Research

BAKER INSTITUTE

ALFRED HOSPITAL

1968

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

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

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

FORTY-SECOND ANNUAL REPORT

of

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

(Including Alfred Hospital Clinical Research Unit)

TWELFTH ANNUAL RESEARCH REPORT

of

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

REPORTS

of

ALFRED HOSPITAL RESEARCH FELLOWS

1968

ALFRED HOSPITAL, PRAHRAN, VICTORIA, 3181, AUSTRALIA

BAKER MEDICAL RESEARCH INSTITUTE

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N. E. JONES, C.M.G.

Sir CHARLES McGRATH

K. N. MORRIS, M.B., M.S., F.R.C.S., F.R.A.C.S.

EDGAR ROUSE, C.B.E.

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, 1968

"Edward Wilson Memorial":	ELIZABETH M. GORDON, M.B., B.S., F.R.C.S.
"Ringland Anderson":	G. W. CROCK, M.B., B.S., M.R.A.C.P., F.R.C.S.
"Dr. Henry Laurie": *"Amelia Haigh Estate (Heart)"*: *"Frederick and Esther Michaelis"*:	A. PITT, M.D., M.R.A.C.P.
"James and Elsie Borrowman": *"Edward Wilson Memorial"*:	F. W. GURR, M.B., B.S., M.R.A.C.P.
"Dr. S. W. Shields": *"George Merriman"*:	R. J. SAWERS, M.B., B.S., F.R.A.C.P., M.C.Path.
"S. W. Jones":	D. A. COVENTRY, M.B., B.S., M.R.A.C.P.
"Sol Green": *"Amelia Haigh (Rheumatoid Arthritis)"*: *"H. M. Black"*: *"Victor Y. and Margaret Kimpton"*:	T. E. LOWE, D.Sc., M.D., F.R.C.P., F.R.A.C.P.
"E. H. Flack": *"Connibere Bequest"*:	PINCUS TAFT, M.D., F.R.A.C.P.
"J. F. Mackeddie": *"R. B. McComas"*: *"A. A. Swallow"*: *"Sartori"*: *"James Richardson"*:	J. A. OWEN, B.Sc., M.D., Ch.B., Ph.D., M.C.P.A.
"William Buckland": *"Collier"*:	A. V. JACKSON, M.D., B.S., F.R.A.C.P., M.C.P.A., F.C.Path.

APPOINTED TO RESEARCH FELLOWSHIPS FOR 1969

"Ringland Anderson":	G. W. CROCK, M.B., B.S., M.R.A.C.P., F.R.C.S.
"Edward Wilson Memorial":	M. A. SHIELDS, F.R.C.S.
"Edward Wilson Memorial": *"Dr. Henry Laurie"*:	D. J. B. St. JOHN, M.B., B.S., M.R.C.P., M.R.A.C.P.
"Victor Y. and Margaret Kimpton": *"William Buckland"*: *"James Richardson"*:	PAULA JABLONSKI, Ph.D.
"George Merriman": *"Collier"*: *"A. A. Swallow"*:	A. V. JACKSON, M.D., B.S., F.R.A.C.P., M.C.P.A., F.C.Path.
"Frederick and Esther Michaelis": *"Connibere Bequest"*:	P. A. LOWE, M.B., B.S.
"Sol Green": *"J. F. Mackeddie"*: *"S. W. Shields"*: *"Amelia Haigh (Rheumatoid Arthritis)"*: *"H. M. Black"*: *"S. W. Jones"*:	T. E. LOWE, D.Sc., M.D., F.R.C.P., F.R.A.C.P.
"Edward Wilson Memorial": *"Amelia Haigh (Heart)"*:	A. PITT, M.D., M.R.A.C.P.
"Edward Wilson Memorial":	R. J. SAWERS, M.B., B.S., F.R.A.C.P., M.C.Path.
"Edward Wilson Memorial": *"E. H. Flack"*: *"R. B. McComas"*:	G. R. STIRLING, M.B., B.S., F.R.A.C.S.
"James and Elsie Borrowman": *"Edward Wilson Memorial"*:	D. J. B. St. JOHN, M.B., B.S., M.R.C.P., M.R.A.C.P. F. T. McDERMOTT, F.R.C.S.
"Sartori": *"J. R. G. and E. McKenzie"*:	PINCUS TAFT, M.D., F.R.A.C.P.
"Edward Wilson Memorial":	J. A. STREETON, M.B., B.S.

TRAVEL GRANTS

"J. H. Patterson Travelling Scholarship":	ELIZABETH SHANAHAN, M.B., B.S., F.R.A.C.S.

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Alfred Hospital Diabetic and Metabolic Unit

Investigations by Research Fellows of Alfred Hospital in Other Departments

INTRODUCTION

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

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

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

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

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

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

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

"It is now generally accepted that research into human disease must be conducted predominantly in close relationship with patients undergoing investigation and treatment. Such research is conducted on two levels. The first is concerned with the basic medical sciences (e.g., at Baker Medical Research Institute), and the second is associated with a study of disease as encountered in the sick person, i.e., clinical research. The organisation of Australian hospitals, which is peculiar to this country, necessitates that the development of the research function of the Hospital be mainly conducted in separate specially equipped units. In addition, many members of the Honorary Medical Staff devote their valuable time to research in their various 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 within the Hospital during the past year illustrating this concept.

BAKER MEDICAL RESEARCH INSTITUTE

STAFF

Director:	T. E. LOWE, C.B.E., D.Sc., M.D., F.R.C.P., F.R.A.C.P.	
Associate Directors:	A. J. BARNETT, M.D., F.R.A.C.P., M.R.C.P. WINIFRED G. NAYLER, D.Sc.	
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Technical:	S. HART (Laboratory Supervisor). A. H. HUCKFIELD (Technical Officer). J. WATSON (Maintenance Engineer). Mrs. B. SKYM (Senior Technologist).	Miss A. CLAYDEN (from 9/9/68). Miss V. MACK. Mrs. E. MORTENSEN. Mrs. R. MUSCUTT. Mrs. W. PRATT (8/7/68 to 21/7/68).
Librarian:	Mrs. M. ZARIFEH.	
Clerical:	Mrs. I. R. ROBINSON. Miss H. M. GAYTON.	Mrs. H. WINSTANLEY.
Laboratory Assistants:	Miss K. CLARKSON. Miss J. DUNCAN. Miss J. FINDLAY. Mrs. R. GILSENEN (to 26/7/68). K. HARVEY. Miss F. HIRSCH.	C. LEWIS. Miss J. LINDSEY-SMITH. Miss B. REEVE. Mrs. D. STUDD. Miss J. THOMPSON. Mrs. M. WILSON (to 8/3/68).
General:	Mrs. E. M. GARNHAM.	C. ANDRENOPOULOS. H. SLUGA.

WARD STAFF

Registrar:	T. J. WILLIAMSON, M.B., B.S.	
Resident Medical Officers:	A. SHIPLEY, M.B., B.S. B. K. FOUNTAIN, M.B., B.S.	A. H. SHARP, M.B., B.S. W. E. STONE, M.B., B.S.
Sister:	J. HOMEWOOD.	
Staff Nurses:	J. JONES (from 13/11/67 to 28/3/68). M. WALDUCK (from 12/2/68 to 8/12/68). F. McKENZIE (from 7/8/67 to 4/8/68). M. GROENWEGAN (from 18/3/68 to 28/8/68). L. REID (from 11/3/68 to 18/8/68).	M. GRAY from 20/4/68). H. CORKHILL (from 17/6/68). M. CLACK (from 5/8/68). G. BAIN (from 19/8/68). M. COOLAHAN (from 16/12/68). R. USTICK (from 16/12/68).

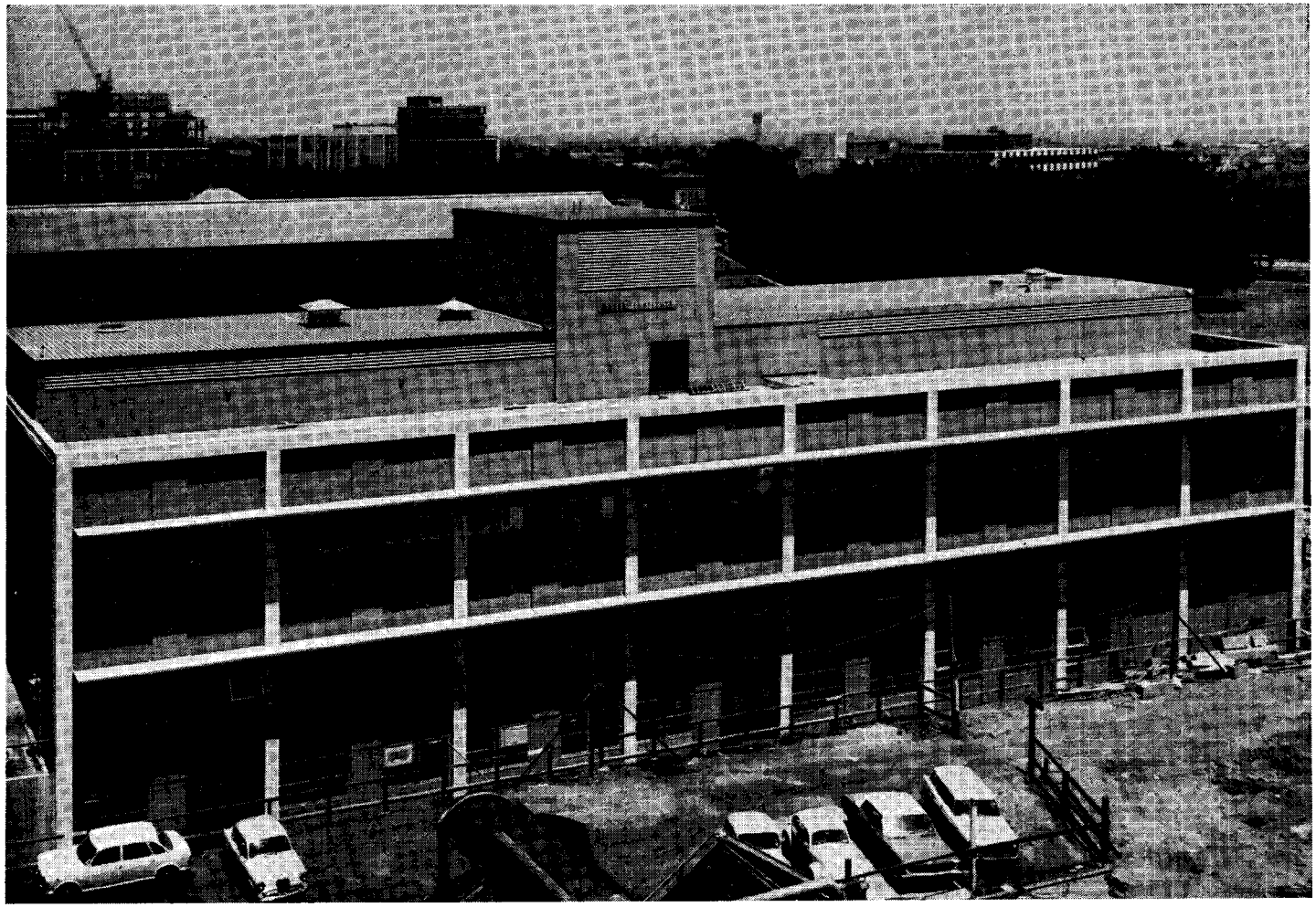
RESEARCH FELLOWSHIPS

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

Honorary:	E. COOPER, M.B., B.S., F.R.A.C.S. D. A. COVENTRY, M.B., B.S., M.R.A.C.P. G. R. STIRLING, M.B., B.S., F.R.A.C.S.
Visiting:	T. W. MOIR, M.D.
Anti-Cancer Council of Victoria ("A. A. Thomas":)	P. E. HUGHES, Ph.D., M.B., B.S.
National Heart Foundation of Australia:	M. ROSENBAUM, M.D., M.R.A.C.P.
"James and Elsie Borrowman":	I. MACDONALD, B.Sc.
"William Buckland Foundation":	P. FANTL, D.Sc., F.R.A.C.I.
"The Lang Fellow":	L. W. WHEELDON, Ph.D.

SCHOLARSHIPS

"Laura Nyulasy (1967)":	P. ZIMMETT, M.B., B.S.
National Heart Foundation: Vacation Scholarship:	K. Y. GAN.



New Building (Stages 1 and 2)

ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

RETROSPECT

Although an organisation dedicated to research should be essentially forward-looking, a retrospective glance can be justified if it helps to clarify objectives and future progress.

By the nineteen-thirties a number of organisations had been set up in the capital cities of Australia to carry out, among other objectives, medical research, and most of them carry some variant of the title — medical research institute. Broadly speaking, these bodies were established in, or in close association with public hospitals or government departments and in addition to the research commitment, were to carry out a service function in some branch of medical care and teaching at some level of medical education. Financial support came to these institutes either from direct government funds, hospital funds or private endowment as the case may be.

Since their foundation there has been considerable evolution of medical care of the community and development of medical science and, as a result, many of the institutes have changed the emphasis placed on their various objectives. In some the service commitment has become very large whereas in others, such as the Baker Institute, it has been taken over by the associated institution. In this same period affiliations between institutes and other bodies have grown up in step with this evolution. In our instance, Alfred Hospital created a clinical research unit and integrated it with the Baker Institute to broaden the resources and fields of activity in medical research. Later affiliation of the Institute and Monash University has permitted development of the teaching objective.

FINANCE

Over this same period a great increase in the financial resources necessary for the support of medical research has occurred and arises from two causes which are unrelated to the volume of work carried out. First, there is the steady depreciation of money values and secondly, increased sophistication of essential equipment and the staff to use it. These changes have affected individual institutes differently, according to the basis of their financial support.

To some the funds granted have kept pace with increasing demands and costs; to others dependent on private endowment their annual income is paying for less and less; to many of the institutes, but not all, the burden has been cushioned by a hidden subsidy of medical research arising from the occupation of buildings neither provided nor maintained by themselves, and the provision of many services, both material and personnel, by the parent institution in support of their service or teaching function. In passing, it should be noted that organisations making grants-in-aid of research have based the level of their grants to some extent on the existence of this hidden subsidy.

The Baker Institute at present receives virtually no such subsidy and because of its very limited financial endowment is dependent on the generosity of the Baker Benefaction for its increasing financial needs. It is also handicapped by the fact that the grants-in-aid received do not cover the expenses incurred in the research they support.

APPEAL

It is against this background that the appeal for funds for endowment of the Institute has recently been made to our friends who are responding so generously as a separate report shows.

To ensure the stability of the Institute it is essential within the next few years to establish additional income to cover the effects of inflationary forces and to match the hidden subsidy enjoyed by others. Failure to do so could result in it being necessary to seek governmental assistance with the possible loss of autonomy.

ROLE OF THE INSTITUTE

In these circumstances an answer is required to the question: "what is the role of an independent medical research institute in Australia in 1969?" Of the many roles such an institute can play, two stand out in the present context.

First, it can initiate research projects without having to convince a group of people outside the institute of the value of the project. This is a most important function, for the assessment of any such group is subjective and although different ones will react differently, committees dispensing public funds tend to be conservative so that, whilst support for a promising or established project may be forthcoming, it may be difficult to obtain initial support for an unusual project.

Secondly, such an institute is ideally organised to combine many disciplines in any project, something which is not easily achieved in the more rigid departmental structure of a hospital or university.

