Research

BAKER INSTITUTE

ALFRED HOSPITAL

1967

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-FIRST ANNUAL REPORT

of

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

(Including Alfred Hospital Clinical Research Unit)

ELEVENTH ANNUAL RESEARCH REPORT

of

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

REPORTS

of

ALFRED HOSPITAL RESEARCH FELLOWS

1967

ALFRED HOSPITAL, PRAHRAN, VICTORIA, AUSTRALIA

BAKER MEDICAL RESEARCH INSTITUTE

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	A. V. JACKSON, M.D., B.S., F.R.A.C.P., M.C.P.A., F.C.Path.
	N. E. JONES
	K. N. MORRIS, M.B., M.S., F.R.C.S., F.R.A.C.S.
	Sir CHARLES McGRATH
	T. H. STEEL, M.D., B.S., M.R.C.P., F.R.A.C.P.
	J. C. STEWART, M.B., B.S., F.R.C.S., F.R.A.C.S. (to 5/10/67)
	DIRECTOR OF CLINICAL RESEARCH UNIT (ex officio)

ALFRED HOSPITAL RESEARCH FELLOWS 1967

"Edward Wilson Memorial":	F. W. GURR, M.B., B.S., M.R.A.C.P.
"James Richardson": *"Sartori"*:	PINCUS TAFT, M.D., F.R.A.C.P.
"Connibere Bequest": *"Sol Green"*:	G. C. ENNIS, M.B., B.S., M.R.A.C.P.
"S. W. Jones":	D. J. B. ST. JOHN, M.B., B.S., M.R.C.P., M.R.A.C.P.
"E. H. Flack":	A. PITT, M.D., M.R.A.C.P.
"Dr. Henry Laurie": *"E. H. Flack"*: *"H. M. Black"*:	J. B. SWANN, M.B., B.S.
"J. F. Mackeddie":	J. A. MIRAMS, M.B., B.S., M.R.A.C.P.
"Victor Y. and Margaret Kimpton": *"Frederick and Esther Michaelis"*:	J. A. OWEN, B.Sc., M.D., Ch.B., Ph.D. (Edin.), M.C.P.A.
"Connibere Bequest": *"R. B. McComas"*:	J. T. SYKES, F.R.C.A.
"Amelia Haigh Estate": *(Rheumatoid Arthritis)* *"George Merriman"*: *"A. A. Swallow"*:	G. BUCKLE, B.Sc.
"Ringland Anderson":	G. W. CROCK, M.B., B.S., M.R.A.C.P., F.R.C.S.

APPOINTED TO RESEARCH FELLOWSHIPS FOR 1968

"Edward Wilson Memorial":	Miss M. E. GORDON, 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 Estate"*: *(Rheumatoid Arthritis)* *"H. M. Black"*: *"Victor Y. and Margaret Kimpton"*:	T. E. LOWE, C.B.E., 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. (Edin.), 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.

TRAVEL GRANT

"J. H. Patterson Travelling Scholarship":	C. W. MORRIS, 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

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Technical:	S. HART (Laboratory Supervisor) A. H. HUCKFIELD (Technical Officer) J. BREMNER (decd 28/4/67) Mrs. B. SKYM (Senior Technologist) Miss J. CHAN	Miss D. CHIPPERFIELD Miss V. MACK Mrs. E. MORTENSON Mrs. R. SABO
Librarian:	Miss M. JOHNSTON	
Clerical:	Mrs. I. R. ROBINSON Mrs. R. BRETT (to 28/4/67)	Miss M. GAYTON Mrs. H. WINSTANLEY
Laboratory Assistants:	Miss K. CLARKSON Miss D. DAVIS Miss J. FINDLAY Mrs. R. GILSENAN Mr. K. HARVEY	Miss F. HIRSCH Miss R. KNOWLES (to 18/8/67) Mr. C. LEWIS Miss M. SHIERS

WARD STAFF

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Resident Medical Officers:	T. P. LONGNEY, M.B., B.S. L. C. CHEN, M.B., B.S.	S. H. CHAN, M.B., B.S. R. HAIN, M.B., B.S.
Sisters:	J. W. SIMON (to 1/10/67) J. HOMEWOOD (from 18/9/67)	
Staff Nurses:	K. M. BECK (from 11/9/67 to 10/12/67) T. A. CANNINGTON (from 12/9/66 to 3/3/67) S. EDGAR (from 29/5/67 to 16/7/67) J. B. HORSFALL (from 4/6/67 to 28/7/67)	J. M. JONES (from 13/11/67 to 31/12/67) F. McKENZIE (from 7/8/67 to 31/12/67) F. A. SULLIVAN (from 17/7/67 to 12/10/67) B. TOLLAND (from 5/6/67 to 10/9/67)

ALFRED HOSPITAL RESEARCH FELLOWS

"Dr. Henry Laurie":
"E. H. Flack":
"H. M. Black": J. B. SWANN, M.B., B.S.

ANTI - CANCER COUNCIL RESEARCH FELLOW

"A. A. Thomas": P. E. HUGHES, Ph.D., M.B., B.S. (on leave)

NATIONAL HEART FOUNDATION RESEARCH FELLOW

M. ROSENBAUM, M.D., M.R.A.C.P.

HONORARY RESEARCH FELLOW

G. R. STIRLING, M.B., B.S., F.R.A.C.S.

BAKER INSTITUTE PRIZE (1966)

R. ATKINS, M.B., B.S.

ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

In recent years great changes have occurred in the conduct of biological, including medical, research both in the range and the complexity of investigations. Much of this change has been brought about by developments in other branches of science which have made available invaluable but expensive tools such as electronic data recording and processing equipment and radio-isotopes with which to trace events even at the cellular level. Further the use of these techniques has emphasized, amongst other things, the need for biological experiments to be carried out under controlled environmental conditions.

NEW BUILDING

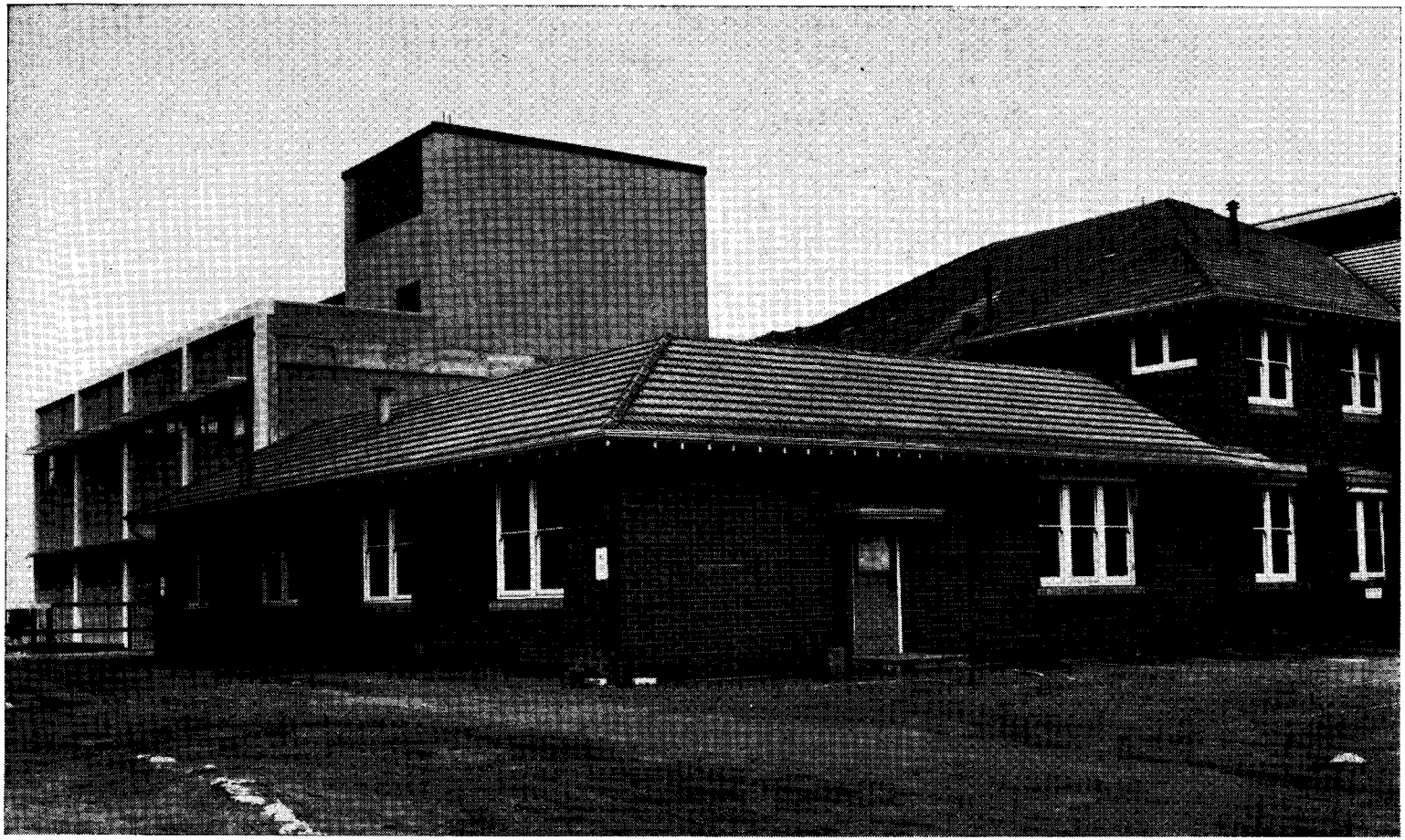
The need for a controlled environment has meant that the laboratory building has become a most important piece of equipment instead of being merely a place in which to conduct an experiment. This influence is seen in both the new building for the Baker Institute, and the new clinical laboratory of the Clinical Research Unit. To provide an adequate environment the entire Institute building has been made dust proof and provided with mechanical ventilation embodying both temperature and humidity control. In addition, within it small laboratories are provided in which the temperature may be further adjusted to any level from 32°F. to 103°F. The clinical laboratory attached to the new ward has been air-conditioned. The first stage of the Institute building was completed and occupied in August and building for the second stage was commenced in November. It is expected that the new ward will be available for occupation early in 1968. The design of the Institute building has been a research project of some duration and is described in detail with the reports of research projects. I thank Messrs Stephenson & Turner, Architects, for their co-operation in translating our ideas into a highly satisfactory laboratory building.

FINANCE

Of the various implications of these changes in research techniques the financial looms large. The cost of rebuilding the Institute and partially re-equipping it will be approximately $1,450,000 and we thank the Government of Victoria for the generous gift of $200,000 towards this and the Baker Benefactions who have generously underwritten the remainder. In addition to making the building an expensive piece of equipment the research changes have increased the running costs beyond that due to depreciation of money values. A decade ago an approximate estimate of the cost of a biological research project was obtained by multiplying the salary of the graduate concerned by 2. In this Institute the multiplier had risen to 2.1 by 1964 and in 1968 it is estimated to be 2.3. This rise is due to the cost of increasingly elaborate laboratory supplies and the maintenance of research equipment including the building.

The depreciation in the purchasing power of money which has gone on steadily for many years has led to an equally steady increase in salaries and wages and, as these form the bulk of any research maintenance budget, has increased the cost of running the Institute so that together with the cost of increasing complexity the average cost per graduate employed now stands at more than $12,000 per annum.

As indicated in the front of this report the main financial support of the Institute is an annual grant from the Baker Benefactions. This grant provides the facilities and the senior staff around which research projects supported by grants-in-aid and the laboratory work of the Clinical Research Unit can be developed. The Trustees of the Benefactions have very generously supported the Institute since its inception and borne these rising costs, but there is an upper limit to their ability to help. That limit is now in sight, so that additional sources of revenue must be sought and ways of



New (Stage 1) and Old Buildings.

appealing to our friends for support are being investigated. It is of interest to note that the monies made available by the Board of Management from the research funds of the Hospital for the Clinical Research Unit and the grants-in-aid of research from bodies such as the Life Insurance Medical Research Fund, National Heart Foundation and Anti-Cancer Council of Victoria, for the support of projects made possible by the facilities provided by the Institute, match approximately the contribution of the Benefactions.

RESEARCH PROJECTS

In 1967, as a consequence of the rebuilding programme, the number of research projects has been somewhat curtailed. This has made the commissioning of Stage I of the new building a fairly smooth operation. However, projects arranged to commence in the New Year will bring activity back to recent levels.

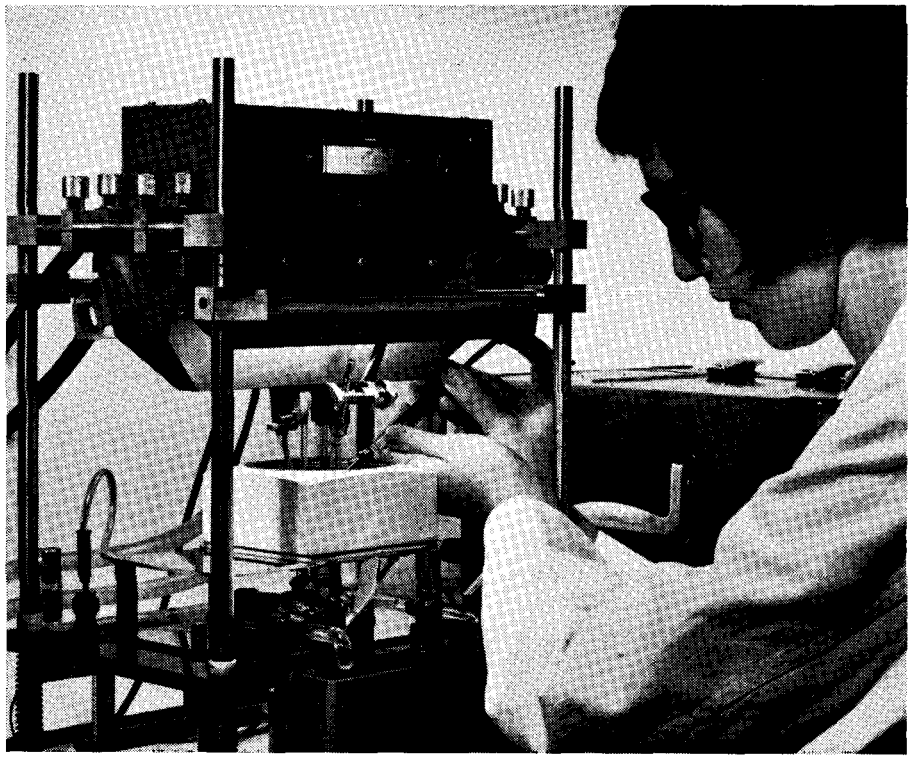
Detailed accounts of research work in progress are given in the Scientific section of this report but a short account of some of general interest is included here. These cover a number of aspects of physiology and pharmacology of the cardiovascular system and clinical investigations into both cardiovascular and gastrointestinal disorders.

CALCIUM AND THE CARDIAC MUSCLE CELL

For a long time digitalis has been known to be a cardiac stimulant in many circumstances but its mode of action has been obscure. Experimentally it can be shown that calcium ions have many actions similar to digitalis; they can increase the mechanical work which the heart can perform in pumping blood and also they can increase the efficiency with which the heart muscle produces the energy for this work. Extensive investigations into the mechanisms which regulate the distribution and concentration of calcium ions in cardiac muscle and in turn regulate myocardial function and efficiency have therefore formed a major part of our cardiovascular studies.

During the past ten years these investigations have established that the force with which the heart contracts and the state of relaxation displayed during each diastole reflects the intracellular concentration of calcium ions in the immediate vicinity of the myofilaments (the contractile elements). Information that has been obtained about the system which is responsible for controlling the intracellular distribution of these ions shows that the system is complex and involves not only the transport of calcium and other ions across the semi-permeable membranes bounding each muscle cell but also the regulated uptake and release of these ions at specific intracellular sites.

