BAKER INSTITUTE

RESEARCH

1964

ALFRED HOSPITAL

The Baker Medical Research Institute derives its main financial support from the Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions. It is also dependent upon donations from private sources. The latter may be allocated to an Endowment Fund.

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

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

THIRTY-EIGHTH ANNUAL REPORT

of

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

(Including Alfred Hospital Clinical Research Unit)

EIGHTH ANNUAL RESEARCH REPORT

of

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

REPORTS

of

ALFRED HOSPITAL RESEARCH FELLOWS

1964

ALFRED HOSPITAL, PRAHRAN
VICTORIA, AUSTRALIA

BAKER MEDICAL RESEARCH INSTIUTE

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Honorary Treasurer:	E. H. CARTER.
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	BRYAN HUDSON, M.D., Ph.D., M.R.C.P., F.R.A.C.P.
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	N. E. JONES.
	K. N. MORRIS, M.B., M.S., F.R.C.S., F.R.A.C.S.
	EDGAR ROUSE.
	T. H. STEEL, M.D., B.S., M.R.C.P., F.R.A.C.P.
	J. C. STEWART, M.B., B.S., F.R.A.C.S., F.R.C.S.
	DIRECTOR OF CLINICAL RESEARCH UNIT (ex officio).

ALFRED HOSPITAL RESEARCH FELLOWS 1964

"R. B. McComas": *"Frederick and Esther Michaelis"*: *"Amelia Haigh Heart Research Scholarship"*: *"Connibere Bequest"*:	A. BAUMGARTEN, M.B., B.S.
Medical Research Fund:	J. M. CALVERT, M.B., B.S., F.R.C.S , F.R.A.C.S.
"E. H. Flack":	J. F. MAINLAND, B.Sc., M.B., B.S., F.F A.R.A.C.S.
"Dr. Henry Laurie":	N. McCONAGHY, B.Sc., M.B., B.S., D.P.M.
"Victor Y. and Margaret Kimpton": *"J. F. Mackeddie"*:	J. NAYMAN M.B., B.Ch. (W'srand), F.R.C.S., F R.C.S. (Edin.), F.R.A.C.S.
"Sol Green": *"Edward Wilson Memorial"*:	P. G. C. ROBERTSON, M.B , B.Ch (St. Andrew's).
"James Richardson": *"Amelia Haigh Rheumatoid Arthritis Research Scholarship"*:	N. KATHLEEN TAYLOR, M.B., B.S.

APPOINTED TO RESEARCH FELLOWSHIPS FOR 1965

"Edward Wilson Memorial": *Medical Research Fund*: *"Collier"*:	MARJORIE BICK, M.Sc.
"Sol Green":	H. D. BREIDAHL, M.D., M.R.C.P., M.R.A.C.P.
"E. H. Flack": *"S. W. Jones"*:	CLINICAL RESEARCH UNIT.
"R. B. McComas": *"J. F. Mackeddie"*:	DIABETIC AND METABOLIC UNIT.
"Sol Green": *"S. W. Jones"*:	B. S. GILLIGAN, M.B., B.S., M.R.A.C.P.
"Dr. Henry Laurie":	A. D. McCUTCHEON, M.D., M.R.A.C.P.
"Dr. Henry Laurie":	J. NAYMAN M.B., B.Ch. (W'srand), F.R.C.S., F R.C.S. (Edin.), F.R.A.C.S.
"Dr. Henry Laurie": *"Victor Y. and Margaret Kimpton"*:	R. J. SAWERS, M.B., B.S., F.R.A.C.P., M.C.Path.
"H. M. Black": *"Amelia Haigh Research Scholarship" (Heart Disease)*:	N. KATHLEEN TAYLOR, M.B., B.S.
"Edward Wilson Memorial":	TAN BOCK YAM, M.B., B.S. (Malaya), M.R.C.P. (Edin.).
"Connibere Bequest":	JEAN TOLHURST, D.Sc.

TRAVEL GRANT

"J. H. Patterson Travelling Scholarship":	R. M. McLELLAN, M.B., B.S., M.R A.C.P.

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Investigations by Research Fellows in Other Departments of Alfred Hospital

INTRODUCTION

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

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

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

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

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

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

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

"It is now generally accepted that research into human disease must be conducted predominantly in close relationship with patients undergoing investigation and treatment. Such research is conducted on two levels. The first is concerned with the basic medical sciences (e.g., at Baker Medical Research Institute), and the second is associated with a study of disease as encountered in the sick person, i.e., clinical research. The organisation of Australian hospitals, which is peculiar to this country, necessitates that the development of the research function of the Hospital be mainly conducted in separate specially

equipped units. In addition, many members of the Honorary Medical Staff devote their valuable time to research in their various specialities and the organised research facilities of our Hospital, namely, Baker Institute and Clinical Research Unit, are at all times available to them in this work. Such an arrangement is in conformity with our objects—treatment of the sick, training of doctors and nurses, and provision of facilities for research."

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

BAKER MEDICAL RESEARCH INSTITUTE

STAFF

Director:	T. E. LOWE, D.Sc., M.D., F.R.C.P., F.R.A.C.P.
Associate Directors:	P. FANTL, D.Sc., F.R.A.C.I. A. J. BARNETT, M.D., F.R.A.C.P., M.R.C.P.
Assistant Director:	WINIFRED G. NAYLER, D.Sc.
Graduates:	Mrs. V. CARSON, M.Sc. D. A. COVENTRY, M.D., M.R.A.C.P. (From 2/11/64). C. C. CURTAIN, Ph.D., M.Sc., F.R.A.C.I. Miss K. KENNEDY, B.Sc. A. D. McCUTCHEON, M.D., M.R.A.C.P. (to 24/7/64). Miss J. PRICE, B.Sc. D. RACE, M.B., B.S. M. ROSENBAUM, M.D., M.R.A.C.P. Mrs. H. STROSBERG, B.Sc. (to 31/8/64). H. A. WARD, M.Sc. R. G. WYLLIE, M.B., B.S.
Technical:	S. HART (Laboratory Supervisor). J. L. BREMNER. Miss J. HOWELLS. Mrs. R. SABO. Mrs. G. WELSH.
Clerical:	Mrs. I. R. ROBINSON. Mrs. B. R. ASHTON. Miss L. CORKE (to 3/4/64). Miss J. GODFREE (from 31/3/64 to 21/9/64). Miss M. GOLDBURG (from 30/9/64). Mrs. R. ROBINSON (to 29/5/64). Mrs. W. WORMALD (from 4/5/64).
Laboratory Assistants:	Mrs. J. BERAN. D. BREEN. Miss E. DENEREAZ (to 6/3/64). Miss J. FINDLAY. K. HARVEY. Miss D. HUGHES Mrs. Y. KNEALE. Miss R. KNOWLES Miss V. MACK. Miss E. MINSTER. Miss M. SHIERS. Miss M. STUCKEY Miss R. E. TURNBULL (from 10/3/64).

WARD STAFF

Registrar:	D. G. ROBERTSON, M.B., B.S.
Resident Medical Officers:	M. W. HEFFERNAN, M.B., B.S. D. L. WEBSTER, M.B., B.S. G. E. McINTOSH, M.B., B.S. J. A. MAUDSLEY, M.B., B.S.
Sisters:	J. W. McCORMICK (to 28/2/64 from 7/9/64). B. HINDLEY (from 1/3/64 to 7/9/64).
Staff Nurses:	E. M. BELL (from 15/6/64). E. K. BRIGDEN (to 14/6/64). A. M. COCK (to 26/7/64). S. H. ROBERTSON (from 27/7/64).

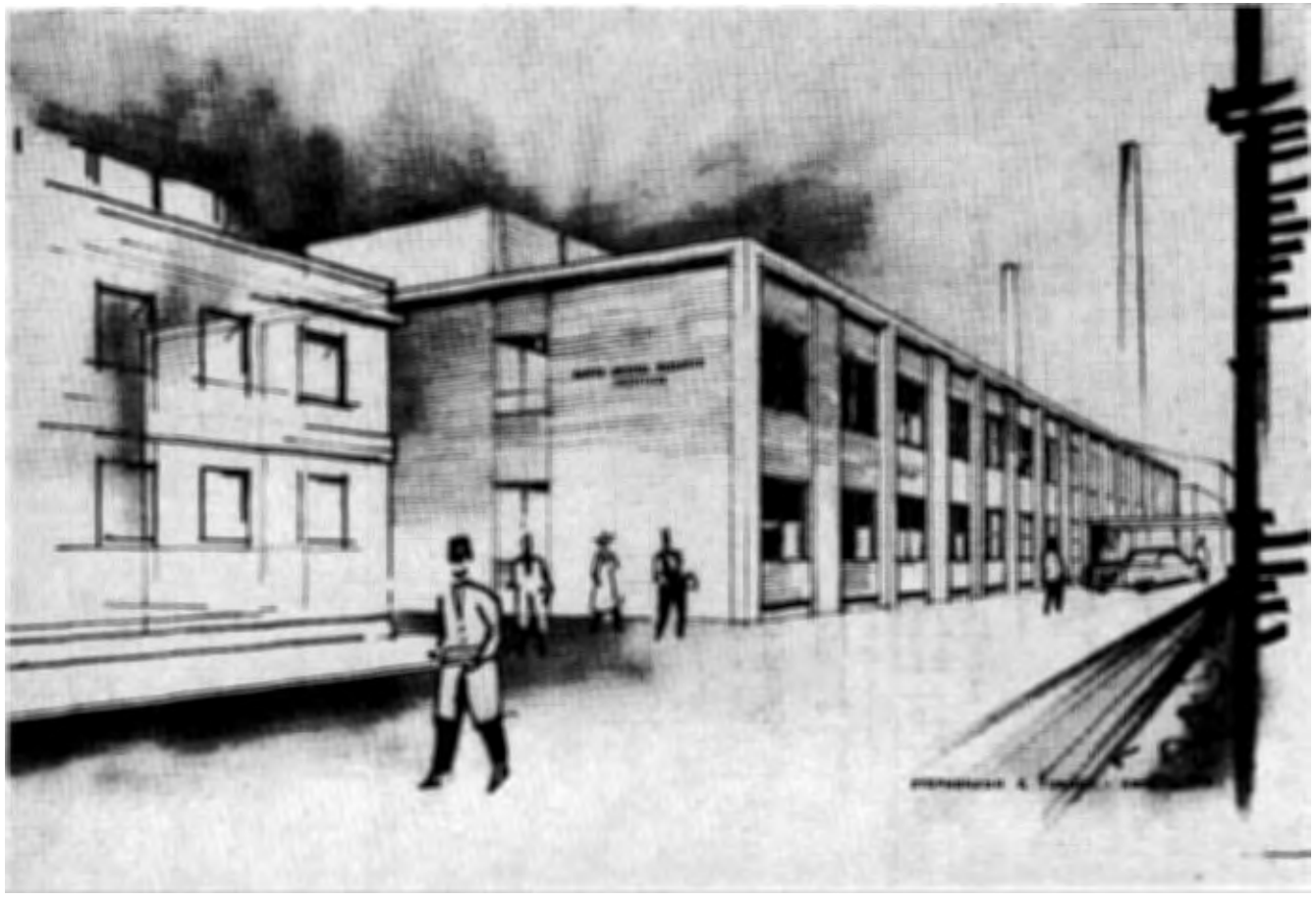
ALFRED HOSPITAL RESEARCH FELLOWS

"R. B. McComas": *"Frederick and Esther Michaelis"*: *"Amelia Haigh Heart Research Scholarship"*: *"Connibere Bequest"*:	A. BAUMGARTEN, M.B., B.S.
"Edward Wilson Memorial": *"Sol Green"*:	P. G. C. ROBERTSON, M.B., B.Ch. (St. Andrew's).

ANTI-CANCER COUNCIL RESEARCH FELLOW

"A. A. Thomas": C. KIDSON, Ph.D. (Lond.), M.B., B.S., B.Sc. (Med.), (on leave).

PROPOSED NEW BUILDING FOR INSTITUTE



BASEMENT AND TWO FLOORS
Floor Area 30,000 sq. ft. Net.

Estimated Project Cost £550,000.

ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

Detailed technical accounts of research in progress are as usual presented in the scientific section of the report but a review of the studies of cardiac surgery carried out jointly by the staffs of the Institute and the Thoracic Surgical Unit of the Hospital since 1945 because of its general interest is included here.

SURGERY OF THE HEART

About 75 years ago the surgeons Billroth and Paget were able to opine that conditions of the heart which required surgical treatment for correction would be forever beyond the reach of the surgeon. Their opinions are not surprising for at that time there appeared to be two insurmountable obstacles to surgical procedures on the heart. In the first place the heart is situated in the centre of the thorax and should the thorax be opened it loses its bellows-like action which moves air to and from the lungs. Secondly, should in some way it become possible to approach the heart, the surgeon would be faced with the the prospect of operating upon a continuously moving organ through which all the blood of the body passed. It is small wonder that in the late 19th century there seemed to be no prospect of surgery on the heart.

However today anaesthetists can maintain breathing, by artificial means, even when the thorax is widely opened and physiologists and surgeons can for limited periods replace by artificial devices either, or both, heart and lungs. The heart can now readily be approached and, after connecting a heart-lung machine, its movement can be arrested to enable the surgeon to work on a quiescent organ.

It should be stressed that in these procedures the breathing of the lungs and the pumping of the heart are replaced by machines, which for efficient control, must be operated by persons who have a detailed knowledge of normal lung and heart function. Automation has not yet reached the stage where they can run themselves. Further, for these machines to work, the clotting activity of the blood must be held in abeyance but it must be returned to normal as soon as the heart and lungs resume activity.

With the rapid development of cardiac surgery in the past three decades it was perhaps inevitable that technical achievements would outrun existing knowledge of cardiac diseases and of the working of bodily organs and their response to surgical intervention. In these circumstances in addition to the surgeon developing his operative techniques, it is imperative for a cardiac surgery team to be seeking answers to the questions in physiology and pathology which continually confront them. Unless the team includes members actively seeking answers it can only follow behind those that do.

This type of development and research into heart surgery began at Alfred Hospital and Baker Institute in 1945 and has continued ever since. It followed publicity about overseas developments and the referring of a number of "blue babies" to Mr. C. J. O. Brown for advice concerning the feasibility of correcting their vascular abnormality by surgery. This condition arises from an abnormality not of the heart but of large blood vessels close to the heart. As the necessary surgical procedures would not encounter the problem of continuous motion of the heart Mr. Brown and Dr. R. H. Orton (Director of Anaesthesia, Alfred Hospital), began at the Institute a study in animals of the problems

involved in anaesthesia and surgical technique for this operation. After this study operations on "blue babies" were frequently performed.

Other operative procedures on the great vessels near the heart were investigated and in 1947 the first patient was operated on for Fallot's Tetralogy and in 1948 the first coarctation of the aorta was corrected.

The surgeon was at this time mainly concerned with the correction of abnormal blood vessel connections and the removal of arterial obstructions. This interest naturally led to arterial surgery in other parts of the body and Mr. K. N. Morris commenced an investigation of the feasibility of replacing blocked arteries in the limbs, first with grafted arteries and later with tubes made from various synthetic materials. These materials were later to be extensively used in cardiac surgery itself.

After much study of anaesthetic agents, procedures and the effects of anaesthesia on the circulation the way to the heart had been opened but to enable any but minimal surgical procedures to be carried out on the heart it had to be stopped beating and rendered bloodless without jeopardising the patient.

One method used reduced the body temperature in order to slow down all processes and the circulation of blood could be stopped for a short while without disaster. Using this procedure—hypothermia—the body temperature of animals was lowered to 28°C and the flow of blood through the heart could be stopped for 10 minutes. Thereby, for a few minutes a bloodless field could be obtained for surgery but the heart, although empty, was still beating. The next step was to stop the beating of the heart by chemical means and to restart it by chemical and mechanical means after this few minutes of circulatory standstill. Although these techniques were feasible in animals they were not really suitable for man. However, the study did provide much information on the effect of limited circulatory arrest on the heart and body as a whole and it enabled surgical procedures to be developed and the use of synthetic materials for grafts and patches to be studied.

During hypothermia sometimes the co-ordinated regular beating of the chambers of the heart was replaced by a disorganised activity—fibrillation—which had no propulsive action. Means of regaining the normal rhythm had to be found in order to restore the circulation. Lengthy investigation of the phenomenon of fibrillation led to an understanding of its production and control so that ultimately it was possible to use fibrillation as an aid to cardiac surgery and restore the normal rhythm at will. Methods of "defibrillation" have now a place in clinical medicine apart from cardiac surgery.

Studies in many centres had now made it clear that a quiescent, bloodless heart for more than a few minutes could not be achieved without a machine to take over the function of the heart and lungs. Consequently in 1956 the development of a pump-oxygenator along the lines described by overseas workers was commenced. This device was a machine which oxygenated blood and pumped it around the body while the heart was shut off from the circulation and could be stopped beating. The first pump-oxygenator constructed was a bulky but simple device. Venous blood was led into a long vertical plastic tube where it was mixed with fine bubbles of oxygen. As this produced frothing it was necessary to defoam the oxygenated blood in a helix which acted as a reservoir from which it was pumped into the arterial system. Many problems

were encountered and detailed studies have been made and are continuing on those relating to gaseous exchange, the damaging effect of the pump on the blood cells and the general reaction of the body to the often inadequate total flow of blood from the pumps. However, by the end of 1956 the group, which now included Mr. G. R. Stirling, found that they could exclude the heart from the circulation for 15 minutes and that extensive human cardiac surgery was within their grasp.

During the next few years other types of pump-oxygenators were investigated and the effect of various anaesthetics on the performance of heart muscle was studied in detail.

By the end of 1959 some fifty human patients had been operated upon with the heart and lungs bypassed by a pump-oxygenator. Some observations were made at this time using a combination of deep hypothermia (cooling to 15°C) and an extracorporeal circulation to replace the heart but the results were considered not to warrant pursuing this technique.

With the techniques already described for using a pump-oxygenator to bypass the heart and lungs the body as a whole could tolerate cessation of cardiac activity for periods long enough to enable most cardiac procedures to be carried out. But with this bypass technique no blood flows through the blood vessels of the heart and the cardiac muscle is deprived of blood. Its capacity to withstand this deprivation has been studied in detail for it places a limit on the duration of the bypass. It was found that to protect the integrity of this muscle for more than very short periods its blood supply must be maintained and this has been achieved by inserting small tubes into the coronary arteries and connecting them into the pump-oxygenator circuit. Adequate time for the surgeon to carry out most feasible procedures on a stationary heart can now be provided. Currently the pump-oxygenator in use uses a spinning disc oxygenator, has five separate pumps for different circuits and heat exchangers to control the temperature of the blood. Monitoring equipment indicates flows, pressures and temperatures in the various circuits.