NEW BUILDING

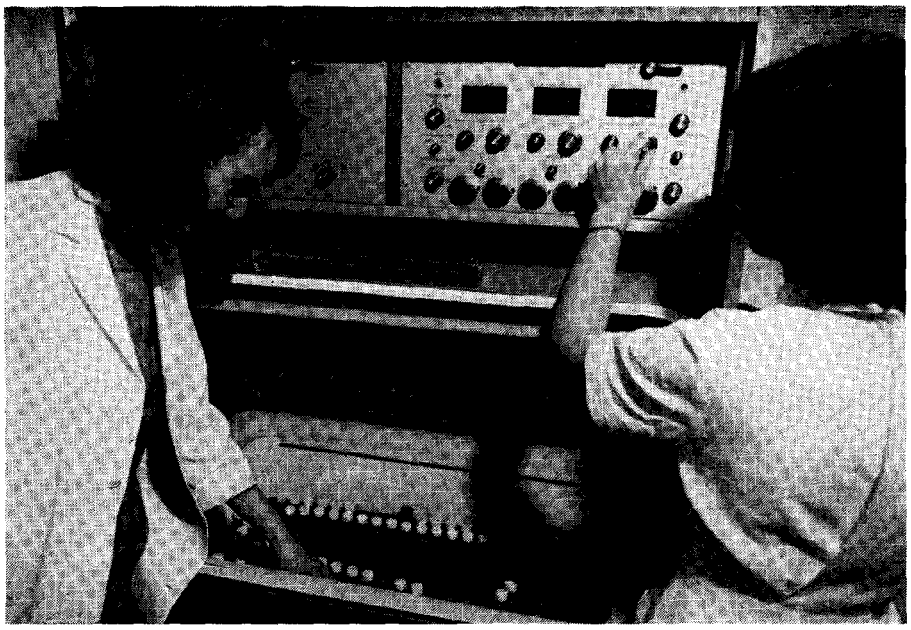
The first half of the new Institute building has been fully operative this year and although contraction of the work of the Institute into half a building has meant some crowding, this has been more than compensated by the excellent facilities provided. In particular it is noted that much equipment is working more efficiently and trouble free in the controlled environment, and this alone has speeded up many projects. The new ward and clinical laboratory of the Clinical Research Unit were occupied early in 1968 and these have provided excellent facilities for clinical investigations. Construction of the second half of the Institute building, commenced in November 1967, is now nearing completion and during the coming year we hope to be able to use its facilities to the full. The design of Stage II follows that of Stage I and provides some special facilities for purposes such as, tissue culture, histochemistry, higher level radioactive studies in metabolism, that have not previously been available. Also, on the lower ground-floor considerable space has been left undivided to provide for future and unplanned needs.

RESEARCH PROJECTS

The detailed account of the research projects given in the scientific section of this report clearly indicates that the restriction of activity enforced on both the Institute and Clinical Research Unit by the rebuilding has largely been removed. These projects in the main are oriented towards problems of the cardiovascular system and illustrate the second of the roles ascribed above to an independent medical research institute. These co-ordinated projects employ the disciplines of physiology, pharmacology, biochemistry, clinical medicine and surgery and to further emphasize the unity of the biological sciences the study of the carcinogenic action of anti-lymphocytic serum has an important relevance to the problems of organ transplantation. A short account of two of these co-ordinated projects is included here.

NEW DRUGS

For many years past the research projects have included studies of new drugs made available to us by various pharmaceutical manufacturers. Before a new drug can be introduced into medical practice its actions must be fully understood, the optimum dosage levels established and any unwanted side effects fully described. To obtain these data the drug is extensively studied on a variety of animals but ultimately it must be tried out in man because the results obtained in one species cannot be assumed to apply in another. Further, if a drug is to be used over a long period a controlled study of its use in such conditions is necessary. The projects recorded this year illustrate all these facets of drug trials. The new drugs LB 46 and ICI 50,172 have an ability to favour-



Automation in Biochemistry

ably influence cardiac arrhythmias and therefore are potentially of great clinical value. It has been possible to show that unlike H 56/28, another new drug of somewhat similar action, they do not (in dogs) at the same time depress the activity of heart muscle either from a physical or chemical viewpoint.

In contrast to these short term experiments is the clinical trial of drugs to treat high blood pressure which has now been in progress for 18 years. This long term study has enabled good comparison to be made of the effects of new blood pressure-lowering drugs, the long term side effects to be studied and above all an adequate documentation of the greatly improved outlook both in wellbeing and expectation of life now available to the sufferer from high blood pressure.

These investigations are essential to the advancement of clinical medicine, they usually do not produce spectacular results but demonstrate the need for close laboratory and clinical co-ordination in the development and introduction of new drug therapy.

CARDIAC SURGERY

Many structural abnormalities of the heart and its blood vessels, whether arising from congenital maldevelopment or acquired from disease, are amenable to correction by surgery. For a great many years various workers in the Institute have been interested in co-operating with the cardio-thoracic surgeons of the Hospital in the study of the surgical problems they encounter. In the past these projects have included investigations into anaesthetic procedures; into ways of stopping the heart beating to provide the surgeon with a quiet field for operation and the subsequent restarting of the heart; into methods of sustaining the viability of the heart and the body during this period of cardiac arrest. To maintain the heart muscle during cardiac arrest it is essential to keep flowing through the blood vessels of the heart (coronary vessels) either blood or a blood substitute for the muscle will tolerate interruption of this flow for no more than about 20 minutes at normal body temperatures. In a number of procedures in cardiac surgery inter-

ruption of the coronary blood flow is inevitable, e.g. in some valve surgery and in transplantation, and this time limit is a severe constraint on the surgeon.

Currently this research group is studying the changes, and their time sequence, which occur in heart muscle when its blood supply is cut off. It is hoped to determine which phenomena become irreversible and, if possible, to lengthen the period before the irreversibility becomes established. Successful solutions of these problems would greatly ease the time constraints on the surgeon and would be an important step towards feasibility of storing hearts for transplantation.

STAFF

There have been a number of changes in the graduate staff during 1968 to report.

Dr. L. W. Wheeldon commenced duty as a Senior Research Biochemist.

Dr. Fedora R. Trinker was appointed as Clinical Pharmacologist and commenced duty in May.

Dr. P. E. Hughes returned from London and re-established the cancer research project in the Institute.

Dr. M. Rosenbaum left to take up a university post in clinical medicine.

Dr. T. W. Moir, Associate Professor of Medicine, Case-Western Reserve University, Cleveland, Ohio, visited the Institute as a Visiting Fellow for 3 months and joined the team investigating kinekard.

Misses J. Chan and D. Chipperfield are to be congratulated on the completion of their courses for B.Sc.

During the year the Trustees of the Institute, to commemorate the generosity of various benefactors, conferred named fellowships on the following members of staff:

The Lang Fellow:
L. W. Wheeldon

Wm. Buckland Foundation Fellow:
P. Fantl

James and Elsie Borrowman Fellow:
I. Macdonald

OVERSEAS VISIT

During August-September Dr. Winifred G. Nayler visited Europe, England and the U.S.A. She presented papers at the Asian Pacific Congress of Cardiology in Tel Aviv, at the European Congress of Cardiology in Athens and was guest speaker at an international symposium held in New York to discuss the action and efficacy of a recently developed hypotensive agent.

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

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

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

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 Common-

wealth 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-operaton during the year.

T. E. LOWE

December 31, 1968

ALFRED HOSPITAL RESEARCH FELLOWS IN THE INSTITUTE

1949 - 1968

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.
Coventry, D. A., 1968.
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, 65-66.
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.
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).
Lumb, F. J., 1960-61 (London).
Marshall, R. J., 1957 (Belfast).
Moir, T. W., 1968 (Cleveland).
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.
Department of Health, New Zealand.
Department of Territories, Canberra.
Halstrom Institute of Cardiology, Sydney.
Instituto de Biologia y Medicina Experimental, Buenos Aires.
Institut Pasteur, Algiers.
Institute of Medicine and Veterinary Science, Adelaide.
Kanematsu Memorial Institute, Sydney.
Medical Research Council, London.
Middlesex Hospital Medical School.
National Heart Foundation, Australia.
National Institute of Nutrition, Japan.
New York State Department of Health.
New York University College of Medicine.
New Zealand Medical Research Council.
Ophthalmic Research Institute of Australia.
Queensland Institute of Medical Research.
Rockefeller Institute, New York.
Royal Children's Hospital, Melbourne.
Royal Melbourne Hospital.
Royal Prince Alfred Hospital, Sydney.
Royal Women's Hospital, Melbourne.
St. Vincent's Hospital, Melbourne.
St. Vincent's School of Medical Research, Melbourne.
South African Institute of Medical Research.
Strangeways Research Laboratories, Cambridge.
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REPORT OF SCIENTIFIC INVESTIGATIONS

PHYSIOLOGY AND PHARMACOLOGY OF THE CARDIOVASCULAR SYSTEM

MYOCARDIAL CONTRACTILITY STUDIES†‡

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According to the sliding filament theory of muscle contraction the development of tension, and hence of shortening, results from the periodic making and breaking of linkages between the actin and myosin filaments of the myofibrils. These interactions between actin and myosin, which probably involve the lateral projections of the myosin filaments, require energy in the form of adenosine triphosphate (ATP) and calcium ions (Ca^{++}). More than ninety percent of the energy-rich phosphate bonds present in cardiac muscle are provided by the two terminal phosphate bonds of ATP and by that of creatine phosphate (CP). A transphosphorylation equilibrium exists between ATP and CP in cardiac muscle so that, whilst ATP serves as the immediate energy source for the myofibrils during the process of contraction, CP functions as a reserve. Since the heart must fulfill its contractile function continuously it must be able to maintain its tissue concentration of ATP above a critical level. ATP is synthesised by 'coupled' oxidative phosphorylation in such a way that 3 moles of high-energy phosphate are produced per gram atom of oxygen consumed. Because of this tight coupling the concentration of adenosine diphosphate (ADP) is an important factor in controlling the rate at which oxygen is used. The absolute concentration of high energy phosphate compounds present in cardiac muscle at a particular time will represent a balance between the rate at which these compounds are being used and the rate at which they are being produced. Hence in the following left ventricular function studies, in which dogs on right-sided cardiac bypass were used, Frank-Starling work function curves were plotted to provide an estimate of the ability of the left ventricle to perform work over a range of work loads and under a variety of conditions. At the same time coronary blood flow and coronary A-V O_2 difference was measured and ventricular biopsies taken so that tissue concentrations of ATP, ADP, CP and IP (inorganic phosphate) could be determined. From these data it is possible to deduce whether a deterioration in the amount of useful mechanical work the ventricle can perform over a range of left ventricular end-diastolic pressures is due to the reduced availability of ATP.

Since the utilization of the high energy phosphate bonds of ATP requires its breakdown by the myofibrillar ATPase enzyme other experiments have been performed to determine whether changes in ventricular function caused by certain drugs can be accounted for in terms of a changed myofibrillar ATPase activity.

The tension developed by cardiac muscle during contraction is proportional to the amount of calcium which is available to the myofibrils. Hence changes in the availability of calcium will influence myocardial function. Information is rapidly accumulating to support the hypothesis that depolarization of cardiac muscle cells is associated with an influx of Ca^{++} and that the quantity of Ca^{++} which enters the cell during a single depolarization is determined by the quantity of calcium on the membrane prior to depolarization and by the duration of that depolarization. These Ca^{++} which are translocated across the cell membrane and transported into the vicinity of the myofilaments may themselves activate contraction; alternatively they may displace Ca^{++} from intracellular storage sites, including the sarcoplasmic reticulum and mitochondria. Since the interaction between actin and myosin proceeds only if the Ca^{++} concentration has reached a critical level, and ATP is available,

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it is not surprising to find that relaxation results from the removal of Ca^{++} from the myofibrils. This is achieved by the sarcoplasmic reticulum. Hence studies on the uptake and release of Ca^{++}, detected as Ca^{45}, by intact cardiac muscle and by isolated mitochondria and sarcoplasmic reticulum have been included as a fundamental part of an experimental series designed to elucidate factors which influence myocardial contractility.

Left Ventricular Function

(a) β-Adrenergic Antagonists and other Antiarrhythmic Drugs. Dogs on right-sided cardiac bypass, perfused under conditions of constant flow or constant perfusion pressure, were used to compare the effects on ventricular function of two recently developed β-antagonists (LB 46 and ICI 50,172) with those already described for *dl* propranolol, KÖ 592 and Trasicor.

LB 46, when used in doses not exceeding 5 mg/kg, displaced the left ventricular function curve to the left, indicating an improvement in ventricular function, whereas higher doses caused the function curve to be displaced to the right. Like *dl* propranolol, KÖ 592 and Trasicor, LB 46 causes an overall increase in the resistance to blood flow in the peripheral circulation, including that of the coronary vasculature. The regional distribution of blood in the peripheral circulation was altered after LB 46 had been administered, so that blood flow through the splanchnic circulation was markedly reduced. Tissue concentrations of ATP, ADP, CP and IP remained unchanged and myocardial oxygen consumption was slightly increased after doses not greater than 4 mg/kg LB 46.

ICI 50,172, unlike other available β-adrenergic blocking drugs does not cause any significant increase in the resistance to blood flow in the peripheral circulation. Dose-response curves showed that doses of ICI 50,172 which effectively antagonized β-adrenergic receptors in the heart failed to have any effect on these receptors in the peripheral circulation. ATP, ADP, CP and IP levels remained unchanged, as they did after the establishment of β-adrenergic blockade with *dl* propranolol, and the ratio between left ventricular 'work' (gm M/minute) and oxygen consumption (ml/minute) remained relatively constant.

Lignocaine (Xylocaine) is increasingly being used in the treatment of cardiac arrhythmias. The effect of this drug has been compared with that of *dl* propranolol and other β-adrenergic antagonists on ventricular function, cardiac stores of high energy phosphates and the regional distribution of blood.

Doses of lignocaine not greater than 4 mg/kg did not cause any significant change in left ventricular function as determined by work-function studies. After 5 mg/kg however, the work-function curve was flattened at high but was not significantly changed at low rates of work. High-energy phosphate stores and myocardial oxygen consumption were not significantly changed after doses of lignocaine of, or up to, 5 mg/kg. Left ventricular efficiency, when calculated as the ratio between left ventricular work and oxygen consumption, remained unchanged after doses of from 0.2-5 mg/kg lignocaine.

Two conclusions have been drawn from these experiments (i) β-adrenergic blockade need not be accompanied by a decline in ventricular function and (ii) the drugs studied do not disturb the balance between the production and utilization of high energy phosphate stores.

(b) **Hypotensive and Antianginal Drugs.** Previous studies had shown Catapres (ST 155) to have a biphasic effect on blood pressure characterized by a slight transient increase in pressure followed by a sustained fall. Both phases of the blood pressure response were accompanied by bradycardia. Left ventricular function and high energy phosphate stores were not modified by the drug, which produced a fall in plasma levels of angiotensin. Investigations showed that the constrictor effect of ST 155 resulted from its direct activation of α-adrenergic receptors in the peripheral circulation whilst the hypotensive effect of the drug resulted from its central inhibition of sympathetic outflow. ST 155 did not depress ventricular function.

In other experiments the effect of a recently developed antianginal drug, amiodarone, on

ventricular function, coronary blood flow, myocardial oxygen consumption and the myocardial level of high energy phosphate stores was studied and the results obtained compared with those already described for *dl* propranolol. Amiodarone caused the left atrial pressure to rise whilst the heart rate slowed and myocardial oxygen consumption fell. Left ventricular function curves were displaced to the right, indicating a decline in the power (gm M/minute) developed by the ventricle at a given filling pressure but the concentration of ATP, ADP, CP and IP in ventricular muscle remained unchanged.

In general these results indicate that left ventricular function can be either increased or decreased whilst the balance between the production and utilization of high energy phosphates in heart muscle remains unchanged.

Atrial and Papillary Muscle

Atrial and papillary muscle taken from patients undergoing open heart surgery has been used to determine whether any correlation exists between the tension developed by these muscles during isometric contraction at a regular rate, their noradrenaline content and their ability to accumulate Ca^{++}. The results obtained so far show that papillary and atrial muscle from patients with Class IV (New York Heart Association classification) failure do develop significantly less tension during isometric contraction, accumulate Ca^{++} at a significantly slower rate and contain less noradrenaline than does heart muscle taken from patients without cardiac failure.

Relationship Between the Inotropic Action of Certain Drugs, Their Effect on Ca^{++} Transport and Myofibrillar ATPase Activity

Dose response curves for a wide variety of β-adrenergic blocking compounds have been established for their effects on the tension developed by human and dog atrial and papillary muscle during isometric contraction at a regular rate. The inotropic effect of the various β-adrenergic blocking drugs on human heart muscle and with their effect on the Ca^{++}-recorded when dog heart muscle was used.

The inotropic effect of the β-antagonists used (*dl* propranolol, KÖ 592, Trasicor, ICI 50,172, LB 46) correlated neither with their effect on myofibrillar ATPase activity nor with their effect on the cardiac stores of high energy phosphates nor with their β-blocking potency. However, the negative inotropic effect of these drugs correlated well with their ability to impair the uptake of Ca^{++} into intact heart muscle and with their effect on the Ca^{++}-accumulating activity of the sarcoplasmic reticulum. These experiments support the hypothesis that the depressant action of β-adrenergic antagonists is due to their ability to interfere with the mechanisms whereby the intracellular concentration of Ca^{++} is regulated to promote contraction.