It has become quite clear that ionized calcium is a primary site of drug action and that certain cardioactive drugs, including the cardiac glycosides, which augment cardiac contractility and efficiency, increase the total intracellular concentration of ionized calcium and hence the number of these ions which are available to activate and potentiate contraction. Other drugs, including some newly developed antiarrhythmic drugs, interfere with the process by which ionized calcium is transported from the extra- to the intracellular phase and therefore decrease the number of ions available for the activation of contraction and hence depress myocardial contractility. Cardiac muscle displays a high degree of specificity towards calcium ions so that other chemically related divalent cations cannot be substituted for them in the process of excitation - contraction coupling. Although cardiac mitochondria are characteristically associated with the system which functions to provide the energy needed to facilitate muscle contraction, current investigations indicate that these mitochondria also influence cardiac muscle contraction by another mechanism — that is, by their ability to accumulate and release calcium ions. It is probable that they play a dual role in maintaining the heart's ability to beat. Because it is clear that calcium ions play such a central role in the functioning of the cardiac muscle cell and its reaction to drugs, these studies are continuing.



Cardiac Muscle Research (Equipment made in Institute Workshop).

CARDIAC SURGERY

Research into a variety of problems connected with surgery of the heart has been carried out in the Institute for the past twenty years and the results have been made available for the treatment of patients through the surgeons of the thoracic surgical unit of the Hospital. At the present time the projects are directed towards the problems of the preservation of cardiac function in hearts used for transplantation. It is hoped that a technique may be evolved which will enable a donated heart to be stored for appreciable periods before being placed in a recipient and so overcome many of the problems, practical and ethical, which present difficulties today.

GASTRO-INTESTINAL DISORDERS

Diseases involving the alimentary canal pose many problems, some of which have been investigated by physicians of the Clinical Research Unit during recent years. Two contributions of importance have been concerned with the development of diagnostic techniques. The first of these has enabled difficulty in swallowing to be investigated by placing in the oesophagus a number of fine polythene tubes, each of which opens to the lumen of the oesophagus 5 cms from the next. These tubes are filled with water and the pressure at the tip is then transmitted to a multichannel recorder. By this means the progress of the peristaltic wave down the oesophagus can be recorded. This test has proved a valuable complement to the clinical, radiological and endoscopic findings in these patients. The second technique enables accurate assessment to be made of small amounts of bleeding from the alimentary tract. It employs a process of tagging a sample of the individual's own red cells with radiochromium, re-injecting them into the blood circulation and subsequently determining the amount of radioactivity in the bowel excreta which can only come from red cells entering the alimentary canal by bleeding. It is proving more satisfactory than the previously available chemical tests for occult bleeding.

STAFF

The temporary reduction in research projects has meant that the number of workers in the Institute this year has been considerably reduced. However, in 1968 there will again be a full complement and on completion of the building some expansion of staff can be anticipated.

Dr. P. Hughes, who has been appointed to the A. A. Thomas Fellowship of the Anti-Cancer Council of Victoria, has been working at the Chester Beatty Research Institute in London and will commence in the Institute in February next.

Dr. D. A. Coventry has completed his term as Assistant Physician to the Clinical Research Unit at the end of 1967 and has been succeeded by Dr. D. J. B. St. John.

Dr. M. Rosenbaum, who holds an Overseas Research Fellowship of the National Heart Foundation of Australia, returned from Philadelphia during the year.

Dr. D. Race has left to join the group investigating the use of modern data processing techniques in hospitals.

Dr. L. W. Wheeldon has been appointed as a Senior Research Biochemist to the Institute and commences early in the New Year.

Dr. P. Fantl, who had been on long service leave, returned to work part-time on his projects concerning blood coagulation.

OVERSEAS VISITS (1967)

In February I visited the U.S.A. as a guest of the Americal College of Cardiology to deliver the Overseas Lecture at their Annual Meeting in Washington, D.C. This lecture was entitled "The Clinical Significance of Kinekard". During the visit the opportunity was taken to discuss problems connected with this project with colleagues in Chicago, Nashville, New Orleans, Philadelphia and Los Angeles. I thank them all for their help and hospitality.

Dr. Winifred G. Nayler visited Europe, England and the U.S.A. during October-November. She presented an account of some of the Institute's research at a symposium on cardiac muscle physiology arranged by the University of Naples. Visits were made to a number of laboratories in each country to discuss with interested workers many of the research projects of the Institute.

Dr. A. J. Barnett has spent the whole of this year overseas as a sabbatical year of study. For many months he studied at the Postgraduate School of Medicine in London with Professor J. Shillingford, and subsequently visited centres in Europe and the U.S.A.

RESEARCH ASSISTANCE

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

It is a pleasure to record thanks for generous donations from those whose names are listed in the various financial reports.

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 Commonwealth Serum Laboratories, Commonwealth X-Ray and Radium Laboratories, and C.S.I.R.O., and also by the Honorary Medical Staff and Departmental Staffs of the Hospital. We thank them all for this continuing interest in our projects and their ready help. Such help as we have been able to give in return has been freely availed of often in the form of lecture and tutorial assistance, particularly to the departments of Biochemistry, Physiology and Medicine at Monash University.

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

December 31, 1967. T. E. LOWE.

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.
Staten Seruminstitut, Copenhagen.
University of Melbourne.
University of Otago, New Zealand.
University of Queensland.
University of Sydney.
Universitatis Mariae Curie Sklodowska, Poland.
Walter & Eliza Hall Institute, Melbourne.
Wellington Medical Research Foundation.
World Health Organisation.

ALFRED HOSPITAL RESEARCH FELLOWS IN THE INSTITUTE

1949 - 1967

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
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)
Robertson, P. G. C., 63-64 (Dundee)
Simpson, F. O., 1958-59 (Edinburgh)
Stevenson, M. M., 1957 (Belfast)
Thomson, J. W. W., 1959 (Edinburgh)

REPORT OF SCIENTIFIC INVESTIGATIONS

PHYSIOLOGY AND PHARMACOLOGY OF THE CARDIOVASCULAR SYSTEM

The continued co-operation between workers in the fields of biochemistry, pharmacology, physiology and surgery, and the integration of their activities has made further investigations into cardiovascular physiology and pharmacology possible during 1967. These investigations have included studies performed (i) at the cellular and sub-cellular level that have aimed at further elucidating the role played by Ca^{++} in regulating the events involved in excitation - contraction coupling in cardiac muscle; (ii) on isolated cardiac muscle in which atria, papillary muscles, intact isolated hearts and right-sided bypass preparations, have been used to gain further information about myocardial function and energy production; (iii) on the peripheral circulation, where heart-lung bypass and isolated arterial muscle preparations have been used to investigate the mechanisms involved in regulating and maintaining the distribution of blood in the peripheral circulation, including that in the coronary vasculature; (iv) on the venous circulation, using a special preparation developed to determine whether or not drug-induced changes in ventricular function reflected alterations in the total capacity of the circulation and hence in venous return to the heart; and (vi) in intact animals which have been used primarily to determine the overall effects of certain recently introduced hypotensive drugs and β-adrenergic antagonists on the cardiovascular system.

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

ROLE OF CALCIUM†‡

W. G. Nayler, J. Price, J. Guthrie and T. E. Lowe

Excitation-Contraction Coupling in Cardiac Muscle

(a) **Inotropic mechanisms.** Since the introduction of the sliding filament theory the hypothesis that progressive interdigitation between actin and myosin filaments is associated with the development and maintenance of tension has become widely accepted. This progressive interdigitation apparently reflects the availability of ionized calcium in the immediate vicinity of the myofilaments for previous investigations have shown the existence of a direct relationship between the amplitude of cardiac muscle contraction and the availability of Ca^{++}, detected as Ca^{45}. This being so, experiments were conducted during 1967 to determine whether or not drugs, which depress myocardial contractility, do so by decreasing the amount of Ca^{++} available to activate the process of contraction. The drugs chosen for study were those which depressed left ventricular function over a wide work load and included the recently introduced hypotensive drug, prenylamine, and the β-adrenergic antagonists, *dl* propranolol, KÖ 592 and Trasicor. Prenylamine (0.1 - 1.0 μg/ml) consistently caused a significant reduction in the intra-cellular concentration of Ca^{++} present in cardiac muscle cells. Further investigations indicated that this decline in the concentration of Ca^{++} reflected a decrease in the concentration of Ca^{++} present in the mitochondrial stores as well as in the sarcoplasmic reticulum. Semi - quantitative experiments, using freshly excised human papillary muscles, have shown that the negative inotropic effect of prenylamine can be correlated with the decline in the total cellular Ca^{++} concentration. Further studies using lipids extracted from the microsomal fraction of dog heart muscle revealed that prenylamine interferes with the lipid facilitated transport of Ca^{++} across an aqueous lipid-solvent interface in much the

same way as do quinidine sulphate and procaine-amide. The decrease in cellular Ca^{++} caused by prenylamine may reflect its ability to interfere with the uptake of Ca^{++} across the lipid-containing cardiac cell membranes during the processes involved in excitation-contraction coupling.

Other drugs which exert a negative inotropic effect on cardiac muscle include the β-adrenergic antagonists, *dl* propranolol, KÖ 592, and Trasicor. These drugs apparently resemble prenylamine in that they exert a negative inotropic effect which is associated with a decrease in the cellular concentration of Ca^{++}. This changed intracellular distribution and concentration of ionized Ca may be associated with the proven usefulness of these drugs for the control of certain cardiac arrhythmias.

(b) **Competition with other divalent cations.** Sarcoplasmic reticulum isolated from skeletal and cardiac muscle cells of the same donor animal differs in its ability to differentiate between Ca^{++} and other divalent cations. Thus sarcoplasmic reticulum which has been isolated from cardiac muscle cells consistently accumulated Ca^{++} in preference to Zn^{++}, whereas reticulum which was isolated from skeletal muscle cells failed to exhibit this discrimination.

MYOCARDIAL FUNCTION‡

W. G. Nayler, I. E. McInnes, V. Carson and T. E. Lowe

β-Adrenergic Antagonists

Because of the increasing interest being displayed in the possible therapeutic use of β-adrenergic antagonists for the treatment of angina pectoris and for the control of cardiac arrhythmias, considerable attention has been given to determining whether or not these drugs impair myocardial function. Isolated atria beating either spontaneously or stimulated electrically to beat at a controlled rate, dog and human papillary muscles, intact Langendorff-perfused hearts and right - sided bypass preparations have been used under a variety of conditions to study and compare myocardial function and energy production before and after adding the β-antagonists. The antagonists used include, *dl* propranolol, KÖ 592, Trasicor, LB 46, ICI 50172 and the partial antagonist, Iproveratril. Dose response curves constructed for human and dog papillary muscle preparations indicated first, that the negative inotropic effect of these drugs is not species specific; secondly, that the prior depletion of as much as 95 per cent. of the catecholamines by reserpine does not significantly modify their negative inotropic activity, from which it can be concluded that their depressant effect cannot be due simply to β-adrenergic blockade; thirdly, that their negative inotropic effect can be separated from their negative chronotropic action, since myocardial depression developed whether or not the heart rate was controlled; fourthly, that left ventricular failure, whether induced by any of the β-adrenergic antagonists listed above, is not associated with a significant fall in the level of high energy phosphate stores or in the level of endogenous catecholamines as occurs after prolonged periods of anoxia.

Other experiments indicated that the depressant action of the partial β-adrenergic antagonist, Iproveratril, must be separated from that of the other β-antagonists because the left ventricular failure induced by it can be reversed by isoproterenol. Iproveratril was found consistently to block the β-adrenergic receptors in the peripheral circulation which mediated vasodilation, whilst the β-receptors in the myocardium which mediate the positive chronotropic and inotropic actions of the catecholamines were not blocked. Correlation of these data with those presented in the first section of this report indicates the probability that left ventricular failure induced by β-adrenergic antagonists may be due not to their β-blocking properties *per se* but rather to a disturbance of the events intermediate between excitation and contraction, probably involving Ca^{++}. A quantitative relationship was found not to exist between the β-adrenergic blocking potency of these drugs and their myocardial depressant action.

PHARMACOLOGY‡

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

Peripheral Circulation

(a) **Coronary blood flow.** Investigations into the mode of action of substances which act

on the peripheral circulation begun in previous years have been continued and extended, using dogs on total heart-lung bypass and isolated strips of arterial muscle. Considerable attention has been given to determining the effect of the recently introduced β-antagonists and some of the hypotensive drugs on coronary blood flow, and on the relationship between coronary blood flow, the amount of oxygen extracted by the myocardium and the amount of useful mechanical work performed by the heart as indicated by left-ventricular 'work function' studies.

Iproveratril (Isoptin, Cordilox, Verpamil) resembles *dl* propranolol Trasicor and KÖ 592 in that it depresses left ventricular function but differs from them in that it reduces the resistance to blood flow in the peripheral circulation, including that of the coronary vasculature. These iproveratril-induced changes in myocardial function and coronary blood flow were associated with a decline in the rate at which the myocardium extracted oxygen. Extensive experiments indicated that Iproveratril may discriminate between the β-adrenergic receptors in the peripheral circulation and those in the myocardium and that it blocks only those in the peripheral circulation, including the coronary vasculature.

The coronary vasoconstrictor effect of *dl* propranolol, Trasicor and KÖ 592 was evident whether or not the heart rate was controlled, indicating that the reduction in coronary blood flow caused by these drugs represents true constriction and not merely the mechanical effect of a change in heart rate. The prior addition of atropine diminished significantly ($p = 0.001$) the coronary constrictor effect of these drugs. Other coronary vasodilator drugs, including Persantin and two of the Persantin derivatives and Iproveratril, induced coronary vasodilation in the presence of either *dl* propranolol, KÖ 592 or Trasicor. Left ventricular work function curves indicated that reduction in coronary blood flow was maintained throughout the whole of the work curves. Other coronary vasodilator drugs used included Prenylamine and Diphenylhydantoin. During these experiments, repeated observations indicated that the coronary constrictor effect resulting from α-adrenergic stimulation was potentiated during the maintenance of β-blockade.

(b) **Other regional blood flows.** Dog heart-lung bypass preparations perfused under conditions of constant perfusion pressure or flow as required were used to establish whether or not all of the β-adrenergic antagonists increase the resistance to blood flow in the systemic circulation. KÖ 592 was found to resemble *dl* propranolol and to differ from either MJ 1999 or Trasicor in causing a marked increase in the resistance to blood flow in the splanchnic circulation.

In other experiments, again using dog heart-lung bypass preparations, the effect of the recently developed hypotensive drugs, Prenylamine, Catapres (ST. 155) and Vialibran on the regional distribution of blood in the peripheral circulation was investigated. Prenylamine and Vialibran both caused a marked reduction in the resistance to blood flow in the systemic circulation. Prenylamine, Vialibran and Catapres all caused a reduction in the resistance to blood flow in the skeletal muscle beds. Other experiments using spinal dogs indicated that Catapres may exert its hypotensive effect via a centrally-mediated pathway. Since the hypotensive effect of Vialibran and Prenylamine persisted after α and β-adrenergic blockade, after the addition of atropine and in the presence of ganglion blockade it is assumed that undefined receptors in the peripheral vasculature can mediate vasodilation.

(c) **Venous blood flow.** Preliminary clinical trials have shown that the hypotensive effect of Catapres (ST 155) in man is accompanied by a change in cardiac output, both at rest and during exercise. Changes in cardiac output can result from either a direct change in myocardial function or from an altered venous return. Left ventricular work function curves showed that in dogs Catapres caused only non-significant displacement of left ventricular function curves to the right. Other experiments have shown that this drug can decrease venous return to the heart by as much as 15-20%. These results have been interpreted to mean that this drug increases the total capacity of the venous circulation. Observations

made during these experiments confirmed the findings of Epstein *et al* that α and β-adrenergic receptors in the venous circulation subserve constriction.

Intact Animal Studies

Intact rabbits and dogs have been used when experiments have been performed to correlate the above data so that the action of particular drugs on the cardiovascular system as a whole could be defined. The drugs used have included Catapres, Vialibran, Prenylamine, Iproveratril and the β-adrenergic antagonists.