Another problem to which much attention had to be devoted arises from the fact that the blood vessels and heart are completely filled with blood. When opened at operation air may enter the system and if carried around with the blood could give rise to "air embolism" in various parts of the body. Methods of preventing this from happening and of closing the system without trapping air bubbles were successfully evolved.

At first the surgical procedures and equipment only permitted abnormalities in the blood vessels or holes betwen the chambers of the heart to be corrected but a major cause of cardiac disability arises from the changes which disease produces in the various valves of the heart; they may become narrowed or incompetent. As the time available for operation lengthened it became reasonable to think about surgical correction of these deformities. In order to understand better the normal behaviour of these valves a device—"cardiac pulse simulator"—was constructed which enabled cinematograph records to be made of the valves in action. Valves obtained at autopsy, human and animal, were inserted in this machine and studied. As a result of these studies heart valves are today being successfully operated upon and in some cases replaced by prosthetic valves.

Although this review touches on only a few of the facets of the research and development of cardiac surgery which have been undertaken here over the

past 20 years, it emphasises the very great amount of investigational work which had to be carried out by a large team of surgeons and laboratory workers before cardiac surgery developed from a dream to a reality.

DEVELOPMENT OF RESEARCH

During the past two decades there has been a marked acceleration in the development of clinical medicine. Modern instruments based on advances in various branches of science have permitted in many instances greater accuracy in diagnosis and improvement in the treatment of patients and a better understanding of many diseases. As examples of these developments the use of electronic recording devices for the diagnosis of heart disease, of radioactive isotopes in various hormone disorders and computers for the assessment of data may be cited.

The consequences of these innovations in terms of additional space to house equipment, of increase in staff often with non-medical skills and of the cost of the treatment of the sick person are apparent to all. However, over this same period and stemming from similar developments a less obvious but no less important change has occurred in the biological sciences to which clinical medicine must look for its advances in understanding and management of disease. This change arises from the development of Molecular Biology as a field of thought and investigation.

The structural unit of organised living tissue is the cell which is an entity of variable size but so small that the unit of measurement used to describe it is the "micron"— one thousandth part of a millimeter. Observation of cells only became possible with the development of the light microscope a little over a century ago. Refinements to the light microscope allowed considerable detail of cell structure to be determined but always with the limitation that structures smaller than about 0.2 micron could not be seen. During this period of increasing knowledge about tissue structure biochemists were studying the behaviour of the cells and aggregates of cells (tissues) as chemical factories which provided the materials and energy for growth and the life of tissues. However, the big difference between the size of the smallest observable structure and the molecules involved in the biochemical reactions made relation of the structure of cells to function possible only to a limited degree.

The introduction, about 20 years ago, of the electron microscope which reveals structures as small as 0.001 micron and of X-ray diffraction analysis which permits measurement down to 0.0002 micron has enabled some of the giant molecules present in cells to be visualised. At the same time the use of radioactive isotopes made it possible to tag molecules so that they can be followed in reactions. As a result the relationships between structure and function of living tissues can now be studied with some precision. This study, called Molecular Biology, embraces morphological, biochemical, biophysical and genetic aspects of living cells.

To some extent this development has moved the clinician and the biological scientist apart for molecular biology studies are still young and as yet their practical application to clinical medicine is slight. One example will however show their importance to clinical medicine. The size and shape of the haemoglobin molecule, which is the oxygen carrying pigment of the red blood cell, has now been determined and its chemical structure has been characterised.

Recognisable variations in this molecule give rise to sickle cell anaemia and resistance to infection with the malaria parasite and they have been shown to be inherited from one generation to the next. There are some who believe that the secret of cancer may be found by studies at this level.

In general,, the impact of this advance on medical research is great. It implies, on the one hand, "that the clinical investigator who hopes to ask and to answer questions of fundamental importance will require advanced scientific training as well as . . . an excellent clinical training" [1] and, on the other, the employment of many new techniques with complex apparatus and workers of diverse and highly specialised skills.

Rebuilding

In particular the impact of the change on the Institute must be described. It is housed in a building which 20 years ago was adequate in size and design but which is now, despite additions, quite inadequate for the work being carried out. As a result our Trustees are proposing to rebuild on the present location and the Board of Management of the Hospital has made available the land presently occupied by Ward 8 as an extension to the present site. Ward 8 will become untenable for patients during the next phase of the hospital rebuilding. Unfortunately this will destroy the physical unity of laboratory and clinical facilities but it is hoped that direct communication between the two parts can be restored before too long. Demolition and rebuilding, without interruption of work, and re-equipping will be expensive and the Institute will need generous help from several sources. A first sketch of the projected new building is shown on page 12.

Monash University

Another result of the change in research pattern is the need for closer collaboration with workers in all branches of science and to this end discussions have been proceeding throughout the year to have the Institute recognised as an educational establishment affiliated with Monash University. This step, it is hoped, will be beneficial to both parties.

Research Projects

Much of the research of the Institute staff is now patterned on the concept of Molecular Biology. The cardiac muscle and blood protein studies are largely carried out at the molecular level and one study, called "Molecular genetics", relates to the proteins of the cell nucleus. The studies on haemodynamics, whilst not at the molecular level, are based on concepts originating in cybernetics and control system engineering. The work on blood coagulation, arterial disease, hypertension and pancreatitis may be classified as the more traditional application of biological knowledge and techniques to clinical problems.

OVERSEAS VISITS

Dr. P. Fantl has been a member of the International Committee for the Standardisation of the Nomenclature of Blood Clotting Factors for the past eight years and during this year he attended its annual meeting in Amsterdam and also visited a number of centres whilst he was away. His term as a member of the committee has now expired and we wish to express our appreciation of the opportunities his membership has given for continual exchange of views with overseas workers.

1 Irving M. London, J. Clin. Invest., Vol. 43 (1964), p. 1222.

Dr. Winifred Nayler was invited to present a paper relative to our projects on cardiac muscle at the First Symposium on Muscle Structure and Function held at the University of Alberta, Canada. Whilst in North America she visited laboratories in Los Angeles, San Francisco, Seattle and Vancouver. Grateful acknowledgement is made of the financial help given by the University of Alberta and the Life Insurance Medical Research Fund of Australia and New Zealand which made this visit possible.

In February, I visited New Zealand to attend the Annual Meetings of the Royal Australasian College of Physicians and the Cardiac Society of Australia and New Zealand and subsequently on behalf of the Life Insurance Medical Research Fund of Australia and New Zealand visited workers in the cardiovascular field in all the major centres. This enabled me to renew contact and to discuss research problems with many colleagues.

STAFF

During the year Dr. A. D. McCutcheon completed his three-year term as a research fellow and was appointed an Honorary Physician on the Hospital Staff. He has been succeeded by Dr. D. A. Coventry.

Miss P. Emery and Mrs. M. W. McCulloch each received the degree of Master of Science for theses on work which they had carried out whilst on the Institute staff.

Dr. C. Kidson has continued to work at the Chester Beatty Research Institute, England and will return to resume his investigations here in January 1965. He has received the degree of Doctor of Philosophy whilst overseas.

RESEARCH GRANTS

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

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

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

Considerable assistance has been given to us through the year by the Heads and Staffs of various departments of the University of Melbourne, Monash University and the Australian National University, also by the members of the Commonwealth Serum Laboratories, Commonwealth X-ray and Radium Laboratory and C.S.I.R.O. 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.

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

T. E. LOWE.

31st December, 1964.

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

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.
Hallstrom Institute of Cardiology, Sydney.
Instituto de Biologia y Medicina Experimental, Buenos Aires.
Institut Pasteur, Algiers.
Institute of Dental Science.
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 Foundation, New York.
Royal Children's Hospital, Melbourne.
Royal Melbourne Hospital.
Royal Prince Alfred Hospital, Sydney.
Royal Women's Hospital, Melbourne.
St. Vincent's Hospital Medical Research Unit, 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.

ALFRED HOSPITAL RESEARCH FELLOWS IN THE INSTITUTE

1949-64

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
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REPORT OF SCIENTIFIC INVESTIGATONS

ENERGY PRODUCTION IN THE MYOCARDIUM†‡

W. G. Nayler, K. Kennedy, J. M. Price, C. C. Curtain,
P. G. C. Robertson and T. E. Lowe

The project directed at the methods by which energy production and utilization by the myocardium is regulated continues to be fruitful.

CALCIUM IONS

Previous investigations have indicated that many drugs with positive inotropic activities including nicotine, caffeine, theobromine, tetraethylammonium chloride, strophanthin-G and digitalis, alter the distribution and exchangeability of calcium within the myocardium. These findings generally substantiate the hypothesis that the exchangeability of cardiac calcium plays a significant role in the mechanisms responsible for the regulation of cardiac contractile activity. During the current year a detailed study has been made of the specificity of the role played by calcium and in addition certain fundamental differences between the exchangeability of calcium in cardiac and skeletal muscle have been explored in an attempt to elucidate the differences between the contractive responses of cardiac and skeletal muscle.

The addition of any one of several multivalent cations to a calcium-free solution bathing frog skeletal muscle restores the potassium-induced contracture after it has been eliminated by the removal of extracellular calcium ions. Cadmium, nickel, cobalt, zinc, manganese and magnesium ions all act in this manner and it has been concluded that these ions evoke contracture because of their ability to displace bound cellular calcium from the *skeletal* muscle stores. Experiments conducted during the current year using *cardiac* instead of skeletal muscle have shown that these divalent cations do not initiate contraction in cardiac muscle in the absence of added calcium ions. Using Ca^{45} it was found that zinc, manganese, cadmium, nickel, cobalt and magnesium all failed to displace any bound cellular calcium from cardiac muscle. The divalent cations barium and strontium, however, did enhance the exchangeability of cardiac calcium, detected as Ca^{45}, and did evoke contracture in cardiac muscle which had been depolarised in the absence of added calcium ions. The divalent cations, accordingly, can be divided into two groups: one group containing barium, strontium and calcium, the other group containing nickel, zinc, cadmium, manganese, magnesium, etc. The former group restore the potassium-induced contracture in cardiac muscle after it has been eliminated by the removal of calcium ions; the latter group fail to do so. The cations of the former group displace bound calcium and enhance the exchangeability of calcium in cardiac muscle; those of the latter fail either to displace calcium or to enhance its exchangeability. There appears, therefore, to be a fundamental difference between the ability of certain chemicals to displace cellular calcium from the two types of muscle, a finding which naturally leads to the need to investigate the sites of storage of calcium in particular muscles.

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

Previous investigations indicated that the potentiation effects characteristic for skeletal muscle do not necessarily occur in other kinds of muscle, including cardiac muscle. Whereas bromide, nitrate and iodide potentiate the contractions of skeletal muscle, they depress those of cardiac muscle. Using Ca^{45} it has been shown that the potentiation effect of these anions on skeletal muscle contractions is associated with an increased influx of calcium ions during depolarisation. Similar studies, using cardiac instead of skeletal muscle, have shown that these anions fail to augment the influx of calcium ions into either resting or depolarised cardiac muscle preparations. These findings again point towards a fundamental difference between the exchangeability of calcium ions in cardiac and skeletal muscle preparations.

FREQUENCY-DEPENDENT PHENOMENON

The fact that the interval between beats affects myocardial contractility has aroused interest ever since 1871, when Bowditch described the stepwise increase in the strength of frog ventricular contractions which occurs when stimulation is begun after a period of rest (the "staircase" or "treppe"). Investigations in these and other laboratories have provided data which substantiate the hypothesis that interval-strength relationships reflect the balance between the influx and efflux of calcium ions associated with each contraction. During the current year mechanical alternans in dog papillary muscle has been studied. Mechanical alternans is characterised by the regular alternation of weak and strong ventricular contractions. Evidence was obtained which indicated that it occurs, at least in dog papillary muscle preparations, in the absence of electrical alternans; it was concluded that mechanical alternans therefore might result from an alternation in the magnitude of the calcium influx associated with each alternate depolarisation.

INOTROPIC ACTION OF d-ALDOSTERONE

The inotropic action of aldosterone on cardiac muscle has been the subject of several conflicting reports. In a series of experiments using papillary muscles isolated from monkeys (*sp. Macaca irus and M. mulatta*) we have found that small doses of d-aldosterone caused a significant increase in tension produced during contraction. This positive response was not blocked by the β-adrenergic blocking drug, Alderlin, and therefore probably does not involve the release of catecholamines from the myocardium. These experiments therefore support the hypothesis that d-aldosterone may play a physiological role in regulating myocardial contractility.

CARDIOACTIVE DRUGS AND Na-K DEPENDENT ATPase ACTIVITY

Particulate fractions derived from the endoplasmic reticulum of cardiac muscle contain a sodium-potassium dependent ATPase enzyme. Investigations into the activity of this enzyme have been continued and have resulted in two significant findings. First, quinidine markedly affects the activity of the enzyme and secondly, there occurs in toad ventricular muscle a seasonal variation in the activity of the enzyme which parallels in time the previously reported seasonal variation in the sensitivity of toad ventricular muscle to added calcium ions and to the cardiac glycosides.

METABOLIC BASIS OF QUINIDINE ACTION

The depressant effect of quinidine on cardiac muscle can be accounted for, in part at least, in terms of an altered exchangeability of ionised cellular calcium. Recent experiments have shown consistently that drugs which enhance the exchangeability of cardiac cellular calcium including nicotine and the xanthines, theobromine, theophylline and caffeine, act as antagonists to quinidine, in that they reverse the depressant effect of quinidine on myocardial contractility. Action potential studies now have shown that the antagonism between the xanthines and quinidine is reflected by changes in the transmembrane potential differences which accompany excitation. 25 μg/ml quinidine markedly prolongs the duration of ventricular action potentials; theophylline, theobromine and caffeine, when added in the presence of quinidine cause a marked reduction in the duration of the action potential as well as restoring the ventricular contractions to the pre-quinidine level.

During these studies it was noted that the xanthines similarly reverse the myocardial depressant activity of Dilantin. This reversal by the xanthines was also found to be associated with a reduction in the duration of the Dilantin-prolonged action potential.

EFFECT OF HYPOTHERMIA ON THE ACTIVITY OF CARDIOACTIVE DRUGS

The responses of normo and hypothermic rat hearts to a series of drugs have been tested in an attempt to establish the relative levels of toxicity at several different temperatures. The strength of ventricular contractions was recorded initially by means of a conventional light-weight lever writing on a revolving smoked drum. This recording apparatus has been replaced with a more sensitive system in which the force of ventricular contractions is detected with a Baldwin-Lima-Hamilton S.E.4 strain gague assembly the output of which is amplified, displayed on a cathode ray oscilloscope and recorded photographically. Contractions of a control series of hearts showed only a small decline in amplitude during three hours' perfusion at 35°C. The contractions of other hearts which, after an initial period of perfusion at 35°C, were perfused at 12°C declined significantly when continued perfusion at 35°C was restored. Comparison of these results with those of the control group, in which the hearts were perfused throughout at 35°C, indicated that perfusion at reduced temperatures has a deleterious effect on mammalian hearts which only becomes apparent when normothermic perfusion is resumed. In this respect hearts isolated from mammals differ markedly from those isolated from poikilotherms. In this latter group perfusion at reduced temperatures apparently does not evoke any deleterious changes in the contractile activity of the muscle.

The effect of aminophylline on the contractile activity of isolated normothermic rat hearts has been compared with that on other rat hearts perfused at either 25 or 12°C as required. The results of these particular experiments provide the basis for the following conclusions: first, hypothermia increases the sensitivity of isolated rat hearts to the inotropic activity of aminophylline; secondly, although a single injection of aminophylline evokes a positive inotropic response, repeated injections of the drug result in an overall decline in the amplitude of contractions.

PHOSPHORYLASE ACTIVITY OF ISOLATED LAMPREY HEARTS

The hearts of lampreys are peculiar in that they contain large amounts of endogenous catecholamines and are insensitive to added adrenaline. There is considerable evidence to suggest that the process whereby the sympathomimetic amines enhance the force of cardiac contraction involves the transformation of the enzyme phosphorylase from the *b* to the *a* form. The lack of sensitivity of the lamprey hearts might, therefore, be due to all the phosphorylase having been converted to the *a* form by the endogenous amines. Experiments performed to test this possibility showed that the percentage of the enzyme in the *a* form in lamprey hearts did not differ significantly from that in toad hearts. The lack of sensitivity of lamprey hearts to added adrenaline therefore cannot be explained in terms of the percentage of the phosphorylase enzyme which already is in the *a* form. It seems unlikely that the endogenous catecholamines in lamprey hearts participate in the natural regulation of the phosphorylase *a/b* ratio.

CARDIOACTIVE PLASMA SUBSTANCE

The beneficial effects of plasma on isolated saline-perfused hearts has been recognised for more than eighty years. A substance which evokes a positive inotropic response in isolated toad hearts and which has a molecular weight of 5,000-8,000 has been isolated from human plasma and some of its properties investigated.

When injected either intra-arterially or intravenously into anaesthetised dogs the cardioactive substance caused a marked rise in systemic blood pressure, an increased coronary flow and an increased cardiac output. Similar results were obtained following the injection of the cardioactive substance into anaesthetised rabbits. The pressor response was independent of the presence of the adrenals. Isolated hind limb perfusion experiments revealed the direct vasoconstrictor action of the substance.

The inotropic action of this substance on isolated mammalian and amphibian hearts was found to be associated with an augmented level of oxidative metabolism and an increase in the percentage of the phosphorylase enzyme which is present in the *a* form. The inotropic effect of the isolated substance is not blocked by the β-adrenergic drug, Alderlin, and accordingly probably does not involve the catecholamines.

HAEMODYNAMIC STUDIES‡

T. E. Lowe, A. J. Barnett, K. H. McLean[1], D. Race, D. Robertson, P. G. C. Robertson, M. Rosenbaum and V. Carson

The object of these studies is to characterise the pressure-flow patterns in the circulation in both normal and abnormal states and it is hoped to deduce therefrom some of the characteristics of the mechanism controlling blood distribution.

