electrophoresis in pH 1.85 and pH 4.7 buffer systems. Two dimensional paper electrophoresis or chromatography gave good isolation but losses of active material were high. Electrofocusing techniques were not helpful because the active substances formed complexes with ampholites.

More recently a procedure using ethanol fractionation has been successfully used to isolate a substance with λ=400nm.

Chemical Characteristics

All of the active fractions prepared absorbed light in the ultraviolet region and two of them had absorption bands in the visible region. In watery solution the absorption maxima were λ=400 nm and λ=320 nm.

The substance with λ=400 nm displayed a maximum fluorescence at 466 nm when activated at 280 or 410 nm. This substance in acidic pH (0.1N HCl) gave λ=380 nm. Acid hydrolysis (6N HCl at 110° for 18 hours) destroyed this substance and resulted in a solution with many absorption bands

(a) in water between 240 and 260 nm

(b) in 0.1N HCl between 245 and 260 nm.

The proportions of naturally occurring amino-acids found by analysis of the purified active fractions indicate a peptide nature for the active substance but the light absorbence studies indicate that it is not a simple peptide.

It is considered that it is likely that an organic compound of some type is attached to some point on the peptide amino acid chain.

Cardiac Muscle

As from time to time it has been reported that cardioactive substances are concentrated within the myocardium (Haberlandt's heart hormone is an example), it was considered desirable to compare any such substances with the cardioactive plasma substances described above.

By selective fractionation of heart muscle homogenate seven fractions have been isolated in the MW range 2,000-10,000 which resemble the components isolated from plasma. Light absorption maxima noted were:

λ=320-340, 400 and 467 nm.

In dog cardio-pulmonary bypass preparations fractions I and II were shown to have vasodilator activity.

E. Cooper, G. C. Shardey[1], D. J. Davies [2] and G. R. Stirling

CARDIAC TRANSPLANTATION

During 1969, 1970 and 1971, an ongoing programme of the study of cardiac allograft rejection has been pursued. For the first two years, production of a heterotopic neck implant to study the effects of untreated and treated cardiac allografts was the main emphasis. During 1971, however, the main emphasis has been given to the preparation and maintenance of an orthotopic canine preparation, both treated and untreated.

From the first two years work, it was evident that unmodified cardiac allografts and also, to a certain extent, allografts treated with immunnosuppressive therapy presented a pattern of rejection. Electrocardiographic arrhythmias, particular degrees of heart block, sinus arrest and nodal rhythm were found to occur early in the rejection phenomenon. Histological confirmation of small round cell infiltration of the sinus and the atrio-ventricular nodal areas was obtained. This infiltration could occur within the first three days following transplantation. Immunocytic cellular infiltration of juxtanodal autonomic neural ganglia was also found to be intense and was also present in the early stages. It was thought that this might be a preferential selection phenomenon, perhaps due to a different type of antigenic makeup of the excitatory and conduction tissues of the heart. However, studies carried out this year by one of us on electronmicroscopy of the excitatory and conductive tissues of the heart undergoing rejection, have suggested that this is not a preferential attack due to any difference in construction of these tissues from other heart tissues, but is probably related to the increased vascularity of the excitatory tissues of the heart. This supposition is based on the fact that surrounding normal cardiac muscle was as infiltrated as the excitatory tissues and also the septal fat and fibrous tissue also appeared to be equally densely infiltrated. Thus, while there is preferential infiltration of the excitatory tissues, it would appear to be not due to any different antigenic makeup from the remainder of the cardiac tissues.

This observation led us to try and prevent rejection by treating orthotopically prepared dogs, on the basis of development of any sinus arrhythmia or for that matter any persistent ventricular arrhythmia during the early stages following transplantation. The animals were placed on a small maintenance dose of azothiaprene 1-3 mg/Kg body weight and hydrocortisone was used as an additive suppressive agent to be increased when the arrhythmias were observed. By carrying out this plan and without increasing the azothiaprene and without using any further immunosuppressive agents, it was possible to keep orthotopic dogs alive up to six weeks until death usually occurred from superadded infection. Examination of the hearts of these dogs showed very little in the way of severe rejection.

This method of treatment is slightly different from that carried out in standard cardiac transplantations as performed overseas, where treatment is more closely related to the height of the R wave in the electrocardiographic tracings. It was noticed also that the height of the R wave quite often varied day by day without increasing or altering the immunosuppressive dosage. Reductions in this height did not always mean rejection. However, we feel now that the occurrence of arrhythmias is a certain indication that rejection is proceeding at an increased pace. We have been disappointed not to gain longer term survivors. However, this appears to be a problem of superadded infection due to the depression of the immunocytic responses of these dogs, and not because the heart has been rejected.

It wou'd appear therefore, that a rationale of immunosuppressive therapy can be worked out based on firstly, the electrocardiographic presence of arrhythmias, secondly on the height of the QRS waves and thirdly, on the exercise

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2 Department of Pathology, Monash University, Alfred Hospital.

performance of the treated animals. In our hands these would appear to be the three most specific indicators of rejection. We have ceased to measure the concentrations of serum lactic dehydrogenase (LDH) and other enzymes supposedly specific for heart muscle. Also, we pay very little attention now to the overall white cell count, although this is still measured daily. The latter tests did not correlate with our other observed indicators of rejection.

During the period of this experiment we have noticed in observing the electrocardiograms of our dogs that in the case of heterotopic neck implants there was a high incidence of synchronisation of the rate of the donated heart with that of the recipient's heart rate. Also, as seen in the donor heart during rejection, when degrees of early conduction defects were produced between the atrium and a ventricle, periods of atrio-ventricular synchronisation would occur. Because it was postulated that this phenomenon is associated with a reflex arc from the aortic arch involving the vagus nerve we felt it was interesting to investigate this phenomenon in the denervated neck implants. Thus, a series of experiments has been prepared and will be carried on in 1972, investigating the factors behind atrio-ventricular synchronisation in denervated hearts. Our preliminary work has suggested that this phenomenon is closely allied to the flow and pulse pressure of blood through the coronary arteries, particularly those supplying the sinus and atrio-ventricular nodes. A preparation has been developed in which an animal can be placed on by-pass and also have a heterotopic heart attached to the carotid artery and jugular vein in the neck. By this method we can produce variable flows, pulse pressures and by blocking the bundle of His we can produce various rates of ventricular contraction by artificial pacing. Also, it is possible to divide the donated atrium from the donated ventricle, thus having an isolated atrial preparation. The experiments at present are only in the preliminary phases.

PULMONARY TRANSPLANTATION

Several lung transplants have been carried out in an endeavour to see whether our method of treating rejecting hearts could be applied to the rejecting lung transplant. Moderate success has been obtained, in that lung transplanted dogs have survived up to nine days. However, these animals had a normal contralateral lung. In a further series of experiments of three dogs, who had been previously pneumonectomised and then the remaining lung transplanted, only one dog survived 24 hours. This one survivor has given us encouragement to pursue this rather difficult problem during the coming year. The problems associated with lung transplantations are not just rejection, but are mainly due to pulmonary vascular alterations and changes in lung compliance.

PERIPHERAL VASCULAR DISEASE

A. J. Barnett, I. A. Ferguson[1], G. C. Gill, D. Rosengarten[2], and K. Stuchbery

ARTERIAL SURGERY

I. A. Ferguson, D. Rosengarten, K. Stuchbery, and A. J. Barnett.

The interest engendered by projects relating to peripheral vascular disease over the past 20 years has culminated in the development by the Hospital of a vascular service to deal with the increased demand for arterial surgery. All the procedures mentioned in the last report are still being used but with growth of experience there is an increased proportion of the more difficult ones such as aorto-iliac bypass and grafts associated with endarterectomy. It is

now unusual for a patient requiring surgery for the relief of severe symptoms to be considered as unsuited for such treatment because of the state of his vessels or technical difficulties. An example of the manner in which such problems may be overcome in a particular case is the insertion of a graft running from an axillary to a femoral artery in a patient with severe leg ischaemia associated with bilateral femoral artery occlusion and whose general condition precluded an aorto-femoral bypass.

The surgical procedures used in the last 123 patients with arterial disease producing lower limb symptoms are listed in Table 1.

TABLE 1

Type of Procedure	No.
Aorto-iliac or aorto-femoral bypass graft	15
Aorto-iliac endarterectomy	13
Femoro-popliteal bypass graft using autologous vein	46
Femoro-tibial or femoro-peroneal bypass graft using autologous vein	7
Femoral, or popliteal endarterectomy	16
Embolectomy and thrombectomy	13
Disobliteration and repair operations involving previous grafts	8
Other by-pass procedures (iliopopliteal, axillo-femoral)	2
Profundaplasty	2
Repair of arterio-venous fistulae	1
Total	123

In addition to arterial surgery for problems of the lower limbs considerable effort is being directed to other regions, for example: carotid endarterectomy, repair of abdominal aneurysms and renal artery surgery.

PATHOGENESIS

A. J. Barnett and G. C. Gill

Clinically important occlusive arterial disease affects three main regions — cerebral, cardiac, lower limbs — and is usually due to atherosclerosis producing narrowing of the lumen and eventual occlusion of arteries. The pathogenesis of atherosclerosis has been the subject of many studies, particularly of a biochemical nature. These latter have mainly revolved about lipid disturbances in the plasma and their modification by dietary control of the intake of fats. More recently attention has been directed to disturbances in carbohydrate metabolism and the effect of dietary control of carbohydrate, particularly sugar, intake.

Most of these investigations have been made on patients with coronary artery disease, and obesity, high fat intake, high plasma cholesterol level, hypertension, lack of exercise and smoking have been identified as risk factors. In general these conclusions have been extrapolated to all atherosclerotic arterial disease.

In order to check the validity of this extrapolation a study based on patients with peripheral arterial disease was commenced and has two aims. First, to seek any aberrations in the metabolism of fat and carbohydrate in the same subjects and to assess the relative importance of any changes. Secondly, to determine whether "risk" factors similar to those in coronary artery disease occur in peripheral artery disease.

Check sheets have been devised for recording relevant data from all new patients with peripheral occlusive arterial disease who are under the age of 65 years. Data are entered regarding family history, obesity, blood pressure, dietary habits, smoking, exercise, plasma lipid and blood sugar concentrations. Table 2 summarises data concerning lipid and blood sugar concentrations of the patients studied.

(See Page 38 for Table 2)

[1] Honorary Surgeon to Outpatients, Alfred Hospital.
[2] Honorary Assistant Surgeons, Alfred Hospital.

TABLE 2

Concentration of	"Normal" value (mg/100ml)	Number of patients with Normal level	Raised level	Total
Plasma cholesterol	⩽ 250	48	16	64
Plasma triglyceride	⩽ 150	29	34	63
Fasting blood glucose	⩽ 110	34	23	57
Peak blood glucose after 50G oral glucose	⩽ 180	14	27	41
Blood glucose 2 hours after 50G oral glucose	⩽ 120	13	28	41

"Normal" values for glucose are those used by diabetic clinics in the diagnosis of diabetes; "normal" values for cholesterol and triglyceride are those stated by Fredrickson.

It is interesting to note that in this series "normal" plasma cholesterol and fasting blood sugar concentrations are more common than "normal" triglyceride or stress glucose concentrations.