Catapres produces a biphasic response causing a transient rise followed by a sustained fall in blood pressure, which is accompanied by bradycardia, a decrease in cardiac output, a decline in peripheral vascular resistance and a raised blood sugar level. The initial pressor response involves α-adrenergic receptor stimulation and is potentiated by ganglionic blockade and the administration of reserpine, atropine and propantheline.

Vialibran produces a sustained hypotension associated with a reduction in the resistance to blood flow in the peripheral circulation and bradycardia.

Prenylamine in intact rabbits causes transient hypotension followed by a mild pressor response. Catecholamine assays have confirmed that prenylamine releases catecholamines from their endogenous stores.

Preliminary experiments with two newly released antagonists, LB 46 and ICI 50172 have shown that both drugs produce bradycardia. ICI 50172 blocks the effect of β-receptor stimulation in the myocardium whilst leaving unblocked the adrenergic receptors which are in the peripheral circulation. This drug differs from *dl* propranolol in that it neither potentiates the pressor effect of α-adrenergic stimulation, nor does it depress myocardial function to any great extent.

Arterial Muscle

Previous experiments have shown that hypothermic conditions modify the effect of β-adrenergic stimulation in the arterial system. During this year these observations have been extended and the effect of 0.1 - 3 μg/ml isoprenaline on the tension produced by thin strips of rabbit thoracic aortae, was investigated at several different temperatures ranging from 37°C. to 15°C. Depending on the temperature, either a contraction or a relaxation occurred. At 27°C., 3 μg/ml isoprenaline (considered a large dose) was always found to contract the aortic strips. At successively lower temperatures, the contraction produced by isoprenaline was reduced, required a longer period of time to develop and to reach a maximum and was sometimes replaced by a relaxation. At 15°C. the only demonstrable effect was a relaxation. In other experiments it was found that the effect of isoprenaline is also concentration dependent. At 37°C. relatively small doses (0.1 μg/ml), if effective, always induced relaxation and intermediate doses (1 μg/ml) reduced either relaxation or contraction (smaller than that produced by 3 μg/ml). The contraction caused by larger doses of isoprenaline was blocked by the α-adrenergic antagonist phenoxybenzamine, and partially blocked by the β-blocker propranolol. It appears then that this contraction is mediated by both α and β-adrenergic receptors and that the β-receptors present in these strips may mediate either contraction or inhibition. It was also found that the contraction produced by 3 μg/ml at 37°C. is not mediated by the release of endogenously stored catecholamines.

KINEKARD†

T. E. Lowe, W. G. Nayler, I. Farrance and V. Carson

Kinekard is the name we have given to a fraction isolated from heparinized blood plasma of mammals, birds, reptiles and fish. Physiologically it acts on two groups of muscle, (a) cardiac, (b) smooth muscle wherever found. It is positively inotropic on cardiac muscle and its action on smooth muscles resembles that of the catecholamines. It can be shown that kinekard is not a catecholamine. In the intact animal kinekard produces both a positive inotropic action on the heart and a pressor response in the circulation.

Preparation

During the past year work has been directed towards improving the methods used for

isolating kinekard from plasma with a view to obtaining the physiologically active component in a purer state and the development of a method suitable for use on a preparative scale.

Previously we have used the method proposed by Curtain and Nayler (1963). This is a two-stage process; the first stage consists of gel-filtration on a large column of polyacrylamide (Bio-Gel P10) and the second stage consists of ion-exchange chromatography on DEAE-methacrylate, after selective adsorption on DEAE-methacrylate of the active principle in fractions obtained from the first gel-tration.

A major problem has been to obtain the kinekard fraction (MW 5,000 - 10,000) free from the plasma proteins because immuno-electrophoresis demonstrated that kinekard prepared by this method contained a significantly high plasma protein content.

The polyacrylamide column now used has a capacity of approximately 8 litres and has dimensions of 20 cm diameter X 1 metre height. In such a large column great care is needed to obtain uniform packing of the column, otherwise very poor separation of the various protein fractions results with a high degree of "tailing". The other crucial change in procedure is thorough washing of the DEAE-methacrylate on which the kinekard is adsorbed before proceeding with the ion-exchange chromatography.

When these precautions were taken it was possible to obtain as a final product from 1 litre of plasma about 15 ml of kinekard solution which had a high inotropic activity and this fraction appeared to be protein-free when tested by immunoelectrophoresis against anti-human serum.

Some exploratory work has also been done to make possible the treatment of plasma in larger batches than can be handled by the method just described and promising results have been obtained using a single ion-exchange step by chromatography on DEAE cellulose. This method would theoretically allow the treatment of ten times the volume of plasma on the same size column as used for polyacrylamide gel-filtration. However, a second concentrating step would probably be necessary.

High Voltage Electrophoresis

Fractions obtained from the modified two-stage process described were concentrated by freeze drying and then subjected to high voltage electrophoresis in pyridine-acetate buffer. The strips were divided longitudinally and one strip was stained with ninhydrin and the other cut into sections along its length, eluted in phosphate buffer and tested for inotropic activity.

Four or five ninhydrin positive bands could be detected. In most cases these did not correspond with the recorded kinekard activity. On testing the eluted areas for kinekard activity two regions on the strip were found which showed inotropic activity together with one or sometimes two regions which showed strong depressant activity in the toad heart assay.

In some cases very faint ninhydrin positive bands could be seen which corresponded to the regions of inotropic activity. These were distinct from the four or five definite ninhydrin bands which showed no inotropic activity.

It therefore appears that the fraction containing kinekard is still far from pure and, if the intensity of the ninhydrin colour is a guide, the other substances are present in far greater quantity than kinekard. Similar high voltage electrophoresis patterns could be shown with fractions obtained by chromatography on DEAE cellulose. An interesting observation using this method was that the depressant substances could be clearly separated from the cardioactive substances in the ion-exchange step.

Assay

The method for assaying kinekard concentration in blood plasma and equating it to noradrenaline has been based on the inotropic action of kinekard on the toad heart. As, however, kinekard has a constrictor action on blood vessels as well as the cardiac inotropic action, it is necessary to determine whether each of these properties provides an adequate basis for the assay of kinekard.

Preliminary experiments have been conducted to confirm that an assay procedure for kinekard based upon its vasoconstrictor pressor action, is practicable.

Clinical Significance

Pending development of a pressor assay technique and better methods of isolation, little further investigation of the clinical significance of kinekard has been carried out this year. Previously we had noted that plasma kinekard concentration did not appear to change with age in children and adults. This age range has been extended by the study this year of 22 new-born babies whose plasma kinekard concentrations (inotropic assay) were within the established normal limits.

Data recently made available to us in Germany indicates that the late Professor C. Rhein almost certainly had isolated the plasma fraction which has now been named kinekard. He had defined some of its pharmacological properties and had accumulated evidence which suggested that this fraction was produced in the liver. As, unfortunately, Professor Rhein had not prepared his results for publication in scientific journals before his death, we greatly appreciate the courtesy extended to us by his colleagues who made available this independent confirmation of much of the kinekard work. We intend to investigate further his conclusions relating to the site of production of this cardioactive fraction.

CONTROL MECHANISMS IN THE CARDIOVASCULAR SYSTEM‡

M. Rosenbaum

This project has been carried out both in the Baker Institute and at the Bockus Research Institute of the University of Pennsylvania, Philadelphia, during the tenure of an Overseas Research Fellowship of the National Heart Foundation of Australia. The broad aim of the research programme at the Bockus Institute is to establish a computer model of the circulation, which would include the changes produced by hypertensive disease. The haemodynamic investigations are centred around quantitating the various components of the circulation and are mainly performed on unanaesthetized, trained dogs, with chronically implanted electromagnetic flowmeters, vascular catheters and pneumatic cuffs which can be used to obstruct various vessels. Investigations are also performed on normal and hypertensive human subjects.

The carotid sinus response: By inflation of a pneumatic cuff around the brachiocephalic artery, the carotid sinus baroreceptor response has been investigated in 17 unanaesthetized dogs. This response affects mainly the total peripheral resistance and heart rate, with little change occurring in cardiac output. Studies made at the Baker Institute on animals on total heart-lung bypass indicate that the peripheral resistance change occurs mainly in the skeletal muscle vascular beds, with lesser changes in the renal and splanchnic beds. Coronary resistance in contrast usually changes in the opposite direction to the skeletal muscle resistance.

Matching between the heart and circulation: By inflation of a pneumatic cuff around the lower aorta, it is possible to vary the total peripheral resistance. This technique has been used to study the effect of total peripheral resistance on cardiac output in dogs, before and after cardiac blockade with propranolol and atropine. Using standard electrical formulae the matching between the heart and peripheral circulation has been calculated from the rate of decrease of cardiac output as resistance rises. The results indicate that before blockade with propranolol and atropine, the heart is well-matched to the circulation and maximum fluid work transfer occurs. After blockade the matching is less good and less transfer occurs.

Human Hypertension: The effect of various pressor stimuli on the blood pressure, heart rate, cardiac output, stroke volume and total peripheral resistance has been studied in 25 hypertensive and 12 normal subjects. There was an overall difference in the pattern of this response between the two groups with the hypertensive subjects responding with a greater blood pressure rise and less heart rate rise than the normal subjects. On the other hand, when the individual subjects were studied, in the majority of normal and hypertensive subjects there was little correlation between the responses to each stimulus. This finding suggests that abnormal circulatory responsiveness is not an aetiological factor in hypertension.

CARDIAC OUTPUT DETERMINATION: XENON^{133} METHOD[1]

A. J. Barnett

The methods currently used for determination of cardiac output have disadvantages. The Fick method is tedious, requires spirometry and disturbs the patient, and therefore is likely to result in a fictitiously high reading for the basal output. The usual dye dilution method requires arterial puncture and considerable time to calculate the results from the dye curves. Further, because of accumulation of dye in the circulation, there is a limitation to the number of times the investigation may be repeated over a particular period. Consequently a method which is simple to perform, which can be repeated frequently at short intervals and from which the result can be quickly obtained would be a valuable investigational tool.

Now that the radioisotope Xenon^{133}, which has a high specific activity, is available it can be used as the basis of a simple method for determining cardiac output.

In this method a brief continuous infusion of a solution of Xenon^{133} is made into the right atrium through a fine flexible catheter introduced percutaneously into a vein at the elbow and then floated into the right heart. During this infusion a steady concentration of Xenon^{133} develops in the pulmonary artery blood. As this blood passes through the lungs 95% of the Xenon^{133} is exhaled and the remainder passes into the systemic circulation and ultimately returns to the right heart and so recirculates. If the concentration of Xenon^{133} in the pulmonary artery blood is determined when the steady state has been reached and whilst recirculation of Xenon^{133} is negligible when related to the amount infused, the cardiac output can be calculated from the formula:

$$C.O. = R/S \times 60{,}000$$

where C.O. = cardiac output (litres/min)
R. = rate of infusion of Xenon^{133} (counts/sec)
S. = specific activity of sample of pulmonary artery blood (counts/ml)

The blood in the pulmonary artery can be sampled through a second fine catheter floated through the right heart.

The feasibility of this method depends on the development of a steady concentration of Xenon^{133} in the pulmonary artery for a period adequate for sampling at a time when the recirculation of Xenon^{133} to the pulmonary artery is negligible. Secondly, the results obtained for cardiac output must be comparable with those obtained by accepted methods.

The following feasibility study was made in a heart model, in animals and in man.

Observations in the Heart Model

The right side of the heart and lungs were simulated by a heart model in which the output could be varied by altering the frequency of a pump activating the "right ventricle" and could be measured by direct collection. Preliminary tests were performed with injection of Coomassie blue dye into the "right atrium" and direct sampling from the "pulmonary artery" through a Gilford densitometer. In this way a continuous concentration curve for dye in the pulmonary artery was obtained, the time for the development of a steady concentration of dye observed (it showed as a plateau in the curve) and the cardiac output then calculated and compared with the direct measurement. It was found that the time for development of a plateau concentration varied inversely with the cardiac output but was not greatly affected by incompetency of the atrio-ventricular or pulmonary valves. With cardiac output in a range of 2—8 litres per minute plateau development occurred within about thirty seconds.

Observations were then made in this model using Xenon^{133} in place of the dye. The cardiac output was calculated and compared with direct measurements, with ranges from 2—8 litres per minute and various degrees of atrio-

[1] The study reported here was done in collaboration with Dr. Y. Kishon with advice from Professor J. Shillingford, during a six months study leave at the Hammersmith Hospital, London.

ventricular and pulmonic valvular insufficiency. It was found that the time for plateau concentration was similar to that established by the dye concentration studies and that by sampling at this time it was possible to calculate cardiac output with an accuracy of $\pm$ 4%.

Observation in Animals

Observations in animals were made to discover whether cardiac output could be similarly measured in the physiological situation. In this situation it is not possible to check the determination of cardiac output by the Xenon^{133} method with direct measurements, but it can be compared with the results of a previously accepted method. Cardiac output in dogs was determined by the Xenon^{133} method and almost simultaneously by a dye dilution method using Coomassie blue dye and a Gilford densitometer. The animals were anaesthetized and in a "resting" state and the cardiac output was either lowered by haemorrhage or the administration of drugs or elevated by the infusion of isoproterenol or adrenaline. There was a good correlation between the results from Xenon^{133} and dye methods.

Observations in Man

To determine whether the Xenon^{133} method was clinically useful it was used in a series of patients in whom cardiac output was being measured for diagnostic purposes by a conventional dye dilution method using Coomassie blue dye and an ear densitometer and by the Fick method. Xenon^{133} in solution containing 1 millicurie per millilitre, was administered by a short continuous infusion over about 30 seconds into the right atrium and blood sampled from the pulmonary artery through fine catheters introduced by an elbow vein. A good correlation between the new method and the conventional methods was noted.

It may be concluded that the Xenon^{133} method for cardiac output determination gives results which are comparable with those from conventional methods and has the advantages of simple, speedy calculation of results and because it is freely exhaled from the lungs, determinations can be repeated at short intervals.

CARDIAC SURGERY

G. R. Stirling, V. Carson and D. Chipperfield

Interruption of the coronary blood flow is frequently necessary during open heart surgery for valve disorders and it is inevitable during cardiac transplantation. The tolerance of heart muscle to anoxia has been extensively studied but correlation of the functional changes that follow with biochemical and structural parameters has not been extensively studied.

In the present investigations a correlation between cardiac function assessed by Starling curves derived from data obtained from dogs with controlled venous return, and estimates of the high energy phosphate stores, glycogen content, catecholamine and ionic concentrations in muscle samples from the right ventricle is being sought. Associated with this project optimum methods of collection, storage and resuscitation of donated hearts are being explored.

Estimation of High Energy Phosphates in Cardiac Muscle

Inorganic phosphate and creatine phosphate are estimated together in a two-stage colourimetric procedure based on the method of Furchgott and de Gubareff (1956), using a trichloracetic acid extract of heart muscle. Adenosine tri-, di- and monophosphate are estimated on the same extract using a single-step paper chromatographic technique, with isobutyric acid-ammonia-EDTA as solvent. Spots are located with an ultraviolet lamp, cut out and eluted with O.IN hydrochloric acid and their ultraviolet absorption measured. Scrupulous attention to detail in washing and preparation of the paper, and in elution methods were necessary to obtain reproducible results.

The success of both the above procedures as a true measure of the high-energy phosphate content in heart muscle is primarily dependent on the method of taking the muscle biopsy, with the aim of preserving the highly labile creatine phosphate and adenosine tri- and di-phosphates.

After many failures a method was evolved using a plastic funnel attached by a short length of plastic tubing to one end of a simple 6 mm. diameter stainless steel cork-borer, the other end of which is placed on the muscle. A fine dacron thread is sutured to the area to be biopsied and the ends of the thread drawn up through the borer and funnel and held taut, thereby making a seal between the heart muscle and the end of the cork-borer. Liquid nitrogen is then poured down the funnel and as the surface heart muscle freezes, a biopsy is performed using a tonsil snare. The biopsy is immediately immersed in liquid nitrogen and so kept until ready for crushing and extraction.

