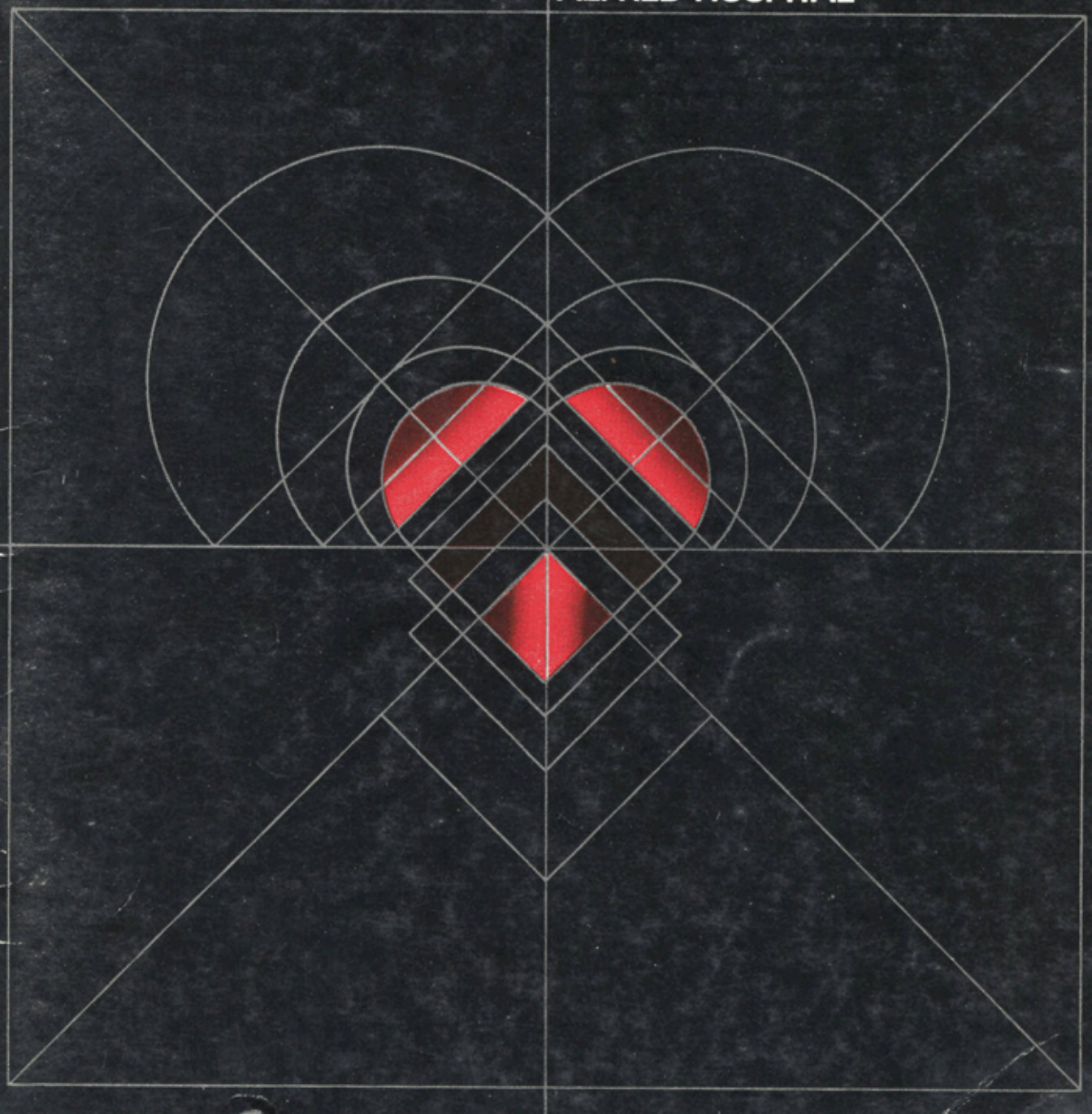
Research 1973

BAKER INSTITUTE

ALFRED HOSPITAL



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

The Clinical Research Unit is a department of the Alfred Hospital whose duty is to conduct clinical research.

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

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

Forty-seventh Annual Report of

The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute

(The Institute is affiliated with Monash University)

Twenty-fifth Annual Report of

The Alfred Hospital Clinical Research Unit

Seventeenth Annual Research Report of

The Ewen Downie Metabolic Unit

Reports of

Alfred Hospital Research Fellows

1973

Alfred Hospital, Prahran, Victoria, 3181, Australia.

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Introduction

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

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

The 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 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 the two units remained functionally integrated until his retirement from the Clinical Research Unit in 1973.

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

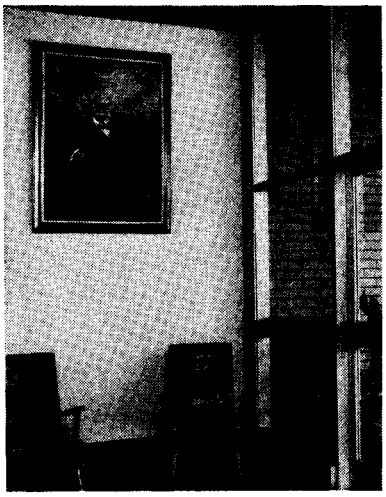
In 1956 the Board of Management formed a Diabetic and Metabolic Unit, which is engaged in investigation of endocrine and allied disorders. In 1969 this unit was renamed The Ewen Downie Metabolic Unit. This has also been placed under the supervision of the Research Advisory Committee.

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

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

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

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



Baker Medical Research Institute

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Staff

Director	T. E. LOWE, C.B.E., D.Sc., M.D., F.R.C.P., F.R.A.C.P.	
Administrative Assistant	R. BLAKEMORE, LL.B.	
Graduates	R. BORLAND, B.Sc., B.V.Sc., Ph.D. J. CANNATA, B.Sc. Miss A. CHANG, B.Sc., Ph.D. (to 17/8/73) E. COOPER, M.B., B.S., F.R.A.C.S. P. DAILE, B.Sc. (to 27/4/73) Miss W. ELSE, M.Sc. (from 1/10/73)	G. C. HARD, B.Sc., B.V.Sc., Ph.D. J. E. MALONEY, M.Sc., Ph.D. EVA MÄSIAR, M.D. P. MÄSIAR, M.D., D.Sc. WINIFRED G. NAYLER, D.Sc. D. M. SHAW, B.Sc. (to 18/5/73)
(Attached)	Miss D. ALCORN, M.Sc. (Ph.D. Student) Miss J. BARWICK, B.Sc.(Hon.) (Ph.D. Student)	B. C. RITCHIE, M.B., B.S., F.R.A.C.P. A WALKER, M.Sc. (Ph.D. Student)
Technical	R. P. STEELE (Laboratory Supervisor) (to 30/3/73) A. H. HUCKFIELD (Acting Laboratory Supervisor) (from 2/4/73) Miss J. DIXON (Senior Technologist) V. BRODECKY	Mrs. H. DILLON (from 16/6/73) Mrs. B. DOBRASTANSKI (to 15/6/73) Miss S. ECKERT (from 30/4/73) R. HORNE (from 15/11/73) D. G. OAKLEY Mrs. J. ORTIZ
(Attached)	R. SMITH (from Monash University)	
Librarian	Mrs. M. RAE	
Clerical	Miss J. CLARK Miss C. MARAZZITA	
Laboratory	Mr. C. BECKER Miss M. COGDON (to 23/11/73) Mr. K. HARVEY Miss D. KELLEHER Miss P. KIRCHNER Miss E. KOHN Mr. C. LEWIS (from 1/4/73)	Mr. R. LOWE (to 15/11/73) Mrs. A. MOORE (to 5/10/73) Mr. E. PARSONS (from 7/5/73) Mrs. E. PAYNE Miss A. SCHUTE (to 22/11/73) Miss C. SCHWARZE
(Attached)	Miss B. CHARLES (from Monash University)	
General	Mrs. M. WRIGHT	

Research Fellowships

Members of the Institute Staff have held the following Research Fellowships

Honorary	E. COOPER, M.B.B.S., F.R.A.C.P. B. C. RITCHIE, M.B.B.S., F.R.A.C.P.
Consulting Fellow	WINIFRED G. NAYLER, D.Sc.
Anti-Cancer Council of Victoria ("A. A. Thomas")	G. C. HARD, B.Sc., B.V.Sc., Ph.D.
"James and Elsie Borrowman"	A. CHANG, B.Sc., Ph.D.
"William Buckland"	R. BORLAND, B.Sc., B.V.Sc., Ph.D.
"The Lang Fellow"	P. MÄSIAR, M.D., D.Sc.
"Laura Nyulasy Scholar"	Miss W. ELSE, M.Sc.

Annual Report of the Director

At the end of 1973 twenty-five years of functional integration of the Institute and the Clinical Research Unit of Alfred Hospital came to a close and 1974 will begin a period of change.

In October I retired from the Directorship of the Clinical Research Unit and an acting director has been appointed. In consequence of these changes the "Research" report this year has the two bodies separately represented and perusal of the projects shows how the Baker projects have depended on CRU integration to give them clinical orientation and **vice versa** how those of the CRU have needed basic biological backing from the Institute. It will also be noted that with the departure of Dr. Winifred Nayler to London last year and my own anticipated retirement the cardiovascular research projects, which have loomed so large in our past work, have been wound up. They cannot easily be revitalised for an additional year. The research projects for 1974 will therefore be arranged around the existing ones which relate to chemical carcinogenesis and cardio-pulmonary developmental physiology.

A development of interest this year has been the evolution of the role of Consulting Fellows of the Institute. Dr. Nayler was in 1972 the first appointee to the rank and during this year much experience has been gained in the ways such Fellows can by visits and modern communications be effective partners in projects. Building on this experience it is planned that in 1974 the chemical carcinogenesis project will become a co-operative effort of three centres with Dr. R. Borland of Cambridge University and Dr. B. W. Stewart of the University of New South Wales as Consulting Fellows.

Current Research Projects

Detailed accounts of the research projects are given in the scientific section of the report and they fall into three major groupings: Cardiovascular System, Carcinogenesis and Respiratory System.

Cardiovascular System

Cardiac muscle provides the motive force for the circulation of blood. It is composed of very large numbers of individual units (cells) which contract in a co-ordinated manner. The efficacy with which these cells convert the energy of this fuel into mechanical energy determines the ability of the heart to do the work required of it. Various research projects in 1973 have been directed towards obtaining a detailed understanding of this energy conversion. These have employed various drugs and chemicals to influence the biochemical processes and ionic movements, they have studied the naturally occurring peptides of the blood stream which appear to influence cardiac muscle contraction and they have investigated the role of the regulatory protein — troponin — within the muscle cell itself.

Carcinogenesis

It has been known for a long time that exposure of living tissues to certain chemicals can provoke a cancer in which unrestrained cell proliferation can lead ultimately to destruction of the host. Exposure to naturally occurring carcinogenic chemicals is so complex a situation that models to simplify and permit control of experimental conditions are essential for progress in an understanding of this process. Such a model in rats has been developed in which one dose of a carcinogenic chemical produces a kidney cancer. It has been possible so far to show that the cancer commences to form within the first 20 hours after exposure to the chemical and possibly within the first four hours.

Respiratory System

In higher mammals the foetus develops in the uterus immersed in liquid — liquor amnii — and its oxygen and carbon dioxide requirements are controlled through the placental blood circulation. At birth the placental circulation ceases and the foetus exchanges its liquid environment for air and controls its oxygen and carbon dioxide requirements by breathing through the lungs.
The development of structure and function of the lung and its timetable in the foetus is therefore of considerable interest, especially if the birth takes place prematurely. It is also possible that some neonatal respiratory troubles may be related to failure of correct development of the structure and function of the lungs.
To study these problems a model has been devised in the sheep by which the development of respiratory function can be investigated during life and this is coupled with structural studies at various periods.

Staff

Apart from various changes in junior scientific and technical staff occurring during the year the following are worthy of individual comment.

Dr. J. E. Maloney made a short visit to the U.S.A. to attend the Sixth Annual Workshop and Symposium on Scanning Electron Microscopy and then visited English centres and the University of Zurich to observe scanning electron microscopy practices.

Dr. A. Chang left to go overseas to take up a fellowship in Paris to further her studies in electron microscopy.

Late in the year Drs. Pavel and Eva Mäsiar completed their three year appointments at the Institute and returned to Europe. Their considerable contribution to the research projects of the Institute is recorded in the Scientific Section of this report.

Dr. R. Borland of the Department of Pathology, University of Cambridge, spent a sabbatical year in the Institute working in the carcinogenesis projects.

Research Assistance

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

It is a pleasure to thank for donations those whose names are listed in the various financial reports and those who have so generously helped with the purchase of equipment. Especially welcome was the recurrent grant from the Government of the State of Victoria towards maintenance expenses.

Many organisations have made gifts to the Institute Library and our thanks are expressed to them, to various libraries that have loaned us journals and particularly to the librarians whose assistance is greatly valued. The continuing close co-operation between the libraries of the Institute, Hospital and Monash University Medical School is of great benefit to our staff.

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

Again I thank the Trustees of the Institute for their continued generous support and members of the staff and research fellows for their co-operation during the year.

T. E. LOWE.

December 31, 1973.

Alfred Hospital Research Fellows in the Institute 1949-1973

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., 1962-73.
Coventry, D. A., 1968.
Daile, P., 1970-71.
Duffy, D. G., 1952-55.
Ferguson, I. A. L., 1957-58, 1973.
Fowler, R., 1953-54.
Francis J. K., 1956-57.
Fraser, J. R. E., 1957.
Gardiner, J. M., 1952.
Goble, A. J., 1951.
Hudson, B., 1952.
Jamieson, K., 1954.
Kay, H. B., 1959-60.
Kincaid-Smith, P., 1959-60.
McCutcheon, A. D., 1959, 1965-66.
McDonald W., 1960-61.
McNeur, J. C., 1955.
McRae, C. J., 1955.
Mäsiar, E., 1972.
Murfitt, L., 1955.
Newman, H. C., 1954.
Parsons, P. J., 1951.
Quinn-Young, M., 1956.
Race, D., 1959-63.
Sawers, R. J., 1953-60.
Silberberg, F. G., 1953.
St. Clair, W. A., 1955.
Stern, W., 1954-55.
Stirling, G. R., 1955, 1969.
Swann, J. B., 1967.
Wagner, G., 1958.

Overseas Fellows

Borland, R., 1973 (Cambridge).
Dawson, J. B., 1961-63 (Oxford).
Emslie-Smith, D., 1955-56 (Dundee).
Hamilton, M., 1954 (London).
Jones, T. G., 1966 (London)
Katz, S., 1971-72 (Montreal).
Lumb, F. H., 1960-61 (London).
Marshall, R. J., 1957 (Belfast).
Moir, T. W., 1968 (Cleveland).
Mommaerts, W. F. H. M., 1971 (Los Angeles).
Nelson, C. V., 1969 (Portland, Maine).
Robertson, P. G. C., 1963-64 (Dundee).
Simpson, F. O., 1958-59 (Edinburgh).
Stevenson, M. M., 1957 (Belfast).
Thomson, J. W. W., 1959 (Edinburgh).

Rouse Library

List of Organisations which have made Gifts to the Library during the Year

Alfred Hospital.
Anti-Cancer Council of Victoria.
Auckland Medical Research Foundation.
Austin Hospital.
A.N.Z.A.A.S.
Australian Medical Association.
Australian National University (John Curtin School of Medical Research), Canberra.
Baylor University, Texas.
Cardiac Society of Australia and New Zealand.
Commonwealth Department of Health, Canberra.
Commonwealth Serum Laboratories.
Department of Public Health, Papua and New Guinea.
Glaxo Laboratories, Greenford, Middlesex.
Imperial Chemical Industries, London.
Institute for Cancer Research, Fox Chase, Philadelphia.
Institute of Medical Research, Brisbane.
Instituto de Biologia y Medicina Experimental, Buenos Aires.
John Innes Institute, Norwich.
Kanematsu Memorial Institute, Sydney.
Medecine d'Afrique Noire, Dakar.
Medical Research Council, London.
Melbourne Medical Postgraduate Committee.
Monash University.
National Heart Foundation of Australia.
Ophthalmic Research Institute of Australia.
Polish Academy of Sciences, Warsaw.
Royal Melbourne Institute of Technology (Appointments Board).
St. Vincent's Hospital, Melbourne.
Societe medicale d'Afrique noire le langue Francaise.
South African Institute for Medical Research, Johannesburg.
South African Medical Research Council, Pretoria.
Statens Seruminstitut, Copenhagen.
Universitas Mariae Curie Sklodowska, Lublin.
University of Melbourne (Appointments Board)
University of Otago, Dunedin.
University of Toronto (Connaught Research Laboratories).
Walter and Eliza Hall Institute.
Wellington Medical Research Foundation.
World Health Organisation.

The changing direction of research at the Institute has resulted in the greater use by the staff of the library's own holdings in physiology monographs and journals. This trend is also reflected in the decreased number of requests for loans and photocopies to other libraries. Another change has been the greatly increased number of requests for loans from other institutions. These have been mainly calls on the library's large biochemical holdings.

Once again the Librarian acknowledges the great help and co-operation given by the closely situated Monash Clinical School Library and the Alfred Hospital Library.

Statistics

Monographs on shelves	755	
Journals received regularly	97	
Enquiries received during 1973 —		
from staff	580	
other libraries	495	
	—	1,075
Requests to other libraries —		
for loans	250	
for photocopies	108	
	—	358

Report of Scientific Investigations

The Cardiovascular System

V. Carson, P. Daile, T. E. Lowe, E. Mäsiar, P. Mäsiar, W. G. Nayler and F. R. Trinker.

Cardiac muscle provides the motive force for the circulation of blood. It is composed of very large numbers of individual units (cells) which contract in a co-ordinated manner. The efficacy with which these cells convert the energy of their fuel into mechanical energy determines the ability of the heart to do the work required of it.

Investigations into various aspects of this energy conversion and its controls have been pursued for many years. Particularly have the controls of this conversion been explored by studying the action on them of various drugs and the biochemistry of various enzyme systems and regulatory proteins and peptides. As some of these projects have been conducted by staff of the C.R.U. detailed descriptions will be found in that section of the report and only brief summaries given here.