HYPERTENSIVE STATES

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

THERAPEUTIC TRIAL

In 1950 a trial of drug therapy in severe hypertension was commenced. The trial was closed at the end of 1968 at which time 190 patients had been admitted to the series. Data collection finished at the end of 1969, since when the information has been put on to punch cards and is being analysed with the assistance of P. Howell of the Monash University Computer Centre.

Last year some of the data and correlations were presented in tabular form and the tables included here extend these in similar form.

TABLE 3 DATA ON PRESENTATION FROM 190 PATIENTS

Age (years)	Under 20	20-29	30-39	40-49	50-59	Over 60	Total
Male	1	3	15	37	35	9	100
Female	—	6	15	32	34	3	90
Total Numbers	1	9	30	69	69	12	190

Type of Hypertension	Benign	Malignant	Renal	Non-Renal
Male	76	24	11	89
Female	75	15	13	77
Total Numbers	151	39	24	166

Blood Pressure

Systolic (mm Hg):	160-179	180-199	200-219	220-239	240-259	260 & over
Numbers	3	21	34	58	42	32
Diastolic (mm Hg):	110-119	120-129	130-139	140-149	150-159	160 & over
Numbers	16	33	48	39	34	20

TABLE 4 CORRELATIONS

Feature Studied	Sex	% Survival at (Years) 1	2	5	10	No. of Patients at Start
Systolic B.P. (mm Hg)						
160-239	M	91	83	68	43	
	F	98	95	88	73	
	M & F	94	89	76	54	116
Over 239	M	75	77	47	29	
	F	81	65	59	56	
	M & F	78	70	53	32	74
Diastolic B.P. (mm Hg)						
110-139	M	89	82	65	39	
	F	98	90	86	62	
	M & F	93	86	73	48	97
Over 139	M	82	77	55	35	
	F	82	73	68	45	
	M & F	82	75	61	40	93
Heart Size						
Normal or slightly enlarged (C.T.I. *less than 0.53)		90	83	69	47	134
Moderately or severely enlarged (C.T.I. greater than 0.53)		82	77	60	36	55

*Cardio-Thoracic-Index.

In general the results are similar to those shown with the smaller series of 100 patients presented in the previous report.

The worse prognosis with the more severe elevation of blood pressure applies both to the systolic and diastolic pressures. In general hypertensive women have a better prognosis than hypertensive men. This is most pronounced in the group with the lower systolic pressures (160-239 mm Hg).

HAEMODYNAMIC STUDIES

(with the assistance of A. Huckfield and K. Harvey)

Although satisfactory control of a raised blood pressure in any particular patient can usually be obtained by clinical trial of various drugs it is believed that a knowledge of the cardiac output and calculated peripheral resistance could help to assess in advance appropriate therapy. This is especially so now that drugs which selectively block α or β adrenergic nervous activity in the cardiovascular system are available.

To overcome many of the problems associated with dye dilution methods of determining cardiac output a study has been made of methods using thermal dilution for this purpose and considerable progress has been achieved by the technical staff of the Institute in designing and constructing the necessary apparatus for this technique.

CLINICAL PHARMACOLOGY

F. R. Trinker, A. J. Barnett, G. Gill and T. E. Lowe

Clinical pharmacology is the name given to a rather diffuse group of studies directed to an understanding of what drugs do to the human body and what the body does to drugs. There is therefore considerable overlap between this discipline and clinical therapeutics, physiology, pharmacology and bio-chemistry. A number of projects reported upon this year (see p. 33) could be classed as clinical pharmacology but are more appropriately described as part of larger projects. The two reports which follow however have a direct clinical import, the first refers to a drug trial in man and the second illustrates the need to use animal models to provide basic data for a study of drug interaction in man.

β-BLOCKING DRUGS

A. J. Barnett

Many hypotensive drugs act either directly or indirectly on the α-adrenergic parts of the sympathetic nervous system and there have been reports that in some hypertensive patients drugs which block β-adrenergic receptors have a hypotensive action. A small trial to test the efficacy of combining α and β blocking drugs has therefore been carried out over the past two years as suitable patients have presented.

The β-blocking drugs propranolol and oxprenolol were added to the patient's previous treatment and the dose gradually increased until a response was obtained or the dose reached 160 mg three times a day. In each patient in the series existing control of blood pressure was inadequate or the drugs being used were producing undesirable side effects and none had any evidence of cardiac failure. A favourable response to the added drug would be either better blood pressure control or reduction in the necessary dose of drugs previously being used.

Table 5 presents the results seen in 14 patients.

TABLE 5

Drug	Response to Drug			
	Good	Fair	Poor	Total
Propranolol	1	1	6	8
Oxprenolol	—	1	5	6
Total	1	2	11	14

The numbers refer to numbers of patients

In most of the patients in this series who had inadequate control of blood pressure with α-blocking drugs no advantage was gained by adding β-blocking drugs to the therapeutic regimen.

DRUG INTERACTIONS

F. R. Trinker

Guanethidine and Clonidine

The efficacy of clonidine as a hypotensive agent has been demonstrated but in common with most hypotensive drugs it is often necessary when treating hypertensive patients to combine it with other hypotensive agents in order to obtain optimal control of blood pressure. To provide basic data as to whether when two such drugs e.g. clonidine and guanethidine, are present together the resultant cardiovascular response shows any drug interaction the circulatory response in the dog has been studied.

(a) When guanethidine is administered intravenously in a dog there is initially a rise in blood pressure, heart rate and ventricular contractile force followed by a sustained fall in arterial blood pressure and heart rate. This hypotensive action is enhanced if the dog is placed at an angle of 45° to the horizontal and this posture has been used throughout this study.

Small doses of clonidine injected into a vertebral artery produce a fall in arterial pressure and heart rate and also a slight decrease in cardiac contractility. When injected intravenously the peripheral vasoconstriction minimises this central hypotensive action. The simultaneous exhibition of guanethidine and clonidine does not alter these actions of either drug.

(b) Guanethidine markedly depletes tissue catecholamine stores but clonidine does not do so. After confirming that these actions occurred in the rat, rats were pretreated with both clonidine and guanethidine. It was found that in these circumstances the catecholamine depleting action of guanethidine is attenuated in comparison to a control series given only guanethidine. The mechanism of this interaction is obscure.

(c) Currently the action of clonidine on the adrenergic neurone blocking action of guanethidine is being investigated.

Tricyclic Antidepressant and Hypotensive Drugs

There is evidence that the tricyclic antidepressant drugs reduce the effectiveness of antihypotensive drugs such as guanethidine. To investigate this interaction imipramine and amitriptyline have been used and studies made in dogs, rats and guinea pigs.

When administered intravenously in dogs both imipramine and amitriptyline produce dose-dependent falls in blood pressure associated with a small increase in heart rate with low doses but a bradycardia with high doses. Changes in cardiac contractile force parallel the changes in heart rate.

(a) The initial sympathomimetic effect of guanethidine i.e. its noradrenaline releasing action, was manifested by rises in systemic pressure heart rate and myocardial contractility. Small doses of imipramine and amitriptyline readily diminished the sympathomimetic actions of guanethidine which were abolished by higher doses of the antidepressants.

The hypotensive action of guanethidine however, appeared to be far more resistant to antagonism by these drugs, although considerable variation existed between dogs as to the

dose required to achieve this effect. A 40-90% reduction in the hypotensive response to guanethidine required 2 to 8 fold differences in the dose of amitriptyline given. Imipramine was less potent than amitriptyline in this respect. Whether the ability of the tricyclic antidepressant drugs to reduce the sympathomimetic action of guanethidine more readily than its hypotensive effect may be a function of time remains to be elucidated.

(b) Guanethidine decreases the heart rate and increases the contractile force in perfused rat and guinea pig heart preparations. With increasing doses a secondary myocardial depressant effect was observed. Imipramine and amitriptyline produced a small transient increase in contractility followed by a prolonged diminution in cardiac contractile force. Furthermore even small doses (200 to 500 mg/ml) of the tricyclic antidepressants frequently evoked cardiac arrhythmias.

(c) The interaction of guanethidine with the tricyclic antidepressants with respect to its adrenergic neurone blocking and catecholamine depleting actions is yet to be investigated.

CORONARY REACTIVITY

I. McInnes

In a special preparation which was deve'oped to allow study of coronary reactivity under carefully controlled conditions, the coronary circulation was provided with a bypass, separate from a systemic bypass circulation, which was perfused under conditions of constant pressure. In both circulations pO_2, pCO_2 and pH were carefully controlled. Left ventricular contractility was monitored by means of a Walton-Brodie strain gauge arch attached to the ventricular wall.

In this preparation it was consistently found that left ventricular function was most effectively maintained if homologous plasma was used to dilute the blood in the coronary circulation, and least effectively maintained if dilution was made with saline.

It was found that relatively small doses of aminophylline injected directly into the coronary circulation abolished or significantly reduced ($p < 0.001$) the vasodilator responses to adenosine, glyceryl trinitrate, persantin and hypoxia. The vasodilator effect of clonidine, however, persisted. This inhibitory effect of aminophylline on the reactivity of the coronary circulation persisted after the positive inotropic and chronotropic effects of the drug had disappeared.

CARCINOGENESIS**

G. C. Hard and M. Shaw

A Model of Tumour Development

Certain chemical carcinogens including the environmental nitroso-compound, dimethylnitrosoamine (DMN), induce in the rat kidney two forms of cancer, adenocarcinomas and mesenchymal tumours. The latter have been erroneously classified as nephroblastoma but they constitute a vascular, sarcomatous neoplasm. This situation in the kidney is somewhat unusual as manipulation of the animal's diet enables a single dose of DMN to induce malignant neoplasms in the kidneys of all rats that survive the acute toxic effects of the agent. Surprisingly little is known of the course of events which take place during the induction of any neoplasm even from the aspect of functional pathology, so that a system which produces a resulting cancer in 100% of cases from a single dose of carcinogen is likely to provide a most suitable experimental model for such studies. Uti'ising this system, an attempt has been made to define at both light and electronmicroscopic levels, the histopathological process by which DMN induces renal cancer. Essential adjuncts to the study have been the technique of perfusion fixation of the kidney and a method whereby whole slices of this organ can be prepared for electronmicro-

scopy so that infrequent and small lesions can be identified by the light microscope and the relevant area of fixed tissue selected for examination in the electronmicroscope.

The results have demonstrated a sequence of events which precede and lead to the appearance of macroscopic tumours in the kidney. These may be described very briefly as mild degenerative changes in cortical epithelial cells and in periglomerular fibroblast-like cells. An increase in mitotic figures in the cortical interstitium is followed by a generalised inflammatory response throughout the cortex which quickly subsides. However, periglomerular aggregates of cells persist beyond this acute phase but become less frequent with time. For the first 8 weeks these lesions are composed predominantly of lymphocytes, plasma cells and macrophages, associated with occasional, abnormal, fibroblast-like cells. Beyond 12 weeks the lesions assume the form of microscopic but unequivocal tumour cell aggregates made up of fibroblast-like cells. These rapidly develop into macroscopic, mesenchymal tumours by 20-25 weeks.

Adenacarcinoma development is preceded by proliferation of lining cells of tubules which either obliterate the tubule lumen or project as papil'ary fronds into the lumen. Such lesions are most frequently seen after 12 weeks, but occasionally occur as early as six weeks. They invariably involve tubules located next to glomeruli and are thus related to the section of tubules most severely affected by the chemical agent in the acute phase of intoxication. Ultrastructural examination of early proliferative lesions identifies many of them as proximal tubule in origin.