Other studies to further investigate the site of action of ouabain depended upon the routine preparation of relatively pure microsomal fractions. The effect of ouabain on the mitochondrial and microsomal concentrations of ionized calcium was studied *in vitro*. Ouabain failed to have any significant effect on the concentration of ionized calcium, detected as Ca^{45}, in the mitochondria but it did reduce the concentration present in the microsomal fraction. This reduction was due to an increase in the rate at which Ca^{++} were released from the microsomes and therefore contrasts with the effect of the β-antagonists, which inhibited Ca^{++} uptake by the microsomal fraction. During these experiments the observation that electrical impulses cause the active release of ionized calcium from microsomal fractions prepared from heart muscle was confirmed.

Effect of Skeletal Muscle Potentiators on Ca^{++}-Accumulating Activity of Cardiac Microsomes

The substitution of either Br^- or NO_3^- for Cl^- in solutions bathing skeletal muscle causes the displacement of Ca^{++} from the sarcoplasmic reticulum and this probably accounts for the increase in the tension produced during contraction. When these procedures were repeated using cardiac instead of skeletal muscle Ca^{++} were not displaced from the reticulum and the tension developed during contraction was not increased. These results have been interpreted to mean that there are qualitative differences between the microsomal fractions of skeletal and heart muscle.

Antiarrhythmic Actions of β-Antagonists

Using ouabain-adrenaline induced ventricular arrhythmias in rabbits experiments have been commenced to determine whether a correlation exists between the antiarrhythmic action of these drugs and their effect on Ca^{++} transport. The results obtained so far indicate that those β-adrenergic antagonists which are effective in controlling arrhythmias induced this way have a marked depressant effect on Ca^{++} transport in heart muscle cells; conversely drugs such as ICI 50,172 which have only weak antiarrhythmic properties have a relatively slight effect on Ca^{++} transport. During the course of these experiments it was noted that the bradycardia caused by ICI 50,172 was consistently more marked than that caused by equipotent β-blocking doses of either *dl* propranolol or Trasicor and was biphasic, showing an initial fall followed by a period of gradual slowing. The commonly accepted explanation for the bradycardia caused by β-blockers, including ICI 50,172, is that it is due to a reduction in sympathetic activity. Such a reduction in sympathetic activity could be mediated centrally, preganglionically or postganglionically. Since the bradycardia caused by ICI 50,172 persisted after propantheline and after bilateral cervical vagotomy it probably is not due to an increase in vagal tone. When injected into animals in which both stellate ganglia had been excised and the cervical vagi and sympathetics cut ICI 50.172 produced an increase in heart rate. The administration of ansolysen, a ganglion blocker, abolished the initial marked drop in heart rate caused by ICI 50,172 but the gradual fall persisted. These results have been interpreted to mean that the initial marked fall in heart rate caused by ICI 50,172 reflects a preganglionic or central effect whilst the prolonged and gradual slowing reflects a postganglionic effect.

H 56/28 (APTIN)—A β-ADRENERGIC ANTAGONIST

F. R. Trinker

The cardiovascular action of this new β-antagonist has been studied in some detail.

Myocardial Function

H 56/28 at doses (0.5 mg/Kg or more) which resulted in complete antagonism of the β-mimetic effect of catecholamines caused a depression in myocardial function but a dose which produced only 60-70% β-blockade caused no concomitant cardiac depressant effect. Myocardial function was estimated in dogs on right-sided bypass and in anaesthetized dogs where myocardial contractility was measured directly by means of a Walton-Brodie strain gauge arch. A significant and quantitatively similar bradycardia was noted at all dose levels of H 56/28 tested.

The negative inotropic effect of H 56/28 did not correlate with any changes in adenosine triphosphate or creatine phosphate levels, i.e. the ventricular levels of ATP and CP did not change significantly. In contrast to other β-blocking drugs the myocardial oxygen consumption was neither decreased nor increased by H 56/28.

Curiously H 56/28 has some inherent β-mimetic property which is only elicited in situations where the sympathetic tone has been reduced, as for example, in isolated Langendorf preparations. In these it produced an initial increase in heart rate, amplitude of contraction and coronary flow. In reserpinized animals transient β-stimulation has been observed by other workers.

Action on Blood Vessels

Coronary Blood Flow was not reduced by H 56/28 at any dose level but there was a slight but insignificant ($p > 0.05$) increase in the coronary flow. During ventricular fibrillation the coronary flow decreases but the addition of H 56/28 increased coronary flow for a period less than 5 minutes.

Peripheral Blood Flow. H 56/28 was found to increase blood flow in all vascular beds provided the systemic pressure was kept constant. Under conditions of constant flow (systemic pressure was allowed to fall) the drug produced minimal changes in blood flow

Supplies of H 56/28 were kindly supplied by Astra Pharmaceuticals (Aust.) Pty. Ltd.

through various regions. On measuring both the ascending aortic flow by an electromagnetic flowmeter and the systemic pressure it was found that H 56/28 produced a moderate increase in aortic flow and a decrease in systemic pressure. These changes indicate a decrease in total peripheral resistance; an effect which lasted less than 5 minutes.

Antiarrhythmic Action. The antiarrhythmic efficacy of H 56/28 was tested during ventricular fibrillation induced by the passage of an 80-100 ma current across the surface of the heart. Only on one occasion did 5 mg/Kg H 56/28 convert the fibrillation to sinus rhythm. This was not reproducible even in the same experiment. Counter-shock by means of the defibrillator was consistently effective in controlling ventricular fibrillation in every experiment.

Summary

The results indicate that H 56/28 does not constrict the coronary or other vascular beds, nor does it cause a change in the high energy phosphate stores or myocardial oxygen consumption. However, H 56/28 causes cardiac depression, an effect found to be common to most β-receptor blocking drugs. It is probable that H 56/28 is effective in certain types of cardiac arrhythmias where an excess of the sympathetic component contributes to the arrhythmia. In ventricular fibrillation this β-blocking drug was found to be ineffective.

KINEKARD †

T. E. Lowe, W. G. Nayler, N. Dorevitch, I. Macdonald and T. W. Moir

Kinekard is the name we have given to a fraction isolated from heparinised blood plasma of mammals, birds, reptiles and fish. The procedure of isolation has been defined and references to kinekard are to this fraction only and not to fractions prepared in other ways which may have cardioactive properties. Kinekard is positively inotropic on cardiac muscle and its action on smooth muscle resembles that of the catecholamines. It can be shown however that the active factor(s) of kinekard is not a catecholamine.

Physiology

(a) Coronary Circulation. Both in the intact beating heart and in a heart perfused at a constant pressure kinekard causes an increase in effective coronary blood flow. This has been interpreted to mean that it causes a decrease in the resistance to blood flow in the coronary circulation. During the present year another technique which allows the coronary flow of isovolumically beating hearts to be measured, has been used to study this effect of kinekard in more detail. In this method the coronary circulation of dogs which were placed on complete heart-lung bypass was perfused with oxygenated blood delivered into the aorta via the left subclavian artery beyond which the aorta was ligated. Pressure in the aortic arch, and hence coronary perfusion pressure, was measured by inserting a long metal cannula down the right carotid artery into the arch of the aorta. Coronary venous blood was drained into a reservoir through a catheter in the right ventricle and the pulmonary artery was occluded. The left ventricle was emptied via a catheter inserted through the left atrial appendage. In contrast to noradrenaline kinekard caused a marked and consistent vasoconstriction under these circumstances. This coronary vaso-constrictor response to kinekard was similar to that caused by angiotensin or pitressin, but was completely eliminated by α blockade in contrast to the constrictor effect or either angiotensin or pitressin which was either unchanged or only moderately attenuated. The effect of kinekard on the coronary circulation, therefore, differs from that of pitressin, noradrenaline or angiotensin.

(b) Plasma Concentration in Normal Man. The plasma concentration of kinekard in terms of both inotropic and pressor activity in mixed venous blood has been measured in a number of normal individuals of both sexes whose ages ranged from birth to young adult life. The concentration does not appear to change with age and the results show a 'normal' distribution. The inotropic activity is 27.5 $\pm$ 3.5 I units (mean $\pm$ S.D.) and pressor activity 9.4 $\pm$ 1.7 P units.

(c) Site of Production. In a series of 8 adults, who were being investigated by cardiac catheterization, blood samples were obtained from the right atrium, hepatic vein and a peripheral artery. Assay of these samples showed that plasma from hepatic vein blood had a kinekard activity of (51.0 ± 6.6) I units which is approximately twice that found in right atrial blood (28.1 ± 3.5) which in turn is consistently a little higher than that found (24.1 ± 2.4) in peripheral arterial blood.

Plasma from two patients from whom the spleen and both kidneys had been removed pending renal transplantation yielded kinekard levels within normal limits. Two patients in whom liver failure had occurred showed a marked lessening of both inotropic and pressor activity of kinekard in mixed venous blood. These levels returned towards normal with recovery of the patients.

These results suggest that the active factor(s) of kinekard enters the general circulation via the hepatic vein and that the kinekard is either produced or activated in the liver.

Plasma obtained from rats which have been hypophysectomized for 15 days contained only 10.8 ± 2.4 (mean ± S.D.) I units and 6.7 ± 1.2 P units of activity, compared with control levels of 29.6 ± 2.9 and 13.7 ± 2.3 units respectively. This indicates that although kinekard is produced or activated in the liver (or alimentary canal) its production is in some way under pituitary control.

Other investigations showed that kinekard could be recovered from red blood cells and urine as well as plasma.

Since the pressor action of kinekard was assayed using rabbit thoracic aorta some additional experiments were performed to determine under what conditions activation of β-receptors in these preparations could result in vaso-constriction. The results obtained indicated that the constrictor effect of large doses of isoproterenol was mediated through α and β adrenergic receptors. The constrictor effect of kinekard, however, was mediated through α receptors and unlike the constrictor effect due to large doses of isoproterenol, was not modified by β-adrenergic blockade.

(d) Correlation Between Plasma Levels of Kinekard and Angiotensin. The drug ST 155 (Catapres), when injected intravenously, caused a significant fall in the plasma level of angiotensin. A series of 15 experiments was performed to determine if the plasma level of kinekard remained constant in the face of a fall in plasma angiotensin. Both the inotropic and pressor actions of kinekard were assayed. The results showed that the plasma level of kinekard remained constant despite a fifty percent, or greater, fall in the level of angiotensin.

Biochemistry

I. Macdonald

Previously it has been reported that kinekard, as originally prepared (i) gives three ninhydrin positive bands on high voltage electrophoresis, only one of which is biologically active; (ii) when incubated with the proteolytic enzymes, pronase and subtilicin, loses its ability to elicit an inotropic response; (iii) comes off the gel filtration column in the same region as substances of molecular weight in the range 6,000-10,000.

This preparation however contained some protein and the ion-exchange step was modified slightly. The kinekard now produced gives two bands with positive inotropic activity on high voltage electrophoresis.

Last year a single step ion-exchange chromatography on DEAE cellulose was investigated as a method for fractionating plasma. This yielded a fraction with kinekard-like activity and with similar inotropically active bands on high voltage electrophoresis (fraction γ). There were also two fractions (α, β) which came off in front of fraction γ that had negative inotropic activity.

These data indicated that the cardioactive properties of plasma might be due to the presence of more than one active component and during the current year efforts have been directed towards characterising these components chemically. The previous work indicated that the two-stage fractionation used to produce kinekard was not so well suited for this investigation as the single stage method and variations of the latter have been used.

Although active fractions produced by this procedure do not fit the definition of kinekard, it is hoped that the investigation of them may lead to an understanding of the nature of the active factor(s) of kinekard.

Multicomponent Nature of the Inotropic Plasma Factor(s). The use of triethanolamine acetate (TEA) pH gradient in the range of 7.3 to 7.1 instead of a sodium chloride gradient to elute fractions from the DEAE cellulose column has produced a set of inotropically active fractions slightly different from those previously described. These are characterised as fraction *a*-(negative inotropic activity), fraction *b*-(positive inotropic activity), fraction *c*- usually positive, but on rare occasions negative, inotropic activity.

Because fractions α and β came off in front of fraction γ only when a large column was used to fractionate a relatively larger plasma sample (40 ml) it was suspected that fractions α and β and also two of the three fractions *a*, *b* and *c* were artefacts arising from the column. However, when the DEAE cellulose used in these experiments was made up into a column and this column eluted with a pH gradient (range 7.3-7.1), without prior addition of plasma following regeneration, no inotropically active fractions were collected.

It had been noted (P. E. Hughes) that when the fraction γ was ultrafiltered, positive inotropic activity passed through the membrane, leaving the solution inside with negative inotropic activity. Using the same membrane (M.W. exclusion limit 10,000) both a sample of plasma and Cohn fraction IV-I were similarly filtered, and in each case the positive inotropic activity measured outside the membrane after filtration was much greater than that of the solution inside. These results indicated the presence of a negative inotropic factor too large to pass through the membrane. As neither the plasma nor the Cohn fraction had been in contact with DEAE cellulose, such contact is not necessary for the production of the negative inotropic factor. Furthermore it was found that a sample of blood plasma which was allowed to stand at 4°C for about three weeks after collection became strongly negatively inotropic as did plasma samples stored frozen for a period of 8-12 months. These observations indicate that this negative inotropic activity arises in the plasma, probably from a plasma precursor, though it has not been definitely proved that DEAE cellulose does not catalyse its production. When fraction *a* was left to stand overnight its negative inotropic activity changed to positive but when this fraction was then made up to 0.9% with sodium chloride the negative activity was restored. The positive inotropic activity of fraction *c* and sometimes that of fraction *b* could also be rendered negative by making these fractions up to 0.9% with sodium chloride. These results, and the lack of negative inotropic activity resulting from DEAE cellulose chromatography of plasma when a salt gradient is not used as the eluent, underline the importance of the presence of sodium chloride in determining the sign of the inotropic activity of the active constituents of any of these fractions.

When fractions *b* and *c* were prepared on a long DEAE cellulose column (the degree of resolution seems to be proportional to length) each fraction gave, on high voltage electrophoresis, a single inotropically active band, which, while in a different region to that given by the other fraction, corresponded to one of the active bands given by kinekard on high voltage electrophoresis. Fraction *c* and its corresponding active band showed pressor activity whereas such activity could not be detected in fraction *b* or in any other region of the two electrophoretograms.

From the above results it has been postulated that plasma contains *inter alia* the following components: a positive inotropic factor *A* comprising two molecular species, one of which has only inotropic activity *Ai* and one which also has pressor activity *Ap*, and an inactive molecular species *Q* which reacts with *A* thus:

$A + Q \rightleftharpoons I$, where *I* is a molecular species having negative inotropic activity.

The above equilibrium would be shifted to the right by increasing concentrations of NaCl.

In view of the postulated di-component nature of A this equilibrium would be better represented thus:

$$Ai + Q \rightleftharpoons Ii \qquad Ap + Q \rightleftharpoons Ip$$

These postulates are supported by the following observations:

(1) When negatively inotropic plasma is dialysed against buffer solution positive inotropic activity soon appears in the solution outside the membrane. Later this activity becomes negative provided that the volume of the outside liquid is not too large. In the latter case this may result in the activity inside the membrane becoming positive also.

(2) If the buffer is dialysed against plasma with negative inotropic activity until the buffer acquiries negative inotropic activity, and if this buffer is then stirred with DEAE cellulose (which takes up the active components) the inotropic activity of the buffer slowly rises to zero and then on up to increasing positive activity.

These results can be explained in the light of the above hypothesis if it is assumed that Q is small enough to pass through the dialysis membrane (though its size would retard its passage), and if it is realised that as A and Q fall in concentration (whether removed as such or combined in the form I) the ratio of A/I is increased, thus increasing the net positive inotropic activity.

The Chemical Nature of the Active Factor(s). Kinekard activity disappears when kinekard is incubated with either pronase or subtilicin. In similar studies using fraction d[1] and chymotrypsin and trypsin as the proteolytic enzymes it was observed that (1) when fraction d was incubated alone, positive inotropic activity remained after incubation; (2) when the enzyme was incubated alone positive inotropic activity was recorded after incubation; (3) when the enzyme and fraction d were incubated together no net inotropic activity could be detected after the incubation.

Now if the active factor was subject to proteolytic breakdown by these enzymes, then in the case of (3) above, the positive inotropic activity of the enzyme should have been observed after the incubation. The only explanation which could be found to fit these observations was that in the case of (3) the active factor of fraction d was not destroyed, but that its inotropic action countered that of the enzyme, resulting in a net inotropic activity of zero. This hypothesis was supported by a subsequent incubation experiment with trypsin and fraction d when it was shown that (i) positive inotropic activity was recorded when trypsin was added after the incubation; (ii) zero activity was recorded when the inhibitor was added before the incubation. Other experiments showed that the inhibitor was destroyed during the incubation.

