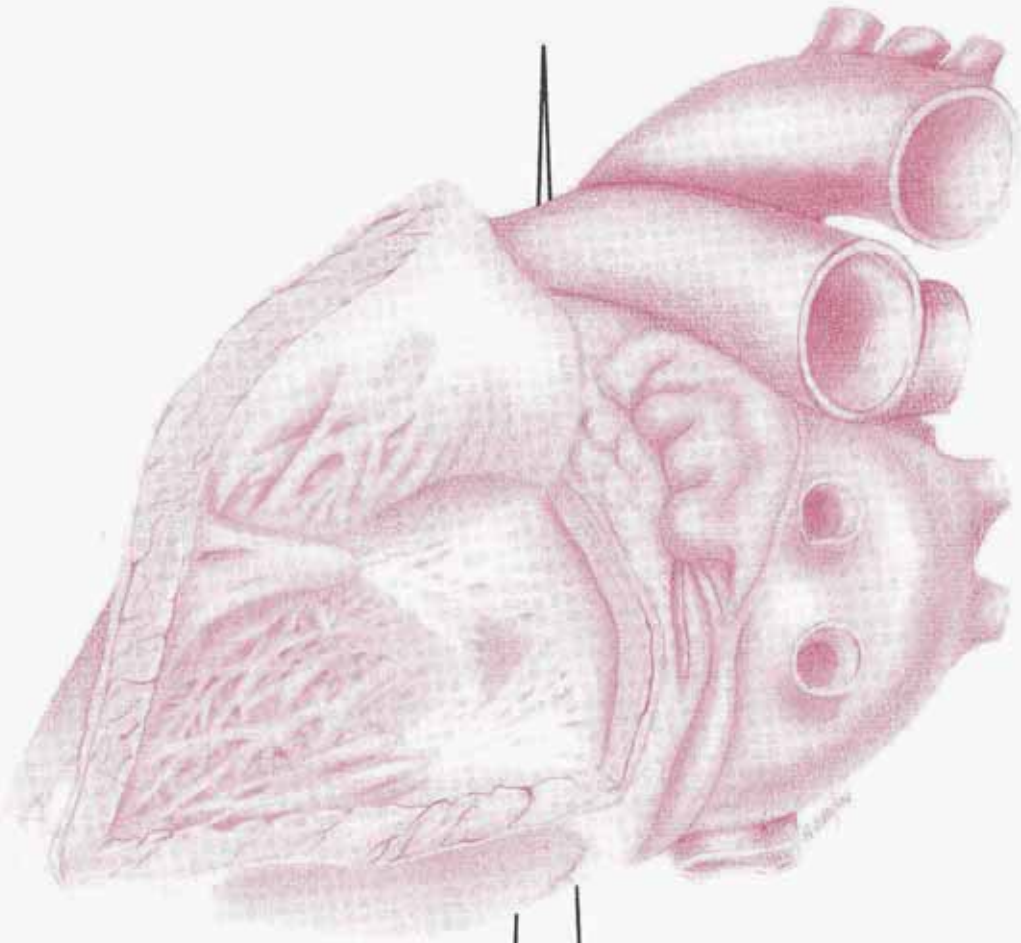

BAKER INSTITUTE

76

ALFRED HOSPITAL

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Fiftieth Annual Report of

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

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Twentieth Annual Report of

THE EWEN DOWNIE METABOLIC UNIT

Report of

C. J. OFFICER BROWN CARDIOTHORACIC SURGICAL UNIT

Foyer of the Institute

HOW TO SUPPORT HEART RESEARCH
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Baker Medical Research Institute

affiliated with
Monash University

ANNUAL REPORT 1976

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PROFESSOR MASAMI IRIKI, M.D., Department of Physiology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan. (S.A. Smith Visiting Research Fellow, Life Insurance Medical Research Fund of Australia and New Zealand).

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Scientific Staff:
B. W. STEWART, M.Sc., Ph.D., Department of Pathology, University of New South Wales (Part-time associate)
J. LEE, B.Med. Sci. (Wisconsin), B.Sc. (Melb), Research Assistant, Anti-Cancer Council of Victoria

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Director's Report for 1976

The Baker Medical Research Institute became 50 years old on 1st April 1976. The fine record of its achievement has been summarized in a definitive history written by my predecessor, Dr. Thomas E. Lowe, which will be published in the first half of 1977. We celebrated the occasion not far from the right date by being host to an International Workshop on Hypertension. Over 50 international visitors and many workers from other Australian institutions participated at this meeting. It provided an opportunity to show our visitors some of the new developments at the Institute and it was clear that the current work was held in high esteem.

Our progress to becoming an exclusively Cardiovascular Research Centre has continued and three major research units have now been established:— (1) *The Circulatory Control and Hypertension Research Unit* (Head: Professor P. I. Korner) which works on how the central nervous system controls the circulation under normal circumstances, and in hypertension; (2) *The Cardiovascular Metabolism and Nutrition Research Unit* (Head: Dr. P. J. Nestel) which is active in the area of basic and clinical research in cholesterol and lipoprotein metabolism, particularly from the viewpoint of coronary artery disease; (3) *The Developmental Biology Research Unit* (Head: Dr. J. E. Maloney) which works on development of the various circulatory and respiratory control systems in the foetus and newborn.

The range of interest covered by these Units is quite unique and apart from the excellence of the individual research effort of each group there is much opportunity for interaction, collaborative effort and serendipity. Details of the nature of the work and advances made during 1976 are summarised in the reports of the individual units.

The *Circulatory Control and Hypertension Research Unit* and the *Cardiovascular Metabolism and Nutrition Research Unit* both use laboratory facilities of the Baker Institute and the clinical facilities of the Clinical Research Unit of the Alfred Hospital. The close proximity between the Institute and Hospital allows for the rapid transfer of ideas developed in the basic laboratories to clinical problems and vice versa. The main work of the two Units is in hypertension and coronary disease. These are the major causes of death and serious incapacity in the Australian community. In financial terms the drug bill alone which is being presented each year to the community for the treatment of hypertension is now well over $80m per annum and we must bear in mind that there are still many people who ought to be treated that are receiving no treatment at all. The economics due to the loss of valuable lives cannot readily be assessed in financial terms but is undoubtedly enormous. In our community at present between one-fifth to one-third of the entire adult population above the age of 40 years is at increased risk from the complications associated with hypertension and coronary artery disease. The susceptibility of young people to these disorders (particularly coronary disease) appears to be increasing. Even if completely satisfactory drug treatment were available now the cost to the community would be much greater than it can be reasonably asked to bear. Hence, there is now more than ever a need to find out the *fundamental causes* of the major circulatory diseases so that *prevention* becomes a preferred method of management rather than the treatment of established disorders. This can best be achieved through giving unstinting support to centres of excellence such as the Baker Institute.

Two distinguished overseas scientists visited the Institute during the year and carried out profitable collaborative programmes with members of the Institute staff. Professor Masami Iriki from Japan stayed here for a period of four months. Professor Michael de Burgh Daly from London visited here for a period of about ten weeks. It is impossible to overemphasise the value of this type of collaboration. Those projects were planned a considerable time ahead and allowed solution of important research problems by a pooling of expertise.

It is a pleasure to record that three of our young scientists won prestigious overseas research fellowships during the year.

Dr. James Angus was awarded the C. J. Martin Fellowship by the National Health and Medical Research Council. Dr. Peter Fletcher was awarded the John Halliday Research Fellowship of the Life Insurance Medical Research Fund and Dr. Peter Blombery was awarded an Overseas Research Fellowship by the National Heart Foundation of Australia and by the National Institutes of Health, U.S. Public Health Service. Details of their awards are given elsewhere in this Report.

Last year I emphasised that the Institute has two important functions. Firstly, and most importantly, it is a centre of excellence for research in several areas of cardiovascular medicine. Secondly, it provides unique opportunities for research training and for postgraduate education. The importance of research training has often been questioned by governments who have asked why cannot such training be obtained overseas? Recently a law was passed by the Congress of the United States of America which will have the effect of making it more difficult for our medical graduates to obtain postgraduate training in clinical research involving patients in the United States. The important message to all of us and to our Government is that a country like Australia whose citizens demand the most advanced patient care just has to be much more self-sufficient in medical research training than we have been in the past. An imaginative national research effort will have the most beneficial and rapid effect on patient care in our hospitals since medical research is still an area of scientific progress where the investigator's ingenuity and ideas are paramount and the instrumentation requirements are relatively moderate.

The Cancer Research Unit remained in the Baker Institute through most of 1976 but left in December to their home in the Department of Pathology at the University of Melbourne. It was good that the Institute was able to help with their accommodation problems in the process of transforming ourselves to a Cardiovascular Research Centre and the assistance of the Anti-Cancer Council of Victoria has been very much appreciated in the support of the research of Dr. Hard's unit.

The year 1976 marked the retirement of Mr. Darren Baillieu from the Board of Trustees of the Institute. Mr. Baillieu served as a Trustee for a period of 25 years. We are enormously in his debt for the wise counsels and unremitting effort on behalf of the Institute, particularly in relation to the crucial decision several year's ago to rebuild the Institute. The decision to rebuild was most important in allowing it to fulfil its present role as a Cardiovascular Research Centre. To show the appreciation of the Trustees and members of the staff a small 'family' party was organised where we entertained Mr. and Mrs. Baillieu and their daughters.

The 1976 also marked the retirement of Professor Rod Andrew as Foundation Dean of Medicine at Monash University. Although Professor Andrew will stop being a Trustee in his capacity as Dean of Medicine he will remain a Trustee in his own right on our Board. It is a pleasure to record the award of the Order of Australia to Professor Andrew and to offer congratulations on behalf of the staff of the Institute. It is a pleasure to record our indebtedness to the National Health and Medical Research Council, the Life Insurance Medical Research Fund of Australia and New Zealand, the National Heart Foundation of Australia and the Alfred Hospital Research Fund as well as all other sources of support which have been acknowledged elsewhere in the Report. We are particularly grateful to the Government of Victoria who made a special grant of $40,000 available to help establish the Cardiovascular Metabolism and Nutrition Research Unit. The new Unit has required the purchase of much new biochemical equipment which will allow it to characterise detailed abnormalities of fat metabolism in patients.

It is perhaps worthwhile to remind ourselves of the cost of the new facilities of the Baker Institute. This can be seen by comparing the annual budget figures for the three years before and the three years after my arrival in Melbourne.

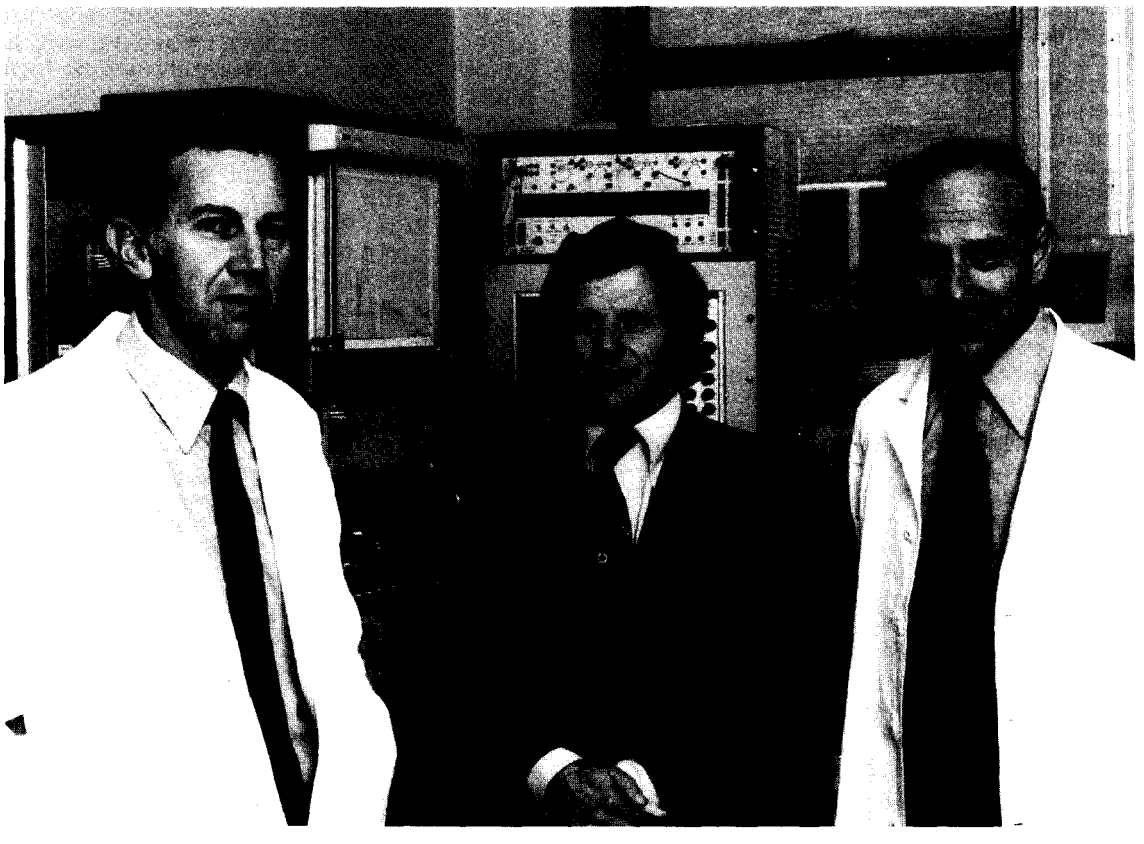
1972	$295,503	
1973	$312,084	
1974	$363,371	
1975	$525,853	
1976	$731,984	
1977	$968,200	(estimate)

The increasing cost is due to the establishment of our two major cardiovascular research units. Both units have been established at a very modest level with only a small senior staff and an

enthusiastic younger staff. The rise in our expenditure has allowed us to use the excellent laboratory facilities of the Institute at an appropriate level for all our three units, though in the two new ones in particular we are still suffering from the current budgetary restraint. At present about half of our total estimated budget depends on renewal of 2-3 yearly research grants and this makes forward planning exceedingly difficult.

We fervently hope for a change in the method of financing of the Baker Medical Research Institute by the National Health and Medical Research Council and also by the Government of Victoria. We badly need more and more reliable finance than is at present the case.

It is important to emphasise how fortunate we are that we have managed to concentrate a very talented research staff at the Baker Institute during the last two years. A research institute such as ours is a delicate plant and does need some long range support by governments. I need hardly say that our expenditure is now levelling off and our major effort will involve the consolidation of the existing units to fulfil their role in research and in postgraduate education. However, we hope that by 1978 it may be possible to establish a small experimental cardiac surgery laboratory the first in Australia. It is useful to remind our various well-wishers that the laboratories of the Baker Medical Research Institute would cost about $2.5m to build today. We are still not using all our facilities and are still renting some laboratory space to the Antarctic Division of the Department of Science to further supplement our income. After two years there is no doubt that the Baker Institute's new role as a Cardiovascular Medicine Centre fills an important need in Australia. We have an obligation to use our facilities wisely and well in the search for new knowledge and in the training of medical research workers.



Left to right: Dr. Paul Nestel, Dr. John Maloney, Professor Paul Korner.

THE FIRST FIFTY YEARS

The Baker Medical Research Institute was founded on 1st April 1926. The inspiration for founding the Institute came largely from Dr. John F. Mackeddie who saw the need for improving the laboratory facilities of the Alfred Hospital. It was important that the Hospital should be able to keep abreast with the exciting new advances that were occurring overseas in relation to the management of diabetes and other metabolic disorders. He was able to persuade Thomas Baker and his wife, Alice and sister-in-law, Eleanor Shaw, to assume financial responsibility for the Institute. They decided that the Institute should not only provide a better laboratory service for the Hospital but should also have facilities for medical research.

Thomas Baker was born in Somerset, England, but had long settled in Australia at the time of establishment of the Baker Medical Research Institute. In partnership with J. J. Rouse he had established a flourishing business for the manufacture of photographic material. They had formed Kodak Australasia Pty. Ltd. by amalgamation with the London Kodak company. The support of the Institute by the Baker and Shaw families continues to this day through the Thomas Baker (Kodak) Alice Baker and Eleanor Shaw Benefaction — a charitable trust which they established in their wills. To-date these trusts have contributed nearly $5m towards the various activities of the Institute. The first Director of the Institute was Dr. W. J. Penfold (1926-1938) and he was succeeded by Dr A. B. Corkill (1938-1948). Both regarded as their main task the establishment of what we now term service departments in biochemistry and other areas of clinical pathology, notably microbiology. Such laboratories were at that time not well established in Australian teaching hospitals. The Institute thus filled the very practical need of providing clinical pathology services for the Hospital and in training staff. It is of interest to note that other research institutes in Melbourne and Sydney started with a very similar function.

