Research

BAKER INSTITUTE

ALFRED HOSPITAL

1969

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

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

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

FORTY-THIRD ANNUAL REPORT

of

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

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

THIRTEENTH ANNUAL RESEARCH REPORT

of

THE EWEN DOWNIE METABOLIC UNIT
(Previously Alfred Hospital Diabetic and Metabolic Unit)

REPORTS

of

ALFRED HOSPITAL RESEARCH FELLOWS

1969

ALFRED HOSPITAL, PRAHRAN, VICTORIA, 3181, AUSTRALIA

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INTRODUCTION

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

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

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

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

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

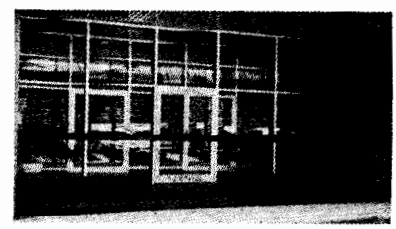
Because of the increasing importance and diversity of the investigational activities conducted in Alfred Hospital, it has been decided to present this report in several sections, indicating the activities of the Baker Institute (including the Clinical Research Unit), the Ewen Downie Metabolic Unit, and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

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

"It is now generally accepted that research into human disease must be conducted predominantly in close relationship with patients undergoing investigation and treatment. Such research is conducted on two levels. The first is concerned with the basic medical sciences (e.g., at Baker Medical Research Institute), and the second is associated with a study of disease as encountered in the sick person, i.e., clinical research. The organisation of Australian Hospitals, which is peculiar to this country, necessitates that the development of the research function of the Hospital be mainly conducted in separate specially equipped units. In addition, many members of the Honorary Medical Staff devote their valuable time to research in their various specialities and the organised research facilities of our Hospital, namely Baker Institute and Clinical Research Unit, are at all times available to them in this work. Such an arrangement is in conformity with our objects — treatment of the sick, training of doctors and nurses, and provision of facilities for reasearch."

The Trustees of the Institute and the Research Advisory Committee are fully aware of the necessity of relating fundamental research to clinical problems, and have pleasure in presenting detailed reports of the research activities during the past year illustrating this concept.

BAKER MEDICAL RESEARCH INSTITUTE



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

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"James and Elsie Borrowman":	Mrs. H. A. JONAS, M.Sc.
"William Buckland":	P. FANTL, D.Sc., F.R.A.C.I.
"The Lang Fellow":	L. W. WHEELDON, Ph.D.

SCHOLARSHIPS

National Heart Foundation Vacation Scholarship:	K. Y. GAN.

Entrance Hall with portrait of Thomas Baker

ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

Nineteen hundred and sixty-nine has been an eventful year for the Institute.

On 15th November Mr. Edgar Rouse, C.B.E,. completed twenty-five years as chairman of the Trustees of the Institute. This is a role which he has filled with distinction, generosity, understanding and kindliness over a period which has included a world war, the rejuvenation and great growth of the Institute. All members of our Staff wish him well.

It is, however, with sorrow that we record the deaths of two former Trustees. Mr. Balcombe Quick, D.S.O., M.B.B.S., F.R.C.S., F.R.A.C.S., who died on September 5, was a Trustee from June, 1940, to June, 1965, and was also Deputy-chairman. Dr. F. Grantley Morgan, C.B.E., M.B.B.S., F.R.A.C.P., M.C.P.A., who died on December 25, was a Trustee from April, 1945, to April, 1965. All those at the Institute who knew these men considered them friends of long standing and we record our appreciation of their devoted guidance.

Early in the year the second stage of the new building for the Institute became available and has been successfully occupied. The pleasure of using this building is a constant reminder of the generosity of the Baker Benefaction in making its construction financially possible. We now have some space both for future expansion and to provide facilities for research workers who may wish to visit us for limited periods. Two visitors from the U.S.A. have worked here this year.

Unfortunately there is now physical separation between the laboratory and clinical facilities of our research group and this must remain until the rebuilt ward block of the Hospital is completed in a few years' time and the planned bridge connection established.

FINANCE

During the year efforts to provide financial stability for the Institute have continued and must continue in the future because the requirements needed to maintain the work of the Institute increase year by year. The increase arises from depreciation of money value, greater sophistication of equipment and the growing volume of research work possible in the new building.

At the same time that these factors act, the money available for medical research from foundations and governmental sources appears to be growing more slowly than the numbers of people with facilities and ability to use such support. Competition for the available funds is consequently becoming more intense and the available money is being more thinly spread.

This situation emphasizes the importance of a substantial endowment of the Institute to buffer rising costs and the fluctuating availability of money for research. So far our generous friends have increased the endowment of the Institute by some $250,000 over the past two years.

The provision of finance for medical research is largely a matter of good-will of donors, whether they be the public-at-large or a more restricted circle of friends. Potential donors must be informed about the objects, progress and needs of research or the desire to help will not be kindled. Until recently the annual report has served this purpose; but, now that it is necessary to widen the basis of support for the Institute, the mass communication media have been kept informed of our activities and we are grateful for their help in publicizing our needs and our progress. The activities of a ladies' committee — The Baker Birds — in addition to their very successful fund-raising, are helping greatly by increasing the public awareness of the Baker Institute.

Details of the generosity of friends is given in a separate report and augurs well for the future, and is an encouragement to those organising this drive for financial stability. Special mention should be made of the generosity of Messrs. D. and E. Baillieu and the Trustees of the William Buckland Foundation. The increased finance made available by the Board of Management of the Hospital on account of the Clinical Research Unit is a valued contribution to this stability.

ASSESSMENT OF RESEARCH

Particularly at a time when financial support is limited priorities must be established between projects and subjective judgements of merit of both projects and workers must be made. The bases on which such judgements should be made are, however, not clear. Although great discoveries often arise from the genius of one man, that man does not work in a vacuum, but in the presence of current knowledge, and because great discoveries are rare it is probably essential to judge projects and workers by their contribution to this background of current knowledge.

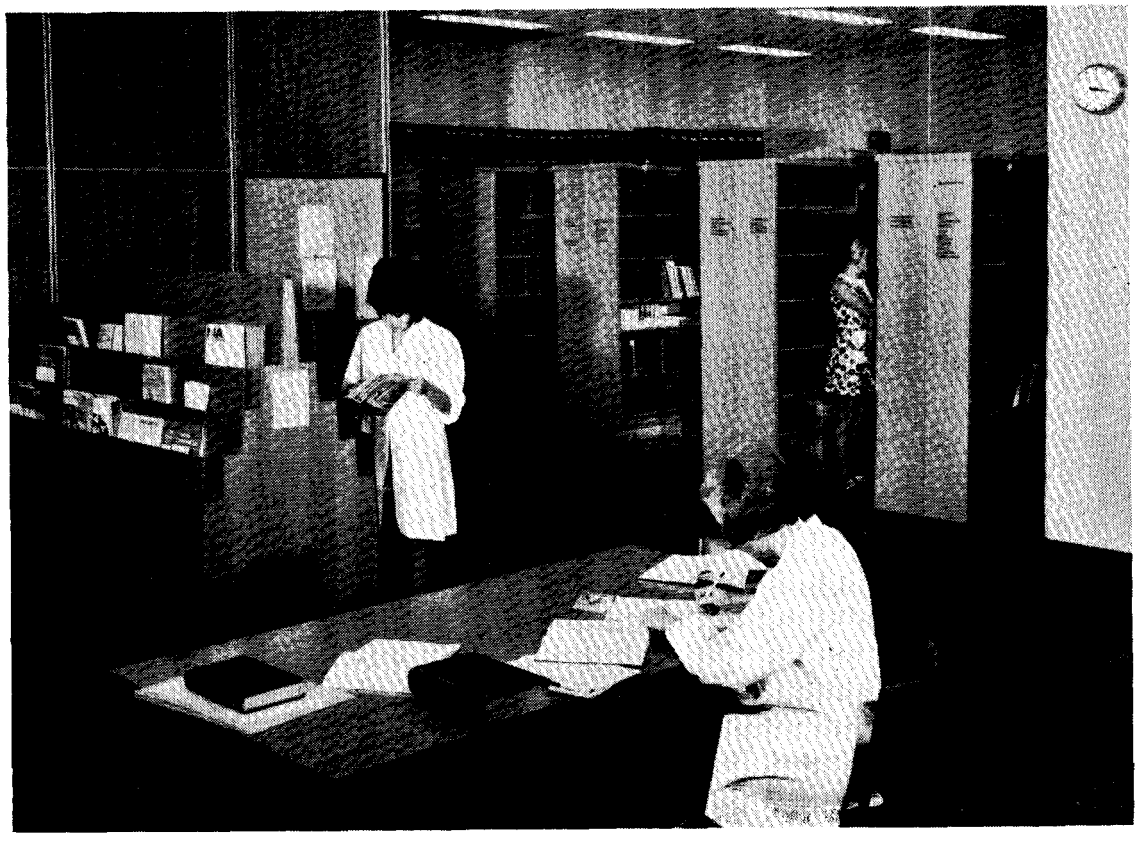
The implied interaction between a research worker, his project and the general community of such workers takes place through papers published in scientific journals, ideas written in monographs, lectures given to groups of colleagues and learned societies and by personal contact with others at scientific gatherings or on visits to other centres. A partial measure of the impact of such interaction is provided by invitations to a worker to contribute reviews, to speak at gatherings and in reference by other authors to his specific work. Imperfect though these criteria are, they give an assessor some guidance, and for these reasons this annual report is set out in a form which provides such information about our workers and their projects.

RESEARCH PROJECTS

For more than 20 years the main emphasis of research in the Institute and Clinical Research Unit has been on problems concerning the cardiovascular system in health and disease, in man and in animals. The evolution of the projects over these two decades shows dramatically the impact on medical research of advances in other branches of science. These advances are reflected in the concepts relating to the cardiovascular system and in the techniques used to investigate it.

The mammalian cardiovascular system consists of a four chambered heart, connected to a complex system of tubes — arteries and veins — through which blood is continuously pumped. The heart is the pump for this system and generates the necessary power from the muscle which forms the bulk of its structure and its valves control the direction of blood flow. An important feature of the vessels of this system is that the resilience of their walls may be changed greatly from place to place and from time to time in response to substances circulating in the contained blood or in response to activity of the nerves supplying them. The amount of fluid which any segment of the circulation can contain at a given pressure therefore can vary with the state of the vessel wall. Co-ordination of these multiple variables is important to the working of this system and is achieved by a control system which involves both the nervous system and circulating hormones and similar substances.

In a system of this type a number of features are important: the pressure of the fluid, the rate of blood flow, the total volume of blood and the freedom from leaks, of the whole and parts must be kept within well defined limits for health. Departures from these limits are usually associated with disease. Excessively high or low blood pressures, too much or too little blood, leaking or blocked vessels are well known disease entities.



The Rouse Library

Our research projects may be broadly grouped on this basis into problems concerning the heart, the blood vessels and the blood contained in them. We have sought to fill gaps in the knowledge of how the various components normally work and how they are affected by disease, and how such effects may be reversed or controlled.

In 1949 a study of congestive cardiac failure was commenced with special reference to the reason for excess fluid in the body. The main investigational tools used were clinical observation, weighing the patient, recording fluid intake and output and simple chemical analyses of body fluids. Over a period of several years this study led to an understanding of the control mechanisms involved and then to a study of diuretic drugs with which to control the fluid accumulation.

Also in 1949 a start was made in applying the then new techniques for examining the cardiovascular system of patients. The recording of pressures at various points in the system by passing fine tubes (catheters) into veins. The measurement of blood flow in the limbs by plethysmography (which involves recording changes in volume of the limbs), the various forms of electrocardiography and heart sound

recording were all investigated, and the most useful techniques were taken over by the Cardiovascular Diagnostic Service of the Hospital in 1952. At this period the research projects did not probe deeply into the minute structure of the components of the system such as the cardiac muscle cell, nor did they probe deeply into the biochemical reactions taking part in the cell nucleus and other intracellular bodies.

Developments in other scientific fields have made available today an array of instruments for these investigations which, in turn, have made feasible the study of the structure of these intracellular structures, of the biochemical reactions which take place there and so to define the action of various drugs and the disorders of disease. It is now possible with electronmicroscopy to obtain magnifications in excess of 200,000 times. It is possible with spectrophotometers to measure chemicals at a microlevel, or with the aid of radioactive tracers to determine even smaller quantities. With an ultracentrifuge small amounts of tissues can be subjected to forces several hundred-thousand times that of gravity and in an analytical ultracentrifuge to photograph their behaviour at the same time.

In 1969 we find, therefore, that the cardiovascular research projects differ in some respects from those of 1949. Today the study of blood vessels is related to the problems of surgical relief of blockage of peripheral vessels, and the treatment of high blood pressure. Heart muscle is being studied at the cellular level to determine all the roles that calcium plays in muscle contraction, the mode of action of cardioactive drugs, the effects of deprivation of blood supply to these muscle cells — a problem of both cardiac infarction and heart transplantation. The total circulation is being studied to more fully understand the control mechanisms which enable this system to work adequately when recumbent, erect, inverted or in a weightless state.

Detailed accounts of the current research projects are given in the scientific section of the report. Although these are reported under the names of the workers directly concerned, each project comes under the supervision of the director and/or the associate directors of the Institute, so that together the various lines of research form a co-ordinated endeavour. They involve the disciplines of histology, biochemistry, physiology, pharmacology and clinical medicine and surgery.

STAFF

A few changes in graduate staff have occurred during the year. Dr. D. J. B. St.John left to take up a university post in clinical medicine and Dr. J. A. Streeton replaces him as assistant physician.

Dr. P. E. Hughes ceased his tenure of the A. A. Thomas Fellowship on December 31 and his assistant, Miss R. Pilczyk, will continue the project at the Melbourne University.

Mr. I. Macdonald left to take up a teaching post.

Dr. T. W. Moir, a Visiting Fellow of last year from Case-Western Reserve University, Cleveland, Ohio, returned for a short period in 1969 to carry out some further studies on the coronary circulation.

Dr. C. V. Nelson, Visiting Fellow from Portland, Maine, commenced some studies on spatial magnitude electrocardiography towards the end of the year.

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

The Lang Fellow:
L. W. Wheeldon, Ph.D.

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

James and Elsie Borrowman Fellow:
Mrs. H. A. Jonas, M.Sc.

RESEARCH ASSISTANCE

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

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

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

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

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

T. E. LOWE.

December 31, 1969.

ALFRED HOSPITAL RESEARCH FELLOWS IN THE INSTITUTE

1948 - 1969

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

OVERSEAS FELLOWS

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

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

Australian Medical Association.
Adelaide Children's Hospital.
Anti-Cancer Council of Victoria.
A.N.Z.A.A.S.
Austin Hospital.
College of Physicians and Surgeons, New York.
Commonwealth Department of Health.
Commonwealth X-Ray and Radium Laboratory.
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 and Eliza Hall Institute, Melbourne.
Wellington Medical Research Foundation.
World Health Organisation.

REPORT OF SCIENTIFIC INVESTIGATIONS

PHYSIOLOGY AND PHARMACOLOGY OF THE CARDIOVASCULAR SYSTEM

MYOCARDIAL CONTRACTILITY†‡

W. G. Nayler, P. Daile, D. Chipperfield, J. M. Price and T. E. Lowe

Calcium Ions

Current concepts of excitation-contraction coupling in cardiac muscle assign a central role to calcium. It seems probable that electrical depolarization of cardiac cell membranes initiates an inward flux of calcium from the extracellular phase across the cell membrane into the intracellular phase, and hence into the vicinity of the contractile proteins and the sarcoplasmic reticulum. In addition depolarization apparently effects a release of stored calcium from the sarcoplasmic reticulum thereby promoting a sudden and marked increase in the concentration of Ca^{++} in the immediate vicinity of the contractile proteins and hence a highly significant increase in the calcium which is available for interaction with the proteins concerned with contraction-relaxation. Recent investigations indicate that these proteins include, in addition to the already well documented actin-myosin complex, a complex of two other proteins — troponin and tropomyosin. Although the interaction between actin and myosin which results in contraction requires Ca^{++}, relaxation is maintained and contraction inhibited by the interaction of the troponin-tropomyosin complex with actin and myosin. This interaction of the actin and myosin complex with the troponin-tropomyosin complex is inhibited by Ca^{++}. Ca^{++}-released into the sarcoplasm in consequence of excitation apparently bind to the troponin complex and in so doing suppress its inhibitory effect on the actin-myosin interaction.

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

During this year troponin has been prepared from cardiac muscle and the properties of this preparation closely resemble those described in the literature for skeletal muscle troponin. Its isolation was a necessary prelude to determining whether its Ca^{++} complexing activity is modified by any of the cardioactive drugs.

Previously we have postulated that the positive inotropic effect of certain cardioactive drugs, including the cardiac glycosides and the xanthine-derivatives, reflected their ability to alter the intracellular concentration of ionized calcium in the immediate vicinity of the contractile proteins. This hypothesis has now been further tested by studying the action of drugs which depress cardiac contractility such as the insecticide, ryanodine and certain anaesthetic agents. As a prelude to an investigation which aimed at elucidating the normal mechanisms whereby the intracellular concentration of ionized calcium in cardiac muscle cells is regulated and what, if any, hormonal factors are involved, the action of the thyroid hormone and of glucagon on calcium exchangeability has been studied.

Troponin

(a) Preparation. Minced dog heart muscle was homogenized with an equal volume of water in a Braun Blender[1]. The solids were collected by centrifugation and mixed with an equal volume of absolute EtOH. The mixture was again centrifuged and the sediment resuspended in four volumes of 1 : 1 EtOH-H_2O. The solids were again collected by centrifugation and resuspended twice in 97% v/v EtOH-H_2O, filtering out the fibres under vacuum each time. Finally the fibres were dried in air at room temperature (21°C).