Excitation - Contraction Coupling in Heart Muscle †

W. G. Nayler and T. E. Lowe

Superficially-located Ca^{2+} storage sites

It is now generally accepted that excitation-contraction coupling in heart muscle cells involves an inwards displacement of Ca^{2+} from the extracellular phase and a displacement of Ca^{2+} from intracellular storage sites. The inwards displacement of Ca^{2+} takes place in response to membrane depolarisation and accompanies the rising and plateau phases of the action potential. Probably some of those Ca ions which enter the cell activate contraction directly but others may function as a "transmitter substance" to evoke a secondary release of Ca^{2+} from intracellular storage sites. These intracellular storage sites are probably located within the sarcoplasmic reticulum. The retrieval of Ca^{2+} from the myoplasm by the sarcoplasmic reticulum almost certainly facilitates the transition from systole to diastole.

Because isotope studies have consistently shown that the Ca^{2+} which is involved in excitation-contraction coupling is heterogeneous in origin, and that some of this Ca^{2+} is derived from superficially-located sites associated with the muscle cell, our experiments this year have been directed towards establishing the importance of the role that this superficially-located store of Ca^{2+} plays in the normal functioning of heart muscle cells. Using lanthanum ions to displace Ca^{2+} from these superficially-located storage sites we have consistently shown that the amount of Ca^{2+} which is stored here for subsequent release is dependent upon

(a) the extracellular Ca^{2+} concentration;

(b) the anionic composition of the extracellular fluid.

Thus the substitution of NO_3^- for Cl^- in the bathing fluid reduces the amount of Ca^{2+} which is stored at these superficially-located sites;

(c) hypoxia, this causing a reduction in Ca^{2+}-storing capacity; and

(d) the presence of certain drugs.

Thus whereas strophanthin-G enhances the Ca^{2+} storing capacity of these superficially-located sites, the compounds perhexiline and verapamil reduce it.

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

Drugs with Negative Inotropic Activity

Because perhexiline and verapamil reduce the capacity of the superficially-located storage sites in heart muscle cells to accumulate Ca^{2+} it was argued that the negative inotropic activity of these drugs, both of which are useful in the relief of angina pectoris, might be overcome by either raising the extracellular Ca^{2+} or reducing the extracellular Na^{+} concentration. Dose response curves were established, using small segments of dog papillary muscle suspended under isometric conditions in aerated Tyrode's solution warmed to 37°C. Under these conditions the negative inotropic effect of both perhexiline and verapamil was proportional to the concentration of Ca^{2+} in the extracellular phase. Thus the negative inotropic effect could be overcome by raising the extracellular Ca^{2+} and by reducing the extracellular Na^{+} concentration.

Inhibitory Effect of Mn^{2+} and Co^{2+} on Ca^{2+} Influx

Because both Mn^{2+} and Co^{2+} interfere with the inwards displacement of Ca^{2+} during the rising and plateau phases of the action potential and because the positive inotropic activity which is exhibited by a wide variety of cardioactive drugs is accompanied by an enhanced influx of Ca^{2+} it was decided to investigate the possibility that these other divalent cations, i.e. Mn^{2+} and Co^{2+}, might interfere with the positive inotropic effect of

(a) the catecholamines,

(b) cardiac glycosides.

The experiments were performed using dog papillary muscle preparations as previously described. Both Co^{2+} and Mn^{2+} interfered with the positive inotropic effect of the catecholamines, in a dose-dependent manner. However, when the cardiac glycosides were used in the presence of either Co^{2+} or Mn^{2+} a sustained contracture developed, a finding which cannot yet be satisfactorily accounted for and therefore requires further investigation.

Effect of Inotropically-active Drugs on the Total Ca^{2+} Content of Cardiac Tissue

As part of a long-term on-going programme aimed at establishing that the inotropic activity exhibited by a wide variety of cardio-active drugs is not necessarily associated with a change in the total (ionised + bound) amount of Ca which is found in heart muscle cells the Ca content of heart muscle which had been exposed to these drugs was determined and compared with that exhibited by control heart muscle not exposed to these drugs. The drugs so far investigated have included isoprenaline (in low doses, to avoid necrosis) glucagon, noradrenaline, verapamil, perhexiline and ouabain. Although the doses of these compounds which were used evoked marked changes in the peak tension developed during contraction, the tissue concentration of calcium remained unchanged. These results, therefore, substantiate the conclusion that changes in the contractile state of the myocardium are related to changes in the amount of **exchangeable** Ca^{2+} which is present, and are independent of the total amount of calcuim which is present.

Cardioactive Peptides

P. Mäsiar and Eva Mäsiar

Kinekard

The finding in heparinised blood plasma of a substance with an impressive inotropic action on heart muscle has been discussed in these reports for several years past. This substance has been described as a polypeptide with a molecular weight variously estimated between 4,000-8,000 daltons. Its inotropic action was reportedly found to be as much as 30 times higher than that of adrenalin. Many other parameters studied and described indicated that the discovery of this substance might have been an important contribution to our understanding of the regulatory mechanism of the heart action.

Although there have been numerous studies of its physiological action its chemical nature has not been determined and at times doubts about the uniqueness of the substance have been expressed.

Some three years ago we decided to endeavour to isolate and determine the chemical nature of this substance which had been already termed **Kinekard.** The following discussion is based on our own results as well as the available published material.

It has been known for a long time that positive or negative inotropic action can be ascribed

to a considerable number of substances present both inside and outside the body. The reaction itself represents a most complex process in the heart muscle and is also highly non-specific. Ringer noted that various compounds, e.g. blood proteins, milk protein, gelatin and some constituents of tap water could exert a positive influence on the contractility of heart muscle and that besides calcium, which he considered essential for cardiac muscle contractility, some organic constituents of protein-free extract of blood plasma could play an important role in the mechanism of heart contractility. He also observed seasonal differences in responses of the frog's heart to various drugs.

Several inotropically active compounds have been produced in recent years by industry and probably the same number are still hidden as trace impurities in "analytical reagents", which when used for the separation of a particular compound can simulate a positive inotropic effect of the separated substance(s) on heart muscle. We observed such an effect when N-ethylmorpholine-HCl buffer was used for purification so that traces of this buffer (which has been found excellent for fractionation) remaining after evaporation had to be removed in a separate experiment, using Sephadex gel filtration and distilled water for elution.

The proper purification of a fraction or a substance having a positive inotropic effect represents, therefore, one of the key problems in the study of the inotropic action of blood plasma fractions. The reports of Curtain and Nayler emphasise this problem. In their studies, considerably different values were found for the inotropic action of isolated fractions from heparinised blood plasma when small and large scale preparation procedures were employed. There are at least two possible explanations of this variation. First, a different fraction from blood plasma was isolated each time; or secondly the tested material was contaminated with some inotropically active material eluted from the columns or chemicals used. (Both possibilities are equally important since the active principle(s) has not been studied as a chemically defined substance(s).)

To avoid these abovementioned possibilities a method with the minimum risk of producing artefacts was needed for our attempts to characterise chemically Kinekard. This involved mainly avoiding the use of chemicals in the first step of the separation from blood plasma of inotropically active non-protein fractions. Therefore membrane ultrafiltration, which has been used to concentrate protein to obtain protein-free filtrates from biological fluids and to separate the products of proteolysis, was used and found essential for this purpose.

The inotropic activities of fractions obtained by this method are rather modest compared to those previously reported and we have not been able to obtain any measurable activity from 1 ml of plasma ultrafiltrate. The limit for fairly detectable activity was about 40 ml of dog blood plasma ultrafiltrate concentrated to 0.1 ml. Similar results were obtained when Tyrode solution was replaced by 40 ml of the respective plasma ultrafiltrate.

Comparing these results to those of Curtain and Nayler, it is possible to conclude that the inotropic action of our "pure" ultrafiltrate was as much as one thousand times lower than their small scale preparation and 2.5 times lower than the material from their large scale preparations.

Our experiments have also shown that further fractionation of the constituents of dog plasma ultrafiltrates by using more effective molecular sieving is possible and results in several physico-chemically different compounds possessing inotropic action when tested on papillary muscle preparations. None of these further fractions nor the purified substances fit completely the description of inotropically active substances given by Curtain and Nayler.

Two of the peptides whose molecular weight would partly correspond to that of Kinekard have been found inotropically inactive.
It should, however, be noted that these new peptides being described here represent only those isolated from dog blood plasma peptides which absorbed in the ultraviolet and a particular part of visible region of the light spectrum. This might lead to the assumption that Kinekard or any other previously described compounds could have remained undetected. In such a case the high molecular weight fraction obtained after Sephadex G-25 gel filtration should have been highly inotropically active. It was found that this fraction displayed only slight inotropic activity when tested on papillary muscle preparations.

From all these data it appears that inotropic activity is a real property of an intermediate molecular weight fraction of heparinised blood plasma. It seems unlikely however that this activity is related to a single compound of a peptide nature.

It is suggested that since the term **Kinekard** has been accepted into the scientific literature that it be used as a generic name for any inotropically active compound of peptide nature isolated from an intermediate molecular weight (1,000-10,000 daltons) fraction of blood plasma and that the active substances be characterised by at least three physico-chemical and chemical criteria, e.g. optical properties, purification procedures and amino acid composition.

It was reported last year that an inotropically active peptide isolated from blood plasma was an octopeptide containing only isoleucine and glycine.amino acids attached to an unknown compound. Its chemical composition has been further elucidated and the octopeptide is now known to be attached to guanosine phosphate and to yield ethanolamine. There still remains, however, an unidentified compound in the molecule.

Seasonal Variation in Inotropic Activity of Heparinised Dog Plasma

In the early reports of observations on the inotropic activity of blood plasma a marked difference of activity between summer and winter plasmas was noted using amphibians as the source of plasma and as activity indicators.

A series of experiments was therefore carried out to determine whether any chemical change could be detected between the intermediate molecular weight fractions isolated from dog blood plasma in summer and winter.

Plasma was fractionated by Sephadex G-25 gel filtration and the composition of the fraction expressed in terms of phenylalanine and tryptophane units (See Table I).

Although it is clear that there is a seasonal change in composition of the fractions the physiological significance of this is not clear.

Table 1

Seasonal Variation of the Concentration of Trace Plasma Peptides over the Period of one Year (expressed in Phenylalanine and Tryptophane Units/100 mls of Dog Plasma Ultrafiltrates)

	Phenylalanine Units found in Fraction						Tryptophane Units found in Fraction				
Season of the Year	A	B	C	D	E	Number of Experiments	A	B	C	D	E
Winter	1.7	3.0	1.5	1.5	0.9	6	0.8	1.7	0.6	1.7	0.8
	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%
Spring	1.8	4.4	2.8	2.6	1.0	13	0.8	2.2	1.5	2.0	1.0
	105%	146%	186%	173%	110%		100%	129%	250%	117%	125%
Summer	1.8	6.3	6.2	5.2	2.4	66	0.8	3.1	3.4	3.6	2.4
	105%	210%	413%	366%	266%		100%	182%	566%	211%	300%

Cardiac Muscle Homogenate

We reported last year that from the N-ethylmorpholine HCl extract of a homogenate of cardiac muscle, five molecularly different fractions were isolated. The fraction with a molecular weight between 1,000 and 10,000 daltons was further fractionated by Sephadex G-25 gel filtration and six different components were obtained. A study of the inotropic activity of these fractions has shown that fractions eluted mainly in the region of molecular weight about 1,000 daltons possessed inotropic activity. The further purification of each of these components was carried out by Sephadex G-10 gel filtration using distilled water as eluent. It was discovered that most of these components were still heterogeneous and only some of the subcomponents were inotropically active.

An attempt to find out whether adrenalin could be considered as one of the active fractions has been made in separate experiments using Sephadex G-10 gel filtration. The pure adrenalin sample prepared either from a pure powder or from an injection solution has been applied to a Sephadex G-10 column equilibrated with water. The subsequent elution with distilled water which had been used in our previous work with cardiac subfractions, was not able to de-sorb the adrenalin from the Sephadex column. The conclusion has therefore been drawn that the inotropic activity observed in the experiments using cardiac subfractions could not be ascribed to adrenalin. The chemical nature of the inotropically-active components isolated from intermediate molecular weight fractions of the heart extracts remains to be characterised.

Myocardial Contractility

Troponin*

P. Mäsiar, Eva Mäsiar and P. Daile

The material basis for the cardiac muscle contraction — relaxation cycle has been found to be similar to that of skeletal muscle. An interaction of specific proteins (actin and myosin) in the presence of ATP, Mg^{2+} and Ca^{2+} is the simplest interpretation of the process. While our knowledge of the actin and myosin and ATP and Mg^{2+} interaction has reached the stage of general understanding, the involvement of Ca^{2+} and the mechanism of its action remains still the subject of discussion. This situation persists partly due to our incomplete knowledge about the protein complex discovered by Ebashi and described originally as the relaxing system and later named **Troponin.** Nearly a decade of intensive work by several

groups has revealed enough information to start informed discussion about skeletal troponin and its subcomponents for it was discovered that the original troponin was not a homogeneous protein. Only a little is known even yet about cardiac troponin.

During the present year the molecular architecture of two types of cardiac and two types of skeletal troponin from dog heart and skeletal muscles has been studied. Preparations of dog (greyhound and mongrel) skeletal and cardiac troponin were made and SDS-polyacrylamide gel electrophoretic studies of these preparations have shown again that some differences between cardiac and skeletal troponin exist. Purification procedures using Sephadex G-200 gel filtration and DEAE-cellulose chromatography have shown that these differences are real.

It is believed that this observation can clarify the problem which has concerned investigators for a long time — that of the number of subunits in the troponin complex. The possible four components found from time to time for skeletal troponin by various authors could result from the existence of two isomers of actomyosin ATPase — inhibitory factor. For the cardiac troponin, both mongrel and greyhound, only one inhibitory component has been found. The peptide-mapping of a tryptic digest of carboxymethylated purified troponin components from dog skeletal and cardiac muscle confirms this conclusion.

It has also been found that two definitely different components in the troponin molecule, which differ in their molecular weights and electrophoretic pattern, do have a certain part of their molecular architecture very similar, if not identical. It is our belief that the primary structure of both might have been derived from a common origin.

Effect of Beta-Blocking and Other Drugs on the Adenyl Cyclase Activity of Cat Myocardium

V. Carson(1)

(See CRU Report, Page 40)

This study which commenced last year has now been completed and it may be said that the **in vitro** blocking effects of the β-blockers on the stimulation of adenyl cyclase activity by adrenalin mirror closely the physiological β-blocking effect and the adenyl cyclase — adrenalin assay would seem to be a useful tool in the evaluation of new drugs for β-blocking properties.

Effect of Mg^{2+} on the Inotropic and Metabolic Responses of the Isolated Perfused Rat Heart To Catecholamines

V. Carson(1)

(See CRU Report, Page 41)

In 1971 Paddle and Haugaard published the results of some experiments which revealed an unexpected action of Mg^{2+} on cardiac muscle metabolism. These observations have been repeated and co-ordinated with assays of cyclic AMP to further study the action of adrenalin on cardiac muscle metabolism.

Distribution of Myocardial Blood Flow in the Dog

F. R. Trinker(1)

(See CRU Report, Page 39)

Cardiac Surgery

Eric Cooper and G. R. Stirling

(See Report of Research Fellows, Page 62)

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(1) Clinical Research Unit, Alfred Hospital.

Carcinogenesis**

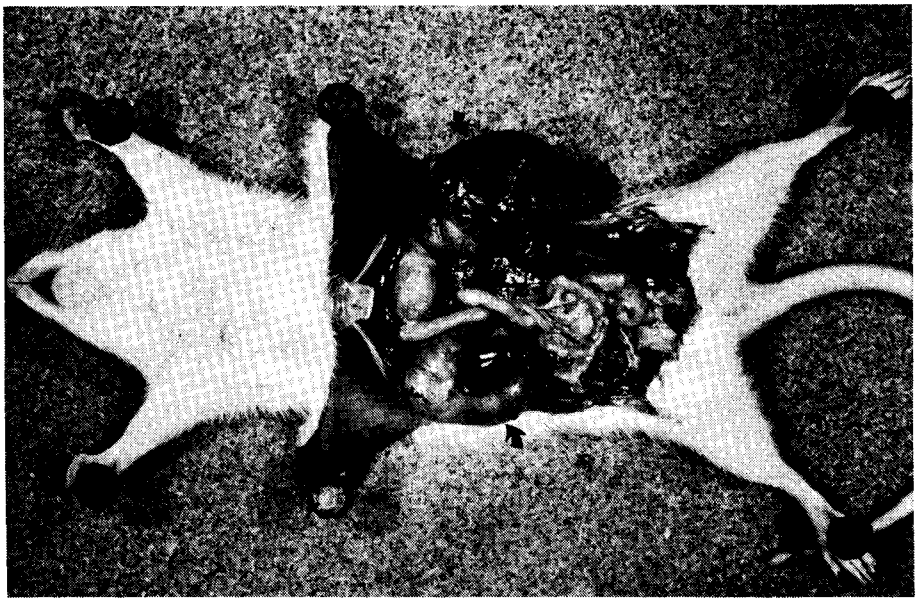
G. C. Hard, R. Borland, D. M. Shaw and B. W. Stewart[1]

Despite the intensity of the cancer research effort throughout the world, very little is yet known of the fundamental tissue and cellular changes which precede the histological or clinical manifestation of neoplasia. Such information, vital to our comprehension of the cancer process, is necessary for the formulation of strategy which might control or prevent this disease. The aim of our present studies is to define some of the phenomena which occur during the induction phase, and are a component of chemical carcinogenesis. The use of a suitable experimental model is a prerequisite. Although the occurrence of many neoplastic conditions in Man is most likely associated with low-level but long-term exposure to chemical carcinogens, in this experimental situation it is advantageous to investigate a model system which relates a single pulse of carcinogen (but necessarily a high dose level) to the induction of a uniform cancer in every treated animal. The action of the nitroso-compound dimethylnitrosamine (DMN) in the dietary-conditioned rat almost fulfils these requirements and provides a unique opportunity for basic research of the nature outlined. A system combining a single pulse with a high tumour incidence facilitates the interpretation of interim lesions with respect to the cause and effect. Furthermore, the time of induction can be firmly established and the relevant phenomena more easily identified. Continued presence of carcinogen resulting from repeated administration of the agent may alter the nature of precursor foci and prohibit their identification because of continued toxic pressure. Examination of the cancer process induced by nitrosamines appears to be particularly relevant as they have been recognised as the most formidable class of experimental carcinogens yet discovered, and represent a potential hazard in Man's environment.