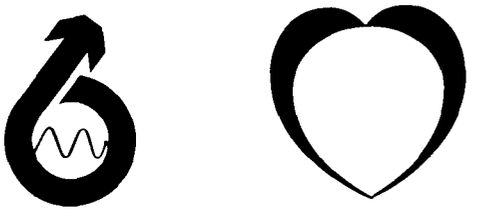
In 1948, Dr. Thomas E. Lowe became Director of both the Baker Medical Research Institute and the newly formed Clinical

The original building and walkway connecting Institute with Clinical Research Unit.

Research Unit of the Alfred Hospital. From this time onwards there was the considerable shift in the nature of the work with a much greater emphasis on research. The various clinical pathology service laboratories became autonomous departments in the Hospital but the Institute continued to help with the establishment of new service requirements and departments. Two examples of what are now flourishing departments of the Hospital:— the Cardiac Diagnostic Service and the Ewen Downie Metabolic Unit started off in the Baker Institute which also provided the facilities for an experimental cardiac surgery Laboratory to allow the introduction of open-heart surgery in the Hospital.

From 1948 to 1974 research in the Institute was in a broad front with the underlying philosophy that aspirant medical research workers should be encouraged in any area where facilities could be provided. Work was done in many research areas including cardiovascular medicine, cardiac surgery, blood coagulation, diabetes and other metabolic disorders, cancer research and gastroentorology. This broad approach meant that each area of research had relatively few workers. Nonetheless the work of the Institute was very well recognised, particularly in the fields of cardiovascular physiology and pharmacology and blood coagulation. Particularly distinguished contributions were made in these areas by Dr. Winifred Naylor and Dr. Paul Fantl. Dr. Lowe retired from the Directorship in 1974 and since then has written a definitive history covering all the major events since the time of the foundation of the Institute to the present. This is currently being published and will be available during 1977.

In 1974 the Trustees of the Institute decided to concentrate on only one major clinically important research area. When Professor P. I. Korner began work at the beginning of 1975 as Director of the Baker Institute and Clinical Research Unit the area chosen was Cardiovascular Medicine. The plan was to utilise the laboratory facilities of the Institute and clinical facilities of the Hospital in a complementary manner. Work at present is in the areas of central nervous control of the circulation, experimental and clinical hypertension, cholesterol and lipoprotein metabolism in relation to atherosclerosis and coronary artery disease and in the field of cardiorespiratory developmental physiology. While each unit is self-contained there is opportunity for much interaction and unexpected developments between units. After two years the prospect for the 'new' Institute appears bright despite the unprecedented economic difficulties. Let us hope that these will be short-lived and that the Institute will flourish as a great Cardiovascular Research Centre.



The original logo of the Baker Institute and the present emblem which underlines that our work is now entirely on research about disorders of the heart and blood vessels.

INTERNATIONAL VISITORS

Professor Masami Iriki was at the Institute from April to July 1976. He was the first S. A. Smith Visiting Fellow of the Life Insurance Medical Research Fund. He is Chairman of the Department of Physiology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan. He is an expert in the field of autonomic neurophysiology and temperature regulation and worked for several years at the Max Planck Institute, Bad Nauheim,

Professor Masami Iriki from Tokyo.

German Federal Republic. While here, he studied the problem of 'resetting' of circulatory baroreflexes. His hobby is work. It was common to see him do experiments on Saturdays and Sundays. All members of the Institute now understand better the concept of Japanese Work Ethic.

Professor Michael de Burgh Daly was at the Institute from July to September 1976. His stay here was made possible by grants from the Royal Society, the Wellcome Society Trust and the Ethel Mary Baillieu Fund. He is Chairman of the Department of Physiology, The Medical College of St. Bartholomew's Hospital, London, England. Michael Daly is one of the world's leading authorities on cardiovascular reflexes. During his stay here we examined the properties of chemoreceptor reflexes in the monkey which has never been done before. For this work he put his hobby (which is mechanical engineering) to very good use.

Professor Michael de Burgh Daly from London.

Because it was not possible to have donor monkeys for priming the pump necessary to perfuse the isolated chemoreceptors he built the smallest bubble oxygenator ever devised. These oxygenators are similar to the oxygenators used for open-heart surgery. However, in contrast to the requirement of a priming volume of 2000-3000 ml of blood in the human oxygenator the bubble oxygenator for use in monkeys required a mere 7-10 ml of blood.

Dr. Jennifer Angell-James has already been mentioned in the 1975 Report. She arrived here in September 1975 and left in September 1976 and spent her sabbatical here as an Edward Wilson Fellow. Her home is in London where she is Senior Lecturer in Physiology in the Department of Physiology in Bartholomew's Medical College. During her visit here she helped establish a cardiovascular neurophysiology laboratory at the Institute and studied the properties of the arterial baroreceptors in chronic hypertension and in relation to treatment by beta blocking drugs. Her hobby is sight-seeing and she saw much of Australia while here.

Professor Walter Kobinger of Vienna stayed here for about a period of two weeks in March 1976. He is Head of the Ernst-Boehringer Institut fur Arzneimittelforschung and one of the leading authorities on centrally acting autonomic drugs.

HYPERTENSION WORKSHOP

The workshop was held in the Baker Institute on 2nd March 1976. The workshop was made possible through the generosity of Ciba-Geigy Limited. It was one of three workshops held after the IVth Meeting of the International Society of Hypertension in Sydney. The main areas discussed and the visiting speakers were as follows:

Integrative Aspects of Circulatory Control and Cardiovascular Reflexes
Chairman: Professor P. I. Korner

Speakers: Professor Bjorn Folkow, Götebörg, Sweden
Professor Peter Sleight, Oxford, U.K.
Dr. Jennifer Angell-James, London, U.K.
Professor E. M. Krieger, Sao Paulo, Brazil
Dr. S. H. Taylor, Leeds, U.K.

Central Transmitters and Cardiovascular Control
Chairman: Professor P. I. Korner

Speakers: Professor J. P. Chalmers, Flinders Medical Centre
Dr. J. L. Reid, Royal Postgraduate Medical School, London, U.K.
Professor D. J. Reis, New York Hospital, Cornell Medical Centre, New York, USA.

Pharmacology of Antihypertensive Drugs Acting on the CNS
Chairman: Professor C. I. Johnston

Speakers: Professor P. van Zwieten, Amsterdam, Netherlands
Professor W. Kobinger, Vienna, Austria
Dr. Wybren de Jong, Utrecht, Netherlands
Professor M. Rand, Melbourne

Renal Hypertension
Chairman: Professor C. I. Johnston

Speakers: Professor M. McGiff, Memphis, Tennessee, U.S.A.
Professor A. C. Guyton, Jackson, Mississippi, U.S.A.
Professor J. D. Swales, Leicester, U.K.
Dr. J. I. S. Robertson, Glasgow, U.K.

The meeting consisted of brief presentations of a controversial topic by one of the speakers and much general discussion which was reinforced by buffett dinners at lunchtime and evening. Several aspects of experimental work were demonstrated to our guests. About as much information was exchanged as is possible to imagine at a meeting lasting one day.

BAKER INSTITUTE WORKSHOP ATTENDANCE LIST

International and Interstate Visitors

K. Abe, Japan
T. Assaykeen, Sydney
P. Baer, Milan
D. Barritt, U.K.
L. Beilin, U.K.
G. Bianchi, Milan
E. Bravo, U.S.A.
J. Brod, West Germany
J. Chalmers, Adelaide
L. B. Coy, Sydney
A. Croxatto, Chile
A. Dalheim, West Germany
C. T. Dollery, U.K.
H. Dustan, U.S.A.
A. Dymond, Sydney
H. A. Emmett, Sydney
C. M. Ferarrio, U.S.A.
A. Fernandez-Cruz, Spain
B. Folkow, Sweden
R. D. Gordon, Brisbane
R. Graham, U.S.A.
R. Gugler, Germany
A. C. Guyton, U.S.A.
A. Gyory, Sydney
C. Hall, Sydney
T. Humphrey, Sydney
S. Huynor, Sydney
W. de Jong, Netherlands
E. M. Krieger, Austria
R. Kolloch, Germany
S. Langer, Argentina
H. G. Langfod, U.S.A.
W. Littler, U.K.
B. Ljung, Sweden
J. McGiff, U.S.A.
G. A. MacGregor, U.K.
J. Maitland, Sydney
H. S. Margolius, U.S.A.
A. Marshall, U.K.
S. Mistilis, Sydney
J. Moehring, West Germany
P. J. Mulrow, U.S.A.
C. Murphy, U.S.A.
G. Nyberg, Sydney
H. Oates, Sydney
W. Oelkers, West Germany
G. Onesti, U.S.A.
W. Pettinger, U.S.A.
K. Poulson, Denmark
B. Prichard, U.K.
D. Pugsley, U.K.
J. Rapp, U.S.A.
I. Reid, U.S.A.
J. L. Reid, U.K.
D. J. Reis, U.S.A.
W. Reiss, Switzerland
J. Robertson, U.K.
M. P. Sambhi, U.S.A.
P. S. Sever, U.K.
P. Sleight, U.K.
J. Steele, New York
G. Stokes, Sydney
J. Swales, U.K.
S. Taylor, U.K.
G. Thomas, U.K.
E. Torok, Hungary
H. Wasir, India
P. Weber, Sydney
M. West, Adelaide
R. Zacest, Adelaide
P. van Zwieten, Netherlands

SCHOLARSHIP WINNERS

Jim Angus graduated with First Class Honours in Pharmacology and obtained his Ph.D. in this field in the University of Sydney in 1973. He came to the Hallstrom Institute, Sydney, as a Postdoctoral Fellow in the middle of the 1973 and 'emigrated' to Melbourne with the rest of the Circulatory Control and Hypertension Research Unit at the beginning of 1975. In his work here he made outstanding contributions to the analysis of the vascular histamine receptors and on several aspects of experimental hypertension. It is appropriate that he should spend his time

Dr. Jim Angus

overseas as a C. J. Martin Fellow with Professor James Black of the Department of Pharmacology, University College — the home of histamine and many other receptors. He relaxes occasionally in all kinds of outdoor activities but there is no doubt that fishing is his No. 1 favourite.

Peter Fletcher graduated B.Sc. Med. (1967) and M.B.B.S. (1970) all with First Class Honours in the University of Sydney. He then spent several years at the Royal Prince Alfred Hospital, Sydney, as a Resident and Medical and Cardiological Registrar. He obtained his M.R.A.C.P. and recently his F.R.A.C.P. and stayed at the Hallstrom Institute as an N.H. & M.R.C. Medical Research Student. He moved south to the Baker Institute and his work here has been in the field of experimental renal hypertension, particularly on the role of salt, and he is completing work for his Ph.D. at Monash University. He was awarded the John Halliday Overseas Fellowship of the Life Insurance Medical Research Fund. Whilst overseas he will work in Professor Eugene Braunwald's Department of

Dr. Peter Fletcher

Medicine at the Harvard Medical School. Melbourne has done a lot for Peter:—he became a model father; became a dog trainer for the Seeing Eye Dogs' Society (one puppy) and showed great aptitude as a villain in the Baker Amateur Dramatic Society's melodrama in 1976.

Peter Blombery graduated as B.Sc. (Med) (1968) and M.B.B.S. (1971) both with First Class Honours from the University of Sydney. He was a Resident and Medical Registrar at Royal Prince Alfred Hospital, Sydney, and started also at the Hallstrom Institute, Sydney, as a Life Insurance Postgraduate Research Scholar in 1974 and then moved south in the general exodus. He is completing his Ph.D. at Monash and his main work has been on how beta-blocking drugs act to lower blood pressure. He was awarded a National Heart Foundation Overseas Fellowship for 1977 and a U.S. Public Health Fellowship for 1978 to spend two years in Dr. Irwin Kopin's laboratory at the National Institutes of Health to work on central neurotransmitters. He has followed the fashion by recently becoming a father, but this seems to have stimulated his creative talent as a musician and a composer.

Dr. Peter Blombery

Circulatory Control and Hypertension Research Unit

MAIN TOPICS

- CONTROL OF THE HEART AND BLOOD VESSELS BY THE CENTRAL NERVOUS SYSTEM
- KIDNEY, SALT AND HYPERTENSION
- DRUGS AND HIGH BLOOD PRESSURE

GENERAL SUMMARY

Abnormally high blood pressure effects about one person in six in the Australian community. With increasing treatment of milder types of hypertension the number where some benefit from treatment might result may even approach to one person in four. However, the cause of high blood pressure is still unknown. Treatment of high blood pressure by drugs is now available but it is important to point out that whilst drug treatment has made a great difference in improving the outlook for patients with hypertension, it is still far from perfect. The mortality in treated hypertensives from stroke, heart failure and coronary artery disease is still considerably higher than in people with normal blood pressure. In Australia high blood pressure heads the list of causes of death or serious economic disability in our community. Research into the fundamental causes of high blood pressure is therefore a matter of the utmost importance. Even if perfect treatment were available now the number of people in our community who might need it would be so great that the cost of such treatment might saddle us with an enormous economic burden. Only by understanding fundamental causes will it ultimately be possible to prevent the disease or to treat it in a less life-long way than is at present necessary.

High blood pressure is a disturbance of one or more of the many control systems available to the body for maintaining the constancy of the cellular environments of the various tissues. The work of the Circulatory Control and Hypertension Research Unit is chiefly concerned with the analysis of the normal function of the most important control systems regulating blood pressure and the study of how abnormalities of such systems might be relevant to the initiation and maintenance of high blood pressure. In this research, members of the Institute work in close collaboration with Professor Colin Johnston's group, Department of Medicine, Prince Henry's Hospital, Monash University. This has been most profitable in that it brings together our expertise in circulatory and autonomic physiology and the expertise of the Prince Henry's group in endocrinology.

Central Nervous Control

One of the most important of the body's control systems involved in the regulation of blood pressure is the central nervous system. This influences blood pressure through the autonomic nerves by increasing the rate and force of the heart beat and the diameter of the small arteries and veins. Blood pressure, it should be remembered, is the amount of blood pumped by the heart x resistance of the blood vessels. In general, resistance is greater the narrower the small arteries of the body. Our Unit has done much research in working out the organisation of the brain pathways which control the heart and blood vessels. In our research we also study the autonomic responses to various environmental stimuli and the site of action in the central nervous system and elsewhere of antihypertensive drugs.

Normally we are not aware of the level of our blood pressure and it was thought, until quite recently, that only the integrative centres of the lower brain stem and spinal cord were involved in its control. The role of higher centers was considered to be small and mainly related to special functions such as exercise or temperature control. We now know from our studies that the centers in the higher regions of the brain, including the cortex and hypothalamus participate in every kind of autonomic response involving the circulation. These studies have established that there is a complex network of brain pathways controlling the circulation with many inputs and outputs. This network controls the activity of the vagus and cardiac sympathetic nerves to the heart, the sympathetic constrictor nerves to the blood vessels and the innervation of the adrenal medulla. The central organisation is illustrated schematically in Fig. 1. The inputs to these pathways convey an enormous amount of information of different kinds. Those entering the brainstem and spinal cord provide information about changes circulatory and respiratory functions such as blood pressure, composition of arterial blood and lung movement. These are

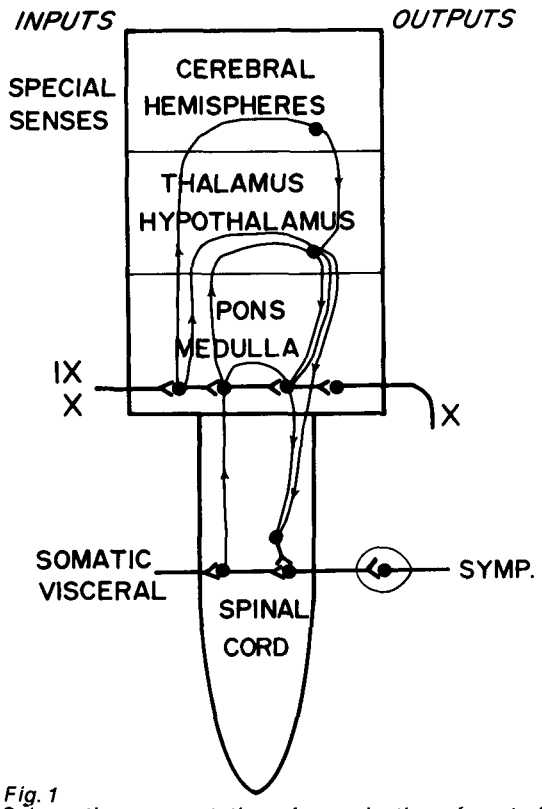


Fig. 1
Schematic representation of organisation of central nervous system autonomic pathways. Inputs carry information from peripheral receptors. Note elaborate loops connecting higher and lower parts of the brain.

signalled through various sensory nerves from a range of strategically placed sense organs or receptors. In addition, inputs to the cortex provided by visual and auditory cues outside the body can also alter autonomic function.