After weighing quickly on a torsion balance, the tissue is crushed in a 50 ml stainless steel centrifuge tube, precooled in liquid nitrogen, into which a stainless steel moulded plunger is hammered using a number of sharp blows. The pulverised tissue is then extracted into ice-cold 5% trichloracetic acid and finally centrifuged in the cold.

Relationship Between Physiological Condition of the Heart and Phosphate Levels in Cardiac Muscle

The methods described above are being used to investigate the effect which varying physiological conditions of the mammalian heart have on levels of inorganic phosphate, creatine phosphate and the adenosine phosphates. In particular, the effects of partial and total heart bypass with and without complete ischaemia of the heart, under normothermic and hypothermic conditions are being studied, using experimental dogs.

Preliminary work indicates that the creatine phosphate falls rapidly to very low levels in the ischaemic heart compared with normal, but returns to control levels when the heart is resuscitated. Periods of ischaemia up to one hour, followed by thirty minutes of resuscitation have been studied.

The fall in creatine phosphate level is accompanied by a rapid rise in inorganic phosphate during ischaemia, which is only partially reversed during the period of resuscitation of thirty minutes. The adenosine triphosphate level falls more slowly during ischaemia and continues to fall during the period of resuscitation.

CARCINOGENS** [1]

P. E. Hughes

Reactions with Cellular Constituents

Chemical carcinogens may be divided into two classes; those molecules which are actually carcinogenic and those which require metabolic activation to form a proximate carcinogen. The former group are carcinogenic for a wide range of organs in many species while the latter group, which includes the aromatic amines, is dependent on the presence in target cells of enzymes capable of producing metabolic activation and hence these carcinogens are usually highly organ and species specific. It appears that with aromatic amine carcinogens this essential step comprises oxidation of the amine nitrogen to give an N-OH compound. Many of these compounds are highly reactive and react with DNA, RNA and protein. In some cases highly specific proteins are involved in carcinogen binding but this specificity may result from a spatial organization within target cells whereby this specific protein just happens to be located near an organelle capable of producing a highly reactive intermediate compound. The binding of carcinogen to a critical macromolecule may thus be just a minute fraction of the total amount of bound carcinogen within a cell.

[1] This is a brief summary of work carried out at Chester Beatty Institute, London in conjunction with G. P. Warwick and Ann P. Weston.

Binding of DAB to Rat and Hamster Liver and Kidney

It has been shown that when tritiated 4-dimethylaminoazobenzene (DAB) is injected intraperitoneally into rats radioactivity becomes bound to DNA, RNA and protein. Within two weeks practically no residual radioactivity is associated with RNA and protein but DNA isolated from the liver of rats killed six months after injection still shows bound radioactive material. This persistent binding of carcinogenic metabolites to DNA could be of critical importance in chemical carcinogenesis and would explain the generally long latent periods that have been observed. The binding of DAB to rat kidney DNA is at a much lower level. DAB is weakly carcinogenic for hamster liver and persistent binding to DNA, but at a much lower level than in the rat, has been found.

At present it is impossible to say if DNA, RNA or protein binding is of crucial importance in chemical carcinogenesis. Various dietary, hormonal and other factors influence carcinogenesis and it is hoped that a study of binding of carcinogenic metabolites to various macromolecular species under different conditions will ultimately establish a series of correlations between a specific type of binding and tumour production.

Carcinogenesis and DNA Repair

There is much evidence for excision and repair of defective DNA. It has been suggested that carcinogens have a dual action of altering DNA and inhibiting its repair. Aniline mustard binds to rat liver DNA but is rapidly excised and the DNA repaired. Rats were given tritiated DAB and after sufficient interval to allow only binding to DNA to remain were given aniline mustard. The excision of aniline mustard was not found to be accompanied by any parallel excision of tritiated metabolites on DAB.

Enhancement of Aminoazo Dye Carcinogenesis by Partial Hepatectomy

The dye 2-methyl-4-dimethylaminoazobenzene (2-MeDAB) has been extensively tested in the past and reported to be non-carcinogenic. Administration of tritiated 2-Me-DAB to rats gives a similar profile of binding to DNA, RNA and protein to that found on giving tritiated DAB. When DAB is fed to rats a marked proliferative response occurs in the liver at about four weeks after the commencement of dye-feeding. The changes leading to tumour production are reversible if dye-feeding is stopped before, but not after, this proliferative response. This proliferative response does not occur with 2-Me-DAB feeding. It has been found that if a proliferative response is supplied, by partial hepatectomy, at or shortly after the commencement of 2-Me-DAB feeding tumours result. The possible enhancement of other weak carcinogens by initial partial hepatectomy and the possibility of obtaining tumours after a single application of protein carcinogen to proliferating tissue is being investigated.

GASTRO-INTESTINAL DISEASES

THE MEASUREMENT OF GASTRO-INTESTINAL BLEEDING

D. J. B. St. John

Chemical tests currently used for the detection of occult gastro-intestinal bleeding are of limited value because they yield both false-positive and false-negative results. It is, however, important for the management of certain patients that the degree of gastro - intestinal bleeding should be assessed.

Qualitative measurement of gastro-intestinal blood loss can be made by labelling a small fraction of the subject's circulating red corpuscles with radioactive chromium, and then comparing the radioactivity of daily stool specimens with the radioactivity of accurately measured volumes of the patient's blood. Although this method was first described in 1954, its satisfactory application in clinical investigation has become possible only with the recent development of large volume scintillation counters. Such a counter was installed in the Cancer Institute during the year and we were kindly given access to it for this investigation. The specimens are counted between two plastic scintillators and volumes of blood

as low as 0.5 ml can be measured accurately. It is therefore possible to measure quite small amounts of intestinal blood loss.

An initial aim of this project is to assess the usefulness of this technique in clinical investigation. Its main use appears to be in the investigation of patients with iron-deficiency anaemia of uncertain cause. So far, four patients with recurrent iron-deficiency anaemia have been investigated. In each patient, bleeding sufficient to account for the anaemia has been demonstrated. In one of these patients this demonstration led to repeated barium studies which revealed a carcinoma of the colon not seen on earlier examination. The investigation, by confirmation or exclusion of gastro-intestinal bleeding, will define the clinical problem with greater precision in patients with unexplained iron deficiency. It should also be a guide to their further management, for if bleeding is shown to be present, repeated barium studies would be indicated. There is some evidence which suggests that even when these barium studies do not reveal a cause for bleeding, laparatomy in this group of subjects will reveal gastro-intestinal tumours sufficiently often to be justified.

Now that it has been demonstrated that this technique is sufficiently sensitive to be of clinical value, it is planned to measure the degree of gastro-intestinal blood loss in normal subjects and also to investigate the effect of drugs, such as aspirin, which are known to produce sometimes some bleeding in the normal person.

COLONIC FLORA IN SMALL INTESTINE

D. J. B. St. John

Both anaerobic and aerobic bacteria are present in the normal flora of the colon. In diseases in which there is either an anatomical abnormality or abnormal motility of the small intestine, the colonic type of bacteria may proliferate in the small intestine and can cause malabsorption.

In collaboration with the Bacteriology Department of Alfred Hospital bacterial cultures have been made from small bowel contents obtained with a Shiner capsule in patients with various types of small bowel disease. The aim of the investigation is to identify patients with malabsorption due partly or wholly to the abnormal bacterial flora in the small intestine and to allow a more rational use of antibiotics and surgery in the management of these cases.

OESOPHAGEAL MOTILITY

D. A. Coventry

Manometric assessment of oesophageal motility in various clinical disorders involving the oesophagus has been used as a complementary diagnostic test in 12 new patients in the past year. A number of these patients have also been studied by means of cine-radiography. Experience with these techniques is growing and it is planned to continue motility recordings in 1968 with particular reference to patients having evidence of disorders of neuromuscular co-ordination in the oesophagus and patients with hiatus hernia.

The recording apparatus has been altered recently so that each open ended tube is now connected to a constant infusion pump delivering fluid through the tube at the rate of 19 μl/minute. This rate of flow does not alter the basal pressure recording. Incorporation of this low - flow constant infusion into the recording circuit should help in the assessment of disorders involving the gastro-oesophageal sphincter.

PANCREATIC FUNCTION

D. A. Coventry

In the past two years 35 pancreatic function tests have been performed using Boot's Secretin intravenously as a pancreatic stimulant. In 33 of these tests the dose of Secretin used was 2.0 units per kilogram body weight as a single intravenous injection. In the remaining two tests Secretin was given intravenously by constant infusion in graded increasing doses in an attempt to establish the dose level at which maximal pancreatic secretion occurred. In all tests the duodenal and gastric contents were aspirated separately through a Dreiling tube and the duodenal contents analysed.

Although considerable overlap has been found between the results in individual patients there appear to be three main groups of

results where the patients are classed according to other clinical data. These groups are: (1) patients apparently normal; (2) patients with presumed or proven chronic pancreatitis; and (3) patients with proven carcinoma of the pancreas. (Patients with carcinoma involving the head of the pancreas causing biliary obstruction, have not been studied). It is planned to continue pancreatic function tests in 1968 with the following aims:—

1. To establish more precise limits to the response to Boot's Secretin in a group of patients with normal pancreatic function.
2. To establish the dose level at which maximal pancreatic secretion occurs in response to Boot's Secretin.
3. To attempt to improve the test by modification of the technique in a series of animal experiments.
4. To analyse the various clinical features of pancreatic disease in all patients who have had pancreatic function tests performed.

BLOOD COAGULATION

P. Fantl

A DEFECTIVE FIBRINOGEN

The penultimate step in the chain of reactions involved in blood coagulation starts with the enzyme thrombin acting on soluble plasma fibrinogen to form a gel-like fibrin clot. Both the amount of fibrinogen and the quality of the fibrin clot influence haemostasis. Insufficient or defective fibrinogen may produce weak clots adequate for the sealing of an injured blood vessel.

During the pre-operative haematological examination of a patient who was to have his prostate removed, it was observed that the thrombin-fibrinogen reaction of the patient's plasma was abnormal. Further investigations revealed that even when potent thrombin preparations of bovine or human origin were used clotting times with the patient's plasma were considerably longer than with normal controls. However, tests for prothrombin time and recalcification gave near normal results but determinations of fibrinogen gave in all instances values below normal, although calcium ions produced somewhat greater amounts of fibrinogen than did thrombin addition . Slight fibrinolysis was observed in the patient's diluted clotted plasma.

After the patient was transfused with human fibrinogen the total fibrinogen in the circulation was at the expected level and the turnover rate of the infused fibrinogen was normal. Immediately prior to the operation the patient was again transfused with fibrinogen and no excessive bleeding during and after the operation was observed.

As it is possible that the slight fibrinolysis seen before the operation could explain the abnormal thrombin-fibrinogen reaction the patient was re-examined at intervals after the operation. During one period of five weeks he received "Lyndiol", which is an oral contraceptive claimed to stimulate fibrinogen production. The thrombin-fibrinogen reaction was studied both qualitatively and quantitatively and although there were some fluctuations, the thrombin clotting time was always prolonged and the plasma fibrinogen level was always far below normal. There was also neither clinical nor laboratory evidence of any liver dysfunction which might be responsible for a reduced fibrinogen production. The possibility that the abnormality in this patient was congenital could not be excluded as there was no clinical evidence to indicate how long it had existed. Therefore a sister (aged 71) and a brother (aged 68) of the patient were examined and although the plasma of each had a normal thrombin clotting time both had plasma fibrinogen levels lower than normal for this age group. It is therefore possible that hypofibrinogenaemia is a congenital trait in this family.

Other possible explanations of the defective fibrinogen were considered. Although the fibrinogen levels in a specimen of foetal blood was of the same order as that of the patient,

the thrombin-fibrinogen reaction was abnormal, so that the persistence of foetal fibrinogen in the patient is unlikely. During the course of this patient's illness he only once showed any evidence, clinical or laboratory, of a tendency to excessive bleeding. The defective fibrinogen seems therefore to be of no clinical significance.

THE NEW BUILDING

L. J. Bishop[1] and T. E. Lowe

The planning and design of a building to be used solely for biological research is itself a research project of some duration and an account of the principles involved is therefore included in this Scientific Report of the Institute.

1926 - 1965

The Institute, which also incorporates Alfred Hospital Clinical Research Unit laboratories, was housed in a two-storey brick building with timber floors and pitched tile roof. Over the years the building had been added to and altered to provide some facilities for the ever increasing demands of medical research. Patients of the Clinical Research Unit were housed in a single storey "cottage ward", which had also been altered on occasions to provide more up-to-date nursing services, treatment and clinical facilities. Neither building was capable of upwards expansion and the location on Alfred Hospital site rendered horizontal expansion impossible. The Baker Medical Research Institute was bursting at the seams.

In 1964 the Trustees of the Institute investigated the possibility of financing a rebuilding programme, and in turn Stephenson & Turner, Architects, were engaged and a research programme began on the project. The Board of Management of Alfred Hospital agreed to absorb the "cottage ward" patients into a hospital ward, thus vacating and allowing demolition of the single storey building. This in turn would free half the site area available for Baker Institute development.

GENERAL

The techniques of biological and medical research are continually changing and the projects carried out are becoming more exacting. It is therefore impossible to predict what changes will be seen in this field within the lifetime of a new building. Great flexibility in use had therefore to be a first priority in the design adopted. This implied laboratories which could be used for almost any purpose.

However, as it was hoped that the building would be fully air-conditioned, economy in operation indicated that the laboratories in which no recirculation of air would be permitted had to be restricted to the top floor and provided with fume cupboards. Biochemical projects producing or using noxious or offensive odours would be confined to them. Variation in laboratory size made possible by combining modules would also add to the flexibility of the laboratories. The uncertainty of future development made it prudent to have some uncommitted space, which has been provided in the lower ground floor, and to design a structure which will allow future alterations and expansion.

A suitable modular form of planning, including a modular arrangement of service lines for present and possible future use — this applies to sewerage, drainage, steam, hot water, cold water, gas, special gases, electric light and power and telephones — was essential. Special equipment and facilities had to be readily accessible to all research workers.

In addition to laboratories and associated ancillaries, other functions had to be provided and include administrative offices, library, staff amenities, animal holding and operating suites, electrically screened rooms, and rooms equipped to handle radioisotopes, general stores, chemical stores, solvent storage and distillation, cold storage, deep freeze storage, isotope storage, workshops, workshop supplies, space for generating and distribution plant for all piped and wired services, including provision to, at any time, temporarily wire from room to room to allow for remote recording and viewing equipment.

[1] Stephenson and Turner, Architects.

PRELIMINARY INVESTIGATION

Before implementing any moves to design and build the accommodation envisaged for development, it was essential to investigate the potential of the available site. This applied not only to space requirements, but to suitability of the ground for foundations and excavation. In conjunction with the Alfred Hospital rebuilding programme, soil investigation work was carried out and rock was found at a depth of 30′ to 35′ below ground level. Drilling continued to prove the rock which was discovered to be a shelf layer only, and even to a depth of some 200′ there was no suitable substratum for bearing or friction/bearing type foundations. The investigation did, however, reveal that a building of up to five storeys could be erected on the shelf of rock.

The site area available for building was 180′ X 75′ or 13,500 sq. ft. Five storeys would give approximately 67,500 sq. ft. gross floor area or some 40,000 sq. ft. nett usable floor area.

It was agreed that some 25,000 sq. ft. nett area was required to provide space for the Institute's research in the foreseeable future. The decision taken, therefore, was to build three storeys over the site area, thus leaving upwards expansion of two further storeys possible in the future. The structure was to be reinforced concrete with brick panel walls and with an external appearance in conformity with the hospital buildings.

As only half the site area could be immediately available, and in order to maintain continuity of work in the Institute, the rebuilding programme had to be carried out in two stages. The Architects' recommendation that the complete building should be planned to suit ultimate needs and then to investigate and if necessary temporarily adapt Stage 1 to accommodate existing facilities during the construction of Stage 2 was accepted.