CLINICAL STUDIES

Using the techniques previously described, intra-arterial and intravenous pressures, cardiac output, forearm and finger blood flows and heart rate have

1 Cardiovascular Diagnostic Service, Alfred Hospital.

been measured in normal subjects and in persons with postural hypotension. From these data, it is possible to derive the mean blood pressure (diastolic BP + ⅓ pulse pressure), stroke output (cardiac output per minute/heart rate per minute), total peripheral resistance (mean BP/cardiac output per minute), forearm and finger vascular resistance (mean BP/blood flow per minute). The changes in these indices during the Valsalva manoeuvre have also been recorded. The haemodynamic response in patients with postural hypotension due to a sympathetic nervous defect has been studied by tilting them to 60° from the horizontal and comparing their response with that of normal subjects. In normal persons there is a decrease in stroke output on tilting by as much as 30 or 40 per cent. but a compensatory rise in the heart rate and peripheral resistance prevents any fall in blood pressure.

POSTURAL HYPOTENSION

In patients with postural hypotension, tilting is followed by a fall in stroke output, which is usually somewhat greater than in normal subjects. If the accelerator mechanism of the heart is intact this is partly compensated by a rise in heart rate, but there is a striking lack of compensatory increase in the peripheral resistance which, in fact, usually decreases. Phasic variations in blood pressure on tilting are observed in patients with partial sympathetic loss, but are absent in those with complete sympathetic loss. The Valsalva manoeuvre produces a marked fall in blood pressure in these patients and the normal "overshoot" is absent. This abnormal response can be consistently elicited.

ANIMAL STUDIES

Pressure-Flow Relationships

The relationship between arterial pressure and blood flow in a number of vascular beds has been studied with an experimental dog preparation (previously described) in which under conditions of total body perfusion it is possible not only to control the total arterial blood flow but also to vary arterial blood pressures over the range of 40 to 200 mm.Hg. and to measure the venous outflow from six vascular beds simultaneously. These beds are referred to as the superior vena cava, the vena azygos, the total coronary venous return, the splanchnic, the renal and the hind limbs.

At arterial pressures less than 80 mm.Hg. the data showed a linear relationship between pressure and the logarithm of flow. Above this level the pressure flow curve flattened indicating that the resistance to flow had increased. In the range of 100 to 140 mm.Hg. this autoregulation of organ flow was demonstrated in all the vascular beds except that of the superior vena cava and the coronary circulations. Above 140 mm.Hg. the responses in the vascular beds altered. In the coronary circulation and the muscular vascular beds the blood flow tended to increase more than the pressure, but in the splanchnic circulation this result was less marked. In the renal circulation in several experiments there was a persistence of autoregulation but in two experiments at pressures above 180 mm.Hg., there was a marked fall in blood flow.

To assess the influence of the sympathetic nervous system on the pressure-flow relationships the ganglion blocking agent "Ansolysen" was administered. This produced a marked increase in blood flow through the muscular beds.

It did not alter the slope of the curves relating blood pressure and flow, except to abolish the effect seen in the renal circulation in the two experiments cited above.

It was concluded that the sympathetic nervous system maintains the tone of the vascular resistance beds in muscle and under our experimental conditions is not primarily concerned with the control of blood flow to the other vascular beds.

Carotid Sinus Effects

The influence of the carotid sinus in pressure-flow relationships was studied using the same preparation modified so that the blood flow to the head through the brachiocephalic artery could be controlled separately from the rest of the body. It was then possible to vary arterial pressure in the head and hence in the carotid sinuses whilst keeping the remaining body blood flow constant so that the influence of the cranially placed baroceptors on the rest of the circulation could be determined.

The ratio of the rise in the carotid sinus blood pressure change to the fall produced in the body was always less than 1.0 and varied from 0.2 to 0.4 in 10 experiments. The range of carotid sinus pressures was 60 to 200 mm.Hg. and that in the remainder of the body 80-140 mm.Hg.

With a decrease in carotid sinus pressure the rise in body pressure was accompanied by a decrease in blood flow in the vascular beds of the vena azygos and hind limbs. The splanchnic and renal blood flows did not change by more than five per cent. In three experiments out of ten a decrease in carotid sinus pressure resulted in an increase in coronary blood flow.

To remove the influence on the body circulation of the aortic arch baroceptors the vagus nerves on each side were cut. In six experiments this resulted in an increase of body blood pressure of 20 to 40 mm.Hg. and an increase in the ratio of pressures mentioned above by an average of 0.2. There was no difference in the responses of the vascular beds to those obtained before vagus nerve section.

At the conclusion of each of these sets of experiments the ganglion blocking drug "Ansolysen" was administered and this abolished the vascular response of the body to changes in carotid sinus pressure.

Lumbar Sympathetic Trunk Stimulation

An important factor in the regulation of blood pressure is the change in peripheral resistance. Because other workers have shown that the muscular resistance vessels are the most reactive, we have examined this resistance by studying the relationships between sympathetic nerve stimulation and perfusion pressure response in an isolated dog hind limb. This preparation was perfused at a constant blood flow with a pump and thus any changes in blood pressure reflected changes in the resistance vessels of the hind limb.

Stimulation of the lumbar sympathetic trunk always resulted in a rise in perfusion pressure. The stimulus rate to produce a maximal rise in perfusion pressure was between 3 and 20 stims./sec. There was a linear relationship between the logarithm of rate of stimulation and rise in perfusion pressure and at a stimulation rate of 1.0 stim./sec. an average of 60% of the maximum response was obtained.

After the onset of stimulation there was a dead time with a range of 0.2 to 2.0 seconds and then an exponential rise with a time constant ranging from 3 to 15 seconds with a mean of 9 seconds. After cessation of stimulation there was a dead time of 2 to 3 seconds and a decline phase with a duration range of 7 to 16 seconds.

Because of the presence of overshoot in some experiments it is concluded that the best description of the relationship, in control engineering terms, is that it is a second order system with a damping factor between 1 and 0.35. The natural frequency in these experiments had a range of 0.04 to 0.025 cps. with a mean of 0.034 cps.

In 11 experiments the nerve stimulation was frequency modulated by a sine wave over the frequency range of 0.001 to 0.4 cps. At frequencies in excess of 0.02 cps. there was a marked decrease in amplitude of the response of the perfusion pressure.

The presence of the overshoot suggesting a second order control system between sympathetic nerve and vascular smooth muscle indicates that the relationship between them is complex and probably involves feed back loops. These feed back loops may be concerned in the maintenance of constant flow through the vascular bed under study.

It may be that instead of the sympathetic system controlling the peripheral resistance outright, thus over-riding local requirements, it alters the autoregulation setting point and therefore it can only modify the extent of local response.

Oscillations in Arterial Blood Pressure

When the lungs of some anaesthetised dogs are inflated and allowed to deflate suddenly there is a rapid fall and return of blood pressure to control levels with the appearance of a damped train of oscillations of the arterial blood pressure around the control level. However in other dogs under the same conditions, there is a slower return of blood pressure to control levels and no oscillation.

The presence of these oscillations can be closely correlated with a dog's ability to maintain its control blood pressure following haemorrhage. Those dogs which exhibited such oscillation would maintain their blood pressure at control levels despite a blood loss of up to 30 ml per Kg. Those dogs with the slow response and no oscillation in response to lung deflation did not hold their blood pressure when they were bled and it rapidly declined.

This phenomenon is of particular interest since it represents oscillations within the control loop of regulation of blood pressure by the baroceptors. One of the requirements of such a control loop is a high degree of gain to ensure good and rapid regulation. However, if the gain is high enough such a system will also oscillate. We conclude that the presence of such oscillations and the excellent blood pressure maintenance are consistent with such a hypothesis. Conversely, those dogs which do not show a rapid response and oscillation also are unable to maintain their blood pressure when bled and we conclude that they have a low gain in their blood pressure control system.

INSTRUMENTATION

Analogue Frequency Modulated Stimulator

This instrument was designed and built for the series of experiments on the response of the hind limb resistance vessels to sympathetic nerve stimulation. It consists of two parts; a conventional phase shift sine wave generator of very low frequency and a triode controlled thyratron pulse generator.

Such an instrument can deliver trains of impulses the time duration between each impulse being determined by the input voltage to the second stage. Thus, when used in conjunction with the sine wave voltage generator, it will deliver frequency modulated impulses with the interval between each impulse obeying a sine wave law.

It can also be used as a partial analogue of the carotid sinus by delivering trains of impulses the interval between each one being determined by the level of the blood pressure at each particular instant.

ELECTRICAL ANALOGUE OF HEART BEAT

Alternation in the strength of heart beats is a phenomenon which has always interested cardiologists. Assuming that each beat produces a release of energy from cardiac muscle it was thought that a study of similar mechanisms in which this sort of energy release occurs might give some clues to the basis of cardiac alternation.

This concept has been represented by an electrical circuit which has two alternate states, each with its own time constant. One state representing systole allows the dissipation of energy from a capacitor and at a certain time, determined by a continuous constant rate impulse representing the A-V node generator, changes to the other state. This state represents diastole and in it the capacitor is now recharged at a different rate until at a point determined by the arrival of the next impulse the machine changes back to the original systolic state.

This very simple model can be made to duplicate a number of abnormal cardiac states including that of pulsus alternans by adjusting the two time constants. A number of limitations have been found in this simple model and we are attempting to overcome these.

SPECTROPHOTOMETRIC DETERMINATION OF BLOOD OXYGEN SATURATION

The spectrophotometric method for the determination of blood oxygen saturation of Roos and Rich has been applied to dog blood and various aspects of the technique studied.

It has been found that temperature control of the spectrophotometer is essential for reproducible results. This has not been explicable on physical or chemical grounds with respect to the reaction itself but rather seems to be related to the physical characteristics of the instrument used for measurement (Beckman DU spectrophotometer).

The absorption curves of oxidised haemoglobin and haemoglobin reduced by physical removal of oxygen in the Van Slyke apparatus and also by the use of sodium dithionite have been studied in some detail. No evidence could

be found to support the hypothesis of Carlier that sodium dithionite produced a haemoglobin derivative with an absorption spectrum different from reduced haemoglobin. Our findings were that the absorption curves in both instances were identical.

We have made numerous comparisons between the results obtained with the spectrophotometric method and those using the Van Slyke apparatus. The differences between the two methods are extremely variable. They range from no difference to as much as 8% saturation, with the spectrophotometric method giving the higher result. No explanation can be offered other than the role of so-called "inactive haemoglobin" in producing an erroneously low value by the Van Slyke method. This concept receives some support from the observation that a similar variability has been observed between the ratio of haemoglobin content determined spectrophotometrically (as oxyhaemoglobin) to haemoglobin content derived from the Van Slyke estimation of oxygen capacity. This ratio has been found to vary from 1.03 to 1.11.

BLOOD PROTEINS

C. C. Curtain and A. Baumgarten

During 1964 this project has continued along two lines. First, a study of hypergammaglobulinaemia in man and secondly, an investigation of the environmental and constitutional factors influencing immunoglobulin production both in the normal subject and in some forms of neoplasia.

These studies ranged from aspects of the chemistry of cryoglobulins to the production of cold haemagglutinins and tissue antibodies in tropical hypergammaglobulinaemia. The volume and complexity of the data obtained necessitated increasing use of electronic data processing methods in its reduction and analysis. Quantitative data printed out from various analytical systems by a solenoid-operated typewriter or qualitative data verbally scored on a dictaphone is transferred to punched cards and then processed at various commercial data service bureaux according to programmes written at the Institute.

STUDIES ON POPULATION GENETICS

When a population is repeatedly exposed to an infectious disease which produces a significant mortality the descendants of the survivors show a resistance to that disease which increases over several generations. Presumably this represents a process of selection of resistant individuals. The genetic factors affecting this resistance depend upon the many genes that affect antibody synthesis and other reactions and any study of immunoglobulin synthesis in man must be directed in part to the identification of the roles played by these genes. This can only be done by the painstaking study of the widest range of genetic markers in populations exposed to the most diverse environmental influences all of which must be defined as precisely as possible. Therefore in addition to our continuing studies on haptoglobin and transferrin genes and the factors affecting their polymorphism in various populations, three projects either completed or commenced this year may be especially mentioned in this field.

Serum Protein Polymorphisms, γ-globulin Levels and Acute Respiratory Infection in Children[1]

We are determining haptoglobulin, transferrin, Gc, Gm and Inv types and the γ-globulin levels of young children admitted to the Royal Children's Hospital suffering from acute respiratory infections and comparing this group with various control groups comprised of sibs, parents and children in hospital for other conditions. During the past winter many fewer children than usual came to the hospital with acute respiratory infections and the collection of blood specimens will need to be resumed next winter in order to bring the groups studied to a satisfactory size.

Attempts have been made during the year to set up a complete Gm and Inv typing system for this project. The existence of these allotypic γ-globulins in man was discovered by Grubb in 1956. He found that the sera of certain individuals suffering from rheumatoid arthritis could agglutinate Rh-positive red cells coated with incomplete Rh antibody and that this agglutination could be inhibited by some normal human sera and that the distribution of the inhibitor followed genetic lines. Sera possessing the inhibitor, which was identified as γ-globulin, were called Gm (A+), while those lacking the inhibitor were called Gm (A—). Subsequently it was found that some normal sera also possessed an agglutinating antibody whose inhibition followed the same genetic distribution as that of the rheumatoid arthritis agglutinator. Work by Harboe, Ropartz, Steinberg and others has resulted in the discovery of a number of allotypic groups among the γ-globulins and this expanding system has been designated the Gm system. An analogous system associated with the γ_1 and γ_2 immunoglobulins is the Inv system. The most important implications of the Gm system lie in its possible association with patterns of infection and resistance with dysproteinaemias (e.g., myelomas) and in genetic and population studies. The major difficulties in the use of this system lie in obtaining suitable reagents as human material of rare incidence must be used and our effort has been aimed mainly at finding such reagents. Using donated sera we attempted to reproduce the characteristic patterns of agglutination. The slide technique of Harboe and standard methods of red cell coating were unsuccessful but a modification in which, following a first incubation in an anti-Rh serum, the cells were washed and re-incubated in the same serum gave satisfactory results. Our standard procedure is now to use 15 minutes for the first incubation and 16 minutes for the second both at 37°C. Tests showed that the serum used in the first incubation had to be of the same Gm type as that used in the second but need not come from the same individual. Gm A+ X− serum could be used satisfactorily in the first incubation and Gm A+ X+ serum in the second gave Gm A+ X+ coated cells. First incubation in Gm A+ X+ serum and second incubation in Gm A+ X− serum also gave Gm A+ X+ sensitised cells. Although it was our impression that the Gm A+ X+ titre was lower in the second case, quantitative studies aimed at resolving this point have not been performed as yet. Other difficulties in investigating the Gm system are attributable to non-specificity or uncharacterised multiple specificity or, perhaps, specificity to an antigen of nearly universal distribution by some antisera and by the equally incompletely characterised composition of the anti-Rh sera used to coat the red cells. This lack of characterisation due to the fortuitous nature of the discovery of suitable pairs

1 In collaboration with Dr. D. Danks, Royal Children's Hospital.

of anti-Rh and anti-Gm antisera suggests caution in interpreting the results obtained with any one pair of sera. Thus, when we attempted to confirm Steinberg's observations that individuals heterozygous for the Gm A characteristic show mixed fluorescence with antisera directed against Gm A and Gm B alleles, we found that the specific fluorescence localised in different cells. Our results would thus suggest that each allelic type of γ-globulin is produced by a different cell line, but this observation needs to be confirmed with other sera.

Distribution of Gc Component in New Guinea

The group specific (Gc) component of Hirschfeld is a serum protein of molecular weight about 50,000 which shows genetically-determnied differences in electrophoretic mobility. The three phenotypes found in the majority of human sera have been characterised as 1-1, 1-2, and 2-2. In a majority of human sera variants of these types have been found, among them the F type found by Kirk in Australian and Papuan indigenes. Following an earlier study of the distribution of the main Gc component among the inhabitants of the Eastern Highlands of New Guinea, where significant differences in incidence of the three types were found in populations speaking different languages, a more comprehensive study was carried out on 483 serum samples obtained by Dr. Eugene Giles from the Markham Valley of New Guinea. The overall incidence of the F component was about 8%. Six different language groups came within this data but the numbers of individuals carrying the F component were too small to establish that the suggested differences in the incidence of this component among the different language groups were real. However, significant differences were found in the distribution of type 1-1 and 2-2 components. These differences were not found between the inhabitants of two sets of villages speaking the same language and close geographically. There was no coherent pattern to explain the existence of these differences. The finding of such differences lies in contrast to the much more uniform distribution of haptoglobin and transferrin types in New Guinea. Elucidation of the role of the Gc component in the body and further data on the pattern of its variation in New Guinea may help to understand the origin of these differences in distribution. Human genes are modified by the forces of selection, drift, mutational pressure and hybridisation and the clines derived from population studies do not yield direct measurements of relationships, even though the characters used are clear cut and consistent in their expression and inheritance. The difficulty arises from the operation of microevolutionary processes upon the populations.

As a result it is important to seek populations whose isolation and simply studied environment enables the problem to be studied by a co-ordinated investigation of genetic characteristics, the effects of both culture and environment on population structure and even the use of the techniques of archaeology, to establish a time scale. Research of this type is enormously complex and very few human populations are suitable for its application.

Microevolutionary Studies

Simmonds, Tindale and Birdsell have shown that the aboriginal populations of the Wellesley Islands of the Gulf of Carpentaria, North Australia, provide marked advantages for this type of microevolutionary research. The Wellesley Islanders comprise the Kaiadilt of Bentinck Island, the Lardiil of Mornington

Island and the Janggal of Forsyth and Denham Islands. The latter are now nearly extinct. The Kaiadilt originally occupied inhospitable Bentinck Island which has an area of about 53 square miles and is surrounded by many reefs. The island is south-east of Mornington Island and is the central island of the group. The Kaiadilt are a raft-using people, but within living memory they have made no direct or enduring contacts with either the Lardiil or Janggal people. The Lardiil of the Mornington Island appear to have some ethnic similarity to the Kaiadilt, but this has been altered by the mainland contacts through the Janggal of Forsyth Island. The physical mixture introduced by these contacts appear to be of the type defined by Birdsell as Carpentarian. Simmonds, Tindale and Birdsell determined the blood groups of Wellesley Islanders and found that the Bentinck Islanders differ physically, and in some blood group gene frequencies, from the Mornington and Forsyth Islanders. The haptoglobin-transferrin data determined in our laboratory show that the Bentinck Islanders possess a significantly higher haptoglobin type 1 frequency (0.35) than either the Kaiadilt or neighbouring mainland aborigines, whose haptoglobin type 1 gene frequencies lie in the range of 0.21 to 0.28. Kirk, Lai and Horsfall found haptoglobin type 1 gene frequencies to occur in a similar range for North Queensland and Cape York Peninsula aborigines. The transferrin D_1 was found in both the Kaiadilt, the Lardiil and neighbouring mainland aborigines and the frequencies observed were similar to those observed by Kirk, Lai and Horsfall in the Cape York Peninsula groups. Glucose-6-phosphate-dehydrogenase deficiency was absent in the populations studied and no abnormal haemoglobins nor the β-thalassaeamia trait were detected by haemoglobin electrophoresis. Much work remains to be done in studying the rate at which genetic drift has occurred among the Kaiadilt. These studies must include the analysis of linguistic differences and the study of larger series of aborigines from adjacent mainland sites, who should be tested serologically and for other genetic markers to provide a baseline for evaluating the difference between the Kaiadilt and Lardiil and Kaiadilt archaeology must be studied by all available methods. In this manner it may be possible to narrow the range of probabilities to provide adequate estimates of the rate at which genetic drift has operated in what Simmonds, Tindale and Birdsell call "this population of opportunity".