[1] Dithiothreitol was present in all solutions throughout.

The residue was extracted twice with KCl (1.0 M, pH 7.0) using 800 ml per 100 gm fibres. The first and second extractions were carried out for 16 hours and 3 hours respectively, centrifuging out the solids each time. The combined crude extracts were then precipitated at pH 4.6 to separate troponin from tropomyosin. When required the precipitate was the starting material for tropomyosin, and the supernatant for troponin.

(b) Purification. The pH 4.6 supernatant was adjusted to pH 7.0 and dialysed overnight against two changes of 10 volumes of distilled water. After dialysis the pH was adjusted to 4.0 and the precipitate collected by centrifugation and redissolved in distilled water, pH 7.0. Ammonium sulphate was added to 40% saturation and the precipitate removed by centrifugation. Ammonium sulphate was added to the supernatant to 60% saturation, again collecting the precipitate by centrifugation. It was redissolved in distilled water, pH 7.0, KCl was added to a concentration of 1.0 M and any contaminating tropomyosin removed by bringing the pH to 4.6 and centrifuging. Finally the solution was dialysed against two changes of buffer containing 60mM KCl, 30 mM imidazole and 2 mM $MgCl_2$, pH 7.0.

(c) Binding of Calcium. The binding of Ca^{++} to troponin was measured by a radiographic method using Ca^{45} as tracer. It is based on the partition of Ca^{++} between the calcium-chelating resin Chelex 100 and the soluble phase containing the calcium-binding protein.

(d) Characterization. Some attempts have been made to characterize troponin. Scans of absorbance in the ultra-violet region have been made on each preparation and consistently a peak was obtained at 260 mμ which is characteristic of nucleic acids. No peak was obtained at 278 mμ. Absorption at this wavelength is characteristic of proteins containing tyrosine and tryptophan groups.

Further work will be undertaken to assess the homogeneity of the preparations using polyacrylamide gel electrophoresis. The enzymic action of troponin-tropomyosin mixtures in the superprecipitation of actomyosin will also be investigated. Molecular sieving may give some indications of the molecular weight of troponin.

Effect of Ryanodine

Vertebrate skeletal muscle characteristically responds to ryanodine by slowly developing an irreversible contracture. In contrast, cardiac muscle responds with a progressive decline in contractile force. The reason for this discrepancy remains obscure. In skeletal muscle ryanodine has been shown to interfere with the re-accumulation of Ca^{++} by the sarcoplasmic reticulum in such a way that the activity of the Ca^{++}-dependent ATPase enzyme of the sarcoplasmic reticulum is inhibited. Studies into the influence of ryanodine on the distribution of Ca^{++} in cardiac muscle revealed that its effect here resembles that described for skeletal muscle in which the activity of the Ca^{++}-dependent ATPase enzyme is enhanced but the Ca^{++}-accumulating activity of the reticulum diminished. At the same time there was an enhanced efflux of Ca^{++} from intact cardiac muscle cells. The Ca^{++}-accumulating activity of troponin prepared from cardiac muscle was not modified. It is concluded that the negative inotropic effect of ryanodine on cardiac muscle results from the loss of Ca^{++} into the extracellular phase. The action of ryanodine on the Ca^{++}-accumulating activity of the sarcoplasmic reticulum and on the activity of the Ca^{++}-dependent ATPase enzyme resembles that described in the literature for skeletal muscle.

Effect of Anaesthetic Agents

Preliminary studies confirmed that the anaesthetic agents chloroform, halothane and sodium pentobarbital all had a negative intropic effect on heart muscle, as gauged by the output from a Walton-Brodie strain gauge sutured to the left ventricular wall.

Sarcoplasmic reticulum which was isolated from control stunned rabbits accumulated significantly less Ca^{++} when either chloroform, halothane or sodium pentobarbital was present than did control preparations which

were not exposed to the anaesthetic agents. Since the depolarization which accompanies excitation in heart muscle is generally believed to result in the displacement of Ca^{++} from the sarcoplasmic reticulum, and since this ionized calcium is thought to play an important role in the development of contraction, it seems probable that the anaesthetic agents depress cardiac contractility by virtue of the fact that they inhibit the accumulation of Ca^{++} by the sarcoplasmic reticulum, thereby reducing the total amount of Ca^{++} stored in the reticulum ready for release.

Effect of Glucagon

Glucagon is a naturally occurring polypeptide which contains approximately 28 amino-acids and has a molecular weight of 3485. It has a positive inotropic and chronotropic effect on mammalian heart muscle. Interest in this polypeptide has been renewed during recent months because of its possible therapeutic use in reversing cardiac failure which is refractory to the cardiac glycosides. Although its positive chronotropic effect is blocked by β-adrenergic blocking agents its positive inotropic effect persists. Because of its ability to stimulate the adenyl cyclase enzyme, which converts ATP to cyclic 3′5′ AMP, it has been generally assumed that an accumulation of 3′5′ AMP is in some way involved in the positive inotropic action of the drug. During the current year experiments were planned to determine if an altered exchangeability of ionized calcium could be involved in the positive inotropic action of glucagon, for previous experiments had shown that the positive inotropic action of other cardioactive drugs, including ouabain and catecholamines, and the negative inotropic effect of certain β-adrenergic antagonists, including propranolol, could all be explained in terms of an altered availability of Ca^{++} at a subcellular level.

Preliminary studies confirmed that the positive inotropic effect of glucagon was associated with an increase in the rate at which tension was developed during contraction. This effect of glucagon together with its positive inotropic effect was abolished by Mn^{++}. Radioisotope studies showed that the increase in tension developed when glucagon was added to isolated papillary muscles which were being stimulated to contract at a regular rate was associated with an increase in the total exchangeability of ionized calcium, so that additional Ca^{++} were accumulated during excitation. Hence, the positive inotropic effect of glucagon is associated with an increase in the amount of ionized calcium which is available for participation in the events associated with excitation-contraction coupling.

Effect of Thyroxine

Although thyroid function has been shown to influence the intrinsic contractile state of heart muscle, as gauged by changes in maximum velocity of shortening, rate of tension development and peak tension developed during individual contractions, the mechanisms whereby the thyroid hormone exerts its effect are poorly understood.

Hyperthyroidism was induced in dogs by the intraperitoneal injection of 1 mg/kg sodium-L-thryoxine for 10 days. Isometric tension studies on papillary muscles from these dogs confirmed that the hyperthyroid state was associated with an increase in maximum tension developed during each contraction, and with an increase in the rate at which tension is developed during contraction. These changes were not associated with an altered sensitivity to catecholamines. Sarcoplasmic reticulum prepared from the heart of thyroxine-treated dogs accumulated Ca^{++} more effectively than did reticulum which had been prepared from control dogs. Moreover, sarcoplasmic reticulum prepared from the thyroxine-treated dogs displaced more Ca^{++} in response to electrical stimulation than was the case for control preparations. These findings imply an increased rate of Ca^{++} turnover in the sarcoplasmic reticulum prepared from thyroxine-treated dogs which may be responsible for the augmented cardiac contractility. The relationship between the augmented adenyl cyclase activity and this increased Ca^{++} turnover has yet to be studied. Electron microscopy of

cardiac muscle from these hyperthyroid animals failed to show evidence of changes in mitochondrial structure and vacuolization, provided that adequate care was taken to prevent the tissues from becoming anoxic during fixation.

Effects of Exercise

One of the normal cardiac responses to exercise is expressed in the relationship between left ventricular end-diastolic volume and myocardial function. Investigators have attempted to explain this in terms of the heart's ultrastructure and have postulated that an increased separation of the actin and myosin filaments should result in more active sites being exposed to calcium. If this argument is correct, then the availability of calcium for interaction with these newly exposed sites should not provide a rate-binding step because there are several ways in which this altered calcium requirement can be met. Firstly, the same calcium ions may react with more active sites; secondly, a greater proportion of calcium ions released from the reticulum during excitation may interact with active sites. Thirdly, the interaction of calcium with the newly exposed sites may be more efficient than interaction with the normally exposed sites, possibly by exposure to troponin, and fourthly, the calcium accumulating and releasing activity of the sarcoplasmic reticulum may be altered. To test this last possibility dogs on right-sided cardiac bypass were used to provide a preparation in which venous return and hence left ventricular end-diastolic volume could be regulated. In this way it was possible to obtain a series of low output hearts in which stroke volume was maintained at 4.4 $\pm$ 0.2 ml and a series of high output hearts, in which stroke volume was maintained at 30.5 $\pm$ 1.0 ml. The concentration of ATP, CP and catecholamines in ventricular biopsies from the low output series was not significantly different from that of the high output series. Sarcoplasmic reticulum from hearts in the low output series consistently accumulated significantly less ($p < 0.01$) calcium than did sarcoplasmic reticulum from hearts in the high output series. Sarcoplasmic reticulum from the low output series released less calcium ($p < 0.001$) during electrical stimulation than did sarcoplasmic reticulum from the high output series.

These results indicate that ventricular function and the Ca-accumulating and releasing activity of the sarcoplasmic reticulum are closely linked.

Effect of Nitroglycerin

Nitroglycerin has been used for many years in the treatment of angina pectoris but its mode of action is not clear.

Although it increases coronary flow in normal subjects, Gorlin was unable to show this increase in patients with coronary artery disease, and suggested that in these patients with angina, the coronary arteries were maximally dilated in response to hypoxia.

The actions of nitroglycerin on the heart itself have been obscured by reflex changes resulting from peripheral actions of the drug.

The isolated rat heart, perfused according to the Langendorf technique, was used to show the direct action of nitroglycerin on the myocardium. A marked reduction in contractile force and in rate of contraction was observed.

Dogs on right-heart bypass were used to investigate the effect of nitroglycerin on the Frank-Starling work curve, and on O_2 consumption. Intravenous injections and infusions of nitroglycerin were given.

In these animals, a fall in systemic blood pressure occurred after the administration of nitroglycerin. The work study was carried out while the BP was low but the work curves obtained after the administration of nitroglycerin were virtually unchanged from control values or slightly depressed. Samples of arterial and coronary venous blood were taken at high and low work levels and indicated that the O_2 consumption was increased by nitroglycerin.

These results do not explain why nitroglycerin is so effective in reducing anginal pain.

Since adrenaline is often released in the situations in which angina occurs, such as exercise and emotional stress, it seemed possible that adrenaline and nitroglycerin might interact.

In the isolated perfused rat heart, adrenaline was perfused at 250 μg/1 followed by nitroglycerin for 10 min. and then the two drugs were perfused together. Under these conditions the positive inotropic response to adrenaline after nitroglycerin was less than the response obtained before nitroglycerin.

However, in experiments with dogs, when cardiac contractions were detected by a Walton-Brodie strain gauge sutured to the heart, the positive inotropic response to adrenaline was not modified by nitroglycerin.

These results have shown that nitroglycerin has a direct depressant action on the myocardium, as demonstrated on the isolated rat heart and in the intact dog using a Walton-Brodie strain gauge.

In the dog on right-heart bypass, myocardial O_2 consumption is increased and the work function curve does not show any improved capacity to do work.

From the work on the isolated heart, it appears that there may be some interaction between adrenaline and nitroglycerin.

CORONARY CIRCULATION‡*

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

Studies undertaken during previous years of substances which act on peripheral blood vessels have been continued.

In addition to the previously described preparations, which included dogs on total heart-lung bypass, and dogs on right-sided cardiac bypass, more specialised preparations have been devised during the past year, to allow a more detailed study of the action of drugs on the coronary circulation. One of these preparations permits perfusion of the coronary circulation under conditions of either constant perfusion pressure or constant flow, whilst the rest of the systemic circulation is being perfused separately under conditions of constant flow or, if required, of constant pressure. It is possible to remove samples of coronary arterial and coronary venous blood for estimating pCO_2, pO_2, pH, Na^+ and K^+. In addition biopsies of ventricular muscle can be taken at any stage of the experiment for determination of myocardial stores of adenosine triphosphate, creatine phosphate, inorganic phosphate, adenosine monophosphate and catecholamines. Ventricular function in this preparation is assessed by measuring the output from a Walton-Brodie strain gauge sutured to the ventricular wall.

A second preparation permits the perfusion of the coronary circulation whilst the remainder of the systemic circulation is occluded.

Effect of ST 155 (Catapres)

Previous investigations have shown that the hypotensive effect of Catapres involves inhibition of a centrally evoked vasoconstrictor response. The drug, also has a direct constrictor action on arteries. More recent investigations, using the techniques described above, have shown that ST 155 reduces the resistance to blood flow in the coronary circulation and that two mechanisms appeared to be involved in the response, a direct and an indirect one, the latter possibly resulting from the central action of the drug. The coronary dilator effect of Catapres depends neither upon an altered heart rate, nor upon change in myocardial contractility, nor was it associated with an increase in the rate at which oxygen was utilized by the myocardium. This coronary dilator effect of Catapres was not blocked by *dl* propranolol nor was it preceded by a transient vasoconstriction.

To study the effect of Catapres on the relationship between mean arterial blood pressure and heart rate, dogs on right-sided cardiac bypass were used to provide a stable preparation in which mean arterial blood pressure could be adjusted as required, and arterial pO_2, pCO_2, pH, Na^+ and K^+ maintained constant. Under these conditions it was possible to show that Catapres does potentiate the pressure sensitive reflex component of cardiac slowing, an effect which is mediated

via the parasympathetic system. The heart rate slowing effect of Catapres is therefore, complex, and involves, in addition to the previously demonstrated sympathetic component, a parasympathetic effect.

Effect of Dopamine

Dopamine is a naturally occurring precursor of noradrenaline and because of its ability to increase cardiac output, raise systemic blood pressure and reduce the resistance to blood flow in renal and mesenteric vascular fields, its use has been advocated in the treatment of hypotension and shock. Investigations into the effect of dopamine on coronary vascular resistance and myocardial function indicated that its primary action is vasoconstriction, the observed vasodilator effect being due to an increased rate of oxygen utilization by the myocardium. Thus, dopamine increased left ventricular function but this effect was associated with a marked increase in the rate at which oxygen was extracted by the myocardium from the coronary circulation, and was accompanied by a fall in the level of high-energy phosphates in heart muscle, reflecting an imbalance between energy utilization and energy production.

Effect of Glucagon

Glucagon has been shown to decrease peripheral vascular resistance by a direct action on the arterioles, but its effect on the coronary vessels is unknown. Experiments were performed to study the direct effect of glucagon on coronary vascular resistance, for this drug has a positive inotropic and chronotropic effect on cardiac muscle which would result in an increase in metabolic demand which could be expected to result in coronary vasodilation. In eight dogs with isolated hearts the coronary arteries were perfused at a constant rate and changes in coronary pressure used as an index of change in resistance. In the beating heart 50 μg/kg glucagon caused a 22% decrease in average coronary pressure. At the same time cardiac contractility increased by 45% and heart rate by 38%. Average coronary venous O_2 saturation decreased from 79.7 to 63.7%. When intracoronary injections of glucagon were repeated after K^+ arrest, there was no change in coronary venous O_2 saturation and no significant decrease in coronary pressure. It is concluded that the decrease in coronary resistance in the beating heart is secondary to the metabolic effects of the increased myocardial contractility and heart rate.

Effect of Salbutamol

Sympathomimetic amines are widely used for the treatment of reversible obstruction of the airways. Isoprenaline has been used intensively but it is accompanied by marked side effects due to stimulation of β-adrenergic receptors in the hearts. A new substance, 2 - 6 - butylamino - 1 - (4 - hydroxy - 3 - hydroxymethyl) phenylethanol (Salbutamol), is a β-adrenergic stimulant with a high specificity of action for bronchiolar smooth muscle. However, when high doses are used (50 μg/kg) it does have a direct cardiac action. Left ventricular work studies showed that this effect of Salbutamol was accompanied by coronary vasodilation, and increase in the ability of the left ventricle to perform mechanical work at a given left ventricular end-diastolic pressure, and hence at a given left ventricular end-diastolic fibre length.

The coronary vasodilator effect of Salbutamol was found to be due in part to a direct effect of the drug on coronary vascular resistance and in part to an indirect effect associated with the increased metabolic demand associated with augmented cardiac contractility. Salbutamol decreased coronary vascular resistance. It failed to have any effect on the myocardial stores of high energy phosphate compounds, nor did it cause catcholamines to be displaced from the myocardium.

Physical Factors

Because changes in blood flow through the coronary vascular bed are governed by many factors, some of which are interrelated, it has been necessary in our investigations to define the contribution made by the physical factors; perfusion pressure, cardiac output and heart rate.

In dogs the left coronary artery was isolated and perfused with blood under a controlled

hydrostatic head of pressure. A square wave electromagnetic flowmeter with an appropriate probe fitted around the artery was used to measure directly the flow in this vessel.

It was found that increases or decreases in perfusion pressure cause a similar change in coronary blood flow, which lasts as long as the perfusion pressure is maintained at the new level. No autoregulation has been observed. This finding, which is not in accord with those of others, may be accounted for by the different experimental conditions and the use of direct measurements of coronary blood flow. Surprisingly even very small changes in perfusion pressure (10 mm Hg) elicited changes in coronary blood flow of comparable magnitude.

When perfusion pressure was kept at a constant level, isoprenaline and aminophylline (both of which normally lower the systemic pressure and give only small rises in coronary blood flow) added to the perfusate produced increases in coronary blood flow markedly greater than either noradrenaline or adrenaline. Angiotensin causes small increases in coronary blood flow when its pressor activity is prominent, but when the perfusion pressure is kept constant, vasoconstrictor action occurs and a reduction in coronary blood flow is observed.