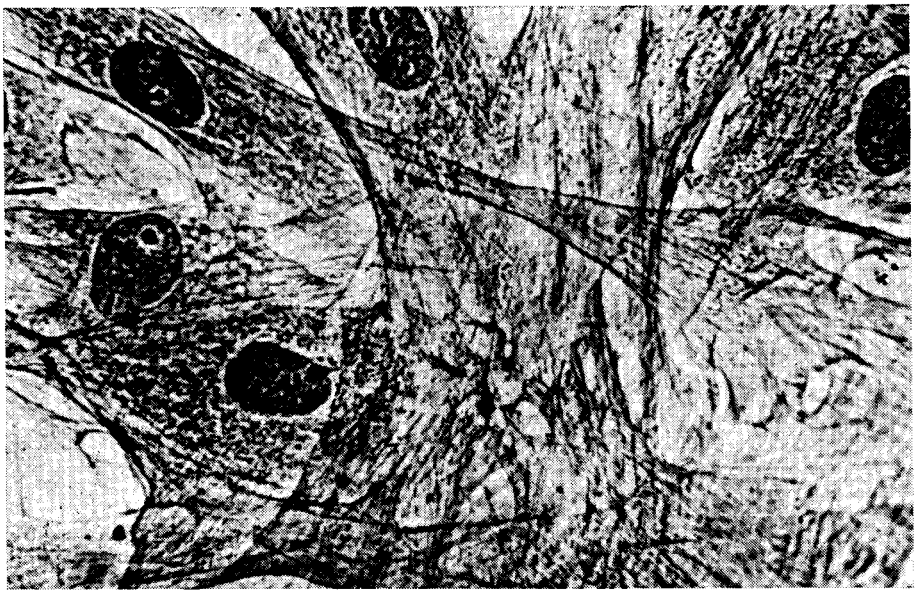
(1) Department of Pathology, University of New South Wales.

DMN-Renal Tumour Model

The intraperitoneal administration of a single dose of DMN to the Porton Down albino variety of Wistar rats (PAW), aged 5-6 weeks, induces a low incidence of kidney tumours. If the rats are pre-conditioned for three days by feeding sugar only (that is a diet high in carbohydrate but lacking in protein) prior to the carcinogen treatment, then microsomal enzyme activity in the liver is suppressed and more DMN is metabolised by the kidney. Under these conditions, all rats surviving the acute toxic effects of a single, intraperitoneal dose of 60 mg/kg, usually between 40-50% of the total number treated, develop renal neoplasia.



Bilateral renal mesenchymal tumour (↑) induced by a single injection of DMN.



In vitro culture of mesenchymal cells established from a renal mesenchymal tumour.

Two types of tumour occur. All survivors bear mesenchymal cancer, often multifocal and bilateral, whilst 30-40% also develop adenomas or adenocarcinomas of the tubule epithelium.

Histologically, the mesenchymal tumour is a very heterogeneous neoplasm consisting of sarcomatous sheets of spindle-shaped fibroblasts, areas of embryonic mesenchyme, tracts of smooth muscle, vasoformative organisation by groups of tumour cells and a matrix of reticulin and collagen. Some tumours also contain mature striated muscle, rhabdomyoblasts, pericytes and areas consistent with haemangioma. An assortment of epithelial structures such as cysts, tubules and islands of transitional epithelium are also a feature of all mesenchymal tumours but light and electromicroscopic examination reveals that these are the remnants of sequestered, pre-existing components of renal parenchyma and invaded pelvis. In the past, this neoplasm has been termed a nephroblastoma but because of its exclusively mesenchymal nature and histogenetic uniformity, the classification of renal mesenchymal tumour is adopted.

It is the mesenchymal tumour which currently forms the basis of our studies. Several stages have been described in the development of this neoplasm. In the first two weeks after DMN treatment an inflammatory reaction occurs in the cortex, followed by a chronic period of 8-10 weeks in which infrequent hypercellular lesions persist in the cortical interstitial space. Consistently, microscopic foci of spindle shaped tumour cells can be identified in the intertubular space at 16 weeks and by 20-25 weeks the tumour cell foci have proliferated to form macroscopic neoplasms.

Radio-Autographic Studies

The inflammatory reaction which is induced in the early phase of the kidney's response to DMN is characterised by an increase in cell numbers within the intertubular space of the cortex, commencing at around day 4, reaching a peak at 1 week and subsiding by 3 weeks. Occasional interstitial fibroblasts display reversible cytoplasmic damage at 24 hours while focal tubule cell degeneration is most evident at 1 week. Lymphocytes and particularly macrophages contribute largely to the increased interstitial cell numbers in the cortex. The kinetics of this early response have been investigated with the technique of autoradiography employing a single pulse of tritiated thymidine (^{3}H-TdR). By embedding slices of perfusion-fixed kidney in resin, the identification of cell types within the various renal zones was facilitated. The pattern evidenced by labelled cells synthesising DNA was matched by that of cells in mitosis so that DNA synthetic activity was indicative of cell proliferation. Apart from an initial depression, the cells of the medulla (with the exception of the outer band of the outer medulla) did not vary from the normal range of activity. Thus the proliferative response was restricted to that

part of the kidney exhibiting focal cell damage and a concomitant inflammatory reaction.

All cell types of all zones were involved in a marked depression of synthetic activity for the first 2-3 days following DMN treatment. Thereafter, the interstitial cells of the cortex exhibited a low peak of increased DNA synthesis at 3 days followed by a major peak at 6 days when the number of labelled cells was 15 times higher than normal. The response at 3 days appeared to involve mainly the resident cortical fibrocytes whereas a major proportion of the cells engaged in DNA synthesis at 6 days were mononuclear inflammatory cells, particularly lymphocytes. In contrast, isotope labelling in proximal and distal tubules reached a peak at 10 days. By 3 weeks, DNA synthetic activity had returned to the normal range.

These data show that the only resident cell types stimulated to an early proliferative response by DMN are the same as those believed to be the source of the two forms of neoplasm which the treatment induces. Electronmicroscopic examination of the autoradiographs is now proceeding in order to attempt quantitative identification of the interstitial cells involved in the proliferative response.

Immunological Studies

The infrequent hypercellular lesions which persist during the second chronic stage of the kidney response to DMN, that is from 3-12 weeks after treatment, consist for the main part of lymphocytes and plasma cells together with a smaller number of macrophages. Occasional fibroblast-like cells also occur in these lesions. Because of their size and nuclear and cytoplasmic character they can be regarded as abnormal constituents of the renal cortex. This histological profile suggests that an immunological reaction is occurring within the renal cortex prior to the development of tumour cell foci. Furthermore, coincident with the stage at which tumour cells organised into foci can be first identified (at 12-16 weeks) there is a disappearance of lesions characterised by lymphoid cells. This pattern of events might be interpreted as the expression of immunological surveillance which, at the critical stage of tumour cell proliferation, may be abrogated by the intrusion of unknown factors (possibly blocking factors). To determine the intrinsic involvement of immunological factors in the developmental stages of DMN renal neoplasia two experiments have been set in progress. One aims to test the effect of immunosuppression on the model system. Specifically, this experiment will show whether tumour incidence is altered at a lower dose of DMN than is required to induce 100% incidence, and whether there is any modification in the manner of development, particularly involving the period of latency. The other experiment will determine the immunosuppressive effects caused by a carcinogenic dose of DMN with particular reference to the sequential stages of tumour development.

The immune competence of DMN-treated rats is being assessed by three tests: the response of spleen cells to phytohaemagglutinin (PHA) stimulation as a measure of cell-mediated immunity, the antibody response to a thymus-independent antigen as a measure of B lymphocyte function, and the antibody response to sheep erythrocytes, an antigen which involves the collaboration of both T and B lymphocytes. Measurement of the latter response is performed by assessing the number of antibody-forming cells in the spleen with the slide-monolayer technique.

It was necessary first to survey the immune function of PAW rats following the application of various immunosuppressive procedures and to select a method which induced a persistently depressed immune response to match the extended interval between DMN treatment and the appearance of tumour cell foci. To assess the normal levels of response, rats received either normal immunoglobulin, sham-thymectomy at birth or a combination of the two treatments. Rats which had been neonatally-thymectomised and/or administered anti-lymphocyte globulin (ALG) were able to mount an agglutinating antibody response to **Brucella abortus** antigen as effectively as control animals. Prior to the testing of T cell function, a standardised and reproducible method for PHA stimulation had to be devised for rat splenic lymphocytes. Culture in RPMI-1640 nutrient medium supplemented with 20% fresh homologous rat serum provided the most suitable conditions. An enriched lymphocyte fraction obtained by centrifugation of spleen cells over a gradient of Ficoll-Urografin was more responsive than crude spleen cell suspensions. ^{3}H-TdR incorporation was highest when culture tubes were incubated at a near to horizontal incline rather than in the vertical position. The addition of purified PHA at a concentration of 10 μg per culture tube produced consistent results.

With this method, spleen lymphocytes from control rats demonstrated a stimulation index of approximately 50 as assessed by the ratio

$$\frac{\text{CPM of cultures with PHA.}}{\text{CPM of cultures without PHA}}$$

Neonatally thymectomised rats and those given two 5 mg injections of ALG were tested at 7 weeks of age (two days after the ALG treatment) and at 15 weeks. Both procedures and a combination of the two induced a marked depression in the response to PHA at 7 weeks, although with ALG alone there was full recovery by 15 weeks, but not so following thymectomy. Combining neonatal thymectomy with weekly injections of ALG depressed the response to the lowest level resulting in a stimulation index of 1.5 at 15 weeks. Within two days of the administration of two doses of ALG the number of plaque-forming cells in the

spleen associated with the antibody response to sheep erythrocytes fell markedly from the control level (1-3 x 10^6) to a mean value of 33,000. As expected, full recovery was complete by 15 weeks. The effect of neonatal thymectomy was less marked on the collaborative response with values between 3 and 5 x 10^5 at 7 weeks and similar levels at 15 weeks. A combination of thymectomy and two injections of ALG reduced the response to the same level as ALG alone at 7 weeks, but by 15 weeks recovery had occurred to the level induced by neonatal thymectomy alone. Neonatal thymectomy, augmented by weekly intraperitoneal doses of ALG, caused prolonged depression of plaque forming cells in the spleen with a mean value of 23,000 remaining at 15 weeks. As well as delineating the range of responses shown by normal and immunosuppressed PAW rats to the three assays, this preliminary study served to illustrate that **Brucella abortus** antigen was an adequate marker of B cell function at the concentration used. Furthermore, for the production of marked long-term impairment of cell-mediated immunity in the rat, neonatal thymectomy combined with repeated injections of ALG was the most effective of the procedures tested.

Cell Culture Studies

Because a single pulse of DMN leads inevitably to the formation of renal mesenchymal tumour by way of a constant sequence of tissue changes although the agent itself is fully eliminated from the body by 24 hours, it might be predicted that the target tissue is already committed to the carcinogenic process within the acute phase of development. To investigate this possibility, cells from the renal cortex of DMN-treated rats have been isolated from 20 hours to 7 days after treatment and supported in nutrient culture medium. This procedure previously has been termed host-mediated, **in vivo-in vitro** assay. The morphology, behavioural properties and ultrastructure of the cultured cells have been traced through successive subcultures. An integral part of this study was the characterisation of the properties of renal mesenchymal tumour cells and of normal cortical cells under **in vitro** conditions.

When normal rat kidney was cultured, both single mesenchymal cells and islands of typical epithelium were found in the primary isolate. The epithelium usually disappeared after the first subculture. Large mesenchymal cells with well developed peripheral sheets of microfilaments and low nucleus/cytoplasm ratio persisted for 3-4 subcultures dying out 3-6 weeks after isolation.

Primary isolates from renal mesenchymal tumour also consisted of mesenchyme-like cells and usually scattered islands of epithelial cells. Establishment of the cultures was more rapid than with normal cells and at confluency, piling up was often noted. Typical epithelium rarely persisted beyond the first subculture. The mesenchyme-like cells grew rapidly and continuously with no apparent finite life span. Some tumours have been maintained in culture now for 12-18 months. The cells were pleomorphic assuming such various forms as bipolar (fibroblastic), polygonal and triangular. Compared to normal cells, the tumour-derived cells were smaller with a higher nucleus/cytoplasm ratio. Some variation in form and behaviour occurred between successive subcultures and between tumour lines but there was a basic pattern common to all. Tumour cells exhibited a high plating efficiency, cytoagglutination when exposed to conconavalin A and were capable of colony formation in methylcellulose gel and on chicken chorioallantoic membrane.

Cells isolated from DMN-treated rats behaved in similar fashion to cells from normal rats for the first few subcultures, excepting for a slower growth rate. Between 5 and 7 weeks after isolation (about subculture 5) transformation was observed in the cultures from DMN-treated rats with colonies of cells piling up into dense foci. Following this stage the cells have been cultured continuously and thus have exhibited a prolonged life span. Compared to the growth of normal cells, DMN-cultures exhibited an increased plating efficiency and rate of mitosis. Like tumour cells they agglutinated in the presence of conconavalin A and were capable of colony formation in methylcellulose and on chicken chorioallantoic membrane. Cultures derived from rats 20 hours after treatment with DMN were as efficient in demonstrating these transformation traits as cells isolated 7 days after the carcinogenic insult.

These results suggest that the target cells responsible for the eventual development of renal mesenchymal tumour may be committed to the malignant process within 20 hours of the administration of carcinogen. Further experiments are in progress to determine the period within the first 24 hours at which target cells have already inherited the potential for transformation. Preliminary results indicate that the critical "switchover" point may lie in the vicinity of 2-4 hours after exposure to DMN.

Biochemical Studies

In addition to investigating the transformation potential of target cells, the host-mediated **in vivo-in vitro** studies have aimed at producing pure populations of precursor cells for analysis at the molecular level. Meaningful **in vivo** biochemical assay of phenomena related to renal carcinogenesis is restricted by the heterogeneous cellular composition of kidney tissue. Moreover, the number of cells relevant to the malignant process might be a quantitatively small proportion of the susceptible target cell population. The host-mediated **in vivo-in vitro** assay offers a valuable opportunity to characterise relatively pure clones of committed cells before and after transformation and at the same time, maintaining data relevance to the **in vivo** situation. Initial studies planned for early commencement include the determination of the rates of synthesis of DNA, RNA and protein in cultures of normal cells, tumour cells and cells from DMN-treated rats and levels of methylated RNA and reverse transcriptase. Repair of DNA damage will also be investigated as well as the metabolism of DMN by the cultured cells.

The Respiratory System *△

D. Alcorn[1], J. Barwick[2], J. Cannata, W. Else, J. E. Maloney, B. C. Ritchie and A. Walker[2].

It is the aim of these projects to increase basic understanding of normal and abnormal heart and lung function in the foetus, newborn and adult using experimental models mainly in the sheep. The projects divide naturally into two major areas — developmental cardio-respiratory physiology and the physiology of the adult cardio-respiratory system.

Development of the Lung and Circulation

Of particular interest during 1973 was a study undertaken to describe the changing architecture of the lung during development. The lung develops from the primitive foregut as an endodermal tube which penetrates the surrounding mesenchyme branching as it grows into the primitive air spaces. Cell differentiation occurs in the upper airways earlier than the lower, so that by 80 days in the sheep (gestation of 147 days) both the cilia and microvilli covered cells are discernable.

The small airways are composed of undifferentiated cuboidal cells at 102 days. The potential gas exchanging surface differentiates later with the appearance of the type I and II alveolar cells. From approximately 125 days in the sheep lamellated bodies appear in the type II cells at the same time as surface active agents are found in the lung liquid which fills the developing airways. At birth the primitive airways occupy approximately 80% of the lung volume in the sheep and only 50% in the rabbit. Both species are viable at birth and the apparent discrepancy is counteracted by an increased capillary density in the foetal rabbit. The rapid growth of the foetal lung which occurs over the last third of gestation has been quantitatively recorded using modern morphometric techniques.

The lung during development produces a liquid of special electrolyte concentration which cannot be accounted for at this time other than by active processes operating across the cell membranes which line the developing airways. Throughout this last year studies have proceeded to elucidate the production and movement of this fluid and to investigate the possibility that the foetus "breathes" **in utero.** Sophisticated experimental models have been designed to continuously follow the development of heart and lung in the foetal lamb from day to day **in utero.** These studies have led to the conclusion that the respiratory muscles are active **in utero** and that the developing foetus makes periodic respiratory movements which change in nature throughout gestation. These movements, however, do not appear to account for the outflow of lung liquid during development. This outflow averages 5 to 10 ml per hour over the last weeks of gestation. Liquid fills the foetal lung prior to air breathing.

1. Department of Physiology
2. Department of Medicine, Monash University.

The co-operation of T. M. Adamson, Department of Paediatrics, Monash University, and of Dr. L. Dugdale, Department of Nuclear Medicine, Alfred Hospital, is acknowledged.

A scanning electron micrograph of foetal rabbit upper airway at 27 days (term: 30 days).

At caesarian section the lungs of the rabbit foetus are removed and immersed in fixative solution. Slicing the parenchyma exposes the inner surface of the airways and surrounding tissue. The epithelium lining the upper airways has differentiated at this stage of gestation, with ciliated and microvilli covered, as well as large cuboidal, cells being prominent.

Alveolar epithelial cell differentiation is only commencing at this time in development.

At the time of air breathing dramatic changes take place in the lung as the liquid in the air spaces is cleared and the pulmonary circulation takes on its adult function. Our group has a special interest in the change which takes place in the circulation at the time of breathing air. Foetal pulmonary blood volume increases by approximately 40% at this time, a transition which depends on the gas breathed. If air is substituted by a mixture of 3% O_2 and 7% CO_2 no significant alteration in pulmonary blood volume occurs. Initially on breathing air, gas and blood flow go to the same region of the lung but when the arterial pressure of oxygen reaches 40 mm Hg the blood flow in the lung reverts to a gravitationally dominated distribution as in the adult. Finally, in the liquid filled lung just prior to birth pulmonary vascular conductance increases as lung volume decreases. Radiographic studies at this time indicate that the diameter of blood vessels initially greater than 500 μm increases as lung volume decreases. Experimental studies in each of these areas will continue in 1974 as we seek to increase our basic understanding of the function of the heart and lung throughout growth **in utero.**

Physiology of The Pulmonary Circulation

The second major area of interest is an elucidation of the factors which are important in the control of blood flow through the lung. To the present the passive effects of vascular pressures within the pulmonary circulation and their relationship to alveolar pressure have been regarded by many as sufficient to explain the regional variations in vascular resistance of the lung. We have examined the control of the pulmonary circulation during double heart bypass, hypoxia and hypovolaemic hypotension. In each study as well as measuring the haemodynamic response of the whole lung to the specific stimulus, the response of regional areas was examined using radioactive isotope techniques. The study during double heart bypass supported the concept that critical closing pressures operate within the pulmonary circulation and demonstrated that factors other than intravascular pressures and lung volume were important in determining the distribution of pulmonary blood flow.
During hypoxia the response of the pulmonary circulation depends on whether the chest is open or closed as does the initial distribution of pulmonary vascular resistance. In both preparations there were marked regional differences in the response of the vasculature to hypoxia, greater increases in vascular resistance being noted in the central lung regions. A single control model is proposed to explain these differences in response. Neuro-humoral mechanisms appear to play a major role in the reaction of the pulmonary circulation to blood loss. This was noted in a study of the reaction of the pulmonary circulation to the removal of twenty-five per cent of the total blood volume of the anaesthetised animal. Studies such as these indicate that a number of modifications will be necessary to previously held concepts of how the pulmonary circulation operates and we are conscious of the need to develop a new model of the pulmonary circulation.
Lung irritants such as particulate matter and cigarette smoke are known to alter airways mechanics in the adult lung but little work has been published on their effect on the pulmonary circulation. A comprehensive pilot study undertaken by the group indicated that particulate matter alone altered regional pulmonary vascular resistance of the lung in a marked contrast to the effect of cigarette smoke. Further work must be undertaken to elucidate the mechanisms operating in this response.