In another series of experiments, samples of fraction d were incubated both with chymotrypsin and on their own. Then the resulting solutions were added to small columns of Dowex 1 x 2 and washed through with buffer. The columns were then eluted by lowering the pH of the buffer. There was no significant difference (in terms of the inotropic activity of the solutions thus eluted) between the series incubated with enzyme and that incubated without enzyme. However when these eluates were made up of 0.9% sodium chloride only those incubated without enzyme became negatively inotropic. Thus the peptide nature of the active components is in doubt.

Stability. When samples of fraction b were incubated at 25°C for half an hour at various pH in the range 2-9 only those incubated in the range 6-8 still showed inotropic activity.

Fraction d has been shown to retain its activity after two months storage (i) in solution at 4°C; (ii) in frozen solution at – 40°C; (iii) dry at – 40°C.

The positive inotropic activity of fractions a, b, c and d is not destroyed by heating at 80-90°C for up to half an hour, not even in the presence of organic solvents.

The Assay. Inotropic activity of the plasma fractions has been assayed on a standardised

[1] Fraction d is an inotropically active fraction prepared from deproteinised plasma on a DEAE cellulose column too small to resolve it into more than one fraction.

procedure using an isotonic toad heart preparation. If the interpretation of the results quoted above is correct this assay will give only the net activity of the positive and negative inotropic active factors present in the sample. In this event the numerical value of this inotropic activity cannot give any indication of the chemical concentration of any of the active molecular species present. The magnitude of the inotropic response should be governed by the relative concentrations of the positive inotropic and the negative inotropic factors, rather than by the absolute concentrations. However dilution studies have shown that even the relationship between the relative concentrations of these two opposing factors and the inotropic response is very complex. While these considerations make this assay unsatisfactory for chemical studies on these fractions they do not effect its ability to evaluate these fractions in biological terms.

Preparation of the Active Factor(s). Up to the present time no satisfactory method of purifying any of these active factors has been developed because any purification step divides the surrounding medium into two phases (e.g. precipitate and supernatant, moving phase and stationary phase, and the tendency is for A to favour one phase in the free state and to favour the other phase when combined with *Q* as *I*. Thus there is the possibility of losing a high percentage of *A* at every step. Furthermore, the limitations of the assay do not permit accurate determination of which fractions of a fractionation procedure contain the active material.

It would seem that *A* can only be effectively isolated if *Q* is removed beforehand. The following observation suggests that this may be possible. When an active fraction is made up to 59% acetone, then evaporated to dryness under reduced pressure at 80-90°C and then made up to its original volume with TEA buffer, there is a striking increase in inotropic activity. This suggests that the inert carrier *Q* is rendered incapable of reacting with active principle *A*. The inotropic activity of such a sample decays in a matter of days, even at 4°C, suggesting that the highly unstable *A* is stabilised by combination with *Q*. This interpretation is in accord with earlier observations that kinekard is rendered less stable by isolation and that when the active principle *A* is separated from *Q* by ultrafiltration it loses activity in a matter of hours at room temperature.

Molecular Size of Active Factor(s). When 1 ml of active sample was passed through a Bio-Gel P 10 column (molecular exclusion 10,000 and total volume 320 ml) activity did not appear until 430 ml and reached a maximum in the region of 570 ml. When this was repeated using a column of Sephadex G 25 (molecular exclusion 5,000 and total volume 366 ml) two active fractions were collected, one in the region of 165 ml and the other in the region of 284 ml. When a 1 ml sample from the 284 ml region of this run was passed through the same column three active fractions were collected with elution volumes in the regions of 120 ml, 212 ml and 305 ml.

These results suggest that a large molecule (*Q* M.W. about 10,000) carries one or more smaller molecules (*A* M.W. 500 or less) through the column in elution volumes which would be expected for compounds in the molecular weight range of 5,000-10,000.

Conclusions. The close similarity between the inotropically active bands given on high voltage electrophoresis by the fractions studied above and kinekard and the ability of the latter to show negative inotropic activity under certain conditions justify the hope that the active components involved may prove to be the same in either case. If so, kinekard would have to be considered as containing a multi-component active principle which endows it with the potential of displaying a range of physiological actions on any specific organ.

BIOCHEMICAL TECHNIQUES

V. Carson

Assay of Tissue Catecholamines

A reliable method for the fluorimetric assay of catecholamines in mammalian heart muscle

has been developed from the method (Crout, 1961) used for the assay of urinary catecholamines.

1-2 g of freshly excised heart muscle is placed in cold Tyrode solution, then blotted and weighed. After mincing finely with scissors it is homogenized very finely in 15 ml of ice-cold 0.2 M sodium acetate buffer, pH 3.5, using a Potter-Elvehjem type of homogenizer; after which 5 ml of ice-cold 20% trichloracetic acid is added and the homogenization continued for a further few minutes. The mixture is filtered through sintered glass, 20 ml of 0.2M Sodium acetate and 2 ml of 0.2M EDTA are added and the mixture brought to pH 8.4 with 5 N and 0.5 N NaOH with constant stirring, and then chromatographed on a column of alumina prepared (Crout) as follows:

1 g of activated alumina in water is added to a glass column of 0.7 cm diameter and allowed to settle.

After the extract has run through, the column is washed with water until the pH is less than 7 and then eluted with 0.2 M acetic acid. After discarding the first ml, 9.5 ml are collected and 0.5 ml 0.2 M EDTA added. The eluate is assayed for catecholamine fluorimetrically by the trihydroxyindole method, using potassium ferricyanide as the oxidant and with the addition of ethylene diamine to the alkaline ascorbate to stabilize the fluorescence. The differential assay of noradrenaline and adrenaline is achieved by carrying out the oxidation at pH 3.5 and 6.5, with inclusion of appropriate standards, essentially by the method of Crout.

Fluorescence is measured in a Turner fluorimeter using a primary filter passing the 365 mμ line and a secondary filter transmitting maximally at 525 mμ (Wratten 58). With this system the fluorescence of adrenaline and noradrenaline are roughly equivalent at pH 6.5 whilst at pH 3.5 the ratio of fluorescence of adrenaline to noradrenaline is about 10 to 1. The fluorescence of adrenaline at both pH is approximately equal.

Contrary to the conclusions of Crout, potassium ferricyanide was preferred to iodine as the oxidant, because the fluorescence obtained with ferricyanide was double that obtained with iodine, the reaction was far less time-consuming and with the use of ethylene diamine to stabilize the fluorescence there were no problems with quenching or stability of the fluorescence up to one hour. Given a more sensitive spectro-photofluorometer, the assay of much smaller quantities of tissue would be possible. Recovery of standard amounts of noradrenaline and adrenaline by the above method is about 78% (mean of six estimations).

Yields of catecholamines from freshly killed normal rat heart have averaged about 0.6 μg/g wet weight (uncorrected for percentage recovery).

In dogs the yields have been 3 μg/g (mean of 2 samples of puppy atrium); 1.2 μg/g (mean of 2 samples of puppy ventricle); 0.75 μg/g (mean of 2 samples of dog ventricle) and 1.6 μg/g (1 sample of dog atrium).

Biochemical Changes Associated with Myocardial Depression

(In association with W. G. Nayler)

As part of a larger study, methods are being developed to investigate a range of biochemical changes accompanying myocardial depression.

Parameters to be studied include myocardial high-energy phosphates, aerobic tissue glycolysis, tissue sodium and potassium, myofibrillar ATPase and tissue catecholamine content.

Methods previously described for the assay of high-energy phosphates (creatinine phosphate, adenosine triphosphate and adenosine diphosphate) will continue to be used.

Catecholamines are being estimated by a method described elsewhere in this report.

Aerobic glycolysis of heart homogenate using fructose-1-6-diphosphate as substrate is being studied in the Warburg apparatus.

The assay of myofibrillar ATPase is proving troublesome owing to the difficulty of isolating and preserving the extremely fragile myofibrils which in the dog seem to be particularly prone to breaking up unless extreme care is exercised in the homogenization process.

CONTROL MECHANISMS ‡

M. Rosenbaum

An attempt has been made to assess the relationship between arterial blood pressure and aortic pulse wave velocity and the possible use of the latter as an indirect measure of systemic arterial pressure. Eight dogs were anaesthetized and arterial blood pressure was measured at the proximal end of the femoral artery by a Statham strain gauge connected to a fine polyethylene cannula. The pulse wave velocity in the aorta was approximated by measuring the time between the R wave of the electrocardiogram and the first detectable rise in femoral artery pressure. Various interventions were then carried out to alter the arterial blood pressure, in particular the valsalva manoeuvre, the administration of pressor amines and sympathetic blocking drugs. The results indicated that although a broad correlation exists between pulse wave velocity and aortic blood pressure, this does not provide an accurate indirect method of measuring systemic arterial pressure. In most animals the scatter between aortic pulse wave velocity and diastolic blood pressure was such that blood pressure could only be predicted with ± 20% accuracy by this method.

MYOCARDIAL CYTOMEMBRANE SYSTEMS

L. W. Wheeldon

The pharmacology and much of the physiology of heart function cannot be understood without reference to processes at the cellular level. Our knowledge of cellular physiology is of two kinds: on the one hand, a great deal is known about the "idealised cell" — information drawn from a great variety of cells and tissues, from bacteria to man; on the other hand, specialised knowledge of particular cells, such as the myocardial fibre, is largely limited to inferences that can be made from pictorial data obtained with the electron microscope. One such inference is that functional specialisation in tissues is allied to the differentiation of uniquely organised cellular membrane systems. However, the assignment of unique functions to these membrane systems depends upon means of isolating them free of extraneous cellular components, an objective which has been achieved satisfactorily for only a few tissues, pre-eminently liver. The goal of the present research has been to develop improved means of isolating cytomembrane material from myocardial tissue and thus permit a more detailed delineation of its composition and functional parameters. Circumstances which have delayed progress in this area are of three kinds: (1) inefficient resolution of membrane fragments derived from mitochondria from those derived from the cytomembrane system; (2) a marked tendency of membrane fragments to aggregate, thus altering sedimentation properties; (3) the existence of a complex cell wall structure, including collagen fibrils and glycoprotein material, which components are likely to contribute to the heterogeneity of cytomembrane preparations.

These problems of resolution have been dealt with by employing centrifugal analysis on density gradients in conjunction with assays for enzyme activities which help to identify the origin of the membrane fragments: cytochrome oxidase, 5'-nucleotidase, esterase, nicotinadenine dinucleotide-cytochrome c reductase and adenosine triphosphatase. Electron microscopy is also being employed in conjunction with these assays. Results show that all types of membrane fragment tend to aggregate and that this property has a dominant effect on rates of both sedimentation and flotation on density gradients. Polyanions retard aggregation, while polycations promote it. The most effective polyanions tested are heparin and dextran sulphate. By including heparin in density gradients to control aggregation, it has been possible to achieve resolution of the three enzyme activities cytochrome oxidase, esterase and calcium-requiring adenosine triphosphatase which are identified respectively with fragments of mitochondrial inner membrane, endoplasmic reticulum and transverse tubular system. Effective resolution depends not so much upon isopycnic banding, as upon differing rates of flotation, which in turn are governed by differing aggregate sizes. We hope to exploit this important technical advance by carrying out further studies on the composition of the sarcotubular adenosine triphosphatase.

EXERCISE TEST OF CARDIAC FUNCTION

A. J. Barnett and F. R. Trinker

In the course of studies of the effect of cardiac disease on cardiac function it has become necessary to investigate the response of the heart to known and controlled amounts of exercise. It is planned to compare the response to exercise of the heart rate, cardiac output and electrocardiogram of patients suffering from cardiac disease with that of normal individuals.

The first requirement is an exercise test severe enough to produce a measurable response but yet not too arduous to be used in patients. For this purpose a bicycle ergometer has been used in two ways: first, starting at a load of 400 kilopond per min. (KPM) and increasing this by 200 KPM every 6 minutes until 1000 KPM is reached; and secondly, maintaining a steady load of 600 KPM for 20 minutes. The former is the more severe test and produces the greater increase in heart rate.

After a marked initial increase the rate of increase of heart rate diminishes but has not completely disappeared after 20 minutes. On cessation of the exercise there is a sudden decrease in heart rate, which is however still above the resting level 20 minutes later.

HYPERTENSIVE STATES

A. J. Barnett, F. G. Silberberg[1] and F. R. Trinker

Analysis of Therapeutic Trial

In the past 18 years 208 patients with severe arterial hypertension have been treated in a special clinic. Previous surveys of these patients have indicated the symptomatic and objective improvement which follows control of the hypertension and the improved life expectancy in malignant hypertension produced by modern treatment.

A start has now been made on a more extensive survey of the course of severe hypertension as influenced by modern treatment. This will have special reference to the duration of life, the symptomatic state, the progress of vascular disease and the complications of treatment. It is important to know how this progress is related to the patient's pretreatment condition with respect to height of blood pressure and the existence of associated cardiac, vascular and renal disease. Such an analysis of numerous variables requires the use of modern data processing methods and a check sheet[2] has been devised for the purpose of coding data from the histories in a form suitable for computer processing and the transfer of data has commenced.

Trial of "Catapres"[3]

Although there has been a steady improvement in the treatment of hypertension with the development of a variety of potent hypotensive drugs, none of these is completely free of side effects and some patients are relatively resistant to treatment. "Catapres" (previously known as ST 155) is a hypotensive drug which previous acute studies have shown to be effective without producing marked postural hypotension or side effects. A clinical trial of this drug is now being conducted and to date eleven patients have been treated, nine with "Catapres" alone initially and two with later addition of cyclothiazide (Doburil, Boehringer). In 7 of the 9 patients treated with "Catapres" alone there was an appreciable fall in blood pressure; the remaining 2 showed a fall on the addition of cyclothiazide and 10 of the total 11 patients are controlled with either "Catapres" or "Catapres" plus cyclothiazide. Side effects have comprised drowsiness (6 cases), dry mouth (2), nightmares in the initial stages only (2).

"Catapres" appears to be a clinically useful hypotensive agent in moderate hypertension, but further study is required.

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[1] Clinical Assistant, Alfred Hospital.

[2] The advice given by D. Race is gratefully acknowledged.

[3] Kindly supplied by Boehringer, Ingelheim Pty. Ltd.

CARDIAC SURGERY‡

G. R. Stirling, W. G. R. M. de Boer[1], V. Carson, E. Cooper and J. Stone

Interruption of the coronary blood flow is inevitable during cardiac transplantation and frequently occurs during cardiac surgery for valve disorders. The study of the effects of ischaemia on cardiac muscle (commenced last year) has continued with particular reference to changes in the content of high energy phosphates and various enzymes in the muscle during the period of ischaemia; and to the ability of the heart to return to normal function after the ischaemia is removed and coronary perfusion is recommenced.

Other facets of the technique of cardiac transplantation that are being explored relate to the storage and resuscitation of donated hearts and their behaviour after grafting to the new host.

ISCHAEMIA

The dog was used as the experimental animal and ischaemia for periods of 30, 60 and 120 minutes was induced by cross clamping the aorta during complete cardio-pulmonary bypass. Before inducing ischaemia the cardiac output was raised in steps by venous inflow control and data were collected for the construction of Starling curves. Biopsies were taken from the right ventricle and these were used for assay of adenosine triphosphate (ATP), adenosine diphosphate (ADP) adenosine monophosphate (AMP) and creatine phosphate (CP). After the period of ischaemia cardiac function was supported for a time by total cardio-pulmonary bypass and then further function studies were performed and biopsies were taken for high energy phosphate content and also for histochemical assay of lactic and succinic acid dehydrogenase (LDH and SDH).

[1] Associate Professor, Pathology, Monash University.

High Energy Phosphates

The techniques of biopsy and assay of ATP, ADP, AMP and CP reported in 1967 have proved to be reproducible and consistent results have been achieved since standardisation of the technique.

Although with prolonged perfusion of the heart with the pump-oxygenator slight falls in CP and ATP occur (possibly from cellular oedema) ischaemia for even a few beats produces a rapid fall of CP to about 10% of the control level. With early resumption of perfusion CP content rapidly returned to the control level and after one hour's ischaemia CP could be restored to 80% of the control level. After two hours ischaemia the CP level remained at 30%.

The return of CP to near normal levels after ischaemia for one hour was noted in hearts incapable of sustained function. However, on the other hand no heart with a CP below 50% of the control level was capable of significant function.