In an open-chest dog with a flow probe around the left coronary artery variations in the perfusion pressure of the coronary circulation were also brought about by the injection of catecholamines, angiotensin and aminophylline into the systemic circulation. Noradrenaline gave a greater pressor response than adrenaline and corresponding increases in coronary blood flow occurred. Isoprenaline produced a marked response and despite the fact that isoprenaline is the most potent of the three catecholamines in its metabolic stimulation of cardiac muscle, the increase in coronary blood flow was far less than those seen with noradrenaline or adrenaline.

When a heart-lung bypass preparation with a constant cardiac output of 100 ml/kg/min was investigated, it was found that the responses, to the catecholamines and aminophylline, of blood flow through the coronary artery and of systemic pressure were significantly less than in experiments where cardiac output was allowed to vary.

In a dog with electrodes attached directly to the heart, pacing the heart at rates above the spontaneous rate produced a decrease in coronary flow and a moderate increase in myocardial oxygen consumption. When the paced heart was allowed to return to its spontaneous rate, the coronary blood flow once more returned to its pre-paced values. Under these experimental conditions no autoregulating mechanism was noted.

The importance of perfusion pressure as a factor governing coronary blood flow raises some doubts concerning the use of isoprenaline to treat patients in shock following myocardial infarction. Objective evidence for its benefits has been lacking and it may be argued that since the coronary vascular bed in cardiogenic shock is already perfused at a reduced systemic pressure, to superimpose a further decrease of pressure produced by isoprenaline, coupled with the concomitant increased oxygen demand by the heart, may in fact be potentially more deleterious than beneficial. Especially as in these series of experiments no evidence of any intrinsic autoregulatory mechanism for coronary blood flow could be demonstrated.

LEFT VENTRICULAR HYPERTROPHY†‡

W. G. Nayler, I. McInnes and V. Carson.

Preliminary investigations undertaken so far have shown that left ventricular hypertrophy in dogs can be induced by placing a tight tape around the aorta immediately proximal to the aortic arch. The amount of hypertrophy has been characterised in terms of an increase in ventricular muscle weight and a changed myocardial performance. Electron microscopy studies are in progress to establish whether or not the changes in ventricular function are associated with structural changes.

β-ADRENERGIC ANTAGONISTS AND OTHER ANTIARRHYTHMIC DRUGS‡

W. G. Nayler, J. Chan, G. M. Picken and J. M. Price.

Stimulation of β-adrenergic receptors in the heart results in an increase in rate, force of contraction and conductivity. Since the discovery 11 years ago of a new class of therapeutic agents which could effectively block the cardiac effects of added or endogenously released catecholamines, there has been a very wide interest in the actions of the numerous compounds with β-blocking capacity which have been synthesized. Clinically these compounds have been used in the treatment of a wide variety of disorders, including angina pectoris, cardiac arrhythmias, hypertension, Fallot's tetralogy, hypertrophic obstructive cardiomyopathy, thyrotoxicosis, certain anxiety states, Parkinsonism, etc. A concept is emerging that different β-blocking drugs may be useful in treating specific disorders. However, selection of a particular β-blocking compound for the treatment of a particular disorder can be achieved only if the pharmacology of these various compounds is known in detail.

Myocardial Depressant Action

As described in previous reports the direct inotropic effect of drugs with β-adrenergic blocking properties has been tested using either dog or human atrial or papillary muscles suspended in aerated Tyrode's solution, isometric conditions being maintained under a constant resting tension. During the current year these investigations have been extended to include ICI 50,172, LB 46, KŐ 1366, KŐ 1313 and H 56/36. Particular attention has been given to the conditions under which the weak positive inotropic effect which some of these β-blocking compounds have can be potentiated. The observation made in earlier studies that the myocardial depressant effect of the β-blocking drugs is proportional to their effect on the uptake of Ca^{++} into the sarcoplasmic reticulum has been found to hold true for all the β-blockers so far investigated. The conditions under which these same β-blockers exert a weak positive inotropic action appears to be related to the presence of some sympathetic support. When a β-blocking drug such as LB 46 exerts a significant positive inotropic effect on the denervated blood perfused heart it exerts, at the same dose, a weak negative inotropic effect if the sympathetic innervation to the heart is still intact.

Bradycardia

The mechanisms involved in the ability of the β-blocking compounds to produce a fall in heart rate could involve three factors: (i) an increase in vagal activity, (ii) diminished activity in the sympathetic nervous system or (iii) a direct depressant action on cardiac conducting tissue.

ICI 50,172, KŐ 1366 and propranolol produced a fall in heart rate after bilateral vagotomy, so that their heart rate slowing effect does not depend simply upon an increased vagal tone. After bilateral stellate ganglionectomy and vagosympathectomy ICI 50,172 and KŐ 1366 produced a small increase, whereas propranolol produces a small decrease in heart rate. The heart rate slowing effect of ICI 50,172 and KŐ 1366 may be due, therefore, to a decrease in sympathetic support. The bradycardia caused by propranolol persisted after bilateral vagosympathectomy and stellate ganglionectomy and therefore may involve depression within the conducting tissue. In pithed rats propranolol caused a fall whereas ICI 50,172 and KŐ 1366 caused a small rise in heart rate.

Species Specificity

ICI 50,172 is noteworthy as a β-adrenergic blocking drug in that it is more effective in blocking cardiac β-receptors than those in the peripheral circulation in man and dogs. In rabbits it seems probable that ICI 50,172 is metabolised to produce a product which blocks β-receptors in the heart and peripheral circulation alike.

Prevention of Arrhythmia

There is a marked difference between the modes of inactivation of the transmitters noradrenaline and acetylcholine following their

release from sympathetic and cholinergic nerve terminals respectively. In the cholinergic system acetylcholine is rapidly destroyed on the post-synaptic membrane by cholinesterase, but most of the released noradrenaline is actively transported back into the adrenergic nerve terminals and stored there for subsequent re-use. Some liberated noradrenaline is enzymically destroyed within the effector cell by monoamine oxidase and catechol-oxymethyltransferase, and some is non-specifically bound at sites such as the ground substance of connective tissue. Noradrenaline which is not bound and inactivated by any of these mechanisms diffuses into nearby capillaries and appears as "overflow" in the venous effluent from the tissue under study.

Many drugs have been shown to interfere with the active transportation of noradrenaline back into the nerve terminal, and include almost all α- and some β-adrenergic antagonists. Blockade of this process has been shown to potentiate the actions of both released and intravenously-infused noradrenaline, and to elevate the overflow of transmitter during stimulation of the sympathetic nerves to organs such as the heart and spleen.

The β-receptor antagonist ICI 50,172 was examined for uptake-blocking activity. The neck-implanted dog heart was used for this study mainly because it can be considered an isolated blood-perfused organ. In this preparation the subclavian branch of the aorta of the donated heart is connected to the carotid artery of the recipient dog thereby perfusing the coronary circulation of the donated heart. This blood drains to the right ventricle and then from one pulmonary artery into the jugular vein of the host. Radioactive noradrenaline was infused into the carotid artery on the afferent side of the donated heart. The accumulation of radioactivity by its right atrium and the amount of radioactivity appearing in the efferent blood during transmural sympathetic nerve stimulation were determined, ICI 50,172 did not modify either the uptake of ^{14}C-noradrenaline or its overflow from the implanted heart, and therefore appears to be a drug with post-synaptic actions only. It will therefore be a useful tool for the investigation of post-synaptic actions of β-blockers, such as their effect on muscle uptake and metabolism of noradrenaline.

Because cardiac arrhythmias have been observed following the administration of adrenaline to patients undergoing surgery under halothane anaesthesia, the anti-arrhythmic properties of the β-blocking agents ICI 50, 172, KŐ 1366 and propranolol were investigated. Bilaterally-vagotomized anaesthetized dogs were challenged with intravenous doses of adrenaline during halothane inhalation. Both KŐ 1366 and propranolol were effective in preventing arrhythmias developing, but ICI 50,172 was much less effective.

The importance of an intact sympathetic supply for the genesis of these arrhythmias was investigated using the neck-implanted dog heart preparation which provided an innervated (host), and a denervated (implanted) heart in the one dog, perfused by the same blood. Electrocardiographs were taken from both hearts and used to measure heart rate. Although the implanted heart appeared consistently less susceptible than the host heart to arrhythmias upon adrenaline-halothane administration, this may reflect only the difference in concentrations of the amine reaching each heart. Adrenaline given into the femoral vein of the host travels first to its own heart, where some is inactivated. Only a fraction of the initial dose is then carried up the carotid artery to the neck-implanted heart. Before further studies can be undertaken, it will be necessary to devise a method whereby both hearts receive the same adrenaline challenge.

Carotid Occlusion Reflex

Shanks and Dunlop have shown that propranolol will inhibit the carotid occlusion reflex in dogs, when the drug is administered daily over a period of six weeks. This effect is thought not to be due to β-blockade, as the tachycardia and fall in blood pressure caused by isoprenaline persists in these animals.

In our experiments, using rats anaesthetized with urethane, these observations were con-

firmed. The rats were given a daily oral dose of 50 mg/kg and 20 mg/kg of propranolol. The effects of various other β-adrenergic drugs may be summarized. ICI 50,172, which blocks mainly cardiac β-receptors, and Isoptin which blocks peripheral β-receptors, inhibited the carotid occlusion reflex. Some slight inhibition was noticed with H 56/28. Prolonged administration of these drugs to the rats did not reduce the arterial blood pressure of the animals.

KINEKARD

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

For several years we have described in these reports investigations aimed at identifying the active principle(s) in blood plasma which are cardioactive. These activities can be found in two fractions of the plasma obtained after gel filtration on Sephadex or 5% cross-linked polyacrylamide gel. One fraction has a molecular weight greater than 60,000 and the other a molecular weight in the range 4,000-10,000. We have applied the name Kinekard to this lighter fraction.

Kinekard has markedly positive inotropic activity on cardiac muscle and actions on smooth muscle resembling catecholmines but it has been shown that the active principle is not a catecholamine. Because the active principle of Kinekard has not been isolated nor chemically characterized a biological assay has been necessary to demonstrate its activity. This assay has measured the change in contractile force of a cannulated isolated isotonically contracting toad ventricle which is perfused with 1.0 ml Ringer solution to which 0.05 ml of plasma fraction prepared under specified conditions is added.

In 1956 we had noted that the response of such a preparation made from a "hibernating" toad differed from that from a "summer" toad. This year this phenomenon has been further investigated and it has been shown that only the summer ventricle responds to Kinekard. It should be noted however, that the hibernating ventricle did respond to the whole human plasma from which the fraction was prepared. The following observations suggest that this response of hibernating toad hearts to unfractionated human plasma may be due to the level of ionized calcium (1.5 mM) in plasma.

(i) Plasma exerts a positive inotropic action on a hibernating ventricle when the perfusing fluid contains 0.65 mM $CaCl_2$ but not when it contains 1.3 mM $CaCl_2$. In the summer heart this difference is not present.

(ii) The addition of disodium EGTA (final concentration 1.0 or 1.5 mM) removes free Ca^{++} from plasma and abolishes its inotropic action on the hibernating ventricle. As EGTA does not remove Ca^{++} bound to plasma protein, levels of plasma EGTA concentration greater than 1.5 mM, endow the plasma with a negative inotropic activity.

(iii) When plasma is subjected to gel filtration on Sephadex G-50 or 5% cross-linked polyacrylamide gel (eluting fluid, "half calcium" Ringer solution), only those fractions containing elevated levels of ionized calcium exert any positive inotropic action on hibernating toad heart.

(iv) Plasma, which has been mixed with sufficient Amberlite CG-120 resin to lower the total plasma calcium level to 0.6 mM, has no positive inotropic action on the hibernating ventricle.

It was also found that hibernating toad heart preparations perfused with "half calcium" Ringer solution are sensitive to any further dilution of the perfusate with distilled water or dilute solutions of inotropically-inactive salts. The positive inotropic action produced by these fluids is blocked by the addition of NaCl to a final concentration of 0.1 M. Since Clark (1913) observed that a reduction in the osmotic pressure or NaCl concentration of the perfusion fluid bathing a frog's heart caused an increase in the amplitude of contraction, it seems likely that the above phenomenon can be explained in these terms.

CYTOMEMBRANE SYSTEMS

L. W. Wheeldon

Last year the rationale and initial experimental steps taken to develop a more detailed knowledge of the biochemistry of myocardial microsomes were outlined and during this year have been developed.

New Class of Cytomembrane Fragment in Myocardial Microsomes

A new type of fragmented cytomembrane material, which has not previously been described for heart muscle or any other tissue, has been discovered. This new finding stems from studies of the aggregation property of heart muscle microsomes and its influence upon flotation of microsomes in density gradients, coupled to analysis of the adenosine triphosphate-splitting activities (ATPases) present in this material.

Recognition of this new class of cytomembrane fragment is based upon three findings. First, flotation of microsomes in sucrose density gradients by a standard technique achieves resolution into two principal components, one recognisable as "microsomal", i.e., showing coincident profiles for esterase, NADH-cytochrome c reductase and oligomycin-insensitive ATPase activities; the other is mitochondrial, being associated with cytochrome oxidase and oligomycin-sensitive ATPase activity.

Secondly, when unwashed microsomes are submitted to flotation in a sucrose gradient containing heparin (conditions which minimise aggregation of the membrane fragments) the oligomycin-insensitive ATPase activity resolves into two components. The "Heavier" ATPase requires Ca^{++} for activity and is sulphydryl-dependent; it is therefore identified with the calcium transport ATPase previously characterised in skeletal muscle microsomes. The "Lighter" ATPase shows neither of these properties. Esterase and NADH-cytochrome c reductase remain co-incident with the heavy ATPase. These activities are low or absent in the light ATPase.

Thirdly, the light component of the microsomal ATPase is active towards all nucleoside phosphates, hydrolysing nucleoside diphosphates (NDP's) at a higher rate than nucleoside triphosphates (NTP's) and among the latter, cytidine triphosphate (CTP) and guanosine triphosphate (GTP) are hydrolysed at faster rates than ATP. The heavy ATPase has a low activity on nucleoside phosphates other than ATP.

Thus, there are two distinct forms of ATPase activity present in heart muscle microsomes. One of these, the light ATPase, is best described as an NTPase. Since they are manifest in components of differing bouyant density, it can be concluded that they represent two distinct types of cytomembrane fragment. The calcium-dependent, heavy component must be derived from those portions of the cytomembrane system which are involved in the regulation of myoplasmic Ca^{++} levels and thus in the dynamics of contraction; the light component would appear to have a wholly different role. The exceptionally high nucleoside diphosphatase activity provides the only present clue to the composition of the light component. This includes activity towards both adenine and non-adenine NDP's. In liver, the former has been localised in elements of plasma membrane[1], while the latter has been found to be associated with polysaccharide synthesising elements, such as Golgi membranes[2, 3]. Efforts will be directed towards further analysis of the light component with regard to these activities.

Control of Nucleoside Phosphate Levels

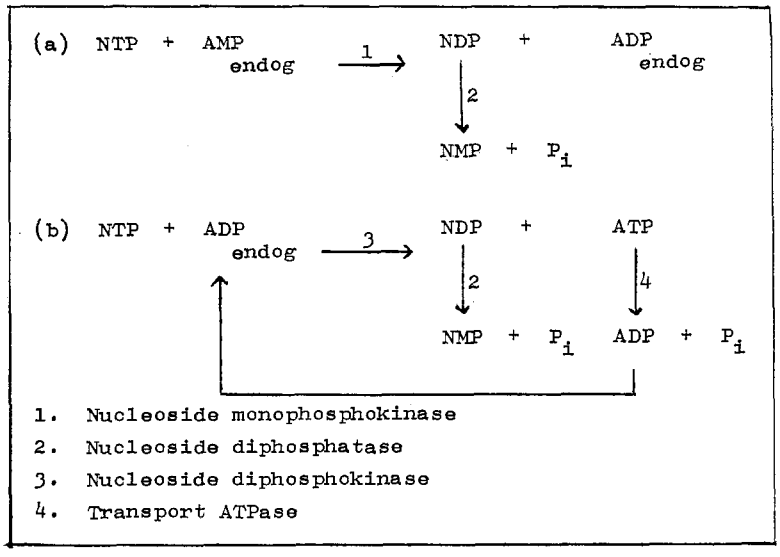
Some studies are presently directed towards an understanding of the nature of the microsomal NTPase activity. A leading observation is that this activity is strongly inhibited by nucleoside mono- and di-phosphates. Moreover, the hydrolysis of nucleoside diphosphates is also inhibited by nucleoside monophosphates. These properties point to a complex system of control, which regulates the synthesis and utilisation of high-energy nucleoside phosphates. Since these compounds have important roles in biosynthesis, such a control system has a pivotal significance for the regulation of biosynthetic pathways in the

heart muscle cell. It is believed that the analysis of mechanisms for the regulation of NTP levels in tissues has so far received scant attention.

Nucleoside Triphosphate Participation in Calcium Transport

Implicit in the distinction between light and heavy ATPases is the dissociation of NTPase activity from direct involvement in calcium transport. Nevertheless, it is widely reported that all NTP's support calcium accumulation in both skeletal and cardiac muscle microsomes. Moreover, Hasselbach[4], who has contributed detailed investigations on this point, refers to the transport system as an NTPase, rather than an ATPase.

Using unfractionated microsomes calcium-dependent hydrolysis of ATP, but not of other NTP's, can be demonstrated. However, a calcium-dependent hydrolysis of GTP and CTP is observed in preparations "uncoupled" by titration with oleate, a treatment which maximises energy utilisation by releasing transport from controls inherent in undamaged membrane systems. This points to the existence of a mechanism whereby NTP's other than ATP can be "called upon" when energy demand is in excess of normal and is consistent with a secondary role of NTP's as an energy source. The most likely form that this might take consists of a transphosphorylation process. This supposes the occurrence in microsomes of endogenous adenine nucleotide, which acts as the primary energy donor. There is published work in support of the occurrence of endogenous adenine nucleotide[5]. As a working hypothesis which might account for the NTPase activity of cardiac microsomes, as well as accounting for the role of NTP's in calcium transport, the following scheme is presented.