HYPERGAMMAGLOBULINAEMIA

Tropical Hypergammaglobulinaemia[1]

(a) **Autoimmune Complement Fixing Antibodies.** Eight hundred and four sera from Melanesians living in New Guinea with varying γ-globulin levels were tested for autoimmune complement fixation with saline extracts of human liver and kidney. Of 250 with γ-globulin levels in the range 0.75 to 1.50 g. per 100 ml, eight gave positive reactions (titres exceeding 1 : 5), compared with four out of 250 European donors with γ-globulin levels in the same range. Of 310 Melanesians with γ-globulin levels in the range 1.51 to 2.50 g. per 100 ml, 10 gave positive reactions and of 220 Melanesians with γ-globulin levels in the range 2.51 to 4.00 g. per 100 ml, 12 gave positive reactions. A possible reason for these findings may be that the tropical environment

1 This work was carried out in collaboration with Dr. D. C. Gajdusek, National Institute of Neurological Diseases and Blindness, Bethesda, Maryland, U.S.A.; Dr. C. Kidson; Dr. J. G. Gorman, College of Physicians, Columbia University, New York, U.S.A.; Dr. L. Champness and Dr. R. Rodrigue, Dept. of Public Health, Territory of Papua and New Guinea.

gives rise to stresses leading to chronic tissue destruction, such as liver damage arising from malnutrition and malaria. In the presence of a heightened capacity for immune response, which may be a condition of individual survival in the face of the infective load incurred in the tropics, this continuous production of tissue break-down products may lead to the production of autoimmune complement fixing antibodies.

(b) **Cold Haemagglutinins in Melanesian Populations.** An unusually high incidence (10%) of cold haemagglutinins, with titres exceeding 1 : 50, was found in sera collected from the Kuanua (Tolai) people of the island of New Britain in Melanesia. Lower incidences were found in other coastal population groups (1%) but these were still far higher than those found either in New Guinea highlands or in the European populations. The cold haemagglutinins varied in their physical properties and in their serological specificity. Forty-seven of the 53 isolated cold haemagglutinins were found to be γ_1 macroglobulins; the remainder being γ_2 7S globulins. Of 45 sera which agglutinated adult cells at 4°C, 39 also agglutinated foetal cells from an I-negative donor. At 4°C all 45 sera agglutinated cells from T^A_J, Lewis (ab) and H-negative donors. It is probable therefore that a single specificity is not involved and that the majority of the haemagglutinins are directed against both I and undetermined antigen(s) present in high frequency.

It was inferred that the agglutinins were not isoagglutinins from the frequent observations of cold autohaemagglutinins in fresh Melanesian blood samples. It is possible that the emergence of cold haemagglutinins depends upon both environmental and genetic influences. There is considerable evidence that the reticulo-endothelial system is in a state of intense activity in individuals exposed to malaria and presumably, to other infections in a tropical environment. Many workers have demonstrated the presence of cold haemagglutinins in malarious patients and in at least one case these disappeared after splenectomy. It is possible that the cold haemagglutinins are produced by some random process occurring during the intense proliferation of the reticulo-endothelial system which occurs during malaria. However, it is difficult to explain the remarkably high incidence of cold haemagglutinins amongst the Kuanua linguistic group from the Gazelle Peninsula. It should be noted that the population about Rabaul where the Kuanua people live is subject to recurrent epidemics of different viral respiratory diseases to a greater extent than most other groups studied. On the other hand, the neighbouring Sulka people, who are also subject to such epidemics have a significantly lower incidence of cold haemagglutinins. Preliminary tests for Eaton's agent antibodies carried out by Professor B. P. Marmion (Department of Microbiology, Monash University) in sera obtained from samples of both Kuanua and Sulka populations have not revealed a significant difference between the two. Nor did individuals with high cold haemagglutinin titres show any signs of recent infection. The Kuanua on the basis of blood groups, differ genetically from other groups in New Britain and this difference, as well as the frequent epidemics of respiratory disease, must be kept in mind as the possible reason for the high incidence of cold haemagglutinins.

(c) **Malaria Antibody Content of γ_2 7S Globulin in Tropical Populations.** A great deal of evidence has accumulated to demonstrate that the raised γ-globulin levels observed in many tropical populations are due, in part at least, to malarial infection. It has been shown, for example, that species-

specific, protective antibodies are associated with the γ_2 7S globulin fraction of sera from immune adults. Using a fluorescent antibody test and a column-immobilised antigen technique we have attempted to determine the proportion of γ_2 7S globulin representing parasite antigen-binding antibody in two groups of individuals living in holoendemic areas of New Guinea and New Britain. One group chosen was from the Sause linguistic group in the Sepik River district and the other from the Kilenge linguistic group of the Gloucester Peninsula in New Britain. Anticoagulated whole blood was obtained from individuals, mostly children, with parasite counts (*P. vivax and P. falciparum*) exceeding 10^5 per cubic mm.

The antigen mixture was conjugated to azobenzyl "Sephadex" which was packed into a column. The sera to be tested were applied to the columns and eluted with 0.9% sodium chloride. Parallel experiments were carried out on columns prepared with unparasitised erythrocytes. All the column effluents were concentrated to 1 ml and these, with the original sera, were then examined for the presence of malaria antibody by the fluorescent antibody test using acid-washed parasitised blood films obtained from both children and adults in various areas of New Guinea to ensure a range of parasite species and strains. It was found that the malaria antibody detectable by the fluorescent antibody technique represented quite a small proportion of the total γ_2 7S globulin (6 to 11%) although it may well be large in relation to the amount formed of specific antibody against other individual types of pathogens. The rise in γ_2 7S globulin with age in the population studied was found to be in accord with the age-dependence of γ-globulin levels which we have observed before in New Guinea populations and it appeared that the amount of γ_2 7S globulin synthesised as malaria antibody remained fairly constant while the total γ-globulin level rose with increasing age. Very little γ_2 7S globulin was adsorbed on columns prepared from the unparasitised erythrocytes, suggesting that antibodies to blood cells did not represent a large part of the high concentration of γ_2 7S-globulin concentration.

(d) **Malaria Antibody and γ-Globulin Levels in Melanesian Children in New Guinea.** In view of the above results it was considered desirable to attempt the correlation of serum γ-globulin levels with malaria antibody titres determined by the indirect immunofluorescence method in individuals exposed to differing intensities of malarial infection.

Sera were obtained from 35 children of Melanesian orderlies at Malahang hospital 7 miles from Lae in the Morobe district of New Guinea. These children received frequent antimalarial drugs (Camoquine). Sera were also obtained from 35 children of the Wampit and Gabensis villages, 30-40 miles up the valley of the Markham River from Lae. These children received only occasional antimalarials. It was found that there was a slight, but significant ($p < 0.05$), rise in both the γ-globulin level and the antibody titre with increasing age in the village group. In the hospital group the number of individuals with antibody and the mean titre of the antibody was low and there was no significant rise in the γ-globulin level with increasing age. In the 0-5 year age group, in which an adequate comparison could be made, the antibody levels in positive village children were higher than the antibody levels in positive hospital children. However, the most significant findings were the marked increase in the numbers of children with positive antibody titres of all ages in the hospital groups and the relatively small differences in γ-globulin levels

between the two groups. While the γ-globulin levels in the 6-10 year hospital group were lower than those in the corresponding village group the actual fall in γ-globulin level was considerably less than would be expected from similar studies in African children. These findings confirm the suggestion that in some situations in New Guinea infectious agents other than malaria play an important role in the production of raised γ-globulin levels even under conditions of very high malaria transmission. Possibly, there is a limit at any age to which the γ-globulin level can rise and in the presence of many other infectious agents the manufacture of malaria antibodies has a limited share of the antibody producing mechanism.

(e) **Relationship of Serum γ-Globulin Level to β-Thalassaemia, Erythrocyte Glucose-6-Phosphate Dehydrogense Deficiency and Malaria in New Guinea.** If competition occurs for antibody producing resources the balance of antibodies produced may well be determined by the balance of individual resistances and environmental pressures. In the case of malaria two of the currently postulated resistances occur in New Guinea. These are erythrocyte-glucose-6-phosphate dehydrogenase (G-6-P.D.) deficiency and thalassaemia. The former has been postulated to protect against malaria by two specific biochemical mechanisms in the erythrocyte, the decreased rate of metabolism of the hexose monophosphate shunt pathway and a deficiency of reduced glutathione. On the basis of its population distribution thalassaemia in its heterozygous form has also been held to protect against malaria, although no evidence for a biochemical mechanism has yet been produced.

In an investigation of the inter-relationship between thalassaemia and erythrocyte G-6-P.D. deficiency to malaria and γ-globulin levels blood specimens were obtained from children under 10 years of age living in three geographically separate areas. These were: the Markham River Valley near Lae, the Sepik River district and the Gazelle Peninsula of New Britain. All are holoendemic malaria areas, the malaria frequency diminishing in the upper regions of the Markham River valley. Of 590 individuals 31 were found to have HbA_2 levels exceeding 4%, suggesting the presence of β-thalassaemia trait, and 38 were found to possess G-6-P.D. deficiency. Twenty-six of the 31 subjects with high HbA_2 levels were found to have blood films characteristic of thalassaemia minor. No individuals were found with both thalassaemia trait and G-6-P.D. deficiency. The mean titre of malaria antibody of the 31 carriers of β-thalassaemia trait was found to be 125 (S.D. 37) and the mean titre of 31 subjects of the same villages matched for age and sex was 124 (S.D. 29). The mean antibody titre of 38 subjects with G-6-P.D. deficiency was 131 (S.D. 36) and the mean titre of 38 matched controls was 133 (S.D. 29). The differences in titre are not significant. The blood films of 11 of the 38 G-6-P.D. deficient children contained parasites compared with 85 of 552 normal subjects and 9 of the 31 children with β-thalassaemia trait. There was no significant difference between the groups. The serum protein values, including the two groups of normal control subjects and those with β-thalassaemia trait and G-6-P.D. deficiency were compared and no significant difference was found between the groups. If the possession of the genes for G-6-P.D. deficiency and β-thalassaemia trait were to confer significant resistance to malaria in New Guinea, then one might expect a reduction in malaria antibody titre and the incidence of parasitaemia, as found in male African children and in the γ-globulin levels of the respective groups; although in view of our previous findings the γ-globulin level alone may be a poor index of malarial infections in New Guinea. Earlier

demographic comparisons between G-6-P.D. deficiency and β-thalassaemia trait in New Guinea and New Britain have revealed a dissociation between the distribution patterns of the two genes. Kidson and Gorman have suggested that different selective forces may be operating to maintain the balanced polymorphism of the two conditions and that malaria may be only a part of a complex of factors involving other endemic diseases, dietary habits and marriage patterns.

Hypergammaglobulinaemia and Proliferative Diseases of the Reticulo-Endothelial System

(a) **Immunocytochemical Localisation of the γ_1 19 S Macroglobulin Cold Haemagglutinin.** A γ_1 macroglobulin with cold haemagglutinating activity of the anti-I type was localised by the fluorescent antibody technique in the bone marrow plasma cells of a patient suffering from lymphoma. No localisation occurred in the naked lymphocytes whose presence in the marrow gave rise to a diagnosis of lymphoma. The plasma cells containing the macroglobulin appeared to be morphologically identical to those containing normal γ_2 7S-globulin as observed by a double-label technique. In no case was the presence of both proteins observed in the same cell. The problem of how the cold haemagglutinins acquire their specificity is important both to our understanding of the factors involved in normal antibody production and in the so-called autoimmune phenomena. The cold haemagglutinins in our study appeared to be produced in plasma cells which were morphologically identical with, but functionally distinct from, the cells producing normal γ_2 7S-globulin. The absence of cold haemagglutinin from the small naked lymphocytes suggests that these are not the direct precursors of the plasma cells although it does not preclude the occurrence of some kind of information transfer, possibly by RNA from abnormal lymphocytes to a plasma cell precursor similar to that observed by Fishman, Hammerstrom and Bond to occur between macrophages and lymph-node cells in normal antibody production.

(b) **The Immunocytochemical Localisation of Cryomacroglobulins.**[1] Using the fluorescent antibody technique a cryomacroglobulin was localised from the plasma cells of the bone marrow and the spleen of a patient suffering from malignant lymphoma of an unspecified type. Cryomacroglobulin was also localised from the plasma cells of the bone marrow of another patient suffering from lymphoma. As in the case of the cold haemagglutinin cryomacroglobulin was not detected in the small lymphocytes with scanty cytoplasm whose presence supported the diagnosis of lymphoma which were observed in relatively large numbers in bone marrow and splenic smears of both patients.

(c) **Mechanism of Cryomacroglobulin Precipitation.**[2] In the course of attempts to purify some γ-19S cryomacroglobulins from serum by cooling and redissolving in physiological saline at 37°C we observed that normal γ_2 7S-globulin appeared to be co-precipitated with the cryoglobulin and that the last traces of this could not be removed by repeated reprecipitation. This led us to consider the possibility that the cryoglobulin might contain a cold-stabilised site capable of combining with some structure common to normal γ_2 7S-globulin and the macroglobulin analogous to the cold-stabilised combination of cold haemagglutinins with the erythrocyte surface. In a series of co-precipitation

1 In collaboration with Dr. M. G. Whiteside, *Honorary Haemotologist*, Alfred Hospital.

2 In collaboration with Mr. J. Pye, Walter and Eliza Hall Institute.

studies it was found that the precipitation of the cryomacroglobulins was brought about partly by their combination on cooling with that portion of the A chain found in the fast component of papain digests of normal γ_2 7S-globulin and of the cryomacroglobulins. Co-precipitation of these fragments presumably occurred because the two halves of the fast fragment are identical and can therefore act as a bivalent element in the precipitation lattice. On the other hand, at high concentrations of A chains, inhibition occurred analogous to the inhibition of antigen-antibody union by low molecular weight haptens. Co-precipitation also occurred to a lesser extent with both the S fraction of papain digests and the B chain, demonstrating that A chain specificity was not absolute. Our experiments throw little light on the location of the binding site which is unique to the cryomacroglobulin molecules studied. Like the active site of the normal macroglobulin antibody and of cold haemagglutinins it was destroyed by low concentrations of thiol.

MOLECULAR GENETICS**

Chev Kidson[1]

The study called molecular genetics aims to elucidate the mechanisms of gene regulation in mammalian cells. Two problems under investigation have yielded information with direct bearing on this. The first is the mode of action of hormones at the molecular level and the second is the patterns of alteration of genetic information during chemical carcinogenesis. Other problems such as the examination of biologically significant alterations in DNA secondary structure and the mechanism of genetic recombination have been investigated in bacterial systems. All these investigations have required new techniques of isolation and fractionation of nucleic acids as well as the development of suitable biological test systems.

The action of steroid and non-steroid hormones at the level of messenger RNA synthesis has been studied in more detail. It now seems clear that this action is selective, different cistrons being affected by different hormones in the same tissue and the same hormone having a different spectrum of action in different tissues, as demonstrated by fractionation profiles of messenger RNA. Using rabbit lymphocytes in vitro, it has been found that the action of cortisol on messenger RNA synthesis is very rapid. Preliminary experiments using inhibitors of protein synthesis suggest that the action of cortisol is dependent upon protein having a rapid turnover. The relevance of this observation to the allosteric repressor theory and to the question of direct action of steroid on DNA is at present under review. Whatever the precise mechanism the demonstration of hormone action on gene transcription, in a selective manner, opens up new perspectives in consideration of regulatory mechanisms in higher organisms.

A more complete picture is now available of the sequence of events occurring during aminoazo-dye carcinogenesis in rat liver. Reversible, selective changes in DNA transcription occur slowly on feeding the carcinogenic dye; irreversible changes appear at 6-8 weeks and continue until tumours appear. In contrast a non-carcinogenic analogue produces fewer changes more slowly, and these remain reversible over a long period. These data suggest that sequential alterations in expression of the genome occur over a wide spectrum

1 On Leave. This report concerns work carried out at the Chester Beatty Research Institute, England.

during the progression from liver to hepatoma cell. Further work is being directed towards correlation of these findings with data on DNA-, RNA- and protein-binding of the carcinogenic dyes, in relation to mutation and regulation theories of carcinogenesis.

Fractionation of bacterial DNA by countercurrent distribution has yielded valuable information concerning alterations of DNA secondary structure and their possible biological roles. Using this technique and pulse-labelling with 3H-thymidine it has been possible to isolate the replication region of DNA obtained from E. coli and B. subtilis by reason of the degree of strand separation occurring in this region. Pulse-chase experiments have shown that partial strand separation also occurs unrelated to DNA replication, and some evidence suggests that it may be related to transcription. Thus, DNA from bacterial cells grown under conditions of aminoacid starvation exhibits a higher proportion of partially-denatured molecules than DNA from bacteria grown in enriched media, which is consistent with their requirement for transcription of many more cistrons.

A similar approach has been used to study the mechanism of genetic recombination during transformation in B. subtilis. Radioactively-labelled donor DNA is observed to become associated with regions of partial denaturation in recipient DNA, and while optimal donor properties appear to depend upon an intact double-helix, optimal recipience appears to depend in part at least on a higher degree of partial strand separation. In contrast to reported data on D. pneumoniae, no free single-stranded donor DNA has been detected. A model has been proposed whereby recombination occurs in regions of partial strand separation in the recipient DNA where one strand is being transcribed into messenger RNA, the donor DNA strand being complemented by hydrogen-bonding with the opposite strand of recipient DNA. Further studies are being undertaken to provide a definitive test of this model in transformation and to assess its relevance to other forms of genetic recombination.