The research currently being developed is designed to interpret the histopathological findings described above in immunological terms and to explore further the significance of the sequential changes. The cellular kinetics of the lymphoreticular system will be followed during the course of tumour induction and the findings correlated with the known renal alterations. Experiments are currently being set up to investigate the effects of immunosuppression, mediated by neonatal thymectomy and treatment with antithymocyte globulin, upon the sequence of events leading to tumour development. Radioautographic techniques will be employed to explore the relationship, if any, between the ce ls responding in the acute phase and the appearance of abnormal cells in later, persisting lesions. Experiments also have been designed to determine whether alternative renal tumour-inducing agents such as ethylmethane sulphonate (mesenchymal tumours) and diethylnitrosamine (adenomas and adenocarcinomas) induce similar sequential alterations throughout the induction phase of neoplasia as does DMN. In addition, an attempt is being made to set up a model utilising 7, 12 dimethylbenz(a)anthracene whereby the development of the tumour classified as nephroblastoma can be elucidated.

Tissue Culture Studies

In correlation with the *in vivo* stages of DMN-induced renal tumour development, renal cortex cells in culture are being studied to determine the stage at which transformed cells can be isolated *in vitro* from the kidneys of carcinogen treated rats. Preliminary results suggest that within one week following a single *in vivo* dose of DMN, clones of renal fibroblasts develop *in vitro* which demonstrate morphologic and growth characteristics altered from the normal. As the experimental model for producing renal tumours upon which all these studies are based has involved only random-bred rats, various inbred lines are now being investigated to determine which strain will prove the most suitable in terms of susceptibility to the carcinogen. This is necessary because the ultimate test for malignant transformation of cells cultured *in vitro* is tumour production following transplantation of suspect cells into syngeneic hosts. The aim of the tissue culture studies is twofold; firstly, to set up a system which will enable the biochemical analysis of relatively pure populations of precursor tumour cells, and secondly, to provide a system for the *in vitro* investigation of the role of cellular immunity in chemical carcinogenesis.

Comparative Renal Tumour Morphology

A comparative study of the ultrastructure of renal tumours of a variety of species will be undertaken, particularly those of the nephroblastoma group. It is possible that the resulting information may throw some perspective upon the relationship that experimental tumours currently under study may have to human counterparts. Such comparative information is needed if the results gleaned from experimental models in laboratory animals are to be extrapolated to the process of carcinogenesis in man.

ELECTRON MICROSCOPY

A. Chang

The electron microscope laboratories came fully into use in the last quarter of the year and their facilities are being used extensively by various project groups in the Institute and Hospital (see pp. 43 and 71).

The Hitachi HU-11E electron microscope installed is capable of magnification up to 300,000 times, with the limit of resolution at 4.5. This instrument and the accessory equipment installed are more than adequate for the current research projects requiring the microscope for ultrastructural studies.

SCLERODERMA

A J. Barnett and B. Bialkower[1]

The clinical study of scleroderma has continued and there are now follow-up data on 70 patients seen over the past 20 years.

The clinical and pathological features of this disease were well established and the broad classification of the various types is generally accepted, but there is still some confusion concerning the use of terms.

However, the aetiology and pathogenesis remain obscure, but as the condition is considered to be a disease of connective tissue and histological study indicates a changed morphology of connective tissue, it has been considered that some clues to its nature might be obtained by biochemical study of the connective tissue. This tissue in addition to cells contains two chief components: collagen and ground substance.

We have commenced a biochemical study of the dermal connective tissue in scleroderma using skin collagen content as the starting point. Specimens of skin from patients with scleroderma have been obtained from a dorsum of the finger (all clinically affected) and from the dorsum of the forearm (sometimes clinically affected, sometimes apparently normal) and the collagen content determined. The results are being analysed.

Prior to the removal of the tissue, the stretch of the skin in response to a lateral force produced by the application of spring loaded calipers, is measured. It is hoped that this may be developed into a test of the mechanical properties of the skin, which may be useful in following the progress of therapy.

Because a marked increase in chromosomal abnormalities has been reported in scleroderma Dr. Margaret Garson of the Genetics Laboratory of the Melbourne University Department of Medicine at St. Vincent's Hospital is carrying out chromosomal studies in peripheral blood in a group of our patients.

[1] Department of Biochemistry, Monash University.

BLOOD COAGULATION

P. Fantl

Evolutionary Trends in Plasma Mercaptalbumin Composition

Last year it was reported that mammals could be grouped according to plasma mercaptalbum reactivity in a thiol-disulphide exchange reaction with di(5-carboxyl-4-nitrophenyl)-disulphide (Ndps) at pH 6.5 and pH 7.8. This year the study has been extended to 21 chordates.

Three classes of plasma thiol compounds occur: (i) non-protein thiol compounds; (ii) mercaptalbumin; and (iii) other protein thiol compounds. In our present series animal plasmas contained between 0.2-1.3 μg SH as non-protein thiol compounds per ml of plasma but higher concentrations were found on several occasions in the plasma of lizards and turtles.

Plasma albumin concentrations in fish were low and mercaptalbumin made up about 5% of this fraction. In toads plasma the albumin concentration was also low with mercaptalbumin about 12% of the fraction.

Amongst the Reptilia, in one case plasma from a turtle contained a very high concentration of non-protein thiol compounds although the albumin concentration was low and consisted of mercaptalbumin only.

In lizards on two occasions a very high concentration of non-protein thiol compounds was noticed with the mercaptalbumin concentration as high as that of mammalian blood plasma.

Snakes had a low albumin concentration and mercaptalbumin content varied between 14-55% of the total albumin in contrast to 50-80% in mammalian plasmas.

The observation in some reptilian plasmas of an excessively high non-protein thiol concentration with a correspondingly high mercaptalbumin concentration suggests that the non-protein thiol compounds which probably consist mainly of cysteine, reduced a cystinyl-disulphide bond of the albumin molecule to mercaptalbumin.

When there is a high concentration of mercaptalbumin in relation to the total plasma thiol concentration as is seen in mammalian plasma, it is justifiable to equate the reactivity of Ndps at pH 6.5 with mercaptalbumin concentration. Varying rates of mercaptalbumin reactivity with Ndps were observed. In one group which included pigs and rats a fast reacting mercaptalbumin, as judged by high reaction rates with Ndps both at pH 6.5 and 7.8, suggests location of the thiol group on the surface of the molecule. In a second group which included the majority of the mammalia there was a fast reaction at pH 7.8, but a slower one at pH 6.5. The guinea pig was in a third group with a low reaction rate at pH 6.5 and 7.8. In this the thiol group in the mercaptalbumin molecule is apparently less accessible to Ndps as compared with other mammalia. It is probably buried in the interior of the molecule. The varying reaction rates were confirmed with isolated albumins.

These data concerning mercaptalbumin concentration and reactivity suggest that during the development of chordates two time-dependent changes modified mercaptalbumin structure. The one is an evolutionary trend which makes its first appearance with bony fishes who have a low mercaptalbumin concentration and continues to reach the highest concentration in mammalian plasma. The other change occurs within each order of animals. In each the early phases of development are associated with a mercaptalbumin in which the thiol group is located on the surface of the molecule. This readily accessible thiol group has a high reactivity in the disulphide exchange reaction. It occurs in the oldest members of that particular family e.g. pig, rat. In more recent development the thiol group has become less accessible, as in most mammals. Finally the thiol group becomes buried within the mercaptalbumin molecule with a consequent low reaction rate in the thiol-disulphide exchange reaction; this was found in the guinea pig.

Thiol Distribution in the Plasmas of Native Australian Mammals

The thiol concentration in the plasma of monotremata is considerably lower than in that of marsupalia. There is some similarity between the thiol concentration of platypus plasma and that of bony fishes and reptilia. These latter have a total protein concentration and albumin distribution comparable to that of mammalia, but their mercaptalbumin concentration is lower. This differs from bird's plasma which has a low mercaptalbumin concentration, that is however approximately 70% of the total albumin.

The marsupalia had a thiol distribution and mercaptalbumin concentration quite similar to that of mammalia. It is interesting to note that two types of mercaptalbumin were observed. Wombat mercaptalbumin contained a thiol group which reacted quickly both at pH 6.5 and 7.8 with Ndps. This indicates that the thiol group is on the surface of the molecule in a peptide environment readily accessible for reaction with Ndps. This type of reactivity has also been found in the mercaptalbumin of pig and rat. On the other hand, the reactivity of possum mercaptalbumin in the disulphide exchange reaction was similarly as slow as that of mammalia including man, bovidae, monkey and dog.

It is possible that mercaptalbumin concentration and reactivity with Ndps are connected with the place in the evolutionary scale, as was suggested for old world mammals. The position of the wombat as an old member of the family phascolomys and the possum as a younger member of that particular family are in accord with this hypothesis.

MALIGNANT HYPERTHERMIA

W. G. Nayler (in conjunction with F. J. R. Hird and M. A. Denborough)

In some animals and occasionally in man the establishment of anaesthesia with halothane results in the onset of malignant hyperthermia. Some Landrace pigs display this phenomenon. The major chemical change that can be regarded as a primary cause of the phenomenon of hyperthermia is the production of excess lactic acid, which occurs 8-10 minutes after the Halothane has been administered. This is accompanied by a simultaneous drop in blood pH and is followed by raised blood calcium and inorganic phosphate levels.

Experiments performed during the past year have shown that the sarcoplasmic reticulum which has been isolated from the affected pigs differs from that of control pigs in that the activity of its Ca^{2+} dependent ATPase enzyme is significantly greater than that attained by control pigs. At the same time the ability of the reticulum to exchange, but not to bind Ca^{2+}, is greater than that present in the control animals. These findings are consistent with the presence of a higher level of Ca^{2+} in the myoplasm. Possibly the primary defect is in that part of the sarcoplasmic reticulum which is associated with maintaining myoplasmic Ca^{2+} at a level which does not stimulate contraction. Halothane resembles other anaesthetic agents in that it inhibits the uptake of Ca^{2+} by the sarcoplasmic reticulum. The primary defect could be an altered protein involved in calcium transport, an altered structural protein of the membrane or an altered phospholipid system.

1 Department of Biochemistry — Melbourne University.

LABORATORY WORKSHOP

A. Huckfield and K. Harvey

An efficient workshop staffed and equipped to build, modify or maintain special mechanical or electronic laboratory equipment is essential today to the conduct of much basic research.

During the present year a number of pieces of equipment were made or modified. To follow the chemical reactions referred to in the studies of the muscle protein troponin a recording spectrophotometer was modified and linked with a pH-stat so that turbidity changes could be recorded in a stirred reaction vessel (optical cell). This included making for the optical cell a dummy partition, lid and side for the cell compartment which incorporated the cell holder and entry points for pH electrodes and the tube delivering the alkaline fluid. It was found that many components for the stirrer and speed reduction of the auto-burette were most adequately obtained from toys. Considerable improvement in both electronic and mechanical design of Walton-Brodie strain gauges has been possible with the aid of a new instrument lathe. Much time has also been spent on the design and construction of thermistor catheters and electronic equipment for thermal dilution measurement of cardiac output.