ATP levels fell with ischaemia but were not restored by even prolonged supporting perfusion. The final level reached seemed directly related to the overall length of time of ischaemia but there was poor correlation between individual changes in ATP and myocardial function. The changes in ATP concentration with ischaemia were little affected by local cooling.

Enzyme Changes in Cardiac Muscle

Histochemical estimates of LDH and SDH showed no significant changes in the tissue content of these enzymes in response to the stress imposed by this experiment.

Effects on Myocardial Function

At normal temperatures ischaemia for 30 minutes results in significant deterioration in function but with support perfusion continuing for 30 minutes there is recovery to near

the control level. After 60 minutes of normothermic ischaemia none of 5 dogs recovered sufficient function to maintain a circulation.

Local hypothermia to 5°C induced by packing the heart in ice chips had a marked protective effect so that 30 minutes of ischaemia had a negligible effect on recovery and good recovery of function was usual after 60 minutes of ischaemia. However, none of the 5 dogs submitted to 120 minutes of ischaemia at 5°C recovered significant function.

It seems apparent that if ischaemia for more than 20 minutes is intended then some form of local hypothermia should be used to preserve function. Although good protection for 1 hour is usual with local cooling it is not invariable and probably some other method would be necessary to preserve function for more than 1 hour of ischaemia.

PERFUSION OF THE ISOLATED HEART

Since it is clear that ischaemia, even in the presence of hypothermia, is progressively damaging to the heart it was decided to study prolonged perfusion of the isolated heart as a possible solution. A Langendorf type of isolated heart preparation has been perfused with oxygenated blood. In this way it has been possible to support such isolated hearts for periods up to 6 hours without obvious deterioration in their contractility. Estimations of high energy phosphates indicate only slight changes with perfusion for 60 and 90 minutes.

The resistance to the flow of blood through the vascular bed of the isolated heart is a subject of continuing study. It is clear that there is a linear relationship between pressure and flow at any instant in time but vascular resistance increases with prolonged perfusion.

With a view to the temporary storage of the donor heart during transplantation, perfusion of its coronary circulation at 5°C was studied and it seems clear that this technique results in more effective preservation of cardiac function than either simple hypothermia, ischaemia or normothermic perfusion. The optimum temperature and the optimum composition of the perfusate for prolonged perfusion are not yet defined and further studies are planned to develop a technique which will allow better preservation of function with prolonged perfusion. Such a technique may allow the storage of donated hearts for human transplantation for periods longer than current techniques which are adequate for preservation of the heart for only a few hours.

CARDIAC TRANSPLANTATION

Experience has been gained with the techniques of transplantation of the dog heart with a view to developing a reproducible successful method to allow study of the rejection phenomenon and also the functional behaviour of the transplanted heart. Many problems have been encountered and it seems that success lies in the conquest of many technical factors rather than in any single fundamental defect in the techniques used. Attention has been directed towards the following problems.

Perfusion of Recipient

The dog tolerates prolonged cardiopulmonary bypass poorly and acidosis develops despite high-flow perfusion. Correction of acidosis and reduction of the time needed to perform the transplant have significantly improved results.

Reduction of "Ischaemic Insult"

The donated heart is exposed to ischaemia in the donor animal before removal, in the period between excision and transplantation and during the process of transplantation itself. It seems crucial that the period of normothermic ischaemia before removal for transplantation should be reduced to a minimum. In our experience no heart which has been ischaemic for more than 20 minutes in the donor animal has functioned effectively after transplantation, but experiments in which the donated heart has been perfused in the donor by cardiopulmonary bypass have resulted in good preservation of function.

Two techniques have been used for preservation of the heart during transplantation. The first (Shumway) involves rapid cooling of the heart in saline at 4°C followed by constant irrigation with cold saline. This technique has proved successful provided meticulous care is taken to thoroughly cool the donated heart after excision and to keep it cool during transplanta-

tion and provided the transplantation can be achieved in less than fifty minutes. The technique appeals in its simplicity but allows little room for error. Studies of the intracavitary temperature in the left ventricle indicate that it rarely falls much below 15°C.

The second technique which has been used is to perfuse the heart, via the aortic arch, with blood at 5°C. This results in efficient and rapid cooling of the heart and the continued supply of oxygenated blood prevents ischaemic damage. However during the period when the aorta is sutured some period of ischaemia occurs. The technique is more elaborate than the first but probably provides more latitude if technical problems are encountered.

Thirdly a combination of the two methods has been effectively used.

Technical Factors and Supportive Measures

Reduction of damage to the myocardium by excessive handling, ventricular distension and air embolism have all contributed to improved results.

Assessment of the function of the transplanted heart by measurement of cardiac output, left atrial pressure and the rate of change of left ventricular pressure shows that all transplanted hearts so far studied have exhibited some degree of failure. Function of the heart in the recipient rapidly improves if supportive perfusion by a pump-oxygenator is provided so that left atrial pressure falls and the cardiac output increases steadily over the first 30 to 60 minutes after transplantation. A period of cardiac support by the pump-oxygenator therefore seems essential.

It has been noted that the transplanted heart responds dramatically to digoxin and ouabain and isoproterenol is of some value in supporting the failing circulation.

The transplated heart, although in sinus rhythm, generally beats at a relatively slow rate (less than 100 per minute) and stimulation by an electrical pacemaker to rates of 120 to 140 per minute has significantly increased the cardiac output.

PERIPHERAL VASCULAR DISEASE

A. J. Barnett, I. A. Ferguson[1], K. N. Morris[2], A. Shanahan[2] and K. Stuchbery[3]

The first arterial graft operation in this hospital was performed in 1950 for the relief of arterial obstruction. Since then the material used for the graft has changed from time to time and other surgical procedures (thrombectomy and endarterectomy) have been introduced.

During 1968 arterial obstructions have been treated by autogenous vein grafts in 56 patients, dacron grafts in 6, endarterectomy in 16 and thrombectomy in 5. A follow-up study on 137 bypass vein grafts inserted before 1st July, 1967 and followed until 30th June, 1968 has been made. This gives a follow-up period of at least 1 year (in some cases 3 years) and Table 1 (Page 34) sets out the results.

Previous studies have shown the patency rates of arterial homografts, teflon grafts and dacron grafts. Although a much longer period is available for study of these earlier types of graft, a comparison (Table 11) of patency rates up to 3 years indicates that vein grafts are superior to the other types.

[1] Honorary Surgeon to Outpatients, Alfred Hospital.

[2] Thoracic Surgical Unit, Alfred Hospital.

[3] Casualty Surgeon, Alfred Hospital.

TABLE I

Results of Vein Grafts					
Time of Review (months)	Discharge	6	12	24	36
Number of Patients	137	137	137	83	40
Lost from follow-up	0	0	3	5	6
Died	3	3	5	4	2
Grafts blocked	10	28	33	25	11
Grafts patent: (a) number	124	106	96	49	21
(b) %	91	77	70	59	52
(c) %	93	79	75	66	66

The numbers in (b) represent the percentage of grafts patent calculated from the total number of patients; in (c) the percentage is calculated from the number of patients available for study at 30/6/68.

TABLE II

Patency Rate for Different Types of Graft					
Time of Review (months)	Discharge	6	12	24	36
Aterial homograft	85 (87)	83 (72)	59 (60)	46 (49)	41 (48)
Teflon	85 (85)	70 (60)	40 (41)	27 (30)	24 (16)
Dacron above groin	92 (97)	77 (91)	70 (87)	51 (75)	47 (73)
,, below groin	75 (78)	48 (50)	37 (38)	24 (26)	24 (24)
Vein	91 (93)	77 (79)	70 (75)	59 (66)	52 (66)

Figures in upper lines represent the patency rate as a percentage of patients operated upon and the figures in lower lines (in brackets) represent the patency rates as a percentage of patients available for examination.

BLOOD COAGULATION

P. Fantl

Hypofibrinogenaemia

In last year's report the results of blood coagulation tests of a patient prior to prostatectomy were recorded and it was indicated that there were probably three independent coagulation defects connected with his condition, namely: hypofibrinogenaemia, a defective fibrinogen and fibrinolysis.

Currently it is believed that the formation of fibrin from fibrinogen is a multistep reaction and when viewed in this light the results obtained with the patient's fibrinogen suggest that the first phase — proteolysis of fibrinogen by thrombin to the fibrin monomer — and also the second phase — the polymerisation of fibrin monomer — proceed normally although with reduced intensity. However the next phase — the conversion of the fibrin polymer to the fibrin gel — is delayed in the patient's plasma. Apparently this step is influenced and accelerated by calcium ions. No evidence for any type of inhibitor could be found. A prolonged clotting time can be due to hypofibrinogenaemia but the clotting times were far more prolonged than would be expected from the amount of fibrin recovered. Therefore it is concluded that a structural defect in the fibrinogen molecule is responsible for the prolonged thrombin clotting time observed. The condition can be classified as a fibrinogenopathy by analogy with the haemoglobinopathies, in which an abnormality occurs in the peptide chain of the globin moiety.

Persistent fibrinolysis was present before and after prostatectomy and as no evidence of liver disease could be found it is likely that small but sufficient amounts of the activator of profibrinolysin (plasminogen) were present and the fibrinolysin so formed lysed the patient's defective fibrinogen. The abolition of fibrinolysis by an injection of fibrinogen supports this contention. Further evidence that fibrinolysis is not responsible for the defects is that two antifibrinolytic drugs, trasylol and ε-aminocaproic acid, did not alter the fibrinogen characteristics. Of practical interest is the fact that approximately 42,000 units per hour of trasylol given I.V. for 72 hours were ineffective in stopping fibrinolysis in this patient, whereas ε-aminocaproic acid was effective in counteracting fibrinolysis both *in vivo* and *in vitro* at a dosage level of 0.12 mg ε-aminocaproic acid per ml plasma. The fibrinogen characteristics neither qualitatively nor quantitatively were altered during the I.V. injection of ε-aminocaproic acid.

It may be concluded that the fibrinogen defects noted are not of serious clinical import nor is the mild hypofibrinogenaemia nor the mild fibrinolysis.

Investigations[1] of the patient's fibrinogen by immunoelectrophoresis revealed a slightly higher than normal cathodic mobility of the patient's fibrinogen. Immunodiffusion revealed no antigenic abnormality.

Optical Characteristics of Human Fibrin Gel

During the above study it was found that thrombin added to the patient's plasma gave prolonged clotting times and an abnormally low yield of fibrin. The addition of Ca^{++} to the plasma resulted in a higher yield of fibrin. On the other hand the turbidity of fibrin gels produced in p'asma-thrombin mixtures was not significantly different from fibrin gels produced from recalcified plasma mixtures when compared with normal plasma although reduced in intensity. Therefore it seemed of interest to determine the influence of several factors on the turbidity of fibrin gels.

Fibrin gels prepared from human fibrinogen and bovine thrombin when measured by transmitted light showed a peak between 227-245μ. This is due to stray light. When the scattering effect is minimised the peak disappears and a smooth curve running from ultraviolet to the visible region is obtained.

Serum proteins, mainly albumin and α-globulin, depress the optical density of fibrin

[1] Carried out by H. A. Ward, Department of Pathology, Monash University.

gels. γ-globulin has no effect. The albumin effect depends also on the composition of the fibrin milieu. In the presence of phosphate and tris buffer albumin shows marked depression of fibrin gel turbidity. However, albumin in the presence of imidazole or cacodylate has little turbidity depressing effect when measured with reflected light.

PHYSIOLOGY AND PHARMACOLOGY OF SMOOTH MUSCLE*

W. G. Nayler, N. Dorevitch and T. E. Lowe

Rabbit Thoracic Aorta

Previous reports have indicated that the reactivity of β-adrenergic receptors in the myocardium differs from that of the β-receptors in the peripheral circulation. The conclusion that some of the β-receptors in rabbit thoracic aorta are excitatory was based on the finding that some of the isoproterenol-induced constriction in this preparation could be abolished by *dl* propranolol. During the current year these experiments have been extended to show that another drug, Verapamil, blocks the β-adrenergic induced constriction in rabbit thoracic aorta. These findings are in agreement with the hypothesis that although Verapamil does not antagonize the β-receptors in the myocardium it does, at least under some circumstances, antagonize β-receptors in the peripheral circulation.

Guinea Pig Tracheal Preparations

JP 428 is a newly developed drug for use in the treatment of asthma. Using isolated chains of guinea pig trachea this drug has been found, over a very wide dose range, to cause an initial constriction which may be as much as 20 percent of the resting tension. The constrictor effect of JP 428 is not antagonized by phenoxybenzamine and it persists for approximately 5 minutes after which relaxation occurs.

CARCINOGENESIS**

P. E. Hughes and R. Pilczyk

CHEMICAL CARCINOGENESIS

Many diverse compounds are now known to cause cancer in man or animals. These compounds react with DNA, RNA and/or proteins in susceptible cells but at present it is not known which, if any, of these reactions is of critical importance in carcinogenesis. It is hoped that by studying a series of carcinogens under different conditions, which either increase or decrease carcinogenesis, a positive correlation may be established between one particular type of macromolecular binding and carcinogenesis.

Binding of 2-Naphthylamine

2-Naphthylamine is a weak carcinogen for livers of mice of the strain CBA but not of the strain C57. A sample of 2-naphthylamine bearing a radioactive (tritium) label of high specific activity was prepared and purified to constant specific activity. This has been injected into mice of each of the above strains and after various intervals samples of DNA, RNA and protein were prepared from liver and kidney and assayed for radioactivity. This was found to be bound to all fractions of both livers and kidneys of both strains and that bound to DNA persists for at least several months.

The level of binding to liver macromolecules has been found to be higher than to their kidney counterparts and the levels bound in CBA mice were higher than those in C57 mice. Thus when either strain or organ susceptibility was varied a good correlation was found between carcinogenic activity and binding to DNA, RNA and protein.

Binding of 3'-Methyl-4-Dimethylaminoazobenzene

Tritiated 3'-methyl-4-dimethylaminoazobenzene has been prepared and is being given to rats under various conditions (partial hepatectomy, altered hormonal states, etc.) known to alter its carcinogenicity, to see if any of these conditions give altered levels of binding to liver macromolecules. In this way it is hoped to establish correlations between levels of binding and carcinogenicity.

Autoradiographic Localization of 2-Naphthylamine

(In association with C. J. Louis[1])

CBA mice have been injected with tritiated 2-naphthylamine and sections of liver and other organs prepared for autoradiography.

Mitotic Responses in Preneoplastic Rat Liver

Feeding of the carcinogenic dye 3'MeDAB to rats results in a wave of mitoses after 4 weeks of feeding. If feeding of the dye is stopped before this response no tumours result but after this time the changes are irreversible and tumours result after cessation of dye-feeding. Rats have been fed the dye for varying periods, and then after an interval without the dye, subjected to partial hepatectomy. After, but not before, 4 weeks of dye-feeding the rats showed a diminished mitotic response to partial hepatectomy.

The dye 2 MeDAB when fed to rats produces neither a mitotic response nor tumours. If however a mitotic response is supplied early in dye-feeding by partial hepatectomy, tumours result. Partial hepatectomy following 2 MeDAB feeding in rats produces a mitotic response similar to that after less than four weeks feeding of 3'MeDAB but rats subjected to a second partial hepatectomy following initial hepatectomy and then 2 MeDAB feeding display a mitotic response similar to that after four or more weeks of 3'MeDAB feeding. A type of mitotic response to partial hepatectomy has been found which appears characteristic of preneoplastic liver once past the irreversible stage.

Action of 4-Nitroquinoline-N-Oxide

(In association with T. Ghose[2])

It appears that carcinogens act on cells at a particular stage during cell division. Synchronized cells in tissue culture are being exposed for short intervals to 4-nitroquinoline-N-oxide to determine which stage of cell replication is vulnerable to chemical carcinogens.

POSSIBLE CARCINOGENIC ACTION OF ANTILYMPHOCYTIC SERUM IN RATS

P. E. Hughes and R. Pilczyk (in association with C. J. Louis[1])

It has been postulated that the body has a general surveillance mechanism against potential tumour cells. Unpublished reports suggest that patients who have been given anti-lymphocytic serum to inhibit rejection of transplated organs show an increased incidence of tumours. An anti-rat-lymphocyte serum is being produced in rabbits and it is intended to test this for carcinogenicity in rats.

[1] Department of Pathology, Austin Hospital, University of Melbourne.
[2] Department of Pathology, Monash University.