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BIOCHEMICAL TECHNIQUES

V. Carson, P. Daile and H. A. Jonas.

Assay of Plasma Catecholamines

The method for the fluorometric determination of tissue catecholamines reported last year has been adapted to permit the estimation of total catecholamines in plasma. A differential estimation of noradrenaline and adrenaline may also be done; however, in normal plasma, the level of adrenaline is below the threshold of the method.

One hundred ml of blood is collected in heparin with 50 mg of sodium metabisulphite, in ice. After centrifuging in the cold for 20 min. at 2,000 r.p.m., 50 ml of plasma is collected in a 100 ml stoppered cylinder. The plasma is brought to 0.4 M with respect to perchloric acid by slow dropwise addition of the concentrated acid with mixing, then shaken for 5 min.

The mixture is quantitively transferred to two 50 ml centrifuge tubes, and the cylinder rinsed with two 5 ml quantities of 0.4 M perchloric acid. The tubes are spun at 15,000 r.p.m. for 10 min.

The supernatant is removed completely to a 100 ml beaker, and for each 20 ml of supernatant, 0.5 ml of 0.2M Na_2EDTA is added.

The mixture is brought to pH 8.4 with 5 N and 0.5 N NaOH with constant stirring and then chromatographed on a thistle-funnel-topped column of 5 mm internal diameter containing 0.3 gm of activated alumina (Crout, 1961). The column is constricted at the bottom, and plugged with a small quantity of glass wool.

After application of the sample, the column is washed with water until the pH is below 7 and then eluted with 0.2M acetic acid. After discarding the first 0.5 ml, 3.5 ml of eluate is collected.

The eluate is assayed for total catecholamines fluorimetrically by the trihydroxyindole method, essentially as described previously. 0.5 ml of eluate is used in a total reaction mixture of 5 ml. For total catecholamines only the fluorescence at pH 6.5 is measured.

The fluorescence was measured in a Turner Fluorimeter using a primary filter passing the 365 mμ line and a secondary filter transmitting maximally at 525 mμ, and the x30 sensitivity setting.

Faded blanks are included but have routinely been zero. The recovery of added noradrenaline was between 45 and 60%.

The mean level of total catecholamines in 16 samples of normal dog plasma was found to be 1.85 $\pm$ 0.19 (S.E.) mg/litre. Values were corrected for an assumed 50% recovery as usually it was impractical to collect sufficient plasma to include a recovery test with each assay.

Assay of Adenyl Cyclase

Work is in progress to investigate the effect of various sympathomimetic drugs and other hormones on the adenyl cyclase system of the mammalian heart.

To this end an *in vitro* assay of adenyl cyclase in heart muscle homogenates is being developed using the micro-radioisotope assay method of Krishna, Weiss and Brodie (1968). The assay is based on the reaction in which labelled ATP is converted to labelled 3-5 AMP by the action of the enzyme adenyl cyclase. The rate of formation of labelled 3-5 AMP is a measure of the enzyme activity, provided the breakdown of 3-5 AMP by phosphodiesterase is prevented by the addition of theophylline to the medium.

However, we have found that aminophylline which consists of 2 molecules of theophylline linked to one molecule of ethylene diamine is a much more efficient inhibitor of phosphodiesterase than the equivalent amount of theophylline and its use results in significantly higher yields of 3-5 AMP. We have confirmed that sodium fluoride is a powerful activator of the reaction.

The optimum conditions for the reaction are being studied and normal values established.

Assay of Calcium

The use of atomic absorption spectrophotometry for the determination of calcium release from, and total calcium content of,

mitochondria isolated from cardiac muscle has involved the development of a diluent which can suppress interferences from proteins, other cations and anions. The existing technique for Ca^{++} analysis in serum by Zittner and Siligson was found to be unsuitable for the extremely low Ca^{++} concentrations encountered. A 2% $La_2O_3/HClO_4/NaOH$ solution and 4% EDTA solution mixture was prepared and, when used in conjunction with a nitrous oxide/acetylene flame, gave maximum sensitivity in Ca^{++} analyses.

Assay of the H_4 Lactate Dehydrogenase Isoenzyme

Mammalian lactate dehydrogenase (LDH) exists in five electrophoretically distinct forms, called isoenzymes. Each isoenzyme is a tetramer composed of four subunits of equal molecular size, and each subunit is one of two types (M or H). These isoenzymes have been labelled H_4, H_3M_1, H_2M_2, H_1M_3 and M_4, according to their subunit composition, or LDH-1, LDH-2, LDH-3, LDH-4 and LDH-5, according to their relative electrophoretic mobility towards the anode, the isoenzyme having the highest mobility being labelled LDH-1 (or H_4).

Normal serum, which contains relatively low levels of LDH, has a characteristic LDH isoenzyme distribution. Tissues from organs such as heart muscle, liver and lung contain high levels of LDH with different isoenzyme distributions characteristic for each tissue. When the cells of a tissue are damaged by disease, the level of total LDH in serum is raised, and the pattern of LDH isoenzymes in serum reflects that of the diseased tissue. Since heart muscle contains a very high proportion of the H_4 isoenzyme, the extent of myocardial necrosis following a heart transplant can be measured by the increase in the level of the H_4 isoenzyme in serum.

The following assay procedure, which is a modification of Hardy's method (Hardy, S. M. (1965) *Nature, Lond., 206,* 933), provides a more rapid, inexpensive quantitative measure of total and H_4 LDH activity. It is a spectrophotometric assay based on the fact that M-type isoenzymes are more readily denatured by urea than H-type isoenzymes. For the estimation of total LDH, the assay medium contained 2.7 ml 0.05 M sodium phosphate pH 7.5, 0.1 ml 0.0093 M sodium pyruvate (in 0.05 M sodium phosphate pH 7.5) and 0.1 ml 0.004 M NADH. Enzyme reaction was initiated by the addition of 0.1 ml of LDH and the activity of the LDH sample was estimated from the rate of decrease in absorbance at 340 mμ. (LDH activity = change in absorbance x 1000 min^{-1}ml enzyme^{-1}). For the estimation of H_4 LDH, the assay procedure was as above, except that the phosphate buffer contained 2.89 M urea and NADH was added exactly 2 min. after the addition of enzyme. Since canine and human H_4 isoenzymes retained 54% activity, (while H_3M_1, H_2M_2, H_1M_3 and M_4 isoenzymes retained zero activity) under these conditions, the LDH activity obtained in 2.6 M urea was multiplied by 1.85 to convert this activity to H_4 LDH activity. The accuracy of this procedure has been confirmed using LDH preparations of known H_4 isoenzyme content.

HYPERTENSIVE STATES

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

Analysis of Therapeutic Trial[1]

The survey commenced last year of the results of long term treatment of severe hypertension has continued. The plan of data collection formulated in 1950 did not envisage that they would be handled by electronic data processing equipment and this has led to some difficulties which have delayed completion of the analysis. Although to date only 60 histories have been coded and 45 of them have been analysed by the computer, interesting results are becoming apparent.

[1] Advice on computer methods was given by Dr. D. R. Race, Director, Hospital and Charities Computer Group.

The following table records the progress of a number of features of the condition in some of these patients. It shows that there is a higher proportion of patients with good blood pressure control and good symptomatic state in the later reviews. Most of the patients have returned to their work and have continued in full employment. Few of the survivors are disabled by cerebro-vascular disease.

Years after commencement of treatment	1	2	5	10	15
Blood pressure control					
Good or fair	17	18	17	13	9
Poor	14	5	1	0	0
Symptomatic state					
Good or fair	26	22	16	12	9
Poor	6	2	2	1	1
Working state					
Full or light work	24	23	17	13	8
Unable to work	5	1	0	0	0
Pensioners	1	0	1	0	0
Grade of cerebral disease					
Nil	30	24	18	12	8
Mild	1	1	1	1	2
Moderate	1	1	0	0	0
Severe	0	0	0	0	0
Grade of ocular disease					
Nil or mild	21	21	15	11	9
Moderate	8	5	4	2	1
Severe	3	0	0	0	0
Grade of cardiac disease					
Nil or mild	17	13	11	0	6
Moderate	12	12	7	8	4
Severe	3	1	1	5	0
Grade of renal disease					
Nil or mild	25	23	18	12	8
Moderate	5	2	1	0	2
Severe	2	0	0	1	0

(Figures represent numbers of patients in each category)

The proportion with renal impairment remains steady and low. By contrast the proportion of patients with moderate or severe cardiac disease is high and affects approximately half of the patients in any review period.

This table indicates the proportions of subjects with the various conditions at a particular time, but does not indicate whether the apparent improvement with time is due to upgrading of individual patients, or to loss by death of the more severely affected.

Trial of "Catapres"[1]

The trial of the hypotensive drug "Catapres" (ST 155) in moderately severe hypertension has continued and the series now contains 18 patients, most of whom have been treated for 12 months.

Initially 75 mcg three times a day is given and the dose gradually increased over a period of 2 months in an attempt to obtain satisfactory blood pressure control. A thiazide drug (cyclothiazide) has then been added to determine whether the combination is more effective.

The results indicate that Catapres alone has a hypotensive effect and that this is potentiated by the thiazide. Some tolerance to the drug appears to develop because the dose of Catapres has required to be increased after the initial stabilization in some cases. Side effects have included drowsiness, dry mouth and disturbed sleep from nightmares. The latter have occurred only in the initial two weeks of treatment. There have been no toxic effects on blood, liver or kidneys.

Although satisfactory blood pressure control was obtained in about half of the patients with "Catapres" alone, this proportion was greatly increased by the addition of a thiazide. Most patients continue to have good blood pressure control when treatment is continued for 12 months.

[1] The "Catapres" and cyclothiazide used in this trial were kindly supplied by Boehringer Ingelheim Pty. Ltd.

The following table summarizes the results with Catapres.

Treatment	Catapres alone	Catapres + cyclothiazide	Catapres + cyclothiazide
Length of treatment (months)	3	6	12
Blood pressure control			
Good ... (Diastolic < 100 mm Hg)	9	14	9
Fair ... (Diastolic < 110 mm Hg)	5	1	1
Poor ... (Diastolic > 110 mm Hg)	3	0	0
Total number of patients ...	17	15	10

Mode of Action of α-Methyldopa

The hypotensive action of α-methyldopa is known to be mediated through an interference with the sympathetic system, but the site of action has not been established. It has been proposed that the amine (α-methylnoradrenaline) formed on metabolism of α-methyldopa takes over the function of the normal transmitter noradrenaline. This hypothesis of a false transmitter requires that the lowering of blood pressure should depend on the α-methylated derivate of noradrenaline exhibiting markedly less pressor activity than the endogenous transmitter. To test this hypothesis, therefore, a comparison of the potency of noradrenaline and α-methylnoradrenaline on blood pressure, heart rate, cardiac output and myocardial contractility at three different dose levels was undertaken in rabbits and dogs. The results indicate that noradrenaline and α-methylnoradrenaline have approximately equipotent actions on cardiac output, heart rate and force of contraction. These findings are explicable on a structure-activity relationship since the presence of a methyl group on the α carbon atom of the side chain will enhance the so-called β-mimetic activity of the catechol nucleus and tends to diminish the action on α-receptors. Similarly the pressor activity of α-methylnoradrenaline was not significantly less than that of noradrenaline in dogs and rabbits; a finding which was previously also observed in rats and cats. It appears therefore that the hypotensive action of α-methyldopa is not mediated by the presence of the false transmitter in the peripheral division of the sympathetic nervous system. This has recently been substantiated by Henning (1969) who gives experimental evidence for a centrally-mediated false transmitter action of α-methyldopa.

CARDIAC SURGERY‡

CARDIAC TRANSPLANTATION

G. R. Stirling, E. Cooper, G. C. Shardey and W. G. R. M. de Boer.

In performing satisfactory cardiac allografts there are two main problems. The first arises from the obligatory period of coronary ischaemia, with or without supportive hypothermia, and the second from the immunological rejection which results in subsequent failure of the transplanted heart.

The first facet has been examined over the past two years during which periods of coronary ischaemia, with and without local hypothermia to 5°C (surface cardiac temperature), were studied. The physical and biochemical recovery of the heart was evaluated and found to be almost complete after ischaemic periods of up to 60 min at 5°C. Following adequate coronary perfusion with whole blood for approximately 20 min, the heart function for a given work load was found to be normal.

On the basis of these findings two satisfactory techniques for canine cardiac allografting have been developed so that the immunological rejection phenomenon could be studied and modified by a variety of immuno-suppressive regimes.

Immunological Rejection

(a) Techniques. The **first technique** used places the donated heart in a heterotopic locus, a formed subcutaneous neck pouch, in the recipient animal utilizing a full sterile surgical technique. The donor animal is completely exsanguinated under anaesthesia, the thorax is then opened and the heart removed inside its intact pericardium after ligating and dividing all the pulmonary venous connections, the superior and inferior venae cavae, and the left pulmonary artery. The arch of the aorta with a 1 cm length of both brachiocephalic and brachial arterial stumps is severed at the junction with the descending thoracic aorta and the right pulmonary artery is divided just proximal to its first bifurcation. Immediately

on removal the donated heart is placed in ice cold normal saline at 4°C. An atrial septal defect is then made by a blind technique in which a blunt instrument is introduced into the right atrium via the azygos vein and pushed through the septum into the left atrium. The azygos vein is then ligated. The neck of the recipient is prepared by making a subcutaneous pouch and isolating the external jugular vein and the internal carotid artery on the same side. The recipient dog is then heparinised and the above artery and vein are clamped and divided. The donated heart is then connected to the recipient by anastomosing its brachial artery stump to the proximal end of the recipient's internal carotid artery and its right pulmonary artery stump to the proximal end of the recipient's external jugular vein. The distal ends of the internal carotid artery and the external jugular vein are then ligated.

Air is expelled from the root of the aorta and brachiocephalic artery of the donated heart by releasing the clamp on the internal carotid artery. The aorta and brachiocephalic stumps are then ligated and the blood of the host is allowed to perfuse the coronary arteries of the donated heart. The clamp is taken off the external jugular vein and the coronary sinus blood returns via the anastomosis between the right pulmonary artery and external jugular vein to the recipient's own heart. The previously constructed atrial septal defect allows thebesian drainage from the left ventricle and left atrium to return to the right heart and thence to the recipient's circulation. In most cases the donated heart, which has been in asystole during the anastomosis, reverts spontaneously to a normal sinus rhythm. If this does not occur a D.C. defibrillating shock is used to start it beating.

The donated heart is only being supported by the recipient and does not contribute in any way to the maintenance of the recipient's circulation. It becomes the target for the rejection process developed by the recipient in response to this whole organ antigen, and allows investigation of the effects of this process without the complications of cardiac failure inherent in the orthotopic transplant. Further, the effects, if any, on the recipient's own heart of the rejection process may be studied.

Separate ECG leads are attached to the right ventricle, left ventricle and right atrium of the donated heart prior to closing the neck pouch. The voltages of the P wave, QRS complex and the T. wave, together with the PR, QRS and ST intervals, are measured daily and correlated with the histological evidence of rejection. Tri-weekly estimations of lactic dehydrogenase isoenzymes are performed and correlated with the histological evidence of rejection.

The **second technique** is the orthotopic implant. The recipient animal is placed on the heart-lung bypass machine and its heart is removed leaving the remnants of the right and left atrium and adequate lengths of the aorta and pulmonary artery for anastomosis to the donated heart which is prepared in the appropriate manner. The technicalities of this method have now been mastered, but there remains one problem related to post-operative bleeding which is causing a high mortality in the first 6 to 8 post-operative hours. This problem is near to solution and long term survivors using this method are expected in the near future.

(b) Untreated Implanted Hearts. Over 40 hearts have now been transplanted to a heterotopic locus and not treated with any immunosuppressive agent. The mean time of survival before rejection has been 8 days (range 7-15). These hearts were palpated daily and as rejection occurred the palpable beat was less in intensity.

Electrocardiograms. QRS and R wave voltages fall abruptly in the first 24 hours then remain steady for 4 to 5 days and finally show a fall terminating in asystole and complete rejection of the implant. Changes in the various ECG cycle intervals were not found to be useful. Arrhythmias are manifest early and late and we are now sure that the early arrhythmias (first 48 hours) are associated

with an irritable myocardium with areas of focal necrosis whilst the late arrhythmias are due to conduction tissue infiltration with immunologically competent cells.

Histology. Implanted hearts were sacrificed at daily intervals to obtain an overall picture of the histological changes associated with the rejection process. Within the first 24 hours there is an infiltrate of polymorphonuclear leucocytes and lymphocytes, associated with an increase in the perimyocyte spaces indicative of cardiac oedema. This infiltrate is probably mainly related to focal anoxic damage associated with the transplant technique.

By the third day, pyrinophilic staining "blast type" cells are seen in the perivascular, sub-endocardial and sub-epicardial regions. Many blood vessels are congested and micro-occlusions are found. Denser areas of immunocytic infiltration are associated with "piecemeal" destruction of the cardiac muscle cells and further focal necrosis occurs in relationship to the microvascular occlusions and perivascular cuffing.

The infiltrate concentrations increase over the next 4 to 5 days so that by 8 days they are widely scattered throughout the myocardium. These blast-like immunocytes appear to have silted on to the vascular endothelium, causing further vascular occlusion, and on to the valvular endothelium.

Of more important interest is what appears to be a preferential infiltration of the areas of the sino-atrial and atrio-ventricular nodal tissue, as well as the conduction tissue. The severity of this infiltration correlates well with the development of late arrhythmias and is believed to be a major cause of the failure of these grafts.