Up till recently it was considered that the input signalling changes in blood pressure always produced a very stereotyped group of reflex responses. These findings were based on the older approach for investigating circulatory reflexes. With these methods stimulation of one particular receptor group was carried out under very carefully controlled conditions whilst the activity from other inputs was maintained as constant as possible. Our research group has shown that this is a very artificial way of looking at circulatory control of the brain. In disturbances which occur in real life there are *simultaneous* changes coming in from very many sense organs. The pathways go to distinctive brain centres and we have found that the properties of the blood pressure control system can be greatly altered by the level of activity in some of the other input channels. We have studied particularly the interactions between the blood pressure receptors or baroreceptors and those from the chemoreceptors which signal changes in arterial blood composition. The central nervous interaction produce resetting of the properties, changing of threshold, sensitivity and magnitude of autonomic responses. During the year we analysed with Professor Masami Iriki the effects of these interactions on the sympathetic nerves going to different organs. In the kidney the sympathetic nerve sensitivity was greatly increased during oxygen lack whilst in the heart it was diminished. This is of advantage to the organism because it reduces the work of the heart and helps to redistribute blood flow to vital regions of the brain and to the coronary circulation. The autonomic nervous system is not just a system for uniform mass action designed to cope with emergencies (though this is one of its roles) but it is also capable of very subtle modes of control with a variety of circulatory blood flow distribution patterns evoked by different environmental and behavioral stimuli.

The properties of the blood pressure control system can be altered considerably during the release of the transmitter noradrenaline in the hypothalamus. This results in a transient marked elevation of the blood pressure lasting several hours. The centrally released noradrenaline has the same composition as the noradrenaline released by the adrenal gland. However, in the brain it is a transmitter which passes messages from one particular group of nerve cells to other cell groups in both higher and lower parts of the brain concerned with blood pressure control. These 'noradrenergic' nerve cells can be experimentally destroyed by injecting a chemical 6-hydroxydopamine into the spinal fluid bathing the animal's brain. This not only alters their blood pressure but also makes them temporarily irritable producing rage responses rather like those of an angry man. In the long run it affects the animal's food habits and appetite and the studies indicate the close association between the many centres controlling the heart and blood vessels in the brain and those controlling behaviour. It seems probable that some emotional stresses or behaviour patterns modify the properties of the blood pressure control system. Whether they play a role in the initiation of high blood pressure is still not known.

Work on the circulatory responses of the monkey by Professor Michael Daly is par-

ticularly important since the monkey's nervous system closely resembles that of man. The responses differ in several respects from those of other species to the same stimuli. When the chemoreceptors of monkey were stimulated under carefully controlled conditions using the classic approach, slowing of the heart and constriction of the small arteries was produced as had previously been found in the rabbit and dog. However, in the monkey the reflex slowing of the heart rate and the constriction of the peripheral vessels is more easily suppressed by increased respiratory activity than in the lower subprimate species. It should be emphasised that all species have built-in safety mechanisms to minimise inappropriately large autonomic responses. These mechanisms appear to be more highly developed in the primate brain than in the lower species. One speculation is that a possible mechanism in hypertension could be impairment in certain individuals of such inhibitory systems.

Kidney and Hypertension

The reason for carefully looking at the role of the kidney in the control of body fluid volume is that there is reasonable evidence suggesting that there is *some* abnormality of renal function in virtually all types of hypertension. In patients with narrowing of the renal artery many develop hypertension. In some of these there is an increased blood level of angiotensin II (A-II), a hormone produced by the kidney that produces constriction of small arteries and also plays a role in the body's salt balance. In other patients A-II may be within normal limits. There has thus been some doubt regarding its role in causing high blood pressure in chronic renovascular hypertension.

Dr. Warwick Anderson and other members of our research team studied a model of experimental renovascular hypertension in dogs which have an inflatable cuff around their renal artery and flowmeters and pressure lines. This way of producing experimental renal hypertension is an old method which was first developed by Goldblatt more than 40 years ago. However, whilst it is an effective way in producing hypertension the mechanism of the hypertension and its relation to A-II is still far from clear. Our recent work has focused on some of the important mechanisms involved in this type of hypertension. When the cuff is inflated to produce quite severe renal artery constriction there is a transient increase in AII production. However, the small blood vessels within the kidney make very rapid adjustment to the disturbance initiated by experimental constriction of the renal artery and the renal circulation becomes rapidly restored almost to normal. Production of AII is turned off and hypertension does not develop. With repeated cuff constriction the capacity for intrarenal compensation diminishes greatly. If the repeated constriction is regularly spaced (say, to twice daily constriction), the AII rises progressively and this is associated with progressive elevation of blood pressure. In this experimental model rise in AII accounts entirely for the hypertension and the blood pressure can be restored to normal by drugs which specifically block AII. In the classic Goldblatt hypertension it is also necessary to repeatedly constrict the renal artery but the spacing is done over an interval of about one hour. Elevation of AII continues for 1-2 days and the blood concentration of this hormone is rapidly restored. These experiments in different models have suggested that the role of AII in the production of renovascular hypertension is permissive. The important underlying mechanism appears to be a reduction in the renal blood pressure with either reduction or redistribution of the intrarenal blood flow and possibly an action of the renin-angiotensin system on the blood vessels of the kidney itself.

Role of Salt

In another group of experimental studies in rabbits Dr. Peter Fletcher and other members of the group have studied the question of the role of dietary salt on the development of experimental renal hypertension. This has always been a matter of great interest. Population surveys in different countries have suggested that there is an association between the incidence of hypertension and the amount of salt in the diet. Moreover, salt balance appears to be an important factor in many types of hypertension due to kidney disease in man. In the rabbit study the animals were maintained for long periods on three different types of diet — very low, normal and high — encompassing a 25-fold range of dietary salt intake. Hypertension was then induced by wrapping both kidneys in cellophane.

Surprisingly the animals developed the same degree of hypertension compared with the control group maintained on iden-

tical diets no matter what their salt intake and they also had similar responses of AII. In all animals the elevation of blood pressure was due to narrowing of the small arteries rather than due to increased amounts of blood pumped by the heart each minute. In all groups, blocking the hormone AII by means of a specific antagonist reduced the blood pressure more in hypertensive than in normotensive animals, probably due to non-specific changes in their circulation. The reason why a given salt intake does not seem to matter in relation to this kind of hypertension appears to be largely due to the compensatory role of the adrenal glands and the autonomic nervous system in the 'low' and 'high' salt groups. When these control systems were removed, but the animals given adrenal hormones adequate for a 'normal' salt intake in the diet, they became salt-sensitive with blood pressure reduced by the low salt diet and increased with high salt. Thus, salt alone does not appear to be a major factor in the production of high blood pressure as long as the function of the adrenals and autonomic nervous system is normal.

Action of Drugs

During the year Dr. Peter Blombery, Dr. Alex Bobik, Dr. Patricia Dorward and other members of the group examined the mechanism of the action of two important drugs used in the treatment of hypertension:— the beta-blocking drug, propranolol, and the new antihypertensive drug, prazosin. Beta-blocking drugs are amongst the most commonly employed drugs in the treatment of hypertension because of their freedom from side effects. Despite this, the way in which these drugs lower blood pressure has not been clearly defined. There have been many theories of possible action. Some have believed that the drugs block the sympathetic nerve endings to the heart, whilst others believe that they act on the central nervous system or alter the sensitivity of the arterial baroreceptors which send messages to the brain. The work has shown clearly in animal studies (which we have also confirmed in man) that the drug has no effect on the central nervous autonomic pathways and that it has minimal, if any, effect on the arterial baroreceptors. Its main action is to block the sympathetic nerves to the heart and lower cardiac output which leads to a gradual reduction in blood pressure once high enough blood concentrations of drug are reached. The new drug prazosin has been said to lower blood pressure by dilating the small arterioles but our work has shown that its main action is to block the sympathetic constrictor nerve endings on the small arteries and veins. Whilst the central nervous system is not necessarily the fundamental cause of high blood pressure it is clear that manipulation of this important blood pressure control mechanism by means of drugs is very effective in the management by present day methods of patients with hypertension. Analysis in conscious animal preparations allows very much more detailed analysis of drug action than is possible in man. This greatly facilitates the introduction of new drugs and more rational use of older drugs.

PROJECTS

1. Baroreceptor 'Resetting' by Arterial Hypoxia in the Renal and Cardiac Nerves.

M. Iriki, P. K. Dorward and P. I. Korner

Renal and cardiac sympathetic baroreflex functions were studied in sodium pentobarbitone anaesthetized rabbits given succinylcholine, during constant artificial ventilation with air and with hypoxic gas mixtures. Mean arterial pressure (MAP) was raised and lowered between values of 40 and 140 mmHg by means of aortic and vena caval perivascular balloons and integrated sympathetic nerve activity (SNA) was recorded. The relationship between MAP and SNA was sigmoid, with upper and lower plateau levels. The curves were defined by calculating median blood pressure, SNA Range and reflex gain. In both renal and cardiac sympathetics section of the carotid sinus and aortic nerves completely abolished the MAP-related changes in SNA. The renal baroreflex curves were reset from control levels during hypoxia. Median blood pressure increased, as did SNA Range and gain. These effects were due to central interactions between arterial baroreceptor, arterial chemoreceptor and vagal afferent activity. The cardiac sympathetic baroreflex curves were shifted in the opposite direction from control with reduction in median blood pressure, SNA Range and the reflex gain. These changes were due to chemoreceptor-arterial baroreceptor interactions. Arterial hypoxia thus evokes a differentiated pattern of baroreflex resetting in the renal and cardiac sympathetic motoneuron pools with different changes in neural response

range and sensitivity to arterial pressure changes.

2. Cardiovascular and Respiratory Effects of Carotid Body Stimulation in the Monkey and Interactions with other Reflexes.
M. de Burgh Daly, P. I. Korner, J. E. Angell-James and J. R. Oliver.

The carotid bodies were stimulated in the pig-tailed anaesthetised macaque monkey (M. nemestrina) using (i) brief injections of cyanide or CO_2-equilibrated bicarbonate solution; (ii) longer perfusion with hypoxic-hypercapnic blood. In spontaneously breathing animals brief stimuli (31 tests, 7 monkeys) increased ventiliation by 97 ± 10% of control, increased pulse interval (PI) by 36 ± 7.5% and increased femoral vascular resistance (FVR) by 44 ± 7%. When the chemoreceptor stimulus was superimposed during apnoea evoked by stimulating either the superior laryngeal nerves (SLN) or nasopharynx respiratory stimulation was minimal but bradycardia and vasoconstriction were enhanced and exceeded the summed responses during individual stimulation of either the chemoreceptors or one of the airways inputs. In spontaneously breathing animals longer chemoreceptor stimulation with asphyxial blood (19 tests, 5 monkeys) increased ventilation by 186 ± 23%, but transient bradycardia occurred in only 8/19 tests. After 20-40s PI was 5.8 ± 0.9% below control and there was either no change or a fall in FVR. When SLN stimulation was superimposed on this response tachycardia reversed to bradycardia and FVR increased above resting. In the monkey the autonomic effects of chemoreceptor stimulation are influenced by the level of respiratory activity: bradycardia and vasoconstriction occur when the level of respiration is low, and tachycardia and vasodilatation when it is high. The organisation of the CNS chemoreceptor autonomic pathways appear to be qualitatively similar in different mammalian species.

3. Role of the Carotid and Aortic Baroreceptors on the Baroreceptor-Heart Rate Reflex
P. A. Blombery and P. I. Korner

The baroreceptor-heart rate reflex was studied in 23 unanaesthetized rabbits by raising and lowering arterial blood pressure above and below resting by means of perivascular balloons. Sigmoid mean arterial pressure (MAP) — heart period (HP) curves were constructed and median blood pressure, threshold, average gain (sensitivity) and heart period range were calculated. Groups of animals were then subjected to one of the following operations: (i) sham-operation; (ii) bilateral section of the carotid sinus nerves; (iii) bilateral section of the aortic nerves; (iv) bilateral combined section of the carotid + aortic nerves. The animals were studied 5 days later. In the sham operated group there was no significant change in blood pressure, whilst all the others developed *neurogenic hypertension.* After section of the carotid sinus alone the rise in mean arterial pressure was nearly double that observed after aortic nerve section alone (i.e. 17.3 ± 1.5mmHg after carotid nerve section compared with 9.4 ± 0.9 mmHg after aortic nerve section). After combined section of both buffer nerves the rise in blood pressure was 19.1 ± 9 mmHg. These results suggest that the afferent activity mediated through the carotid sinus nerve has a greater disinhibitory effect on the central nervous blood pressure control system. The baroreceptor-heart rate reflex was also affected more markedly after carotid sinus nerve section. Thus after sham-operation gain of the reflex was 12.5 ± 1.4 ms/mmHg, after carotid sinus nerve section it was 2 ± 0.3 ms/mmHg, while after aortic nerve section it was 5.2 ± 0.5 ms/mmHg. The HP changes were completely abolished after cutting both sets of nerves. Similarly the heart period range was 187 ± 15 after sham operation, 67 ± 13 after cutting the carotid nerves, 104 ± 10 after cutting the aortic nerves. These findings indicate that in unanaesthetized rabbits the carotid sinus nerve has greater effects on vagal + sympathetic heart rate motoneurons. In each animal the properties of the sympathetic motoneurons were studied by giving methylscopolamine and the results showed that the carotid sinus nerve exerted more marked effects on this group of motoneurons also.

4. Role of Carotid and Aortic Baroreceptors on the Valsalva Constrictor Reflex
P. A. Blombery and P. I. Korner

Reflex vasoconstrictor responses were evoked by a Valsalva-like manoeuvre in unanaesthetized rabbits with previously implanted Doppler flowmeters. Valsalva pressures (VP) from 2.5-20 mmHg were applied for periods of 30s to a respiratory valve and cuff around thorax and abdomen. The changes from resting in mean arterial pressure (MAP), right atrial

pressure (RAP), cardiac output (CO) and total peripheral resistance (TPR) were determined during the last 5-7s of the manoeuvre. Animals studied included (i) sham-operated rabbits; (ii) rabbits with bilateral section of the carotid sinus nerves; (iii) rabbits with bilateral section of aortic nerves; (iv) rabbits with bilateral combined section of both sets of nerves. CO fell by 20.3 ± 0.9%/10 mmHg VP in sham-operated animals and by similar amounts after section of carotid sinus nerves alone (21.7 ± 0.6%/10 mmHg VP) and after section of the aortic nerves alone (20.3 ± 0.6%/10 mmHg).

Reduction in CO was significantly greater after combined section of both sets of nerves. In sham-operated rabbits and in those with section of carotid sinus nerves alone blood pressure had become completely restored by the end of the manoeuvre. After section of the aortic nerves alone it was 88 ± 2.3% at that time, and restoration was much reduced after combined section of both sets of nerves (44 ± 4%). The rise in TPR at VP 20 mmHg was to 134 ± 2.2% of control in sham-operated animals. It was similar in animals with carotid section alone 149 ± 7.1%) but the effect was significantly less pronounced in animals with section of the aortic nerves alone (115 ± 2.9%). After combined section of both nerves TPR was reduced below resting to 73 ± 6.7%, closely similar to the findings after 'total' autonomic block. The results indicate that the aortic arch baroreceptors play a major role in the Valsalva-constrictor reflex, contrasting with their relative unimportance in the baroreceptor-heart rate reflex.