A preliminary study of estimated accommodation requirements indicated that the full 75′ width of the site should be used for building. This in turn suggested that a double corridor type plan be considered. Apart from utilising the site to the maximum, this type of plan presented the opportunity to plan laboratories and offices around the entire perimeter of the building and to utilize the central core for special instrument and service rooms, opening on both sides of the building. This type of plan makes maximum use of daylight for regular working areas, whereas facilities not requiring daylight could be located in the central core. These include special instrument rooms, warm rooms, cold rooms, photographic dark rooms, toilets, lift, stairs, ventilating ducts, etc. (see Fig. 1).

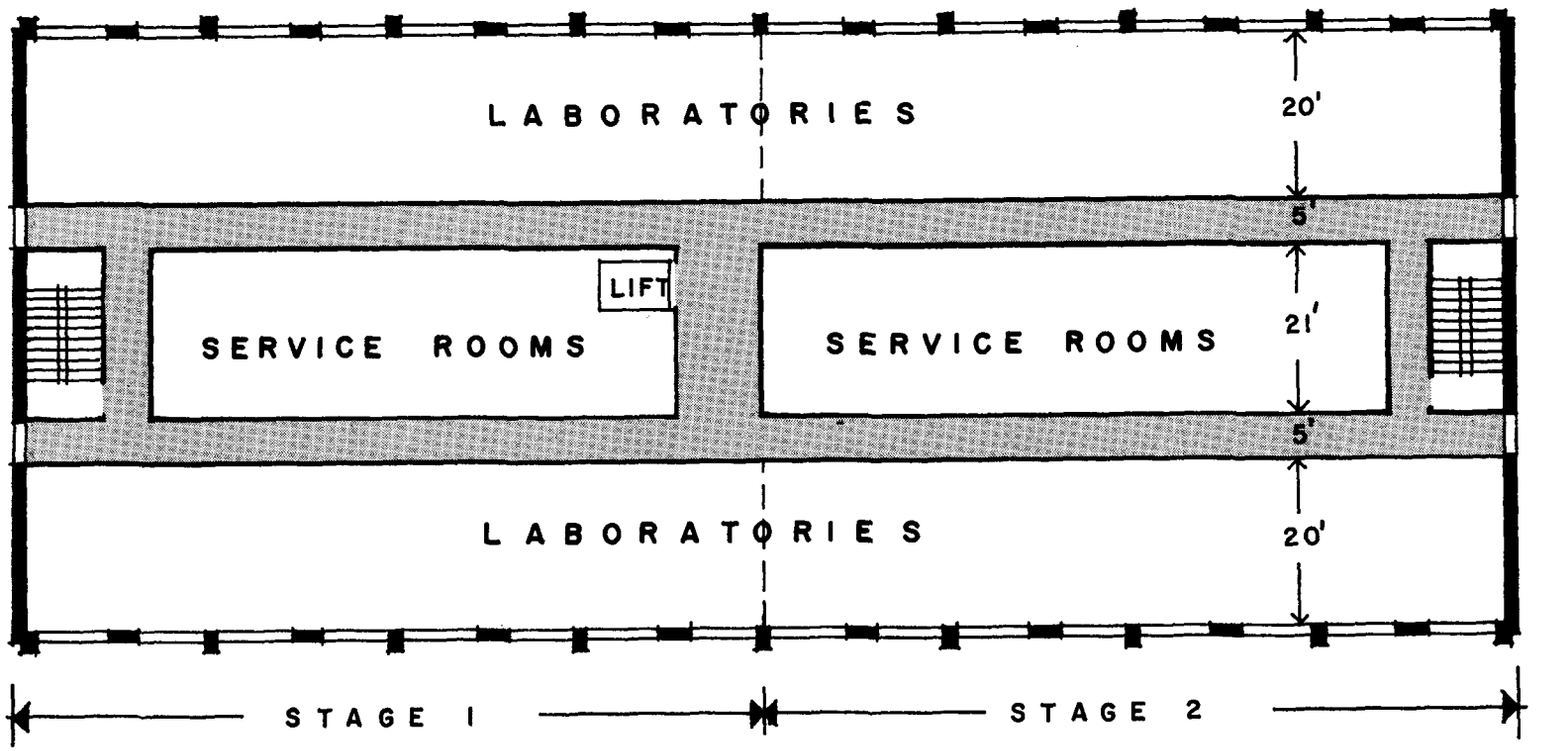
The proposed Alfred Hospital Main Block development provides for stores to be delivered to basement level and as the truck turning area for this would also be available to the Institute the opportunity offered to plan storage areas and loading dock at basement level.

The floor by floor arrangement of accommodation became simple and logical in that stores, heavy equipment, workshops, staff amenities and mechanical plant could be located at basement level with the loading dock on the north side of the building. Administrative offices, director's suite, board room, library, seminar rooms, main lobby, etc., could be located at ground floor level with the main entrance off the existing hospital roadway on the south side. It was also possible to locate the animal operating suite at ground floor level with the necessary separate external access at the eastern end of the building. The plan area available allowed the physiology laboratories, with associated offices and service rooms, to be located on the north-west side away from other functions at this floor level.

Having located all general and administrative functions on the two lower floors, the entire third storey or first floor was available for laboratories, offices for research workers, special instrument rooms and service rooms.

LABORATORIES

The laboratory accommodation — the central purpose of the building—would naturally determine the structural frame and modular pattern of the planning. Having decided on the double corridor type plan, it was then necessary to determine dimensions for the laboratories related to the facilities and fittings required in each.



DOUBLE CORRIDOR PLAN

FIG. 1

Considerable research was carried out prior to deciding these critical issues. The aim was to plan a standard combination of small and large laboratories with small offices opening off each laboratory to provide comfortable and quiet conditions for reading, writing and using the telephone. It was also important to ensure an alternative means of escape from all laboratories in the event of an accident or emergency.

Each small laboratory was required to be fitted with 13′ of 24″ wide working bench space with sink; 6′ of 30″ wide bench for equipment, a 6′ X 24″ deep fume cupboard, 6′ of 30″ washing up bench with sink, a water still, draining rack, scribble board, notice board and coat cupboard. The large laboratories required the same basic fittings with twice as much benching and fume cupboard space.

Many alternative arrangements were planned and the approved plan was set up in mock-up form on the floor of the old main lobby, before the main decision was taken. It was agreed that all laboratories be 20′ deep from the external wall to corridor wall; that small laboratories be 11′ wide and the large laboratories 22′ wide (see Fig. 2).

This then set the pattern of the plan and determined the structural grid at 22′ column bays along the length of the building. Further investigation determined that 5′ wide corridors would be sufficient, therefore the structural grid across the width of the building was set out to suit 20′ deep laboratories and 5′ corridors which, with the width of the building available, allowed 21′ for the central service core.

To permit the desired flexibility of piped and wired services, a vertical service duct was included in each structural bay along the external wall with a further vertical duct adjacent to each fume cupboard along the corridor wall. All vertical service ducts were fitted with removal access panels.

LABORATORY DETAIL

Having determined the standard layout for the laboratories, it was then necessary to decide on detail design of fittings and the materials to be used in their manufacture. Samples of fittings, materials and finishes were tested for resistance to numerous chemicals, resulting in a decision to use ¾″ thick dense asbestos cement sheeting for bench tops with an applied grey coloured epoxy resin finish.

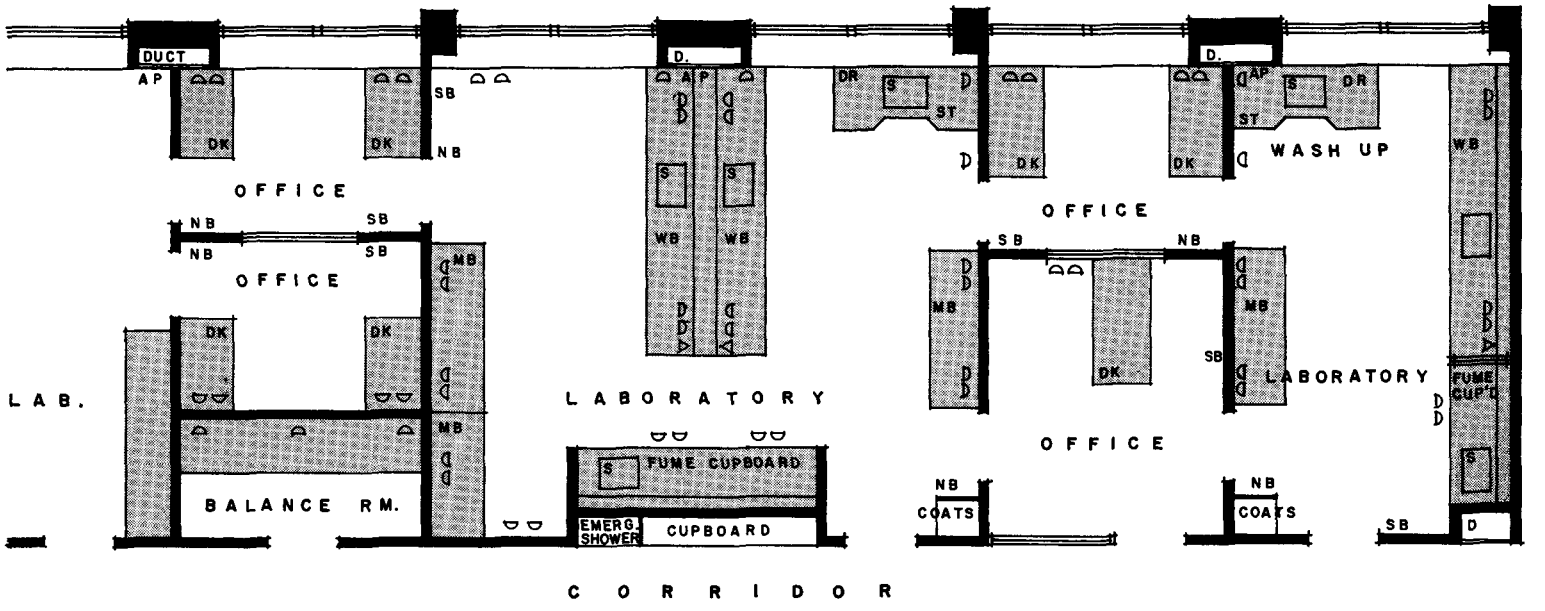
Similarly, after testing various grades of stainless steel, plastic, porcelain, china, etc., it was decided that all sinks be 21″ X 15″ of vitreous china and that the draining board for the wash-up sink in each laboratory be laminated timber with applied clear epoxy resin finish. To provide for the variety of glassware, a special plastic coated peg and grid type draining rack was designed.

Again with flexibility in mind, underbench cupboards were designed as standard interchangeable units of three basic types of modular dimension. These fittings were suspended below the benching and kept off the floor for easy cleaning. At any time a nest of drawers can be replaced by a cupboard or a cupboard and drawer unit, or alternatively a fitting can be placed in store if additional knee space is required.

Fume cupboard design was critical in that safety regulations must be met and exhaust ventilation must be maintained when creating both heavy and light vapours, when the front slide is open or closed, all without upsetting the balance of ventilation in the laboratories. To help maintain desired conditions and allow individual usage, it was decided to install a separate locally - switched exhaust system for each fume cupboard — some 20 systems in all — including the chromatography rooms.

As a safety measure the use of naked gas flames was reduced to a minimum and electric mantle or sealed element heating provided for. Remote control master switches and gas taps (where used) were provided on all benches. In the chromatography rooms a further safety device was the protection of workers from noxious vapours by an air curtain in front of the work bench. Protection from the hazards of static electric charges was provided by the controlled humidity of the building air.

Protection of laboratory benches from mechanical vibration was important, as some equipment requires the use of long exposure



TYPICAL LABORATORY LAYOUT FIG. 2

L E G E N D :

AP	ACCESS PANEL	NB	NOTICE BOARD
D	SERVICE DUCT	SB	SCRIBBLE BOARD
DK	DESK	S	SINK
DR	DRAINING RACK	ST	WATER STILL
MB	MOVABLE EQUIPMENT BENCH, SHELVES OVER	WB	WORK BENCH
			ELECT. OUTLET
			GAS OUTLET

photography. This was achieved by installing all the heavy equipment in the building on vibration-free mountings, including a floating floor for the centrifuge rooms, and in critical areas providing mass concrete stands for recording equipment.

SERVICES

The cost component of electrical, mechanical and general piped services in laboratory buildings is very high, representing one third of the total cost. It was therefore most important to consider carefully the number of and location of electrical, gas and other outlets still bearing in mind the need to allow flexibility for the future. To provide for this a horizontal service duct with removable cover was run along the back of all wall benches with outlets as now required, but with sufficient line capacity in pipes and wiring for future tapping.

In view of the immediate and possible future usage of the laboratories, it was agreed that sinks in every laboratory may be subject to acid disposal which in turn requires waste to be neutralised prior to entry into the main drainage system. Past practice has been to install a neutralising trap immediately adjacent to each sink unit, or group of units; however, after investigation it was decided to install a P.V.C. waste pipe system throughout the building connected to one large neutralising pit outside the building at ground level, thus limiting the required regular maintenance and eliminating inconvenience in the laboratories.

The double corridor type plan demands balanced mechanical ventilation to the internal or central core area. This demand, together with further investigation, proved that by extending the plenum and exhaust systems and warming the filtered supply air a dust free internal atmosphere would result, as well as a cheaper form of heating in winter. Investigations went beyond these basic systems to full air conditioning throughout. It was agreed that for the controlled environment required in much biological work, and for the fine degree of accuracy required from the instruments, a reasonable even level of temperature and humidity should be maintained throughout all seasons of the year. It was therefore decided to design the plenum, or air supply system, for full air conditioning to be installed either initially or in the future. Documentation allowed for alternative tenders and when prices were obtained, the Trustees of the Institute agreed to proceeding with the full air conditioning scheme.

Because the demands on ventilation in different areas of the building varied from continuous to day-time on working days only, from complete airchange to recirculation and from heavy to light heat loads, it was agreed that nine separate air conditioning systems be installed, each supplying separately zoned areas.

The more recent commercial buildings are usually fully air conditioned, and as they have as much glass as possible, double glazing or some means of protection from external heat and cold are fitted in order to minimise temperature transmission into the building and so to reduce running costs of the air conditioning plant.

In laboratories wall space is valuable for benching, equipment, etc., and in view of the need to minimise temperature transmission through external glazing, it was decided that windows be limited to the minimum required by regulations and window sill heights in all laboratories were set at a height of 5′ above the floor. Aluminium louvred eyebrows were fitted at window head level along the north facade to gain sun protection during the summer months.

Provision has also been made for a gangway connection with Alfred Hospital New Ward Block and the Institute will be connected to all parts of the Hospital through a fully automatic pneumatic tube system. This direct link makes easy access to the patients of the Clinical Research Unit and so that they could, if necessary, be brought to the laboratories for special investigations — a full-sized bed/passenger lift has been incorporated in the Institute instead of a goods hoist.

Stage **1** of the rebuilding scheme was completed and occupied in August. The old Institute building has now been demolished and the Stage **2** building is scheduled for occupation in December, 1968. Experience over the past few months indicates that a successful solution of the design problem has been achieved.