PUBLICATIONS IN 1971

PHYSIOLOGY AND PHARMACOLOGY OF CARDIOVASCULAR SYSTEM

Role of Calcium†‡

NAYLER, W. G. and N. C. R. MERRILLEES. — "Cellular Exchange of Calcium in 'Calcium and the Heart'." Academic Press (1971) P. 24.

NAYLER, W. G., N. C. R. MERRILLEES, D. CHIPPERFIELD and J. B. KURTZ. — "Influence of Hyperthyroidism on Uptake and Binding of Calcium by Cardiac Microsomal Fractions and on Mitochondrial Structure." *J. Cardiovasc. Res.* Vol. 5 (1971) P. 469.

NAYLER, W. G., J. STONE, V. CARSON and D. CHIPPERFIELD. — "Effect of Ischaemia on Cardiac Contractility and Calcium Exchangeability." *J. Mol. Cell. Cardiol.* Vol 2 (1971) P. 125.

NAYLER, W. G. and J. SZETO. — "Effect of Sodium Pentobarbital on Calcium in Mammalian Heart Muscle." *Amer. J. Physiol.* In Press.

NAYLER, W. G. and J. SZETO. — "Effect of Verapamil on Contractility, Oxygen Utilization and Calcium Exchangeability in Mammalian Heart Muscle." *J. Cardiovasc. Res.* In Press.

Pharmacology‡

NAYLER, W. G. — "Some observations on the Cardiovascular Effects of Salbutamol with Particular Reference to the Cardiovascular System." *Postgrad. Med. J.* Vol. 47 (1971) Supp. P. 16.

NAYLER, W. G. — "β-Adrenoceptor Agonists and Antagonists." Sandoz Ltd. In Press.

NAYLER, W. G. — "Comparative Inotropic Activity of β-adrenoceptor antagonists. *Brit. Pharmac.* Submitted.

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

NAYLER, W. G. and J. TAY. — "Effect of 0-2-hydroxy-3-(tert. butylamino) propoxybenzonitrile HCl (KO1366) on beta-adrenergic Receptors in the Cardiovascular System." *J. Pharm. Therap.* In Press.

TRINKER, F. R. — "The Significance of the Relative Potencies of Noradrenaline and α-methyl-Noradrenaline for the Mode of Action of α-methyldopa." *J. Pharm. Pharmac.* Vol. 23 (1971) P. 306.

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

TRINKER, F. R. and V. CARSON. — "Pharmacological Effects of H56/28 — a new β-antagonist on the Cardiovascular System." *Cardiovasc. Res.* Vol. 5 (1971) P. 383.

Myocardial Function‡

NAYLER, W. G. and V. CARSON. — "Effect of Phentolamine on Myocardial Function, Efficiency and Noradrenaline Levels in Blood Plasma." *Cardiovasc. Res.* In Press.

NAYLER, W. G. and I. McINNES. — "Salbutamol and Orciprenaline — induced Changes in Myocardial Function." *Cardiovasc. Res.* In Press.

NAYLER, W. G., I. McINNES, J. STONE, V. CARSON and T. E. LOWE. — "Effect of Dopamine on Coronary Vascular Resistance and Myocardial Function." *Cardiovasc. Res.* Vol. 5 (1971) P. 161.

WHEELDON, L. W. and K. GAN. — "Resolution of Fragments of Plasma and Sarcotubular Membranes in Heart Muscle Microsomes." *Biochem. Biophys. Acta* Vol. 233 (1971) P. 37.

BLOOD COAGULATION

FANTL, P. — "Protection from Hg-Inactivation of Factor XIII by Mercaptalbumin." *Thrombosis et Diathesis Haemorrhagica.* Vol. 24 P. 351.

FANTL, P. — "Thiol Distribution and Mercaptalbumin Reactivity in the Blood Plasma of Different Vertebrates." *Aust. J. exp. Biol. med. Sci.* Vol. 48 (1971) P. 631.

FANTL, P. — "Evolutionary Trends in Plasma Mercaptalbumin Composition." *Comp. Biochem. & Physiol.* In Press.

FANTL, P. — "Thiol Distribution in the Plasmas of Native Australian Mammalia." *Aust. J. exp. Biol. med. Sci.* In Press.

MISCELLANEOUS

MÄSIAR, P., D. MORRISON and D. SHAW. — "Two Molecular Forms of an ATP-guanidine Phosphotransferase." *Proc. Aust. Biochem. Soc.* Vol. 4 (1971) P. 78.

LECTURES DELIVERED DURING 1971

"Relative Specificities of Anti-leptospiral Axial Filament Immunoglobulins and their Taxonomic Significance." — *Society for Microbiology, Brisbane.*	A. CHANG
"Contraction of Cardiac Muscle with an Emphasis on the Role of Troponin" — *Hospital Scientists' Association.*	P. DAILE
"Physics and Medicine" — Cecil E. Eddy Memorial Oration, *Australasian Institute of Radiography.*	T. E. LOWE
"The Changing Face of Cancer Research." — *Anti-Cancer Council of Victoria.*	T. E. LOWE
"Some Possibilities to obtain Trace Plasma Peptides by means of Membrane Filtration." — *Australasian Biochemical Society, Brisbane.*	E. MÄSIAR
"Two Molecular Forms of an ATP-guanidine Phosphotransferase." — *Australasian Biochemical Society, Brisbane.*	P. MÄSIAR
"A New Technique for Isolating from Blood Plasma Trace Amounts of Proteins and Peptides with Inotropic Activity." — *Cardiac Society of Australia and New Zealand.*	P. MÄSIAR
"Effect of Antiarrhythmic Drugs on Noradrenaline Release in Response to Sympathetic Nerve Stimulation." — *Cardiac Society of Australia and New Zealand.*	W. G. NAYLER
"Importance of Membrane-bound Calcium in Cardiac Muscle Contraction." — *Australian Physiological Society.*	W. G. NAYLER
"Effect of Salbutamol on Myocardial Function." — *Symposium on Salbutamol, London.*	W. G. NAYLER
"Myocardial Function during β-adrenoceptor Blockade." *University of Singapore.*	W. G. NAYLER
"Mitochondrial and other Studies in Australian Landrace Pigs affected with Malignant Hyperthermia." — *Symposium on Hyperthermia, Toronto, Canada.*	W. G. NAYLER et al.
"Myocardial Uptake of Isoprenaline." — *Australian Physiological Society.*	G. M. PICKEN
"Influence of Sympathetic Nerve Stimulation on Coronary Blood Flow." — *XXV International Congress of Physiological Sciences, Munich, Germany.*	F. R. TRINKER

SEMINARS HELD DURING 1971

Cardiac Muscle Cell (5 May)

Fine Structure of the Cardiac Muscle Cell.	N. C. R. MERRILLEES
Troponin and its Regulatory Role in Contraction.	P. DAILE
Excitation-contraction Coupling.	W. G. NAYLER
Production of Energy by the Cardiac Muscle Cell.	C. L. GIBBS
The Role of Cyclic 3, 5 AMP.	B. JARROTT
Sympathetic Innervation of the Cardiac Muscle Cell.	L. B. GEFFEN
Structure-activity Relationships for Adrenergic Agonists and Antagonists.	G. A. BENTLEY
The Effect of β-Receptor Blockade on Myocardial Function.	F. R. TRINKER
The Effects of Denervation (by Transplantation) on Myocardial Function.	E. COOPER
Review of Seminar.	P. KORNER

β-Adrenergic Blockade (7 October)

Method of Assessing β-Blockade.	J. HAMER
A Comparison of Propranolol and Tripranolol.	G. SLOMAN
Exercise after Myocardial Infarction.	M. ROSENBAUM
Effect of β-Adrenoceptor Blockade on Noradrenaline Release.	W. G. NAYLER
Pharmacology of the β-Receptor.	M. BARRETT
Long Term Effects of some Antihypertensive Drugs on the Sympathetic Nervous System.	G. BURNSTOCK
Effects of β-Receptor Antagonists at Neuro-effector Junctions other than on β-Receptors.	E. MYLECHARANE
An Analysis of Rabbit Sino-atrial Potentials and the Action of Catecholamines.	S. E. FREEMAN and R. J. TURNER

Friday Seminars

Fats, Sugar and Arterial Disease.	A. J. BARNETT
Adenyl Cyclase.	V. CARSON
Leptospiral Axial Filaments.	A. CHANG
New Approaches to Cardiac Surgery including Coronary Artery Grafts.	E. COOPER
Interactions of Actomyosin and Tropomyosin, Troponin and Fractions of Troponin.	P. DAILE
The Nitroblue Tetrazolium Test.	B. DOWTY
Conformation of Mammalian Mercaptalbumins.	P. FANTL
Cyclic AMP Metabolism in the Brain.	S. KATZ
Effect of Aminophylline on the Action of Coronary Vasodilator Drugs.	I. McINNES
The Significance of a Protein and Peptides in Connection with Heart Failure.	P. MÄSIAR
Biochemistry of Muscle Contraction.	W. F. H. M. MOMMAERTS
Energetics of Muscle Contraction.	W. F. H. M. MOMMAERTS
Innervation and Muscle Proteins.	W. F. H. M. MOMMAERTS
The Effect of Isoptin on Excitation-contraction Coupling in Cardiac Muscle.	W. G. NAYLER
The Uptake and Metabolism of Noradrenaline by Cardiac Muscle.	G. PICKEN
Ventricular Function after Bilateral Adrenalectomy.	J. SZETO
The Effect of Rates of Sympathetic Stimulation on Coronary Blood Flow.	F. R. TRINKER

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

Revenue Account for the Year ended December 31, 1971

EXPENDITURE		INCOME		
Salaries and Wages	$162,859	Donations from Baker Benefactions —		
Laboratory Supplies and Isotopes	21,697	Transfers from Restricted Fund		$142,000
Library Maintenance	7,293	Grants in Aid of Research —		
Postage and Telephone	1,736	Anti-Cancer Council	$8,459	
Printing and Stationery	3,848	National Heart Foundation of Australia	16,296	
Light and Power	17,600	Life Insurance Medical Research Fund of Australia and New Zealand	8,500	
Insurance	5,332	The James and Elsie Borrowman Research Trust	4,500	
Repairs and Renewals	6,062	The William Buckland Research Fund	3,000	
Animal House Contribution	4,000			40,755
Sundries	2,952	Interest from Investments —		
Travelling Expenses	1,621	Held by Trustees of The Baker Institute Grant Trust	$1,700	
Public Relations	862	Other Income	33,859	
Electron Microscope Supplies	536			35,559
		Sundry Sales, Recoveries and Refunds		15,789
		Deficit for Year		2,295
	$236,398			$236,398

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

Balance Sheet as at December 31, 1971

FUNDS AND LIABILITIES		
Funds —		
Accumulated Revenue brought forward	$389	
Less Deficit for year	(2,295)	
Accumulated Deficit	($1,906)	
Restricted Fund	88,613	
Endowment Fund	564,664	
Development Fund	15,616	
William Buckland Research Fund	20,870	
Laura Nyulasy Research Scholarship Fund	3,940	
Lang Research Scholarship	4,246	
		$696,043
Current Liabilities —		
Sundry Creditors and Accrued Expenses		6,948
		$702,991

ASSETS		
Fixed Assets (Note 1)		
Investments —		
Held by the Trustees of the Institute:		
Commonwealth Inscribed Stock	$2,937	
M.M.B.W. Stock	7,202	
S.E.C. Stock	6,556	
Treasury Bonds	5,000	
Argo Investments Co. Ltd. Shares	29,806	
Softwood Products Treatment Co. Pty. Ltd. Shares	100	
Short-Term Deposits	72,786	
Mortgage Loans	50,000	
	$174,387	
Held by the Trustees, Executors & Agency Co. Ltd.:		
Laura Nyulasy Research Scholarship Fund	3,940	
William Buckland Scholarship Fund	20,870	
Endowment Fund	464,063	
		$663,260
Current Assets —		
Cash at Bank	$29,320	
Sundry Debtors	10,411	
		39,731
		$702,991

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, 1971, including the building, totalled $1,700,000.