5. Brain Amines and Circulatory Reflexes

P. I. Korner and J. R. Oliver

Fluorescence histochemical studies by Dahlstrom and Fuxe and others have revealed the presence of neurons in the brain which contain noradrenaline, adrenaline and serotonin in substantial amounts. The cell bodies of these neurons are mostly in the pons and medulla. From some axons ascend to cerebellum, parts of the upper brainstem, hypothalamus, basal ganglia and cortex, while descending pathways from other cells go to the sympathetic motoneurons of the spinal cord. There has been surprisingly little study of the role of those neurons in the various circulatory reflexes. Hence the object of this study was to examine the effects of giving 6-hydroxydopamine (6-OHDA) which destroys mainly noradrenergic neurons on (i) the baroreceptor-heart rate reflex; (ii) the 'smoke' nasopharyngeal reflex; (iii) arterial hypoxic-chemoreceptor relfex, as well as on resting haemodynamics. After injecting 600 ug/kg 6-OHDA the animals develop a on resting haemodynamics. After injecting 600 ug/kg 6-OHDA the animals develop a marked hypertension due mainly to peripheral vasconstriction (Fig. 2). The rise in pressure of about 35-40 mmHg lasts for 2-4 hours and is frequently associated with supraventricular tachyarrhythmias. There is little impairment of sensitivity and range of the baroreceptor-heart rate reflex at this time. The major study was done one week after intracisternal injection when most of the noradrenergic neurons have been destroyed. There were quite minor changes in all reflex function suggesting compensatory effects by other neuron systems. Initially a pronounced effect on the Valsalva reflex was noted but this was mainly due to unwillingness of animals to eat and drink — a constant behavioral accompanient after administration of 6-OHDA, and requiring daily feeding through an indwelling gastric tube. Preliminary studies in pontine animals suggest that the ascending and descending noradrenergic neurons have antagonistic effects.

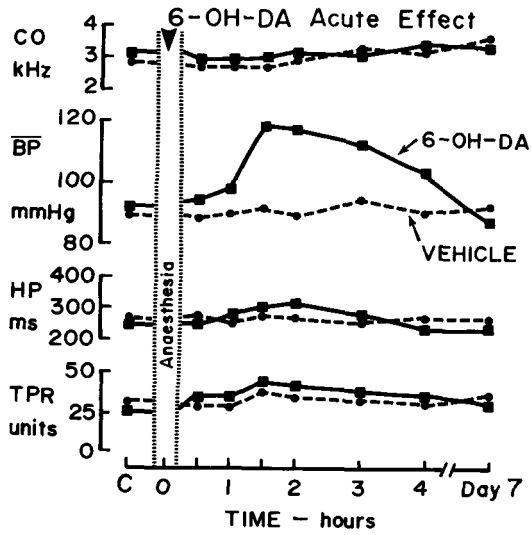


Fig 2
Effects of 6-hydroxydopamine (6-OH DA) or vehicle (control) on cardiac output (CO), blood pressure (BP), heart period (HP) and total peripheral resistance (TPR). Note the large rise in BP after injecting 6-OH DA.

6. Mechanisms of Hypertension in Renal Artery constriction

W. P. Anderson, J. A. Angus, P. I. Korner, C. I. Johnston* and D. J. Casley*

The experiments have been performed in unanaesthetized dogs trained to lie quietly in the laboratory. At a preliminary operation performed several weeks before the study a rubber balloon cuff is placed around the left renal artery for subsequently constricting the vessl. A Doppler ultrasonic flowmeter is placed around the left renal artery for measuring renal blood flow and catheters are implanted for measuring distal renal blood pressure and systemic blood pressure. The right kidney is removed and cardiac output is measured by means of an electromagnetic flowmeter.

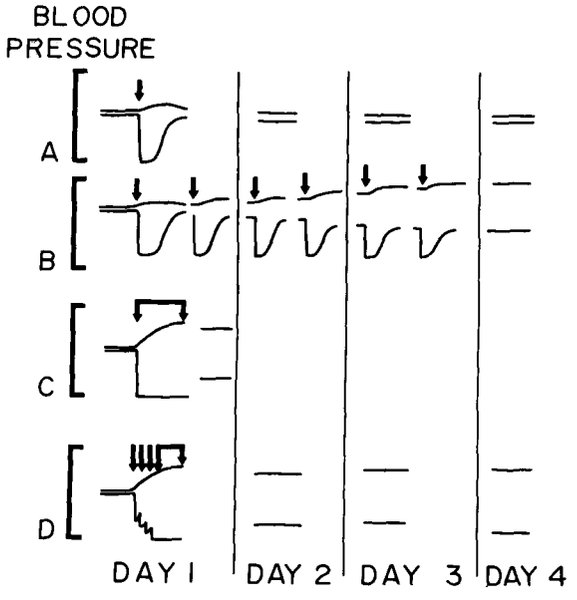


Fig 3
Four models (A-D) of inducing renovascular hypertension, showing effect of renal artery constriction on systemic blood pressure (top variable) and distal renal artery pressure (lower variable) in each model. Description in text.

Four different models of 'one-kidney' hypertension have been studied (Fig. 3).

Model (A). In this model renal blood pressure (RBP) was rapidly lowered from the normal day's systemic blood pressure (SBP) of about 90 mmHg to 60, 40 and 20 mmHg, the latter being well below the autoregulatory range. Pressures were maintained at this level for 30s and the cuff tubing was then clamped. It was found that the local renal circulation had a remarkable capacity for adjusting to these large reductions in perfusion pressure and renal haemodynamics, plasma renin activity (PRA) and angiotensin II concentration (AII) became rapidly restored even with the most severe degree of constriction. Elevation of blood pressure did not develop over the next three days.

Model (B). This model was designed to examine the limit of compensation by the kidney for the initial reduction in pressure. The renal artery was repetitively constricted. The basic procedure was as in model (A) with similar graded reduction of RBP for 30s. However, the RBP was lowered to the specified values of 60, 40 and 20 mmHg twice daily, thus providing a repetitive stimulation the renin-angiotensin system. The haemodynamic effects were measured each day 18 hours after the last cuff adjustment. Over the next three days there was now a graded progressive rise in systemic arterial pressure which averaged 19, 34 and 52 mmHg in each of the groups. Rise in BP was highly correlated with the rises in PRA AII and proof that the hypertension was indeed 'renin-dependent' came from the demonstration that it was almost completely reversed within 10-12 min by infusing the competitive AII antagonist 1-sar-8-ile-AII. In these animals RBP was below control levels after the first day's adjustments with a progressively increasing SBP-RBP gradient in each of the groups. Cardiac output did not alter and the rise in systemic blood pressure was through elevation of TPR. Deflating the cuff restored blood pressure within 24 hours.

Model (C). In this model RBP was maintained at either 20 or 60 mmHg over a period of 1 hour and SBP reached a steady plateau level which was almost equivalent to that corresponding to model (B) at the end of one hour. Model (B) provides a repetitive open loop stimulus to the renin-angiotensin system by bringing renal blood pressure eventually below initial control twice daily and measures the intervening restoration of the renal circulation between cuff adjustment. On the other hand, model (C) examines continuing open-loop stimulation of the kidney where RBP is maintained at a specified level independently of any homeostatic adjustments. The hypertension was reversed completely within 24 hours after deflating the cuff.

*Department of Medicine, Monash University.

Model (D). This model was designed to mimic the classic Goldblatt hypertension. The cuff was applied repetitively over a period of 1 hour at the end of which RBP was reduced to 30 and 20 mmHg. The cuff was then clamped and the renal and systemic circulatory changes observed. The conditions produced more marked reduction in renal blood flow than any of the other models. Hypertension developed over the next 5 days as in the classic Goldblatt hypertension. PRA and A-II were elevated at the beginning of the study but returned close to control within 2-4 days when RBP was only slightly below initial control. Letting the cuff off restored blood pressure within 24 hours.

These studies indicate (i) that the renal circulation has an enormous capacity for restoring its haemodynamics after reduction in renal perfusion; (ii) unless steps are taken to keep lowering RBP the hypertension becomes rapidly renin-independent without influencing the overall level of blood pressure; (iii) when renal artery pressure is maintained below control the duration of the renin-dependent phase is prolonged so that this model provides the means of analysing some of the factors that turn A-II off in relation to the level of blood pressure. A given degree of renal artery constriction produces similar levels of systemic 'one kidney' hypertension that can be either renin-dependent or renin-independent, depending on the exact method for constricting the renal artery. Plasma A-II concentration is elevated only as long as RBP remains below initial control; whether the hypertension is renin-dependent or renin-independent it can always be reversed by releasing the constricting cuff. Thus, except in special circumstances where the cuff is applied so as to keep renal blood pressure below control, peripheral PRA or A-II activity does not contribute to the maintenance of renovascular hypertension. However, in the renin-independent phase these hormones could still act either as a trigger factor stimulating the autonomic nervous system or the antidiuretic hormone system of the brain, or could act locally in the kidney.

7. Dietary salt and Renal Hypertension

P. J. Fletcher, P. I. Korner, C. I. Johnston* and D. J. Casley*

*Department of Medicine, Monash University.

Rabbits were maintained on different sodium diets (low, normal and high, covering a 25-fold range of daily intake). The animals remained on each diet for several weeks till they had become thoroughly familiar with it and were thriving and gaining weight. Both kidneys were then wrapped in cellophane in one group of rabbits, whilst other animals on the same three diets were subjected to sham-operation, and the effects on blood pressure and cardiac output (thermodilution) were studied. In addition, the effects on plasma renin activity and urine catecholamine excretion were also observed. The last is an index of autonomic nervous system activity, whilst plasma renin activity reflects the hormonal (AII) responses of the kidney which are activated by reduction in body salt and/or by lowering the renal blood pressure.

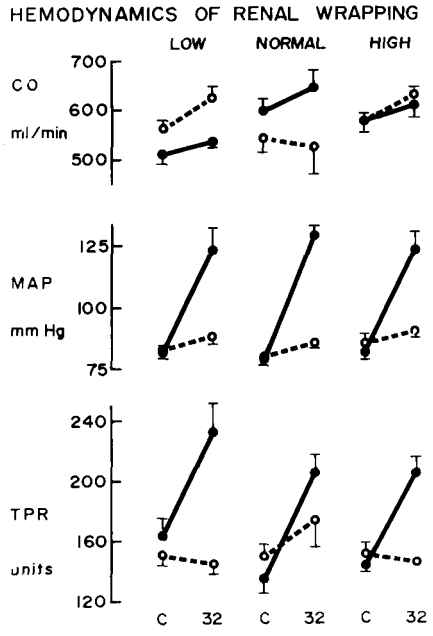


Fig 4
Effect on cardiac output (CO), mean arterial pressure (MAP) and total peripheral resistance (TPR) of either cellophane wrapping of both kidneys (closed circles, solid lines) or sham operation (open circles, interrupted lines) in rabbits chronically maintained on low, normal or high salt diets.

The blood pressure increased by the same amount in all the renal hypertensive groups independently of the dietary salt intake (Fig. 4). In each group the rise in blood pressure was due to increased total peripheral resistance and there was no change in cardiac output in any of the groups. The rise in plasma renin activity (PRA) and AII was identical in the wrapped

and sham-operated animals on each diet, with the greatest rise on low salt intake. Administration of the selective AII antagonist 1-sar-8-ala AII was associated with a greater fall in blood pressure in the hypertensive animals (Fig. 5). This can best be explained by a difference in vessel architecture between normotensive and hypotensive animals, similar to our previous findings after total autonomic block. The results suggest that the renin-angiotensin system contributes nothing to the elevation of blood pressure in this type of hypertension but that it is of the utmost importance in body salt homeostasis.

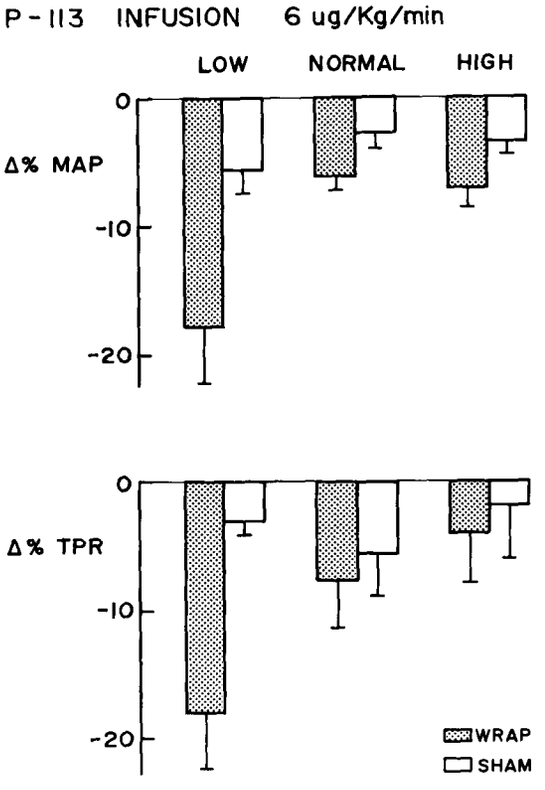


Fig 5
Effect of infusing AII antagonist P113 on mean arterial pressure (MAP) and total peripheral resistance (TPR) in cellophane wrapped or sham-operated rabbits maintained on low, normal or high salt diets.

The findings that dietary salt intake had no effect on the level of blood pressure was surprising in the light of the epidemiological findings. Experiments are in progress to examine whether the animals become more salt sensitive after (i) reducing their renal mass; (ii) after inactivating two major control systems by studying the responses of adrenalectomized + guanethidine-sympathectomized rabbits maintained on fixed steroids. Preliminary results suggest that removing one kidney has only a minor effect with the difference in blood pressure between animals on high and low sodium diets only about 10 mmHg. However, the adrenalectomized-autonomically blocked animal becomes very salt sensitive with great reduction in blood pressure on low salt and rise on high salt. In these preparations there seems again to be no difference between wrapped and sham-operated group. The findings therefore suggest that the above homeostatic systems are important in normal maintenance of body salt and water, but make no contribution at all to the maintenance of this type of hypertension.

8. Role of brain Renin-Angiotensin System in Hypertension

J. S. Hutchinson

The renin-angiotensin system is known to be involved in certain types of experimental hypertension but it is not thought to play a significant role in essential hypertension. In 1972, Goldstein and colleagues found a close positive correlation between an angiotensin-like material in the cerebrospinal fluid(CSF) and the level of blood pressure in patients with essential hypertension. Since then the cerebral angiotensin-like material has been the subject of many experimental projects. Interest was further stimulated when Ganten, Hutchinson and Schelling showed that the blood pressure can be restored to near normal value in rats with genetic hypertension, by injecting an angiotensin antagonist into the CSF.

The angiotensin-like material in CSF is generated in brain tissue but its identity to the angiotensin in blood has not been shown. The nature of the angiotensin-like material in CSF was examined by using a combined polyacrylamide gel radioimmunoassay-bioassay system. It was found that immunoreactive angiotensin I and II (AI, AII) are present in CSF of rats, dogs, sheep and man. The AI-like material is the same as intact molecular AI with the occasional presence of some additional immunoreactive material that may be the des-asp'-angiotensin I fragement which is detected in the AI radioimmunoassay at concentrations 10 times less than those of intact molecular AI. In CSF from normal dogs the AII immunoreactive material has

now been shown to be the des-asp'-angiotensin II fragement both by bioassay and radioimmunoassay of material separated on polycrylamide gel electrophoresis. The high concentrations of AI and AII immunoreactivity previously found in brain tissue has been shown to be due to an enzymatic artefact. A tissue method has been developed which has eliminated the artefact and now levels of immunoreactivity are low. As the method used also precipitates protein we are currently using procedures that dissociate hormones like AI and AII from proteins to examine the possibility that the low levels may be due to co-precipitating AI and AII with a protein. The action of angiotensin perfused through the brain ventricles is to raise blood pressure. By using conscious diabetes insipidus rats it was shown that this blood pressure increase is due to the release of the pituitary hormone vasopressin into the blood stream. This model is currently being used to investigate the role of vasopressin in the blood pressure response to other agents given into the brain ventricles

9. Effect of Histamine Bolus Injections and continuous infusions on the H_1-and H_2-receptors in the hindlimb vessels of the rabbit.

J. A. Angus, A. Bobik and P. I. Korner

Hindlimb vascular resistance (HVR) was continuously measured after pharmacological block of the autonomic effectors in unanaesthetized rabbits with previously implanted Doppler ultrasonic flowmeters. Histamine bolus injections caused a dose-related short lived fall in HVR followed by a more sustained rise. The fall was due to H_2-receptor stimulation (blocked by burimamide or metiamide) and the rise to H_2-receptor stimulation (blocked by mepyramine). At the doses of histamine tested the magnitude of the H_1-mediated vasoconstriction had a larger peak effect than the H_2-mediated vasodilatation. Histamine infusions up to 200 μg kg^{-1} min $^{-1}$ did not alter HVR significantly but both increases and decreases in HVR were observed after giving H_2- or H_1- antagonists respectively. From the double reciprocal plots of 1/ peak HVR change and 1/dose of histamine the magnitude of the predicted H_1- and H_2-mediated peak HVR effects at large doses were the same. This suggested that the number of H_1-H_2-receptors were similar in the hindlimb vascular bed, in agreement with the infusion data.

10. Regional Vascular Resistance and Heart Rate responses mediated through H_1-and H_2-Histamine receptors in the rabbit.