BLOOD COAGULATION

P. Fantl, H. A. Ward, H. Strosberg, K. N. Morris[1] and P. de V. Meiring[2]

CLOTTING IN HEPARINISED BLOOD

Heparinised blood is used clinically mainly for pump oxygenators and extensive experience in this hospital has shown that adequate heparinisation of blood prevents loss of fibrinogen during thoracic surgery. However, it is necessary to inspect bottles of heparinised blood before use because clots occasionally form. As careful collection of the blood or admixture with various fluids does not obviate this it is of considerable practical importance to know the conditions in which clots will form in heparinised blood. On two occasions in a disc oxygenator, which had been primed with a mixture of blood containing 4 units of heparin per ml., dextrose and Hartman's solution, considerable clots appeared and the operation had to be abandoned. The following investigations which have been carried out provide some data concerning this problem.

1 Thoracic Surgical Unit, Alfred Hospital.
2 Resident Medical Staff, Alfred Hospital.

Clot Promoting Compounds

It was found that the addition of dextrose, d-fructose, mannose, 2-desoxy-glucose, saccharose, mannitol or glycine to heparinised blood or platelet-containing plasma induced clot formation readily and sodium chloride and thiourea were the least active compounds of any tested. The amount of clot formed is proportional to the concentration of the clot-inducing compound present and incubation time used. Clots are formed in the presence of adequate numbers of platelets but the process is not associated with their glycolytic system and the presence of calcium ions and the components of the prothrombin complex is necessary. Decalcified heparinised blood, citrated blood, prothrombin-deficient blood from patients under treatment with oral anticoagulants and heparinised plasma from haemophilia A patient (factor VIII deficiency) incubated with dextrose did not clot.

Dextrose and Thrombin-Fibrinogen Reaction

It was established that in this unexpected clotting no appreciable loss of heparin occurred but there was a reduction of heparin co-factor (anti-thrombin II). The thrombin clotting time of heparinised plasma which contained dextrose, mannitol, saccharose, glycine or thiourea was shorter than in the presence of sodium chloride but in contrast, the thrombin clotting time of citrated plasma was not shortened by any of the above compounds except glycine.

Incubation of plasma containing between 1 and 1.6 heparin units per ml., without further additions, produced a high yield of plasma clots. Addition of thrombin to such heparinised plasma reduced the clotting time more in platelet-rich plasma than in platelet-poor plasma. The yield of plasma clot was dependent upon the thrombin concentration but a low concentration of thrombin added to heparinised platelet-containing plasma caused more fibrin formation than in platelet-poor plasma. A low concentration of thrombin added to platelet-containing plasma deficient in factor II (prothrombin), factor VII (proconvertin), factor X (Prower-Stuart factor) gave a low yield of fibrin. The difference of fibrin yield between platelet-containing and platelet-poor plasma was seen with both commercial and purified bovine thrombin preparations. Very dilute human brain extract added to heparinised platelet-poor plasma gave, despite a very long clotting time, high yields of fibrin.

Relevant experiments indicated that heparin co-factor activity is greater in plasma than in serum.

Dextrose and Heparinised Blood

In another series of experiments heparinised whole blood was mixed with 5% dextrose in a disc oxygenator similar to that in clinical use. The discs were well siliconised and were rotated at low speed throughout the experiment. At the beginning of the experiment, and again after 60 and 120 minutes incubation time at 37°C, samples were drawn from the oxygenator for the determination of fibrinogen, pH, plasma haemoglobin and cell volume and a careful inspection was made for the presence of clots. Practically no clots were seen in most of the experiments. Progressive determinations of the fibrinogen content of the mixture did not show the fall expected, should clotting have occurred, in contrast to the fall seen after the incubation of platelet-rich heparinised plasma with 5% dextrose in the test tube. There was in fact a rise in the concentration of fibrinogen in the mixtures with an associated increased cell volume and a rise in the plasma haemoglobin level.

These experiments indicate that the clotting process in heparinised blood requires all the components of the blood clotting system which are essential for thrombin formation and physico-chemical factors which influence clotting play a significant part in the activation process. Clotting of mixtures of heparinised blood with dextrose or sodium lactate occurs to a significant degree in contact with glass or stainless steel; clotting was increased by agitation of stainless steel rods in the mixture and it was abolished when the surface of the rods and tubes were siliconised. In addition to this clot-promoting effect, dextrose caused an increase in the red cell volume, increased red cell fragility and increased levels of plasma haemoglobin. The action of dextrose upon the erythrocyte is independent of the clot-promoting action and occurs in both siliconised and non-siliconised containers, whereas the clot-promoting effect is only seen in non-siliconised containers. Five per cent. dextrose is slightly hypotonic (0.28 M) but even when isotonic dextrose (0.3 M) was used fragility of the erythrocytes was very pronounced, particularly when the mixture was centrifuged at 2-4°C.

Clot Accelerating Activity of Intravenous Fluids

The observations that a number of compounds can induce clotting in heparinised blood and the occasional occurrence of clots in heart-lung machines charged with mixtures of heparinised blood and various diluents suggested that the components of fluids in common clinical use for intravenous therapy might accelerate clotting. Distilled water and 0.15 M sodium chloride were autoclaved in glass bottles and these sterile solutions were then concentrated. From them a silica-containing deposit was obtained which produced a marked shortening of the coagulation time of both platelet-containing and platelet-poor plasmas. This indicates that it might be desirable either to use resistant glass or plastics as containers for intravenous fluids in order to prevent clot-accelerating glass components entering the circulation.

Heparin Solution Free of Preservatives

In some operations large amounts of heparin have to be used and commercial heparin preparations usually contain tricresol as a preservative. In such cases the amount of cresols used may not be harmless because phenols counteract antithrombic activity and cresols in larger quantities are toxic. Recently heparin solutions free of preservatives have become available (Commonwealth Serum Laboratories) and preliminary tests have shown them to be satisfactory for use in oxygenators.

Conclusions

From the results of these investigations it may be said that the exposure of heparinised blood to 5% dextrose will certainly result in haemolysis and may result in fibrin formation. The possibility of clotting can be minimised by reducing the concentration of dextrose or sodium lactate used in the priming of pump oxygenators by partial substitution with 0.9% sodium chloride. This will lessen haemolysis and clotting. To use isotonic sodium chloride alone for priming may give too great a sodium load to the patient, so currently at this hospital a solution containing 0.45% sodium chloride and 2.5% dextrose is used for priming the oxygenator. The mixture of blood and diluent should be kept in the oxygenator for as short a period as is practical before commencing bypass and the discs of the oxygenator should be well siliconised.

BLOOD COAGULATION AND PROSTATIC CARCINOMA

The occurrence of a severe haemorrhagic diathesis in a patient with carcinoma of the prostate was investigated during the year.

During the height of the haemorrhagic state one-stage prothrombin tests gave prolonged clotting times but prothrombin assays were well within the normal range. The one-stage prothrombin time in plasma specimens taken 5 minutes after intravenous transfusion of fibrinogen was shortened. This was also observed in vitro after the addition of oxalated plasma treated with $BaSO_4$ (a source of fibrinogen and factor V) to the patient's plasma. Assays of factor V were carried out on two occasions while fibrinolysis was positive and showed 65% and 100% of normal factor V activity indicating that despite active fibrinolysis factor V was not significantly reduced. This is in contrast to the observations of Cosgriff and Leifer (1952) who have found significant factor V reduction in a case of carcinoma of the prostate.

The prolonged one-stage prothrombin times in our case are explained by a reduction in fibrinogen concentration and are not due to a reduction of the other factors which influence the one-stage prothrombin time, namely factor V, factor VII, factor X or coagulation inhibitors. The influence of fibrinogen on the one-stage prothrombin time becomes apparent at fibrinogen concentrations below 100 mgms%. Transfusions of isolated human fibrinogen raised the low fibrinogen in the patient's blood to a level which corresponded with the concentration calculated from mixing of the patient's plasma fibrinogen with transfused fibrinogen. However, there was rapid loss of plasma fibrinogen giving a half-life of less than 20 hours in contrast to the normal value of 96-114 hours. Fibrinolysis has been recognised as one cause of the haemorrhagic tendency in carcinoma of the prostate and we have previously shown that a blood fibrinolytic enzyme of low activity occurs after electrical shock or muscular exercise and attacks fibrin preferentially. The more labile fibrinogen is not affected.

The proteolytic activity of the plasma of both this patient and a second case of prostatic carcinoma was studied and it was found that fibrin gels produced in concentrated plasma by bovine thrombin and incubated at pH 7.4 disappeared in from 2-18 hours at 37°C but that fibrinogenolysis was insignificant in that period. On the other hand transfused fibrinogen disappeared at an increased rate from circulating blood. It is not known whether this discrepancy between the in vitro tests and the behaviour of fibrinogen in the circulation occurred because fibrinogen in the circulation was continually converted into fibrin or because the proteolytic activity was far greater in the circulation than the in vitro tests indicate or because the isolated fibrinogen preparation is more labile than plasma fibrinogen. Fibrinogen concentrations calculated from the thrombin clotting time agreed reasonably well with physicochemical determinations of fibrinogen and this agreement of two independent techniques indicates that excessive amounts of antithrombin were not present in the patient's plasma, for this would lead to prolonged thrombin clotting time and consequently to an apparently lower fibrinogen concentration. Other clotting tests carried out during the period of active fibrinolysis, namely partial thromboplastin time and assays for factor VIII, another labile plasma component, were normal. A deposition of cryofibrinogen was noted in the patient's plasma throughout the time of observation. It is worth pointing out that the whole blood clotting time was at all times normal despite the subnormal fibrinogen levels.

The patient was treated with stilboestrol and the one-stage prothrombin tests and the number of platelets became normal during the recovery phase after fibrinogen levels rose above 100 mgm per 100 ml plasma but fibrinolysis was still present.

PHOSPHORUS CONTENT OF FIBRINOGEN AND FIBRIN

Previously we have reported that mammalian fibrinogens and fibrins can be grouped into three categories according to their phosphorus content but the degree of adsorption of phosphorus-containing compounds on the fibrin clot was not investigated. This has now been studied as follows. In one series of experiments plasma was diluted before clotting in order to reduce adsorption of non-fibrin material on the clot, and in another series isolated fibrinogen was used.

In immunoelectrophoretic tests it was found that fibrin obtained from plasma diluted to approximately 10 mg/100 ml gave a single band, indicating absence of other proteins. However, when fibrin obtained from such diluted plasma was extracted with ethanol-ether the phosphorus content of the fibrin was reduced and the ethanol-ether extract contained phospholipids which must have come from lipoproteins. The amounts adsorbed on the plasma clot are apparently too small to be detected by the immunological technique employed and do not significantly affect the quantitative protein determination in the clot, but the contamination is sufficient to influence the phosphorus results. In contrast to plasma, purified fibrinogen gave fibrin the phosphorus content of which was not altered by ethanol-ether extraction. Phosphorus in fibrin prepared from purified fibrinogen preparations was probably present as O-phosphoryl serine. It was observed that the ratio of the phosphorus content of human fibrin and fibrinogen was approximately 0.5. Experiments with concentrated and dilute fibrinogen preparations showed that the phosphorus content in fibrin was practically identical in both cases, thus excluding adsorption of phosphorus-containing fibrinopeptides on the fibrin clot.

One possible contaminant of fibrinogen and thrombin preparations is fibrinolysin (plasmin). Experiments were carried out in the presence and absence of epsilon-aminocaproic acid in concentrations which inhibit fibrinolysis but not to interfere with thrombin action.

The phosphorus content of fibrinogen and also of fibrin was practically unaffected by the presence of epsilon-aminocaproic acid, indicating that the decreased phosphorus content of human fibrin compared to that of fibrinogen is not due to fibrinolytic action but is the result of proteolysis by thrombin alone.

HYPERTENSIVE STATES

A. J. Barnett, D. Robertson and P. G. C. Robertson

MALIGNANT HYPERTENSION

It was intended originally that the clinical trial of long-term treatment of severe hypertension with hypotensive drugs should study the effect of treatment in malignant hypertension over a period of 5 years and to compare the results with the known prognosis (uniformly fatal) for this period without treatment. This period has now been long exceeded in the majority of patients as it is 14 years since the trial was commenced.

The results of treatment of the first 64 patients have been studied in detail and are presented here. A diagnosis of malignant phase hypertension was made on the basis of papilloedema in 32 patients and another 32 had comparably raised blood pressure but without papilloedema.

The following table shows the number of patients surviving at various times after the commencement of treatment.

Category	Number of Patients				
	Initial	Survivors			
		1 yr.	2 yrs.	5 yrs.	10 yrs.
Malignant	32	23 (6)	17 (3)	14 (0)	6
Severe Benign	32	27	25	18	12

Figures in parenthesis are expected numbers without treatment.

It is remarkable that patients previously in the malignant phase have survived as long as 14 years with drug treatment. In general the management of these patients is not more difficult than in patients initially in the benign phase and the dose of drugs is not particularly high. Most of the surviving patients are leading happy, useful lives.

NEW HYPOTENSIVE DRUGS

Pargyline Hydrochloride[1]. The study of the hypotensive effect of pargyline, an amine oxidase inhibitor, has now been concluded. In the original plan, four investigators were each to treat 6 patients, with placebo, guanethidine and pargyline, using a double blind technique—the order of treatment being randomized and the particular treatment being unknown to the observer. However, only 2 of the investigators (A. J. Barnett and M. C. Davis[2]) were able to carry out their part of the trial.

Average blood pressure falls for 11 patients completing the trial are as follows.

Drug	Cases with fall in "mean" B.P. (mm.Hg.)			
	> 50	25-49	15-24	< 15
Pargyline				
Lying	1	4	2	4
Standing	1	7	0	3
Guanethidine				
Lying	1	0	5	5
Standing	0	4	2	5

1 Kindly supplied by Abbott Laboratories Pty. Ltd.

2 Honorary Physician, Alfred Hospital.

Side effects were blurred vision (3 cases), postural faintness (2), drowiness (3), dry mouth (1), all of minor degree. One of the investigators (M.C.D.) also used pargyline in 12 patients who had proved resistant to other forms of drug therapy and obtained a satisfactory response in 9. It was concluded that pargyline appears to be an effective hypotensive agent and that its dose varies from 12.5 to 75 mg. (given once daily or divided into two doses). Further its effect is greater on standing than lying and side effects are insignificant. It appeared that pargyline would be a useful hypotensive agent, but a warning given by the makers of the possibility of adverse reactions which might occur in patients taking the drug after eating cheese or other substances containing amines has precluded its routine use in the hypertension clinic.

Bethanidine[1]. Further experience has been obtained in the use of the new hypotensive drug bethanidine, which is believed to act like guanethidine in preventing the release of transmitter substance from sympathetic nerve endings but to have the advantage of more rapid onset and offset of action. This should permit hypertension to be more rapidly controlled and the effects of overdosage more rapidly corrected.

To date bethanidine has been used in 16 patients, 8 of whom are still under treatment. The reasons for discontinuing treatment have been: the death of the patient in 2 cases, default of patient in one, treatment was no longer required in one and severe side effects in 4. The time under treatment has been less than one month in 3 cases; 1-6 months in 7 cases, longer than 6 months in 6 cases. The drug has been given 12-hourly, usually in evenly divided doses. That some tolerance to the effect of the drug develops is shown by the rise of the average daily dose from 52.5 mg. at one month after commencing treatment to 107.5 mg. at six months. Doses for individual patients have ranged from 15 to 240 mg. per day.

Of the 16 cases, bethanidine was substituted for guanethidine in 10, for methyldopa in one, used as the main hypotensive treatment in 4, and added to other treatment in one. In the patients in whom bethanidine was substituted for guanethidine, blood pressure control was generally similar to that with guanethidine.

Nine patients complained of one or more side effects and in four these were sufficiently severe to require early withdrawal of the drug. Individual side effects were faintness and weakness (7), blocked nose (2), dry mouth (2), headache (2), nausea (1), vomiting (1), palpitation (1), impotence (1), poor mental concentration (1). Of the 10 patients in whom substitution of bethanidine for guanethidine was made, 7 experienced side effects, whereas only three of these 10 had had side effects with guanethidine.

Our experience indicates that bethanidine is a hypotensive agent with similar potency to guanethinide but that its side effects are more troublesome than those of guanethidine.

1 Kindly supplied by Burroughs Wellcome & Co. (Aust.) Ltd.

DISEASES OF BLOOD VESSELS

A. J. Barnett, V. Carson, K. N. Morris[1], B. Aarons[1], and I. A. Ferguson[2]

RECONSTRUCTIVE SURGERY

The study of the treatment of obliterative arterial disease of the lower limbs is continuing and, as indicated last year, the procedures used are adapted to the type of lesion instead of relying mainly on arterial grafting as in the early years. Currently arterial grafting, endarterectomy, thrombectomy and patch angioplasty alone or in various combinations are being used. Reconstructive arterial surgery is usually combined with lumbar sympathectomy.

Our experience shows that the ideal material for an arterial graft has not yet been found and the synthetic materials have been associated with complications such as infection, narrowing of the channel by false intima, dehiscence of the suture line and thrombosis. At present autogenous vein grafts are being used particularly in patients with short arterial blocks or when a "repeat" operation is necessary following failure of a synthetic graft.

LIPIDS IN ATHEROSCLEROSIS

Atherosclerosis is commonly associated with raised levels of cholesterol and triglyceride in the plasma and many regard these increased plasma lipids as playing a causative role in the development of atherosclerosis.

Previous studies have indicated that the level of lipids in the plasma may be lowered by dietary measures, by nicotinic acid or by triparanol. The unattractiveness of the diet and the side effects of the drugs have however prevented the sustained use of these methods. A new drug, "Atromid", was found to be effective in lowering plasma lipid levels and well tolerated but expensive. It is a combination of androsterone and chlorphenisate. Although androsterone was at first considered to be the active agent, recent work has indicated that chlorphenisate alone is just as effective as the combination.

As women with normal ovarian function are less subject to atherosclerosis than men, but lose this protection after ovarectomy a recently produced oestrone derivative, "Atheran", which is stated to have anti-atherogenic properties but very little oestrogenic activity has been investigated to see whether it can lower the raised plasma cholesterol and triglyceride levels in patients with atherosclerosis.