SCLERODERMA

A. J. Barnett, W. G. R. M. de Boer[1] and D. A. Coventry

During the past 18 years a variety of patients with ischaemic episodes in the fingers and scleroderma have been studied and were reviewed[2] in 1959. Three years ago a further study of such patients was commenced. This is based on the records of 61 patients of whom 31 could be further investigated.

[1] Associate Professor, Pathology, Monash University.
[2] BARNETT, A. J., "Scleroderma and Raynaud's Phenomenon" Alfred Hospital Clinical Reports 1959 page 9.

For descriptive purposes the cases were divided into Type 1, with skin changes confined to the digits, Type 11, with skin changes spreading slowly beyond the digits, and Type 111 with skin changes diffuse from the onset or spreading rapidly. There was evidence of visceral involvement and progression in all 3 groups, justifying the term 'progressive systemic sclerosis'. However the rate of progress and the eventual outcome varied greatly between the groups. Scleroderma Type 1 did not usually affect life expectancy. Patients with Type 11 and Type 111 are likely to die from the disease; the life expectancy being short in Type 111, long in Type 11. Although there is no specific treatment sympathectomy was found to be helpful in respect to the circulatory troubles in approximately half the patients.

Detailed study of the 31 patients currently available showed a high incidence of visceral disturbances in all 3 types of scleroderma. We could find no evidence of an hereditary basis, infection or exposure to toxins in the aetiology of scleroderma. In some instances there was a history of uncertain significance of psychological trauma. Positive auto-immune tests occurred in a proportion of the cases but their occurrence is not related to the severity of the disease nor do the findings prove that scleroderma has an auto-immune basis. However auto-immunity phenomena may be responsible for some of the manifestations.

GASTROINTESTINAL DISEASES

D. J. B. St. John

MEASUREMENT OF GASTROINTESTINAL BLEEDING

Using the radiochromium[1] method previously described measurement of occult gastrointestinal blood loss has been performed on 20 patients in the last 14 months. Most of these patients had unexplained iron-deficiency anaemia. Demonstration of chronic blood loss and then repeated radiological investigation led to the diagnosis of two previously undetected gastrointestinal neoplasms. Demonstration of blood loss associated with aspirin ingestion, combined with the absence of basal blood loss and the history of consumption of large quantities of aspirin has enabled the diagnosis of aspirin-induced anaemia to be made in four cases. In six other cases it was shown that there was chronic blood loss sufficient to account for the anaemia, but in some of these cases further investigation has failed to demonstrate the site of bleeding.

The value of this investigation is that it enables accurate division of cases of unexplained anaemia into three groups. In the first group occult gastrointestinal bleeding is present and repeated radiological and endoscopic investigations are imperative. In the second group, significant blood loss is present only when aspirin is ingested. This finding, combined with a history of analgesic abuse would give strong support for a diagnosis of aspirin-induced anaemia. In the third group, the absence of occult blood loss and the absence of a history of analgesic abuse would indicate that studies of diet, or iron absorption or of other possible disturbances of iron balance should be pursued.

[1] Counting facilities have kindly been made available at the Cancer Institute.

EFFECT OF DRUGS ON THE GASTRIC MUCOSA

After aspirin administration acute ulcers may develop in the gastric mucosa and 80% of normal subjects show significant occult gastrointestinal blood loss when given aspirin. Epidemiological studies have shown an association between massive upper gastrointestinal haemorrhage and recent aspirin ingestion; the association is greatest in the large group of patients where investigation after the haemorrhage fails to reveal a chronic peptic ulcer. In many patients in this group, an acute gastric ulcer can be identified either by gastroscopy or laparotomy. Regular consumption of large quantities of aspirin may cause chronic gastric ulceration. This hypothesis has received strong

support from epidemiological studies in Townsville, Newcastle and Sydney. It is possible that aspirin produces acute and chronic ulceration by different mechanisms.

In an attempt to learn more about these mechanisms two projects have recently been started. In the first project aspirin-induced blood loss is being measured in patients with pernicious anaemia using the radiochromium technique. Resistance to aspirin bleeding is present in 20% of the population, and it is possible that this resistance occurs in subjects with achlorhydria, whether due to pernicious anaemia or to simple atrophic gastritis.

A second project being undertaken in collaboration with Mr. F. T. McDermott of Monash University Department of Surgery, is a pilot study on the effect of aspirin on the rat stomach and the mouse stomach and in 1969 it is planned to measure the cellular kinetics of the gastric mucosa, using autoradiographic techniques. The aim of this project is to establish whether the cellular kinetics are altered by acute and prolonged aspirin administration and to relate any changes in cellular kinetics to the development and healing of ulcers.

PUBLICATIONS IN 1968

PHYSIOLOGY AND PHARMACOLOGY OF CARDIOVASCULAR SYSTEM

Role of Calcium†‡

CHIPPERFIELD, D. — "The Effect of Ouabain on Mitochondrial Calcium *in vitro*". *Life Sciences*, In Press.

NAYLER, W. G. — "Ion Movements in Heart Muscle",—for "Membrane Metabolism and Ion Transport", edited by E. Edward Bittar. In Press.

NAYLER, W. G. and D. CHIPPERFIELD — "Effect of Skeletal Muscle Potentiators on Ca^{++} in Cardiac Sarcoplasmic Reticulum". *Amer. J. Physiol.* In Press.

NAYLER, W. G. and T. E. LOWE — "The Effect of Heart Failure and of Drugs with Negative Inotropic Activity on the Uptake of Ionized Calcium by Human and Canine Heart Muscle". *Circulat. Res.* Submitted.

Pharmacology‡

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DOREVITCH, N. — "The Adrenergic-Receptor Blocking Action of Verapamil". *Arch. int. Pharmacodyn.* Submitted.

NAYLER, W. G. — "Biochemical Aspects of Prenylamine Action—Preliminary Communication". in "Biochemical Aspects of Prenylamine". Ed. F. Cedranglo, *Biochimica Applicata.* Vol. *14* (1968) p. 305.

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NAYLER, W. G., I. McINNES, V. CARSON, J. STONE and T. E. LOWE — "The Effect of Lignocaine on Myocardial Function, High Energy Phosphate Stores and Oxygen Consumption. A Comparison with 'Propanolol' ". *Amer. Heart J.* In Press.

NAYLER, W. G., I. McINNES, V. CARSON, J. B. SWAN and T. E. LOWE — "The Combined Effect of Atropine and β-adrenergic Receptor Antagonists on Left Ventricular Function and Coronary Blood Flow". *Amer. Heart J.* In Press.

NAYLER, W. G., I. McINNES, J. B. SWANN, J. M. PRICE, V. CARSON, D. RACE and T. E. LOWE — "Some Effects of Isoptin (Iproveratril) on the Cardiovascular System". *J. Pharm. exp. Therap.* Vol. *161* (1968) p. 247.

NAYLER, W. G., I. McINNES, J. B. SWANN, D. RACE, V. CARSON and T. E. LOWE — "Some Effects of Diphenylhydantoin and Propranolol on the Cardiovascular System". *Amer. Heart J.* Vol. *75* (1968) p. 83.

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NAYLER, W. G., I. McINNES, J. B. SWANN, D. RACE and T. E. LOWE — "Effect of Adrenergic Beta-Receptor Blocking Drugs on Blood Flow in the Coronary and Other Vascular Fields". *Cardiovasc. Res.* Vol. *2* (1968) p. 371.

NAYLER, W. G., J. M. PRICE, J. STONE and T. E. LOWE — "Further Observations on the Cardiovascular Effects of ST 155 (Catepres)". *J. Pharm. exp. Therap.* In Press.

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NAYLER, W. G. and J. M. PRICE — "Effects of Skeletal Muscle Potentiators, including Uranyl Ions, on Cardiac Muscle". *Amer. J. Physiol.* Vol. *213* (1967) p. 1459.

Kinekard†

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Control Mechanisms‡

BARNETT, A. J. — "Idiopathic Orthostatic Hypotension: A Pharmacological Study of the Action of Sympathomimetic Drugs". *Med. J. Aust.* Vol *i* (1968) p. 213.

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BARNETT, A. J. — "Role of the Physician in Management of Obliterative Arterial Disease". *Clinical Medicine.* Vol. *75* (1968) p. 20.

BARNETT, A. J. and S. CANTOR — "Observations on the Hypotensive Action of 'Catapres' (ST 155) in Man". *Med. J. Aust.* Vol. *i* (1968) p. 87.

CANTOR, S. — "Angina Drug Trial". *Med. J. Aust.* Vol. *ii* (1967) p. 983.

CARDIAC SURGERY‡

STIRLING, G. R., K. N. MORRIS, K. H. McLEAN, J. A. MIRAMS, D. RUBINSTEIN and G. R. WAGNER — "Surgical Aspects of Pulmonary Embolism". *Med. J. Aust.* Vol. *i* (1968) p. 571.

MOLECULAR BIOLOGY

BAUMGARTEN, A., E. GILES and C. C. CURTAIN — "The Distribution of Haptoglobin and Transferrin Types in North-East New Guinea". *Amer. J. Physical Anthropol.* Vol *29* (1968) p. 29.

KIDSON, C. and J. BALDWIN — "Recognition of DNA Base Sequence Difference among Vertebrates". *Nature* Vol. *217* (1968) p. 1256.

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BLUNCK, J. M., P. E. HUGHES and J. G. SCROGGIE — "Some Aromatic Fluorine Compounds". *J. Medicinal Chem.* In Press.

MISCELLANEOUS

BAIRD, I. McL., D. J. B. ST. JOHN and S. MASSER — "Role of Occult Blood Loss and Aspirin Bleeding in Anaemia after Partial Gastrectomy". *Gut.* Vol. *9* (1968) p. 364 (Abstract).

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BARNETT, A. J. and COVENTRY, D. A. — "Scleroderma I, Clinical Features, Course of Illness and Response to Treatment in 61 Patients". *Med. J. Aust.* In Press.

BARNETT, A. J. and D. A. COVENTRY — "Scleroderma II. Incidence of Systemic Disturbance and Assessment of Possible Aetiological Factors". *Med. J. Aust.* In Press.

BARNETT, A. J. — "Diuretics in Heart Failure". National Heart Foundation. *Notes on Cardiovascular Disease* (1968).

LOWE, T. E. — "The Control of Body Fluid Volume Regulation". in *Biological Basis of Medicine.* Ed. R. A. Farrand, Academic Press, London. In Press.

McCUTCHEON, A. D. — "A Fresh Approach to the Pathogenesis of Pancreatitis". *Gut.* Vol. *9* (1968) p. 296.

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ST. JOHN, D. J. B. — "The Role of the Dietitian and Diet in the Management of Gastrointestinal Disorders". *J. Dietetic Assoc. Victoria.* In Press.

ZIMMETT, P., H. D. BREIDAHL and W. G. NAYLER — "Plasma Ionized Calcium in Hypomagnesaemia". *Brit. med. J.* Vol. *i* (1968) p. 622.

LECTURES DELIVERED DURING 1968

"Modern Management of Occlusive Arterial Disease" — *College of General Practitioners.*	A. J. BARNETT
"Blood Coagulation (I) and (II)" — *Melbourne University.*	P. FANTL
"Modern Concepts of Chemical Carcinogenesis" — *Monash University, Melbourne.*	P. E. HUGHES
"Observations on the Hypotensive Effect of ST 155 (Catepres)" — *Symposium on ST 155 (Catapres) Auckland, New Zealand.*	W. G. NAYLER
"The Pharmacology of Catapres (ST 155)" — *Symposium on Catapres (ST 155), New York, U.S.A.*	W. G. NAYLER
"β-Adrenergic Antagonists and Ventricular Function" — *Ciba Research Laboratories Basle, Switzerland.*	W. G. NAYLER
"Ventricular Function During β-Adrenergic Blockade" — *Asian-Pacific Congress of Cardiology, Tel Aviv, Israel.*	W. G. NAYLER
"Observations on the Effect of β-Adrenergic Antagonists on Myocardial Function" — *11th European Congress of Cardiology, Athens, Greece.*	W. G. NAYLER & T. E. LOWE
"Myocardial Function during beta-Adrenergic Blockade" — *Australian Physiological Society, Canberra.*	W. G. NAYLER
"Observations on the Cardiovascular Effects of Xylocaine (Lignocaine)" — *Australian Society of Clinical and Experimental Pharmacologists, Sydney.*	W. G. NAYLER & I. McINNES
"Some Observations on the Effect of Amiodarine (SKF 33134) on Myocardial Function and Coronary Blood Flow" — *Australian Society of Clinical and Experimental Pharmacologists, Sydney.*	J. M. PRICE
"The Role of the Dietition and Diet in the Management of Gastrointestinal Disorders" — *Dietetic Association, Victoria.*	D. J. B. ST. JOHN
"The Role of Occult Blood Loss and Aspirin Bleeding in Anaemia after Partial Gastrectomy" — *British Society of Gastroenterology.*	D. J. B. ST. JOHN & I. M. BAIRD
"The Surgical Treatment of Pulmonary Embolism" — *Melbourne Medical Post Graduate Committee.*	G. R. STIRLING
"Electrolyte Disturbances during Open Heart Surgery" — *Cardiac Society of Australia and New Zealand, Sydney.*	G. R. STIRLING

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

Revenue Account for the Year ended 31st December, 1968

EXPENDITURE		INCOME		
Salaries and Wages	113,412	Donations —		
Laboratory Supplies and Isotopes	25,491	Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions		100,069
Library Maintenance	4,782	Grants in Aid of Research—		
Postage and Telephone	901	National Heart Foundation of Australia	18,038	
Printing and Stationery	1,796	Life Insurance Medical Research Fund of Australia and New Zealand	17,851	
Light and Power	9,626	William Buckland Research Fund	645	
Insurance	3,631			36,534
Repairs and Renewals	526	Interest from Investments —		
Animal House Contribution	4,000	Held by Trustees of the Estate of the Late Thomas Baker	1,700	
Sundries	2,686	Other Income	11,255	
Travelling Expenses	1,227			12,955
Surplus for year	413	Sundry Sales, Recoveries and Refunds		18,933
	$168,491			$168,491

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

Balance Sheet as at 31st December, 1968

FUNDS AND LIABILITIES			ASSETS		
Funds —			Fixed Assets (Note 1)		
Accumulated loss brought forward	306		Investments —		
Deduct surplus for year	413		Held by the Trustees of the Institute		
Accumulated revenue	107		Commonwealth inscribed stock	21,760	
Restricted Funds	13,819		M.M.B.W. stock	7,201	
Endowment Fund	79,319		S.E.C. stock	6,556	
Development Fund	286,904		Treasury bonds	8,020	
William Buckland Research Fund	16,200		Argo Investments Co. Ltd. shares	27,738	
Laura Nyulasy Research Scholarship Fund	3,940		Softwood Products Treatment Co. Pty. Ltd.	100	
Lang Research Scholarship Fund	3,860		Short term deposits	243,574	
		404,149		314,949	
Current Liabilities —			Held by The Trustees, Executors & Agency Co. Ltd. —		
Sundry creditors and accrued expenses		2,893	Laura Nyulasy Research Scholarship Fund	3,940	
			William Buckland Research Fund	16,200	
					335,089
			Current Assets —		
			Cash at bank on current and deposit accounts	70,163	
			Sundry debtors and prepayments	1,790	
					71,953
		$407,042			$407,042

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 31st December, 1968 other than the building totalled $100,000. The cost of the present building to 31st December, 1968 totalled $1,108,280.

(2) Retention monies paid to 31st December, 1968 in respect of the contract for a new building amount to $29,375, and have been deposited in a bank account. The release of these funds to the builders or sub-contractors is dependent on the satisfactory completion of the contracts.

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

(4) During 1968 the Institute conducted an appeal for funds, the proceeds of which are currently held in a separate fund. The proceeds of the appeal have not yet been transferred to the Institute and are not, therefore, reflected in the above balance sheet.

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 31st December, 1968.

Melbourne.
26th February, 1969.

PRICE WATERHOUSE & CO.
Chartered Accountants.

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

Year Ended 31st December, 1968

DEVELOPMENT FUND

Balance at 31st December, 1967		429,635
Add —		
Baker Benefactions	174,510	
Victorian Government Grant	100,000	
Interest Received	20,308	
		294,818
		724,453
Deduct —		
Building Costs	404,400	
Furnishing and Equipment Costs	33,149	
		437,549
Balance at 31st December, 1968		$286,904

ENDOWMENT FUND

Balance at 31st December, 1967		75,751
Add —		
Donations	8,528	
Income from Investments	2,730	
		11,258
		87,009
Deduct —		
Transfer to Maintenance	3,830	
Transfer to Lang Research Scholarship Fund	3,860	
		7,690
Balance at 31st December, 1968		$79,319

DONATIONS

Apart altogether from donations to the Appeal and the second instalment of $100,000 of the donation of $200,000 by the Government of Victoria towards the cost of the new building, both of which are recognised elsewhere in the report, the Institute received the following gifts towards its funds during 1968.