An interesting and important side observation is the extensive infiltration of viable neural ganglia, presumably parasympathetic, in the proximity of the SA and AV nodes. This finding, hitherto not reported, strongly suggests that viable neural tissue is as antigenic as cardiac muscle so that any neural allografts can be expected to suffer rejection at an intensity at least as great as that seen in cardiac allografts.

(c) Recipients' Hearts with Untreated Implants. Standard ECG tracings of the recipients' hearts made daily have revealed non-specific ST elevation in some dogs but there have not been any consistent histological abnormalities noted.

(d) Treated Implanted Hearts. Two main therapeutic regimes have been used. In the **first regime** standard chemical immunosuppressive therapy (IST), using azothioprine (2-4 mg/kgm/day) and hydrocortisone (20-40 mg/kgm/day), has been used with success in 7 dogs producing survivors up to 37 days.

In the **second regime** a single dose of bovine anti-dog thymocyte globulin[1] (ATG), has been given. Although the *in vitro* potency of this ATG is acceptable by international standards, *in vivo* tests using both canine skin allografts and cardiac allografts have been disappointing. In the latter case these grafts failed to survive longer than 8 days and the mean survival time in 10 treated dogs was 7 days. The failure of this regime appears to be related to the thrombogenic properties of the ATG and further efforts are under way to purify the existing supply and produce only the IgG fraction.

Changes with First Regime. In those animals treated with azothioprine and cortisone an early voltage drop in the ECG is again seen but there is prolongation of the following steady period. During this phase transient falls of the voltage of the R wave can be abolished by increasing the dose of cortisone. These transient falls are most probably acute exacerbations of the rejection process. Finally the voltage falls prior to asystole of the implant. Arrhythmias occurring during the period of steady voltage may also be abolished by increasing the IST suggesting that they are due to increased infiltration of the cardiac conductive tissue as a consequence of exacerbation of the rejection process.

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[1] Made with the co-operation of the Department of Pathology, Monash University.

Histologically the main differences between these hearts and those from the untreated animals is the reduced concentration of the immunocytic infiltration in the first 10 days. By 37 days scattered intense foci of small lymphocytic type cells, mainly in the sub-endocardium and in the conduction and neuronal tissue, are seen.

Immuno-fluorescence studies on the longer surviving hearts demonstrate the presence of anti-dog antibody in the vessels. This shows that a humoral antibody has developed and is playing a part in the rejection process.

In the recipient's heart in IST treated dogs, non-specific ST changes are again seen but the histology is not greatly altered from the normal.

Changes with Second Regime. In addition to the early fall in R and QRS voltages there appeared to be ECG evidence of ischaemia in most of the hearts. No clear period of steady voltages could be demonstrated. Arrhythmias were mainly early and were most likely related to focal ischaemia and irritability. Further numbers of ATG-treated hearts are needed before any significant conclusions can be made.

Histological studies showed that the immunocytic cellular infiltrate was almost completely absent even in the 8-day surviving hearts in this series. This suggests that the immuno-suppressive action of the prepared ATG is satisfactory. However, many micro-vascular occlusions were seen and these are presumably due to the thrombogenic properties of this bovine ATG. An interesting histological observation in these hearts is a blast-like change seen in the nuclei of the cardiac myocytes and the conduction tissue. No reason for this observation can be provided as yet.

Some of the recipients' hearts in these ATG-treated dogs showed small vascular occlusions at microscopical level with focal necrosis. ECG changes, however, were not consistent with these histological findings.

Summary and Prospect

From these findings a pattern of cardiac allograft rejection has emerged which may assist in the planning of immuno-suppressive therapy. Further work using the orthotopic transplant is planned utilizing standard chemical immuno-suppressive therapy and differing batches of anti-dog thymocyte and lymphocyte globulin. Studies on the behaviour of cultures of leucocytes taken from recipient animals at various stages in the untreated and treated rejection cycles are planned and pilot studies suggest that these may shed further light on the process of immunological rejection.

STORAGE OF THE ISOLATED HEART‡

J. Stone

In the procedure of cardiac transplantation the donated heart is exposed to ischaemia of varying degree and varying duration and this is progressively damaging to it. The study of methods to preserve cardiac function during this period which was commenced last year has been continued, and a method[1] of storage of canine hearts using hypothermia and an aqueous perfusate has been explored.

The initial perfusate consisted of a modified Krebs solution containing 70,000 MW dextran, hydrocortisone and insulin. Our results using this perfusate in a Langendorf preparation over periods of between 24-72 hours were disappointing. Only some 65% of these hearts could be revived, and then only worked poorly, on rewarming with whole blood from a support dog.

The hearts appeared extremely oedematous and as a rule gained between 30-50% in weight. Histological evidence showed an increased extracellular space, some destruction of myofibrils and capillary damage. It was found that the initial flushing of the heart immediately after removal from the donor with normal saline, Krebs solution or even whole blood, produced increases in weight of an order of 10%. Subsequent experiments showed that following immediate neck implantation these hearts returned to their original weight after about six hours.

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[1] British Medical Journal, Vol. 2 (1968), p. 296.

A number of modifications to this method of storage have led to greater success in reviving the hearts. A 5μ millipore filter was incorporated in the perfusion circuit to remove any crystalline substances from the perfusate and any cellular debris which may accumulate. All parts of the apparatus are now made of stainless steel, stainless steel with teflon coating or silastic (silicone rubber) tubing. Changes have been made in the perfusate and a five-fold increase in the concentration of dextran in the solution has largely abolished the weight gains. A small amount of Aminofusin has been added to provide physiological levels of essential amino-acids and vitamins to supplement the nutrient value of Krebs solution. Also added are heparin, chlorpromazine, which acts as a cell membrane stabilizing agent, and penicillin to act as an antibiotic. A small amount of phenol-red dye has been used to give colour to the solution and to act as an approximate pH indicator. Trimethamine buffer has been used to supplement the phosphate and bicarbonate buffers already present in the Krebs solution without adding appreciably to the total sodium ion concentration.

There has been some difficulty in regulating pH, pO_2 and pCO_2 in the solutions because of the high solubility of the gasses, particularly CO_2, at 4°C. However, these problems have been overcome by the use of increased amounts of buffer and very small amounts of CO_2 when aerating the perfusate. The perfusate has a volume of 1.5 - 2.0 litres and is constantly circulated by a roller pump. Perfusion pressure is 15-20 mm Hg.

The revival rate of these hearts, whether stored for 24 or 48 hours, has been about 90%, an increase of some 25% on the previous method. Although some of these hearts have not beat well many have sustained good co-ordinated beating over a period of hours when used as a neck implant (see p. 35). None have been able however to support the circulation of a recipient.

As it has been previously shown that hearts given periodic flushing only can survive longer than hearts which are left soaking in the perfusate, a study has begun using an automatic valve that will provide only six minutes of perfusion every hour in the hope that it will improve the survival of the hearts. These periodically flushed hearts do not have the accumulation of extracellular fluid noted to follow continuous perfusion.

PERIPHERAL VASCULAR DISEASE

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

In 1953 we commenced a register of patients who had reconstructive surgery for occlusive arterial disease of lower limbs. This year these patients have been reviewed. The review which includes most of the reconstructive arterial operations performed in the Alfred Hospital in this period concerns 490 patients with occlusive arterial disease affecting 560 limbs. The patients concerned have been followed from the time of operation until death, or until final blockage of the graft or treated vessel, or, if still patent, until December, 1968. This period has varied from one to fifteen years. A few patients were lost from the survey.

[1] Honorary Surgeon to Outpatients, Alfred Hospital.
[2] Thoracic Surgical Unit, Alfred Hospital.
[3] Assistant Surgeon, Alfred Hospital.

Aorto-iliac Disease

In aorto-iliac disease the main procedures employed were thrombo-endarterectomy and dacron grafts. The operative mortality was 7 in 123 operations (5.7%). Three years after operation in approximately 80% of the survivors the grafts or treated vessels were patent. However, there was a considerable fall-out due to late deaths, probably not related to the treatment. It appears therefore that successful restoration of vessel patency is likely from treatment of aorto-iliac occlusive arterial disease, but because of a high operative mortality and high death rate in the early post-operative years, operation should only be performed for severe symptoms.

Femoro-popliteal Disease

In femoro-popliteal disease, the procedures used were thrombo-endarterectomy, arterial homografts, teflon grafts, dacron grafts and autogenous vein grafts. The operative mortality was 5 in 383 cases (1.3%). Patency rates for survivors at 3 years were: thrombo-endarterectomy, 38%; arterial homografts, 45%; teflon grafts, 10%; dacron grafts, 17%, and vein grafts 68%. The incidence of late deaths is less than in patients with aorto-iliac disease. It is apparent that the results from vein grafts are much better than from the other procedures which we now consider to be outmoded.

The results in patients with chronic distal ischaemia were less favourable than in those with claudication only. In the aorto-iliac disease group diabetic patients fared worse than non-diabetic ones. However, in the patients with femoro-popliteal disease the results in diabetics at one year are almost the same as for the group as a whole.

Because of the low operative mortality, relatively fewer late deaths and the reasonably good patency rate with the present procedure of vein grafting, operation may be advised in suitable cases with symptoms less severe than would be required if they had aorto-iliac disease.

The conclusions from this survey are gratifying in that they show steady improvement in results with new methods of treatment in femoro-popliteal disease. However, these results are still far from ideal.

HISTOLOGY OF CARDIAC MUSCLE

J. B. Kurtz

(in association with N. C. R. Merrillees[1] and P. M. Robinson[1])

Structural changes in cardiac muscle cells such as hypertrophy and hyperplasia have been noted in disease conditions since cardiac muscle was first studied by light microscopy. In recent years the ultrastructure of this tissue has been investigated by electron microscopy and some changes observed in disease states. Attempts have also been made to combine these various microscopic techniques with chemical reactions so that the localization within the cell of various macromolecules, such as enzymes, can be made.

During the past year papillary muscle and specialized conducting tissue of the ventricle of normal dog and rat heart has been studied both by electron microscopy and by light microscopy of ultra-thin araldite-embedded sections. Similar studies were made on hypertrophied ventricular muscle, ventricular muscle from hyperthyroid dogs and on isolated papillary muscle perfused in calcium-free conditions.

Preparation

Results obtained using buffered paraformaldehyde (1-4% in Pearse phosphate, Tyrode or Dulbecco buffers) as a fixative by coronary artery perfusion were consistently superior to fixation with gluteraldehyde or gluteraldehyde/paraformaldehyde.

Post-fixation with osmium tetroxide (1-2%) was followed by specimen dehydration with acetone and then embedment in araldite.

Morphology

The T tubule system was demonstrated in papillary muscle. Longitudinal and transverse elements of the reticulum system were clearly shown to form a branching network which surrounded the myofibrils and approached closely to T tubules and mitochondria in both papillary and conducting tissue cells.

No structural change was discerned in hearts from dogs with hyperthyroidism.

[1]Department of Anatomy, University of Melbourne.

After left ventricular hypertrophy had been produced by constricting the ascending aorta thickening of some Z-bands was seen in papillary muscle cells.

When strips of papillary muscle kept under tension were perfused in calcium-free Tyrode solution which contained EGTA separation of intercalated discs and damage to mitochondria was seen. These changes were absent in control strips which had been perfused with Tyrode solution and under similar tension.

Histochemistry

Histochemical studies to demonstrate acetylcholinesterase and monoamineoxidase activities revealed specific acetylcholinesterase activity on the plasma membranes of axons running in the conduction tissue and associated with the basement membrane of the specialized muscle cells nearby. Pseudocholinesterase activity was demonstrated in association with mitochondrial and nuclear membranes. On the outer membrane of some mitochondria in both papillary muscle and conduction tissue cells monoamine oxidase activity was seen.

BLOOD COAGULATION

P. Fantl

Vitamin-K Dependent Clotting Factors

Because thiol compounds, of which 2,3-dithiopropanol (BAL) is particularly potent, delay the one stage prothrombin time of mammalian plasma it was concluded (Fantl and Nance, 1947) that the activity of prothrombin (factor II) is dependent on S-S centres in the molecule.

However, since these experiments were carried out it has been recognised that other plasma components (factors V, VII, X) in addition to prothrombin are essential for extrinsic thrombin formation and that vitamin K is required for the production of factors II, VII, IX, X. If the factors II, V, VII, X are reduced interaction with tissue thromboplastin and calcium ions will result in diminution or delay in thrombin formation. Similarly, reduction of the factors II, V, IX, X will affect thrombin formation in blood. It is therefore necessary to reassess the validity of the original conclusion and to determine which of the several factors involved are affected by thiol compounds.

Our experiments now indicate that all of the four vitamin-K-dependent factors (II, VII, IX, X) are reduced by BAL or dithiothreitol to SH derivatives. However, in the presence of the reducing agent in very diluted plasma factor II (prothrombin) can be converted into thrombin with a reasonable yield provided that active factors VII and X are present. This implies either, that prothrombin is reactive in the reduced -SH form but that the -SH forms of factors VII and X are not, or that -SH prothrombin but not -SH factors VII and X is reoxidised under the condition of the prothrombin assay. The reduced state of factors II, VII, and X in these experiments was demonstrated by blocking their -SH groups with N-ethyl maleimide to produce products which were inactive as thrombin precursors or in special tests. Reoxidation of the reduced form of all of the four vitamin-K-dependent clotting factors could be achieved by absorbing the -SH form on barium sulphate and then eluting with citrate.

The -SH forms of factors VII and X are inactive and produce neither active thromboplastin when combined with brain factors for factor VII estimation nor thrombin when combined with bovine prothrombin, Russell's Viper venom and calcium ions for factor X estimation.

Although these observations underline the close chemical relationship of the four vitamin-K-dependent clotting factors the quantitative differences between the recovery of -SH derivatives to active compounds indicate definite structural differences between them. The original conclusion that factor II has active -SH centres can now be extended to factors VII, IX, and X.

PHYSIOLOGY AND PHARMACOLOGY OF SMOOTH MUSCLE*

W. G. Nayler and J. Chan

BRONCHODILATOR EFFECT OF ISOPRENALINE

Na^+/K^+

So far these experiments have shown that if the Na^+ concentration in fluid bathing isolated segments of bronchial smooth muscle is allowed to exceed 178 mM, whilst tonicity is maintained by deleting sucrose, the bronchodilator effect of isoprenaline is significantly ($p < 0.001$) reduced. This is not a species specific phenomenon and has been shown for guinea pig, rabbit and rat bronchiolar muscle. Current experiments indicate that the Na^+/K^+ ratio is a vital factor in determining how effective isoprenaline is in relaxing bronchiolar muscle.

pH

pH has an important effect on the ability of bronchiolar muscle to relax in response to added isoprenaline. If the pH is allowed to fall below 6.3 then the relaxing effect of isoprenaline and of orciprenaline is greatly ($p < 0.001$) reduced.

Steroids

DOCA and 9-α-fluorohydrocortisone, when present in microgram amounts, potentiate the dilator actions of isoprenaline on guinea pig and rabbit bronchiolar smooth muscle. Progesterone is less effective ($p < 0.01$) than 9-α-fluorohydrocortisone but still does potentiate the dilator effect of isoprenaline and orciprenaline.

SALBUTAMOL

This drug is a β-agonist. It has approximately 1/500 the action of isoprenaline on cardiac β-receptors and has little effect on β-receptors in the peripheral circulation, including those in coronary blood vessels. It is a potent bronchodilator and current experiments suggest that bronchiolar smooth muscle may develop tachyphylaxis to it. Its bronchodilator action has been shown to be potentiated by 9-α-fluorohydrocortisone.

CHEMICAL CARCINOGENESIS**

P. E. Hughes and R. Pilczyk

Chemical carcinogens react with many macromolecules in cells and alter the cell in some way so that it escapes from growth control and gives rise to a cancer.

It is generally considered that binding to DNA is of importance in carcinogenesis for it could give rise to faulty base-pairing when the cell divides and so alter genetic code in the cell. Although cells have an efficient mechanism for excising and repairing defective DNA after division with faulty DNA base-pairing the DNA-base-carcinogen complex would be replaced by a different, but naturally occurring, base and so be outside the scope of the DNA repair mechanism.

For a substance to be carcinogenic *per se* this hypothesis requires that it must (a) bind covalently to DNA, (b) inhibit the repair enzyme mechanism, (c) induce a proliferative response whereby faulty base-pairing occurs and the DNA change so induced cannot then be excised and repaired. In this scheme carcinogen binding to RNA and protein would be incidental. The studies reported here relate to (a) and (c).

Binding of 2-Naphthylamine

2-Naphthylamine is carcinogenic for livers in strain CBA but not C57 mice and not for mouse kidney. A study of the binding of tritiated 2-naphthylamine to liver and kidney DNA, RNA and protein of each strain shows that binding occurs to all macromolecules but higher levels are found in liver than in kidney and in the susceptible than in the resistant strain. Binding to DNA of liver persists for at least three months after injection but that to protein and RNA rapidly decays.

Autoradiographic studies show a selective binding to the central parts of the liver lobules.

Binding of 3'Methyl-4-Dimethylaminoazobenzene

The dye 3'MeDAB is a potent liver carcinogen in the normal rat and is even more carcinogenic to regenerating rat liver. Tritiated 3'MeDAB has been prepared and injected into normal rats and into rats immediately after partial hepatectomy. DNA, RNA and proteins have been prepared from these rats when killed at various intervals after injection and these have been assayed for bound radioactivity.

It is hoped that when completed this study will establish correlation between levels of binding and carcinogenicity.

Proliferative Response in Aminoazo Dye Carcinogenesis.

Hooded Wistar rats have been fed 3'MeDAB for from 1-6 weeks and then observed for subsequent tumour development. Under the conditions used five or more weeks of dye-feeding was necessary for maximum tumour development. Estimations of levels of protein-bound dye did not correlate in time with the necessary period of dye-feeding but mitotic figure counts on the livers showed that the time of dye-feeding necessary to produce tumours was also that required to produce a proliferative response in the rat livers.