A clinical trial was devised to determine and compare the effectiveness of "Atromid S"[3] (chlorphenisate) and "Atheran"[4] in lowering the raised plasma cholesterol and triglyceride levels in patients with atherosclerosis. To exclude the possibility that any observed changes might be due to seasonal variation a group of patients was treated with placebo. The trial is of a "cross-over" type involving 3 groups of 6 patients each of whom is treated with "Atromid S", "Atheran" and a placebo—the order being varied between groups, and the groups receiving different drugs at the one time. A preliminary observation

1 Thoracic Surgical Unit, Alfred Hospital.

2 Honorary Surgeon, Alfred Hospital.

3 Kindly supplied by Imperial Chemical Industries of Australia and New Zealand Ltd.

4 Kindly supplied by Difrex (Aust.) Laboratories Pty. Ltd.

period of 4 weeks is followed by treatment periods of 14 weeks with intervals of 8 weeks between treatments. The dose of "Atromid S" has been 9 capsules (2.25 g.) per day and of "Atheran" 3 tablets (6 mg.) per day. The effects of the drugs are assessed by plasma cholesterol and triglyceride levels, the general clinical state and serial electrocardiograms. The first stage of this trial shows that the six patients treated with "Atromid S" all showed falls in plasma triglyceride and cholesterol levels. The triglyceride fell by a mean value of 48 mg. per 100 ml. (28%) and in 4 of the 6 cases the falls were of statistical significance ($p < 0.05$). Cholesterol fell significantly in all 6 patients. The mean value was 61 mg. per 100 ml. (20%). Of the 6 patients treated with "Atheran", 4 showed a mean rise of 19 mg. per 100 ml. (11%) in triglyceride and of 9 mg. per 100 ml. (3%) in cholesterol levels. One patient had a significant fall and one had a significant rise in these levels. In the placebo-treated patients the changes were insignificant.

These results show a significant response to treatment with "Atromid S" but not with "Atheran" in the dosage used. The dose of "Atheran" used has been increased to 6 tablets (12 mg.) per day.

CARDIAC SURGERY†

K. N. Morris[1], Fay Kincross[1], I. McInnes[1], G. R. Stirling[1]

HYPOTENSIVE AGENTS AND VENTRICULAR FUNCTION

Following the studies in 1963 of the circulatory effects of "Hypaque 85" we have investigated the behaviour of the dog's heart after the administration of two hypotensive agents "Arfonad" and "Ansolysen". The systemic blood pressure was reduced to half its normal level by administration of one of the drugs and at this level an assessment of myocardial function was made from the pressure responses in the left atrium and aorta to a graded series of flow loads. The data obtained were plotted as ventricular work and the curve obtained was compared with the curve expressing "control data". In some experiments total coronary blood flow and myocardial oxygen consumption were measured.

In all instances it was evident that the ventricular performance was impaired under conditions of low cardiac output when hypotensive agents were administered. There was a good correlation between low coronary blood flow and impaired ventricular performance.

At high levels of cardiac output the behaviour was less predictable but there was a tendency towards improved ventricular function and an increase in coronary blood flow. In some experiments the dog's heart was exposed to a very high cardiac inflow so that temporary left ventricular failure occurred. Under these circumstances "Arfonad" administration resulted in a fall in peripheral resistance and a dramatic fall in left arterial pressure. At lower levels of systemic arterial pressure the cardiac inflow could then be further raised by 30% above the level which had caused left ventricular failure in the control experiment. This relationship invites further study.

1 Thoracic Surgical Unit, Alfred Hospital.

TOTAL BODY PERFUSION

Although no dramatic changes have been made in perfusion technique certain important evolutionary trends have allowed safer and longer perfusion.

Reduction in the amount of blood required to prime the oxygenator circuit, by adding a greater proportion of aqueous perfusate, has had several advantages. In the first instance there has been a considerable reduction in post-operative bleeding and, probably, an increased cardiac output. The economic gain is obvious. We have had some experience with "bloodless" perfusion using the Baxter disposable oxygenator and have established the value of this technique for emergency perfusion.

Coronary perfusion for aortic valve surgery has been further developed at a technical level and has now reached a satisfactory position.

Haemolysis is no longer a clinical problem.

CONTROL OF BODY FLUID VOLUME

T. E. Lowe, A. J. Barnett and D. Robertson

Although some work was continued on the miniaturisation of an electrode system for continuous recording of pNa in the blood stream the major project has been clinical assessment of new diuretic drugs.

TRIAL OF NEW DIURETIC DRUGS

With the development of new diuretics over the past 10 years the control of oedema has become much more satisfactory and severely oedematous patients are much less frequently seen in hospital wards. Previous experience has shown that where control of oedema with one diuretic is unsatisfactory an adequate response can usually be obtained by combining two or three diuretics. However, in some cases difficulties in treatment arise from electrolyte disturbances, the necessity for combination of drugs and occasionally from the need to use Mersalyl parenterally.

Two new diuretics which are reputed to be highly potent and effective when given orally have therefore been tested. These are ethacrynic acid[1] [2,3 dichloro 4(2-methylene butyryl-phenoxy) acetic acid] and frusemide[2] [4-chloro-N-(2 furylmethyl)-5-sulphamoyl-anthranilic acid].

Fifteen patients with oedema which was proving refractory to other treatment were treated with ethacrynic acid or frusemide or with ethacrynic acid and frusemide in succession.

Eight patients received ethacrynic acid, 3 received frusemide and 4 both frusemide and ethacrynic acid in succession. The new diuretics were given in addition to other routine therapy for the underlying condition such as cardiac failure or cirrhosis of the liver, and sometimes in addition to other diuretics to which the patient had been unresponsive and sometimes in place of other diuretics.

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1 Kindly supplied by Merck, Sharp and Dohme (Aust.) Ltd.

2 Kindly supplied by Hoechst Pharmaceutical Pty. Ltd.

Response is briefly summarised in the following table.

Diagnosis	Drugs Used			
	Ethacrynic Acid		Frusemide	
	No. treated	No. with good response	No. treated	No. with good response
Congestive cardiac failure	5	5	1	1
Nephrotic syndrome	1	1	1	1
Cirrhosis of liver with ascites	2	2	2	2
Nephritis and pyelonephritis	3	0	2	0
Malignant ascites	1	0	1	0
	12	8	7	4

There was a similar good response from each drug in congestive cardiac failure, nephrotic syndrome and cirrhosis of the liver with ascites. Two of the three patients with nephritis or pyelonephritis who were treated with ethacrynic acid were also treated with frusemide, with similar lack of response to both drugs. These were patients with gross renal failure and it is unlikely that they were capable of responding to any diuretic. The patient with malignant ascites also failed to respond to either drug.

The usual dose of ethacrynic acid was 100-150 mg./day and of frusemide was 80 mg. on alternate days. Larger doses were used on occasions in the non-responsive patients.

The response was similar with each drug and the resulting diuresis was associated with a weight loss of about 1 kg/day. The excretion of water was associated with a loss of sodium, but the potassium excretion was uniformly less than sodium excretion (usually in ratio of 1/2 or 1/3) and there were no instances of hypokalaemia. In one case (with cirrhosis of the liver) ethacrynic acid produced a further diuresis when the effect of frusemide had worn off.

In one patient with a nephrotic syndrome, treatment was commenced with ethacrynic acid 300 mg./day for three days and this produced a massive diuresis with loss of 10 kg. weight in the three days. As the patient felt weak, treatment was continued with a smaller dose (100 mg./day) with slower weight loss but this was associated with arterial hypotension, collapse and renal failure. One patient while being treated with chlorothiazide (1 g./day) and frusemide (80 mg. on alternate days) developed a low electrolyte state requiring treatment with hypertonic saline.

Apart from these instances of ill-effects from excessive response, no toxic effects were observed.

These experiences indicate that both ethacrynic acid and frusemide are potent diuretic agents by which rapid loss of fluid can usually be achieved without electrolyte disturbance or toxic effects, but care should be exercised to avoid this being excessive.

PANCREATITIS

A. D. McCutcheon and D. Race

Previously the effectiveness of the proteolytic enzyme inhibitor "Trasylol"[1] in preventing experimental pancreatitis has been shown and it is now being used in the treatment of human pancreatitis. A new proteolytic enzyme inhibitor "Iniprol"[2], extracted from pancreatic tissue has become available and has been compared with "Trasylol", which is obtained from beef parotid gland.

Blind duodenal loops were made in eight dogs and each dog was given a continuous intravenous infusion of 5% dextrose containing 1 million units of "Iniprol" per litre. One dog developed generalized acute haemorrhagic pancreatitis but the intravenous drip had not functioned well during the early post-operative hours. One dog showed pancreatitis in one limb of the pancreas, the other limb being relatively normal. One dog showed multiple small abscesses in pancreatic interstitial tissue, with relatively normal acini. The other 5 had a normal looking pancreas.

These results compare favourably with the previous results in a group of 7 dogs, which were not given proteolytic enzyme inhibitors, where 4 had severe pancreatitis and 3 had mild to moderate pancreatitis. It appears therefore that "Iniprol" has a protective effect similar to "Trasylol" in preventing the development of experimental pancreatitis in dogs.

CELLULAR ENZYMES**

R. G. Wyllie

METHODS IN ENZYME HISTOCHEMISTRY

The developing front of enzyme histochemistry has left behind problems which limit the application of histochemistry in a number of situations. Chief among the problems is the occurrence of artefacts and false localisation of hydrolysed products. During the year experiments have been made to determine the nature of the artefacts that occur with the Gomori lead sulphide method for demonstrating hydrolytic enzymes. This procedure, which can be used to show acid phosphatase, glucose-6-phosphatase and the nucleotide phosphatases, has been described as the as the most fickle stain in enzyme histochemistry. It is a simple stain. Sections are incubated in buffered substrate containing lead ions. The phosphate, hydrolysed from substrate by the enzyme, is precipitated as lead phosphate and subsequently changed to lead sulphide, which is black and visible, by passage through dilute ammonium sulphide. Each step of this reaction has been examined. It has been found that the following factors are responsible for artefactual staining with the Gomori lead sulphide method when it is applied to unfixed frozen sections.

Amount of Enzyme Present. When tissues containing large amounts of enzyme with high rates of activity, as for example acid phosphatase in human prostate gland, lead phosphate can be formed faster than the ability of the

1 Kindly supplied by Faben Fabriken Bayer, W. Germany.
2 Kindly supplied by Choay Laboratories, France.

adjacent tissue to hold the precipitate. The precipitate then diffuses beyond its site of formation to produce extensive non-specific staining. This artefact can be overcome by soaking the section in saline to hydrate tissue sites adjacent to the enzyme which will then hold the precipitate when it is formed. This artefact does not occur when the enzyme content or rate of activity is low.

Thickness of Section. As section thickness increases beyond 10 μ in tissues incubated at pH 7.0 or more some areas containing lead phosphate fail to react normally with ammonium sulphide. This artefact may be due in part to the local formation of Liesegang's rings.

Concentration of Substrate. Aberrant deposition of lead sulphide, often with a pronounced affinity for nuclear structures, occurs if the concentration of substrate exceeds a critical level. This artefact does not occur if the concentration of substrate is reduced.

pH of Substrate Solution. pH 7.0 is the mid-point in the frequency and extent of false staining. Below this level artefactual staining progressively decreases. It does not occur at pH 5.0, the optimum level for the activity of acid phosphatase. Conversely as the pH increases beyond pH 7.0 artefactual staining increases.

Purity of Ammonium Sulphide. Ammonium sulphide is a mixture which also contains ammonium hydrosulphide and on standing the solution slowly oxidizes to ammonium polysulphides. Aged solutions of ammonium sulphide, where significant oxidation has occurred, will produce artefacts, especially when the enzyme content of a tissue is high. Close observation to these factors enables the Gomori lead sulphide method to be used accurately with freedom from artefact.

MAST CELLS

The morphological changes which occur in lymph nodes following antigenic stimulation include fluctuations in the mast cell population. These changes have been studied in rats and mice using metachromatically staining thionin and Gomori's aldehyde fuchsin. Gomori described this reagent as specifically staining elastic tissue and mast cells. His original method has been modified to increase its selectivity in respect to these tissues.

The first metachromatic cells to appear are lymphocytes or mesenchymal cells. They arise in the cortex and stain red with aldehyde fuchsin. These cells are oriented in cords. Granules are secreted which condense, within or adjacent to the cell, into elastic tissue fibres. The staining reaction of granules and fibres is the same and structuraly they are continuous. In this way an interlacing network of elastic tissue fibres arises between the cortical cells. These network define the future medullary sinuses.

While this development progresses a different series of metachromatically staining cells, which stain green with the aldehyde fuchsin method, arise in relation to the margin of forming elastic tissue. After maturation these cells migrate between the elastic tissue fibres and release granules which lose their staining properties and appear to play an important part in breaking down the union between adjacent cells. Once released the cells are carried away to leave a definite sinus which brings cortex into communication with medulla of the lymph node. This process has enabled definition of the elastoblast cell and division of metachromatically staining mast cells into two populations, each with a different function.

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"Malignant Hypertension"—*Symposium, Melbourne Medical Postgraduate Committee.*	A. J. BARNETT
"The Molecular Basis of Cold Haemagglutination"—*Australasian Society of Haematology, Sydney.*	C. C. CURTAIN
"Population Genetics in Melanesia" — *Seminar, University of Melbourne.*	C. C. CURTAIN
"Cyroglobulinaemia: a Failure in Communication" — *Australian Society of Immunologists, Canberra.*	C. C. CURTAIN
"The Biochemistry of Blood Coagulation"—*Monash University.*	P. FANTL
"Laboratory Diagnosis of Haemorrhagic Disorders"—*Stanford University, California.*	P. FANTL
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"Diuretics" — *Victorian Division, Royal Australasian College of Physicians.*	T. E. LOWE
"The Pathogenesis of Pancreatitis"—*Central Middlesex Hospital, London.*	A. D. McCUTCHEON
"The Use of Trasylol in Experimental Pancreatitis" — *Central Middlesex Hospital, London.*	A. D. McCUTCHEON
"The Pathogenesis and Treatment of Pancreatitis"—*Westminster Hospital, London.*	A. D. McCUTCHEON
"Calcium and Other Divalent Ions in Contraction of Cardiac Muscle"—*First Symposium on Muscle Structure and Function, University of Alberta, Canada.*	W. G. NAYLER
"A Cardioactive Principle in the Plasma"—*Australian Physiological Society, Melbourne.*	P. G. C. ROBERTSON
"Periodic Fluctuations in Blood Pressure"—*Victorian Group, Cardiac Society of Australia and New Zealand.*	M. ROSENBAUM
"Pulmonary Insufficiency after Open Heart Surgery"—*Royal Australasian College of Surgeons, Hobart.*	G. R. STIRLING
"Implantable Cardiac Pacemaker"—*Society for Medical and Biological Electronics.*	G. R. STIRLING
"Complete Heart Block — Surgical Treatment"—*Royal Hobart Hospital Clinical Society, Hobart.*	G. R. STIRLING

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

Revenue Account for the Year Ended 31st December, 1964.

EXPENDITURE		INCOME		
Advertising	£32 2 11	Donations—		
Drugs, Chemicals, Provisions, etc.	2,654 8 6	Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions	£35,414 10 0	
Fuel and Lighting	353 5 7	Other Donations as per attached schedules	1,532 15 6	
Instruments and Glassware	3,263 8 9			£36,947 5 6
Insurance	1,154 19 5	Grants in Aid of Research—		
Library and Publications Cost	3,306 12 9	National Health and Medical Research Council	1,741 0 0	
Postage, Telephone, Printing and Stationery	1,413 16 5	National Heart Foundation of Australia	7,174 0 0	
Repairs and Renewals	1,906 6 0	Anti-Cancer Council of Victoria	4,609 10 0	
Salaries and Wages	40,369 19 9	Life Insurance Medical Research Fund of Australia and New Zealand	3,763 0 0	
Travelling Expenses	991 18 1			17,287 10 0
Sundries	2,640 3 3	Interest from Investments—		
Cost of Animal House	1,000 0 0	Held by Trustees of the Estate of the late Thomas Baker	850 0 0	
Surplus for Year	174 17 4	Endowment Fund	1,198 14 7	
				2,048 14 7
		Interest from Commercial Bank of Australia Ltd.		158 12 5
		Sundry Sales		2,819 16 3
	£59,261 18 9			£59,261 18 9

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

Balance Sheet as at 31st December, 1964.

LIABILITIES			ASSETS		
Endowment Fund	£24,935 16 10		Endowment Fund Investments—		
Capital Grants and Gifts	1,431 10 3		Inscribed Stock—		
Accumulated Revenue	2,047 3 0		Commonwealth Government	10,880 0 0	
		£28,414 10 1	Treasury Bonds—		
			Commonwealth Government	4,010 0 0	
Current Liabilities—			Shares in Companies	10,057 7 9	
Bank Overdraft	£235 12 6			£24,947 7 9	
Sundry Creditors	2,329 15 2		Cash at Bank (overdrawn)	11 10 11	
		2,565 7 8			24,935 16 10
			Restricted Funds (represented by Cash at Bank)—		
			Capital Grants and Gifts		1,431 10 3
			Fixed Assets—		
			Furniture and Fixtures		3,206 12 4
			Current Assets—		
			Cash on Hand	£10 0 0	
			Sundry Debtors	1,395 18 4	
					£1,405 18 4
		£30,979 17 9			£30,979 17 9

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

In our opinion the above Balance Sheet is properly drawn up to show a true and fair view of the state of the Institute's affairs at 31st December, 1964, according to the best of our information and the explanations given to us and as shown by the books of the Institute.

FLACK & FLACK,
Chartered Accountants,
Honorary Auditors.

Melbourne,
22nd February, 1965.

NOTE: In addition to receiving interest from the Investments as shown on the Balance Sheet, the Institute receives the income from 5% Commonwealth Government Inscribed Stock face value of £17,000, which is inscribed in the name of the Trustees of the Estate of the late Thomas Baker for the benefit of the Institute.

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

Year Ended 31st December, 1964.