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"The Pathogenesis of Liver Failure" — *Alfred Hospital Clinical Society.*	D. A. COVENTRY
"The Use of the Fibrescope" — *Alfred Hospital Clinical Society.*	D. A. COVENTRY
"Blood Coagulation" — (a) *Department of Biochemistry, Melbourne University;* (b) *Department of Physiology, Monash University.*	P. FANTL
"Clinical Significance of Kinekard" — (a) *American College of Cardiology, Washington, D.C.;* (b) *Royal Australasian College of Physicians, Melbourne.*	T. E. LOWE
"Kinekard" — (a) *Northwestern University Medical School, Chicago, U.S.A.;* (b) *Vanderbilt University Medical School, Nashville, U.S.A.;* (c) *Hahneman Medical College, Philadelphia, U.S.A.;* (d) *Tulane University Medical School, New Orleans, U.S.A.;* (e) *Cedars of Lebanon Research Department, Los Angeles, U.S.A.*	T. E. LOWE
"Some Effects of Isoptin on the Cardiovascular System" — *Australian Society of Clinical and Experimental Pharmacologists, Melbourne.*	W. G. NAYLER
"Effect of Isoptin and other Beta Blocking Agents on Myocardial Function and the Peripheral Vascular Bed" — *Cardiac Society of Australia & New Zealand, Victorian Branch, Melbourne.*	W. G. NAYLER
"Innervation of the Vertebrate Heart". *A.N.Z.A.A.S., Melbourne.*	W. G. NAYLER
"Some Factors Involved in the Maintenance and Regulation of Cardiac Contractility" — *International Symposium, "Catecholamines and Cardiovascular Physiology", Canberra.*	W. G. NAYLER
"Some Observations on the Effect of the Hypotensive Drug ST 155, on the Cardiovascular System" — *Australian Physiological Society, Adelaide.*	W. G. NAYLER
"Some Observations on the Pharmacology of the Coronary Circulation" — *Cardiac Society of Australia and New Zealand, Hobart.*	W. G. NAYLER
"Inotropic Mechanisms in Cardiac Muscle" — *Seminar — Institute for Muscle Disease, New York, U.S.A.*	W. G. NAYLER
"Observations on the Effect of ST 155 and KÖ 592 on the Cardiovascular System" — *Boehringer Research Laboratories, Ingelheim, Germany.*	W. G. NAYLER
"Beta-Adrenergic Blockade and the Cardiovascular System" — *Wellcome Research Laboratories, England.*	W. G. NAYLER
"The Effects of ST 155 on the Cardiovascular System" — *Geigy Research Laboratories, New York.*	W. G. NAYLER
"Biochemical Aspects of Prenylamine Action" — *Symposium on "Antiarrhythmic Drugs", University of Naples, Italy.*	W. G. NAYLER
"Hypertension — A New Approach to an Old Problem" — *Victorian Medical Women's Society, Melbourne.*	W. G. NAYLER
"The Effect of β-Adrenergic Blockade on Myocardial Function" — *Cardiac Society of Australia and New Zealand, Victorian Branch, Melbourne.*	I. E. McINNES
"The Regulation of Blood Flow, Particularly in the Peripheral Circulation" — *Faculty of Anaesthetists, Royal Australasian College of Surgeons, Melbourne.*	D. RACE
"Haemodynamic Responses in Normal and Hypertensive Subjects" — *Royal Australasian College of Physicians, Melbourne.*	M. ROSENBAUM
"Circulatory Control" — *Department of Physiology, Monash University, Melbourne.*	M. ROSENBAUM
"Effect of Systemic Resistances on Left Ventricular Output in the Unanaesthetized Dog" — *Australian Society of Medical Research, Melbourne.*	M. ROSENBAUM
"Radiochromium Studies in the Investigation of Occult Gastrointestinal Bleeding" — *Alfred Hospital Clinical Society.*	D. J. B. St. JOHN

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

Revenue Account for the Year ended 31st December, 1967

EXPENDITURE		INCOME		
Salaries and Wages	89,903	Donations —		
Laboratory Supplies	18,826	Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions		80,969
Library Maintenance	6,560	Grants in Aid of Research —		
Isotopes	874	National Heart Foundation of Australia	14,083	
Postage and Telephone	1,093	Life Insurance Medical Research Fund of Australia and New Zealand	15,220	
Printing and Stationery	1,137			29,303
Light and Power	4,989	Interest from Investments —		
Insurance	2,939	Held by the Trustees of the Baker Grant Trust	1,700	
Repairs and Renewals	1,966	Other Income	3,396	
Animal House Contribution	4,000			5,096
Sundries	2,742	Sundry Sales and Recoveries		24,481
Travelling Expenses	4,769			
Data Processing	162			
Deficit for year	(111)			
	$139,849			$139,849

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

Balance Sheet as at 31st December, 1967

FUNDS AND LIABILITIES			ASSETS		
Funds —			Fixed Assets (Note 1)		—
Accumulated Loss brought forward	(195)		Investments, at cost —		
Add, Loss for year	(111)		Commonwealth Inscribed Stock:		
Accumulated Loss	(306)		Development Fund 100,000		
Restricted Funds	14,499		Endowment Fund 21,760		
Endowment Funds	75,751			121,760	
Development Fund (Note 2)	429,635		M.M.B.W. and S.E.C. Stock (market value, $6,805)	6,821	
William Buckland Research Fund	13,200		Treasury Bonds	8,020	
Laura Nyulasy Research Scholarship Fund	2,868		Shares in Listed Company (market value, $32,202)	25,154	
		535,647	Unlisted Investments	100	
Current Liabilities —			Held by Trustees Executors and Agency Co. Ltd	16,068	
Sundry Creditors and Accrued Expenses		1,725	Short-Term Deposits	325,930	
					503,853
			Current Assets —		
			Cash at Banks on Current and Deposit Accounts	33,409	
			Sundry Debtors and Prepayments	110	
					33,519
		$537,372			$537,372

AUDITORS' REPORT TO THE TRUSTEES OF THE THOMAS BAKER, ALICE BAKER AND ELEANOR SHAW MEDICAL RESEARCH INSTITUTE

In our opinion, the above Balance Sheet, to gether 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, 1967.

Melbourne.
10th March, 1968

FLACK & FLACK, Chartered Accountants,
Honorary Auditors.

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, 1967, other than the building, totalled $100,000. The cost of the present building to 31st December, 1967, inclusive of outlay in 1966, totalled $703,880.

(2) At 31st December, 1967, the Institute had a capital commitment, not shown above, in respect of a contract for stages 1 and 2 of its new building $663,000. In future periods this expenditure is to be charged to the Development Fund, which at balance date stood at $429,635.

(3) Retention monies paid to 31st December, 1967, in respect of the contract for a new building, amount to $40,000, 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 contract.

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

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

Year Ended 31st December, 1967

DEVELOPMENT FUND

Balance at 31st December, 1966		668,117
Add—		
Baker Benefactions	189,126	
Victorian Government Grant	100,000	
Interest Received	22,649	
		311,775
		$979,892
Deduct—		
Building Costs	522,959	
Furnishing and Equipment Costs	27,298	
		550,257
Balance at 31st December, 1967		$429,635

ENDOWMENT FUND

Balance at 31st December, 1966		56,279
Add—		
Donations	30,450	
Profit from Sale of Investments	68	
Income from Investments	2353	
		32,871
		89,150
Deduct—		
Transfer to Maintenance	$2353	
Transfer for Purchase of Equipment	11,046	
		13,399
Balance at 31st December, 1967		$75,751

DONATIONS

The following donations to the Institute's funds were received during 1967:—

Sunshine Foundation	$6000.00
Beckworth Court Pastoral Co. Pty. Ltd. $5000, and William Buckland Foundation $3000 (Trustees Executors & Agency Co., Ltd.	8000.00
Appel Family Estate (Trustees Executors & Agency Co. Pty. Ltd.	2275.00
Estate of H. and L. Hecht (Hedderwick Fookes and Alston)	2000.00
Estate of James & Elsie Borrowman Research Fund (Trustees Executors & Agency Co. Pty. Ltd.)	2000.00
Australian Hoechst, Ltd.	1550.00
The William Angliss (Victoria) Trust Charitable Fund	1500.00
Selpharm Laboratories Pty. Ltd.	1250.00
Boehringer Ingelheim, Pty. Ltd.	1162.00
The Truby & Florence Williams Charitable Trust (Trustees Executors and Agency Co. Pty. Ltd.)	1000.00
Marian and E. H. Flack Trust (Trustees Executors & Agency Co. Pty. Ltd.	700.00
Riker Laboratories of Australia Pty. Ltd.	600.00
Estate Alfred Edments (Trustees Execu- & Agency Co. Pty. Ltd.)	600.00
Mr. and Mrs. Frank Crane	500.00
George F. Little Trust (The Equity Trustees Executors & Agency Co. Ltd.)	474.00
Mr. and Mrs. Edgar Rouse	110.00
Pethard Tarax Charitable Trust (Marquand & Co.)	100.00
A. C. Goode & Co.	100.00
Library Grant	100.00
Mr. Siegfried Meyer	100.00
Mr. Herbert Weyl	50.00
A. V. Jennings Industries, Australia, Ltd.	50.00
Mr. R. Blakemore	5.50
Mr. R. M. Wright	5.00
Miss N. E. Cameron	5.00
Mr. S. Hart	5.00
Mr. H. Stevens	2.00
	$30,243.50

Generous contributions towards overseas travel expenses were received from:—

Hoechst-Emelfa SpA, Ciba Ltd., Parke Davis Inc (U.S.A.) and Boehringer Ingelheim, A.G.

DONATIONS, in memory of:—

Baron Snider; E. A. Lucas; Henri Mallard; W. J. Porter; Hugo Fletcher; J. P. Mitchell; M. P. Weetman; Captain J. K. Davis; Sir Mortimore McCarthy; William Crane; Gilbert Weller; Wilhemina Habersberger; Wilfred Broadhead; George Enstein; Jack Ellis; John Vantin; J. C. Armstrong; Mary Louise Falkiner; W. T. Summer; Eric Redwood; Norman Goode; Noble Pennell; Professor W. A. Osborne; Walter A. Grove; W. J. Davis; Reginald St. John; Marjorie Cameron Thorpe; Francis Henry Wright; Charles Burke; Percy Thomas; Arthur Donne; Harold Bruce; Gilbert Rumble; Maude Mary Thompson; Leonard Shepherd.

were received from:—

Mr. Edgar Rouse; Mr. Charles Donne; Eagle Star Insurance Co. Ltd.; Mr. George E. Knox; "The Cronies"; Mr. and Mrs. J. C. Habersberger; Mrs. and Miss Tovell; Mr. R. Blakemore; Mr. and Mrs. B. K. Phelan; Misses D. & J. Jeffrey; Mr. and Mrs. K. Allen; Kodak (Australasia) Ltd.; Miss N. E. Cameron. — Total $206.20.

Grand Total — $30,449.70

Donations in kind were made by:—

Melbourne Wire Works Pty. Ltd. and I.C.I.A.N.Z. Ltd. (Plastics Technical Services Laboratory).

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

1967

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:	P. ZIMMET, M.B., B.S.
Biochemists:	DORA WINIKOFF, M.Sc. JUNE SHEATH, M.Sc. DORIS PAGE, B.Sc.
Technical Staff:	Mr. W. HUDSON Miss I. EKKEL Miss R. WITCHELL Miss J. KINDLER Mrs. F. RABOLD (part-time)
Secretary:	Miss J. SHARP

DIABETIC CLINIC

Clinical Assistants:	MARGARET SANDERS, M.B., B.S. GORDON ENNIS, M.B., B.S., M.R.A.C.P.
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. GORDON ENNIS, M.B., B.S., M.R.A.C.P. J. R. STOCKIGT, M.D., M.R.A.C.P.

RESEARCH FELLOWS

E. H. Flack Medical Research Scholarship:	J. R. STOCKIGT, M.D., M.R.A.C.P.
Burroughs Wellcome Research Fellow:	GORDON ENNIS, 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

During this year, in the course of their investigation and treatment, 235 patients with various endocrine disorders have passed through our acute ward and 393 through the convalescent ward. There have been approximately 800 attendances at the Endocrine Outpatient Clinic and some 1300 patients have paid in excess of 700 visits to Diabetes Clinics. Clinical problems often present themselves — problems of diagnosis and of treatment, which are of direct concern to the patient as they affect his present and future health.

From the biochemical, biological and ecological patterns of disease as we see them in patients presenting, certain problems become posed and hypotheses constructed to explain them. Experiments can then be designed to test these hypotheses.

Systematic review of the large numbers of diagnostic tests, old established and newly developed, undertaken in the laboratory of the Unit, when considered in relation to the clinical history of patients undergoing these studies, define not only the scope and usefulness of such investigations and help clarify their diagnostic role, but also may define unusual and unexpected patterns which add to knowledge regarding the genesis and evolution of disease.

The application of new techniques of investigation to old problems is often helpful in advancing knowledge although sometimes, unfortunately, the new information obtained compounds pre-existing confusion.

In a variety of situations, increasing demand for facilities calls for modification of procedures in order that service requirements can be met. The diagnostic tests undertaken, if modified for this reason, must be so monitored as to be certain that reliability of results can be assured.

The reporting of findings occupies an integral role in research, for in this way the experience of all is shared and the constantly growing reservoir of knowledge can be tapped freely by all.

These various approaches to clinical investigation have particular application to the Diabetic and Metabolic Unit and are described in the report of research activities. In the main, the investigative work undertaken relates closely to the service responsibilities of the department. It is closely integrated, too, with a teaching responsibility in this aspect of Medicine. During the past year Dr. Cheah Jin Seng has spent some five months with us studying methods of radioimmunoassay as part of a postgraduate study programme prior to his return to Singapore to resume duties in the Department of Medicine. We have been able to offer terms of training in clinical medicine to medical students taking elective terms in the Unit. This year Mr. D. Ireland spent six weeks with us during which time, among other duties, he made ophthalmometric measurements on patients with normal thyroid function to serve as a control group for comparison with similar measurements made in patients suffering from various thyroid disorders. Dr. Gordon Ennis, who has worked as Burroughs Wellcome Research Fellow for the past two years studying glycerol metabolism, has been awarded the Cleveland Fellowship and has taken up duties in the Department of Medicine in Western Reserve University, Cleveland, U.S.A.

No report of the research activities of the Diabetic and Metabolic Unit would be considered complete without acknowledgement of the support—material and moral, which is given us by organisations and individuals. It is with a deep sense of the significance of their gifts that this acknowledgement is made. To the members also of the Honorary Medical Staff of the Alfred Hospital and of the Head and Staffs of the departments of Medicine, Surgery and Biochemistry of Monash University, grateful acknowledgement is made of their continued collaboration and assistance.

31st December, 1967. PINCUS TAFT.

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

Alfred Hospital Research Funds.
Boots Pure Drug Co. (Aust.) Pty. Ltd.
Burroughs Wellcome & Co. (Australia) Ltd.
Mrs. M. Clark.
Difrex Laboratories Pty. Ltd.
Dunklings Pty. Ltd.
Eli Lilly & Co., Indianapolis, U.S.A.
Hoechst Pharmaceuticals.
Pfizer Corporation.
Sandoz Ltd.
Syntex Pharmaceuticals Ltd., Berkshire, U.K.
The Upjohn Company, Kalamazoo, U.S.A.
William R. Warner & Co. Pty. Ltd.

THE METABOLISM OF GLYCEROL

Gordon Ennis and Doris Page

Studies in glycerol metabolism have continued during this year.

Three obese female patients were studied. Their metabolic clearance rates of glycerol were determined by constant infusion of H_3 glycerol. 50 μCi glycerol-2-H_3 were infused over one hour, and blood taken at 30, 40, 50 and 60 minutes. During the later stages of the infusion it was shown that a constant plasma level of glycerol-2-H_3 had been achieved. Thus it could be assumed that at this stage a glycerol space had been uniformly labelled with the tracer substance and that the rate of removal of labelled and stable glycerol from the pool was equal to the rate of its introduction and production.

Under these circumstances Metabolic Clearance Rate (MCR) is derived from the formula

$$MCR = \frac{\text{cpm/day infused}}{\text{cpm/litre plasma}}$$

In the three patients the MCR of glycerol were 5800, 5500 and 5600 litres per day under the standard conditions of an overnight fast.

After a week of complete caloric starvation the procedure was repeated in two of the patients and the MCR was found to be unaltered. This was to be expected since the clearance rate is a function of those organs which remove and metabolise glycerol, and not of the state of the adipose tissue.

However, it is known that the liver, the kidneys, and the intestinal wall are the only tissues which contain α-glycerokinase, the enzyme required to activate glycerol to α-glycerophosphate, a process that is necessary for the further metabolism of glycerol, so that it would be expected that the MCR would not exceed the combined blood flow through these organs. We assume, therefore, that the results we have obtained are erroneously high, as it is unlikely that this figure would be more than about 2500 to 3000 litres per day, even in the grossly obese.