Mitotic Responses to Partial Hepatectomy

In normal rats partial hepatectomy produces a peak in mitosis in the remaining liver at 28 hours. A further subsequent partial hepatectomy is performed either before or after the subsequent proliferative response. Partial hepatectomy prior to this proliferative response gives a peak in mitosis at 24 hours while partial hepatectomy after this proliferative response gives a peak in mitosis at 60 hours.

The dye 2-methyl-4-dimethylaminoazobenzene (2MeDAB) is normally noncarcinogenic to rat liver. Although it binds to macromolecules it does not induce a proliferative response on continued feeding. If however, a proliferative response is supplied by partial hepatectomy at the start of the 2MeDAB feeding, tumours result. Partial hepatectomy in rats fed 2MeDAB gives a mitotic peak at 28 hours. However, a second partial hepatectomy in rats which had previously had partial hepatectomy immediately followed by feeding of 2MeDAB induced a mitotic response with a peak at 60 hours.

Thus, normal rats and rats subjected to carcinogen for a period insufficient to produce tumours showed a mitotic response to partial hepatectomy with a peak at 28 hours while partial hepatectomy in rats whose exposure to aminoazo dyes is such as to produce tumours showed a delayed type of mitotic response to partial hepatectomy with a peak at 60 hours.

Aminoazo Dyes in Partially Hepatectomized Animals

Partial hepatectomy at the commencement of dye-feeding converts the non-carcinogenic dye 2MeDAB into a carcinogen. Similarly the carcinogenic dye 3'MeDAB is even more effective as a carcinogen in regenerating liver. Experiments are being carried out in which regenerating liver is subjected to 3'MeDAB for short periods to see if there are differences in tumour incidence if the dye is present during DNA replication or actual cell division. Other feeding experiments are being made to try to elicit information as to whether 2MeDAB inhibits DNA repair mechanisms.

Conclusion

The hypothesis presented needs much further testing. Binding of carcinogens to DNA is well documented and much evidence is accruing regarding the role of a proliferative response and experimental studies of inhibition of DNA repair by carcinogenesis will be very important. It appears that these three aspects may be separable in many instances and, if so, current tests to identify carcinogens could be quite inadequate because many substances could be carcinogenic for rapidly growing tissues but not for mature tissues as they themselves could not initiate the necessary proliferative response.

SCLERODERMA

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

The review of the clinical types, visceral involvement, and course of scleroderma mentioned in the last report has been completed.

A proportion of these patients have shown positive tests indicating an auto-immune process in scleroderma, and we have suggested that auto-immunity may play a part in some of the clinical manifestations.

In two fatal cases studied this year, heavy deposition of IgM. has been revealed in renal arterioles and glomeruli by an immuno-fluorescence technique. In view of this we are considering a trial of more vigorous immuno-suppressive treatment in patients with scleroderma type III which has a poor prognosis.

Plasma Viscosity

It has previously been demonstrated that vascular phenomena in scleroderma are associated with narrowing of small vessels. It was postulated that this might be due to deposition of material from the blood, and that this deposition would be more likely to occur if the blood flow were slowed by increased viscosity.

The kinematic viscosity of the plasma at 37°C and 20°C was measured therefore in a group of 12 patients with scleroderma and 12 control subjects. Parallel observations were made on the levels of the various plasma proteins in these patients. Evidence for dermal auto-immunity was looked for in 20 patients.

No difference of kinematic plasma viscosity at 37°C, 20°C or the change in viscosity with temperature between the patients with scleroderma and the control subjects was demonstrated. Compared with the control subjects, scleroderma patients had lower total plasma protein and serum albumin levels, but no significant differences were demonstrated in respect to the other plasma proteins.

No dermal auto-immunity peculiar to scleroderma was demonstrated in the skin biopsies.

[1]Associate Professor, Pathology, Monash University.

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LECTURES DELIVERED DURING 1969

"The Unnatural History of Hypertension" — *Alfred Hospital Clinical Society.*	A. J. BARNETT & F. G. SILBERBERG
"Observations on the Chronotropic Actions of ICI 50,172" — *Australian Physiological and Pharmacological Society, Melbourne.*	J. CHAN
"The Effect of Ouabain on the Distribution of Ionized Calcium in Cardiac Muscle Cells" — *Australian Physiological and Pharmacological Society, Melbourne.*	D. CHIPPERFIELD
"Observations on the Coronary Vasodilator and Positive Inotropic Effect of Glucagon" — *Australian Physiological and Pharmacological Society, Melbourne.*	W. G. NAYLER
"Calcium and the Heart" — *Physiology Department, University of Melbourne.*	W. G. NAYLER
"Mechanisms of Contraction in Normal and Failing Heart Muscle" — *Royal Prince Alfred Hospital, Sydney.*	W. G. NAYLER
"β-Adrenergic Blockade and Myocardial Function" — *Royal Melbourne Hospital, Melbourne.*	W. G. NAYLER
"A Correlation between Drug-induced Changes in Myocardial Function and the Availability of Ionized Calcium" — *Cardiac Society of Australia and New Zealand, Brisbane.*	W. G. NAYLER & T. E. LOWE
"The Effect of Dopamine on Coronary Vascular Resistance, Myocardial Function, Ventricular Efficiency and High Energy Phosphate Stores" — *Australian Society of Clinical and Experimental Pharmacologists, Melbourne.*	W. G. NAYLER I. McINNES & J. STONE
"The Coronary Vascular Effects of Glucagon" — *American Heart Association, Dallas, U.S.A.*	T. W. MOIR & W. G. NAYLER
"The Effect of Kinekard on the Coronary Circulation of the Isolated Heart" — *American Society of Experimental Biology, Atlantic City, U.S.A.*	T. W. MOIR & W. G. NAYLER
"Interplay of various Factors affecting Coronary Blood Flow" — *Australian Physiological and Pharmacological Society, Melbourne.*	F. R. TRINKER
"Pharmacological Agents affecting Myocardial Function" — *A.N.Z.A.A.S., Adelaide.*	F. R. TRINKER

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

Revenue Account for the Year Ended December 31, 1969

EXPENDITURE	
Salaries and Wages	$143,566
Laboratory Supplies and Isotopes	25,199
Library Maintenance	6,801
Postage and Telephone	1,605
Printing and Stationery	2,283
Light and Power	13,000
Insurance	4,877
Repairs and Renewals	4,964
Animal House Contribution	4,000
Sundries	3,466
Travelling Expenses	590
Surplus for Year	258
	$210,609

INCOME		
Donations —		
Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions		$127,071
Grants-in-Aid of Research —		
Asthma Foundation	$4,310	
National Heart Foundation of Australia	19,185	
Anti-Cancer Council of Victoria	9,066	
Life Insurance Medical Research Fund of Australia and New Zealand	18,338	
The James and Elsie Borrowman Research Trust	4,500	
The William Buckland Research Fund	—	
		55,399
Interest from Investments —		
Held by Trustees of The Baker Institute Grant Trust	$1,700	
Other Income	9,839	
		11,539
Sundry Sales, Recoveries and Refunds		16,600
		$210,609

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

Balance Sheet as at December 31, 1969

FUNDS AND LIABILITIES		
Funds —		
Accumulated Revenue brought forward	$107	
Add, Surplus for year	258	
Accumulated Revenue	$365	
Restricted Funds	57,086	
Endowment Funds	356,004	
Development Fund	69,151	
William Buckland Research Fund	16,200	
Laura Nyulasy Research Scholarship Fund	3,940	
Lang Research Scholarship Fund	3,860	
		$506,606
Current Liabilities —		
Sundry Creditors and Accrued Expenses		1,516
		$508,122

ASSETS		
Fixed Assets (Note 1)		
Investments —		
Held by the Trustees of the Institute:		
Commonwealth Inscribed Stock	$1,907	
M.M.B.W. Stock	7,202	
S.E.C. Stock	6,556	
Treasury Bonds	6,000	
Argo Investments Co. Ltd. Shares	27,738	
Softwood Products Treatment Co. Pty. Ltd.	100	
Short Term Deposits	77,451	
	$126,954	
Held by The Trustees, Executors & Agency Co. Ltd.:		
Laura Nyulasy Research Scholarship Fund	3,940	
William Buckland Research Fund	16,200	
Endowment Fund	303,298	
		$450,392
Current Assets —		
Cash at banks on current and deposit accounts	$55,123	
Sundry debtors and prepayments	2,607	
		57,730
		$508,122

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 has been charged against appropriate funds, grants or revenue accounts.
   The insured value of all assets at December 31, 1969, other than the building, totalled $120,000. The cost of the present building to December 31, 1969, totalled $1,300.000.
2. Retention monies paid to December 31, 1969, in respect of the contract for a new building amount to $45,800 and have been deposited in a bank account. The release of these funds to the builders or sub-contractors is dependent on the satisfactory completion of the contracts.
3. In addition to receiving income from investments shown above, the Institute receives interest on $34,000 5% Commonwealth Inscribed Stock which is held by the Trustees of The Baker Institute Grant Trust for the benefit of the Institute.

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

In our opinion, the above balance sheet, together with the notes thereto, is properly drawn up to show a true and fair view of the state of the Institute's affairs at December 31, 1969.

PRICE WATERHOUSE & CO.,
Chartered Accountants.

Melbourne. March 10, 1970.

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

Year Ended December 31, 1969

DEVELOPMENT FUND

Balance at December 31, 1968		$286,904
Add —		
Transfer from Appeal	$2,000	
Income from Investments	9,121	
		11,121
		$298,025
Deduct —		
Building Costs	$185,804	
Furnishing and Equipment Costs	43,070	
		228,874
Balance at December 31, 1969		$69,151

RESTRICTED FUND

Balance at December 31, 1968		$13,819
Add —		
Donations	$67,637	
Income from Investments	2,128	
		69,765
		$83,584
Deduct —		
Transfer to The Trustees, Executors and Agency Company Limited	$6,000	
Transfer to Revenue Account	14,467	
Equipment Costs	6,031	
		26,498
Balance at December 31, 1969		$57,086

ENDOWMENT FUND

Balance at December 31, 1968		$79,319
Add —		
Baker Benefactions	$156,919	
Transfer from Appeal	131,000	
Donations	8,088	
Income from Investments	2,419	
		298,426
		$377,745
Deduct —		
Transfer to Revenue Account		21,741
Balance at December 31, 1969		$356,004

DONATIONS

During the year $133,000, which is included in the financial statements, was derived from the Appeal and has been acknowledged elsewhere.

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

William Buckland Foundation (Trustees Executors & Agency Co. Ltd.)	$6,000
Edward Wilson Estate	5,000
James & Elsie Borrowman Research Fund (Trustees Executors & Agency Co. Ltd.)	4,500
Estate of H. & L. Hecht (Hedderwick Fooks & Alston)	4,000
Appel Family Estate (Trustees Executors & Agency Co. Ltd.)	3,700
The William Angliss (Victoria) Charitable Fund	3,000
Estate of the late Alfred Edments	750
Marion and E. H. Flack Trust	700
George F. Little Trust (Equity Trustees Executors & Agency Co. Lt.)	474
The Ian Potter Foundation	410
Mr. and Mrs. F. Crane	250
Mr. Walter P. Ham	200
Mr. and Mrs. Edgar Rouse	110
Pethard Tarax Charitable Trust	100
Mr. Siegfried Meyer	100
Cr. Roy Morgan (Lord Mayor's Fund)	50
George Turner Pty. Ltd.	10
Miss N. E. Cameron	5
	$29,359

DONATIONS, in memory of —

Dr. J. Adam, J. Allendorf, Mrs. M. Archer, Hugh Bartram, V. Butler, W. R. Clarkson, Mrs. D. Cornish, Dr. S. Crawcour, G. J. Cuming, Georgina C. Dixon, Alan Dowes, Alex Downie, M. Fawcett, Mrs. M. Fullager, Mrs. Jane Gardiner, L. C. Giddings, Very Rev. P. Gleeson S.J., W. Harbutt, E. M. Hooper, K. E. Hosken, Bruce Jackson, C. N. Jeffrey, R. H. Keon Cohen, G. Kilburn, Mrs. E. Lefoe, Sir Charles Lowe, Lady Lowe, Dr. C. MacDonald, J. Mardling, Kathleen N. Melville, Maude Monteith, L. J. Morgan, Mary Noble, Constance Oliver, J. E. Page, Colin A. Perry, Betty Poulsen, Harry Poulton, Balcombe Quick, J. F. Ryan, Harry Routh, Emily Elizabeth De Serville, A. M. Sheppard, Mary Reid Smith, Bruce Sutherland, M. Welten, B. P. Werner, A. R. L. Wiltshire, Mrs. S. Wright, F. De Zilva.

were received:

Associated Broadcasting Service Ltd., R. Blakemore, Sir Wm. Bridgeford, Miss N. E. Cameron, M. A. Cuming, Eagle Star Insurance Co. Ltd., Goulburn-Murray TV Ltd., J. C. Habersberger, A. Holper, Misses D. and J. Jeffrey, Kodak (Australia) Pty. Ltd., Kodak Senior Staff Association, S. J. A. Kemp, G. Knox, Nancy McLean, G. Mirgard, Noel Monteith, D. G. L. Newbigin, Alma Parmenter, N. Payne, Edgar Rouse, H. E. H. Tobin.

Totalling $349.50

EWEN DOWNIE METABOLIC UNIT
1969

STAFF

Honorary Consulting Physicians:	EWEN DOWNIE, M.D., F.R.C.P., F.R.A.C.P. BRYAN HUDSON, M.D., Ph.D., F.R.C.P., F.R.A.C.P.
Honorary Consulting Biochemist:	JOSEPH BORNSTEIN, D.Sc., M.D., F.R.A.C.P.
Physician-in-Charge:	PINCUS TAFT, M.D., F.R.A.C.P.
Honorary Physician:	HARALD BREIDAHL, M.D., M.R.C.P., F.R.A.C.P.
Registrar:	R. DARGAVILLE, M.B., B.S.
Biochemists:	DORA WINIKOFF, M.Sc., F.A.A.C.B. JUNE SHEATH, M.Sc., F.A.A.C.B. J. S. C. CHAN, B.Sc.
Technical Staff:	Mr. W. HUDSON. Miss I. EKKEL. Miss R. WITCHELL. Mrs. J. UPTON. Mrs. F. RABOLD.
Secretary:	Mrs. N. STEWART.

DIABETIC CLINIC

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

RESEARCH FELLOW

Burroughs Wellcome Research Fellow: J. HERINGTON, B.Sc.

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

It is with a great deal of satisfaction that this research report comments on the change in name of the Unit to the Ewen Downie Metabolic Unit. This change honours the man whose vision and effort was responsible for its establishment. Dr. Downie's continued active interest in the Unit, and his counsel in its activities, is highly valued. He was personally closely involved in the early studies in the Diabetic and Metabolic Unit of the insulin inhibitors referred to in this report.

His work and influence is perpetuated in current investigations since in the past twelve months the activities of the Unit have become involved again with studies of circulating inhibitors of insulin. These were shown in earlier work in the Unit to have been dependent for their presence *in vivo* on an intact pituitary. Work undertaken by Professor Bornstein since taking his position at Monash has extended knowledge of these substances and their action. It has been shown that a fragment of growth hormone of molecular weight approximately 2000 (code named In-G) has effects *in vitro* which mimic the diabetogenic action of intact growth hormone — impaired glucose uptake by liver and muscle, increased lipolysis and diminished lipogenesis. A further fragment of growth hormone (code named Ac-G) has the capacity to negate these effects.

During the last year *in vivo* studies in human volunteers — diabetic and non diabetic — have been undertaken using Ac-G. As is recounted in the research report a fall in blood glucose was induced in the diabetic patients and an increment in the hypoglycaemia produced by insulin in the normal patients. These results have been regarded as suggesting a possible role for fragments of growth hormone in the maintenance of blood sugar levels normally and in the genesis of the hyperglycaemia of diabetes.

Further work is planned for the coming twelve months in learning more of the human pharmacology of Ac-G. Dr. P. Zimmet, a former registrar in the Unit, is to work as a N.H.M.R.C. research fellow on this project.

This avenue is one of a number currently being explored in all parts of the world in the search for aetiological factors in the genesis of diabetes — the variety and number of studies being done reflecting the perplexing nature of the problem.

As always, the Unit is indebted to Medical Staff of Alfred Hospital, the clinical departments at Monash University, and the Biochemistry departments at Alfred and Monash for their collaborative help. Again, too, grateful acknowledgement is made to generous donors of grants-in-aid and in kind.

PINCUS TAFT.

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

Alfred Hospital Research Funds.
Burroughs Wellcome & Co. (Australia) Ltd.
Estate of the late Mrs. E. E. Bishop.
Hoechst Pharmaceuticals.
Mr. Bert Hotchkiss.
Monash University.
Syntex Pharmaceuticals Ltd., Berkshire, U.K.
William R. Warner & Co. Pty. Ltd.

THE DIABETOGENIC ACTION OF GROWTH HORMONE

P. Taft and J. Bornstein[1]

For a number of years it has been known that certain serum fractions are capable, *in vitro*, of inhibiting the action of insulin on various tissues — muscle, liver and fat. These studies have been made since recognition of the fact that insulin is commonly present in the plasma in diabetes — not infrequently in above normal amounts after a glycaemic stimulus. Growth hormone is a known diabetogenic stimulus, capable of inducing permanent diabetes in a substantial percentage of animals to whom it is administered chronically. In the past few years work done at Monash University by Bornstein *et al* has resulted in a fragment of growth hormone being prepared by acid hydrolysis (In-G) which has been shown to possess all the diabetogenic effects of the whole growth hormone molecule. A further fragment also separated from growth hormone by acid hydrolysis (Ac-G) inactivates the diabetogenic fragment.