2. In addition to receiving income from investments shown above, the Institute receives interest on $34,000 5% Commonwealth Inscribed Stock, which is held by the Trustees of The Baker Institute Grant Trust for the benefit of the Institute.

AUDITORS' 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, 1971.

PRICE WATERHOUSE & CO.,
Chartered Accountants.

Melbourne. March 30, 1972.

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

Year ended December 31, 1971

DEVELOPMENT FUND

Balance at December 31, 1970		$70,675
Deduct —		
Building Costs	$47,085	
Furnishing and Equipment Costs	7,974	
		55,059
Balance at December 31, 1971		$51,616

RESTRICTED FUND

Balance at December 31, 1970		$148,292
Add —		
Transfer from Estate of Thomas Baker	$258,054	
Income from Investments	10,102	
Further Donations from Victorian Government relating to Electron Microscope and Suite	52,554	
Donations — Other	54,569	
Sundry Receipts	352	
		375,631
		$523,923
Deduct —		
Transfer to Revenue Account	$142,000	
Equipment Costs (including $52,554 for Electron Microscope and Suite)	123,346	
Transfer to the Endowment Fund	169,764	
Payment of Baker Prize (J. Tay)	200	
		435,310
Balance at December 31, 1971		$88,613

ENDOWMENT FUND

Balance at December 31, 1970		$373,528
Add —		
Donations	$21,576	
Transfer from Restricted Fund	169,764	
Sundry Receipts	543	
		191,883
		$565,411
Deduct —		
Losses on Redemption of Investments		747
Balance at December 31, 1971		$564,664

DONATIONS

The following gifts to the Institute were received during the year:—

Victorian Government	$52,554.00
"Baker Birds"	6,033.91
Ian Potter Foundation	3,000.00
Edward Wilson Estate (T. E. & A. Ltd)	2,750.00
Edgar Rouse	2,032.50
Ciba Co. Pty. Ltd.	1,000.00
Sir Wm. Angliss Charitable Trust	1,000.00
Truby and Florence Williams Charitable Trust (T. E. & A. Ltd.)	1,000.00
Alfred Edments Estate (T. E. & A. Ltd.)	750.00
Carlton & United Brewery Ltd	750.00
M. and E. H. Flack Estate	700.00
George F. Little Trust	475.00
J. C. Habersberger	250.00
Mrs. N. Stallman	200.00
Roche Products Pty. Ltd.	200.00
Dr. Arnold Cooper	100.00
Siegfried Meyer	100.00
Pethard Tarax Charitable Trust	100.00
C. A. Gordon	100.00
H. D. Stewart	100.00
W. A. J. Baker	100.00
W. J. Ould	100.00
Stuart Sanderson	75.00
Specialty Press Ltd.	50.00
Mrs. W. Baker	50.00
Sandoz Australia Pty. Ltd.	45.20
Darren Baillieu	32.50
Dodds Consolidated Industries Ltd.	25.00
Yarra Grange Co-op Housing Society	14.03
George Turner Pty. Ltd.	10.00
Miss N. E. Cameron	10.00
Miss C. Gates	10.00
P. and P. Shaw	10.00
N. E. Payne	10.00
Mrs. M. Rae	4.00
D. G. Oakley	2.88
	$73,744.02

Further contributions were received from:—

Eagle Star Insurance Company Ltd., R. Blakemore, Miss N. E. Cameron, Kodak (Australasia) Pty. Ltd., Mrs. Donna Habersberger, Edgar Rouse, Associated Broadcasting Services Ltd., Miss J. and D. Jeffrey, J. C. Habersberger, Goulburn Murray Television Ltd., H. E. Tobin, George E. Knox, Miss J. Hall.

In memory of:

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Totalling $454.80

$74,198.82

During the year the Wellcome Trust made a gift to the Trustees of a Scintillation Spectrometer, costing $15,159.

The Trustees again record their great appreciation of the work of the two Women's Groups, The Baker Birds and "B" Group.

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ANNUAL REPORT

In this past year the 50th anniversary of the discovery of insulin has been celebrated. Although by now the lives of many millions of diabetics have been saved and made useful and for millions more treatment has become convenient with the use of oral hypoglycaemic agents, many of the original problems concerned with diabetes remain unsolved. These include importantly the mode of action of insulin and the integration of its actions on various tissues, the factors regulating insulin secretion and release, the modes of action of oral hypoglycaemic agents and the factors determining the development of the long term complications of diabetes.

In October an International Symposium "The Impact of Insulin on Metabolic Pathways" was held at which the writer was privileged to be present. Although much new work in all these areas, and many others, was reported and discussed, it did not appear that answers to these questions were yet being provided. One of the big problems in study remains the fact that in human diabetes a spectrum of metabolic disorders ranging from asymptomatic hypoglycaemia and glycosuria through to ketoacidosis and diabetic coma is seen. Treatment of diabetes may be by diet, oral agents or insulin and the complications may or may not appear affecting some systems more than others — after a longer or shorter time. The question as yet unresolved is as to whether this clinical spectrum is representative of one or more diseases. Its relationship to insulin secretion, release, circulation and action demands an investigative approach based on the widest possible premises. Hence it was not surprising that 500 representatives of 27 countries met to present their work at this Conference.

It is as always a source of great pleasure to acknowledge collaboration with and assistance from clinical and laboratory colleagues at Alfred Hospital and Monash University. And too to place on record our debt to those who have made and continue to make grants-in-aid and in kind to facilitate our work.

PINCUS TAFT,

Physician in Charge.

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

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GROWTH HORMONE DERIVED POLYPEPTIDES, SOMANTIN (InG) AND CATAGLYKIN (AcG), AND THEIR ROLE IN CARBOHYDRATE AND FAT METABOLISM

P. Zimmet[1], P. Taft, F. Ng, and J. Bornstein

In last year's report, we outlined studies on the possible role of somantin (InG), a growth hormone (GH) derived peptide, in human diabetes. This polypeptide, originally obtained by acid hydrolysis of GH, has *in vitro* actions which simulate the diabetogenic effects of GH i.e. impaired glucose utilisation, impaired fat synthesis, and accelerated lipolysis. The isolation of a polypeptide from blood with similar structure and biological effects suggested that somantin might have a role in glucose homeostasis *in vivo*.

Previous studies have shown that another GH derived polypeptide, cataglykin could reverse the metabolic effects of somantin, and that it caused an increased sensitivity to insulin in both normal and diabetic subjects. Thus it became important to demonstrate whether cataglykin could be isolated from human biological fluids.

A method has been developed to isolate cataglykin from human urine. Ultra-filtrates of urine (either normal or diabetic) were chromatographed on Dowex 50 and CG-50 columns. The polypeptide obtained by these procedures had similar actions to the growth hormone derived cataglykin.

It reversed the inhibition of glyceraldehyde-3-phosphate dehydrogenase induced by somantin from a number of sources (ovine GH, human GH, plasma, urine and synthetic somantin). The polypeptide was absent from the urine of an hypophysectomized diabetic.

Cataglykin from urine was injected into rabbits and was shown to potentiate insulin action *in vivo* during intravenous glucose tolerance tests. A relative hypoglycaemia was seen from 75-180 minutes in rabbits who received cataglykin, as compared to a control group.

Studies have been made on glucose uptake *in vitro* by the isolated rat hemidiaphragm. Plasma somantin causes inhibition of glucose uptake by the isolated diaphragm and this effect can be reversed by the addition of urinary cataglykin to the medium.

At present, studies relating to the action of these polypeptides on fat metabolism are in progress. GH derived somantin has been shown to cause accelerated lipolysis and decreased lipogenesis *in vitro* in the rat epididymal fat pad. Cataglykin reverses these effects, and it is proposed to find out whether the polypeptides from blood and urine have a similar effect.

One of the most important aspects of this study is to be able to obtain meaningful data about plasma levels of somantin in normal and diabetic subjects. The results of a small scale study were mentioned in last year's report and these data were obtained from a bioassay. Work has commenced in order to develop a radioimmunoassay for somantin. One of the main difficulties will be to obtain a completely pure sample of the polypeptide to test for the presence of antibodies, as will be the problem of iodination because the polypeptide contains no tyrosine or histidine.

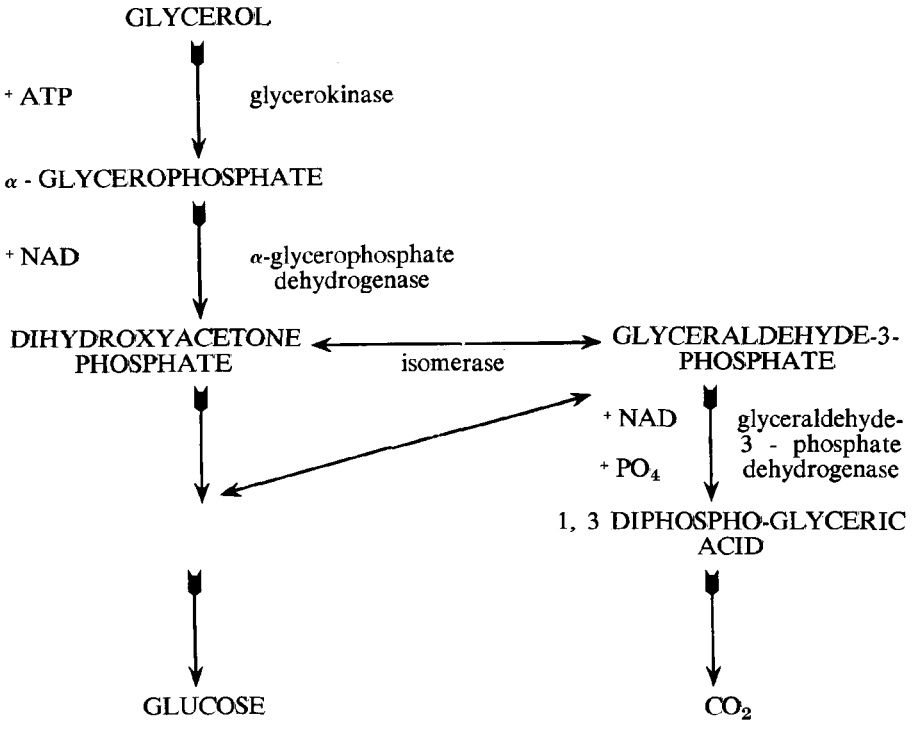
The possible role of these polypeptides in glucose homeostasis and the pathogenesis of diabetes was discussed in last year's report. The work done during this year has provided further evidence for such a role.

[1] N.H.M.R.C. Post-graduate Research Scholar.