CAPITAL GRANTS AND GIFTS

Balance at 31st December, 1963		£1,986 16 1
Add		
Donations—		
Dr. T. E. Lowe and Associates		33 19 6
Truby and Florence Williams Charitable Trust for recording spectrophotometer		1,000 0 0
Anti-Cancer Council of Victoria, for recording spectrophotometer		1,500 0 0
National Heart Foundation, for digital clock, pulse generator and recording paper		675 0 0
Estate, Late Irene Alice Kiddle		100 13 4
Special Donation, Baker Benefactions		1,785 10 0
		£7,081 18 11
Deduct		
Equipment—		
Room Conditioner	£198 17 9	
Pulse Generator	477 0 0	
Spectrophotometer	4,853 0 0	
Additional cost for Scanner	121 10 11	
		5,650 8 8
Balance at 31st December, 1964		£1,431 10 3

ACCUMULATED REVENUE

Surplus at 31st December, 1963	£672 5 8
Add	
Transfer Provision for Guest Speaker	1,200 0 0
Surplus for Year	174 17 4
Surplus at 31st December, 1964	£2,047 3 0

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

OTHER DONATIONS RECEIVED DURING YEAR TO 31st DECEMBER, 1964.

The William Angliss Charitable Fund	£400	0	0
Marion and E. H. Flack Trust	350	0	0
Mr. and Mrs. Edgar Rouse	160	10	0
George F. Little Trust	148	8	6
Eagle Star Insurance Co. Ltd.	126	5	0
Kodak (Australasia) Pty. Ltd.	100	0	0
Siegfried Meyer	50	0	0
Mr. and Mrs. Lawrence Simpson	20	0	0
Mr. J. C. Habersberger	10	10	0
Dr. and Mrs. John Rouse	10	10	0
Mr. John C. Ingleton	10	0	0
Miss N. E. Cameron	2	2	0
In Memory of Sir John Latham	10	5	0
„ „ „ Sir William Angliss	10	0	0
„ „ „ John Clemenger	9	0	0
„ „ „ George Drummond Cree	5	14	0
„ „ „ Sir Josiah Francis	5	5	0
„ „ „ John V. Inglis	5	5	0
„ „ „ Frederick Ernest Manning	5	5	0
„ „ „ Charles D. Seabrook	5	5	0
„ „ „ Dr. James Erskine Sewell	5	5	0
„ „ „ Lord Brabazon	5	0	0
„ „ „ Sir Norman Carson	5	0	0
„ „ „ Alexander Warren Mason	5	0	0
„ „ „ William Lewis Morgan	5	0	0
„ „ „ Albert Perier	5	0	0
„ „ „ Udo Waldeman Seppelt	5	0	0
„ „ „ Alfred Henry White	5	0	0
„ „ „ Molly Blakeney	4	4	0
„ „ „ Henry Thomas Clarke	4	4	0
„ „ „ Alured Kelly	4	4	0
„ „ „ Elsa Nesbitt	4	4	0
„ „ „ Florence Muriel Williams	4	4	0
„ „ „ Gertrude Elizabeth Brown	4	0	0
„ „ „ Alice Clegg	4	0	0
„ „ „ Shirley Hollis Johnston	4	0	0
„ „ „ Major-General Colin Simpson	4	0	0
„ „ „ Frederick Robert McDougall	3	10	0
„ „ „ Elsie Street	3	3	0
„ „ „ Nettie Zeikel	3	3	0
„ „ „ Harry Trevor Walker	1	10	0
	£1,532	15	6

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

1964

STAFF

Honorary Consulting Physicians:	EWEN DOWNIE, M.D., F.R.C.P., F.R.A.C.P. BRYAN HUDSON, M.D., Ph.D., M.R.C.P., F.R.A.C.P.
Honorary Consulting Biochemist:	JOSEPH BORNSTEIN, D.Sc., M.D., F.R.A.C.P.
Physician-in-Charge:	PINCUS TAFT, M.D., F.R.A.C.P.
Honorary Physician:	HARALD BREIDAHL, M.D., M.R.C.P., M.R.A.C.P.
Honorary Clinical Assistants:	A. P. DOREVITCH, M.D., M.R.A.C.P. K. J. CATT, M.D., M.R.A.C.P.
Registrar:	J. R. STOCKIGT, M.B., B S.
Biochemists:	DORA WINIKOFF, M.Sc. JUNE SHEATH, M.Sc. DORIS HEYMAN, B.Sc.
Technical Staff:	Mr. W. HUDSON. Miss I. EKKEL. Miss W. DAVIES. Miss R. WITCHELL. Mrs. F. RABOLD (part-time).
Secretary:	Miss J. SHARP.

DIABETIC CLINIC

Clinical Assistants:	MARGARET SANDERS, M.B., B.S. A. P. DOREVITCH, M.D., M.R.A.C.P. IAN BURR, M.B., B.S., M.R.A.C.P.
Chiropodist:	MAIDA O'CONNOR, F.Ch.A.V., M.Ch.I.A.

RESEARCH FELLOWS

Medical Research Fund:	N. KATHLEEN TAYLOR, M.B., B.S.
Burroughs Wellcome Research Fellow:	IAN BURR, M.B., B.S., M.R.A.C.P.

HONORARY RESEARCH FELLOWS

MARGARET SANDERS, M.B., B.S.

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

ANNUAL REPORT

This report of research in the Diabetic and Metabolic Unit is an account of the specific tasks undertaken by members of the Unit.. Some of them refer to the development of techniques for methods of measurement and others to the natural consequence—a study of the influence of disease on various parameters of adrenergic organisms, and have reported on the diagnostic confusion which may arise. A clinical study of thyrotoxic heart disease is being undertaken. The composition from the point of view of treatment needs of the diabetes out-patient clinic is being analysed. A long-term course of thyrotoxic patients treated with radioactive iodine is being re-examined. From such studies will emerge not only answers but further questions to be answered, and again methods will have to be evolved and disordered parameters of function evaluated. The cycle of clinical research is never ending. The passing parade of patients never fails to evoke enquiry.

During this year Dr. Breidahl attended the 5th Congress of the International Diabetes Federation in Toronto and the 2nd International Congress of Endocrinology in London. He had the opportunity during the course of his travels to visit various departments in the United States, Canada, United Kingdom and in Europe. The stimulus of his experiences has naturally been imparted to the Unit.

Distinguished visitors to the Diabetic and Metabolic Unit have included Dr. Peter Bishop, Guy's Hospital, London; Professor Robert Loeb, New York; Dr. J. K. W. Ferguson, Connaught Laboratories, Toronto; and Dr. V. Stevens, Ohio State University, Ohio. Our thanks are due to them for helpful advice and valuable criticism.

Generous assistance and support has been given by members of the Honorary Medical Staff in clinical advice and in their referral of patients. The close association with the Monash University Departments of Medicine and Biochemistry continues to provide a valued forum for exchange of technical aid and clinical opinion.

Once again I must acknowledge the gifts in money and in kind from individuals and organizations whose interest in our work is a constant source of encouragement and without whose help we would be considerably hampered in our efforts.

PINCUS TAFT.

31st December, 1964.

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

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

STUDIES OF GLUCOSE UTILISATION

Ian Burr, W. Hudson and D. Heyman

We have been investigating glucose utilisation using a small priming load (1.126 G./Kgm.) calculated in the average person to raise blood sugar by the order of 40-60 mgm./100 ml. followed by continuous infusion at two levels — 250 mgm./min. and 350 mgm./min. These tests have been performed on a total of 146 occasions in the following groups of patients:

Normal adults.

Normal pregnant women—1st, 2nd, 3rd trimester and some post partum.

Mild diabetics—defined as mild by virtue of purely dietary or dietary and oral hypoglycaemic tablet control.

In the normals, graphs plotting blood sugar against time at 250 mgm./min. rate of glucose infusion show an almost straight line after initial 15-25 minute settling period, the mean of the difference between maximum and minimum blood sugar values being 6 mgm.% with a standard deviation of 4.2 mgm.%, there being no tendency to steady rise or steady fall in blood glucose at such rates. The rate of glucose infusion was based on previous estimates of glucose disappearance rates in these normal people at blood sugar levels 40-60 mgm. above fasting. There was an average rate of disappearance of glucose of 250 mgm./min.

In the pregnant state the data in the 1st trimester are inadequate, very small numbers of patients being studied. However, in the 2nd trimester results were similar to the non-pregnant state using the criteria of mean difference between maximum and minimum values, this being 8.5 mgm.% with standard deviation 5.3 mgm.%—a statistically identical population to the "normal non-pregnant group" (using "t" and student tests). However, there was a tendency—exhibited by three of the 16 subjects—to show a steady fall in blood glucose during infusion. When the infusion was repeated at 350 mgm./min. these patients showed steady glucose levels and the "swings" observed were again within the normal range. In the 3rd trimester swings were greater — mean 13.4 $\pm$ 6.2 mgm.% at infusion rates of 250 mgm./min. and an even larger percentage showed steady fall in blood glucose over infusion time. Again, when repeated at 350 mgm./min. these controls showed steady blood glucose level and the "swings" were in the same range as for the 250 mgm./min. infusions (12.2 $\pm$ 5.3 mgm.%).

Although investigations are not yet completed, these results suggest that during the third trimester of pregnancy there is an increased capacity to handle intravenous glucose, and that those factors responsible for the "swings" in glucose levels at constant infusion rates also alter during pregnancy.

The third group—mild diabetics—infused at 250 mgm./min. showed mean swings of 32 mgm.% with standard deviation 12.2 mgm.% — a population totally different from any of the above. However, these cannot be directly compared with the pregnant state and it is intended to perform further similar tests on diabetics in pregnancy to obtain comparable data. Clinically "pre-diabetic" pregnant patients are also being studied, for their detection by other biochemical means are not currently available.

During the coming year it is hoped to investigate the reason for the large "swings" detected in the diabetic patient as against the normal controls.

Finally, the work on "glucose utilisation" is being correlated with plasma insulin determinations—an assay method for this having been developed in the Unit.

DOUBLE ANTIBODY IMMUNOASSAY FOR INSULIN

Doris Heyman

Theory:

1. Insulin reacts with anti-insulin antibody in the serum from insulin immunized guinea-pigs to form a soluble complex.
2. This complex can be precipitated by an anti-guinea-pig γ-globulin serum prepared in rabbits.
3. Using a fixed quantity of I^{131}-insulin as tracer, the amount of radioactivity in the precipitate is a function of the concentration of unlabelled insulin, provided insulin is in excess of its antibody.

Method of Hales and Randle (Biochem. J., Vol. 88, 1963, p. 137).

1. Anti-insulin serum (suitably diluted) was incubated with anti-guinea-pig γ-globulin serum for 16-24 hours at 4°C. to precipitate the anti-insulin.
2. Standard insulin solutions or buffer or plasma was added and incubation continued at 4°C. for 6 hours.
3. I^{131}-insulin was added and the mixture incubated 16 hours at 4°C.
4. Free insulin was separated from antibody bound insulin by filtration through cellulose acetate membranes. These were then washed, dried and assayed for radioactivity on a Nuclear Chicago gas-flow counter.
5. Standard curves were constructed by plotting counts per minute on the membrane against concentration of unlabelled insulin.

Results:

We confirmed Hales' and Randle's findings that (a) preliminary precipitation of the anti-insulin gave us a steeper standard curve than that obtained by complexing insulin with its antibody and subsequently precipitating the complex with anti-γ-globulin, and (b) addition of I^{131}-insulin several hours after incubation of unlabelled insulin with the precipitated antibodies also increased the sensitivity of the assay. The standard curves, constructed in the range 0-160 microunits per ml., were fairly steep and reproducible. However, (a) reproducibility of plasma assays from one to another were poor; (b) recovery of insulin added to plasma was quantitative in some but not all cases; (c) plasma dilutions gave unpredictable results; (d) the range of insulin levels in fasting individuals was 10-100 μU/ml.

Other groups using this assay method reported similar difficulties, often attributing their unreliable results to the variable porosity of the oxoid membranes. Thus we abandoned the Hales and Randle method in favour of that of Morgan and Lazarow ("Diabetes", Vol. 12, 1963, pp. 115-126).

Method:

1. Unlabelled insulin (standard or assay sample) is incubated with I^{113}-insulin and guinea-pig anti-insulin serum (1 : 10,000) at 4°C. for 24 hours.

2. The complex is precipitated by anti-guinea-pig γ-globulin serum. Normal guinea-pig serum (1 : 100) is added to increase the volume of the precipitate. The reaction is carried out at 4°C. for one hour.
3. The precipitate is separated by centrifugation, washed and the precipitate and supernatant plus washing fractions counted.
4. The standard curve is constructed by plotting per cent. radioactivity bound:

$$\frac{\text{counts/min. in precipitate} \times 100}{\text{counts/min. in precipitate} + \text{counts/min. in supernatant}}$$

against concentration of unlabelled insulin.

Our experiences with this method and modifications introduced:

1. Contrary to our findings with the Hales and Randle method, preliminary precipitation of the anti-insulin did not increase the sensitivity of the method.
2. We found that equilibration of unlabelled insulin with anti-insulin prior to the addition of I^{131}-insulin gave a more sensitive assay.
3. Following the report of Soerldner at the Fifth Congress of the International Diabetes Federation in 1964 we lengthened the period of incubation of insulin with its antibody from 24 hours to 48 hours and the precipitation step from 1 hour to 36 hours. Further (see 2.) we added I^{131}-insulin 24 hours after incubation of unlabelled insulin with anti-insulin.

   We obtained better reproducibility particularly in the plasma assays using these lengthened incubation periods. We have also found that 24 hours for the precipitation step gives quite satisfactory results.
4. Where I^{131}-insulin of sufficiently high specific activity was supplied a steeper standard curve was obtained using 5 μg. in place of 10 μg. of I^{131}-insulin.
5. Using the scintillation (Nuclear Chicago) counter we found that in hundreds of assay samples counts in precipitate plus counts in supernatant equalled total counts in an aliquot of I^{131}-insulin, within the counting error. Thus we removed the supernatant fraction and washing by suction, counted the precipitate and calculated per cent. bound from

$$\left(\frac{\text{counts in precipitate}}{\text{total counts added}} \times 100\right).$$

6. By transferring an aqueous suspension of the precipitate to a planchette by means of a Pasteur pipette and drying at about 30°C. we were able to use a Nuclear Chicago gas-flow counter which has the advantages of a much higher counting efficiency and automatic sample changing and recording devices.

Results:

The standard curves obtained were steep and generally reproducible, the steepest curves being obtained with anti-insulin at 1 : 8000 dilution.

The assay is sensitive to 0.5 μU of insulin. Repeated assays of several plasma samples gave closely reproducible insulin values.

Insulin levels in plasma undiluted or diluted with buffer albumin 1 : 2, 1 : 5, 1 : 10 became progressively higher with increasing dilution. However, in dilutions up to 1 : 20 the insulin level did not increase above levels found at 1 : 10 dilution.

Insulin added to plasma diluted 1 : 10 was recovered quantitatively (90-110%), whilst only 70% of insulin added to a 1 : 2 or 1 : 5 dilution of plasma was recovered.

Both these findings seem in accordance with the suggestion by many workers that there is some inhibitor in plasma which is removed on dilution. For these reasons we have chosen to use 1 : 10 plasma dilution for assay.

Mean fasting plasma insulin level in normal individuals was 14 μU/ml. with a range of 0-40 μU/ml. These levels agree with those found with most immunological techniques.

Plasma insulin levels rise to approximately double the fasting values during the slow infusion of 25% glucose and fall to fasting or below fasting levels at the end of the infusion.

One patient with high blood sugar levels throughout the infusion had low insulin values throughout the test whilst a patient with particularly low blood sugar levels maintained a high plasma insulin throughout the infusion.

One acromegalic patient studied had a higher than fasting plasma insulin and maintained high insulin levels throughout the infusion.

ESTIMATION OF FATTY ACIDS IN PLASMA

June Sheath

A new method for the estimation of plasma fatty acids has been developed and by it non-esterified fatty acids (NEFA). In addition, by this method total triglyceride and cholesterol ester fatty acids were measured. By extraction of total lipids and their fractionation by thin-layer chromatography, the relevant fatty acids of these pure fractions were determined. This was achieved by saponification, liberated fatty acids being assayed by the same colorimetric procedure as that employed for NEFA. This was achieved by the formation and estimation of a copper complex.

For the estimation of NEFA it was found that various solvent systems extracted different lipids and all of those systems investigated extracted NEFA, but some to different extents. It was found that two of these systems extracted maximum amounts of NEFA, but by their use other lipids were also extracted and these interfered in the colorimetric estimation. However, when aliquots of a single plasma specimen were extracted by either of these solvent systems and the NEFA extracts were separated and identified by thin-layer chromatography, identical values were obtained after colorimetry. This would indicate that true NEFA values were obtainable by the method developed.

INTERMEDIARY METABOLISM IN DIABETES UNDER TREATMENT WITH BIGUANIDE

June Sheath and Pincus Taft

A close examination of blood pyruvate and lactate levels, fasting and after food, in a number of maturity onset diabetic patients given progressively increasing doses of dimethyl biguanide has been made. Levels of these metabolites did not vary significantly from control levels (with the dose of the drug reaching a maximum of 3 grams per day) with but one exception.

Lactate-Pyruvate ratio remained relatively constant. Although these parameters of metabolism were relatively unchanged there was an influence on carbohydrate metabolism reflected by depression of blood sugar levels below control values. This study is continuing.

THYROID SURVEY

Kathleen Taylor

This survey conducted in conjunction with Dr. C. Nugent of the Department of Medicine in Salt Lake City, U.S.A., has continued. Computer analysis of selected symptoms and signs in 359 patients (including 117 patients from the Alfred Hospital) suspected of hyperthyroidism has now been made.

The diagnostic significance of 7 symptoms and 13 signs has been examined. Although a highly confident diagnosis ($P < 0.01$ or > 0.99) is being recorded by the computer in 60% of cases of euthyroidism and hyperthyroidism it appears that the present analytic scheme is making unjustifiably confident diagnosis in some instances.

A revised version of the computer programme is being examined in an effort to improve performance. Further work will continue and further case records be added to the study.

THYROID FUNCTION STUDIES UNDER THE INFLUENCE OF ORAL CONTRACEPTIVES

Dora Winikoff, Kathleen Taylor and Wanda Davies

An investigation of the influence of oral contraceptives on thyroid function tests is in progress.

The following indices have been used: protein bound iodine, I^{131}-triiodothyronine resin uptake, hormonal iodine, electrophoresis with added I^{131}-thyroxine, and in some instances oxygen consumption and 4-hour uptake of I^{131} by the thyroid gland.

The results to date confirm the well known facts of elevation of protein bound iodine, and depression of T_3 resin uptake.

Our investigations have also disclosed an increase in oxygen consumption as recorded by B.M.R. and an increased binding capacity of thyroid binding globulin demonstrated by electrophoretic studies. A consistent increase in "hormonal iodine" estimated by dowex resin separation which parallels the rise in PBI has also been observed.