The latter material, prepared from sheep growth hormone, has been given intravenously to five volunteer untreated diabetics in whom it was shown that endogenous insulin was present. In all patients a fall in blood sugar was achieved, with a mean fall of 51 mgm% from fasting levels at 80 minutes after injection, the fall in blood sugar beginning 20 minutes after the injection of Ac-G. Insulin levels were unchanged. These results suggested that the action—under the circumstances of the experiment—of this growth hormone fragment may have been to have diminished a resistance to the action of endogenous insulin conferred by the presence of an excess of the inhibitory fragment.

Support for this hypothesis has been gained from a study in six normal volunteers. A small dose of insulin was given intravenously and the hypoglycaemic effect observed. On a subsequent occasion the same insulin dose was given and concomitantly a dose of the acceleratory fragment of growth hormone was administered intravenously. A mean increment of 17mgm% in the maximum hypoglycaemic effect of insulin was observed at 30 minutes after insulin injection with Ac-G, the degree of hypoglycaemia being increased from 10 minutes after injection and the duration of the hypoglycaemic action of insulin being extended by some 20 minutes.

Further studies are planned to examine the dose/effect relationship, possible modes of administration, and effects in different diabetic states over longer periods of administration.

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[1] Professor of Biochemistry, Monash University.

CONTRIBUTION OF GLYCEROL TO PLASMA GLUCOSE

Jenny Herington, P. Taft and J. Bornstein

Fifty years ago it was shown that an oral glycerol load would increase the blood sugar concentration in normal subjects and in some diabetics. However, since then little, if any, work has been carried out to measure the extent of this conversion and its importance as a source of glucose for metabolism.

This project, then, is designed to measure:—

(a) The turnover rate of plasma glycerol
(b) The turnover rate of plasma glucose
(c) The amount of glycerol converted to glucose in a given time
(d) The percentage of plasma glucose derived from glycerol under particular conditions.

It is proposed to compare these parameters in normal subjects with those of maturity onset diabetics prior to, and during, treatment with the Growth Hormone fractions suspected of controlling this form of diabetes.

The technique for making these measurements involves a single I.V. injection of either C^{14} glucose or C^{14} glycerol. Samples of blood are then taken periodically and the amount of radioactivity in the glucose and glycerol pools is determined.

Most of this year has been spent developing methods for the isolation of glucose and glycerol from the plasma and estimation of their concentration and radioactivity.

Glucose and glycerol are assayed in a protein-free plasma extract by specific enzyme reactions linked to pyridine nucleotide dehydrogenase reactions.

C^{14} glycerol is isolated from the protein-free plasma by thin layer chromatography on silica gel after desalting and concentration steps. The concentration of glycerol eluted from the silica gel is assayed and from this the recovery of plasma glycerol is calculated.

C^{14} glucose is isolated from the protein-free plasma by the preparation of its penta-acetate derivative. The recovery is calculated from the weight of glucose penta-acetate produced.

It is important to calculate the recovery of both glycerol and glucose since it is not constant using these methods and allowances must be made for differences. C^{12} and C^{14} recoveries however are identical in any one sample.

Preliminary experiments have been carried out on rats, rabbits and dogs. From these we can say that —

(a) Plasma glycerol turnover time is very fast (under 10 minutes).

(b) Plasma glucose turnover time is much slower than that of glycerol (approximately 2-4 hours).

(c) Approximately 40% - 60% of the metabolized glycerol is converted to glucose.

(d) Under fasting conditions, close to 100% of the plasma glucose is derived from glycerol.

At the time of writing this report, we are performing these experiments on normal volunteers and it appears that the results are similar to those above.

CARBOHYDRATE METABOLISM IN URAEMIA

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

There has accumulated in the last half century a substantial amount of evidence for a deranged glucose tolerance in the uraemic patient. The origin of this aberration, however, is still surrounded by a great deal of controversy.

The delayed rate after oral glucose of fall of insulin and glucose levels in the uraemic patient after a normal rate of rise has stimulated our interest to examine the role of insulin in the observed impaired carbohydrate tolerance.

The influence of urea on glucose uptake and utilisation has been investigated under *in vitro* conditions simulating a uraemic environment. Work has been performed, using rats, to study the effect of insulin, in the presence and absence of urea in the incubation media, on C^{14} glucose uptake into muscle, liver and epididymal fat pads, and C^{14} glucose incorporation into muscle and liver glycogen and fat pad lipid, and fatty acid, have been measured.

Initial results from such radioactive experiments have indicated little or no effect of urea on the C^{14} glucose uptake into the muscle and liver, and, in fact, there was a slight enhancement of the action of insulin in the urea containing media. Experiments with the fat pads yielded contrary results however, and showed an inhibition of insulin action by urea. This work is being extended to confirm preliminary results. It is proposed to later look at the pancreases of these rats and observe insulin production under various conditions. It is hoped from such *in vitro* studies to obtain information of the site of the biochemical disturbance. Questions to be borne in mind are:

Is there a defective hepatic glycogen storage, a defective glucose uptake by peripheral tissues, or does there exist an antagonist(s) to the action of insulin? Whilst all these questions are asked, it must not be forgotten that the insulins assayed immunologically may not be a true reflection of their biological activity.

Such *in vitro* preliminary studies will progress to using normal and artificially induced uraemic rats, and studying the glucose uptake by *in vitro* and *in vivo* methods in them.

It is then proposed, after work on the animal is done and necessary information is obtained, to proceed to the uraemic patient, uncomplicated by diabetes or other carbohydrate metabolic defects not due to his uraemic condition, and establish the role of insulin in glucose intolerance in ureamia in man.

THYROID INVESTIGATIONS

Dora Winikoff (with technical assistance of Rosalind Witchell and Janis Upton)

THE THYROID AND THE PILL

A survey of patients suspected of having thyroid disease while taking oral contraceptives was carried out in preparation for a paper delivered during an overseas thyroid conference.

It transpires that the mean values for all thyroid parameters used by us were significantly different for the euthyroid, hyperthyroid and hypothyroid groups.

Nevertheless the diagnosis in a single patient requires a full "thyroid profile" of tests — in our case the assay of P.B.I., T_3 — resin uptake, Electrophoretic Index, in some instances the serum thyroxine assay, and the measurements of the total binding capacity of thyroxine binding globulin (TBG) being employed.

With the advent of new contraceptive preparations containing small doses of oestrogen, the deviation of thyroid indices from normality is less conspicuous and often confined to only one parameter. In the case of thyrotoxicosis the P.B.I. or serum thyroxine are still significantly elevated; the presence of hypothyroidism is harder to detect and may require also the TSH Stimulation Test.

Our trial of the long-term use of the oral contraceptives continues. An interesting observation is the occurrence of an "adaptation" state in some women whose thyroid function tests return to normal levels in spite of the intake of the same dose of oestrogen.

TOTAL THYROXINE ASSAY

The method of competitive binding based on the "Tetrasorb" Kits (Abbott Laboratories) was further modified in order to eliminate the three washing steps and perform the final count automatically. At the final stage after the ice bath incubation, 0.5 ml of water is added before the withdrawal of the plastic plunger and the contents are well mixed. Using a bulb pipette with a curved outlet, the sponge is depressed and 0.5 ml of supernatant withdrawn and placed in an automatic counting tube. The number of counts are multiplied by a factor of three. A suitable standard curve is prepared, in the same manner.

The recovery varies with the extraction procedure. We obtain 85% (mean) while using 95% alcohol. This value can be increased up to 100% when alcohol-ammonia (95% alcohol, 4% water and 1% ammonia), in proportion of 4:1 to serum, is employed.

Since the thyroxine iodine (using 95% alcohol) closely follows our P.B.I. value, we use this method of extraction without correction. Our normal range is from 5-12μg% with a small overlap of 1μg% at the upper limit. Up to date we have performed with our modification over 600 assays in duplicate with satisfactory results at all hormonal levels.

TRIIODOTHYRONINE-RESIN UPTAKE

The method developed in the Unit (1967) has been further modified. Instead of weighing

out the amberlite resin we deliver it with a bulb pipette from a suspension in saline containing Teepol (detergent), under continuous stirring. This not only simplifies the procedure but improves the precision of the method. We now use T_3^{125} with a longer half life. However, this has the disadvantage that our normal range has been narrowed, the lower limit having risen by 3%. This we attribute to radioactive impurities which are trapped on the resin.

At the request of the Commonwealth X-Ray and Radium Laboratory, we also evaluated commercially available Kits for T_3-Resin Uptake assay, namely "Thyopac" (Radiochemical Centre, England), "Charcoat" (Nuclear, New England, U.S.A.), and "Trilute" (Ames, Israel).

Our findings indicate that the first two have the great disadvantage of being awkward to handle, and there is the difficulty of avoiding spilling the radioactive substances on the operator's fingers. The results of the T_3 tests are certainly no better and the reproducibility far worse than we obtain with our own method.

The "Trilute" Sephadex Kits, however, can be highly recommended for the simplicity of operation and a very wide range of normal values. On the negative side, the mode of delivery of T_3^{125} from a plastic squeeze bottle is laborious, and the small amount of serum required (0.05 ml) tedious to deliver with accuracy. If these small disadvantages were overcome, we would regard the "Trilute" Kits as the most suitable of all commercially available products, particularly if only a small number of assays are involved.

TOTAL BINDING CAPACITY OF THYROXINE BINDING GLOBULIN (TBG)

This assay, recently introduced in the Unit, is essentially based on our "Electrophoretic Index" (E.I.) technique, but the residual binding capacity of TBG is saturated by the addition of a 100μg% of thyroxine to serum. This comprises the radioactive tracer as well as the cold T_4. The manipulation requires the utmost precision, and we measure out the small quantities of hormones with "Oxford" automatic pipettes.

The age of the radioactive thyroxine (Abbott Laboratories) is also of great importance. The result tends to have an upward trend if a batch of T_4^{125} is used longer than four weeks after its issue.

An ordinary E.I. of a standard thyrotoxic pooled serum and of a normal "loaded" serum is always included in each run, and the results are corrected to the mean values for these sera based on multiple runs. The normal range (preliminary) is from 30-40 μg thyroxine per 100 ml of serum.

TBG binding capacity =

$$(\text{serum thyroxine} + \text{added thyroxine}) \times \frac{\text{E.I.}}{100}$$

Elevated or decreased TBG values in patients taking drugs or hormones can help greatly in diagnosing thyroid disease when R.U. and E.I. are only marginally affected and remain in the normal range of values.

After perfecting this technique we hope to be able to utilize it in studying the puzzling month-to-month fluctuations in thyroid parameters during intake of oral contraceptives.

MATERNAL AND FOETAL FAT METABOLISM DURING LABOUR AND AT DELIVERY

J. Sheath, J. C. Grimwade[1], E. C. Wood[1], P. Taft, M. Bartlett[1]

As reported last year, the study of maternal and foetal fat metabolism during labour and at delivery is being carried on in conjunction with the Department of Obstetrics and Gynaecology at Monash University.

[1] Department of Obstetrics and Gynaecology, Monash University.

Reproducible and sensitive ultra micromethods for the assay of plasma glycerol and non-esterified fatty acids (NEFA) have been set up and are now being routinely employed for these assays in maternal and foetal plasma samples.

Sample collections have been made during labour in maternal venous and in foetal scalp

blood, also at delivery in umbilical arterial and umbilical venous blood.

Primarily it was necessary to determine certain data. The technique of cord blood collection was investigated in 20 subjects, using liquid or dried heparin, to ascertain whether a significant difference was produced in the values of glycerol and NEFA. No significant difference was produced thus eliminating any possibility of error due to variation in collection technique. Also the influence of anaesthetics on the blood levels of glycerol and NEFA was studied in six non-pregnant patients at three stages of anaesthetic administration. (This was done with the helpful co-operation of Dr. M. Griffith, Director, Department of Anaesthesia, Alfred Hospital.) Blood samples were taken immediately before anaesthetic administration, and at 15 and 30 min. after administration. A variable response was found, but the glycerol:NEFA ratios remained fairly constant between zero time and 15 min. after the anaesthetic was administered. This was done as a control against the proposal to make some studies during Caesarian section.

So far the following points have emerged in this study regarding plasma glycerol and NEFA concentrations:—

(a) It has been shown that maternal levels of these substances are three to four times higher than the foetal levels.

(b) The cord venous value is statistically significantly higher than that in cord arterial blood.

(c) The arterio-venous difference appears to be greater with increasing foetal maturity.

(d) Foetal levels (scalp capillary blood) taken 0.5-3 hrs. before delivery are similar to cord arterial levels.

(e) Maternal levels appear to fluctuate to a greater extent in repeated estimations in the same patient than do foetal levels, but fewer data have been obtained for the foetus than the mother.

(f) So far, no obvious correlation between maternal and foetal levels of glycerol and NEFA has been demonstrated.

(g) Finally, preliminary data suggest that there is a higher ratio of glycerol to NEFA in the maternal than in the cord blood, and this ratio is lowest in foetal scalp blood. Theoretically, it could be anticipated that if the plasma NEFA concentration only represented the turnover of triglycerides, one molecule of glycerol and three molecules of NEFA would be liberated. However, turnover of lipids other than triglycerides could contribute to the plasma NEFA concentration thereby producing a glycerol:NEFA ratio greater than 1:3.

LUTEINISING HORMONE BIOASSAY AND THE PREPARATION OF F.S.H.

I. Ekkel and P. Taft

During the past year work has continued on the examination of the uterine weight augmentation assay introduced by Donini. Satisfactory assays have been performed using ovine F.S.H. as the uterine primer. Although there is a significant L.H. contamination in the material we have used (N.I.H. Standard), ovine L.H. in these quantities does not appear to invalidate the assay.

Supplies of F.S.H. now available have too high an L.H. contamination and it has become necessary to prepare our own F.S.H. Ovine F.S.H. is being prepared by the method of Jiang and Reichert (B.B. Acta **93**, 436, 1964) using ammonium sulphate fractionation and in addition urea treatment of the extract at 40° for 24 hours.

Human material is being prepared from post-menopausal urine. The sample is extracted by the Johnson method followed by Albert's B extraction. This extract is run on a G100 sephadex column followed by further sephadex separation with sodium dodecyl sulphate or with pretreatment by carboxymethyl cellulose or DE 32.

Preliminary results suggest that biologic activity is found in the F.S.H. separated and that there is an acceptably low L.H. activity.

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"Pitfalls in the Management of Diabetes" — *Box Hill & District Hospital Clinical Society.*	H. D. BREIDAHL
"Results of I^{131} Treatment for Thyrotoxicosis" — *Alfred Hospital Clinical Society.*	H. D. BREIDAHL
"Recent Advances in the Management of Diabetes" — *Pharmaceutical Society of Victoria.*	H. D. BREIDAHL
"Results of ^{90}Y Implantation at Alfred Hospital" — *Monash University Medical School, Alfred Hospital.*	H. D. BREIDAHL
"Food" —*Australian Chiropody Association.*	H. D. BREIDAHL
"Diabetes" — *Melbourne Medical Post-Graduate Committee, Sale.*	H. D. BREIDAHL
"The Pathogenesis of Diabetic Acidosis" — Symposium "Recent Advances in Diabetes" — *Royal Australasian College of Physicians.*	PINCUS TAFT
"The Pituitary and Diabetes" — *Centenary Scientific Programme, Prince Henry's Hospital, Melbourne.*	PINCUS TAFT
"Diabetes and Obesity" — *Post-Graduate Seminar, Dietetic Association, Victoria.*	PINCUS TAFT
"Cushing's Syndrome — A Follow-up Study of Response to Treatment" (with F. I. R. Martin, R. A. Melick) — *Royal Australasian College of Physicians.*	PINCUS TAFT
"Recent Advances in Endocrinology" —*Society of Hospital Pharmaceutical Chemists of Australia.*	PINCUS TAFT
"The Basis of Thyroid Function Tests," Symposium on "The Thyroid" — *Alfred Hospital Clinical Society.*	PINCUS TAFT
"New Trends in Thyroid Function Tests" — *Department of Medicine, University of Singapore, Singapore.*	DORA WINIKOFF
"The Impact of Oral Contraceptives on Routine Biochemical Tests" — *Endoctrine Society, Helsinki, Finland and Tel Aviv, Israel.*	DORA WINIKOFF
'The Diagnosis of Thyroid Disease during Intake of Oral Contraceptives" — *European Thyroid Conference, Athens, Greece.*	DORA WINIKOFF
"The Electrophoretic Index in Diagnosis and Research" — *Thyroid Group, Haifa, Israel.*	DORA WINIKOFF
"The Relative Value of Thyroid Parameters" — *Radioisotope Department of G.S. Medical College, Bombay, India.*	DORA WINIKOFF
"Radioactive Isotopes and Thyroid Function Tests" — *Radioisotope Study Group, Melbourne.*	DORA WINIKOFF
"Electrophoresis Theory and Practice" — *Royal Melbourne Institute of Technology.*	DORA WINIKOFF
"Interpretation of Thyroid Function Tests based on Radioiodine" — *Royal Melbourne Institute of Technology.*	DORA WINIKOFF

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

RETINAL ANGIOGRAPHY

G. W. Crock[1]

The inventions, original observations and various applications which have come out of the Retinal Angiography Unit since it began in 1966 have established Melbourne as a leading centre in this field of clinical research.

In 1967 Parel perfected the automatic retinal camera, which still provides more detailed angiographic data than any other fundus camera in use.

The first detailed observations of circulatory events on the optic disc in health and disease were made in Melbourne and published by the Lancet.

During 1968, stereo-angiography became possible as a routine investigation, again due to Parel's technical skill. The scientific exploitation of this process was begun late in that year.