Trophoblast Survival: An Immunological Paradox?

R. Borland

In the pregnant animal the trophoblast layer of the placenta is in immediate contact with or actively invades the maternal tissues. It has two main functions. First, it provides the means whereby nutrients are transferred from mother to foetus. Secondly, it forms a "barrier" between maternal tissue and the foetal tissue (an allograft).

Although the precise nature of the "foetal-maternal" barrier is not yet clear, we do know that the human trophoblast cells have transplantation (Hl-A) antigen discriminants on their surface and that the mother is immunised to trophoblast and foetal antigens. Nevertheless the trophoblast and the foetus survive this active maternal immunological response. There has been much speculation in the literature concerning the manner by which this survival is achieved and it now appears that the "foetal-maternal" barrier may be some form of sialomucin coat which works by preventing the maternal immune reaction from damaging and destroying the "foreign" trophoblast and foetal cells.

Human chorionic gonadotrophin (HCG) which is a sialoglycoprotein produced by human trophoblast cells, has a possible protective role in the survival of transplanted trophoblast tissue and a continuing study of this has been completed by a series of experiments carried out this year. In these experiments the killing effect of rabbit lymphocyte sensitised against human trophoblast target cells was studied and showed that human trophoblast cells were not susceptible to this killing action. If, however, the HCG layer around the trophoblast cells was first removed by enzymatic action then they were killed by the sensitised lymphocytes. Where such trophoblast cells after the enzymatic treatment were treated with HCG their resistance to specific lymphocyte damage was restored.

The findings provide further evidence for the possible key role of HCG in the prolonged survival of the human trophoblast and foetus in pregnancy.

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Hard, G. C. and D. M. Shaw "Proliferative Response in the Rat Kidney Induced by a Single Carcinogenic Dose of Dimethylnitrosamine". **Cell & Tissue Kinetics.** In Press.

Morphology and Physiology of Respiratory System *△

Morphology

Alexander, I. G. S., B. C. Ritchie and J. E. Maloney "The Scanning Electron Microscopy of Pulmonary Alveolar Capillary Vessels". **Thorax** Vol. 28 (1973) p. 222.

Alexander, I. G. S., P. M. Capicchiano, B. C. Ritchie and J. E. Maloney "Plastic Embedding and Surface Erosion Techniques in Soft Tissue Scanning Electron Microscopy". **J. Micr.** Vol. 99, Pt. 1 (1973).

Alcorn, D., I. G. S. Alexander, J. E. Maloney and B. C. Ritchie "Scanning Electron Microscopy of Developing Lung Surfaces". **Aust. Paed. J.** In Press.

Alcorn, D., I. G. S. Alexander, J. E. Maloney and B. C. Ritchie "Morphology of the Foetal Lung". **Proc. A.P.P.S.** In Press.

Alcorn, D., I. G. S. Alexander, J. E. Maloney, B. C. Ritchie and A. M. Walker "Morphological Development of the Lung: A Review". **Aust. Paed. J.** In Press.

Physiology

Adamson, T. M., T. Lambert, Susan Cranad, V. Brodecky, J. E. Maloney and B. C. Ritchie "Lung Liquid Production and Composition in Chronic Foetal Sheep". **J. Physiol.** Submitted.

Korman, M. G., J. Hansky, B. C. Ritchie, J. McK. Watts and J. E. Maloney "The Disappearance of Gastrin Across the Lung". **Aust. J. exp. Biol. Med. Sci.** Vol. 51 (1973) p. 670.

Maloney, J. E., T. M. Adamson, B. C. Ritchie and A. Walker "Distribution of Pulmonary Blood Flow during Controlled Perfusion of the Intact Lung". **Aust. J. exp. Biol. Med. Sci.** Vol. 51 (1973) p. 655.

Maloney, J. E., Daine Alcorn, A. Walker and B. C. Ritchie "Regional Vascular Response to Hypoxia in the Lung of Anaesthetised Sheep". **Cardiovasc. Res.** Submitted.

Maloney, J. E., J. Cannata, T. Davey, A. Walker and B. C. Ritchie "Regional Changes in Pulmonary Vascular Resistance during Hypovolemia in Anaesthetised Sheep". **Cardiovasc. Res.** Submitted.

Walker, A., B. C. Ritchie, D. Alcorn and J. E. Maloney "Postural Influence on Tissue Density in Newly Ventilated Lamb Lungs". **Proc. A.P.P.S.** In Press.

Miscellaneous

Mäslar, P. and D. C. Shaw "Two Isoenzymes of Arginine Kinase from **Panulirus longpipes**". **Biochim. Biophys. Acta** Vol. 30: (1973) p. 308.

Hard, G. C. "Examination by Electron Microscopy of the Interaction between Peritoneal Phagocytes and **Corynebacterium ovis**". **J. Med. Microbiol.** Vol. 5 (1972) p. 483.

Lectures Given During 1973

D. Alcorn	"Scanning Electron Microscopy of Developing Lung Surfaces".	*Paediatric Research Society of Australia, Canberra.*
D. Alcorn	"Morphology of the Foetal Lung".	*Australian Physiological and Pharmacological Society, Sydney.*
R. Borland *et al.*	"**In vitro** Transformation of Kidney Cells following Isolation from Dimethylnitrosamine (DMN) Treated Rats".	*Australian Society of Experimental Pathology.*
R. Borland	"Dimethylnitrosamine-induced Carcinogenesis Investigated using a Host-mediated **In Vivo/ In Vitro** System".	*Cancer Institute.*
G. C. Hard *et al.*	"Kidney Tumour Induction in Rats by a Single Dose of DMN, as a Model for the Study of the Carcinogenic Process".	*New Zealand Society of Oncology, Christchurch.*
G. C. Hard *et al.*	"Autoradiographic Analysis of DNA Synthesis in the Rat Kidney following a Carcinogenic Dose of Dimethylnitrosamine".	*Australian Society of Experimental Pathology.*
J. E. Maloney	"Lung Mechanics".	*Royal Australasian College of Surgeons.*
J. E. Maloney	"Gas Exchange and Control of Respiration".	*Royal Australasian College of Surgeons.*
J. E. Maloney	"Respiratory Physiology".	*Two Lectures, Melbourne Medical Post-graduate Committee.*
J. E. Maloney	"An Introduction to Respiratory Physiology".	*Seven Lectures, Monash University.*
J. E. Maloney	"Lung Function".	*Three Lectures, Australian College of Physiotherapy.*
J. E. Maloney	"Respiratory Physiology".	*Eight Lectures, University of Melbourne.*
J. E. Maloney	"The Developing Lung".	*University of Melbourne.*
P. Mäsiar	"The Chemistry and Physiological Significance of an Intermediate Molecular Weight Fraction of Blood Plasma from Cardiac Muscle".	*John Curtin School of Medical Research, Canberra.*
W. G. Nayler	"Regulation of Myocardial Contraction".	*Conference of the Myocardium, N.H.F., Canberra.*
B. C. Ritchie	"Surfactant and the Respiratory Distress Syndrome".	*University of Melbourne.*
B. C. Ritchie	"Structure of Type II Cells".	*Monash University.*
A. Walker	"Postural Influence on Tissue Density in Newly Ventilated Lamb Lungs".	*Australian Physiological and Pharmacological Society, Sydney.*

Seminars Held During 1973

Friday Seminars

D. Alcorn	The Morphology of the Developing Lung.
A. J. Barnett	Scleroderma.
R. Borland	(i) Studies on the Trophoblast and its Tumours. (ii) Dimethylnitrosamine-induced Carcinogenesis Investigated Using a Host-mediated **"In Vivo/ In Vitro"** System.
V. Carson	Factors Affecting the Adenyl Cyclase Activity of Cardiac Muscle.
E. Cooper	Sinus Node Control.
G. C. Hard	Immunological Responses of Normal and Immuno-depressed Rats.
T. D. Lewis	Gastro-oesophageal Reflux.
T. J. Lugg and K. R. Kerry	Antarctic Division — Biology and Medical Research.
J. E. Maloney	(i) Control of the Pulmonary Circulation. (ii) Cardio-respiratory Activity and Lung Liquid Mechanics in the Developing Lung.
P. Mäsiar	(i) Quo Vadis Biochemistry? (ii) Some Aspects of the Biochemistry of Troponin.
W. G. Nayler	Regulation of Myocardial Contraction.
B. C. Ritchie	Surface Tension and the Lung.
M. Shaw	An Autoradiographic Study of DNA Synthesis in Rat Kidney following a Carcinogenic Dose of DMN; Acute Phase.
F. R. Trinker	The Interaction of Tricyclic Anti-depressants with Adrenergic Neurone Blocking Drugs — Fact or Fallacy?
A. Walker	The Pulmonary Circulation in Foetal and Newborn Lambs.

Respiratory Workshop (October 13)

The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute

Revenue Account for the Year Ended 31st December, 1973

EXPENDITURE		INCOME		
Salaries and wages	$208,570	**Donations from Baker Benefactions**		
Laboratory supplies and isotopes	40,554	Statutory amount	$11,569	
Library maintenance	9,208	Transfers from Restricted Fund	125,000	$136,569
Postage and telephone	1,180			
Printing and stationery	3,620	**Grants in aid of Research Projects**		
Light and power	19,408	Anti-Cancer Council	$22,716	
Insurance	5,609	The Australian Tobacco Research Foundation	7,938	
Repairs and renewals	8,845	Life Insurance Medical Research Fund of Australia and New Zealand	12,792	
Animal house contribution	4,000	National Health and Medical Research Council	8,184	
Sundries	5,124	**Other Grants**		
Travelling expenses	5,128	The James and Elsie Borrowman Research Trust	4,500	
Public relations	492	The William Buckland Research Fund	1,655	
	$311,738	Victorian State Government	25,000	
Surplus for year	346	Laura Nyulasy Research Scholarship	1,800	84,585
		Interest from Investments		
		Held by Trustees of The Baker Institute Grant Trust	$1,700	
		Other income	63,530	65,230
		Sundry Sales, Recoveries and Refunds		25,700
	$312,084			$312,084

The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute

Balance Sheet as at 31st December, 1973

FUNDS AND LIABILITIES		
Funds		
Accumulated (deficit) brought forward	($2,296)	
Deduct, Surplus for year	346	
Accumulated (deficit)	($1,950)	
Restricted Fund	63,483	
Endowment Fund	925,874	
William Buckland Research Fund	20,870	
Laura Nyulasy Research Scholarship Fund	3,940	
Lang Research Scholarship	4,852	
		$1,017,069
Current Liabilities		
Sundry Creditors and accrued expenses		9,255
		$1,026,324

ASSETS			
FIXED ASSETS (Note 1)			
INVESTMENTS			
Held by the Trustees of the Institute:			
Government and Semi-Government Stock	$19,811		
Shares in companies	55,885		
Short-term deposits	73.720		
Mortgage loans	292,000		
		$441,416	
Held by the Trustees, Executors & Agency Co. Ltd:			
Shares in companies	$58,986		
Trust units	473,719		
		532,705	
Scholarship Funds (managed by Trustees, Executors & Agency Co. Ltd.):			
Laura Nyulasy Research Scholarship Fund	3,940		
William Buckland Research Fund	20,870		
		24,810	
			$998,931
CURRENT ASSETS			
Cash on hand		$100	
Cash at bank		20,835	
Sundry debtors		6,458	
			27,393
			$1,026,324

Notes to the Balance Sheet

1. Expenditure included in present or past periods on fixed assets, including laboratory equipment, motor vehicles, buildings, improvements and furniture and fittings have been charged against appropriate funds, grants or revenue accounts.
   The insured value of all assets at December 31, 1973, including the building, totalled $1,825,500.
2. In addition to receiving income from investments shown above, the Institute receives interest on $34,000 5% Commonwealth Inscribed Stock which is held by the Trustees of The Baker Institute Grant Trust for the benefit of the Institute.
3. There is a commitment amounting to $726 for the balance of the purchase price of investment in quoted ordinary shares.

Auditors' Report to the Trustees of the Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute

As an audit procedure it was not practicable to extend our examination of contributions and donations beyond the accounting for the amounts received as shown by the books and records of the Institute.

Subject to the above reservation in our opinion the above balance sheet together with the notes thereto is properly drawn up to show a true and fair view of the state of the Institute's affairs at 31st December, 1973

PRICE, WATERHOUSE & Co.
Chartered Accountants.

Melbourne.
February 11, 1974

The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute

Year Ended 31st December, 1973

RESTRICTED FUND		
Balance at December 31, 1972		$39,078
Add:		
Transfer from Baker Benefactions	$253,272	
Transfer from Revenue Account for equipment ordered but not delivered at balance date	20,200	
Income received in advance from Trustees, Executors & Agency Co. Ltd. investments	19,050	
Donations — other	17,050	
Sundry receipts	1,992	
		311,564
		$350,642
Deduct:		
Transfer to Endowment Fund	$122,218	
Transfer to Revenue Account	136,569	
Equipment costs	28,372	
		287,159
Balance at December 31, 1973		$63,483
ENDOWMENT FUND		
Balance at December 31, 1972		$784,740
Add:		
Donations	$17,153	
Transfer from Restricted Fund	122,218	
Accretion of Trustees, Executors & Agency Co. Ltd. Investments	1,192	
Interest — bank and investment — short-term	2,171	
		142,734
		$927,474
Deduct:		
Transfer to Ethel Mary Baillieu Fund	$994	
Transfer to Lang Scholarship Research Fund	606	
		1,600
		$925,874

Donations

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

Victorian Government	$25,000.00
H. & L. Hecht (Perpetual Executors & Trustees Association of Australia Ltd.)	5,000.00
"Group B"	3,460.00
The Ian Potter Foundation	3,000.00
Edgar Rouse	2,000.00
Edward Wilson Estate (Trustees, Executors and Agency Co. Ltd.)	2,000.00
William Angliss (Victoria) Charitable Trust	1,500.00
Appel Family Bequest (Trustees, Executors & Agency Co. Ltd.)	1,100.00
Bell Charitable Trust	1,000.00
Truby & Florence Williams Charitable Trust (Trustees, Executors & Agency Co. Ltd.)	1,000.00
Alfred Edments Estate (Trustees, Executors & Agency Co. Ltd.)	700.00
Marian & E. H. Flack Estate	700.00
J. C. Habersberger	600.00
Carlton & United Breweries Ltd.	500.00
George F. Little Trust (Equity Trustees Executors & Agency Co. Ltd.)	475.00
J. B. Were & Son	300.00
Mrs. R. Hewgill	200.00
Roche Products Pty. Ltd.	200.00
General Motors-Holden Pty. Ltd.	150.00
Pethard Tarax Charitable Trust	120.00
Stanley E. Watkin & Son	100.00
Siegfried Meyer	75.00
S. Sanderson	75.00
Specialty Press Limited	50.00
Mrs. N. G. Baker	40.00
IBM Australia Limited	26.71
Alan Drayton	25.00
P. Rosenbaum	22.00
R. Moser	20.00
George Turner Customs Pty. Ltd.	20.00
M. Levinson	9.00
Mr. & Mrs. Peter Shaw	5.00
Miss P. Lyster	4.50
	$49,477.21

Further contributions were received from —

Australian Eagle Insurance Company Limited, Reginald Blakemore, Miss N. E. Cameron, Mrs. E. Cooper, Mr. and Mrs. Charles Donne, J. C. Habersberger, Misses D. and J. and Mr. R. Jeffrey, Kodak (Australasia) Pty. Ltd., A. L. Lazer, Noel Monteith, Neil Payne, Reliance Grocery Pty. Ltd., and Edgar Rouse.

In memory of —

Mrs. E. Angliss, W. A. Bain, F. Calhoun, E. D. Casper, A. Walwell, Dr. A. Cooper, Ada Flannagan, Graeme Grove, S. R. Hillman, J. C. Hodge, H. B. Horswell, J. H. Lindell, Mrs. M. McInerney, Mrs. M. Mead, Eric Morris, J. Moynahan, Mrs. W. M. Porter, Sir Raymond Purves, Sir Alan Ramsay, S. G. Reeve, Ethel Smith, J. N. Smith, Alec Thompson, K. R. Veevers, W. G. West, Mrs. M. N. Wilson.

Total: $237.08.

Alfred Hospital Research

Research Advisory Committee

W. S. PHILIP, C.M.G., M.C., F.C.A. (Aust.)
Chairman.

B. G. FIRKIN, B.Sc.(Med.), M.B., B.S., F.R.A.C.P.

J. S. GUEST, O.B.E., V.R.D., B.Sc., F.R.C.S., F.R.A.C.S.

J. C. HABERSBERGER, B.Comm.

A. V. JACKSON, M.D., B.S., F.R.A.C.P., F.C.Path., M.C.P.A.

H. B. KAY, M.D., B.S., F.R.C.P., F.R.A.C.P.

Sir CHARLES McGRATH, O.B.E.

EDGAR ROUSE, C.B.E., F.R.A.C.R.(Hon.)