J. A. Angus and P. I. Korner

The effects of histamine infusions (10-100 μg/kg/min) on heart rate and hindlimb, carotid, mesenteric and renal vascular resistance were investigated in unanaesthetized rabbits after 'total' autonomic effector block to abolish reflex effects. Histamine caused a rise in heart rate that was predominantly due to stimulation of H_2-receptors (blocked by metiamide). Hindlimb and carotid vascular resistance did not change significantly during histamine infusion. However, after blocking H_2-receptors with metiamide histamine infusions produced dose-related vasoconstriction in these beds while after H_1-receptor block with mepyramine histamine caused dose-related vasodilatation indicating that H_1 and H_2-receptors mediated opposite vascular effects which were of similar magnitude. By contrast, histamine infusion caused vasodilatation in both the mesenteric and renal vasculature before giving antagonists. This dilatation was mediated by both H_1 and H_2-receptors as either receptor antagonist attenuated the response. These studies suggest that H_1-receptors in the same species mediate vascoconstriction in some beds and vasodilatation in others while H_2-receptors mediate vasodilatation in all the beds studied and also account for most of the increase in heart rate.

11. Guanethidine induced Vasodilatation in the rabbit mediated by Endogenous Histamine

J. A. Angus, A. Bobik, P. I. Korner and M. T. Stoneham

The effects of guanethidine (0.5-4mg/kg i.v.) on arterial pressure, hindlimb blood flow and hindlimb vascular resistance (HVR) were compared in unanaesthetized rabbits subjected to 'total' autonomic block. Evidence that this response was mediated by histamine release was that (a) ^{3}H-labelled histamine levels in the hindlimb venous blood rose substantially after guanethidine; (b) infusion of exogenous histamine caused an inhibition of the guanethidine induced vasodilatation; and (c) competitive antagonism of the response by the H_2-antagonist burimamide. There was good

correlation between the ^{3}H-labelled histamine release and the time course of the vasodilator response. Glyceryl trinitrate infusions that lowered HVR substantially, did not cause release of histamine. Reserpine, desipramine and indomethacin pretreatment did not alter the vasodilator response to guanethidine. The guanethidine vasodilator response was not influenced by the H_1-antagonist mepyramine or by other H_2-antagonists metiamide or cimetidine. The vascular receptors stimulated by endogenous histamine may be distinctive from those stimulated by exogenous histamine, or the action of guanethidine may involve greater production of histamine at an intracellular site that is more readily reached by burimamide than by the other H_2-antagonists.

12. Guanethidine Vasodilatation in different regional beds in the autonomically blocked rabbit

J. A. Angus, P. J. Fletcher and P. I. Korner

Injection of guanethidine causes dose related falls in blood pressure and hindlimb vascular resistance in autonomically blocked rabbits. Similar falls in vascular resistance occur in the common carotid bed, but in the renal and mesenteric beds the falls were slight and were not dose related. Previous analysis referred to above has shown that the action of guanethidine is through the release of endogenous histamine acting on a site that is distinctive from the vascular H_2-receptor which is stimulated by exogenous histamine. In support of that is that stimulation of vascular H_2-receptors results in dose related vasodilatation in hindlimb, common carotid, renal and mesenteric beds. Radioactive microspheres were used to analyse in detail the flow and vascular resistance changes in the different beds. The main site of the vasodilator action of guanethidine was the skeletal muscle bed, where resistance at a dose of 4 ug/kg fell by more than 75% of resting, compared with falls of 8.3% in skin, 18% in kidney, 21% in small intestine, and 24% in brain. The major source of histamine released by guanethidine thus appears to be in skeletal muscle.

13. Effect of Propranolol on Haemodynamics of normal and Baroreceptor Denervated Rabbits

P. A. Blombery, P. I. Korner and A. Bobik

There is controversy about the mechanisms of action of the antihypertensive beta-blocking drug, propranolol. The question at issue is whether the drug acts by blocking the tonic effects of cardiac sympathetic thus lowering cardiac output or whether it acts on central nervous autonomic mechanisms including the sympathetic constrictor pathways. We have investigated the effects of how different doses of the drug in normal rabbits and in rabbits with neurogenic hypertension induced by surgical denervation of the arterial baroreceptors. Resting haemodynamics and reflex vasoconstrictor responses have been studied by means of a Valsalva-like manoeuvre in the unanaesthetized rabbit. Two doses of drug were studied:— (i) the 'low' dose giving levels of about 100 ng/ml plasma, i.e., levels well in the therapeutic range for treating hypertension; (ii) a 'high' dose giving average plasma concentrations of 250 ng/ml plasma. In normal rabbits, the 'low' dose of propranolol caused a significant fall in blood pressure (12 mmHg) due to a reduction in cardiac output (Fig. 6). In animals with neurogenic hypertension, the fall in pressure was larger and was also associated with a reduction in cardiac output. There were no effects upon reflexly stimulated changes in vascular resistance with the Valsalva manoeuvre. The high dose of propranolol had no further hypotensive effect in normal animals but caused further falls in neurogenically hypertensive animals associated with both reduction in resting total peripheral resistance and cardiac output. The effect on resistance in the denervated animals could be partly the result of the Bayliss effect consequent upon the large reduction in pressure. In animals with intact baroreflexes the hypotensive action of propranolol is entirely due to lowering of cardiac output and there is no attenuation of the effects mediated through the sympathetic constrictors.

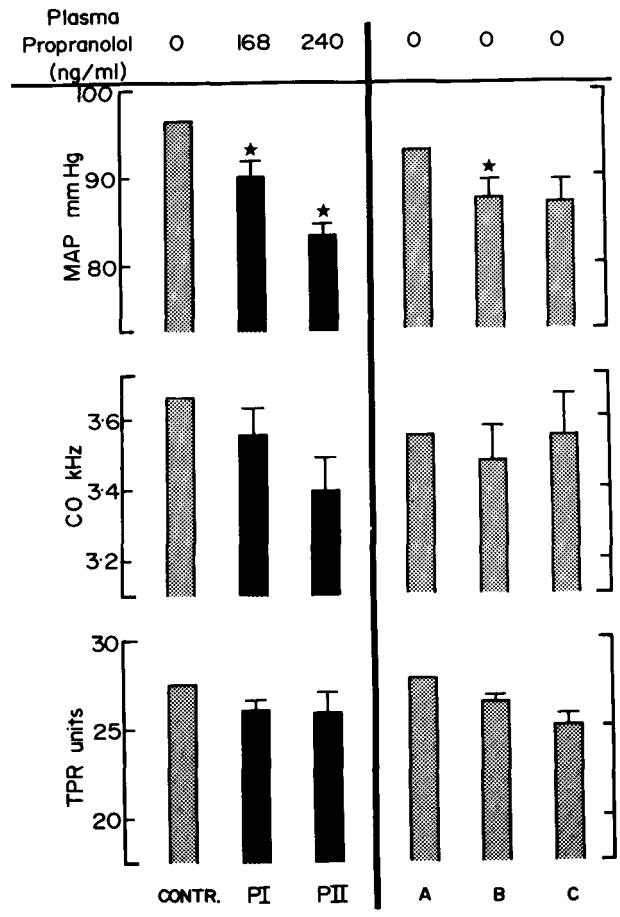


Fig 6

Right Panels: *Effects on resting mean arterial pressure (MAP), cardiac output (CO) and total peripheral resistance (TPR) during control period and after infusing two levels of propranolol for one hour before observations.*

Left Panels: *Control animals infused with dextrose solution. The highest level of propranolol produced a greater fall in blood pressure than in the control group which was due to a fall in cardiac output.*

14. Effect of Propranolol on Arterial Baroreceptor Function

P. K. Dorward, A. Bobik and P. I. Korner

Effects of propranolol on baroreceptor function is being studied in anaesthetised immobilised rabbits. Integrated aortic nerve activity and activity of single units dissected from the aortic nerve are being used to characterise baroreceptor function. Curves of activity against mean arterial pressure (MAP) are obtained by inflating perivascular balloons implanted around the abdominal and thoracic aortas and inferior vena cava at a previous operation.

Three control stability curves of integrated baroreceptor activity (IBA) were obtained from measurements taken over a 2½-3 hr period in five rabbits. The preparation was very stable providing an adequate control for the experimental series. Propranolol infusions to produce plasma concentration of approximately 180 ng/ml and 450 ng/ml were substituted for saline infusion during the 2nd and 3rd recording periods. In a further five rabbits the MAP-IBA curves showed a small but significant shift downwards during path propranolol infusions, indicating less baroreceptor activity for any given pressure. There was no further shift at the higher dose of drug.

Single units studied to date have shown no apparent change in the firing frequency — MAP relationship due to propranolol. A study of a larger unit population is currently in progress.

Cardiovascular Metabolism and Nutrition Research Unit

Establishment of New Unit

Most members of the new unit had previously worked together in the Department of Clinical Science at the John Curtin School of Medical Research, Australian National University, Canberra. The members of the Unit are: Dr. Paul Nestel (Head and Deputy Director of the Institute), Dr. Noel Fidge (Senior Biochemist), Dr. Norman Miller (Clinical Research Fellow), Dr. Mike Reardon (Postdoctoral Fellow), Dr. T. Ishikawa (Visiting Scientist) and four graduate research assistants. The first arrivals of the new Unit included Mike Reardon and Andrea Poyser who arrived in Melbourne in October 1976. Norman Miller arrived in October 1976 and the entire unit has been fully operational since the beginning of January 1977. The establishment of the new Unit has required greatly increased biochemical facilities at the Institute and we have been helped in a very important way by a special grant of $40,000 from the Victorian Government to help establish the Unit. In addition to its research activities the Unit will be responsible for an important clinical service in the Alfred Hospital where Dr. Nestel is Deputy Director of the Clinical Research Unit.

Research Activity

The work of the Unit is predominantly in clinical research, mainly in the field of lipid metabolism. Each of the senior investigators, Dr. Nestel, Dr. Fidge and Dr. Miller, will pursue some projects that are independent of the others. However, most of the long-term studies will be joint and will relate to the causation of hyperlipidaemia and the manner in which this predisposes to coronary heart disease.

It is possible to measure the amounts of different lipids such as cholesterol and triglyceride which are produced each day.

For instance, one can measure the amounts of cholesterol that are absorbed, synthesized, excreted and disposed of in several ways each day. People with hypercholesterolaemia (high blood levels of cholesterol) may suffer from this disorder because of:—

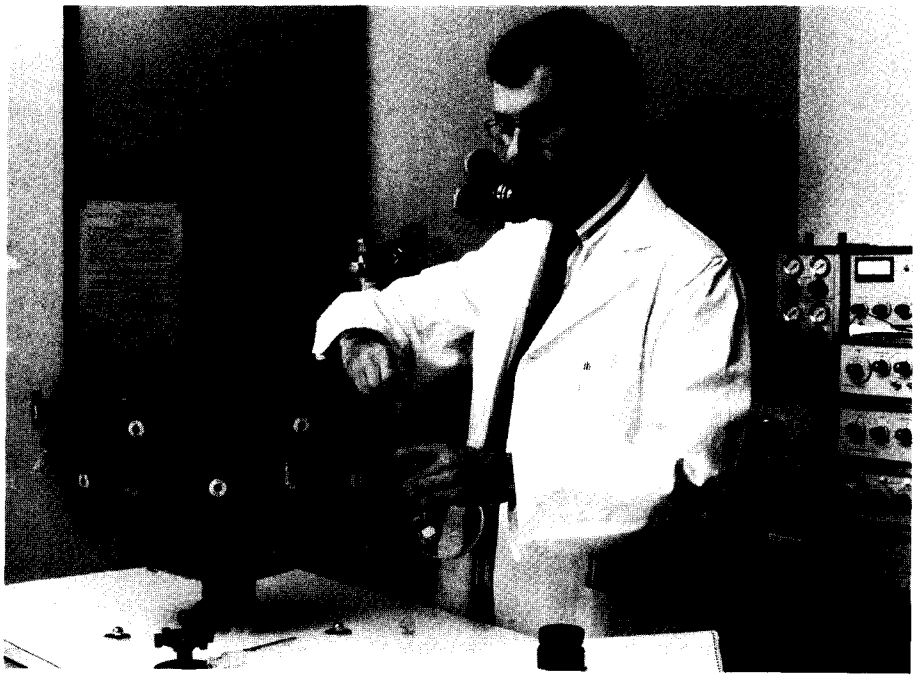
Back Row (from left to right): Dr. Paul Nestel, Dr. Norman Miller, Dr. Noel Fidge, Dr. Toshi Ishikiwa, Elaine Fagarazzi, Jane Ma, Dr. Michael Reardon; Front Row (from left to right): Margaret O'Connor, Andrea Poyser, Liz Leembruggen, Lyn Taylor.

1. An inherited defect in clearing cholesterol from the blood;
2. Overproduction of cholesterol in body tissues;
3. Inefficient re-excretion of dietary cholesterol, etc.

Our studies can identify these problems and the basic mechanisms that lead to disordered function. We can also define the ways in which specific diets and drugs contribute to or overcome hyperlipidaemia.

There are various proteins associated with the blood lipids (called lipoproteins) that control the formation and disposal of the lipids. We have developed techniques for measuring the functions of these proteins in man as well as in isolated cells. Deficiency of some of these proteins is responsible for some varieties of hyperlipidaemia.

Another theme which will be pursued is the regulation of the cholesterol content of tissues and how this is influenced by the composition and relative concentrations of the different plasma lipoproteins. Particular attention will be paid to the high density lipoprotein (HDL), which tissue culture and clinical studies have indicated may play a central role in transporting cholesterol out of cells, including the cells that make up the atherosclerotic plaque. Cholesterol is deposited in those cells from the cholesterol-rich low density lipoprotein (LDL) of plasma. The balance between cholesterol moving in and out of cells may therefore be a function of these two lipoproteins. Interest in HDL in this regard was recently stimulated by our observation that the amount of cholesterol in the tissues of man, is inversely related to the plasma HDL cholesterol concentration, suggesting that the efficiency with which cholesterol is removed from tissues depends on the amount of HDL in plasma. Attention was subsequently drawn to the presence of low HDL concentrations in patients with existing clinical CHD and in subjects with characteristics known to be associated with increased susceptibility to CHD such as obesity. This raises the possibility that a low HDL concentration might be an important unrecognised factor in accelerating the progression of coronary artery disease. We shall be studying this interesting new area by metabolic studies in hypercholesterolaemic subjects and also in isolated cells grown in culture systems.

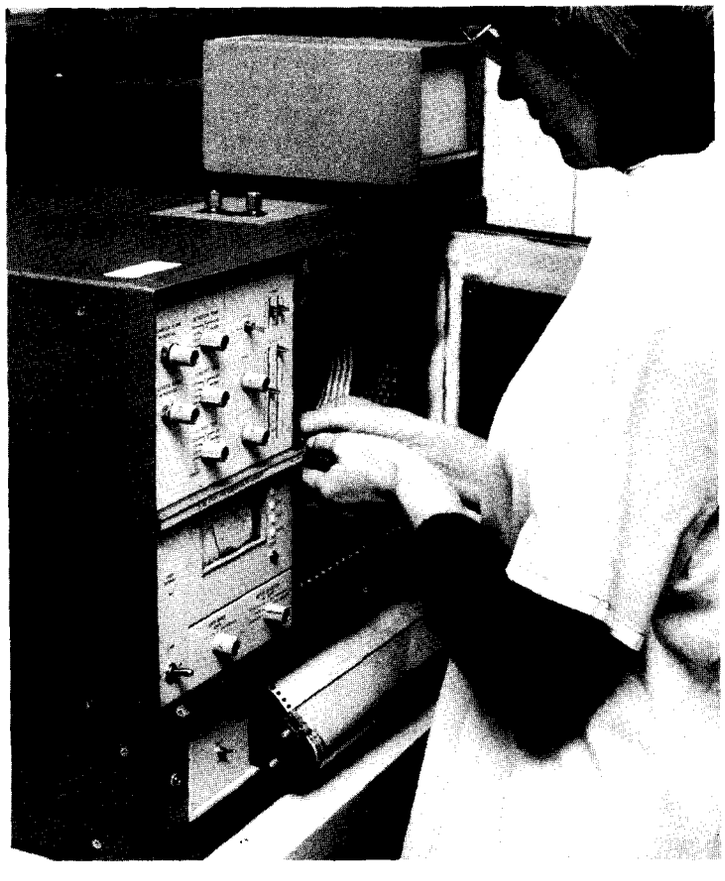


Freeze Dryer and Gas Chromatograph for analysing lipid patterns in blood.