GLYCEROL METABOLISM IN DIABETES

J. Herington, P. Taft, M. K. Gould[1] and J. Bornstein[1]

Last year P. Zimmet **et. al** reported the biochemical actions of the Growth Hormone derived peptides InG and AcG.



Two important actions of InG are the ability to inhibit the enzymes α-glycerophosphate dehydrogenase and glyceraldehyde-phosphate dehydrogenase, both of which are important in glycerol, as well as glucose, metabolism. The accepted pathway of glycerol metabolism is as follows:

From this pathway it can be seen that in a diabetic, with high levels of InG, both glycerol conversion to CO_2 and glycerol conversion to glucose would be expected, on the basis of the known effects of InG, to be at least partially inhibited.

As we reported last year, parameters of glycerol metabolism in normal and diabetic subjects have been studied using an injection of radioactive (^{14}C labelled) glycerol, and following the blood ^{14}C glycerol level over a period of time.

1 Many of the biochemical investigations have been carried out in the Department of Biochemistry, Monash University, under the supervision of these authors.

Analysis of these results has shown wide variations in both the normal and diabetic groups and no difference in either the rate of glycerol metabolism or in the rate of conversion of glycerol to glucose is observed.

This year a biochemical explanation for these observations has been sought.

It was postulated that an alternate pathway may exist in liver which, in the case of the diabetic, may have sufficient activity to retain the normal rate of glycerol metabolism whilst by-passing the InG inhibited reactions. Three approaches have been taken to demonstrate this "new" pathway.

The first approach was to isolate the intermediates of both the known and the new pathway from a tissue such as rat liver so that if a pulse of ^{14}C glycerol was applied to the tissue the path it took could be identified by the presence of radioactive carbon in the intermediates.

However the intermediates involved, namely glycerol, glyceraldehyde, glyceric acid, dihydroxyacetone and their phosphates are chemically very similar and this makes their separation by either thin-layer or paper chromatography almost impossible. For this reason this line of investigation has been time-consuming and unsuccessful.

The next approach was to look for the enzymes which would be ncessary for the alternate pathway. Two such enzymes have been demonstrated in rat liver homogenates. The first, which is tentatively called glycerol dehydrogenase, catalyses the reaction glycerol to glyceraldehyde, although *in vitro* only the reverse reaction has been demonstrated. This is often a problem with dehydrogenase enzymes which can only be forced in the aldehyde direction when they are extensively purified. It is postulated that the glyceraldehyde so formed is then phosphorylated to glyceraldehyde-3-phosphate so by-passing the InG block of α-glycerophosphate dehydrogenase.

We also have indirect evidence to show that the enzyme glyceraldehyde dehydrogenase exists in the rat liver. The action of this enzyme is to convert glyceraldehyde to glyceric acid. The glyceric acid so formed can then be phosphorylated by the enzyme triokinase forming one of the phosphoglyceric acids beyond the InG block at glyceraldehyde-3-phosphate dehydrogenase.

Attempts to purify these enzymes have been partially successful, particularly in the case of "glycerol dehydrogenase" which has many similar properties to the enzyme alcohol dehydrogenase which is abundant in liver. However it has been found that with purification, both enzymes rapidly lose activity, probably due to the removal of a stabilising factor.

The third approach has been to incubate rat liver slices with a mixture of ^{14}C and ^{3}H glycerol such that, if the alternate pathway does not exist all the ^{3}H will be lost during conversion of the glycerol to glucose and then to glycogen, but if the alternate pathway is functioning, ^{3}H will be incorporated into liver glycogen.

Preliminary results using this system have shown that approximately twice as much ^{3}H-glycerol is incorporated into glycogen as ^{14}C-glycerol, suggesting that the new pathway not only exists but that it has a large flux of glycerol passing through it.

The effect of InG on this system will be studied in the near future.

COMPUTER ANALYSIS OF GLUCOSE AND INSULIN TOLERANCE TESTS

D. Feiglin and H. D. Breidahl

One mathematical model for the glucose tolerance test level leads to two simultaneous constant coefficient second degree differential equations.

Solution of these equations leads to a time/concentration equation for both glucose and insulin. The morphology of the equation is a damped oscillatory curve with four indepen-

dent parameters. These are (i) the fasting level of either glucose or insulin; (ii) the damping of the curve or the rapidity of return of values to base-line; (iii) the period of the curve or the time of return to base-line values; (iv) the curve amplitude, or the theoretically maximum value that can be obtained.

Determination of these parameters is complex and requires the use of a computer, in our case a Hewlett-Packard 2114A, 8K model.

Applying discriminant analysis to these parameters leads to a single value for each particular curve whether normal or diabetic.

Results. The results of our study have been encouraging and may be recorded as follows:

(i) Two types of curve morphology or shape emerge in normals: (a) a highly damped non-oscillatory type with rapid rise of glucose to "abnormal" levels $\approx$ 180mg% with equally rapid fall within one hour occurring in $\approx$ 26% of the population. The gene for diabetes is said to occur in 25% of the population and we speculate as to whether this type of curve is diagnostic of this group of patients. Follow-up studies will help determine this. (b) the remaining were "normal" oscillatory curves. Bi-peaked curves which have been reported did not appear in our series.

(ii) There is a high degree of separation between normal and diabetic populations, in terms of the discriminant analysis value, to a level $> 98\%$.

(iii) The theory of the model implies that both glucose and insulin should have the same damping and period parameters; this was borne out. The clinical and physiological implications in the few cases where there has been a significant difference is unclear.

(iv) Our analyses suggest that the critical time for determining curve morphology are at the 30, 60 and 120 minute time intervals and that the longer the study is carried out the more accurate the value of discriminant analysis i.e. a 3 hour G.T.T. is optimal.

The study is being used to help in cases where tolerance test values lead to an equivocal result and clinical assessment is inconclusive. The aim is to state that the patient is either normal or diabetic; we are following a number of these cases to determine if the computer assessment is correct.

THYROID INVESTIGATIONS

Dora Winikoff, Malvina Malinek and Jennifer Ross

During the last year we have channelled our efforts in the main towards improvement and simplification of our techniques in order to undertake a more complete investigation into the changes of binding capacity of thyroxine binding globulin (TBG) in the following circumstances: (a) during normal and abnormal pregnancy; (b) in women prone to habitual abortions; (c) in women taking oral contraceptives for long periods; (d) in sick patients with abnormal TBG levels which result in discordant thyroid function tests.

TBG assay by Dextran coated charcoal

This method initiated and reported last year has now been modified so that it can be carried out at room temperature throughout the whole procedure. The overall normal range is fortunately very close to our electrophoretically determined TBG capacity, i.e. 26-40 $\mu g T_4$ per 100 ml serum (mean 33 μg T_4 per 100 ml). However, there appears to be an age and possibly a sex determined difference with young females having values at the lower end of the range.

The following categories of subjects are being investigated:

A. Normal controls (Preponderance of young females):
   Range: 27-36 $\mu g T_4\%$ (mean 31 μg $T_4\%$)

B. Euthyroid patients (Preponderance middle age, male and females):
   Range: 31-41 $\mu g T_4\%$ (mean 35.7 $T_4\%$)

C. Normal pregnancy: > 52 μg T_4%

D. Women on oral contraceptives of the combined type: > 35 μgT_4%

The correlation between the two methods is particularly good at the low binding level. The value of this method lies mainly in its simplicity of execution, and in its application to large batches of samples.

T_3 Resin Uptake (RU) by a "closed system"

This method is essentially the same as our previous one, the difference being that instead of separating the supernatant from the resin we pack the resin inside the plastic stopper by reversing the tube and centrifuging at 4000 rpm. The supernatant is then counted after gently turning the tube to its original position and allowing the liquid to drain away to the bottom. This is facilitated by the addition of one extra drop of the detergent "Teepol" 5% v/v.

The polystyrene tubes used are similar to the ones employed in Tetrasorb tests. The count rate in these taller tubes is not significantly increased by the presence of the resin at the top. The normal range remains unchanged, 80-110% of normal pool and the two techniques correlate well. In the presence of a high TBG however, which previously resulted in RU below the normal range, the new modification shows slightly elevated values. In the event of divergent tests over the whole range of values, the result of the new modification was more consistent with other parameters of that particular patient.

This closed system minimises the handling of the radioactive material, virtually eliminates the pipetting error, and simplifies the calculation.

Serum Thyroxine (T_4) Assay by the "Tetralute" kits (Ames)

In our experience, this technique appears to be equally as good as that of "Tetrasorb" kits (Abbott Laboratories), as modified by us, while its execution is much simpler. As it is to be expected both methods have their disadvantages, e.g. "Tetrasorb" necessitates alcohol extraction and the results are not always consistent, while "Tetralute" has a more limited working range. Values below 1 μgT_4 per 100 ml with "Tetralute" are not very reliable, and above 10 μg T_4 per 100 ml cannot be estimated, except by using half volumes (thereby increasing the error).

The normal range for our modified "Tetrasorb" technique and that for "Tetralute" as quoted by the manufacturers are identical, i.e. 5.0-12.0 μgT_4 per 100 ml serum.

The radioactivity on "Tetralute" sephadex columns can be successfully counted only on the specially designed "Thyrimeter" counter. In patients taking oral contraceptives, the thyroxine values are often lower when estimated by "Tetralute." We intend to study this phenomenon and compare it with PBI levels.

Free Thyroxine Index (FTI) and "Binding Ratio" (BR)

Our new parameter the "Binding Ratio" introduced last year and derived mathematically from Resin Uptake (RU) and Electrophoretic Index (EI), BR = RU/EI, was compared to the long accepted "Free Thyroxine Index" (FTI = PBI x RU) in patients in whom the TBG binding was outside the normal limits.

In 148 of such patients the diagnostic accuracy was greatly enhanced (see Table 1).

TABLE 1
ABNORMAL BINDING OF TBG
DIAGNOSTIC ACCURACY

	Correct %	Incorrect %	Doubtful %	No. of Patients
FTI	66	18	16	148
BR	86	5	9	148

applies with low thyroxine levels due to myxoedema and low TBG binding respectively (see Table 2).

We have found that the F.T.I. does not invariably, as claimed, cope with the diagnostic problem of binding abnormalities and that the concomitant use of the B.R. helps to delineate more accurately the all too frequent difficult interpretation of discordent tests.

It is our opinion that only a full thyroid profile can resolve the doubt about the thyroid status in such patients.

	FTI	BR
Thyrotoxic		>2·0
Myxoedema		<1·0
Low TBG	normal	>1·8
High TBG	normal	<1·1
Normal Range	2.4–8.3	1.0–2.0

TREATMENT OF DIABETIC RETINOPATHY

H. D. Breidahl, J. B. Foster and P. Taft

A special and separate clinic for the assessment and treatment of diabetic retinopathy has been established. The prime purpose of the clinic is to examine the role of retinal photocoagulation by laser beam in the treatment of retinopathy.

Criteria for acceptance of patients into a trial of this treatment have been established and include the presence of bilateral symmetrical disease with vision 6/18 or better in patients with reasonable life expectancy who give informed consent to the treatment of one eye chosen at random. All patients are submitted to general health assessment and review of diabetes. Retinal photography is undertaken on both eyes before and at review visits. General medical and diabetic assessment is made.

Patients who do not fulfil the criteria for such treatment or who do not elect to have it, serve as a pool of patients in whom the natural history of the condition is being studied or in whom treatment with clofibrate is being prescribed. Again in these groups careful general and diabetic assessment is being made and follow-up including retinal photography undertaken.