T. H. STEEL, M.D., B.S., F.R.C.P., F.R.A.C.P.

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

DIRECTOR OF CLINICAL RESEARCH UNIT (ex officio)

Alfred Hospital Research Fellows, 1973

E. Cooper	"Edward Wilson Memorial"
	"James Richardson"
I. A. Ferguson	"William Buckland"
	"A. A. Swallow"
B. G. Firkin	"E. H. Flack"
B. Ford	"Sol Green"
	"Ian Gideon McLean"
P. Garcia-Webb	"J. F. MacKeddie"
M. W. Johns	"Edward Wilson Memorial"
T. E. Lowe	"H. M. Black"
(Clinical Research Unit)	"Paul Henry Christie"
	"Amelia Haigh (Heart)"
	"Amelia Haigh (Rheumatoid Arthritis)"
	"Dr. Henry Laurie"
	"R. V. Sartori"
	"Connibere"
	"Collier"
J. E. Maloney	"R. B. McCormas"
	"S. W. Shields"
A. Pitt	"S. W. Jones"
D. S. Rosengarten	"James and Elsie Borrowman"
J. R. Stockigt	"E. H. Flack"
H. P. Taft	"Frederick and Esther Michaelis"
	"Victor Y. and Margaret Kimpton"
	"J. R. G. and E. McKenzie"
	"George Merriman"

Appointed to Research Fellowships for 1974

A. J. Barnett and M. H. Briggs	"S. W. Jones"
	"R. B. McComas"
M. H. Briggs	"William Buckland"
	"Frederick and Esther Michaelis"
B. B. Davis	"James and Elsie Borrowman"
I. A. Ferguson	"Collier"
J. Koutts	"Connibere"
	"S. W. Shields"
J. McMichan	"Sol Green"
	"Victor Y. and Margaret Kimpton"
A. Pitt	"E. H. Flack"
	"A. A. Swallow"
D. S. Rosengarten	"James Richardson"
	"J. F. MacKeddie"
J. R. Stockigt	"Dr Henry Laurie"
	"Ian Gideon McLean"
N. D. Yeomans	"Edward Wilson Memorial"
	"Patterson Travelling"
Clinical Research Unit	"Edward Wilson Memorial"
	"Amelia Haigh (Heart)"
	"Amelia Haigh (Rheumatoid Arthritis)"
	"H. M. Black"
	"George Merriman"
	"Paul Henry Christie"
	"Sartori"
	"J. R. G. and E. McKenzie"
	"C. B. and G. E. Miller"

The Clinical Research Unit

Staff

Director	T. E. LOWE, C.B.E., D.Sc., M.D., F.R.C.P., F.R.A.C.P. (retired 24/10/73)
	A. J. BARNETT, M.D., F.R.A.C.P., M.R.C.P. (Acting Director from 25/10/73)
Graduates	VALERIE CARSON, M.Sc.
	T. D. LEWIS, M.B.B.S., M.R.A.C.P.
	F. G. SILBERBERG, M.B.B.S., M.R.A.C.P.
	FEDORA R. TRINKER, M.B.B.S., Ph.D. (to 18/5/73)
Technical	A. H. HUCKFIELD (Technical Officer on loan to Baker Institute from 1/4/73)
	D. G. BRUCE
	Mrs. R. MUSCUTT
Clerical	Mrs. E. KERN
Laboratory	P. BENNETTS (to 8/4/73)
	Miss M. COGDON (from 23/4/73)
	R. LOWE (from 16/11/73)
	Miss M. ROSIER (to 15/11/73)

Ward Staff

Registrar	B. JACKSON, M.B., B.S.
Resident Medical Officers	M. D. GOODYEAR, M.B., B.S.
	K. P. GULLIFER, M.B., B.S.
	RENATE KALNINS, M.B., B.S.
	Y. L. LIM, M.B., B.S.
Ward Sister	Mrs. P. NEWELL

The publication of the current annual report marks the end of an epoch in the history of the Clinical Research Unit. Since its inception, the Clinical Research Unit has been closely linked with the Baker Medical Research Institute, both bodies having the same Director and with offices and laboratories in the same building. In fact it was often difficult to distinguish them, the main division being whether members of the combined body were engaged mainly in clinical or laboratory research. The contributions of members of the Clinical Research Unit over the past twenty-four years, particularly in respect to hypertensive states, peripheral vascular disease and scleroederma, have been summarised in the 1972 report.

With the retirement of Dr. Lowe as Director of the Clinical Research Unit, the directorship of the two organisations is no longer by the same person and it would seem appropriate at this time to reappraise the function of a clinical research unit.

There is a belief in some quarters that worthwhile medical research can be done only by scientists and that clinical research is no longer important. Although it is probably true that fundamental discoveries can now only be made by teams of scientists with skills not available to the clinician, I believe there is still an important place for clinical research.

The stimulus to study a particular medical problem must arise from contact with human disease and the relevance of any discovery, particularly if related to management of disease, can only be assessed by its application in patients. I suggest the following as proper functions of a clinical research unit.

1. A Centre within the Hospital for Research in Humans into Problems of Human Disease

This implies a close contact with patients so that investigators may be alerted to the main problems needing study and to have sources of patient material available when necessary to carry out the study. Although research activities may be carried out to some extent by other departments, these studies are secondary to other commitments such as patient care, teaching or routine diagnostic procedures. Although research is the prime function of a clinical research unit, patient care is necessary to keep in touch with patient problems. Also a certain amount of teaching is advisable both for the benefit of the student, who is appraised of the scientific side of medicine, and the teacher who is stimulated by the questions of the students. However, the patient care and teaching loads should not be so heavy as to exclude time for research.

Continuing research projects by established staff are necessary to keep the unit alive and also to make available expertise to other research workers who, from time to time, maye be associated with the unit.

Facilities could also be made available to other people, such as recipients of Alfred Hospital Research Scholarships or Ph.D. students engaged in a one-year or two-year project. Such facilities could include office and laboratory space, advice by the established staff and, in suitable cases, control of a small number of beds, under the supervision of the Director.

2. A Nucleus for the Developing and Later Budding Off new Specialities and Services

A research activity may grow to such an extent that it is later budded off as a separate department. The origins of the study of endocrine diseases (by the E.D.M.U.) and cardiovascular diagnosis (by the C.D.S.) and vascular disease as a speciality in the Alfred Hospital can be traced to development of interests in these problems by workers belonging to or attached to the Baker Institute or C.R.U.

3. As a Link Between Non-Clinical Medical Research Departments and Clinical Medicine

Although, as already mentioned, more fundamental research is likely to be done by scientists without medical qualifications than by clinicians, the scientists need contact with the clinicians to determine the subjects most deserving of the research and the significance of the problem. Also the clinician needs to be able to contact the scientist for help and advice in his investigations.

For ease of communication it is desirable that at least one party, the scientist or clinical investigator, should be familiar with the languages both of science and of medicine.

Although the administrative link between the Baker Institute and the Clinical Research Unit has now been severed, it is hoped that the close association between these bodies in respect to research interests will continue. Indeed their activities may be complementary, with the Clinical Research Unit developing in the clinical field ideas and procedures developed in the animal laboratory, and members of the Baker Institute elucidating clinical problems by investigations in the animal by methods not applicable to man.

Also, it is hoped that a close link will be established between the Clinical Research Unit and the Department of Biochemistry. A possible field of co-operation is in problems of drug action. An example of a condition in which this may be employed is in the treatment of hypertension. The Clinical Research Unit has the patients being treated with the drugs; the Department of Biochemistry has the equipment and can develop the techniques for measuring the blood levels of the drugs. Another example is the clinical features and management of drug overdose in relation to blood levels of the drugs.

In the ensuing year, it is intended to continue investigations on the types of problems studied in the past, but with a different emphasis. In respect to hypertension it is intended to study (in association with the Biochemistry Department) the effect of treatment in relation to blood levels of drugs and probably to their haemodynamic action. In scleroderma, the clinical features and course are now fully described, and it is hoped to commence a study of the more fundamental biochemical disturbance. Study of abnormalities of collagen metabolism are being carried out extensively in other centres and we hope to turn our attention to connective tissue ground substance which may be of equal or perhaps greater importance. In peripheral vascular disease assistance will be given to a study by the Peripheral Vascular Unit in relating blood flow determinations in limbs to the results of arterial surgery. It is hoped to commence (in association with the Biochemistry Department) a study of clinical aspects of drug overdoses in relation to blood levels of drugs.

A. J. BARNETT.
Acting-Director.
December 31, 1973.

Report of Scientific Investigations

Hypertension

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

The results of the long-term treatment of hypertension, of which a preliminary description was given in the 1972 report, have now been published. These involved 191 patients with severe complicated hypertension admitted to a clinic commenced in 1951 and reviewed up to December, 1969.

Most patients achieved good or fair blood pressure control and symptomatic state and approximately 80 per cent remained in full work. There was an overall survival of 60 per cent at 5 years, 43 per cent at 10 years and 21 per cent at 15 years. Factors influencing survival included the height of the systolic and diastolic blood pressures (systolic being more important than diastolic), sex (being better for females), age (being better for younger patients) and the degree of involvement of ocular fundi, heart and kidneys. Eighty six patients died, the commonest causes of death being cerebral vascular accident (21), myocardial infarction (24) and renal failure (21). Most of the cerebral and renal deaths occurred in the first five years of treatment, most of the deaths from myocardial infarction after five years. These results are in accord with those of others, indicating a beneficial effect of treatment in severe hypertension. However, comparison of survival curves of the hypertensive patients with those from the general population leaves no cause for complacency. They indicate the need for early treatment of hypertension and elimination of other risk factors for arterial disease.

Low Sodium Diet

In spite of high doses of modern drugs, some hypertensive patients still remain resistant to treatment. In a small group of these, treatment with a low sodium diet (40 mEq per day) has been used with favourable results in the patients in hospital under "balance" conditions, with an accurate record of the sodium intake and excretion. However, the results in the outpatient clinic have been less favourable and have been associated with a relatively high 24-hour sodium excretion, indicating the patients are not restricting their sodium intake to the figure prescribed.

Amiloride ("Midamor")[1]

As part of their treatment, most hypertensive patients receive a thiazide diuretic or similar drug promoting salt and fluid loss. An undesirable side effect of this treatment is excessive urinary excretion of potassium resulting in low body potassium reflected in a low serum potassium level. One method of counteracting this effect is to supply a large amount of potassium salts orally, usually in the form of tablets. An alternative method is to administer concurrently a drug such as amiloride, which suppresses potassium excretion.

A trial was set up for the comparison of treatment with amiloride and administration of additional oral potassium in maintaining or increasing the serum potassium levels. According to the trial plan, selected patients were to be divided into two groups, one of which was to be treated first with amiloride for three months followed by a crossover to an oral potassium preparation for three months and the other was to be treated first with a potassium preparation and then amiloride for similar periods.

Unfortunately, due to irregular patient attendance and other factors, the trial was not completed as planned. Thirty patients have been treated with amiloride for more than one month (periods of one to six months, mean 3.5 months). Of these only 14 have cqnformed to the plan with initial and three monthly serum potassium levels with each treatment course. The mean level of serum potassium was slightly higher with amiloride treatment in an average dose of 9.5 mg per day, than with potassium supplementation, in an average dose of 34 mEq per day (3.36 mEq per l compared with 3.23). The serum potassium level rose in seven instances and fell in seven instances during potassium supplementation, whereas with amiloride the rises and falls were respectively nine and five.

In the thirty patients treated with amiloride from one to six months, the mean of the last serum potassium level on potassium supplementation and on amiloride are compared in the following table.

1. Supplies of "Midamor" were generously donated by Merck, Sharp and Dohme (Australia) Pty. Ltd.

Treatment Group	Serum K [mEq per l] Following K Supplementation	Following Amiloride
(A) Potassium supplementation first (18)	3.45	3.47
(B) Amiloride first (12)	3.29	3.56
(A) Plus (B)	3.39	3.50

It is seen that the mean serum potassium level is slightly higher following amiloride treatment than following treatment with potassium supplements. The greatest difference is in the group given amiloride treatment first.
There were no side effects or toxic effects which could be attributed to treatment with amiloride.

The results suggest that in the dose used (usually 5-10 mg per day) amiloride is slightly more effective than oral potassium salts in a dose of 28-42 mEq per day in maintaining serum potassium levels in hypertensive patients treated with diuretic drugs.

Scleroderma

A. J. Barnett

Scleroderma has continued to arouse my interest for many years and in the 1972 Report there is a brief summary of a follow-up study of 78 patients seen over the period 1950-1972.
The number of patients with this condition of whom I now have personal records amounts to 100.
During 1973, I have completed a monograph, commenced two years previously, based on a study of my personal series and a survey of the literature. This is now in press and publication is expected early in 1974.
Although much is known about the clinical features and course of this disease and much interesting work is in progress on the biochemical disturbance in the connective tissue, the aetiology remains unknown and there is no specific treatment.

Recently new treatments have been described by overseas workers, with the claim that they favourably influence the collagen disturbance and produce amelioration of the clinical features. One of these is with "Gestagens"[1] (progesterone-like drugs), used in certain gynaecological disturbances and another with salazopyrin[2], used in the treatment of ulcerative colitis.
A trial has been commenced to study the effectiveness of these treatments, using as criteria not only symptomatic relief but also objective tests of the function of affected organs, histological examination of skin biopsy specimens and excretion of glycosaminoglycans and hydroxyproline in urine.
The trials are as yet incomplete, but none of the patients have as yet shown any striking symptomatic benefit.

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Distribution of Myocardial Blood Flow in the Dog

F. R. Trinker

The methodology using labelled microspheres was described in last year's report. ^{46}Sc labelled microspheres (15 μ diam.) were used to establish, by radiation counting, coronary blood flow through the myocardium under control (baseline) conditions. ^{85}Sr labelled microspheres of the same size were injected into the left atrium 2-4 hours following ligation of either the anterior descending or left circumflex coronary arteries. Systemic pressure, cardiac output and heart rate were monitored simultaneously.
Cardiac output and systemic pressure decreased following the ligation of either coronary artery reaching significant levels after two hours. Changes (i.e. a decrease) in heart rate were minor and arrhythmias occurred in two instances only—in the latter situation, the experiment was discontinued. Table 1 gives the result of these observations.

Table 1

N = 7	Control	After Ligation of Arterior Descending Artery
Blood Pressure (mmHg)	96	86
Cardiac Output (ml/min)	1064	646
Heart Rate (beats/min)	168	162
N = 7	Control	After Ligation of Left Circumflex Artery
Blood Pressure (mmHg)	88	68
Cardiac Output (ml/min)	1830	970
Heart Rate (beats/min)	161	139

Following the ligation of the anterior descending coronary artery, changes in coronary blood flow in certain regions occurred as tabulated below (Table 2). The right ventricular septal region had areas of ischaemia and hyperaemia as did the left ventricular septal regions and the adjacent left ventricular endocardial areas.

Table 2

Region	Control	After Ligation of Anterior Descending Artery
RVS2	977* ± 235	1625 ± 247
	1869 ± 480	1013 ± 35
LVS2	1233 ± 79	1679 ± 243
	1728 ± 256	1159 ± 191
LVS4	1164 ± 58	1679 ± 136
	1725 ± 217	1350 ± 208
LV4	1414 ± 162	2146 ± 274
Endocardial	1616 ± 203	1117 ± 174

Table 3

Region	Control	Ligation of Left Circumflex
RV	1322 ± 78	2283 ± 179
RVS	1885 ± 183	3178 ± 266
LVS	2091 ± 174	2926 ± 278
LV1 endoc.	1873 ± 295	736 ± 320
LV1 epic.	1734 ± 336	418 ± 113
LV4 endoc.	2094 ± 436	3682 ± 202
LV4 epic.	2132 ± 360	3611 ± 362

* In Tables 2 and 3 units are cpm/g wet weight.

No significant changes were observed in the distribution of blood flow through the other regions of the myocardium.

When the left circumflex artery was ligated, the right ventricle, right and left ventricular septal regions and parts of the left endocardial and epicardial areas were hyperaemic. Only two areas were ischaemic (left endo- and epicardial zones). (See Table 3.)

It would appear from these experiments that ligation of a major coronary vessel produces patches of ischaemia that are in closest proximity to the ligated vessel. Adjacent areas exhibited marked hyperaemia; probably an early compensatory mechanism becomes operative.

Further studies on the significance of neuronal control and the effects of vasodilator and antiarrhythmic drugs on the distribution of coronary blood flow through the myocardial tissue were anticipated prior to the termination of this project.

Cardiac Muscle

V. Carson

Effects of Beta-Blocking and Other Drugs on the Adenyl Cyclase Activity of Cat Myocardium Homogenates

The study of the effect of β-blocking and other drugs on the adenyl cyclase activity of cat myocardium and on the stimulatory effect of adrenalin, using the methods previously described (1972 Report, p. 23), has been completed.

In this **in vitro** system adrenalin caused an increase in 3′ 5′ cyclic AMP (cAMP) production of approximately 100% compared with control levels.
It was found that oxprenolol at a concentration of 10^{-5}M completely blocked the stimulation of the adenyl cyclase system caused by adrenalin (10^{-4}M). This blocking effect was still almost complete at 10^{-6}M oxprenolol and was completely eliminated at 10^{-8}M oxprenolol. Oxprenolol alone had no effect on cAMP production at any of these concentrations.

In the case of prindolol significant blocking was obtained with 10^{-6}M and there was some reduction (not significant) in the stimulatory effect of adrenalin at 10^{-7}M prindolol. Further experiments revealed that complete blocking was obtained with 10^{-5}M prindolol and that this effect was completely eliminated at 10^{-8}M.

Thus under these **in vitro** conditions of test, the two drugs would appear to have a similar potency with respect to their effective blocking concentration. However, only 10-fold changes in concentration were tested. If smaller changes in concentration had been tested, it is possible that the reported greater potency of prindolol **in vivo** may have been revealed.

As previously reported verapamil, at concentrations as high as 10^{-4}M, was found to have no effect on the adenyl cyclase activity of cat heart homogenates, either in the presence or absence of adrenalin.
To test the specificity of the reaction for β-blocking drugs, a known α-blocker, phenoxybenzamine, was tested in the same system. The results of six such experiments showed that phenoxybenzamine at concentrations from 10^{-3}M to 10^{-5}M had no effect on the adenyl cyclase activity of the cat heart homogenates, nor did it affect the stimulation of the system by adrenalin.

Thus the **in vitro** blocking effects of the β-blockers on the stimulation of adenyl cyclase activity by adrenalin mirror closely the physiological β-blocking effects and the "adenyl cyclase-adrenalin" assay would seem to be a useful tool in the evaluation of new drugs for β-blocking properties.

The Effects of Mg^{2+} on the Inotropic and Metabolic Responses of the Isolated Perfused Rat Heart to Catecholamines

Paddle and Haugaard (1971) published a paper in which they reported that adrenalin caused a positive inotropic response in a Langendorf preparation of rat heart perfused with Krebs-bicarbonate medium containing 1.2 mM Mg^{2+}. This was accompanied by an increase in phosphorylase **a** activity, a decrease in glycogen content and an increase in lactate production together with small changes in ATP, ADP, and AMP and glycolytic intermediates.