The opportunity will also be taken to collaborate with other groups in the Institute and in the Hospital. Projects include the study of the association of hypertension and hypercholesterolaemia, the effect of treating one condition on the other, the biochemical and metabolic derangements in hypertension, etc. Links have already been formed with the Cardiovascular Diagnostic Service to relate disease in different parts of the coronary circulation to specific disorders of lipids.

Dr. Miller intends to continue his studies into heart muscle metabolism, in particular the sources of energy available to the heart that is deprived of oxygen and the deleterious effect of fatty acids on heart muscle function. Collaborative studies are also under way with the Ewen Downie and Metabolic Unit: many endocrine disorders also show abnormal lipid metabolism.

The Unit will also participate actively in teaching within and outside the Hospital. It will provide clinical advice and specialized biochemical services for hyperlipidaemic individuals referred widely from within Victoria.



Gas Chromatograph for analysing lipad in the blood.

Risk Evaluation Clinic

A new clinic is to start at the Institute early in 1977. The clinic has been established jointly with the National Heart Foundation (Victorian Division). It will offer a service to identify risk factors for heart disease in apparently healthy individuals. This *Risk Evaluation Service* will be located in the Baker Institute. It will be available to the community in general. Subjects found to have high blood pressure or high blood fats will be offered appropriate advice and treatment or referred to their own doctor. The main purpose of this project is to provide a community service that can be seen to be a direct extension of and practical application of our research. It is important for medical research groups to maintain an 'open house' to the public so that the relevance of their research becomes evident.

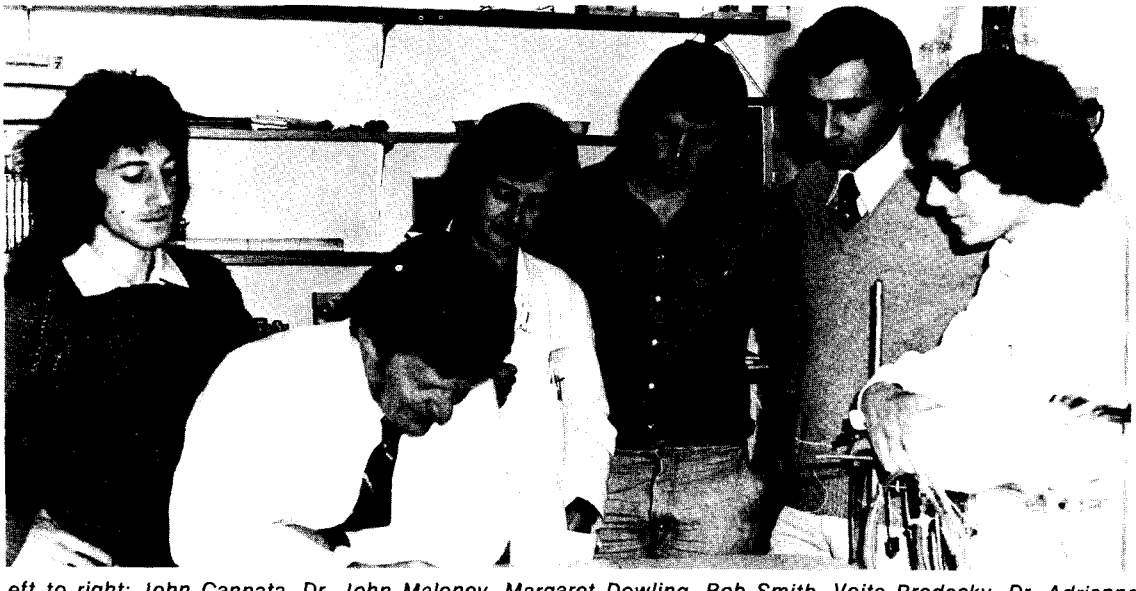
Developmental Biology Research Unit

Main Topics

- Development of Respiratory System in the Foetus and Newborn
- Control of Cardiac Function in the Foetus and Newborn
- Management of Heart and Lung Disorders in the Human Infant

General Outline of Work:
Within the last six years the Developmental Biology Research Unit has initiated a number of interdisciplinary studies on the development of the heart and lungs throughout life 'in utero' and in the immediate postnatal period. The work divides into two basic streams, the first centred on laboratory studies in animals and the second on human infants in the clinical situation of the Neonatal Special Care Nursery in conjunction with the Paediatric Department, Monash University. Animal studies are concerned mainly with sheep in which the function of the heart and future air-breathing lung can be investigated from day to day over the last third of gestation throughout birth and into the newborn period. With the Special Care Nursery cardiovascular and respiratory function may be studied in babies at term and in prematurely born infants. Problems of special interest in this area are the recurrent cessation of breathing in the premature newborn baby and the general problem of the early detection of a malfunction in the heart and respiratory systems.

Not only in our own unit but in several others around the world the structure and the development of the cells which line the airways of the adult lung have come under review. Studies of the Developmental Biology Research Unit have described the changes which take place in the trachea (Fig. 7) of the early newborn and the manner in which the future glands of the lung are formed. Detailed analyses of the structure of the depths of the lung are being made with an emphasis on the effects of its natural fluid environment on future development. Some structural defects which occur in the human lung during development can be mimicked in these studies and examined at the microanatomical level. Since it has been discovered that the muscles of respiration are active 'in utero' and that the baby makes breathing movements well before birth an examination is proceeding on the control of these movements and the interaction between them and the systems which sense the amount of oxygen and carbon dioxide in foetal blood. New techniques for detecting muscle activity have been developed and it is hoped that computer analysis of the pattern of this activity will lead to a better understanding of the development and maturation of the



Left to right: John Cannata, Dr. John Maloney, Margaret Dowling, Bob Smith, Vojta Brodecky, Dr. Adrianne Walker.

Director's Report

The Clinical Research Unit continues to provide special diagnostic investigations for the Alfred Hospital and also performs clinical research in cardiovascular medicine that is closely integrated with the programme of the Baker Institute. The main services provided during 1976 were through the *C.R.U. Hypertension Evaluation Clinic* and through quantitative exercise testing in patients with ischaemic heart disease. In addition a special *Hyperlipidaemia Clinic* will be operating from the beginning of 1977, under the direction of the new Deputy Director of C.R.U., Dr. Paul Nestel. Its role will be to provide a diagnostic and consultative service for patients with disorders of lipid metabolism. The clinic will have the services of a dietitian on the staff of the hospital, Miss Denise Winters, who will also assist with the work of the *Risk Evaluation Clinic* at the Baker Institute.

The *C.R.U. Hypertension Evaluation Clinic* has been expanding its activities under the direction of Dr. G. L. Jennings. There is close collaboration with Dr. J. Stockigt of the Ewen Downie Metabolic Unit and Dr. J. Sabto of the renal unit so that an integrated diagnostic service and a consultative service for difficult therapeutic problems is provided through the clinic. Members of the staff working in the clinic included Dr. Peter Blombery, Dr. Peter Fletcher and Dr. John O'Sullivan from the Department of Social and Preventive Medicine. Dr. O'Sullivan provided the clinic with much needed background of the problems faced by general practitioners in the management of hypertension. A number of new tests are now available through our Biochemical Pharmacology Laboratory which works under Dr. Alex Bobik and is located in the Baker Institute. These include a radioenzymatic assay for total plasma catecholamines, measurements of urinary adrenaline, noradrenaline, metanephrine and VMA concentrations. The laboratory also determines acetylator status in patients on hydrallazine therapy, which is an important factor in assessing the maximum 'safe' dose of this anti-hypertensive drug. Other assays currently available for special problems include the measurement of plasma concentrations of several beta-blocking drugs including propranolol, prindolol and timolol. The unit is also investigating the use of frequent ambulant blood pressure measurements by the patient in the course of ordinary daily activity using the Remler semi-automatic blood pressure recorder.

From 1977 special biochemical investigations of lipoprotein disorders will be available through the Biochemical Laboratory of the *Cardiovascular*

Staff of C.R.U. ***Left to right*** *(sitting) Denise Winters, Ursula Gregorek; (standing) Professor Korner, Sr. Anne Hewett, Dr. Alex Bobik, Sr. Denise Chambers, Sr. Sue Seally, Peter Ashley, Dr. Garry Jennings, Dr. Peter Jenkins, Moham Bangah, Dr. P. Nestel, Helen Skews.*

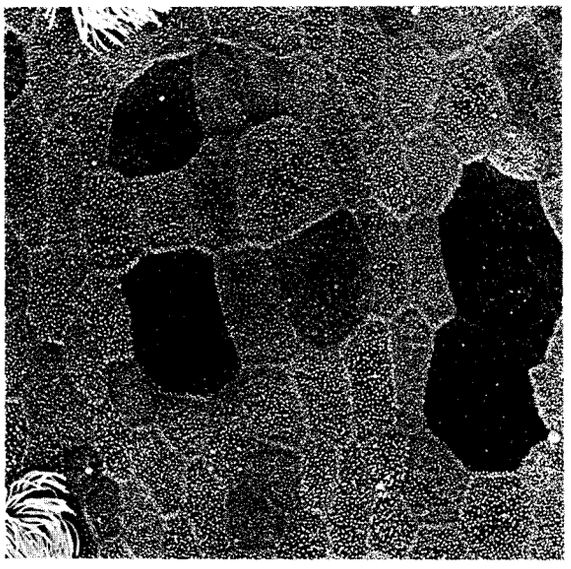


Fig 7 A scanning electron micrograph of the anterior and lateral aspects of the trachea in the early phase of postnatal development.

respiratory system prior to birth. The survival of the infant at birth depends not only on a sufficiently mature respiratory system but also on a functioning cardiovascular system capable of responding to the stresses of birth and the immediate neonatal period. Little is known of the development of the nervous systems which control the function of the heart in the foetus of the degree to which they are important in survival of the newborn. Extensive studies have been undertaken during this past year on the control of blood pressure in the foetus and newborn and the changes which take place in these systems at birth. This has required the use of special techniques for manipulating foetal blood pressure in order to analyse the contributions of the various components of the nervous system to variations in this pressure. While there is little difference in the response of the foetus and the newborn to transient increases in blood pressure following the infusion of phenylephrine there is a considerable difference in response when the blood pressure is lowered in both situations. As knowledge accrues from these investigations it is hoped that our understanding of the responses of the human newborn to the stresses of intrauterine/extrauterine life will be enhanced. Apart from these investigations on the structure, function and control of the respiratory and cardiovascular systems 'in utero' and in the newborn period the unit spent some time in pilot studies on the possibility of direct observation and measurement of the foetus over the middle third of gestation (Fig. 8). Because of the sensitivity of the

Fig 8
A prototype of a young foetus moving in the extra uterine compartment. This compartment allows direct observation of the foetus and this traction to changing environmental conditions.

tissues and the smallness of the foetus in this period long-term measurements have proved extremely difficult. In collaboration with members of the electronics laboratory and research workshop and the staff on the experimental operating theatres a novel technique has been developed in which a plastic window is attached to the abdominal wall of the ewe and grafted into the pregnant uterus. A removable section in this window allows the undisturbed foetus to be placed into a water tight extrauterine compartment for direct observation. The compartment has been designed so that intrauterine conditions can be simulated and that fluid composition, pressure, and temperature can be controlled. At the end of the observation period the foetus can be returned to its natural uterine environment where it continues to develop until it is observed at a later time. Such observations may be repeated from time to time throughout life 'in utero'.

Of the problems which present themselves, clinical studies on the human newborn baby are especially important. The initial problems in this area revolve around systems for data acquisition and analysis which will enable early warning of the impending collapse of the heart and lung. At this level the Unit is working on the development of non-invasive techniques for the measurement of the function of the respiratory and cardiovascular systems. Monitoring techniques of respiratory patterns are being developed and these are being used in conjunction with normal electrocardiographic apparatus to describe the relationship between cardiovascular and respiratory functional patterns in preterm and full term human infants. Eventually these analyses may be of predictive value in signalling abnormalities and the patterns may become of value in the early recognition and prevention of failure of respiratory and cardiac function in these babies.

PROJECTS

1. Maturation of the Respiratory System in the Foetus and Newborn

J. E. Maloney, B. C. Ritchie, T. M. Adamson, A. M. Walker, V. Brodecky and M. H. Dowling.

The ability to measure the activity of the respiratory system in foetal sheep 'in utero' affords an opportunity to examine how this system develops and what the methods are important in its control at birth. Techniques are being developed to denervate the aortic and carotid sinus nerves of the foetus 'in utero' in order to remove the peripheral chemoreceptor input to the respiratory centre. The normal pattern of respiration will be examined in these foetal sheep and the response to variations in oxygen and carbon dioxide contents will be measured and contrasted with control situations. The influence of vagotomy on respiratory activity will also be analysed. Pilot studies in our laboratory suggest that its vagal afferent input to respiratory centre is unimportant as far as respiratory activity of the foetus is concerned but that it may influence the structural development of the lung (see below). Because of the nature of the electromyographic signals which are recorded from the diaphragm and the amount of information available, attempts at developing automatic data acquisition and analysis systems are being made. With the available systems analogue circuitry has been developed in collaboration with the electronic development laboratory which enables the automatic analysis of the electromyographic information. The versatility of this system is limited and preliminary work is now proceeding on a digital computer data acquisition and analysis system.

2. The Development of an Extra Uterine Compartment for Foetal Observation

J. E. Maloney, V. Brodecky, J. Baird and Jan Dixon

Throughout this past year work has continued on the development of a technique for the direct observation of the foetus in the unanaesthetised state. This involved firstly, the implantation of a plastic window which could be secured to the abdominal wall and enable direct access to the foetus 'in utero' and secondly, the development of a compartment into which the foetus could move and be directly observed. Within the compartment measuring instruments could be attached to the foetus to examine cardiac and respiratory activity whilst the foetal environment was altered. This technique has now been developed and promises to be of great value in our programme for non-invasive study of foetal function. The process of establishing an extra-uterine

compartment involved control of pressure, temperature and filtration in a large volume of fluid which had to be maintained under sterile conditions. Fig. 12 illustrates an earlier prototype of the system with the unanaesthetised foetus in the viewing compartment.

3. The Development of the Upper Respiratory Epithelium of The Rat

J. J. Smolich, Bernice F. Stratford, J. E. Maloney and B. C. Ritchie

The development of the upper respiratory epithelium of the rat has been investigated from birth onwards with light and scanning electron microscopy. Detailed analyses of the micrographs from these studies has revealed the following information.

Two divisions, termed the *early postnatal and mature* periods, have been recognized. The early postnatal period, occupying the first 3 weeks after birth, is characterized by the presence of abundant and atypical mucin-containing cells, lesser numbers of 'low electron responsive' and ciliated cells, infrequent brush cells and primary cilia. Regional differences in the morphology and distribution of the different cell types also occur. The mature period is divided into *early* and *late phases.* In the early phase, adult mucin-containing cells appear for the first time in development, brush cells increase in number and displayed adherent mucous granules of various sizes. In the late phase, 4 types of epithelium occur in the laryngeal, cartilaginous and intercartilaginous zones, classified on the basis of differences in the ciliated to non-ciliated cell ratio and the nature of the non-ciliated cells. Most often the epithelium appears to be formed mainly by ciliated cells, but variations occur both between and within animals. The membranous zone is corrugated in many regions and in general ciliated cells predominate. However, areas where these cells are few or absent also occur. All cell types of the epithelium display filamentous strands between their apical projections. These appear to be extensions of the glycocalyx. Cell borders separated the cells of the epithelium and, on non-ciliatec cells other than brush cells, have a characteristic structure which altered with maturation. It has been proposed that: (i brush cells may be active in the absorption of mucus; (2) scanning electron microscopy of biological tissue surfaces demonstrates the glycocalyx and (3) the electron response of a tissue surface may be related to the density of exposed potential acid radicals in the glycocalyx.

4. Morphological Effects of Phrenectomy in the Foetal Lamb Lung

Daine Alcorn, T. M. Adamson, J. E. Maloney, B. C. Ritchie and P. M. Robinson

The foetal lamb demonstrates both spontaneous phrenic nerve activity and diaphragmatic electrical activity indicating that breathing movements 'in utero' involve the respiratory muscles. The role of this activity in intrauterine pulmonary development has been investigated in this study using chronically phrenectomized foetal lambs. Three foetal lambs (110 days) were partially delivered by caesarian section using aseptic techniques. Following bilateral phrenectomy, the lambs were returned to the uterus. Three weeks later the lungs were fixed. Two foetal lambs underwent the same procedures except for the section of the phrenic nerves. Three normal foetal lamb lungs were fixed at 130 days without prior surgical techniques. The lungs of the phrenectomized lambs were decreased in weight and volume. This was not accounted for by their diminished lung liquid when compared with both sham operated and normal controls. Phrenectomized lambs' lungs had a decreased percentage of future air spaces and on histological examination this was seen to be a result of thickened walls as well as future air space collapse. The proliferation of potential alveoli was reduced. Cellular differentiation, however, had advanced as indicated by the frequency of the alveolar type II cell. These experiments demonstrate that the integrity of the phrenic nerve is essential for normal pulmonary development. The precise mechanisms of this relationship are not known.