The Ian Potter Foundation	$10,000.00
Estate of H. & L. Hecht (Hedderwick Fookes & Alston)	5,000.00
The William Buckland Foundation (Trustees Executors & Agency Pty. Ltd.)	3,000.00
The James & Elsie Borrowman Research Fund (Trustees Executors & Agency Pty. Ltd.)	2,500.00
The William Angliss (Victoria) Charitable Fund	2,000.00
The Sidney Myer Trust	1,500.00
The Truby & Florence Williams Charitable Trust (Trustees Executors & Agency Pty. Ltd.)	900.00
Estate Alfred Edments (Trustees Executors & Agency Pty. Ltd.)	800.00
Marian & E. H. Flack Trust	700.00
George F. Little Trust (Equity Trustees Executive & Agency Co. Ltd)	474.00
Watson Victor Ltd.	450.00
Imperial Chemical Industries of Australia and New Zealand	400.00
Mr. Mark Cowen	400.00
Mr. and Mrs. E. J. Rouse	110.00
Pethard Tarax Charitable Trust (Marquand & Co.)	100.00
Mr. Siegfried Meyer	100.00
Carlton & United Breweries Ltd.	50.00
Mrs. I. R. Robinson	27.96
Dr. T. W. Moir	25.00
Dr. T. E. Lowe & Associates	20.00
Merbein High School	10.00
Mack Thomson Pty. Ltd.	5.25
Christian Women's Evening Fellowship	4.00
	$26,476.21

DONATIONS, in memory of:—

Dr. Eric Angliss; Lady Archer; Adrian Barnett; Roy Banfield; H. A. Beauchamp; C. Cartey-Salmon; R. A. Cooper; John Fitts; Lord Florey; Charles Russell Gabb; J. F. Gooley; A. E. Hacker; H. Harman; A. Haugh; Alan Read Hill; Mrs. Elsie Hudson; Mrs. S. K. Jacobs; Arthur Keers Jnr.; S. Kilburn; F. Levy; T. Luxton; Mrs. A. C. Middleton; J. Mish; C. L. Mutton; J. Mullumby; R. S. Nesbitt; H. W. Norman; Miss Pascoe-Webb; George Prince; E. J. Richards; Kenneth Sutherland; H. Tatt; Dr. T. Tyrer, Air ViceMarshall A. Walters; Mrs. H. M. Werner; Colin Wilkinson.

were received from:—

Mr. Edgar Rouse; Kodak (Australasia) Ltd.; Mr. and Mrs. Noel Monteith; Mrs. Maude Monteith; Mr. George Knox; Mr. Reginald Blakemore, Eagle Star Insurance Company Ltd.; Alfred Hospital Residents' Club, Mr. Charles Donne; Mr. J. Habersberger; Miss N. E. Cameron; Melbourne University Rugby Football Club. — Total $272.10.

Generous support for overseas travel was given by Ciba Ltd. (Basle, Switzerland); Boehringer Ingelheim Pty. Ltd. (Australia) and Geigy Chemical Corporation (U.S.A.).

Donations in kind were made by:—

Philips Electrical Pty. Limited and Fielding Springs Pty. Ltd.

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

1968

STAFF

Honorary Consulting Physicians:	EWEN DOWNIE, M.D., F.R.C.P., F.R.A.C.P. BRYAN HUDSON, M.D., Ph.D., M.R.C.P., F.R.A.C.P.
Honorary Consulting Biochemist:	JOSEPH BORNSTEIN, D.Sc., M.D., F.R.A.C.P.
Physician-in-Charge:	PINCUS TAFT, M.D., F.R.A.C.P.
Honorary Physician:	HARALD BREIDAHL, M.D., M.R.C.P., F.R.A.C.P.
Registrar:	N. J. RADFORD, M.B., B.S.
Biochemists:	DORA WINIKOFF, M.Sc., F.A.A.C.B. JUNE SHEATH, M.Sc., F.A.A.C.B. DORIS PAGE, B.Sc.
Technical Staff:	Mr. W. HUDSON. Miss I. EKKEL. Miss R. WITCHELL. Miss J. KINDLER. Mrs. F. RABOLD (part-time).
Secretary:	Mrs. N. STEWART. Miss J. SHARP (resigned March, 1968).

DIABETIC CLINIC

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

ENDOCRINE CLINIC

Clinical Assistants:	D. P. CAMERON, M.B., B.S., M.R.A.C.P. J. R. STOCKIGT, M.D., M.R.A.C.P.

RESEARCH FELLOW

Burroughs Wellcome Research Fellow: S. MENAHEM, M.B., B.S., M.R.A.C.P.

HONORARY RESEARCH FELLOWS

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

ANNUAL REPORT

For a period of some three months late in 1967 and early in 1968 I had the privilege of working collaboratively with Physicians in Singapore. We studied some aspects of endocrine function in a number of patients with proven cirrhosis of the liver being prompted so to do by the frequency of the occurrence of testicular atrophy and gynaecomastia in these patients.

Some of the laboratory studies were made in Singapore and some in this Unit, and the results of the investigation are included in the Research Report. These comprise a significant segment of the work of the Unit this year, some aspects being presented at the Third Asian-Pacific Congress of Gastroenterology, an appropriate forum for a Singapore/Australian venture.

Three aspects of significance in clinical investigation are illustrated in this study. Firstly that collaborative work can usefully be done by groups with similar or complementary interests despite great geographical separation and that such collaboration is of mutual benefit. Secondly that investigation can be geared by posing the appropriate questions to the facilities available for the pursuance of the inquiry and lastly that investigation should be made to seek new information and to add to knowledge.

Also this year it was my privilege to represent the Unit at an International Symposium on chlormadinone acetate held in Cambridge. Work done over the years by Mrs. Winikoff and Dr. Taylor on the effects on thyroid function tests of the hormones contained in various oral contraceptive agents was reported. The invitation to speak is regarded as a tribute to the Unit and in particular to the workers concerned.

A reference is regularly made in this Annual Report to the cordial relationships existing with the medical staff of the Alfred Hospital and with the clinical departments of Monash University and with the biochemistry departments at the Alfred and Monash. This acknowledgment is made again, with an expression of appreciation for the many assistances afforded. This Report would be incomplete without grateful acknowledgment to those generous donors of grants in aid and in kind which enable our work to proceed.
31st December, 1968.

PINCUS TAFT.

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

Alfred Hospital Research Funds.
Burroughs Wellcome & Co. (Australia) Ltd.
Difrex Laboratories Pty. Ltd.
Hoechst Pharmaceuticals.
Pfizer Corporation.
Syntex Pharmaceutical Ltd., Berkshire, U.K.
William R. Warner & Co. Pty. Ltd.

ENDOCRINE STUDIES IN LIVER CIRRHOSIS

MELBOURNE: **W. Hudson, D. Page, J. Sheath and P. Taft.**

SINGAPORE: **J. S. Cheah, K. L. Chua, G. S. Lee, K. T. Lee, C. S. Seah and B. Y. Tan.**

Prompted by the frequency of diabetes, testicular atrophy and gynaecomastia in cirrhotic patients, a study of endocrine function in twenty-five patients with proven cirrhosis was undertaken. The patients were studied in the Thompson Road and Singapore General Hospitals, Singapore. Laboratory studies were made in the Biochemistry Department, Singapore General Hospital and in the Diabetic and Metabolic Unit.

CARBOHYDRATE METABOLISM:

Twenty-five male patients suffering from cirrhosis of the liver proven by chemical tests of liver function and by liver biopsy were studied. Their ages ranged from 27 to 66 years the aetiology of the cirrhosis being alcohol in 13, post hepatitic in 3 and cryptogenic in 9.

Glucose Tolerance:

(a) Oral glucose tolerance was studied using a load of 100gm and diabetes diagnosed where the blood glucose level exceeded 190 mgm% at 90 or 120 minutes. Diabetes was excluded with a fasting level less than 85 mgm.% and a 120 minute level less than 115 mgm.%. Intermediate results were termed "impaired".
Twelve patients were normal (48%) 11 were diabetic (44%) and two had impaired tolerance (8%).

(b) Intravenous glucose tolerance tests using the technique of Amatutzio confirmed the oral findings.

(c) The plasma insulin was above the normal range for this laboratory in all 15 patients studied at some point during the test and in only three patients—all non diabetic—did the insulin level fall significantly at the end of the test. This insulin pattern is that seen in maturity onset diabetes.
Insulin resistance determined by the calculation of the rate of removal of glucose following intravenous insulin was demonstrated in these patients being greater in the diabetic cirrhotics. It was of interest to note too that a delayed rate of removal of insulin from the plasma after intravenous injection was observed in six of the eight patients in whom it was studied. No correlation between the rate of insulin removal and carbohydrate tolerance was noted.

Glucagon Response:

Following Glucagon, 30 micrograms/Kg. subcutaneously, the blood glucose increment was measured. The increment in diabetic cirrhotics was similar to that of normal and diabetic non cirrhotic control patients, being higher than the increment observed in non-diabetic cirrhotics. This significant liver glycogen content in untreated diabetic cirrhotic patients was a surprising finding, is possibly steroid induced, and is referred to in the section on adrenal function.

ADRENAL FUNCTION:

Plasma Cortisol — diurnal rhythm:

The mean 8 a.m. plasma cortisol level was 18.5 micrograms % in the diabetics and 14.9 micrograms % in the non diabetic cirrhotics ($0.05 > p > 0.02$). Normal diurnal rhythm in plasma cortisol was present in all but one patient.

A.C.T.H. Stimulation:

The **plasma cortisol** response to 25 units of A.C.T.H. intravenously was equal and adequate in all patients indicating a normal adrenal responsiveness in both diabetic and non diabetic cirrhotics.

The urinary excretion of hydroxy steroids was however lower in the diabetic patients when the levels on the two days of A.C.T.H. stimu-

lation, 25 U twice daily, and the day following, were compared. The means were 8.8 mgm./day for the diabetics and 24.7 mgm./day for the non diabetic cirrhotic patients. ($0.01 > p > 0.001$).

The higher plasma cortisol level of the diabetic due possibly to the delayed removal of steroid suggested by the urinary figures, is invoked as a factor in the genesis of the diabetes of cirrhosis. The high liver glycogen—a feature of steroid diabetes is supporting evidence for this contention.

PITUITARY FUNCTION:

Gonadotrophin excretion. In all 15 patients studied, "general" gonadotrophin, measured by the mouse uterus assay was present. In three patients, borderline high values were recorded, but in only one of these was testicular atrophy present. There was thus evidence that in this series of patients impotence and testicular atrophy—evidences of gonadal failure—were not due to gonadotrophic insufficiency.

Adrenocorticotrophin Secretion. This was tested using the insulin hypoglycaemia stress test, the plasma cortisol increment being used as the parameter of response. In seven of fifteen patients the rise in plasma cortisol fell below the 10 micrograms % expected in the normally responsive patient. Only two of these patients were diabetic. These results suggest some impairment of adrenocorticotrophin reserve. There was some correlation though not complete with diurnal A.C.T.H. rhythm assessed by diurnal cortisol fluctuation.

Growth Hormone Secretion. Also assessed by the response to insulin hypoglycaemia this was normal in all but one patient with increments above 6 nanograms per ml. Four patients had a supernormal response with increases greater than 60 nanograms per ml.

THYROID FUNCTION:

Studied under basal conditions (P.B.I. and I_{131} uptake) and after T.S.H. stimulation thyroid function was normal in all patients.

CONCLUSIONS

The conclusions reached thus far are:

1. The frequency of the occurrence of diabetes in liver cirrhosis is confirmed.
2. The plasma insulin response to glucose in these patients is similar to that seen in the maturity onset diabetic.
3. The liver glycogen content of the diabetic cirrhotics is paradoxically elevated and this fact associated with the higher plasma cortisol level in the diabetic patients (vide infra) is suggestive of an adrenal role in the genesis of the diabetes.
4. While a diurnal rhythm of plasma cortisol is present in all patients the mean 8 a.m. level is higher in the diabetic patients. This may be due to the slower urinary excretion of hydroxy steroids seen in these patients after A.C.T.H. administration.
5. Pituitary function as judged by gonadotrophin excretion, thyroid function and growth hormone increments following hypoglycaemia appears to be normal. However the A.C.T.H. reserve appears limited as assessed by the cortisol response to insulin hypoglycaemia stress.

THYROID FUNCTION TESTS

Dora Winikoff and Associates

Last year we reported the use of "Isopor" resin as an ancillary technique to supplement the P.B.I. assay.

This in our hands greatly improved the accuracy of diagnosis in patients who were taking iodine in an inorganic form.

However, after ingestion or organic-iodinated drugs the results were not satisfactory and in the presence of X-ray contrast media even the application of the "thyroxine by column" technique (reported by us in the past) which measures the hormonal iodine, did not give a clear cut discrimination between the euthyroid and hyperthyroid group of patients and were distinctly misleading in the presence of hypothyroidism.

We would like to present here our experience with two new techniques both measuring the true hormonal iodine which gave most encouraging results.

HORMONAL IODINE ASSAY

Dora Winikoff and Rosalind Witchell

The use of a cation exchange resin Dowex 50W x 2 introduced by Dutch workers[1] permits the assay of hormonal iodine (H.I.) even in the presence of iodinated radio-opaque media or other organic iodo-compounds which spuriously elevate P.B.I. levels.

The original method was modified by introducing larger size columns 3 cm. x 0.6 cm. and the passage of 0.5 ml. of serum through the resin bed four times for better absorption. After the application of borate buffer pH 8.6 the thyronines (T_4 & T_3) are eluted from the column with 5 ml. of 5N Ammonia. Following this, since we apply an ashing method for the iodine assay, 0.5 ml. of 12% glycine in 2N KOH are added which prevent the losses of iodine during drying and the incineration process.

[1] E. T. Backer, T.H.J. Postems & J. D. Wiener, Clin. Chim. Acta 1967, **15**, p. 77.

The percentage recovery of radioactive thyroxine I^{125} added to serum ranges from 86-97%. When the technique is applied to the patient's sera, however, the iodine assay renders no more than 80% of P.B.I. value in euthyroid and hyperthyroid individuals.

The tests are run in triplicate since the reproducibility and accuracy are definitely inferior to an ordinary P.B.I. assay in our hands. Normal range is 2.5-6.0 μg%. Nevertheless we found that in contaminated sera in the presence of 100 μg% iodine or more the H.I. reflects the true functional state of the gland and in such cases the technique described has a definite place in a diagnostic laboratory. The operation is simple, rapid and does not require elaborate equipment or standardized conditions. Furthermore changes in binding capacity have no effect on the results as is the case in T_3-resin uptake.

TOTAL THYROXINE ASSAY

Doris Page and Dora Winikoff

The determination of total thyroxine, based on the properties of thyroxine binding globulin (T.B.G.) and competitive binding employing an anion exchange resin plus radioactive thyroxine I^{125} is the basis of the method used by Abbott's in "Tetrasorb" kits.

A standard curve is constructed by the use of known amounts of "cold" thyroxine which displaces the T_4^{125} from its binding sites on T.B.G. The radioactivity is measured on a sponge containing the anion exchange resin.

We encountered many difficulties in employing the "Tetrasorb" kits and had to introduce a number of modifications. The most important of these are:

(1) Increase of alcohol to serum ratio (for the extraction of thyroxine from patient's serum) to 4:1.

(2) The original count to be carried out in preincubation period. Only a few random samples need to be counted.

(3) The plastic plunger provided should be left on the sponge for uniform drainage until washing commences.

The Abbott's working standard of aqueous thyroxine solution containing albumin was the only one which gave consistent results. This curve varied little from day to day within the expiry period limits. On the whole the results were reproducible and of satisfactory accuracy providing the procedure was timed and executed with utmost care. The percentage recovery is approximately 85%. Good discrimination is obtained in all categories of patients and contamination of any origin does not interfere.

The limitations of the method are the many steps necessary, the alcohol extraction of serum which causes most of the errors and the need for most meticulous handling and timing, and temperature dependence. Its main ad-

vantage is its applicability in all thyroid states, regardless of the presence of iodine of non hormonal nature.