When the concentration of Mg^{2+} in the perfusion fluid was changed to 20 mM there was an 85% drop in the force of contraction together with a marked bradycardia but no significant changes in the metabolic responses compared with the control situation.

When adrenalin was added, still in the presence of 20 mM Mg^{2+} there was a significant rise in the contractile force, back to control levels. There was also a significant rise in phosphorylase **a**. However, this was not accompanied by any change in lactate, glycogen, ATP or glycolytic intermediates.

These results were somewhat unexpected and no satisfactory explanations could be offered. As no cyclic AMP determinations were included in their study, it was thought worthwhile to try to duplicate Paddle and Haugaard's results with respect to the physiological responses, phosphorylase activity and glycogen content, but also including estimations of cyclic AMP levels, in the hope that some logical explanation for the observed changes could be proposed.

The method of perfusion of the rat hearts was similar to that of Paddle and Haugaard except that in our experiments the two-way stopcock for interchange of perfusion fluids was placed above the 37° water-bath surrounding the glass perfusion tubing, whereas in their experiments, it was placed just above the level of the cannula into the aorta. Thus in our apparatus the change from one composition of fluid to another was much more gradual, owing to diffusion effects, than in the case of Paddle and Haugaard.
Control perfusions were carried out for 10 minutes.

The infusion of adrenalin, in either the control medium or the high Mg^{2+} medium with a trace of ascorbic acid added, was made by means of a Harvard syringe, connected through fine nylon tubing to a tube inserted into the cannula just below the level of the aorta and carried out at a rate of 1 ml/min for one minute and was always done after 10 minutes of control perfusion.

The high Mg^{2+} perfusions were done after 10 minutes of control perfusion and were carried out for one minute after the commencement of the effect on the contractile force.

The spontaneous rhythmic contractions were recorded in the usual way through transducers, etc., the transducer being connected to the apex of the heart by a thread and a Palmer clip.

Experiments were terminated by quickly dropping the whole heart into liquid nitrogen in which the heart was stored until the biochemical estimations were carried out.

Cyclic AMP was determined on the frozen tissue by the method of Gilman (1970) after purification of the tissue extract by Zn SO_4-$Ba(OH)_2$ precipitation and chromatography on Dowex 50. The percentage of phosphorylase **a** was determined by the method of Cori and Illingworth (1956), proteins by the method of Lowry **et al.** (1951) and tissue glycogens by the method of Seifter **et al.** (1950).

This study is not yet completed but the following results have been obtained.

Under control conditions of perfusion (seven experiments) the mean percentage of phosphorylase **a** was found to be 21.9 ± 0.95 (S.E.) % and the mean level of cyclic AMP was 9.7 ± 1.00 (S.E.) pM per mg protein.

The infusion of adrenalin at a concentration of 5 μg/ml was found to cause a short-lived rise in the force of contraction followed by a return to normal force and a bradycardia.

It was thought that this whole effect could be due to the dose of adrenalin being too high and it was therefore reduced to 0.625 μg/ml at which concentration the positive inotropic response and slight tachycardia lasted from ½ to 1 minute. At one minute the experiments were terminated.

Under these conditions in seven experiments compared with controls there was a rise in the mean percentage of phosphorylase **a** to 63.7 ± 6.56% (S.E.) ($p < 0.001$) and a rise in the mean tissue cyclic AMP to 15.6 ± 1.31 (S.E.) pM per mg protein ($p < 0.005$).

When the concentration of Mg^{2+} in the perfusion medium was changed to 20 mM there was a gradual decline in the force and rate of contraction over a period of 10-15 seconds to about 60% of the control values; then the heart slowed markedly and abruptly stopped beating.

It was found necessary to reduce the concentration of Mg^{2+} to 10 mM in order for the heart to continue to beat at a reduced force and rate of contraction for one minute at which point the experiment was terminated.

Six experiments using 10 mM Mg^{2+} showed no change in the mean percentage of phosphorylase **a** (20.1 ± 1.7 (S.E.)). However, the mean tissue cyclic AMP level fell to 4.8 ± 0.57 (S.E.) picomoles per mg protein ($p < 0.001$) compared with controls.

The response of individual rat hearts to 10 mM Mg^{2+} was somewhat variable, this concentration appearing to be a critical one which, in about 20% of cases, caused the heart to stop beating within one minute. Such hearts were not included in the results. The same variability was seen when the hearts were subjected to the high Mg^{2+} concentration followed by infusion with adrenalin (0.625 μg/ml) in the high Mg^{2+} medium. The most common result was that the force and rate of contraction decreased but under the action of adrenalin the control force and rate were restored or even exceeded. Occasionally the heart stopped after one minute of perfusion with the high Mg^{2+} but the beats could be restored by immediate infusion with adrenalin. Biochemical analyses were done in both instances.

Full discussion of the results must await the completion of the adrenaline-high Mg^{2+} series and the glycogen analyses; but it would appear that the infusion of adrenalin in these cases also causes a marked rise in phosphorylase **a** activity and an even greater rise in cyclic AMP levels.

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Interaction Between Amitriptyline and Guanethidine in Rat Heart

F. R. Trinker

The initial **in vivo** experimental work in dogs and on the isolated perfused rat Langendorff heart preparation was described in last year's report. Based on the findings that tricyclic anti-depressants (TCA) and guanethidine are both inhibitors of noradrenalin (NA) uptake into adrenergic neurones and that in doing so these drugs themselves are taken up by adrenergic tissues, it has been postulated that the TCA can prevent the pharmacological effects of guanethidine. The antagonism of guanethidine by TCA drugs, however, is not uniformly observed, clinically or experimentally. For this reason in a model the NA uptake mechanism and the NA depleting action of guanethidine were studied simultaneously.

Tracer doses of (36 μCi) radioactive NA (^{3}HNA) were infused for 10 minutes in rats, pretreated with guanethidine at various dose levels and time intervals, to study the active uptake of NA into myocardial tissue and the NA depleting effects of guanethidine.

Table 1 gives a summary of the effects of guanethidine and amitriptyline pretreatment on the NA content and uptake systems.

Table 1

	Control	Guanethidine			Amitriptyline	
		30 mg/kg 1 hr.	50 mg/kg 1 hr.	30 mg/kg 18 hrs.	30 mg/kg 1 hr.	30 mg/kg 18 hrs.
NA content (μgm/gm)	0.92 ± 0.08	0.54 ± 0.03*	0.58 ± 0.6*	0.26 ± 0.06*	0.91 ±0.07	0.72 ± 0.08
^{3}HNA uptake μCi/gm	3.7 ± 0.48	0.88 ± 0.38*	2.1 ± 0.3	2.07 ± 0.4	1.36 ± 0.2*	3.42 ± 0.64

Table 2

	Control	Guanethidine		
		10 mg/kg	20 mg/kg	30 mg/kg
NA content (μgm/gm)	0.92 ± 0.08	0.17 ± 0.0*	0.14 ± 0.0*	0.12 ± 0.0*
NA uptake (μCi/gm)	3.7 ± 0.48	3.3 ± 0.15	1.18 ± 0.41	2.2 ± 0.39

Table 3

	Guanethidine 30 mg/kg 18 hours	Amitriptyline (30 mg/kg) + Guanethidine (30 mg/kg 18 hours)			
		Together	½ hour	1 hour	2 hours
NA content (μgm/gm)	0.26 ± 0.06	0.32 ± 0.02	0.29 ± 0.02	0.27 ± 0.04	0.23 ± 0.05
NA uptake (μCi/gm)	2.07 ± 0.47	2.6 ±0.4	2.6 ± 0.24	1.05 ± 0.30	2.7 ± 0.16

* significance: $p \leqslant 0.05$ in all Tables.

As the duration of guanethidine pretreatment was increased to 18 hours the NA content decreased significantly further whereas the inhibition on the uptake mechanism became less marked. Amitriptyline significantly depressed the uptake mechanism after one hour, but this was not maintained after 18 hours. Chronic pretreatment of rats with guanethidine (i.e. 3-11 days) at three different dose levels gave the following results (Table 2).

To investigate the importance of a possible interaction between guanethidine and amitriptyline, the effect of guanethidine or amitriptyline alone was compared with both drugs administered simultaneously and at ½, 1 and 2 hours intervals apart with amitriptyline given first (Table 3).

These results demonstrate that amitriptyline whether given together with or at various time intervals apart from guanethidine, neither modified the NA depleting action of guanethidine nor its uptake into myocardial tissue. In some way during the 18 hours, guanethidine was able to exert one of its pharmacological actions, i.e. its ability to deplete NA stores.
Chronic administration of guanethidine at a a lower dose (10 mg/kg) together with amitriptyline (30 mg/kg) produced impairment in the NA depleting action of guanethidine (Table 4).

Table 4

	Control	Guanethidine 10 mg/kg > 3 days	Guanethidine (10 mg/kg) + Amitriptyline (30 mg/kg) > 3 days
NA content (μgm/gm)	0.92 ± 0.08	0.17 ± 0.0*	0.35 ± 0.03*
NA uptake (μCi/gm)	3.7 ± 0.48	3.3 ± 0.15	2.66 ± 0.4

Hence amitriptyline produces antagonism of the NA depleting action of a low dose of guanethidine.
The acute early (1 hour) effects of the amitriptyline/guanethidine interaction at various time intervals and at a reduced dose of the latter was studied, the results of which are shown in Table 5.

Table 5

	Guanethidine 10 mg/kg	Amitriptyline (30 mg/kg) + Guanethidine		
		(30 mg/kg) Together	(30 mg/kg) ½ hour later	(10 mg/kg) ½ hour later
NA content (μgm/gm)	0.54 ± 0.03	0.80 ± 0.05*	0.65 ± 0.06	0.95 ± 0.11*
NA uptake (μCi/gm)	0.88 ± 0.38	1.16 ± 0.15	1.31 ± 0.31	1.34 ± 0.24

It appears that amitriptyline and other tricyclic anti-depressant drugs may antagonise some of the effects of guanethidine and other related adrenergic neurone blocking drugs **initially** if they are administered simultaneously and/or the dose of guanethidine is reduced to a level where this drug can no longer effectively compete for the same uptake mechanism as the tricyclic anti-depressant group of drugs.

Amputation Survey

A. J. Barnett, B. Ford[1], E. Twist[2] and A. Balfe[3].

In 1967, Barnett, Guthrie and Crawshaw[4] published a review of 59 patients with lower limb amputations performed in the Alfred Hospital during the year 1964. The findings were very depressing in that there was a high death rate. About two-thirds of the survivors had received prosthesis and about two thirds of these used them. Since then an amputee clinic has been established and there are better facilities for treating amputees, although the follow-up is still inadequate.
It was considered of interest to review more recent amputees at the Alfred Hospital to determine if there were any improvement in the outlook with the newer approach and what further help could be given to these patients.
A list was compiled of patients who had received amputation during the years 1970, 1971 and 1972, and an attempt was made to contact the survivors by letter and arrange for interviews and medical examination and, in the case of deceased patients, obtain information on survival following operation from local doctors and relatives.
There were 114 patients registered (approximately 38 per year). To date only 91 have been studied, but these are sufficient to indicate certain trends.
The first point of interest is that amputation is now less frequent (average 38 per year against previous 59). It would seem likely that this is due to the more active treatment of peripheral vascular disease.
The type of amputation is shown in Table 1.

Table 1

Amputation Site	1964	1970	1971	1972
Above knee	33	12	21	18
Through knee	13	7	4	2
Below knee	12	7	10	13
	(19%)	(26%)	(29%)	(39%)
Other	1	1	1	—
Number of Amputations	62	27	34	32
Number of Patients	59		91	

The number of amputations is more than the number of patients due to some patients receiving more than one amputation in the study period.

Above-knee amputations remain the most common. Through-knee amputations have become progressively less popular and are now rarely performed. Below-knee amputations are becoming more popular.
Comparison of the two series in respect to number of survivors and supply and use of prosthesis is shown in Table 2.

Table 2

	1964 Series	1970, 71, 72 Series
Deceased	26	53
Survivors	33	38
Prosthesis supplied	21	23
Prosthesis used satisfactorily	14	17

The high death rate continues (the higher proportion of deceased in the recent series is due to the longer follow-up period of six months to 3½ years compared with six months to 1½ years).
A disappointing finding is that the proportion supplied with and using prosthesis is even lower in the recent than in the earlier series.
Two of the 30 below-knee amputations in the recent series were subsequently converted to above-knee and one of the 51 above-knee amputations needed revision indicating that the choice of site of amputation was usually correct.
However, below-knee amputation was associated with better prospect of mobility than above-knee. Fourteen of 16 surviving below-knee amputees were mobile either with prosthesis alone or prosthesis and sticks; whereas only five of 18 surviving above-knee amputees were mobile in this way.

1. Director of Rehabilitation.
2. Rehabilitation Medical Officer.
3. Medical Social Worker, Alfred Hospital.

4. BARNETT, A. J., D. J. GUTHRIE, and J. CRAWSHAW. "A Review of Lower Limb Amputations at the Alfred Hospital in 1964". **Alfred Hosp. Clin. Reports** Vol. 14 (1967) p. 21.

Gastro-Oesophageal Reflux

T. D. Lewis

Gastro-oesophageal reflux is not uncommon, producing the symptom of "heartburn". The acid refluxed, as well as producing classical symptoms, may also produce atypical local pain, and may also induce oesophagitis.

The presence of intermittent asymptomatic acid reflux has been shown to be common in normal people in the upright position, but only occurs in the recumbent position in those with symptoms.

The following methods are used to help in the diagnosis of abnormal reflux:

(i) **Clinical history.** With straight-forward symptoms appears to be the most reliable.

(ii) **Barium swallow and meal.** If reflux is demonstrated, this is definitely abnormal, as a large volume of gastric contents has to be refluxed before it is obvious on x-ray screening. However, this is often unhelpful. (The presence of hiatus hernia alone is not really helpful, as hiatus hernia may be present without reflux and reflux may occur without hiatus hernia.)

(iii) **Oesophagoscopy** (with or without biopsy) will demonstrate oesophagitis, if present, and may also demonstrate an incompetent lower oesophageal sphincter which will allow reflux.

(iv) **Oesophageal manometry** will show the pressure developed in the lower oesophageal sphincter, which is a major barrier to reflux.

(v) **Intra-oesophageal pH studies.** A 3 mm wide pH electrode, with the flex attached to a pH meter, is swallowed and can be positioned 5 cm above the gastro-oesophageal junction. Normal oesophageal pH is approximately 6 and a pH drop to below 4 signifies acid reflux. Various "stress" manoeuvres (e.g. straight leg raising, coughing, deep breathing, etc.) can then be performed by the patient to induce reflux.

For practical purposes "significant" reflux is accepted if free reflux occurs (i.e. without straining) or if reflux during stress occurs on more than one occasion.

(vi) **Acid infusion test (Burnstein).** 0.1M hydrochloric acid is infused into the lower oesophagus and the patient's pain response is compared with that to water infusion. A positive response is pain produced by acid but not by water and is a measure of the sensitivity of the gullet to acid. A positive test is good supportive evidence for reflux being present.

(vii) **Acid clearing test.** This is a measure of the ability of oesophageal peristalsis to remove refluxed acid. 0.1M hydrochloric acid is infused as a bolus into the lower part of the oesophagus and then the number of swallows counted until pH rises above 4 (the normal number is approximately 10).

To further assist in the diagnosis of oesophageal reflux, I have been using a pH electrode (Titron) for the past 18 months for intra-oesophageal pH measurement. This particular method of investigation has been shown to give the best results for the presence or absence of significant reflux. The major indications for use of this test are —

(i) to help in the investigation of the cause of chest pain, which may be due to reflux;

(ii) as one means of assessing the presence of reflux.

The accompanying table shows a comparison of the various investigations. Unfortunately there is no absolute standard with which to compare results, but pH probe testing correlates well with the acid infusion test and these two tests can be performed together. These two also sometimes give positive results when other investigations for reflux (especially radiological) fail to demonstrate it. However, I think the results here show that pH probe testing for gastro-oesophageal reflux is a useful addition to the tests already used for a condition which may mimic cardiac pain and which may also insidiously progress to stricture.

Symptoms	Barium Meal		Oesophagoscopy (Inflammation)	pH Test	Acid Infusion Test (+ = pain)	Acid Clearing Test (No. of Swallows) (n ⩽ 10)	Conclusion
	Reflux	Hiatus Hernia					
1. Chest pain	—	+	N.D.	—	—	N.D.	No
2. Heartburn	+	—	— (I.C.)	+	N.D.	N.D.	Yes
3. Angina ? Heartburn	—	—	+	—	+	N.D.	Yes
4. Epigastric pain	+ (minimal)	—	—	+	+	16	Yes
5. Heartburn	+	+	+	+	N.D.	10	Yes
6. Scleroderma (no oesophogeal symptoms)	—	—[1]	N.D.	+	N.D.	N.D.	Yes
7. Chest pain	N.D.	N.D.	N.D.	—	—	N.D.	No
8. Chest pain	+	—	N.D.	+	+	10	Yes
9. Recurrence heartburn after repair	+	+	+	+	+	N.D.	Yes
10. Chest pain	—	—	N.D.	—	—	13	No
11. ? Reflux	+	—	— (I.C.)	±	±	> 20	Yes
12. Reflux	—	—	—	+	—	> 20	Yes
13. Chest pain	—	+	±[2]	+	N.D.	N.D.	Yes
14. Recurrent pain after repair	—	—	N.D.	—	N.D.	N.D.	No
15. Angina ? Reflux	—	—	N.D.	+	+	20	Yes
16. ? Reflux	—	—	±[2]	—	—	9	? Yes
17. Reflux	+	+	+	±	N.D.	N.D.	Yes

+ = positive result (e.g. oesophagitis, reflux present, etc.)

— = negative result.

N.D. = test not done.

I.C. = incompetent cardia.

1. = poor motility seen.

2. = thickened opacified oesophageal mucosa seen consistent with reflux changes, but no definite oesophagitis.

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Lectures Given During 1973

A. J. Barnett	"Classification and Course of Scleroderma (Progressive Systemic Sclerosis)".	*Royal Australasian College of Physicians, Melbourne.*

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Annual Report

This year a paper was published which reported experience over a twelve month period in the treatment of patients suffering from diabetic coma and pre-coma who were admitted to our care. These patients were selected for inclusion in the report because they had been under medical observation prior to their admission to hospital for treatment. The report was made because it was apparent that in instances of delay in recognition of the nature of the illness, or of institution of effective treatment, the course of the disease was less favourable. The message of the paper was an educational one presenting the rationale of early effective treatment against a background of the morbid biochemistry and physiology of the severe insulin deficiency causing diabetic ketoacidosis.