We have as yet studied no normal subjects by this method. However, the results we have obtained by continuous infusion of unlabelled glycerol (a method which for other substances gives higher values for MCR than when calculated from radioactive tracer infusions) were in the range of 1500 to 3000 litres per day, which is within the expected range of values.

The possible sources of error in our results using tracer techniques could lie either in falsely high calculation of the rate of infusion of the radioactivity, or a falsely low estimation of the plasma level of H_3 glycerol achieved during the constant infusion. All investigations of these possible sources of error have so far failed to reveal the reason, and they are being continued.

SEPARATION OF PLASMA GLYCEROL BY THIN LAYER CHROMATOGRAPHY

Gordon Ennis

Until recently, the method for measurement of tritiated glycerol in this laboratory has been that of Havel, which involves oxidation of glycerol, and collection and counting of the released formic acid containing the tritium. Because, in certain experiments, we were obtaining values for plasma H_3-glycerol which were possibly lower than expected, it was decided to compare results obtained by chromatographic separation of glycerol-2-H_3 from other plasma substances which would be expected to contain tritium from the metabolism of H_3-glycerol — namely, glucose, the organic acids and water.

METHOD

Plasma samples are deproteinized with trichloracetic acid and the excess trichloracetic acid extracted by washing with ether. Plasma

lipids are removed by shaking the deproteinized extract with chloroform, and the glucose is removed with copper sulphate and calcium hydroxide. The resultant extract is then treated in the manner described by Nikkala and Ofala (Life Sci. 3, 1964, 234).

The extract is dried by lyphilization and redissolved in methanol. The salt free filtrate is then evaporated and applied quantitatively to a silica gel-G thin layer chromatography plate with methanol-water 1:1. The chromatography solvent is ethyl acetate, 58 : isopropylalcohol, 25 : water 17. The glycerol spots are identified by spraying the plate with ammoniacal silver nitrate. The spots are eluted with methanol-water and after evaporation Bray's scintillator is added and the samples counted in a liquid scintillation counter.

RESULTS

This method has been found to give satisfactory separation of glycerol from the organic acids. Recovery of added radioactivity is in the range of 50 - 60% and the precision of the method is ± 5% in individual samples.

This method will now be applied to routine separation of H_3 glycerol during studies of glycerol metabolism.

THYROID FUNCTION TESTS

Dora Winikoff[1]

A considerable time in 1967 has been devoted to reviewing and streamlining our routinely used thyroid function tests for the diagnosis of thyroid disease. The ever increasing demand on our services has forced us to seek more efficient methods and introduce automation.

PROTEIN BOUND IODINE (PBI).

The use of small tubes in the manual ashing technique has increased output fourfold. In addition, one standard curve (based on a blank and three quality control sera in duplicate) is constructed for two batches of patients' samples. The ashing step is carried out at the one time for all samples in the run. At the colorimetry stage (which has to be completed within two hours) the standards are split and one aliquot of each duplicate is used for each batch of samples (60 tests).

TRIIODOTHYRONINE RESIN UPTAKE (RU)

The procedure described last year has been modified to include an automatic sample changer[2] for the count on the supernatant solution. The results are expressed in terms of resin uptake.

This technique has been compared with the commercially available "TBI" (Mallincrodt) and "Triosorb" (Abbotts) kits for T_3 resin uptake.

It was found that although these simpler techniques were suitable for routine use, the results were not as consistent and the normal range much narrower than for our own method. Consequently the overlap, particularly for the hypothyroid patients, was much greater.

The same difficulties were encountered when bulk T_3 I^{125} was substituted for the bulk T_3 I^{131} which we are currently using.

ELECTROPHORETIC INDEX (EI)

This parameter of thyroid function (the method being described in 1965) has proved of inestimable value. With the advent and use of many new hormonal preparations (oestrogens, androgens, corticosteroids and oral contraceptives) the results of PBI and RU tests are often contradictory, deviating in opposite directions from the normal range, but the use of EI will, by defining abnormal thyroxine binding, indicate the fact that hormones are being administered to the patient.

[1] With technical assistance of R. Witchell and J. Kindler.

[2] This instrument was kindly donated by "Syntex Pharmaceuticals Ltd.".

Two individual tests (or one in duplicate) can therefore be run on each strip. Again the automatic and more sensitive counter made the use of narrow strips possible without the need to increase the amount of radioactive thyroxine since this has been shown to alter the thyroxine bound globulin-albumin ratio.

The long lasting isotope T_4 I^{125} (Amersham) was tried and proved of equal value although the normal range is slightly displaced.

THE USE OF "ISOPOR" RESIN FOR THE PBI ASSAY

In recent years the value of PBI estimation has greatly diminished. Among other explanations the use of a large number of iodine containing drugs adversely affect this parameter of thyroid function by adding exogenous iodine to the endogenous thyroxine source. We investigated the "Isopor" anion exchange resin "Permutit De-Acidite FF-IP" which removes iodine from contaminated samples. The difference in PBI level before and after a passage through the resin gives a guide to the level of true hormonal iodine in the patient's blood. The procedure is simple. 2.5 ml. of serum are shaken mechanically for 15-20 min. with 0.5 gm. of resin. The serum is separated and PBI estimation commenced immediately.

Following the intake of iodides the use of "Isopor" was shown to achieve a fall in PBI to a fraction of the original level. Organic iodine compounds are harder to remove, some contaminated sera being unaffected. In normal samples, PBI before and after treatment with resin does not vary more than 10%. We have taken a difference of 20% or more to indicate the presence of contamination by exogenous iodine.

In 100 patients with PBI higher than expected the above outlined procedure contributed to correct diagnosis in over 60% of cases. Moreover, in many instances it avoided the necessity to undertake more complicated and time consuming additional diagnostic tests.

THE THYROID AND THE "PILL"

Dora Winikoff

The work on the effects of oral contraceptives on thyroid function tests which commenced in 1964 is continuing. We have established that

(1) The ovulation inhibitors invariably alter the thyroid parameters from pretreatment values;

(2) The magnitude of deviation can be correlated with the amount and length of administration of the oestrogen component; and

(3) The progestogens, apart from norethynodrel, have no effect and do not synergise the action of oestrogen.

During the current year we have studied the effects on thyroid function tests of long-term administration of cycle-to-cycle variations and of patient-to-patient variations.

It was found that interpatient variations are far greater than cycle-to-cycle fluctuations, which are also considerable. With this experience we feel that we are able to diagnose patients with thyroid disease while on the "pill".

This investigation has also extended to the use of Chlormadinone at 0.5 mg. levels for contraceptive purposes. The results are being correlated with other endocrine effects of this steroid.

Work is also in progress on the establishment of a "free thyroxine" assay in order to study the influence of oral contraceptives on this metabolically important component of the plasma pool of thyroxine.

PLASMA INSULIN LEVELS IN PATIENTS WITH SUSPECTED INSULINOMA

Doris Page

During 1966-67 the fasting plasma insulin concentration and the insulin levels during standard intravenous tolbutamide tolerance tests have been measured in 19 patients in whom the diagnosis of insulinoma was suspected. At subsequent operation, six of these patients were found to have an insulinoma.

The mean fasting insulin level of the 13 patients in whom a negative diagnosis was made was 13 ± 3 μU/ml. with a range of 6-17 μU/ml. This is directly comparable with that found in our series of 50 normal volunteers, where the mean fasting insulin level was 16 ± 7 μU/ml., and the range 0-30 μU/ml. However, 5 of the 6 patients who were subsequently shown to have an insulinoma had fasting insulin levels far in excess of the upper limit of the normal range. The levels were 50, 117, 55, 84 and 250 μU/ml. The fasting insulin concentration in the remaining patient was measured on three occasions, the highest level recorded being only 29 μU/ml.

Plasma insulin levels were determined at intervals during one hour after intravenous tolbutamide. The maximum insulin response occurred within 5 minutes in 18 of the 19 patients. The mean rise in plasma insulin five minutes after tolbutamide in patients with no insulinoma was 45 ± 26 μU/ml. (range 14-107 μU/ml.) and in patients with an insulinoma was 121 ± 57 μU/ml. (range 56-> 250 μU/ml.). The plasma insulin level in two of the patients with insulinoma rose to beyond the upper limit of the assay. The large variation in the insulin response to tolbutamide seen in the normal group makes this a less reliable indication of an insulinoma than the fasting insulin level.

A fasting insulin value in excess of 30 μU/ml. (mean normal + 2 S.D.) and a rise in the plasma insulin level of more than 71 μU/ml. (mean normal + 1 S.D.) five minutes after a standard tolbutamide load appears to be diagnostic of an insulinoma.

However, one patient who had an insulinoma and had fasting insulin levels below 30 μU/ml. had an abnormally elevated response to tolbutamide by the above criteria in only one of the three tolbutamide tests performed. Some low fasting blood sugar levels were measured but no abnormal depression of the blood sugar was noted during any of the tolbutamide tests. Apparently in this particular patient, the tumour produced an insulin or insulin-like substance which differed immunologically to native insulin and did not respond to tolbutamide.

Two patients with an insulinoma in whom grossly elevated fasting plasma insulin levels were noted had normal fasting insulin levels on another occasion. This observation is in agreement with the clinical finding of episodic hypoglycaemia in patients with insulinoma, and it indicates that the estimation of more than one fasting plasma insulin may be necessary to detect an abnormally elevated insulin level in the presence of an insulinoma.

FOETAL INSULIN LEVELS AND THE TRANSPORT OF INSULIN ACROSS THE HUMAN PLACENTA

Doris Page and P. Taft

(in conjunction with P. S. Paterson and E. C. Wood, Monash University Department of Obstetrics and Gynaecology, Queen Victoria Hospital)

Although much larger molecules than insulin, such as some gamma - globulins, are known to cross the human placenta, the results of studies on transplacental insulin transport are conflicting. The use of Saling's ingenious technique for obtaining blood samples from the foetal scalp during labour, and the employment of a sensitive immunoassay for measuring insulin levels has enabled us to study the movement of unlabelled insulin

across the placenta during labour, rather than as in earlier studies, observing the movement of radioactively labelled insulin and making all observations at the time of birth, when acute changes may be taking place.

Response of mother and foetus to maternal insulin injection

During the first stage of labour, 7 normal patients received 0.1 U/kg. insulin intravenously and blood samples were taken from a maternal vein and from the foetal scalp before and after the injection. Both the maternal and foetal glucose levels decreased, but although the mean maternal insulin concentration rose to more than four times the pre-injection level, the rise in foetal insulin was insignificant. Thus exogenous insulin does not cross the placenta during labour.

Response of mother and foetus to maternal glucose load

A further 11 normal patients received 25 gm. glucose intravenously in an experiment similar to that outlined above. A large and similar rise in maternal and foetal glucose levels was noted, demonstrating the free passage of glucose across the placenta. The maternal hyperglycaemia evoked a large insulin response in the mother but no significant rise in the mean foetal insulin level was noted.

Thus neither did the endogenous insulin produced by the mother cross the placenta to the foetus, nor did the foetus show any acute insulinogenic response to foetal hyperglycaemia.

Experiment	Mean Change in Glucose Levels (mg. %)		Mean Change in Insulin Levels (μU/ml.)	
	15 min.	60 min.	15 min.	60 min.
Maternal	+125	+16	+45	+43
25 gm. Glucose I.V.				
Foetal	+120	+20	+3	+3
Maternal	—35		+64	
0.1 Insulin/kg. I.V.				
Foetal	—17		+3	

Concentration of insulin in cord plasma in relation to maternal insulin levels — basal values

We have found no significant difference between the mean cord and maternal insulin levels in both normal patients and diabetic patients on dietary control. However, in diabetic patients receiving insulin therapy, the cord insulin level was much higher than the maternal concentration. The plasma from insulin treated patients often contains insulin antibodies which interact with the immunoassay system, leading to the finding of erroneously high insulin concentrations.

However, this does not invalidate the observation of a large difference between maternal and cord insulin levels. No explanation can be offered at this time for the higher foetal insulin level.

Patients	No.	Mean Maternal Insulin Concentration μU/ml. $\pm$ S.D.	Mean Cord Insulin Concentration μU/ml. $\pm$ S.D.
Normal Controls	7	17 ± 7	14 ± 6
Diabetic Patients on Diet Alone	7	30 ± 8	31 ± 24
Diabetic Patients on Insulin Therapy	9	79 ± 63	170 ± 95

We found no statistical correlation between individual maternal and cord insulin levels in any of the groups, thus supporting the notion that the placenta is impermeable to insulin.

PLASMA IONIZED CALCIUM IN HYPOMAGNESAEMIC STATES

Paul Zimmet, Harald Breidahl and Winifred Nayler[1]

For some years, it has been known that in clinical situations where hypocalcaemia and hypomagnesaemia coexist, that administration of magnesium (orally or parenterally) will correct both of these abnormalities. This is most commonly seen in patients with malabsorption syndromes.

We have studied two patients with hypocalcaemia and hypomagnesaemia — the first patient suffered from intestinal lymphangiectasia, the second from adult coeliac disease. Both patients exhibited tetany, and were shown to have very low plasma ionized calcium levels.

The patients were given intravenous infusions of magnesium lasting two hours, and plasma levels of magnesium, calcium (both total and ionized) and phosphate, were measured pre-infusion, hourly for four hours, and at 6, 8 and 22 hours. Ionized calcium levels were measured by a biological technique using the isolated frog's heart.

During infusion, total calcium levels rose significantly ($>$ 1.5 mEq/L.) — the rise being accounted for wholely by an increase in the ionized calcium fraction.

It would appear that the administered magnesium causes mobilization of ionized calcium from either the exchangeable bone pool, or muscle cells, in hypomagnesaemic individuals.

The true significance of this can only be assessed after similar infusions have been performed in hypocalcaemic, normomagnesaemic individuals, and ionized calcium levels measured. These studies are in progress at present. However, overseas workers have reported no change in total plasma calcium levels during magnesium infusion, so it is unlikely that any change in ionized levels does occur.

POTASSIUM DEPLETION AND CARBOHYDRATE MEABOLISM

J. R. Stockigt

The aim of this programme has been to investigate the effect of potassium depletion on insulin release and carbohydrate metabolism. A mild diabetic state has frequently been observed in association with potassium depletion. It has been proposed that potassium depletion impairs the release of insulin from the pancreas.

An isotope dilution method of measuring exchangeable potassium with K^{42} was established, and this gave a precise index of the total body potassium status. This measure-

[1] Baker Medical Research Institute.

ment was made several times in each subject, and the result correlated with standard tests of insulin release and carbohydrate tolerance, i.e., oral glucose tolerance tests (G.T.T.), intravenous G.T.T., and intravenous tolbutamide test.

After basal studies in normal subjects potassium depletion was induced with oral ion exchange resin, and the subjects retested. Two patients with Conn's syndrome were studied preoperatively while potassium depleted and after correction of potassium depletion by removal of the aldosterone - producing adenoma.

It has been demonstrated that potassium deficits of 300-500 milliequivalents are associated with delayed and diminished plasma insulin response to oral glucose, and with an abnormal glucose tolerance test resembling that found in maturity onset diabetes. This effect is less marked following intravenous glucose. Preliminary data suggest that potassium depletion diminishes the effect of intravenous tolbutamide.

DIABETIC ACIDOSIS

P. Taft, P. Zimmet, J. Sheath, G. Ennis and J. Owen[1]

The main purpose of this work is:—

(1) To define the acid radicles contributing to the acidosis.

(2) To assess degree of osmolar changes before and during treatment, and to define major contributing factors (i.e., sugar, urea) to the increase in osmolality.

(3) To measure base deficit in an attempt to rationalise the basis of alkali replacement in acidotic individuals.

(4) To study the distribution of keto acids before and during treatment.

In thirteen patients presenting in diabetic acidosis, the following levels were measured at 0, 4, 8 and 24 hours.

(1) Blood pH and gases.

(2) Serum electrolytes and urea.

(3) Blood sugar.

(4) Osmolality.