It compares favourably with the previous method in the state of myxoedema or low binding capacity of thyroxine binding globulin inherent or induced by drug or illness, when the P.B.I. is low or in cases of extreme high levels caused by contamination.

We have little experience in assessing the value of either methods in patients with atypical thyroid hormone production when a high P.B.I. is coexistent with its low calorigenic action and the presence of subthyroidism.

TRIIODOTHYRONINE RESIN UPTAKE

Dora Winikoff and Janis Kindler

A comparative study was carried out using the Abbott "Triosorb" kits[1] with the resin uptake method developed in our Unit. The discrimination between all categories of patients was equally good with both methods. However the overlap zone was much greater for the "Triosorb" procedure and the normal limits less sharply defined. This occurred in many cases of myxoedema or hyperthyroidism fhen the resin uptake was much lower or higher respectively when tested with "Triosorb" kits, than with our method. We have no explanation for this phenomenon.

[1] Australasian Bulletin of Medical Physics and Biophysics N34 December 1, 1967, p. 18, supp. p. 11.

THE THYROID AND THE PILL

Dora Winikoff

The study of the effects of oral contraceptives on thyroid parameters during a long-term administration is continuing. We have established that considerable variations from cycle-to-cycle occur in each individual while taking the same preparation throughout the period of observation. An occasional result within normal limits, reflected in one or more parameters has been also observed. With the aid of the improved methods for the hormonal iodine assay now developed, and the electrophoretic technique, we hope to be able to investigate whether this is a result of significant changes in the total binding capacity of the T.B.G. or fluctuation in the hormonal output of the thyroid gland. By studying women who are taking ovulation inhibitors of the combined type, women on a regimen of low dosage of progestogen only, and normal controls without medication, we intend to gather more information concerning the cumulative effects of endogenous and exogenous oestrogen which we believe is responsible for these fluctuations.

BIOLOGICAL ESTIMATION OF LUTEINISING HORMONE

I. Ekkel

Although measurement of luteinising hormone (L.H.) by radio-immunoassay is widely undertaken there is yet a need for cross-checking data with bioassay results. In view of the difficulties experienced with animal supply for the Parlow O.A.A.D. assay, the mouse uterine augmentation assay (Donini 1968) has been examined. This assay is based on the prin-

ciple that with a constant dose of biologically pure F.S.H., increasing amounts of L.H. induce an increase in uterine weight which is proportional to the L.H. activity.

N.I.H. F.S.H. has been used as the uterine "primer" in a dose of 2-3 I.U. Nine to 10 gm. 21 day old female mice are used, 5 injections of standard (2nd IRP) and unknown being given over 72 hours, the log dose interval being 0.301.

The slope has been satisfactory and parallel line assays achieved with a λ of 0.16 - 0.28.

Estimations have been made on urine extracts, values in normal males ranging from 6.4 - 10.7 (2nd IRP) per 24 hours. In 6 patients with oligospermia of non endocrine origin, values of 6.0 - 37.8 I.U. have been recorded.

Further examination of this assay is proceeding.

Donini et al 1968, Acta Endocr. **58,** p. 463.

ESTIMATION OF GLYCEROL AND NEFA BY MICROANALYSIS

J. Sheath

In conjunction with the Department of Obstetrics and Gynaecology at Monash University, it is proposed that foetal fat metabolism be examined during labour and comparisons with maternal metabolism made. Since very small quantities of foetal blood are obtainable from the scalp an appraisal of micro methods for the estimation of plasma non-esterified fatty acids (NEFA) and glycerol has recently been made.

Macro methods, previously employed in this laboratory for the estimation of these substances, could not be satisfactorily adapted to micro use. For the estimation of NEFA an ultra-micro method has been set up which requires 0.05 ml. plasma. This is a colorimetric procedure based on Elphick's method[1] and recoveries of palmitic acid added to plasma are between 95% and 98%. The estimation of plasma glycerol is enzymatic and for this 0.1 ml. plasma is required. Preliminary studies of the recovery of glycerol added to plasma are of the order of 100%.

[1] J. Clin. Path. 1968, **21,** p. 567.

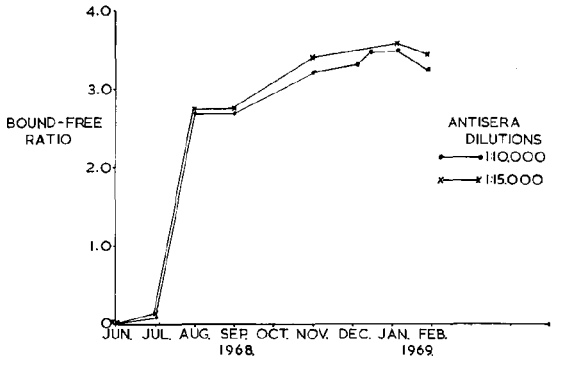
PREPARATION OF HUMAN GROWTH HORMONE ANTISERUM

J. Sheath and W. Hudson

During the past year the solid phase growth hormone immunoassay devised by Catt has been established and is providing satisfactory results. We have thus begun preparation of the growth hormone antiserum which has been produced by injecting a rabbit with a mixture of human growth hormone and Freund's complete adjuvant at two to four weekly intervals for five months, after which the animal has been given booster intramuscular injections of growth hormone at biweekly intervals. The booster injections are being continued as preliminary trials appear to confirm that this antiserum is satisfactory for the radioimmunoassay of human plasma growth hormone.

The efficiency of the antiserum was assessed by taking small quantities of blood from the animal at intervals and estimating the binding capacities of the samples by radioimmunoassay. The binding capacity, expressed as the bound-free ratio of radioactivity, has proges-

FIGURE. I.



CHANGE IN BINDING CAPACITY OF RABBIT ANTISERUM DURING IMMUNISATION WITH H.G.H.

sively increased as shown in Figure I. Results obtained using antisera dilutions of 1:10000 and 1:15000 are shown and higher dilutions of the antiserum may even be more suitable for use.

During the administration of the booster injections, blood samples were taken within seven days after the injections, except on the last occasion at the end of January, 1969, when blood was drawn immediately before the booster. A slight decrease in the bound-free ratio was observed with both dilutions, suggesting that serum collected within a few days of the booster injection may have a higher titre of antiserum.

A STUDY OF GONADOTROPHIN EXCRETION IN SECONDARY AMENORRHOEA

I. Ekkel and P. Taft

In the majority of patients suffering secondary amenorrhoea, neither pituitary nor ovarian disease or severe non-endocrine disorders —recognised causes of amenorrhoea—can be demonstrated. In such patients, the urine contains gonadotrophin (measured by the mouse uterus assay as general gonadotrophin) in normal amount. The urinary oestrogen excretion ranges from post-menopausal levels to early menstrual cycle levels. The aetiology of the amenorrhoea is obscure. An examination of the urinary general gonadotrophin (G.G.), follicle stimulating hormone (F.S.H.), and luteinizing hormone (L.H.), has been made in nine such patients, in four of whom urinary oestrogen excretion was at post-menopausal levels and in five at low normal levels. General gonadotrophin was present in all, in three at the upper limit of normal levels. The 24 hour urinary F.S.H. excretion ranged from 3.9 I.U. to 27 I.U., and L.H. excretion from 4.2 I.U. to 29.4 I.U., the F.S.H.:L.H. ratio ranging from 0.66 to 0.95 in eight, and being 2.1 in the remaining patient. The only correlations of significance were those existing between the levels of F.S.H. and G.G. ($r = 0.76$, $p < 0.01$), L.H. and G.G. ($r = 0.80$, $p. < 0.01$) and F.S.H. and L.H. ($r = 0.91$, $p < 0.001$). No clear explanation for the amenorrhoea of these patients can be derived from this study, but argument for an hypothalamic aetiology appeared most cogent in light of the clinical features and natural history of these patients.

DIABETIC ACIDOSIS AND ALKALI THERAPY

P. Zimmet, J. A. Owen, J. Sheath and P. Taft

The production of metabolic acids in patients with severe diabetic acidosis has been studied to determine the contribution of various acids and to reassess the need for alkali in individual patients. Plasma lactic pyruvic acetoacetic β-hydroxy butyric and free fatty acids were determined before and during the first twenty-four hours of treatment. In addition, estimates of base deficits were made using the alignment nomogram devised by Siggaard-Ambersen. Patients were given alkali therapy in the form of sodium bicarbonate solution.

In 11 patients with severe diabetic acidosis, the mean total concentration of determined acids was 14.5 mEq/litre (range 11 - 21), while the mean calculated base deficit was 22.5 mEq/litre (range 17 - 25). The mean amount of bicarbonate actually administered was 185 mEq (range 88 - 264), while the mean bicarbonate requirement predicted from the formula 0.3 x base deficit x body weight was 371 mEq (range 285 - 485).

The difference between the measured acids and the "acid load" computed as base-deficit could be explained by:—

(1) the renal excretion of organic acid as anion.

(2) the production of undetermined organic acids.

(3) partial failure or renal hydrogen ion excretion in the face of increased production of sulphuric and phosphoric acids from increased metabolism of protein and phospho lipids

(4) invalidity of the nomogram

The difference between the administered and predicted bicarbonate could be explained by:—

(1) reduction in bicarbonate requirement due to concurrent metabolism of organic acids in response to therapy.

(2) invalidity of the formula used to predict bicarbonate requirement.

The therapeutic implications of this study are that the predictive formula over-estimates bicarbonate requirement and that bicarbonate therapy should be limited to pH restoration to a value below 7.4.

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"Hormone Therapy" — *Australian College of General Practitioners.*	H. D. BREIDAHL
"A Study of Gonadotrophin Secretion in Secondary Amenorrhoea" — *Endocrine Society of Australia, Canberra.*	PINCUS TAFT
"Pituitary — Adrenal Function in Hepatic Cirrhosis" — *Third Asian Pacific Congress of Gastroenterology, Melbourne.*	PINCUS TAFT
"The Effects on Thyroid Function Tests of Oral Contraceptives With and Without Ovulation Inhibitory Properties" — *Institute of Hormone Biology, Cambridge, England.*	PINCUS TAFT
"The Biosynthesis of Thyroxine and its Regulation" — *Royal Australasian College of Surgeons, Melbourne.*	PINCUS TAFT
"The 'Pill' and the Thyroid" — *Aust. and N.Z Association of Clinical Biochemists, A.N.Z.A.A.S., Christchurch, N.Z.*	D. WINIKOFF
"Modern Trends in the Interpretation of Thyroid Function Tests— A Review of 1000 Consecutive Cases" — *Endocrine Society of Australia.*	D. WINIKOFF
"How to Deal with the 'Pill' " — *Australian Association of Clinical Biochemists.*	D. WINIKOFF
"The Application of Radioactive Isotopes in Chromatography and Electrophoresis" — *Royal Melbourne Institute of Technology.*	D. WINIKOFF

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

CORONARY BLOOD FLOW

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

Selective injections of Xenon^{133} into left and right coronary arteries enable left and right myocardial blood flow to be calculated from the rate of wash-out of this radioactive indicator as obtained with a precordial detector. Patients being studied are those with acquired and congenital heart disease undergoing cardiac catheterisation, including coronary angiography. Previous studies have indicated that left myocardial blood flow per unit mass of perfused tissue is greater than right myocardial blood flow. Work in the dog has indicated that right myocardial blood flow is dependent on right ventricular pressure and right ventricular stroke work. The current project is being undertaken to establish whether there is in man a relationship between the ratio of left and right myocardial blood flow and left and right ventricular pressure and stroke work.

LONG-TERM RESULTS OF IMPLANTED PACEMAKERS

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

Sixty patients who have had a pacemaker implanted are being reviewed to assess the long term results of this therapy. Individual makes of batteries are being assessed as well as a comparison of epicardial and endocardial leads. The clinical indications and efficacy of ventricular triggered units are also being studied.

HAEMODYNAMIC AND ANGIOGRAPHIC CONSEQUENCES OF MITRAL AND AORTIC VALVE REPLACEMENT

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

Patients who have undergone aortic or mitral valve replacement are being studied clinically and by cardiac catheterisation. Haemodynamic, phonocardiographic and angiographic data are being used to assess the functional changes which result after this form of surgery.

PROPHYLACTIC USE OF XYLOCAINE IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

H. Lipp and A. Pitt[1]

A single blind trial is being used to study the place of an intraveous infusion of Xylocaine prophylactically to patients with an acute myocardial infarction. To date over one hundred patients have been admitted to the trial and the incidence of various arrhythmias in patients receiving therapeutic doses of Xylocaine is being compared with those on 5% dextrose alone.

[1] Cardiovascular Diagnostic Service.

EFFECT OF VARIOUS GASTRODUODENAL EXTRACTS ON OUTPUT OF HEPATIC BILE

Elizabeth Gordon, Paula Jablonski, M. C. Douglas, J. Toouli, J. A. Owen and J. McK. Watts[1]

Studies have been perfomed on the factors responsible for the regulation of hepatic bile secretion.

Pig livers weighing 600 - 800 grams were perfused in a pump-oxygenator circuit, with diluted homologous compatible blood. The hepatic artery and portal vein were both used for perfusion, the total flow rate being about 1 ml./gram weight of liver/minute. The enterohepatic bile acid circulation was mimicked by constant taurocholate infusion. The cystic duct was ligated, and the bile collected from the cannulated common bile duct. Test substances were infused or injected into the circuit after basal conditions had been established.

Secretin, cholecystokinin/pancreozymin, gastrin I and gastrin-pentapeptide and aqueous and ethereal extracts of porcine antral mucosa were tested for their effect on the volume and composition of hepatic bile.

Secretin was found to be a potent hydrocholeretic and cholecystokinin produced a choleresis with increase in bile acid output when given in large doses. Neither antral mucosal extracts nor pure gastrin produced any change in bile flow or composition in this preparation, although similar preparations increase hepatic bile flow in animals with duodenal cannulae.

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

ULTRA-THIN SECTIONS FOR HISTOLOGY

A. V. Jackson[1]

In 1968 a project to produce ultra-thin sections of plastic-embedded biopsy tissues for histological examination was commenced and the technical details are briefly recounted here.

Biopsy tissues are fixed very shortly after operational removal in 10% buffered formalin or in Helly's fluid. The specimen is then washed in phosphate buffered saline, post-fixed in 1% "osmic acid" (osmium tetroxide) and, finally, embedded in "araldite" (CIBA epoxy resin). After hardening, the araldite block is cut with a Huxley microtome into sections 0.4μ thick.

Various staining techniques have been tried but the best results have been obtained with a commercial stain, of uncertain composition, known as Paragon. This, we further modify by preliminary treatment of the section with borax.

The technique has produced, at times, very good sections indeed, though there are quality variations which, so far, we have been unable to control.

Tissues examined in this way have included small bowel, normal skin and skin cancer, normal thyroid and carcinoma of the thyroid, normal stomach and polypoid carcinoma of the stomach, bone marrow aspirate, spleen and lymph nodes. The increased detail seen in these tissues, compared with the routine paraffin embedded sections, is, at times, astonishing.

Many of the things observed are difficult to interpret because we are using a new dimension of histology which is somewhere between old routine and new electron-microscopic. In particular, the method seems promising with spleen and lymph node biopsies of lymphomas, and with bone marrow aspirate sections. These can be compared with "squash" preparations of spleen and lymph node, and bone marrow smears and with paraffiin sections of both. In these we are still observing and recording, rather than drawing conclusions. Attention has already been drawn (Eastham and Essex) to the suitability of thin sections of renal biopsies for demonstrating minimal glomerular changes in podocyte and basement membrane, and so the method is already of practical clinical value. It will probably also prove useful in gastroenterology. Microvilli in small bowel mucosa can be seen and minimal changes in some early cases of malabsorption syndrome should be detectable.

In short, the results of the technique are quite exciting and we intend to continue work in 1969.

[1] Morbid Anatomy Department, Alfred Hospital.

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"Intravenous Xylocaine in the Management of Ventricular Arrhythmias" — *i Royal Australasian College of Physicians, ii Alfred Hospital Clinical Society.*	S. T. ANDERSON and Aubrey PITT
"Coronary Arteriography" — *Alfred Hospital Clinical Society.*	Aubrey PITT
"The Effect of Beta Sympathetic Blockade on Left Ventricular Function" — *Cardiac Society of Australia and New Zealand.*	A. PITT and S. T. ANDERSON
"The Results of Reconstructive Surgery for Mitral Incompetence" — *Asian-Pacific Congress of Cardiology, Tel-Aviv, Israel.*	A. PITT, R. ZIMMET, S. T. ANDERSON, K. N. MORRIS, and G. R. STIRLING