This educational role is one regarded by the staff as being an extremely important aspect of its activities. It is, however, a less tangible feature of the operation of the unit than that which can be recorded in its clinical and investigative operations. In endocrinology and diabetes the scene has changed dramatically in the last decade and is continuing to change at a rapid rate in respect to both concepts and techniques.

Education at all levels is an integral part of the function of the Unit. We take our place alongside others in undergraduate teaching and are fortunately placed in respect to a combination of Honorary and "on the spot" staff to give close advice to the frequently changing junior medical officers who serve as house officers on the Unit ward, in what is a new and often bewildering medical environment.

Clinical and seminar type teaching is ongoing throughout the year to serve those studying for post-graduate degrees and the facilities of the Unit will continue to be available for the Senior Post-graduate undertaking specialised training in clinical and laboratory endocrinology. Tabulation of the representative list of lectures given by members of the staff of the Unit is indicative of yet another aspect of the educative function of the Unit.

The increasing sophistication of endocrine investigation produces a considerable service load in performing assays which only a few years ago were regarded almost entirely as research procedures. We are currently running seven radioimmunoassays which are all required for routine investigation of patients although each is applied to both fundamental and clinical research.

Table of Current Assays

Assay	Some Clinical Applications
Luteinising Hormone	Hypogonadism Premature Puberty Pituitary Assessment
Growth Hormone	Acromegaly Short Stature Pituitary Assessment
Thyroid Stimulating Hormone	Hypothyroidism Atypical Hyperthyroidism Pituitary Assessment
Tri-iodothyronine	T_3 thyrotoxicosis Monitoring of Therapy "Euthyroid sick"
Insulin	Islet Cell Tumour Insulin binding in Diabetics
Aldosterone	Hyperaldosteronism Hypoaldosteronism Cyclical Oedema
Renin	Renal Hypertension Hyperaldosteronism Hypoaldosteronism Classification of Hypertension

While this list is extensive it does not provide complete evaluation of endocrine patients. For instance we lack an ACTH assay for optimum evaluation of Cushing's syndrome and a parathyroid hormone assay for evaluation of patients with suspected hyperparathyroidism or renal osteodystrophy. Further diversification is one alternative but it is predictable that Endocrine Units will become increasingly interdependent as the scope of necessary technology increases.

During 1973 Dora Winikoff retired from the staff. She has given long and valued service to the Unit in both investigative and service roles. She was a pioneer in the methodology of the first generation of thyroid function tests and of their introduction into clinical practice. On these methods and their applications she has written widely. Her contribution to the Unit over the years is warmly acknowledged.

Collaboration with colleagues in clinical and laboratory departments at Alfred Hospital and Monash University continues to be a pleasant feature of our work. Valued relationships are maintained too with the Endocrine Group and Medical Research Centre at Prince Henry's Hospital and with the Howard Florey Institute at Melbourne University. Support for our activities in the form of financial assistance and gifts in kind have again been made and grateful acknowledgement is made to those whose names follow.

PINCUS TAFT.
Physician-in-Charge.
December 31, 1973.

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

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Report of Scientific Investigations

Affinity Chromatography and Immunoadsorption

J. R. Stockigt, I. Ekkel and W. Hudson

Attachment of a specific antigen to a solid polymer will still allow an antibody to react specifically with that antigen. The antibody-antigen bond can then be broken at low pH and high ionic concentration and a purified antibody obtained. This technique has direct application to the technique of radioimmunometric assay in which the antibody rather than the antigen is iodinated. This procedure is useful in the assay of peptides which cannot be iodinated and for the assay of steroid hormones. The purified antibody can itself be attached to the solid polymer and used for specific extraction procedures.

The initial phase of this work has been in the attachment of tri-iodothyronine and cortisol hemisuccinate to activated Sepharose. Both have been successfully linked by the carbodiimide reaction and antibodies can be removed from rabbit serum onto the solid phase. This reaction is a specific antibody reaction rather than non-specific adsorption of antibody as determined by lack of removal of angiotensin I or aldosterone antibody by Sepharose-T_3. However, it is difficult to recover specific binding from the solid phase because small quantities of antigen are also eluted. Techniques for extensive preparation of the solid phase are necessary in order to prevent antigen removal at the time of antibody elution.

The conventional technique for iodination of peptides and proteins is the Chloramine T reaction. This strong oxidising agent damages many proteins and an alternative iodination procedure with Lactoperoxidase has been established. This is applicable to the iodination of both the tracers currently used in radioimmunoassay and to the iodination of purified antibodies.

Renal and Adrenal Hypertension

J. R. Stockigt, E. Cukier and I. H. Jurk

Laboratory Methods

Techniques for the determination of aldosterone and plasma renin have been established in 1973. Both methods are radioimmunoassays using rabbit antisera with dextran-charcoal to separate free from antibody-bound hormone. The renin method involves quantitation of angiotensin I generated **in vitro** in the presence of inhibitors of the plasma enzyme which converts angiotensin I to angiotensin II. The method can be performed either with endogenous substrate (plasma renin activity) or with addition of an excess of heterologous renin substrate from nephrectomised sheep (plasma renin concentration). The latter method is the most sensitive renin assay yet reported.

The measurement of aldosterone in urine involves acid hydrolysis at pH 1 after pre-extraction of cross-reacting steroids. This method is much less costly and time consuming than the double isotope derivative assay and is suitable for analysis of multiple samples. The specificity of the method has been examined in detail in adrenalectomised or Addisonian subjects on maintenance steroid therapy and after suppression of aldosterone with deoxycorticosterone acetate. Specificity is comparable to the double isotope assay and the simplified method gives good correlation with the more complex method.

Significance of These Studies

There are now over 10 varieties of surgically curable renal and adrenal hypertension. The most common types are primary aldosteronism (Conn's Syndrome) and renal artery stenosis. The laboratory techniques for aldosterone and renin measurement are essential for the proper selection of patients for surgery. Typical findings in primary aldosteronism are elevated aldosterone which is non-suppressible by sodium loading, associated with marked suppression of plasma renin. Surgically correctable renal hypertension is identified by predominant renin secretion from the ischaemic kidney with associated suppression of contralateral renin secretion.

Radioimmunoassay techniques have greatly shortened the study periods. A patient with suspected primary aldosteronism can be evaluated in four days and assessment of renal hypertension requires only overnight admission to hospital.

Clinical Investigation

A. Adrenal Disease

The major unsolved problem in primary aldosteronism is the pre-operative distinction between a unilateral adenoma and bilateral hyperplasia. A patient with a unilateral adenoma can usually be cured of hypertension by unilateral adrenalectomy and has normal adrenal function remaining on the other side, while a patient with bilateral hyperplasia is rarely cured of hypertension but has the problem of iatrogenic Addison's disease after bilateral adrenalectomy. While pre-operative distinction is vital it has been difficult by standard techniques. The problem is heightened by the small size of many adenomas (0.5 - 2 cm) which may not be visible until the adrenal is removed. These problems have been approached in three ways:

(i) Documentation of the degree of **renin suppression** and determination of **aldosterone suppressibility** after treatment with exogenous mineralocorticoid (deoxycorticosterone acetate or 9-fluorohydrocortisone). The chances of a unilateral adenoma are greatest if renin suppression is gross and if aldosterone is resistant to suppressive manoeuvres.

(ii) **Adrenal vein catheterisation** (in collaboration with Dr. N. Sacharias). The left adrenal vein has a fairly constant position as a tributary of the left renal vein. With skill and patience it is possible to pass a catheter from the femoral vein into this vessel and measure the hormone effluent from this gland. This may identify a left sided adenoma or will indicate left sided suppression of secretion in the presence of hormone excess (by inference a right sided adenoma). The right adrenal vein is inconstant in position and has so far defied catheterisation.

(iii) **Adrenal Scanning** (in collaboration with Dr. L. Dugdale). By giving ^{131}I cholesterol as a steroid precursor it is possible to visualise the adrenal glands and to identify a local source of hormone overproduction. Several days after the dose a gamma camera is used to visualise the glands and computer analysis is used to quantitate the uptake of ^{131}I by the adrenals.

In the latter half of 1973 nine patients have been evaluated for primary aldosteronism. Three patients have been successfully operated upon, one is pending and three are being treated medically with Spironolactone.

B. Renal Hypertension

Renal vein renin determination has become widely established as a means of predicting benefit from surgery. It is now available as a routine technique and our clinical research has been directed to two unsolved problems.

(i) Renin secretion is intermittent and significant lateralisation may be missed if renal vein samples are taken during a non-secretion phase. The drug Diazoxide (Hyperstat) produces a prompt and reproducible outpouring of renin and is being used to confirm and accentuate the lateralisation of renin secretion. This technique is applicable to segmental renal infarcts, unilateral hydronephrosis with hypertension and intrarenal vascular malformations as well as to renal artery stenosis.

(ii) When an ischaemic lesion affects only a segment of one kidney the blood draining this segment is diluted by a high volume of effluent blood from normal renal tissue in which renin secretion may be suppressed. When renin samples are drawn from the common renal vein a significant lesion may be missed. It has been demonstrated that a renin sample drawn from the appropriate segmental renal vein improves the accuracy of defining such a lesion. In collaboration with Dr. N. Sacharias the technique of segmental renal vein renin sampling has been established. Because renal venous anatomy is highly variable it has been useful to perform a preliminary renal venogram to define the anatomy of renal vein branches. Fifteen patients have been evaluated for possible renal hypertension. Three have been operated upon and several are awaiting further evaluation.

Characterisation of Human Pituitary Gonadotrophin

I. Ekkel, P. Taft and R. Pepperell[1]

In the treatment of human infertility—male and female—gonadotrophin prepared from human pituitaries has been found to be clinically effective. A major problem in the treatment of anovulation in women is that of choice of dosage such that multiple ovulation is avoided. Quantitation of potency of hormone is of advantage in treating patients with different batches and was in Australia originally limited to F.S.H. bioassay. In this study the patient's response was compared to the bioassay and immunoassay potency of various batches of hormone used therapeutically.

Potency variations were observed between batches as assessed by all methods. It was of interest that the patient response or "patient bioassay" correlated well with radioimmunoassay potency for F.S.H. and L.H. but not for either of two types of F.S.H. bioassay.

One batch with good F.S.H. radioimmunoassay potency was inactive in the patient and was without F.S.H. bioassay potency. It was also shown to have a very short plasma half life when compared with a biologically active hormone. Thus although retaining immunologic competence this batch had lost its biologic potency. Such a lack of immunological and biological correlation and difference in time of disappearance from plasma was also noted in successive fractions of hormone eluted from the preparative column during hormone production. Loss of sialic acid from the molecule is suspected.

Thus in order to predict clinical potency, it is necessary to have radioimmunoassay evidence of the presence of F.S.H. Bioassay activity although not correlating well with patient response seems to be indicative of the totality of the F.S.H. molecule and predictive of its survival for a time to permit biological activity.

1. Medical Research Centre, Prince Henry's Hospital.

Pituitary Growth Hormone Fragments and Carbohydrate Metabolism

G. T. Seiler[1], **F. Ng**[2] **and P. Taft**

Reference was made in last year's report to the absence of consistent increase in insulin sensitivity in rats treated with cataglykin (the growth hormone fragment for which an insulin sensitising role is proposed) although the effect was seen in rabbits. Studies made this year in a further examination of this phenomenon suggest that this effect is seen, but only in the mature animals. This is comparable to Young's original observation that the diabetogenic effect of crude pituitary extract was confined to mature dogs.

Much of this year's effort has been invested in the extraction from human urine of cataglykin, collected into a sufficiently large supply such that its effect might be studied in man. Its influence in potentiating the action of insulin, endogenous or exogenous, could be examined as a possible means of modifying treatment.
During collection of urine from normal subjects, diabetics both "genetic" and due to haemochromatosis or pancreatitis and from patients suffering from pituitary disease, the opportunity was taken to study both the rhythm of appearance and total amounts of cataglykin extracted. Inadequacy of precise methods of quantitation limit the interpretation of data. They suggest, however, that cataglykin is highest in concentration after food and lowest in the fasting state. They indicate also lowest total excretion in diabetic ketoacidosis and hypopituitarism. These data are compatible with a physiological insulin facilitatory role.

Further toxicity studies have been undertaken this year using mice and both human urinary and synthetic cataglykin (the sequence used being that proposed by Niall) have been shown to be without harmful haematologic effects or pathologic influence on the wide spectrum of tissues examined.

1. N.H. & M.R.C. Post-graduate Research Fellow.
2. Department of Biochemistry, Monash University.

Thyroid Methodology

M. Malinek, J. R. Stockigt, I. H. Jurk, V. Feller, J. Roy and D. Winlkoff

Samples from about 60 patients per week are received for investigation of thyroid function. While this is a very significant service commitment it also offers great scope for clinical investigation and identification of patients with unusual forms of thyroid disease. The current thyroid methodology was reported in detail in the 1972 Annual Report but during 1973 two radioimmunoassays have been added to increase the scope and precision of our assessment of thyroid function.

Triiodothyronine (T_3)

Evidence is increasing that this is the most important and active thyroid hormone although its concentration in plasma (10^{-7} g/100 ml) is far lower than that of thyroxine (T_4 : 10^{-5} g/100 ml). The direct measurement of T_3 in plasma by radioimmunoassay has been developed using a potent rabbit antiserum developed during 1973 which is capable of detecting 10^{-11} g T_3 and which shows less than 1:200 cross-reaction with T_4.

The major problem in direct T_3 measurement is to eliminate the binding of T_3 to thyroxin-binding globulin present in the serum sample. Numerous approaches to this problem have been reported and include complex extraction procedures. We have produced a simple valid assay using a high concentration of merthiolate which inhibits binding of T_3 to thyroxin binding globulin without inhibiting the antibody reaction. A normal range of 75-175 ng/100 ml has been established and about 500 samples have so far been assayed. After several thousand samples have been assayed we will be in a position to answer the following questions:

(i) What improvement in assessment occurs when T_3 assay is added to a routine thyroid function profile?
(ii) Can T_3 assay alone be used as a test of thyroid function?
(iii) What is the prevalance of T_3 toxicosis in the Melbourne population?

About 10 patients have been identified with T_3 toxicosis who by conventional thyroid function tests would have been regarded as euthyroid. A group of patients recently described as "The Euthyroid Sick" is emerging who have low levels of T_3 associated with normal conventional thyroid function tests. These patients have severe illness or liver disease with a possible defect in the conversion of T_4 to T_3 in peripheral tissues. It is at present uncertain whether treatment of this group with T_3 would be appropriate.

During 1973 a patient with myxoedema coma (rectal temperature 28°C) was successfully treated with parenteral triiodothyronine. Levels of T_3 before and during treatment have given useful guidelines to appropriate dosage which has previously been controversial.

Thyroid-Stimulating Hormone (TSH)

Using antiserum and purified TSH obtained from the National Institutes of Health, Bethesda, Md. U.S.A., and standard hormone from National Institute for Medical Research, Mill Hill, London, a radioimmunoassay for TSH has been established. This assay has been shown to be the most sensitive index of borderline hypothyroidism and is useful in the assessment of the numerous patients who have marginally subnormal conventional thyroid function tests. A group of patients can be defined who may merit long-term thyroid replacement although they have low-normal conventional tests.

Thyrotropin releasing hormone (TRH), a synthetic tripeptide first identified in the hypothalamus in 1970, is a potent stimulus to pituitary TSH release. Intravenous doses of 200-400 μg of TRH with subsequent measurement of serum TSH is a short safe definitive test of pituitary function. The use of this test has simplified the assessment of pituitary-hypothalamic function and has improved the assessment of borderline hypothyroidism and atypical thyrotoxicosis. In hyperthyroidism the TSH response to TRH is absent while in subthyroidism it is accentuated.

The Predictive Value of a Thyroid Test Profile in Spontaneous Abortion

D. Winikoff, M. Malinek and V. Feller

The investigations of the thyroid test profile in women prone to miscarriage, started some years previously, have been concluded.

An evaluation of each of the usual thyroid parameters employed in our laboratory namely serum thyroxine (ST_4) estimation, triiodothyronine resin uptake (RU), electrophoretic index (EI) and thyroxine binding globulin maximum binding capacity of T_4 (TEG) at regular intervals of gestation, allowed the mean values of each to be plotted against weeks of gestation. The changes occurring with the advancement of pregnancy were then compared in three groups of women:

(i) normal pregnant controls;

(ii) pregnant women with a past history of two or more spontaneous abortions; and

(iii) women at the time of an actual spontaneous miscarriage.

The most striking parameters were found to be RU, EI and TBG. In the normal group, the mean RU had dropped by as early as 4-6 weeks, while in the second group only at 12-14 weeks, and the third group even later.

EI values in normal pregnancy also become elevated very early, rising above a characteristic 80% by the 6th week, but the mean of the habitual aborting group (except those who later aborted) reached that level at 14 weeks, while the third group (including those of group 2 who aborted later) never reached that value. The rise in TBG in the second and third groups similarly lagged behind the normal groups.

ST_4 showed little significant difference between the three groups. However, at 14 weeks there was a distinct elevation of the mean ST_4 only in the normal group, above the normal range of values. As expected, the two indices, Free Thyroxine Index (FTI) and Diagnostic Thyroxine Ratio (DTR) were non-contributory.

Therefore a complete thyroid function test profile as carried out in our laboratory, in the early stages of pregnancy, may indicate the necessity for treatment, particularly in those women with a past history of spontaneous abortions.

Several patients have been maintained on small doses of thyroxine after initial investigations have indicated delay in TBG and EI elevation.

Their parameters eventually attained normal pregnancy levels and they carried to term. The results of this investigation will be presented at the 5th S.E. Asia and Oceania Congress of Endocrinology, January, 1974, in Chandigarh, India.

Publications in 1973

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Taft, Pincus, Charles C. Varley and H. D. Breidahl "The Management of the Sick Diabetic — The Physician's Role in the Prevention of Diabetic Coma". **Med. J. Aust.** Vol. 1 (1973) p. 1197.

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Zimmet, P. Z., F. M. Ng, H. P. Taft and J. Bornstein "A Role for Growth Hormone Derived Polypeptides Somantin and Cataglykin in the Pathogenesis of Diabetes Mellitus". **Proc. 8th Congress International Diabetes Federation,** Brussels (1973) p. 145.