The duration of the effect of oral contraceptives has been followed by serial testing, on and off medication. It was found that the full effect was not

apparent by the end of the first or second cycle. On the other hand, after long term administration, thyroid function tests did return to normal for many weeks.

These observations are of significance in the interpretation of thyroid function tests in patients taking oral contraceptives—an increasing number of whom are coming to our attention.

By the combination of all indices used in our laboratory we have been better able to assess thyroid function in these patients as well as in early pregnancy where rise in PBI and depression of T_3 resin uptake is also seen.

It is hoped to extend this study into the field of utilisation of thyroid hormones and the long range effect of oral contraceptives on iodine metabolism.

THYROID HORMONE CARRIAGE AND BINDING CAPACITY

Kathleen Taylor and Dora Winikoff

Electrophoretic study of the binding of thyroid hormone to plasma protein using the technique of electrophoresis on cellulose acetate with barbital buffer, pH 8.6 has been initiated. Radioactive thyroxine is added to the sera under study and the radioactivity present in the various protein fractions measured.

The fraction of radioactivity found in association with thyroxine binding globulin (TBG) has been expressed as a percentage of the total radioactivity bound to all the proteins. By these means the ratio in hyperthyroidism was found to be less than 62%, in euthyroidism between 62-75%, and in hypothyroidism, pregnancy and, under influence of oestrogens, greater than 73%. The overlap is very small.

This ratio reflects the capacity of thyroid binding globulin to bind additional thyroxine and provides indirect information regarding the amount of endogenous thyroxine bound in carriage in the plasma. Inference can then be drawn regarding the functional activity of the thyroid gland.

This technique also provides an additional tool in the elucidation of problems of thyroid diagnosis under circumstances of unusual thyroxine binding where PBI level may not truly reflect thyroid functional status.

MICRO IODINE ASSAY

Dora Winikoff

1. Studies with Dowex Resin and Sephadex Gel.

Serum or plasma is passed through columns of Dowex and Sephadex and the eluate, after washing the columns with acetic acid and water respectively, is collected in a series of fractions. Iodine assays are performed on these fractions. Characteristic patterns of recovery of iodine in these fractions have been observed with different types of iodine administration to the patient under study. It is under such circumstances of iodine contamination that these techniques have their place in thyroid diagnosis.

With uncontaminated sera the first three fractions at pH 1.4 collected from Dowex columns contain the "hormonal" iodine and from Sephadex columns the iodine value of the first fraction closely approximates the PBI.

Findings in Situations of Iodine Administration.

Iodine	Dowex	Sephadex
None	Iodine in fractions I-III (Hormonal iodine) > 99% of PBI in euthyroid, > 85% of PBI in hyperthyroid and > 70% of PBI in myxodema. Nil unabsorbed (fraction 0) or in fractions IV-VI.	Iodine in fraction I 96% of PBI. Practically none in fractions II-V.
Inorganic Iodine	Iodine in fractions I-III > 75% of PBI. In presence of large quantities some may be found in fraction 0.	Slight elevation in fraction I, increasing quantities in subsequent fractions.
Organic Iodine	Iodine in fractions I-III > 75% of PBI. Iodine found in fraction 0 and may be found in fractions IV-VI.	Large bulk of iodine contaminant found in fraction I. In time and with splitting of the organic compound, iodine is found in fractions II-V.

2. A new modification in the assay of hormonal iodine on Dowex resin has been adopted.

The initial absorption on the columns is now performed at alkaline pH 11-12 by using 0.8 N ammonia for equilibration. The elution of thyronines from the column is executed with 10 N acetic acid.

The percentage recovery with this modification is from 95-105%. Moreover, some contrast media after a passage through the column remain in the unabsorbed fraction, and do not interfere with the rest of the procedure.

This improved technique has been of great value in difficult diagnostic problems where iodine contamination invalidates the PBI results.

ESTIMATION OF PLASMA GONADOTROPHINS

Ida Ekkel and Pincus Taft

Previously the assay of gonadotrophic activity of human plasma has been confined to that of post-menopausal women or to people with suspected high levels. However, because of insufficient sensitivity it was not possible to determine the lower levels which could be expected in normal subjects of reproductive age. The maximum volume of plasma extract which could be injected into mice without toxicity was 5 ml. prepared from 10 ml. plasma.

During the investigation, a method has been devised which will detect lower levels of gonadotrophic activity as found in plasma of men and women of reproductive age. By this technique it is possible to administer to mice extracts equivalent to 100 ml. plasma without toxic effects.

Briefly, the plasma extract is prepared for injection in the following way. Heparinised plasma is adjusted to pH 5 and the proteins are precipitated by the addition of acetone. The precipitate is then extracted with an alcoholic ammonium acetate solution. Gonadotrophins are finally precipitated by absolute alcohol saturated with ammonium acetate. After washing and drying, the precipitate is dissolved in distilled water and estimated by the mouse uterine weight assay.

In this study plasma pools of normal men and women of various age groups were assayed. These age groups were: male, 20-45 years; male, 45-60 years; female, 20-40 years; female, 50-60 years. Results show that gonadotrophic activity is present in all groups studied, the highest being demonstrated by females of the post-menopausal group. Significantly lower values were obtained for the other groups. In comparison with the male group (20-45 years), slightly higher values were found for males (45-60 years) and slightly lower values for females (20-40 years).

PAPERS PUBLISHED DURING 1964

BREIDAHL, H. D.—"The Glucose Tolerance Test in Pregnancy". *Aust. & N.Z. J. Obstet. & Gynaec.*, Vol. 4 (1964), p. 90.

ENNIS, Gordon, Richard GORDON and Bryan HUDSON—"A Synthetic Polypeptide (Ciba 30'920-Ba) with Adrenocorticotrophic Properties". *Med. J. Aust.*, Vol. 1 (1964), p. 590.

TAYLOR, Kathleen, Dora WINIKOFF and Wanda DAVIES—"Resin Uptake of I^{131}-Labelled Triiodothyronine as a Test of Thyroid Function". *Aust. Ann. Med.*, Vol. 13 (1964), p. 240.

LECTURES DELIVERED DURING 1964

"Diabetes in Pregnancy"—Malcolm Gillies Memorial Lecture, *Royal North Shore Hospital Clinical Society.*	PINCUS TAFT
"Studies of Gonadal Function in a Patient with Male Pseudohermaphroditism"— *Endocrine Society of Australia.*	PINCUS TAFT
"Pregnancy and the Physician"—in a Symposium for the *Melbourne Medical Post-Graduate Committee.*	PINCUS TAFT
"Thyroid Disorders in Childhood"—in a Symposium—*Royal Children's Hospital Clinical Society.*	PINCUS TAFT
"Osteoporosis — a Geriatric Problem"—*Alfred Hospital Residents' Graduates Association.*	H. D. BREIDAHL
"Diabetes in Pregnancy"—*Seminar to Medical Students, University of Wisconsin, Madison, Wisconsin.*	H. D. BREIDAHL
"The Growth and Development of Children Born to Mothers with Diabetes"—*Read by title, 5th Congress of the International Diabetes Federation, Toronto.*	H. D. BREIDAHL
"The Management of Obesity" — *Royal Perth Hospital Residents' Society.*	H. D. BREIDAHL
"Testicular Biopsy in the Diagnosis of Hypogonadal States"—*Princess Margaret Hospital for Children, Perth.*	H. D. BREIDAHL
"Diabetes in Pregnancy"—*King Edward VII Memorial Hospital for Women, Perth.*	H. D. BREIDAHL
"Diabetes in Pregnancy"—*Melbourne Medical Post-Graduate Committee Meeting at Warrnambool.*	H. D. BREIDAHL
"Thyroid Function and Disorders in Pregnancy"—*Royal Women's Hospital, Melbourne.*	H. D. BREIDAHL

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

STUDIES ON CHEMOTHERAPY[1]

J. C. Tolhurst and G. Buckle

For many years past infections by the organism *Staphylococcus aureus* have been increasingly troublesome all over the world and especially so in hospitals. Many of the antibiotics now in use have been produced mainly to combat this organism.

During the last two years it has come to appear possible that the problem has nearly been solved, to a large extent by the use of the antibiotic Methicillin. This drug is highly efficient and no strains of *Staphylococcus aureus* resistant to it have been encountered here yet. Methicillin has been so effective that there has been no opportunity to test the clinical value of two other anti-staphylococcal antibiotics which, though known from laboratory work to be useful, are not expected to be as good as it.

While the staphylococcal infections have been decreasing, infections by gram-negative bacilli have been increasing. These events appear to be related. Just as the multiplication of antibiotic-resistant staphylococci is favoured by the accidental elimination of the antibiotic-sensitive staphylococci through the agency of antibiotic dust and droplets of antibiotic solution which are split or sprayed about, so is the usage of antibiotics entangled with incidental, unsought effects on the gram-negative bacili.

Much thought, observation and experimentation is being directed toward a wider view of the effects of antibiotics on micro-organisms.

Only one new substance, Nalidixic acid, has become available for use against gram-negative bacilli recently. It is useful in urinary tract infections.

The second edition of the monograph on antibiotics has been well received and has been praised by reviewers. The National Health and Medical Research Council of Australia has asked us to prepare a third edition by the end of 1965.

STUDIES ON MYCOBACTERIA[1]

J. C. Tolhurst and G. Buckle

In 1948, *Mycobacterium ulcerans,* a new organism infecting humans, was reported by a team which included members of this Department. The infection has been called "Searl's ulcer" after the physician who first took note of it, or "Bairnsdale Disease" after the town in which it was first recognised.

This infection has continued to appear in Gippsland and has been seen in other parts of Victoria, in New South Wales, Queensland and the Northern Territory, in Mexico and in the Belgian Congo.

In the past few years several reports have come from another part of Africa – Uganda – where many cases of a similar infection have been found and a similar organism cultivated. Recently a report appeared which stated that the organism from Uganda differed from *Myco. ulcerans* in various details.

1 Department of Bacteriology.

This claim is being examined. It appeared from the beginning to be a defect that the distinguishing points were based on the reactions of a single un-named strain of *Myco. ulcerans* and preliminary results have shown that the reactions in question are variable and therefore probably of no significance.

WOUND INFECTION[1]

G. Buckle and A. Perceval

A Register of Wound Infections has been kept in the Hospital according to a system which has been unchanged since 1958. It shows a continued decrease in infections to a rate which, for all wounds, is half what it was.

The greatest decrease from 14.7% to 3.3% is in the section called Clean Cases. This is the area in which improvements in material arrangements play a large part and in which laboratory investigations showing the necessity or the desirability of alterations in such things as ventilation, sterilizing methods, etc., gave support.

Other sections of the Register are much more influenced by the attitudes of and the care exercised by personnel. The results in these sections have not been as good and some aspects have deteriorated. This department has been engaged therefore in dissecting the results in order to draw attention to this in an attempt to stimulate endeavours to equal or surpass the best previous result.

SUBARACHNOID HAEMORRHAGE

James M. Calvert[2]

In the first year of randomized treatment of single intracranial aneurysms with proven subarachnoid haemorrhage, 69 patients were referred for treatment or investigation.

Bilateral carotid angiography was performed on 61 patients. The other 8 died shortly after admission. Vertebral angiography was performed in addition on 25 patients.

Twenty-three patients qualified for admission to the treatment study. No vascular malformation was found in 21 patients undergoing vertebral and bilateral carotid angiography.

Fourteen patients were disqualified from the study for various reasons:—

3 — Multiple aneurysms.

2 — Emergency surgery for evacuation of haematoma.

2 — Angiomatous malformation.

2 — Previous operative treatment.

4 — Referred from other hospitals for specific treatment.

1 — Aneurysm without haemorrhage.

Three patients were considered to have suffered arteriosclerotic intracerebral haemorrhage with rupture into the ventricles.

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1 Department of Bacteriology.

2 Neurosurgical Unit.

Randomized Treatment

Treatment	Total	Mortality
Rest in bed	6	1
Drug-induced hypertension and rest in bed.	5	2
Carotid Ligation	8	2
Direct Approach	4	3
	23	8

No significant figures have yet emerged from this co-operative study.

ACTIVITY OF RESPIRATORY MUSCLES

J. F. Mainland[1]

The electrical activity of the respiratory muscles has been studied following cholecystectomy and gastrectomy.

(a) Silver disc electrodes, held in place by suction and "Steridrape" for electromyograph recording from the intercostal muscles were designed along the lines used by Campbell (1958). These were tested and found to work well but considered to be unsatisfactory for use on patients being cared for intensively.

(b) Stainless steel wire electrodes, similar to those used for cardiac stimulation, were sewn into the diaphragm of patients undergoing upper abdominal operations. The electromyograph was recorded daily for up to eight post-operative days.

Results have shown a decrease in rhythmic activity of the diaphragm post-operatively for about three days, after which time full rhythmic activity of the diaphragm returns. This finding is considered sufficiently important to continue investigations with this type of recording. Further, the technique can be regarded as satisfactory, safe, and without discomfort at all to patients on whom it is used.

(c) Design and construction of an integrating pneumotachograph is continuing. Two breathing heads were designed and constructed and subsequently tested with the assistance of Commonwealth Industrial Gases Ltd. One of these heads was found to be linear up to 525 litres per minute, i.e., about double the velocity of any previously published pneumotachograph head. This head will be used for studying coughing in post-operative patients.

It is hoped that the complete pneumotachograph will be ready for clinical use before the end of 1964.

1 Department of Anaesthesia and Resuscitation, in conjunction with Department of Surgery, Monash University.

SOCIAL AND PSYCHOLOGICAL ASPECTS OF CARDIOVASCULAR DISEASE‡

B. B. Thomas[1] and H. L. Martin[1]

The social and psychological aspects of a first coronary occlusion have been studied, at the time of onset of symptoms, in 142 male patients aged between 30-65 years. Patients were referred from three public teaching hospitals, one non-teaching public hospital, from private and consultative practice and were included in the sample of patients according to specified medical criteria. Data were gathered by one worker in a series of interviews with patients, families and doctors in the acute stage of the illness. These were supplemented by detailed job studies and by review interviews with patients and families at three and six months after the onset of symptoms. This has allowed an assessment to be made of the rehabilitation of the patient to work, of attitude and leisure patterns at three and six months after the illness.

Qualitative analyses have been made of:—

(i) The psychological reactions noted in patients and families in the first six months after the coronary episode.

(ii) Factors contributing to failure and success in rehabilitation at three and six months after the coronary occlusion.

(iii) Formal conditions of employment possibly affecting the employment and re-employment of disabled cardiac workers.

A detailed review of the literature relating to the social and psychological aspects of coronary artery disease, with particular reference to rehabilitation has been completed. A quantitative analysis of the employment situation in Victoria has been made to cover the period of study up to November, 1964.

Statistical analysis of the relative weight of certain variables thought to affect rehabilitation is in progress, and some recommendation will be made as to principles of rehabilitation management of cardiac patients, based on the observations obtained in this study on this series of patients.

AUTOTRANSPLANTATION OF KIDNEY

J. Nayman[2]

The technique of transplantation of the left kidney to the right iliac fossa (with subsequent right nephrectomy) has been undertaken.

The technical problems have been overcome and dogs are surviving for up to 10 months, with a single renal autotransplant.

HAEMODIALYSIS

J. Nayman[2]

DYNAMICS OF DIALYSIS

The efficiency of dialysis has been improved by increasing the flow rate of the dialysing fluid, employing a new pump. Studies comparing dialysance utilising these two pumps have been completed.

‡ This project is supported by a grant from the National Heart Foundation of Australia.

1 Medical Social Work Department
2 Haemodialysis Unit and Department of Surgery, Monash University.

WEIGHING BED

An improved design has been developed and is proving to be more satisfactory than the original model.

ARTERIO-ARTERIO-VENOUS SHUNTS

A new technique for cannulation which may be of help in the long-term cannulation for periodic haemodialysis is being investigated.

The artery and vein are anastomosed directly to each other at the level of the wrist, and a prosthesis is inserted in the radial artery in the middle third of its course in the forearm.

WOUND HEALING IN RENAL FAILURE

J. Nayman[1]

This project is continuing and the effect of dialysis in the dog in relation to wound healing in renal failure has commenced.

RENAL BIOPSY

J. Nayman[1]

This technique has been evaluated and a modified biopsy needle constructed.

1 Haemodialysis Unit and Department of Surgery, Monash University.

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JOHNSON, D., J. A. BARNES and E. McLEOD—"A Case of Endocarditis Treated with Ampicillin". *Med J. Aust*

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"Gram-Negative Bacilli"—*A.N.Z.A.A.S., Canberra.*	G. BUCKLE
"Torulosis in Victoria"—*Seminar for U.C.L.A. Group visit, Monash University.*	G. BUCKLE
"Some Aspects of Renal Failure"—*Alfred Hospital Clinical Society.*	J. NAYMAN
"Surgery and Renal Failure"—*Repatriation Hospital, Melbourne.*	J. NAYMAN
"Haemodialysis"—*Australian College of Pathologists, Melbourne.*	J. NAYMAN
"Use of Regional Heparinization in Haemodialysis"—*Western Reserve University, Cleveland, U.S.A.*	J. NAYMAN
"Acute Renal Failure in Surgical Practice and Some Recent Developments in the Use of Haemodialysis" — *University of Witwatersrand, Johannesburg, South Africa.*	J. NAYMAN
"Wound Healing and Renal Failure"—(a) *Alfred Hospital Clinical Society,* (b) *Portland, Oregon, U.S.A.,* (c) *Georgetown University, Washington, U.S.A.*	J. NAYMAN
"Technology of Arteriovenous Shunts"—*University of Seattle, Seattle, U.S.A.*	J. NAYMAN
"An Arteriovenous Shunt for Use in Acute Renal Failure"—(a) *American Society for Artificial Internal Organs, Chicago, U.S.A.* (b) *Veterans Hospital, Boston, U.S.A.,* (c) *Peter Bent Brigham Hospital, Boston, U.S.A.*	J. NAYMAN
"Dialysis"—*Georgetown University, Washington, U.S.A.*	J. NAYMAN
"Open Renal Biopsy"—(a) *Walter Reed Army Hospital, Washington, U.S.A.,* (b) *Veterans Hospital, Boston, U.S.A.*	J. NAYMAN
"Review of Renal Homotransplantation"—*Melbourne University.*	J. NAYMAN
"Use of a Simplified Sub-Culture Apparatus" — *Veterans Hospital, Boston, U.S.A.*	J. NAYMAN
"Tracheostomy Humidification" — *Workshop Conference, Monash University.*	J. F. MAINLAND

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