In patients with haemorrhagic retinopathy unsuited for other treatment a trial of pituitary suppression with synthetic progestagens and/or chlorpromazine is being made. Studies of growth hormone and ACTH secretion in response to insulin hypoglycaemia before and after such suppressions are being undertaken to check the effect of pituitary function so as to relate these effects to any observed retinal changes.

STUDY OF MUCOPOLYSACCHARIDE (GLYCOSAMNIOGLYCAN) SYNTHESIS IN VITRO BY HUMAN LEUCOCYTES

R. Dargaville and J. Sheath

It has been demonstrated that human leucocytes are capable of incorporating labelled sulphate and glucosamine into intracellular mucopolysaccharides (glycosaminoglycans or GAG) particularly chondroitin-4-sulphate. Therefore it was suggested to us that leucocytes could be used as a readily available human cell model for investigation of GAG synthesis *in vitro* under different conditions.

A role for abnormal GAG metabolism in diabetes has been suggested in such findings as altered GAG composition of diabetic glomerular basement membrane and diabetic vitreous humour, although the basis for these abnormalities has not been well defined. Also it has been demonstrated that GAG synthesis is subject to the influence of hormones including insulin and growth hormone, so that abnormal GAG synthesis may play some role in the genesis of diabetic complications, particularly in relation to permeability of vascular walls.

We have separated leucocytes from human blood (utilising blood from haemochromatotic patients for preliminary work) by a dextran sedimentation technique. Following lysis of residual erythocytes, leucocytes have been papain digested and GAG's precipitated with cetyl pyridinium chloride (CPC). Separation of nucleic acids and glycopeptides from GAG and partial separation of individual GAG has been performed using the varying solubility in CPC solutions by centrifugation of a Celite supporting medium. Following acid hydrolysis hexosamine concentration has been estimated by a colorimetric reaction.

It is planned to incubate leucocytes in a medium containing labelled sulphate and to measure GAG synthesis (particularly chondroitin sulphate) by calculating the activity per unit of hexosamine. Such synthesis is to be measured under a variety of metabolic conditions such as might be expected to occur in diabetes.

Theoretically the inhibition of glucose oxidation and increased gluconeogenesis induced by the pituitary peptide InG described by Prof. Bornstein's group may cause a shunting of substrates to the uronic acid pathway and to amino sugar synthesis, thus tending to an increased synthesis of GAG. We hope to be able to examine the effect of pituitary peptides on leucocyte GAG synthesis in this system.

TREATMENT OF THYROTOXICOSIS BY ^{125}I

P. Taft, H. D. Breidahl, L. Dugdale, H. G. Burger[1] and Y. Patel[1]

That radioactive idodine therapy is a convenient safe and effective means of reducing the capacity of the thyroid hormone and thus to control the symptoms and signs of thyroxine excess is uniformly agreed. There appears to be a direct correlation between the cure rate in the first year after radio-iodine therapy and the occurrence of hypothyroidism in this period. There have also appeared a number of reports of the appearance of subthyroidism after initial apparent control in increasing frequency with the passage of time (1). It is suggested that the reason for this high post-radiation hypothyroidism is the result of an irreversible depletion of secretory cells following radiation damage to cell nuclei with consequent failure of renewal by replication.

^{125}I has been suggested as an alternate to ^{131}I. Since its energy emission — achieved by electron capture and soft gamma rays — would theoretically be concentrated at the apex of the cell where hormone synthesis occurs, the more basally situated nucleus would be spared and

late hypothyroidism might be avoided. Preliminary accounts of the use of ^{125}I have been given ([2], [3], [4], [5], [6]). Two features have emerged from these papers (a) there is a wide divergence of view as to what constitutes an appropriate dosage, (b) that in the short term follow up (3 to 21 months) patients have remained thyrotoxic, have become euthyroid or have become hypothyroid. Thus the theoretical promise held for ^{125}I did not seem to be fulfilled.

Some 12 months ago a trial of ^{125}I was begun independently at Alfred and Prince Henry's Hospitals and this report pools the results. Selection of ^{125}I dosage was based on various approaches to calculation and the amounts administered ranged from 5-18 millicuries. All but three patients have had one dose only. Patients were regarded as still toxic if supplementary medication (antithyroid drugs, propanolol) was required to maintain control. It can be seen from the table of results that each group received similar dosage. Where control was achieved, the time to reach this state was in general less than the follow up period of patients remaining thyrotoxic. Three patients had become subthyroid during the period of the study. Three patients complained of marked and quite persistent pain over the thyroid after the therapy.

Within the limits of this study, ^{125}I seems to offer no advantages over ^{131}I for the treatment of thyrotoxicosis since it has the same problems of dosimetry, produces early hypothyroidism at a similar rate, causes a painful radiation thyroiditis in some, and provides difficulty in assessment of progress by *in vitro* tests because of the persistent blood radioactivity associated with the long half-life of the isotope. It is yet to be determined if delayed hypothyroidism will pose the same problem as with ^{131}I treated patients and observation of these patients is continuing.

TABLE 3

Gland Size	Final Clinic State			
	Toxic	Euthyroid	Subthyroid	
Large (60 gms. +)	1	1	1	3
Moderate (40-60 gms.)	3	-	1	4
Small (up to 40 gms.)	7	11	1	19
Impalpable	2	2	-	4
Total	13	14	3	30
Follow up (months)	4.8	2.9	6.3	
Range	(2-7)	(1.5-5)	(4-8)	
Dose (mci)	10.9	9.1	10	
Range	(6-18)	(5-18)	(5-15)	

References:

(1) Leading Article, Lancet Vol. 1 (1965) p. 637.

(2) Greig, W. R., J. F. B. Smith, F. C. Gillespie, J. A. Thomson and E. M. McGirr (1969) Lancet Vol. 1 (1969) p. 755.

(3) Lewitus, Z. Lancet Vol. 2 (1969) p. 1368.

(4) Weiner, J. D. Lancet Vol. 1 (1970) p. 783.

(5) McDougall, I. R., W. R. Greig, H. W. Gray and F. C. Gillespie Lancet Vol. 2 (1970) p. 840.

(6) Werner, S. C., P. M. Johnson, P. N. Goodwin, J. D. Wiener and G. A. Lindeboom, Lancet Vol. 2 (1970) p. 681.

1 Monash University Department of Medicine, Prince Henry's Hospital.

PUBLICATIONS IN 1971

BURR, I. M., H. P. TAFT, W. STAUFFACHER and A. E. RENOLD. — On the Role of Cyclic A.M.P. in Insulin Release II. Dynamic Aspects and Relations to Adrenergic Receptors in the Perfused Pancreas of Adult Rats. Ann N.Y. Acad. Sci. 185:245.

BREIDAHL, H. D. — "Clinical Experience with Glibenclamide — Daonil" in Symposium on Diabetes Mellitus and Oral Hypoglycaemic Drugs. *Aust. N.Z. J. Med.* Vol. 1 (1971) Sup. 2. P. 63.

BREIDAHL, H. D. and P. TAFT. — "Hypophysectomy for Diabetic Retinopathy." *Aust. N.Z. J. Med.* Vol. (1971) P. 286 (Abstract).

MARTIN, F. I. R. and P. TAFT. — "Hypophysectomy for Diabetic Retinopathy during Pregnancy." (In Press).

TAFT, P. — "Summary of Symposium on Diabetes Mellitus and Oral Hypoglycaemic Drugs." *Aust. N.Z. J. Med.* Vol. 1 (1971) Sup. 2 P. 63.

TAFT, P. — "Endocrine Disease in Pregnancy." *Med. J .Aust.* (In Press).

TAFT, H. P., I. EKKEL, J. R. BROWN and J. H. EVANS. — "Urinary Gonadotrophins — General, FSH and LH and Urinary Oestrogens in Dysfunctional Amenorrhoea." *Aust. N.Z. J. Obstet. Gynaec.* Vol. 11 (1971) P. 27.

TAFT, P., P. ZIMMET, F. M. NG and J. BORNSTEIN. — "Role of Growth Hormone Derived Peptides in Human Glucose Homeostasis." Proceedings of International Symposium commemorating the 50th Anniversary of Insulin. "Impact of Insulin on Metabolic Pathways." Jerusalem 1971.

ZIMMET, P, F. M. NG, J. BORNSTEIN, J. McD. ARMSTRONG and H. P. TAFT. — "Insulin Antagonist of Pituitary Origin in Plasma of Normal and Diabetic Subjects." *Brit. Med. J.* Vol. 1 (1971) P. 203.

ZIMMET, P., F. M. NG, J. BORNSTEIN and P. TAFT. — "Somantin (In-G), Cataglykin (Ac-G), and Glucose Homeostasis In Man." *Horm. Met. Res.* (In Press).

ZIMMET, P., F. M. NG, J. FUNG, J. BORNSTEIN and P. TAFT. — "The Insulin-like Action of Growth Hormone." *Horm. Met. Res.* (In Press).

WINIKOFF, Dora. — "Oral Contraceptives and Thyroid Function Tests. The diagnosis of thyroid disease." *Med. J. Aust.* Vol. 1 (1971) P. 1059.

LECTURES GIVEN DURING 1971

"The Use of Oral Hypoglycaemic Agents in The Management of the Pregnant Diabetic." — *4th Asian and Oceanic Congress of Endocrinology. Auckland N.Z.*	H. D. BREIDAHL
"Thyroid Nodules." "Hypophysectomy for Diabetic Retinopathy." "Serum Calcium Levels in Pancreatitis." — *Alfred Hospital Centenary Meeting.*	H. D. BREIDAHL
"Hypophysectomy for Diabetic Retinopathy." — *Royal Australasian College of Physicians. Annual Meeting, Melbourne.*	H. D. BREIDAHL and P. TAFT
"Types of Obesity and Their Management." — *Melbourne Medical Post-graduate Committee, Colac.*	H. D. BREIDAHL
"Pitfalls in the Management of Diabetics." — *Royal Perth Hospital.*	H. D. BREIDAHL
"The Current Controversy on the Aetiology of Diabetes Mellitus." — *Alfred Hospital Centenary Meeting.*	P. TAFT
"The Treatment of Thyrotoxicosis by ^{125}I". — *Endocrine Society of Australia, Canberra.*	P. TAFT et alii
"Growth Hormone Derived Peptides and Glucose Homeostasis" — Symposium "Impact of Insulin on Metabolic Pathways." Jerusalem.	P. TAFT et alii
Graduation Address, *College of Chiropody of Australia, Melbourne.*	P. TAFT
"Electrophoretic Index" (EI) and Binding Ratio (BR) as Aids to Thyroid Diagnosis in the Presence of Binding Abnormalities of TBG." — *4th Asian and Oceanic Congress of Endocrinology. Auckland, N.Z.*	D. WINIKOFF
"No Short Cut to Thyroid Profile." — *Annual Meeting. Australian Association of Clinical Biochemists. Melbourne.*	D. WINIKOFF
"InG — A Growth Hormone Derived Insulin Antagonist in the Blood of Normal and Diabetic Subjects." — *4th Asian and Oceanic Congress of Endocrinology, Auckland, N.Z.*	P. ZIMMET
"Growth Hormone and the Aetiology of Diabetes Mellitus." — *Alfred Hospital Centenary Meeting.*	P. ZIMMET
"Biological Activity of Cataglykin (Ac-G) — a Pituitary Polypeptide Isolated from Human Urine." — *Endocrine Society of Australia, Canberra.*	P. ZIMMET

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

ULTRA-THIN SECTIONS FOR HISTOLOGY

A. V. Jackson[1]

During the course of this year, the project has changed somewhat, both in "depth" and "direction." Its original aim was to establish whether light microscopy of ultra-thin plastic-embedded tissue could provide a useful addition to the techniques already available to routine histopathology. With certain tissues — particularly spleen, lymph nodes, jejunum and kidney — very good preparations were "sometimes" obtained. In these slides, a precision of histological detail was achieved which was much beyond anything previously seen with paraffin embedded material. However, "sometimes" was the operative word because the method was frustrating in its variability, and it appears, on enquiry and from the literature, this has been the experience of other laboratories using this approach.