Lectures Given During 1973

H. D. Breidahl	"Diabetes in Pregnancy".	*Royal College of Obstetrics & Gynaecology, Melbourne.*
H. D. Breidahl	(i) "The Future of the Infant of the Diabetic Mother". (ii) "The Use of Sulphonylureas in Diabetic Pregnancy".	*8th Congress of the International Diabetes Federation, Brussels.*
H. D. Breidahl	"Diabetes Mellitus".	*Postgraduate Committee Meeting, Mildura.*
H. D. Breidahl	"Diabetes Mellitus".	*Royal Australasian College of General Practitioners, Ballarat.*
J. R. Stockigt	"Adrenal Physiology".	*Commonwealth Serum Laboratories, Melbourne.*
J. R. Stockigt	"Endocrine Tumours and Hypertension".	*Melbourne Medical Postgraduate Committee.*
J. R. Stockigt	"Suppressibility of Aldosterone in Hypertension".	*Howard Florey Institute.*
J. R. Stockigt	"Renin-angiotensin and Aldosterone in the Assessment of Hypertension".	*Alfred Hospital Clinical Society.*
J. R. Stockigt	"Aldosterone: Excess and Deficiency".	*Royal Melbourne Hospital.*
J. R. Stockigt	"Primary Aldosteronism".	*Prince Henry's Hospital.*
J. R. Stockigt	"Diagnosis of Curable Hypertension".	*St. Vincent's Hospital.*
P. Taft with I. Ekkel and R. Pepperell	"Characterisation of Human Pituitary Gonadotrophin".	*Endocrine Society of Australia, Adelaide.*
P. Taft with Y. Patel *et al.*	"Therapy of Thyrotoxicosis with ^{125}I".	*Royal Australasian College of Physicians, Adelaide.*
P. Taft	"Aetiology of Diabetes".	*Diabetes Week, Adelaide.*
P. Taft	"Whither Diabetes?"	*Bruce Hunt Memorial Oration, University of Western Australia.*
D. Winikoff	"Use of Radioisotopes of Iodine in Diagnosis and Treatment of Thyroid Disease. (Series of three lectures)".	*Royal Melbourne Institute of Technology.*
P. Z. Zimmet	"Diabetes Mellitus — In Search of a Cause and a Cure".	*Annual General Meeting, Victorian Diabetes Association.*

Reports of Investigations by Research Fellows of Alfred Hospital in other Departments

Biochemical Techniques

P. Garcia-Webb[1]

Gas Chromatography

Various methods for the analysis of pharmacologically active compounds in body fluids have been investigated. Derivative formation has been selected for some compounds using for example trifluoroacetic-anhydride, while this is not necessary for other compounds.

Analytical methods for the following compounds —alcohol, barbiturates, other hypnotics, phenothiazines and tricyclic antidepressants are under investigation.
It is hoped to introduce these methods for general use in 1974.

Mass Spectrometer

The instrument was originally installed in the Biochemistry Department, Alfred Hospital, but was temporarily relocated in the Baker Institute during the first half of the year. Work has concentrated on investigating the properties of various interfaces connecting the gas chromatograph to the mass spectrometer. With some assistance from the manufacturers various designs have been used using a Watson Bieman frit and the Llewellyn and Littlejohn membrane separator. The use of a double Ryhage has not been excluded. The solid sample probe has been used to enable mass spectrographs of various compounds to be investigated under differing ionising voltages.

1. Biochemistry Department.

Chronic Disability

B. Ford[1]

The aim of this project is to establish the numbers of persons, who upon discharge from the Alfred, are seen to have some permanent incapacity and to evaluate the influence of this incapacity upon the patient's life style, and on the demand it creates for community health and welfare services, such as rehabilitation, domiciliary services or permanent nursing care. A full-time research assistant, Sister Anne Koutte, B.Sc., who is a trained statistician, has been engaged on funds supplied by an Alfred Hospital research fellowship. She collects data on every patient prior to discharge. The data schedule contains items, under the main headings of General Statistics, Diagnosis, Functional Level of Disability, Ability to return to work or usual activity, Source of ongoing care after discharge, Community Services required after discharge, Degree of social support and so on. These data are being correlated by the Hospitals and Charities Commission computer and the effect of the many variables on each other and on the medical and social outcome of the patients' incapacity are presently being studied. Already the partially analysed data are showing some interesting trends in hospital utilisation such as the very high proportion of old people with permanent incapacity being treated; of the influence of age on the utilisation of bed days, the patterns of medical management after discharge and the influence these have on hospital resources, such as outpatient services, the types of incapacity most commonly causing disruption of the patients' life style and employment. The study proceeds for one year and will be completed in January. In the following year a sample of discharged patients will be followed-up in the community and the predicted outcome of their incapacity checked against the actual outcome, and the patients' attitude to the medical services provided will be evaluated. A grant for this follow up study has been provided by the National Health and Medical Research Council.

1. Director of Rehabilitation Services.

Arrhythmias and Myocardial Infarction

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

A study of the incidence and significance of arrhythmias in ambulatory patients in the first year after myocardial infarction is being made utilising Avionics portable tape recorders and a modified Avionics replay system.

Patients who have suffered a myocardial infarction are monitored for 24 hours at the following periods:

(a) in the coronary care unit;

(b) when ambulatory prior to discharge; and

(c) at 3, 6, 9 and 12 months after admission.

The aim of the project is to assess the incidence, types and prognostic significance of arrhythmias in ambulatory patients during the first year after myocardial infarction. It is hoped to identify certain subsets of arrhythmias, particularly

1. Cardiovascular Diagnostic Service.

of ventricular tachyarrhythmias and conduction disturbance, to see if these carry specific prognostic significance. The relationship of in hospital to late arrhythmias, effect of daily activities and the effect of β-adrenergic blocking agents are being specifically documented. Currently 50 patients are continuing in the trial, the number being added to as suitable new patients enter the coronary care unit. Comparison of the initial period of monitoring has shown a considerable difference in the frequency of detection of disturbances of rhythm as compared with conventional coronary care unit monitoring. Analysis of recording taken prior to discharge from hospital have shown a high incidence of rhythm disturbances, 45% having significant ventricular premature beats including salvos, multifocal beats and bigeminy or trigeminy.

Peripheral Arterial Disease

I. A. Ferguson[1]

In spite of an apparently successful operation, a small proportion of arterial graft procedures fail for poorly understood reasons. It would seem probable that grafts most likely to fail would be those with low blood flow, either due to increased resistance in the graft or in the patient's vessels, either proximally or distally. The flow cannot be judged clinically as the graft may be fully distended and have good pulsation in spite of low flow. It is necessary to measure the flow using a flowmeter. A good flow indicates low resistance and would be expected to carry a good prognosis. A low flow indicates a high resistance. The site of the resistance can only be determined by pressure measurements. Measurement of the blood flow at operation has therapeutic as well as prognostic implications. If the measured flow is low and resistance can be demonstrated in the graft, the defect may be remedied at the time.

An E.M. electromagnetic flowmeter has been obtained for measurement of the flow. The first problem was to calibrate the probes and demonstrate that they could be used to measure flow in arteries and graft materials such as saphenous veins. This preliminary work was done with the help of Baker Institute and CRU staff. Calibrations were done by inserting a segment of human vein or artery or animal artery in a circulation model and comparing the instrument reading over a wide range of flows with the flow measured by collecting the blood over a timed interval into a measuring cylinder. There was a linear relationship of flowmeter reading to measured flow. The accuracy of the procedure over a large number of experiments was ± 10%.

The instrument has since been used to measure flow through grafts at operation. In seven bypass operations (six femoral to popliteal, one femoral to tibial), the mean flow has ranged from 50 to 145 ml/min. In one case the flow through the femoral artery before operation was also measured and found to be 40 ml/min and the subsequent flow through the bypass was 145 ml/min.

In one case the flow through the common carotid artery following endarterectomy has been measured with the following results:

Internal carotid flow (external carotid clamped) 300 ml/min.

External carotid flow (internal carotid clamped) 175 ml/min.

External and internal (no clamp) 400 ml/min.

The numbers are too few and the follow-up time inadequate to determine at this stage whether the blood flows measured at operation after reconstructive procedures are related to their ultimate success or otherwise.

1. Vascular Service.

Post-Operative Deep Vein Thrombosis

D. S. Rosengarten[1]

In 1972 it was found that a combination of perioperative low dosage heparin and intraoperative electrical calf stimulation abolished post-operative venous thrombosis in a high risk group of elderly patients with malignant disease.

In 1973 this regimen was tested in the field by studying 250 consecutive patients in the same high risk group. Again no thromboses were detected which confirmed our earlier findings. This regimen being cost effective and of low morbidity is recommended for routine use on all patients undergoing operation to prevent thrombosis and its sequelae of pulmonary embolism and the postphlebitic syndrome.

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

A study of the natural history of thrombosis after splenectomy, using the radioactive fibrinogen uptake test for diagnosis, showed that only 20% (12 out of 58 patients) developed thrombosis.

This incidence is low as compared to an overall incidence of 30% in abdominal operations. However in 25% of the patients with thrombosis (9 out of 12) the thrombosis extended out of the calf at which time the patient was anticoagulated. This extension is much higher than the expected rate of 30%. As extended thrombi have a high risk of pulmonary embolism, these findings are compatible with the long known high incidence of pulmonary embolism after this operation.

A prospective randomised comparative study on the prevention of post-operative thrombosis after total hip replacement was commenced.

Preliminary results show that the incidence of thrombosis is 50% without prophylaxis, that aspirin does not reduce the incidence, that intraoperative calf stimulation either alone or in combination with aspirin, or low dosage heparin alone, reduces the incidence to only 25%. However a combination of low dosage heparin and calf stimulation abolishes thrombosis.

Cardiac Surgery

Eric Cooper and G. R. Stirling[1]

Control of Sinus Rhythm in the Transplanted Heart

Using the technique of acute heterotopic cardiac transplantation as described in last year's report (p. 27) it has been possible to evaluate the effect of an increase in aortic root pressure (A.R.P.) on the rhythm of the isolated heart. This variation has been performed at normal right atrial pressures (R.A.P.) defined as 4 mmHg or less and at an elevated R.A.P. being 6 mmHg or greater. Normal sinus rhythm at between 80-110 beats/min. was found to occur with a mean A.R.P. of 106 mmHg (SD ± 15.6) and a mean R.A.P. of 4.8 mmHg (SD ± 1.7). Bradycardia (rate less than 80 beats/min.) occurred at a mean A.R.P. of 116 (SD ± 23) and a mean R.A.P. of 5.4 (SD ± 6.7). There is no statistically significant difference between these two groups of figures.

If the R.A.P. was kept in the normal range and the A.R.P. elevated, two basic changes in sinus rhythm occurred. The commonest were multiple ventricular extra-systoles followed by multiple atrial extra-systoles, nodal tachycardia, ventricular tachycardia and finally sinus tachycardia. The group of tachycardias occurred with a mean A.R.P. of 180 ± 8.1 mmHg, while the mean R.A.P. was 3.5 ± 0.7 mmHg. The irregular ventricular rhythms had a mean A.R.P. of 160 ± 14.5 mmHg and a mean R.A.P. of 4.4 ± 0.45 mmHg. The difference between the aortic root pressures with normal sinus rhythm and with either tachycardia or irregular ventricular beats is statistically significant ($p < 0.001$ in each group). If the R.A.P. is raised approximately three times, it was found that the same arrhythmias could be produced at a lower A.R.P. For example with an R.A.P. mean of 11.7 ± 5.5 mmHg various tachycardias were produced with a mean A.R.P. of 138 ± 35.7 mmHg (compare R.A.P. 3.5 ± 0.7 mmHg and A.R.P. of 180 ± 8.1 mmHg).

These differences are statistically significant ($p < 0.01$). Also with a mean R.A.P. of 11.5 ± 5.6 mmHg (compared to a mean R.A.P. of 4.4 ± 0.45 mmHg) multiple ventricular arrhythmias could be produced with a mean A.R.P. of 153 ± 29.6 mmHg (compared to a mean A.R.P. of 160 ± 14.5 mmHg). The difference between the venous pressures is highly significant ($p < 0.001$) while the difference between the arterial pressures is less significant ($p < 0.05$). These findings show that rises in the A.R.P. with normal R.A.P. produce a multitude of ventricular arrhythmias ranging from multiple ventricular extra-systoles to sinus and nodal tachycardias. These effects can be produced with a lower mean rise in the A.R.P. if the R.A.P. is significantly raised.

One explanation for these findings is an increase in the pressure in the sinus node artery and also the vessels supplying the atrio-ventricular node. In the former there will be a direct effect on the sinus node tissue due to the anatomical arrangement of the sinus node around this artery. This may be the mechanism for altered rhythms. The added effect of an increase in the pressure in the right atrium causing these arrhythmias with a lower aortic root pressure may also be explained by the tension effect on the sinus and atrio-ventricular nodes. Estimation of serum catecholamines in arterial and coronary sinus blood before and after these mechanical changes did not suggest any other explanation.

These experiments show that two of the mechanisms of control of normal sinus rhythm are the variations in the aortic root and right atrial pressures.

1. Department of Cardio-Thoracic Surgery.

Autologous Tissue in Valve Surgery

This project has continued successfully from 1972. A group of 10 dogs have undergone successful mitral valve surgery. Two dogs have died at two and four weeks post operatively from pulmonary oedema following rupture of the autologous grafted mitral valve tissue. The histology of the re-sutured mitral valve tissue has suggested destruction.

One of the surviving eight dogs has evidence of chronic cardiac failure due to mitral valve incompetence, while the remaining seven dogs are well at 6-10 months. These dogs will be sacrificed within the next two months and their grafted mitral valves studied.

Effects of Trimethoprim on Folate Metabolism

J. Koutts[1]

The mechanism of haematological side effects produced by Trimethoprim (Bactrim or Septrin) was studied. Trimethoprim acts as a bacteriostatic agent by virtue of its competitive inhibition of bacterial dihydrofolate reductase. The interference of folate-dependent DNA-thymine synthesis could be studied in the bone marrow by assessing the incorporation of tritiated thymidine into DNA. In four normoblastic patients studied, the effects of therapeutic levels of trimethoprim on DNA-thymine synthesis was negligible. In contrast to this, patients who were megaloblastic, due to either folic acid or Vitamin B12 deficiency, showed a potentiation of abnormal DNA-thymine synthesis by trimethoprim in therapeutic concentrations. Moreover, in these megaloblastic patients the addition of appropriate haematinics (folic acid or Vitamin B12), in the presence of trimethoprim, failed to correct the metabolic defect. It is probable that trimethoprim interferes significantly with the folate dependent methylation of deoxyuridine to form DNA-thymine, and that this contributes to the adverse haematological effects of this agent. Furthermore, it appears that many of the haematological side effects which have been reported to occur following the use of trimethoprim have resulted from the use of this agent in patients with subnormal folate or Vitamin B12 stores. It is suggested that the use of Bactrim or Septrin is contra-indicated in any situation in which folate or Vitamin B12 stores are diminished without prior supplementation. Trimethoprim is excreted largely unchanged in the urine resulting in higher plasma levels in patients with renal impairment. Since **in vitro** studies show a progressive concentration-dependent impairment of DNA-thymine synthesis by trimethoprim, additional caution is suggested in patients with renal disturbance. Certainly, trimethoprim, for prolonged courses, or in debilitated, elderly or uraemic patients should be supplemented with folate and B12 or with folinic acid. These findings are important because of the widespread use of this excellent antibiotic in clinical practice.

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Publications in 1973

Bremmer, A. S., G. S. Wagner, S. T. Anderson and J. J. Morris	"Transvenous, Transmediastinal and Transthoracic Ventricular Pacing: A Comparison after Complete Two-Year Follow-up". **Circulation.** In Press.
Briggs, M. H. and P. Garcia-Webb	"Problems in the Chemical Analysis of Blood Alcohol". **Search** Vol. 4 (1973) p. 385.
Cooper, E., G. Stafford and G. R. Stirling	"Injury to the Heart and Great Vessels". **Aust. N.Z. J. Med.** (Abstract) Vol. 3 (1973) p. 71.
Cooper, E., P. O. Williams, W. A. Osborne, D. Challis and G. R. Stirling	"Aortic and Mitral Fascia Lata Valve Replacements. A Three-Year Follow-up". **Aust. N.Z. J. Med.** (Abstract) Vol. 3 (1973) p. 71.
Ferguson, I.	"Arterial Injury in Association with Fractures of the Lower Limb". **Aust. N.Z. J. Surg.** In Press.
Pitt, A., P. G. Habersberger and S. T. Anderson	"The Effect of Anti-coagulants on Venous Thrombosis in Acute Myocardial Infarction". Submitted.
Siu, K. and I. Ferguson	"Bilateral Cervical Rib and Subclavian Aneurysm". **Aust. N.Z. J. Surg.** Vol. 42 (1973) p. 245.

Lectures Given During 1973

E. Cooper	"Injury to the Heart and Great Vessels".	*Royal Australasian College of Physicians, Adelaide.*
E. Cooper	"Aortic and Mitral Fascia Lata Valve Replacements. A Three-Year Follow-up".	*Royal Australasian College of Physicians, Adelaide.*
E. Cooper	"Injury to the Heart and Great Vessels".	*3rd Asio-Pacific Congress of Diseases of the Chest, Bangkok.*
E. Cooper	"Aortic and Mitral Fascia Lata Valve Replacements. A Three-Year Follow-up".	*3rd Asio-Pacific Congress of Diseases of the Chest, Bangkok.*
E. Cooper	"The Surgical Treatment of Left Ventricular Aneurysms and Dyskinetic Areas. A Report of 18 Cases".	*3rd Asio-Pacific Congress of Diseases of the Chest, Bangkok.*
I. Ferguson	"Management of Arterial Injuries in Association with 'Fractures of the Lower Limb'."	*Royal Australasian College of Surgeons.*
D. S. Rosengarten	"A Regimen Designed to Abolish Post-operative Venous Thrombosis".	*Royal Australasian College of Surgeons, Singapore.*
D. S. Rosengarten	"The Incidence of Venous Thrombosis after Open Heart Surgery".	*Royal Australasian College of Surgeons, Singapore.*
D. S. Rosengarten	"Thromboembolism".	*Melbourne Medical Post-graduate Committee.*
J. Whitford	"Inadequacy of Conventional Coronary Care Unit Monitoring".	*Cardiac Society of Australia and New Zealand, Adelaide.*
J. Whitford	"Electrocardiographic Monitoring of Ambulant Patients".	*Alfred Hospital Clinical Society.*