5. Development of Baroreflex Activity in the Foetus and Newborn

J. E. Maloney, B. C. Ritchie, J. Cannata, Margaret H. Dowling and A. M. Walker

In earlier studies within the group the activity of the baroreflex was measured in unanaesthetised foetal sheep over the last one third of gestation and in newborn lambs. Blood pressure in these experiments was increased by bolus injections of phenylephrine and the results indicated that the baroreflex pathways were active by at least day 105 in the foetal

lamb. With this transient method the average sensitivity of the reflex was 7.8 ± 0.9% msec/cmH_2O and was unchanged throughout the last third of gestation and into the newborn period. Within the past year an alternative method for increasing blood pressure has been explored. This method consists of placing an inflatable balloon around the thoracic aorta in the foetus or newborn which upon rapid inflation transiently increases blood pressure and stimulates the baroreflex arc. Experiments in this series indicate that while the reflex is unchanged throughout gestation it is substantially less sensitive in the newborn period. The mean foetal sensitivity was 3.03 ± 0.19 msec/cmH_2O whilst the mean newborn sensitivity was 0.91 ± 0.11 msec/cmH_2O. The two methods of measurement provide significantly different values for the sensitivity of the reflex arc and indicate that the interpretation of results in this area needs an element of caution. The range of receptors stimulated by these two methods is different and control integration of this input is most probably important in determining the sensitivity of this reflex.

6. The Response of the Autonomic Nervous System to Transient Decreases in Blood Pressure in Foetal and Newborn Lambs

A. M. Walker, J. Cannata, Margaret H. Dowling, B. C. Ritchie and J. E. Maloney

The reflex changes in heart rate induced by hypotension were analysed in three conscious, chronically instrumented foetal lambs over the last third of gestation and in four newborn lambs of 1-22 days of age. Beta-adrenergic blockade produced by propranolol (1mg/kg) and cholinergic blockade effected by atropine (0.2-0.3 mg/kg) were used to examine the autonomic efferent components of the changes. Graded arterial hypotension was produced by varied inflations of a liquid-filled balloon implanted around the thoracic inferior vena cava. Results of a total of 99 balloon inflations in newborn lambs show a relationship between the magnitude of the tachycardia and the decrease in mean arterial pressure. The heart rate increase reached 64 ± 6 bpm (33 ± 3%) when mean arterial pressure was reduced by 39 ± 1 cmH_2O (32 ± 1%). The tachycardia was partially prevented by either atropine or propranolol, indicating that both sympathetic and parasympathetic influences contributed. In foetal lambs 134 experiments showed no progressive tachycardia with progressive arterial blood pressure decreases. Changes in average heart rate were small (11 ± 3 bpm) when mean arterial pressure was reduced by 8.3 ± 0.4 mmH_2O. The tachycardia was smaller after propranolol, indicating a sympathetic nervous contribution. Further decrement in mean arterial pressure to 23 ± 1 cmH_2O (40 ± 1%) led to a small mean decrease in heart rate of 11 ± 5 bpm (6 ± 3%). Reversal of this bradycardia by atropine showed that parasympathetic tone was increased by this degree of hypotension. Thus the autonomic nervous system mediates reflex heart rate changes in both foetal and newborn lambs during hypotension, but the pattern of nervous influence is different. Whereas parasympathetic + sympathetic influences both contribute to tachycardia in the newborn, these influences are antagonistic in the foetus.

7. Autonomic Components of the Heart Rate Response to Hypoxia in the Foetal and Newborn Lamb

A. M. Walker, J. Cannata, Margaret H. Dowling, B. C. Ritchie and J. E. Maloney.

Hypoxia causes tachycardia in the newborn lamb but the response is bradycardia in the foetal lamb. This study compared the relative parasympathetic and sympathetic nervous control in these two situations. Conscious, chronically instrumented animals comprising nine foetal lambs of gestational age 109-142 days (term = 147 days) and eight newborn lambs of 2-28 days old were used. Autonomic influences on heart rate were quantified by measuring changes in heart rate following injection of atropine (0.2-0.3 mg/kg) or propranolol (1mg/kg). The change in the pattern of autonomic influence induced by hypoxia was assessed by comparing measurements made during hypoxia with measurements made at normal arterial PO_2. Results in foetal lambs (20 hypoxia experiments, 30 normoxia experiments) show an antagonistic increase in both sympathetic and parasympathetic tone occurs when the foetus becomes hypoxic; the net bradycardia reflects the predominance of parasympathetic restraint of heart rate.

In contrast, newborn lambs (11 hypoxia experiments, 8 normoxia experiments) show a decrement in parasympathetic restraint

of the heart rate together with a synergistic increment in sympathetic tone; both changes contribute to the net tachycardia shown by the hypoxic newborn lamb.

8. Autonomic Control of Basal Heart Rate in Foetal and Newborn Lambs

A. M. Walker, J. Cannata, Margaret H. Dowling, B. C. Ritchie and J. E. Maloney

Development of autonomic influence on the heart rate of conscious unstressed foetal and newborn lambs was studied. Heart rate (HR) and blood pressure were measured before and after injection of atropine (0.2-0.3 mg/kg) and propranolol (1mg/kg). Animals studied were 9 foetal lambs (59 experiments) of gestational age 93-141 days (term — 147 days), and 8 newborn lambs (30 experiments) of 1-29 days old. Parasympathetic influence on basal heart rate in both foetal and newborn periods was shown by an increased heart rate following atropine injection. This parasympathetic HR restraint was small early in gestation (HR increase 7.2 ± 6.4 bpm at 90-104 days) but increased progressively toward term (HR increase 36.8 ± 7.6 bpm at 135 days — term) and showed a further increment after birth (HR increase 60.1 ± 7.5 in newborn lambs).

Sympathetic influence on HR was present at all ages studied. In contrast to the parasympathetic system, there were no differences in sympathetic influence over the gestational period 90 days — term, though an increment was apparent after birth. Propranolol injection reduced foetal HR by 12.8 ± 2.9 bpm and newborn HR by 37.6 ± 12.3 bpm. Heart rate after combined parasympathetic and sympathetic blockade (intrinsic HR) was not different during foetal gestation nor changed after birth, averaging about 185 bpm over the entire age range studied. Thus differences in basal heart rate during the last third of foetal gestation and following birth in lambs reflect differences in autonomic influence on the heart. An increasing parasympathetic restraint of the heart account for the progressive showing of foetal HR in late gestation.

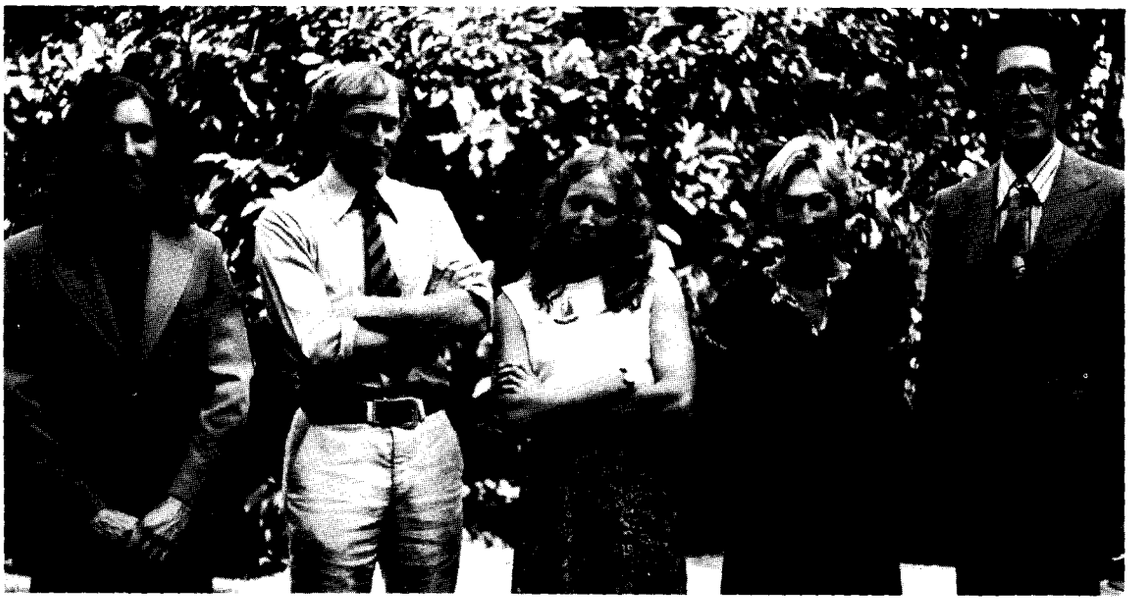
Cancer Research Unit

GENERAL SUMMARY

The programme of studies continued by the Chemical Carcinogenesis Group is aimed at examining in depth, the key events which occur during the course of development of chemically-induced cancer. The current emphasis remains centred on the investigation of a potent animal model of nitrosamine-induced kidney cancer. The long-term intention however, is to compare the pertinent findings in this single-pulse system with those obtained from similar studies utilizing other models of chemical carcinogenesis such as multiple low-dose systems inducing cancer at other tissue sites. The underlying significance of such experimental investigation is to provide information that will aid the understanding of the cancer process in man.

The experimental system utilizes a single, high dose of dimethylnitrosamine (DMN) administered by intraperitoneal injection to young rats which have been preconditioned by a diet lacking in protein but high in carbohydrate. The regimen results in 100 percent incidence of kidney tumours in those rats which survive the acute hepatotoxic effects of the carcinogen.

Studies completed previously have outlined the sequential, morphological events at light-and electronmicroscopic levels which occur in the kidneys from the first day following DMN administration up to the stage at six months when macroscopic neoplasms are present. The data from these initial studies have provided a broad foundation for a series of studies which have attempted to answer several avenues of enquiry posed by the model. Autoradiographic studies with tritiated thymidine were conducted to identify those target cell populations in the kidney stimulated by the carcinogen to engage in waves of proliferative activity during the early phase of tissue reaction. Experiments aimed at investigating the possibility of establishing an *in vitro* culture system which would in itself represent in terms of relevant target cell populations and behaviour, the situation pertaining *in vivo,* were also undertaken. Such an *in vitro* system would provide access to relatively purified populations of target cells, an additional advantage for biochemical characterization. Immunological studies have been directed towards determining the role if any, of the carcinogen in facilitating the cancer process by immunodepression.



The Chemical Carcinogenesis Unit, now transferred to the garden setting of Melbourne University.
From left to right: *John Lee, Dr. Gordon Hard, Helen King, Paula Hayward and part-time collaborator Dr. B. W. Stewart.*

Autoradiographic analysis of proliferating cell populations in the chronic phase of DMN renal carcinogenesis.

G. C. Hard

The response of the kidney to a carcinogenic dose of DMN is characterized by an initial period of two to three weeks during which an inflammatory reaction occurs in the outer zones, the cortex and outer band of outer medulla. This is followed by a chronic phase extending to approximately the twelfth week during which isolated hypercellular lesions persist in the same zones. The third phase, that of tumour cell proliferation commences at around the sixteenth week when unequivocal foci of tumour cells can be identified in the appropriate sites of the kidney.

Our prevoius autoradiographic studies concentrated on the events occurring in the acute phase of inflammatory reaction but the present studies are now oriented towards characterizing the various cell types present in the persistent lesions of the chronic phase in terms of proliferative capacity. In particular, it is necessary to determine whether the large mesenchymal cell characterized by certain cyctoplasmic and nuclear abnormalities present in the chronically-persisting lesions is capable of replication. To do this, the rat is pulsed with a single dose of tritiated thymidine at intervals between 3 and 12 weeks post-DMN treatment and the kidneys processed for autoradiography one hour later. In this way the proliferative character of the various cell types within the chronically persisting lesions can be determined.

If a suspect cell type proves to be incapable of replication thus representing an end cell, then it is not candidate for giving rise to the rapidly proliferating foci of tumour cells developing later.

Autoradiography also provides the possible means for relating the populations of cells specifically stimulated by DMN in the acute phase to cells within the persisting chronic-phase lesions and even early developing tumour cell foci. The work of Leblond and co-workers (Lab. Invest. 1959, *8,* 296) offers the possibility of devising such studies in the kidney by providing data on cell renewal systems. This data showed that autoradiographically labelled cells could be found in the kidney several months after pulse-labelling with tritiated thymidine indicating firstly, that DNA stability was such that the radioactivity did not change significantly over that period of time, and secondly, that the kidney was constituted normally by fairly permanent but expanding cell populations. In our earlier study it was found that only those resident cell populations from which the tumours were believed to be later derived, were stimulated into waves of proliferation by DMN during the acute phase of the response. Thus, the resident cortical fibrocytes of the intertubular spaces of zone 1 engage in a relatively synchronous wave of proliferative activity at day three whereas those of zone two reach a peak of activity at day nine. On the other hand, amongst the various epithelial subpopulations of the nephrons, only the cells of the proximal and distal tubules are stimulated to increased proliferative activity and this peaks at day ten. The findings of Leblond and co-workers suggest that the fate of cells of the various populations labelled during their most active replicating periods after DMN stimulation may possibly be traced in the ensuing months if the number of divisions into daughter cells does not exceed a minimum compatible with the retention of enough radioactivity to produce an autoradiographic image. To this end, rats are being injected with tritiated thymidine at three, nine and ten days after DMN administration and the kidneys processed by perfusion fixation for autoradiography at periods of one to several months after the isotope pulse.

In this way it may be possible to determine whether any interrelationship exists between proliferating populations in the acute phase, cells of the chronic phase lesions, and cells of the earliest tumour foci.

Parameters of behaviour in kidney cell cultures derived from DMN-treated rats

G. C. Hard

Following a series of experiments studying the behaviour *in vitro* of kidney cells that have been derived from rats injected with a single dose of DMN sufficient to induce 100 percent kidney tumour incidence, it is possible to review collectively the growth characteristics of surviving cell populations. Consistently, cells from treated rats express morphological transformation and persist indefinitely in artificial culture conditions, traits that characterize cultures isolated from the living animal from 1 hour to 7 days after the intraperitoneal injection of

DMN. For the first few subcultures, cells from DMN-treated rats behave in identical fashion to cells derived from normal, untreated rats. However, the latter usually reach senescence by subculture four although on occasions, control cultures have persisted until the ninth or fourteenth passage before expiry. In contrast, cells isolated 4 or more hours after the DMN stimulus *in vivo,* express morphological transformation consistently at subculture five visualized as the development of densely-staining, macroscopic foci of piled-up cells. These observations indicate the emergence of an altered population of cells characterized by a loss of density-dependence and contact-inhibition. In cultures derived from DMN-treated rats at intervals less than 4 hours following the carcinogenic stimulus, the expression of morphological transformation is delayed progressively with decreasing exposure time. Thus, transformation usually becomes manifest at subculture six in the 2 and 3 hour test cultures and at subculture seven in the 1 hour isolate.

Such observations suggest that fewer cells with the potential for transformation exist in the 1 hour isolate than do in the 2 and 3 hour preparations and that these in turn contain fewer altered cells initially than the 4 hour isolate. That a graded effect is exerted on the target cells by DMN with increasing exposure time *in vivo* is suggested further by the finding of progressively increased numbers of morphologically transformed foci at subculture five from the 4 hour up to the 8 hour isolate, the point to which the serial hourly observations were taken. The demonstration of a graded target cell effect is compatible with the known secondary metabolic changes occurring in the kidneys of protein-deprived rats correlated with the degradation of DMN. Thus, it has been shown by other research groups that methylation of nucleic acids, inhibition of protein and RNA synthesis and structural damage to DNA are evident within 1 hour of DMN administration and progress with increasing severity over the ensueing 8 to 12 hour period.

Concomitant with the expression of morphological transformation, the test cells also acquire a greatly enhanced proliferative capacity relative to the normal cells in culture. This can be clearly demonstrated by means of an assay for DNA synthesis based on tritiated thymidine incorporation as discussed in a separate section of this report. Test cultures at the transformation passage are capable of levels of tritiated thymidine incorporation (13,557-65,762 DPM per 10^5 cells) some 5 to 24 times higher than the upper limit of the control cell range (690-2699 DPM per 10^5 cells). Preliminary autoradiographic analysis indicated as expected, that this dramatic upsurge in isotope incorporation is a function of greater replicating pool size within the test cell population; that is a greater proportion of the altered cell population engages in DNA synthesis than is characteristic of normal cell cultures. In this system therefore morphological transformation implies enhanced proliferative capacity associated with a significantly increased fraction of contributing cells.