By early 1969, the validity of stereophotogrammetric analysis of fundus angiographs had been settled and the Melbourne findings became the highlight of the International Symposium on Fluorescein Angiography held at Albi, France, in July.

The angiographic unit now has a large library of material awaiting detailed analysis. It contains hitherto unreported findings of many conditions, but the most notable areas of interest and study concern diabetic retinopathy, tumours of the eye and brain and the optic nerve.

[1]Department of Ophthalmology, University of Melbourne.

ULTRA-THIN SECTIONS FOR HISTOLOGY

A. V. Jackson[2]

The technique, which was described in last year's report, for cutting ultra-thin sections, is progressing well and other hospital departments have shown interest in the fresh light these very thin sections can throw on the morphology of diseases. It seems likely that this will eventually be regarded as part of a "routine service," but in the meantime it is being carried out at a "research level." Perhaps more importantly in the long run, the technique is training our technical staff to handle tissues in the same way as is required for full electron microscopy. I hope that in the relatively near future we will be able to move on to electron microscopy of, at least, renal biopsies.

[2]Morbid Anatomy Department.

TEMPERATURE CHANGES UNDER ANAESTHESIA

Peter A. Lowe[1]

Over the past 10 months changes in oesophageal and skin temperature under halothane, nitrous oxide and oxygen anaesthesia in 25 patients have been studied.

Method

Two electrical thermometers have been constructed to record these temperatures. To measure blood levels of halothane a Pye Unicam gas/liquid chromatograph has been set up. Initially a 5 ft. long column packed with glass beads was used but a 15% dinonyl phthalate on diatomaceous brick dust column, also 5 ft. long, was found to provide more reproducible results. A flame ionisation detector is being used with nitrogen as the carrier gas. It remains to calibrate this equipment and to standardise methods of extracting halothane from the blood samples.

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

It was initially proposed to limit all observations to varicose vein operations, this has not been possible and observations have been made on patients having other operations suitable for halothane, nitrous oxide and oxygen anaesthesia. The pattern of temperature changes does not appear to be altered by this change in plan. This variation has been allowed for in the statistical analysis of the results.

Results

To date the results show a statistically significant fall in oesophageal temperature during the course of the anaesthesia at 25 min. and at 60 min. The observations on skin temperature show an initial rise in all cases and then either no further change till the end of the operation, when the temperature falls toward the pre-operative level, or a gradual fall averaging 0.3°C, till again at the end of the operation there is a more rapid return to pre-operative levels.

THE METABOLISM OF BROMSULPHTHALEIN (BSP)

THE MODIFIED BSP TEST

R. Coates[2], P. Jablonski[1] and J. A. Owen[1].

Many different investigative procedures have been adopted in determining the aetiology and evaluating the severity and prognosis of hepatobiliary lesions. One of these procedures has been the study of the removal by the liver of bromsulphthalein (BSP) after an intravenous injection of this dye. The dye is conjugated with glutathione in the liver and both the conjugated and free forms are excreted in the bile. However, if bile secretion is impaired some conjugated dye enters the circulation. The standard BSP test measures total BSP in plasma, but a study of the relative proportions of free and conjugated BSP may yield a more sensitive test of liver function. A rapid method for the determination of this proportion has been developed and a study of two groups of patients was undertaken: patients with parenchymal cell lesions and patients with extra-hepatic biliary obstruction. A group of healthy volunteers was also tested. The discriminatory capacity of the modified BSP test was determined and was compared to the discriminatory capacity of the standard BSP test and tests of other liver function.

[1]Biochemistry Department.
[2]Student.

METABOLISM

P. Jablonski[1], J. A. Owen[1], E. Gordon[2] and J. McK. Watts[2].

This is a continuation of earlier studies on the metabolism of BSP by the isolated perfused pig liver. The effect of hydrostatic pressure on the biliary tree was studied and it was shown that at low pressures, when bile flow was not impaired, there was an entry of conjugated BSP into the perfusate. This entry ceased if pressures were normalized. At higher pressures bile flow ceased and the regurgitation of conjugated BSP could not be reversed by lowering the pressure.

BSP is also taken up by extra-hepatic tissues, especially the kidney. The metabolism of BSP in the hepatectomized, in the hepatectomized/nephrectomized and in the nephrectomized pig was studied. The kidney also conjugated the dye but no regurgitation of conjugated dye could be demonstrated in hepatectomized animals.

[1]Biochemistry Department.
[2]Department of Surgery, Monash University, Prince Henry's Hospital.

Perfusion of isolated pig kidneys was also attempted to study BSP metabolsm in the kidney, but preparations were viable for short periods (1 hour or less) only before hypertention developed. These kidneys removed and conjugated BSP adequately and excreted both conjugated and free dye in the urine.

The BSP test was also applied to pigs with transplanted livers. It was found that the test was normal even when there was histological evidence of rejection.

BACTERIOLOGICAL RESEARCH

G. Buckle and A. K. Perceval[1]

Sterility of Bed Pans

We have been working with the Hospital Engineering Department on an improved bed-pan washer. It would be of some value to make the emptying, cleaning, and disinfection of bed-pans a simple, automatic process for aesthetic reasons but improved asepsis is our main motive. Many patients now are susceptible to infection by organisms that are comparatively harmless to the healthy and the primary source of these organisms is in the large bowel. A prototype pan-washer has been made and installed in a large ward and is being tested; so far with good results.

Operating Theatre Ventilation

A laminar flow overhead ventilator has been used in the Department of Surgery, Monash University, in connection with the treatment of burned patients in an attempt to keep the patient surrounded by sterile air. Routine testing has shown this to be satisfactory from the bacteriological point of view.

Much ingenuity has been expended in the past on supplying a sterile air flow and using this flow to remove contaminants liberated in the vicinity of an aseptic field. For some time effective filters have been available which will remove all particles of bacterial size or larger, but there always have been difficulties caused by the venturi effect of any stream of air which causes it to draw into itself the neighbouring air, so that the process becomes chiefly one of mixing and the gradual dilution of contamination. Although in operating theatres, it is usual to supply sterile air at the rate of 20 times the volume of the theatre per hour, so that the air might be changed every three min., there is a chance that any infective particle may be swept around and around the room, settling and being lifted again by fluctuations in the airstream. At any time it may settle and remain on an area which needs to be kept sterile.

In so-called laminar flow, as applied to whole rooms, the whole of one wall is made the source of the air and the whole of an opposite wall used for the removal of air. Under these circumstances the air moves fairly uniformly in straight lines from the source to the exhaust. The wall which serves as source is composed entirely of bacterial filters. If the velocity of air-flow is above 35 ft./min. (usually about 90-100 ft./min. the equivalent of walking pace), skin scales, lint, the droplet nuclei expelled when speaking, i.e. the common bacteria laden particles, remain suspended and are swept into the exhaust and caught in the bacterial filters as the air is circulated. The air can be filtered two or three hundred times an hour without causing inconvenience from draughts, and the air of the room is sterile two or three seconds after the blowers are turned on. Infected particles created in the room when carried downstream, do not, in most cases (depending on their mass), have enough momentum to hit and stick to any objects in their path, but are swept around the obstruction and go on. If the obstruction is large, however, it may create eddies and dead spaces beyond it, of which notice must be taken.

In the application of the laminar flow principle to a single bed for the care of a patient who is highly susceptible to infection, it has

[1]Bacteriology Department.

been possible so far to produce a unit which maintains sterile air and rapidly removes contamination. The patient is, however, isolated behind plastic curtains and exposed to the constant noise of the blowers while the medical and nursing staff are inconvenienced by the curtains in anything they have to do for the patient. If the curtains are removed the former difficulty, entrainment of unsterile air, recurs. It may be possible to use an air-curtain: two encircling slots through which higher velocity streams are blown, so that while there will be entrainment from the surrounding air to the first stream, and some entrainment from the first stream to the second stream, there may be little from the second stream to the bed-area. But it is probably wrong to fiddle with such a device. Patients who are so susceptible are very likely to need intensive care in other ways and it will be more desirable to use laminar flow in its simplest form, ventilating the whole room, not just separate beds.

This ventilating principle has been used in factories, where fine instruments are made, and scrupulously clean work is carried out. These are places where the capital cost of the ventilation has been borne willingly. Its application to hospitals is just beginning at a time when we know enough of it to wish to see it incorporated into intensive care units, but not enough to form an opinion yet whether it should be built into operating theatres. In the last there are much larger obstructions and there are reasons, e.g. anaesthetic gases, for avoiding air recirculation.

Antibiotics

Several new antibiotics have come into use since we last reported. Another new penicillin, carbenicillin, is useful against gram-negative organisms resistant to many antibiotics, or sensitive only to some toxic ones. Some tests have been done with 7-chloro-lincomycin, a derivative of lincomycin. Cephaloridine, the penicillin-like substance, has been in use here for several years. We now have another of the group, cephalothin, and expect soon to start testing a third, cephalexin. One of the most interesting new antibacterial drugs is trimethoprim, a substance which interferes with the synthesis of folic acid by bacteria, obstructing a later stage than is interfered with by sulphonamides. The combination of trimethoprim and a sulphonamide has a much greater effect than either drug alone, in almost all cases. We have been interested in two uncommon, but severe infections — nocardiosis and melioidosis, in which sulphonamides have been the basis of the most effective treatment available. It appears that trimethoprim plus sulphonamide should give a better result, for the combination synergises *in vitro*.

It has been reported that EDTA (ethylenediamine-tetra acetic acid) synergised with benzyl penicillin against penicillin-resistant staphylococci. This report has been checked and extended, showing that the effect was due to the removal of calcium and magnesium ions by EDTA. In the presence of sufficient EDTA the staphylococci could no longer make penicillinase, and were destroyed by the penicillin, but EDTA seemed to be of no value in treating experimental infections in mice.

Mycobacterium Ulcerans

There has recently been a patient in Alfred Hospital suffering from an ulcer on the wrist caused by *Myco. ulcerans*, Curiously, another such infection was found at Prince Henry's Hospital the next day and a specimen received from a patient in Papua a few days later.

In conjunction with Mr. Reznikov in the Microbiology Laboratory of the Department of Health, Queensland, all the available cultures are being examined with a view of determining whether there is a single type of some variation, and in order to subject them to a variety of new tests which have been used to differentiate the atypical mycobacteria cultivated from lung and cervical lymph node lesions.

Further attempts are being made to find the source of *Myco. ulcerans* infections. The disease has been found now in Australia, New Guinea, Malaya, Africa and Mexico, and may well exist in other countries; but wherever a

substantial number of cases has been found, they have been confined to circumscribed areas where the disease is relatively common, though rare or never seen a few miles away. Few patients have had contact with each other and there is no reason to believe that the disease is transmitted from patient to patient. Therefore it seems that there may be some host, and perhaps a vector, which has a distribution that is at the same time wide, but localised. The various mycobacteria can cause disease in a number of species of animals but all other mycobacteria have one host that is preferred. We are investigating the possibility of bats being the host of *Myco. ulcerans* because some species of these mammals have a distribution that includes the countries named, yet they tend to congregate in particular localities. Furthermore, some bats have body temperatures below 37°C, suitable for the growth of the organism. Bats are inconspicuous and the disease could be rife amongst them without its being known. The susceptible experimental animals, rats and mice, are not likely to be the natural hosts, for they are spread too widely, and the centres of their population are not the places where *Myco. ulcerans* infections occur.

Gram-negative Bacilli

The occurrence of R factors among gram-negative bacilli cultivated from patients in the hospital is being studied. These factors are particles transmissible from one bacterium to another by an infective process, and they carry genes which confer resistance to antibiotics. An R factor may carry resistance to up to six different antibiotics. All of the resistant coliform bacilli tested were found to be carrying R factors, as did also about two-thirds of the *Klebsiellae* tested. R factors are capable of intergeneric transfer e.g. between the two genera named. A preliminary examination showed that staff and patients in wards carried in the gut strains resistant to antibiotics because of the presence of R factors, whereas laboratory staff did not. As was shown earlier in connection with antibiotic-resistant *Staph. aureus* carried in the nostrils, exposure to antibiotics is correlated with resistance.

PROPHYLACTIC USE OF LIGNOCAINE IN ACUTE MYOCARDIAL INFARCTION

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

Lignocaine is now widely used in the treatment of ventricular tachyarrhythmias, occurring in patients with acute myocardial infarction. However, its use as a prophylactic agent is at present unknown. A trial was therefore undertaken to assess the efficacy of Lignocaine given prophylactically to patients admitted to the Coronary Care Unit. Lignocaine was given intravenously in a statim dose of 1 mgm/kg body weight and then by intravenous infusion at 150 mg/hour for 48 to 72 hours.

One hundred and fifty-six patients with myocardial infarcts proven by both electrocardiogram and serum enzymes were admitted during the trial period. Forty-nine patients were excluded from the trial, 23 because of the presence of ventricular tachyarrhythmia on admission, 14 who had cardiogenic shock and 12 whose infarcts occurred more than 24 hours prior to admission. The remaining 107 patients entered the trial and were randomly allocated either Lignocaine or an intravenous infusion of Dextrose without Lignocaine.

The patients were classified according to the absence (class 1) or presence (class 2) of cardiac failure. Thirty-six patients of class 1 received no Lignocaine. Eight (22%) developed a ventricular tachyarrhythmia within the first 48 hours, either ventricular extrasystoles more frequent than 1 in 10 or ventricular tachycardia. Thirty-one patients of class

[1]Cardiovascular Diagnostic Service.

1 received Lignocaine and only 2 (6%) developed a ventricular tachyarrhythmia. Thirty-nine patients of class 2 received no Lignocaine and 13 (33%) developed tachyarrhythmia. Thirty-two patients of class 2 received Lignocaine and 4 (12%) developed a ventricular tachyarrhythmia.

Of the 43 patients who received Lignocaine, 16 developed side effects mostly drowsiness and in 4 epileptiform seizures occurred. All side effects were rapidly abolished by modification of the Lignocaine dosage.

Although the trial is still at a preliminary stage, the results at this stage are encouraging that Lignocaine may be of use in preventing serious ventricular arrhythmias in patients suffering from acute myocardial infarction. There may be a place for the routine administration of Lignocaine to all patients admitted to a coronary care unit with this diagnosis.

HAEMODYNAMIC AND ANGIOGRAPHIC CONSEQUENCES OF MITRAL AND AORTIC VALVE REPLACEMENT

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

Studies on patients who have had replacement of their mitral valve are now completed and the data are being analysed and collected for publication. A project has now started on studying patients who have had replacement of their aortic valve and it is expected that this study will continue through 1970.

[1]Cardiovascular Diagnostic Service.

AUTOMATED STORAGE AND RETRIEVAL OF MEDICAL INFORMATION UTILIZING A NARRATIVE COMPUTER LANGUAGE

T. J. McQuade, S. T. Anderson and A. Pitt[1]

A computer based information storage and retrieval system for medical data in the form of case reports written by physicians is of necessity a compromise between two mutually exclusive requirements.

1. The more regular the structure of the data the greater the ease, and efficiency and reliability of information storage.
2. Free English at present cannot be handled by a computer and therefore restrictions are placed on the physician generating reports. Minimization of such restrictions is desirable.

We have defined a medical reporting language which has a fairly rigid structure yet is a subset of English so that reports can be written in this language which are also acceptable to physicians not utilizing this system. The subset has been designed to allow relatively free English usage by the generating physician and allow for additions to the languages as the need arises.

Cardiac catheterisation reports of the department which include a clinical history, physical examination, results of special investigations and a diagnosis were chosen as a model for the system. Following analysis of 100 such reports, 25 sentence structures have been defined. Each sentence has many optional parts which must be used in a definite order and must contain computer identifiable key words or phrases.

Because of its resemblance to normal English usage, the time involved in learning this system is minimal. The typing of the report will generate the computer input on punch tape, thus eliminating the need for intermediate translation with resulting saving in time and cost.

[1]Cardiovascular Diagnostic Service.

DECOMPRESSION WITH RELIEF OF INTESTINAL OBSTRUCTION

M. A. Shields[1]

Decompression of the small bowel at the time of relieving obstruction has become a common practice. Usually some form of suction device is introduced through an enterotomy. It is agreed that the operation is made technically much easier by doing this, but there is little experimental evidence or controlled clinical evidence that normal bowel function returns any more quickly as a result.

In this project rabbits are used. The small bowel is obstructed, and later, relieved of obstruction, with and without decompression of the bowel, and with stripping of the small bowel content into the caecum, making three groups. Bowel wall oedema, using wet and dry weights, bowel motility, and bacterial contamination of the peritoneum are being studied.

Efforts are being made to develop a long term re-usable model in the dog, using twin Thiry Vella loops.

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

SWELLING IN SMALL BOWEL ANASTOMOSES

M. A. Shields and H. A. F. Dudley[1]

The literature and experienced surgeons from time to time comment on cases of extremely oedematous anastomoses in small bowel. The causes are not known, but are often ascribed to large quantities of i.v. fluid on a *post hoc ergo propter hoc* basis.

A preliminary study in rabbits is in progress in which the small bowel is cut and anastomosed. The fluid content of the anastomoses and undisturbed bowel is measured using wet and dry weights at set times after the operation has been done. It is hoped that the pattern of development of oedema in a healthy animal, not given any special treatment, will be determined. This group will be compared with a similar group given i.v. fluids and it is possible that detailed studies into the nature of the excess fluid will be started later.

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

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LECTURES DELIVERED DURING 1969

"The Uptake and Excretion of Bromsulphthalein by the Isolated Perfused Pig Liver" — *Australian Biochemical Society, Adelaide.*	Paula JABLONSKI
"Uptake and Metabolism of B.S.P. by the Isolated Perfused Pig Liver" — *Australasian Surgical Research Society, Dunedin.*	Paula JABLONSKI
"Temperature Changes in Anaesthesia" — *Australian Society of Anaesthetists, Brisbane.*	Peter A. LOWE