For these reasons we were pleased to be able this year, to move into the deeper (though indeed much more exacting and time consuming) level of study provided by electron microscopy. By arrangement between the hospital and the Baker Institute, we have had access to the newly installed Institute Hitachi microscope (see p. 44). In changing depth we have also changed direction somewhat and are now concentrating on breast cancer. There is some evidence that the classical subdivision of mammary cancer into scirrhous and medullary, is also valid at E.M. levels. Scirrhous carcinoma, arising from myoepithelial cells, will show in E.M. an investment of cell-membrane associated ATPase — whilst medullary cancer, arising from lining ductal cells, may be more precisely defined by the demonstration of microvilli. We are just commencing this study but hope to continue it, more vigorously, next year in 1972.

1 Morbid Anatomy Department.

MEASUREMENT OF CARDIAC GLYCOSIDES

S. T. Anderson and A. Pitt[1]

The immunoassay technique for measuring the level of cardiac glycosides in serum was investigated and pilot runs undertaken. Due to pressure of routine work in the Cardiovascular Diagnostic Service as yet the measurement of cardiac glycosides cannot be made available for routine use within the hospital.

1 Cardiovascular Diagnostic Service.

BONE DENSITOMETRY

H. A. Luke[1] **and L. M. Dugdale**[2]

Early in 1970 Mr. P. Currer commenced research into, and construction of equipment for radio-isotope and radiographic bone densitometry. The principle of both techniques is that bone mineral absorbs radiation. In the isotope technique, an Americum source gives rise to mono-energetic λ rays which pass through the limb which is immersed in a water bath and the transmitted radiation is detected by a scintillation system. The source and detector, with the narrowly collimated beams, are scanned across the limb and the transmitted count rate recorded on magnetic tape. This is subsequently analysed on the computer in the Department of Nuclear Medicine. Present

1 Department of Radiology.
2 Department of Nuclear Medicine.

results show good correlation between computed mineralisation and actual bone mineral per unit length of bone as assayed in animal bone. There is also a high degree of sensitivity and reproducibility, with variation of ±3% of mineralisation of bone detectable. Further development of the equipment is underway to make it easily used on patients.

Radiographic bone densitometry has been studied in principle. This basically involves radiography of an immersed limb under standard conditions with subsequent densitometric comparison of standards against the bone concerned. Equipment is under development to carry out this investigation. Up to the present there have been no published comparisons between radiographic and radio-isotope methods of bone densitometry and this study is aimed in this direction as well as towards providing a rapid method of bone densitometry in patients.

ACUTE LEUKEMIA IN ADULTS

M. B. Van Der Weyden[1]

The mechanisms involved in the production of anaemia in patients with acute leukaemia were studied using standard ferrokinetic techniques, indices of red cell metabolism and determination of ^{51}Cr red cell survival. Gastrointestinal blood loss was measured in a number of patients. This study has demonstrated that there is no fixed correlation between the status of erythropoiesis, as judged by marrow morphology, and ferrokinetic data. Erythropoietin activity appears to behave normally in the leukaemic state and mechanisms of increased haemolysis as related to indices of red cell metabolism remain obscure. Significant gastrointestinal blood loss occurs in a number of patients and is related to thrombocytopenia.

Factors involved in the production of thrombocytopenia in acute leukaemia were examined using standard techniques of platelet aggregation, platelet factor III (PF_3) availability as related to platelet survival. It has been demonstrated that, during the phases of active disease or incomplete remission, abnormalities of platelet function — as reflected by abnormal platelet aggregation and PF_3 availability — occur commonly. This is associated with a reduced survival of such defective platelets. During the phase of complete remission, platelet function and survival appear normal.

The aetiology of megaloblastosis in acute leukaemia has been examined by determination of various parameters of pyrimidine metabolism. Deoxyuridine suppression of ^{3}H-TDR incorporation into DNA, a sensitive index of impaired folate metabolism, has been examined in marrow cultures of patients with acute leukaemia and it has been demonstrated that folate deficiency is relatively uncommon. The erythrocyte enzymes — orotidylic pyrophosphorylase and decarboxylase — responsible for the conversion of orotic acid to uridine monophosphate, are generally normal in acute leukaemic, but increased activity is apparent in patients with erythroleukaemia and those exhibiting megaloblastosis of erythroid precursors.

The chemotherapeutic trial using cytosine arabinoside and rubidomycin has disclosed that a remission rate of 30-35% can be achieved using these agents.

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

EXPERIMENTAL GASTRIC ULCERATION

D. J. B. St. John[1]

Investigation of the effects of acetylsalicylic acid (aspirin) on the gastric mucosa of the rat was continued in 1971. Histological and autoradiographic studies in 1970 had shown an altered mucosal response to aspirin with reduction in mucosal damage when the drug was given in repeated daily doses. To examine this further, methods for measurement of hydrochloric acid secretion, pepsin, hexosamines, fucose and sialic acid were established. Aspirin absorption and hydrochloric acid secretion were not reduced with repeated aspirin and there was no significant change in secretion of gastric mucus. These results indicate that there is a partial mucosal adaptation in response to repeated administration of aspirin.

Little information was available about the sequential events in regeneration of the gastric mucosa following drug-induced injury. Histological and autoradiographic studies with ^{3}H-thymidine were performed on groups of rats killed at intervals after a single dose of aspirin, and on further groups after 14 daily doses.

The findings indicated that the speed and pattern of regeneration depend upon the depth of the initial mucosal damage. Widespread shedding and necrosis of surface mucous cells was present up to 30 minutes after aspirin, but extensive recovery occurred within 4 hours before evidence of cell proliferation was found. Healing of deep mucosal erosions was accompanied by increased cell proliferation, but took up to 7 days, when the initial damage penetrated the progenitor zone. The morphological features were consistent with a topical effect of aspirin with focal mucosal destruction and repair. The mucosal response to aspirin was altered by prior administration of the drug, confirming our previous observations. There was a reduction in further acute damage but the pattern of healing of individual lesions was unchanged.

An experiment on the effect of long-term administration of aspirin on the rat stomach will be completed in February 1972. Some rats have died from massive upper gastrointestinal haemorrhage. In the remaining 50 rats, mucosal morphology and function will be examined, either immediately after completion of a 6 months' course of aspirin, or after a further interval of time, to assess possible mucosal recovery. A preliminary experiment on four rats showed widespread de-differentiation and dysplasia in the glandular stomach after aspirin for 14 weeks, with antral ulcers in three of the rats.

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

THE DIAGNOSTIC PROCESS

J. F. McL. Oldham[1]

Over the past two years an attempt has been made to define and investigate the short cuts used by surgeons while making a diagnosis in a patient with acute abdominal pain.

The investigation was carried out in three major phases. The first involved definition of the processes used in real life situations; whole diagnostic interviews were transcribed and analysed; parellel to this a traditional checking routine was developed. The second phase of the study required the construction and testing of a model of this strategy. The final stage involved transferring the strategy to a computer and developing a program that would allow the model to "learn" with experience. A computer based routine could allow parallel comparison between surgeon and machine, thus the process used by the surgeon could be elucidated.

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

PUBLICATIONS IN 1971

BETT, J. H. N. and S. T. ANDERSON. — "Plastic Catheter Embolism to the Right Heart: Non-Surgical Removal." *Med. J. Aust.* Vol. 2 (1971) P. 854.

HANSKY, J., M. G. KORMAN, C. SOVENY and D. J. B. St. JOHN. — "Radioimmunoassay of Gastrin: Studies in Pernicious Anaemia." *Gut* Vol. 12 (1971) P. 97.

LIPP, H., S. T. ANDERSON and Aubrey PITT. — "Long Term Pacing in the Management of Bradyarrhythmias." *Med. J. Aust.* Vol. 1 (1971) P. 574.

PITT, Aubrey, H. LIPP and S. T. ANDERSON. — "Lignocaine given Prophylactically to Patients with Acute Myocardial Infarction." *Lancet,* Vol. 1 (1971) P. 612.

ROBERTS, P. D., D. J. B. St.JOHN, R. SINHA, J. S. STEWART, I. M. BAIRD, N. F. COGHILL, and J. MORGAN. — "Apparent Folate Deficiency in Iron Deficiency Anaemia." *Brit. J. Haem.* Vol. 20 (1971) P. 165.

St.JOHN, D. J. B., F. T. McDERMOTT, W. G. R. M. de BOER. — "Response of the Gastric Mucosa to Continued Administrations of Aspirin: Histological and Radioautographic Studies in The Rat." *Gastroenterology* Vol. 60 (1971) P. 712.

VAN DER WEYDEN, M. and B. G. FIRKIN. — "The Management of Aplastic Anaemia in Acute Leukaemia." *Brit. J. Haem.* In Press.

VAN DER WEYDEN, M., M. ROTHER and B. G. FIRKIN. — "Megaloblastic Maturation masked by Iron Deficiency: A Biochemical Basis." *Brit. J. Haem.* In Press.

VAN DER WEYDEN, M., M. ROTHER and B. G. FIRKIN. — "Variation in Pyrimidine Metabolism in Megaloblastic Anaemia." Submitted.

VAN DER WEYDEN, M., M. ROTHER and B. G. FIRKIN. — "Metabolic Significance of Reduced Serum B_{12} in Folate Deficiency." Submitted.

YEOMANS, N. D., D. J. B. St.JOHN, W. G. R. M. de BOER. — "Regeneration of the Gastric Mucosa after Aspirin-induced injury in the Rat." *Proc. Aust. Soc. Med. Res.* Vol. 2 (1971) P. 443.

LECTURES GIVEN DURING 1971

"The Effects of Continued Administration of Aspirin on the Gastric Mucosa: Histological and Autoradiographic Studies in the Rat." — *Annual Meeting, Gastraenterological Society of Australia.* D. J. B. St.JOHN

"Regeneration of the Gastric Mucosa after Aspirin-induced Injury in the Rat." — *Annual Meeting, Australian Society for Medical Research.* D. J. B. St.JOHN

"Anaemia in Acute Leukaemia." — *Royal Australasian College of Physicians. Melbourne.* M. VAN DER WEYDEN

"Platelet Abnormalities in Acute Leukaemia." — *Asian-Pacific Division of the International Society of Haematology. Melbourne.* M. VAN DER WEYDEN

"Megaloblastic Maturation masked by Iron Deficiency: A Biochemical Basis." — *Australian Society of Medical Research Melbourne.* M. VAN DER WEYDEN

DISSEMINATION OF RESEARCH RESULTS

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.