(5) Ketone bodies (acetone, aceto-acetic acid and β-hydroxy butyric acid).

(6) Free fatty acids.

(7) Glycerol.

(8) Lactate and pyruvate.

Several interesting points arise from the work.

(a) The high levels of serum osmolality seen in untreated diabetic acidosis do not appear to bear any direct relationship to blood glucose, urea or serum electrolyte levels but probably are a reflection of the dehydration seen in these patients.

(b) Judging from the base deficit shown in these patients, the amount of alkali replacement necessary (either sodium lactate, or bicarbonate) is probably less than is usually given in clinical practice.

(c) Before treatment, the β-hydroxy butyric acid:aceto acetic acid plus acetone ratio is 1.7, but this ratio is reversed following treatment, being 0.60 at 24 hours.

(d) The estimated acid load (lactate, pyruvate, ketones, etc.) that we have measured, does not seem to account for the total base deficit in these patients. There may be some other acid product (that we are not measuring) that might account for this. A method for measuring citric acid levels has been established and interest will centre on levels of this acid in future patients studied.

[1] Biochemistry Department.

URINARY GONADOTROPHIN EXCRETION IN SECONDARY AMENORRHOEA

Ida Ekkel and Pincus Taft

In the majority of patients suffering secondary amenorrhoea in whom no organic cause can be found, the estimation of urinary "general" gonadotrophins (using the immature mouse uterine assay) indicates a normal excretion of these hormones. A study of 13 such patients with normal urinary gonadotrophins has been made in which the urinary follicle stimulating hormone (FSH) and luteinising hormone (LH) levels have been individually measured along with urinary oestrogen. The patients appeared to fall into two groups in that ovarian activity as judged by oestrogen levels was normal in half and inadequate in the remainder. However, the ratio of FSH to LH was essentially similar in the two groups, being approximately 1 to 1.3. This observation appears to exclude the possibility that an abnormal ratio of pituitary gonadotrophin secretion of the individual hormones is responsible for the existence of amenorrhoea in face of normal "general" gonadotrophin urinary levels.

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LECTURES DELIVERED DURING 1967

"Treatment of Thyroid Disease" — *Melbourne Medical Postgraduate Committee.*	H. D. BREIDAHL
"Medical Uses of Radioisotopes" — *Med. Women's Soc. of Victoria.*	H. D. BREIDAHL
"Diabetes — Cradle to Grave" — *Queen Victoria Hospital Clinical Society.*	H. D. BREIDAHL
"Obesity" — *Queen Victoria Hospital Clinical Society.*	H. D. BREIDAHL
"Modern Therapy of Diabetes" — *College of General Practitioners Victorian Faculty.*	H. D. BREIDAHL
"The Diabetic and Exercise" — *6th Congress of International Diabetes Federation.*	H. D. BREIDAHL
"The Endocrine Profile in Secondary Amenorrhoea" — *Royal College of Obstetricians* and *Gynaecologists' Australian Regional Council Meeting.*	PINCUS TAFT
"The Influence of Human Pituitary and Chorionic Gonadotrophin on the Testis in Oligospermia" — *Endocrine Society of Australia.*	PINCUS TAFT
"The Site and Mode of Action of Insulin in Intermediary Metabolism" — *Royal Australasian College of Surgeons.*	PINCUS TAFT
"The Clinical Uses of Human Gonadotrophins" — *Royal Australasian College of Physicians.*	PINCUS TAFT
"Pitfalls in Estimation of Protein Bound Iodine" — *Association of Hospital Scientists.*	D. WINIKOFF
"Diagnosis of the Thyroid Function in the Laboratory" — *Annual Meeting of the Australian Association of Clinical Biochemists, Victorian Branch.*	D. WINIKOFF
"Oral Contraceptives and Thyroid Function Tests. The Role of Progestogens" — *Annual Meeting of the Endocrine Society of Australia.*	D. WINIKOFF
"Counting Techniques and Laboratory Practice. Electrophoresis — Application to Thyroid Disease" — *Royal Melbourne Institute of Technology.*	D. WINIKOFF
"Intestinal Lymphangiectasia with Hypomagnesaemic Tetany" — *Royal Australasian College of Physicians.*	P. ZIMMET and H. D. BREIDAHL

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

OPTIC ATROPHY

G. W. Crock[1]

During 1967, the Melbourne University Department of Ophthalmology was greatly assisted by a Grant from the Ringland Anderson Fund. The Grant was used to continue our work on ocular angiography.

We have made the first detailed study of vascular changes in the optic nerve of patients suffering from optic atrophy. The results were reported in the Lancet, July 1967.

J. M. Parel, of the Melbourne University Department of Ophthalmology, designed and built an automatic retinal fundus camera with the assistance of Australian Optical Manufacturers. This apparatus is based on the Zeiss fundus camera of Dr. Hans Littman, the Chief Designer for Carl Zeiss, Oberkochen, who has personally undertaken the construction of certain lenses in Parel's design.

Prior to fluorescein angiography, only subjective data on optic atrophy could be recorded. With the automatic fundus camera, objective measurements of the vascular changes at the disc are possible. A much greater understanding of the pattern of vessel changes involved in optic atrophy can now be obtained from the improved data recording facilities.

In 1968 the programme will continue with the development of automatic intravascular injection equipment, and electronic monitoring of the fluorescein passage through the retina and optic nerve.

It is intended to apply this new equipment to a clinical study of vascular changes at the optic nerve in several blinding diseases, particularly in chronic simple glaucoma.

THE RESULTS OF PLASTIC REPAIR OF THE MITRAL VALVE

Aubrey Pitt, Rena Zimmet and S. T. Anderson[2]

This study aims to evaluate results of repair of the mitral valve in patients suffering from mitral incompetence.

Preliminary results indicate that marked improvement may result haemodynamically although only a partial relief of mitral incompetence is achieved.

STUDIES IN LEFT VENTRICULAR FUNCTION

Aubrey Pitt and S. T. Anderson[2]

Left ventricular function is altered by graded doses of Angiotensin which increase left ventricular afterload. Curves are constructed relating left ventricular stroke work to left ventricular end - diastolic pressure.

Ten patients have been studied and preliminary results indicate that β-sympathetic blockade using Inderal or Trasicor depresses the abnormal left ventricle. In patients with normal left ventricular function there is little change following β-sympathetic blockade.

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[1] Department of Ophthalmology, Melbourne University.

[2] Cardiovascular Diagnostic Service.

THE RELATIONSHIP OF POTASSIUM IN THE GENESIS OF VENTRICULAR ARRHYTHMIAS FOLLOWING MAJOR CARDIAC SURGERY

S. T. Anderson and Anne Shanahan[1]

One hundred patients are being studied to assess the effects of potassium added to the pump prime and intravenous fluids during cardio-pulmonary bypass surgery.

Before potassium was added 42% of fifty patients had episodes of ventricular ectopic beats, 4% had ventricular tachycardia and 16% had ventricular fibrillation.

After the potassium supplements were used only 16% of fifty patients had evidence of ventricular ectopic beats and there was a 2% incidence of ventricular tachycardia.

THE USE OF XYLOCAINE IN THE TREATMENT OF VENTRICULAR ARRHYTHMIAS

S. T. Anderson and Aubrey Pitt[1]

To date twenty-five patients have received intravenous Xylocaine for the treatment of ventricular arrhythmias, including ventricular extra-systoles and ventricular tachycardia. Xylocaine has proved successful in 80% of these patients. No cardiac effects have been noted. With large doses of Xylocaine, drowsiness may result and in two patients muscular twitching was noted. These side effects are readily reversible if the dosage of the agent is reduced.

A survey is currently being undertaken to assess the usefulness of Xylocaine in preventing arrhythmias in patients admitted to the Coronary Care Unit with acute myocardial infarction.

TREATMENT OF ANGINA PECTORIS WITH TRASICOR

Aubrey Pitt[1]

Trasicor is a new β-sympathetic blocking agent. Its usefulness in the treatment of angina pectoris in a group of Out-patients is being assessed using a double blind control study.

STUDIES IN CLINICAL CHEMISTRY

J. A. Owen[2]

BIOMETRICAL STUDIES

The aim of this project, which is continuing, is to obtain information on the frequency distribution of biochemical changes in disease, which will aid the interpretation of biochemical findings in individual patients. Frequency distribution of the results of various tests in healthy persons and in patients have been prepared using computer techniques and the fit of the data as normal, log-normal and three parameter log-normal distribution examined. Data so far obtained have already led to a modification of the "normal range" of some tests.

A study (carried out in conjunction with Mr. R. Bywater) involving the results of 1000 oral glucose tolerance tests, has provided data which could lead to a re-evaluation of the significance of a particular result. We have failed to obtain evidence of a bimodal distribution of glucose tolerance which has been claimed to exist in the population at large.

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[1] Cardiovascular Diagnostic Service.
[2] Biochemistry Department.

In these studies, and in the studies on laboratory error, we are greatly indebted to Dr. C. Bellamy, of the Computer Centre, Monash University, and to his staff for help on numerous occasions.

STUDIES ON LABORATORY ERROR

This is a continuation of early studies and is being carried out in conjunction with Dr. D. G. Campbell, Royal Women's Hospital, and various staff members of the Biochemistry Department of the Alfred Hospital and the Royal Women's Hospital.

The aim of the project is an improvement in the accuracy of biochemical tests. Studies have comprised defining and measuring the components of laboratory error. Random and systematic error has been examined by preparing large pools of plasma and analyzing portions daily, or more frequently. Most of the work to date has concerned measurement of plasma electrolytes. In these tests systematic error between batches accounts for about 50% of the error.

Other studies are concerned with errors arising out of the use of Auto-Analysers and with the incidence and causation of gross blunders. An investigation into the efficiency of quality control based on patients' results has shown that this is not as effective as conventional quality control achieved by including known material in each analytical batch.

BIOCHEMICAL STUDIES ON THE FUNCTION OF ISOLATED PIG LIVER

These studies are being carried out jointly with members of the Monash University Department of Surgery (M. C. Douglas, P. Jablonski, J. McK. Watts), and are a continuation of previous studies. Since the last report the role of conjugation of bromsulphthalein and its excretion by the liver, and to a lesser extent, the kidney, has been extensively studied. Use of ^{75}Se-methionine has shown that there is fairly rapid incorporation of the amino-acid into plasma protein. This ability of the liver could form the basis of a liver function test for use in everyday clinical work.

DERMAL NECROSIS TEST FOR DETECTION OF BACTERIAL ENDOTOXINS

F. W. Gurr[1] and R. C. Atkins[2]

Local clinical experience in the diagnosis and management of patients suffering from severe bacterial infection has emphasized the need for a reliable and sensitive laboratory test capable of detecting the presence of circulating bacterial toxins. Although many methods for assay of bacterial toxins have been reported all depend on a biological response and are thus beset by inherent problems of time lag, methodology and standardization.

However, Thomas in 1956 described a dermal necrosis response to bacterial endotoxins which has been shown to possess advantages over other tests in terms of sensitivity and reproducibility. This test is performed in rabbits by simultaneous intravenous injection of endotoxin and intradermal injection of adrenalin. A positive response is marked by the development of an area of haemorrhagic dermal necrosis at the site of intradermal injection. The response appears within 4 hours and is fully developed by 24 hours. Characteristics of this test which favour its clinical application include ease of performance, all or none positive response and a relatively short latent interval.

A review of reports describing the rabbit dermal necrosis response to bacterial endotoxin administration suggested that laboratory characterization was incomplete and revealed that results of attempted clinical evaluation were inconclusive. In particular, although negative results have been recorded in patients with proven bacteraemia, no attempts to increase the sensitivity of the test have been described.

[1] Director, Dialysis Unit.
[2] Research Assistant.

A research study was therefore begun to evaluate the dermal necrosis test in the laboratory, study of its clinical application and increase its sensitivity. All investigations were matched by appropriate control studies and measures were adopted to avoid contamination of samples or apparatus by bacteria, bacterial toxins and detergents. Initial studies on selected rabbits using commercially prepared endotoxin and adrenalin tartrate were performed with particular care in relation to details of technique and definition of criteria determining recognition of a positive response.

Positive Dermal Necrosis Response

The characteristic skin lesion was observed following intravenous injection of bacterial endotoxin and was reproducible at certain dose levels. Descriptive criteria for recognition of the lesion were developed in detail.

False Positive Responses

Skin lesions developing at the site of the intra-dermal adrenalin injection in control animals were studied as a source of error in interpretation of results. It was found that in certain rabbits an atypical reaction to intradermal adrenalin injection alone resulted in skin necrosis, but that the appearance of such lesions permitted differentiation from the dermal necrosis response to endotoxin administration.

Local Variation in Dermal Reactivity

Simultaneous intradermal adrenalin injection at varying sites in rabbits receiving intravenous endotoxin showed a complete lack of dermal reactivity in relation to this test when the ear was chosen as the site of intradermal adrenalin injection. This local absence of dermal reactivity occurred despite the simultaneous production of well developed dermal necrosis on abdominal wall sites of adrenalin injection.

Sensitivity of Standard Dermal Necrosis Test

Performance of the test using endotoxin from four species of gram negative organisms demonstrated that, while dermal necrosis occasionally followed administration of 1mcgm of endotoxin, consistently positive responses did not occur until endotoxin dosage was elevated to 100 mcgm.

Variation in adrenalin dosage did not appear to influence sensitivity of the test.

Histological Studies

Examination of histological sections from areas of dermal necrosis following endotoxin injection showed predominant eosinophil infiltration. This finding, which was confirmed by specific staining, stands in contrast to previous reports describing predominant polymorphonuclear leucocyte infiltration. Preliminary immunofluorescent antibody studies failed to demonstrate endotoxin localized at the site of dermal necrosis.

Thorotrast Augmented Dermal Necrosis Test

Failure to produce dermal necrosis in rabbits following injection of plasma from patients with symptomatic bacteraemia has been recorded in several studies. The possibility is thus suggested that the standard dermal necrosis test as described by Thomas cannot detect levels of circulating endotoxin at lower concentrations in the clinically significant range. Although preliminary inactivation of the reticuloendothelial system by thorotrast injection has been shown to greatly enhance many biological properties of endotoxins, application of this effect has not been recorded in relation to the dermal necrosis response.

Experiments demonstrated that the preliminary administration of thorotrast increased the sensitivity of the dermal necrosis test by a factor of 100 to yield consistently positive responses to endotoxin at dosages of only

1 mcgm. Histological examination of the lesions produced showed an appearance identical to that of dermal necrosis following the unmodified test. Further experiments were performed to determine optimal dose and time factors for Thorotrast administration.

CLINICAL EVALUATION

Simultaneous evaluation of the standard dermal necrosis test and the thorotrast augmented dermal necrosis test was performed using plasma from septic patients and healthy controls. Results support the belief that this investigation will prove of value in the definitive and early diagnosis of systemic toxaemia following bacterial infection. A higher incidence of positive responses in relation to the thorotrast augmented test suggests that the increased sensitivity demonstrated in the laboratory is maintained under clinical circumstances.

SUMMARY

The necessity for a reliable test to detect circulating bacterial toxins prompted the laboratory and clinical evaluation of the rabbit dermal necrosis test described by Thomas. Details of technique and characteristics of the lesions have been studied to permit standardization of the procedure and reduce errors in interpretation of results.

Augmentation of the test by a factor of 100 has been achieved by preliminary thorotrast administration and clinical application of the dermal necrosis test has yielded results supporting its value in the early and definitive diagnosis of bacterial toxaemia.

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"Clinical Studies on the Effect of a β-Blocking Agent on Left Ventricular Function" — *Cardiac Society of Australia and New Zealand.* — Aubrey PITT

"Coronary Arteriographic Findings in Young Adults" — *Cardiac Society of Australia and New Zealand.* — Aubrey PITT

"Comparison of Left and Right Myocardial Blood Flow Determined by Xenon^{133}" — *Australian Society for Medical Research.* — Aubrey PITT

"Patho-physiology of Heart Failure" — *Melbourne Medical Postgraduate Committee.* — Aubrey PITT

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