Following the expression of morphological transformation, the test cultures exhibit a prolonged and possibly unlimited life span, maintaining the enhanced proliferative capacity as measured by DNA synthesis assay, through each successive subculture. The ability to proliferate into colonies at reduced seeding rates, that is at very low cell densities, is acquired several subcultures after expression of morphological transformation, usually at subculture eight or nine. The loss of anchorage dependence, expressed as the ability for colony formation in semi-solid media is delayed further still, usually to subculture fourteen. Results just to hand indicate that the altered cells are capable of producing tumours when injected into host rats at a later subculture still, thus confirming the malignant nature of the transformation process.

This series of experiments demonstrates that a few target cells committed by DMN to express morphological transformation *in vitro* are present in the rat kidney within 1 hour of the carcinogenic stimulus. Furthermore, it is likely from the results to hand, that increasing numbers of cells become committed with increasing exposure time although these studies have not attempted to establish the point at which this effect levels out. It is apparent also that cells isolated in culture from DMN-treated rats do not acquire all of the behavioural changes at one time. Rather, there seems to be a stepwise evolution of these altered growth properties during continuous *in vitro* culture.

Morphological character of renal cell cultures derived from DMN-treated rats.

G. C. Hard

A total of 20 cell lines derived from rats treated with a single carcinogenic dose of DMN have been prepared for *in vitro* culture in this laboratory and followed through serial passage to a stage beyond the expression of morphological transformation. Each culture has been characterized with respect to the morphological identity of the cell populations surviving in culture, and compared with a series of more than 40 normal rat kidney cell cultures.

In all cases, the primary kidney isolates from both control and DMN-treated rats consist of two major cell populations-islands of cohesive epithelium-like cells presumably derived from the nephrons, with attenuated or flattened mesenchyme-like cells of presumed origin from the intertubular connective tissue interspersed between. With the exception of two cultures from DMN-treated rats to be described later, epithelium in control and test cultures does not survive beyond subculture one, under the growth conditions employed. In these early stages, cells from treated rats exhibit signs of cytotoxicity such as vacuolation, nuclear fragmentation and various forms of mitotic anomaly which distinguish them from control cultures. However, for several passages subsequent to subculture one, control cultures and those from DMN-treated rats are indistinguishable, consisting of monolayers of relatively large mesenchymal cells, irregularly shaped or elongate, consistent with the form of fibroblast-like cells *in vitro.* In subcultures three and four, control cultures usually undergo senescence marked by an enlargement of cells to very expanded non-proliferating mesenchymal types incapable of forming confluent monolayers. In contrast to this inability to passage normal cells further, those from DMN-treated rats persist through subcultures three and four in a form closely resembling the immediately preceding passages. At subculture five, the morphological character of cells from DMN-treated rats changes abruptly with the expression of morphological transformation. The macroscopic foci represent densely-crowded, multi-layered aggregates of rapidly proliferating cells piled up in criss-cross fashion. This change is never seen in the control cultures regardless of the period of their life-span. The transformed cells within the foci, are mesenchymal in type but of smaller dimensions and with increased nucleus to cytoplasm ratio in comparison to the normal fibroblast-like cells of the population which forms a monolayer between the foci. Abnormal mitoses including chromosomal displacement, multipolar spindles and lagging chromosomes are prominent within the transformed foci.

Beyond the stage of transformation expression, succeeding subcultures are composed entirely of small mesenchyme-like cells similar to those of the dense foci. Although they are a relatively uniform population of polygonal cells, some pleomorphism is evident with forms including spindle-shaped, fusiform, triangular and giant cells, the latter with polymorphic or multiple nuclei. Consistently, a percentage of abnormal mitoses characterize the persisting populations. This general pattern of morphology in transformed renal cell populations originating from DMN-treated rats therefore closely resembles the *in vitro* appearance of tumour cells isolated from the DMN-induced renal mesenchymal neoplasms as has been described in earlier reports.

In 2 of the 20 test cultures a significant difference was noted from the remainder in that islands of cohesive epithelial cells survived serial passage beyond subculture one. By subculture four sheets of distinctive epithelial cells became progressively ascendant over the remaining mesenchymal cell population. The epithelial population displayed a marked variation in cell size and irregularity in shape with nucleolar hypertrophy, multi-nucleate giant cells, increased intercellular gaps and mitotic anomalies frequent. Thus the persisting epithelial cells were also of altered morphological character contrasting with the epithelium of primary isolates from normal rats. In fact these cells displayed altered behavioural properties as well in plating and cloning assays, consistent with morphological transformation. During serial passage of these 2 test cultures, altered mesenchyme also persisted and just beyond subculture ten, became the dominant population possessing the same morphological character as cells of the other 18 transformed mesenchymal lines.

Nevertheless, epithelial cells survived in very low numbers through succeeding subcultures indicating that longevity was retained in the face of culture conditions which were more favourable to the proliferation of the mesenchymal cell population.

The involvement in morphological transformation of mesenchymal cells in all test cultures and the prolonged survival concomitantly of altered epithelium in 2 of the 20, must be viewed in the light of events occurring *in vivo*. The dose of DMN used in these experiments induces tumours of mesenchymal type in the kidneys of all surviving rats along with epithelial tumours of renal tubule origin in approximately one-third. Thus the events *in vitro* reflect in terms of the surviving population type, the general incidence anticipated *in vivo*. It appears that the culture system represents a promising *in vitro* correlate of the animal model.

Application of a bioassay for DNA synthesis in populations of rat kidney cells in vitro.

B. W. Stewart and G. C. Hard

As described previously, an assay system has been developed for the appraisal of rates of DNA synthesis by means of thymidine incorporation. This assay has been used to assess the growth characteristics of cells cultured from the kidneys of DMN-treated rats as well as from untreated animals and DMN-induced renal mesenchymal tumours. The study has now been completed. Beyond the immediate, detailed quantitative data, sufficient cell populations have been studied to permit the following generalizations. (a) The thymidine incorporation assay has been shown to be a far more sensitive measurement of transformation than any parameter directly related to cell number. The change upon transformation in isotope incorporation is at least an order of magnitude greater than changes in cell number. This greater margin is of critical importance in assessment of any atypical transformation induced for example by chemicals of unknown biological activity. (b). The thymidine incorporation has been shown to be remarkably consistent between cell populations at the same biological stage but isolated from different animals. (c). There is a precise correlation between gross morphological characteristics and thymidine incorporation. This applies not only to the time of morphological transformation but to those occasions on which a predominantly epithelial culture rather than an exclusively mesenchymal culture develops. These correlations encourage experiments in which small changes in thymidine incorporation — in the absence of any morphological change — are sought as the earliest events preceding transformation. (d). In undertaking this study, sufficient cell populations have been studied to allow a first order quan-

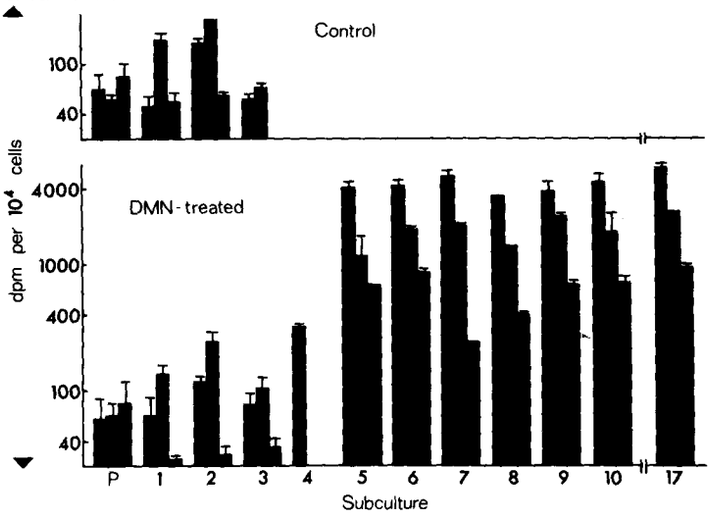


Fig 9
DNA synthesis assessed by tritiated thymidine uptake in sequential subcultures of kidney cells isolated from normal (control) rats and DMN-treated rats. Thymidine incorporation has been assessed on the first, second and fifth days after seeding of the cultures. Simultaneously with the expression of morphological transformation at subculture five, the proliferative capacity of the cells from carcinogen-exposed rats is markedly elevated at each successive passage, in contrast with their early subcultures and those from normal rats which reached senescence at subculture 3.

titation of rarer events in the present experimental system. For example, approximately 5 percent of control cultures persist beyond subculture four, confirming the value of a biochemical assay as an adjunct to any simple assessment based merely on the ability of the cells to survive *in vitro.* (Fig. 9).

DMN metabolism by rat kidney cells in culture

B. W. Stewart and G. C. Hard

In a series of experiments in which the toxic effects of DMN added to cultures of rat kidney cells is assessed by morphological and biochemical criteria, the initial results have indicated that such cells grown *in vitro* could not be considered as the exact counterparts of cells growing in kidney tissue *in vivo* in terms of their susceptibility to nitrosamine toxicity. At physiologically toxic concentrations, DMN has minimal effect on the renal cells of epithelial or mesenchymal types. The concentration of carcinogen was therefore increased and some slight toxic effect could be detected after the addition of very high doses of DMN to the culture medium e.g. 4000 ug per ml.

There was no obvious difference between renal mesenchymal tumour cell lines and cells recently isolated from normal rat kidneys. The data imply that the culture process is associated with a loss in microsomal demethylase activity. This biological event has critical importance in terms of the minimal requirements for *in vitro* cell culture systems used for assessment of carcinogenic activity. Experiments in which the "S9" fraction of rat liver homogenate, which contains demethylase activity is added to renal cells in culture, are in process. It has been established that the "S9" fraction in itself has a toxic effect on the growth characteristics of the kidney cells as demonstrated by the assay system for DNA synthesis utilizing incorporation of tritiated thymidine. Careful adjustment therefore of both the "S9" and carcinogen fractions is necessary for an optimum biological effect. In spite of these considerations, the effect of DMN on rat renal cell populations is minimal when compared with the toxicity demonstrated in isolated cells from other tissues under apparently the same conditions. The studies, considered overall, are virtually the only experimental system in which *in vitro* phenomena can be directly related to *in vivo* biology in terms of the chemical, the tissue of origin of the cells, and the types and growth characteristics of the particular tumours. It is desirable therefore to obtain as "complete" a picture as possible of all aspects of the *in vitro* addition of DMN to renal cells.

Reactivity of normal and neoplastic rat kidney cell populations with human smooth muscle antibody in vivo and in vitro.

G. C. Hard and B. H. Toh

Smooth muscle antibody (SMA) has been detected in the blood of some cancer patients indicating that actin-like contractile microfilaments may be present in many tumour cells. Previous work in the Department of Pathology and Immunology, Monash University Medical School has shown that in certain human tumours and chemically-induced experimental neoplasms there is an emergence of or increase in smooth muscle-associated antigen accompanying the neoplastic state. It has therefore been postulated by this group that the presence of antigen in the form of actin-like contractile protein may be involved with local tissue invasion by tumour cells. Extension of these studies to the DMN-rat renal tumour model holds advantages beyond that of simply exploring a system in which sequential development of the increased reactivity can be traced both *in vivo* and *in vitro* against a background of known tissue alterations. Any differential staining characteristics might aid the identification of various cell populations especially in the *in vitro* situation where distinction between epithelium and connective tissue mesenchyme is not altogether straightforward. Cells established in culture from DMN-induced renal mesenchymal tumours frequently acquire an epithelioid form which compounds their precise identification, particularly in view of the fact that the parent tumours contain numerous epithelial tubule profiles representing sequestered, pre-existing nephric elements. Thus determination of the reactivity with SMA of the various cell populations constituting the kidney tumours can aid in the interpretation and understanding of these complex neoplasms.

In the studies conducted, SMA serum obtained from a patient with active chronic hepatitis was used. This material gave a titre of 1 in 256 against rat smooth muscle and on specificity testing with muscle proteins was confirmed to be specifically

anti-actin in nature. The reactivity of the tissues under study was assessed in standard 'sandwich' immunofluorescence tests.

Reactivity with SMA in normal rat kidney tissue is localized to three main sites, the brush border and base of proximal renal tubules, the capillary loops and mesangial cells of glomeruli and the endothelium and smoother muscle of intertubular blood vessels. The resident interstitial cells of the outer zone show only weak, non-descript staining.

In contrast, the neoplastic cells of DMN-induced renal mesenchymal tumours in tissue section display a strong immunofluorescent staining of filamentous nature. Consistent with the epithelium of normal parenchyma, sequestered tubules are unstained but for the apical border of the constituent cells where the presence of microvilli would be expected.

Turning to the staining properties of these various populations transferred to cell culture conditions, we have to date examined control kidney cells, cell lines established from DMN-induced renal mesenchymal tumours and cells derived within 24 hours from rats receiving a carcinogenic dose of DMN after stage of morphological transformation expression. Cultures from normal rat kidneys consist by subculture two of monolayers of large, irregularly-shaped mesenchymal cells with branching processes suggestive of fibroblasts. These cells show fluorescence as fine parallel filaments extending throughout the long axis of each cell, a pattern confirming their suspected fibroblastic identity. In comparison, the highly pleomorphic cell populations established in long-term culture from renal mesenchymal tumours which include, bipolar, spindle, fusiform, stellate, triangular, polygonal and multinucleate giant cell forms show increased intensity of staining over their normal counterpart but with variation in fluorescence pattern dependent upon cell morphology. For example, spindle cells and some giant cells show a diffuse cytoplasmic fluorescence; polygonal cells show mainly peripheral staining, while other giant cells contain prominent fluorescent cytoplasmic filaments. In addition, nucleolar staining, and diffuse cytoplasmic fluorescence is a feature of some tumour cells. The increased intensity of staining with SMA was retained in those tumour cell cultures which had assumed a typically epithelioid appearance, indicating that the population had not altered in type from its mesenchymal origin nor that a previously "silent" epithelial cell population had suddenly become dominant. SMA reactivity in transformed cell lines derived from DMN-treated rats, like the tumour cells, is demonstrable as increased staining intensity when compared with normal renal fibroblasts in culture. The pattern however is more usually of thick, randomly-oriented overlapping filaments, which also distinguishes these cells from normal fibroblasts. Nevertheless, the reactivity confirms that these morphologically transformed cell lines are mesenchymal in nature.

Our continuing studies are directed towards a more detailed analysis of the staining patterns of individual cell forms in the mesenchymal tumours and to further characterization of SMA reactivity as a means for descriminating between renal epithelial and renal mesenchymal cell populations. Study of the staining patterns in cultures isolated from DMN-treated rats through serial subculture will, in conjunction with electron microscopic observations, provide some information on the acquisition of increased SMA reactivity.

Comparison of the range of childhood renal tumours with rat renal tumours

G. C. Hard

The histological range of rat kidney tumour types by a variety of chemical agents in general reflects an apparently similar morphological spectrum in man. The occurrence of a comparable group of tumour types in experimental animals, especially in high incidence situations such as in the DMN model where cellular origin and course of development can be investigated, raises the issue of what implications such animal models might have for the better interpretation and understanding of human kidney tumours. Determination of the relationships between experimental animal tumours and human cancers furthermore helps to provide some basis for possible extrapolation of animal data concerning the process and mode of cancer induction, to man.

Through the courtesy of Dr. P. Campbell, Royal Children's Hospital, Melbourne, the range of morphology presented by a series of 32 renal neoplasms of childhood has been examined.

These tumours have been compared with two particular neoplasms of the rat; firstly renal mesenchymal tumour as induced by such chemicals as DMN, nitrosomethylurea, ethyl methanesulphonate and preparations from various cyad species which include the compound sycasin; and secondly, true nephroblastoma, a neoplasm induced in low incidence by dimethylbenzanthracene under special conditions, or occurring spontaneously on occasion. Comparison of their respective histology, described in a previous Annual Report, demonstrates major differences between rat mesenchymaltumour and rat nephroblastoma, the one being a connective tissue neoplasm without encapsulation and the other epithelial, usually with capsule formation.

To date the two rat neoplasms have not been described as simultaneous or mixed tissues and separate existence is likely to imply separate cell origins. Investigations into the histogenesis of DMN-induced mesenchymal tumours in the rat indicate that they are derived from mesenchymal cells of connective tissue form present within the interstitial space of the outer zones of the kidney. Such an interstitial origin is consistent with the entirely mesenchymal nature of the ultimate tumour tissue. The histogenesis of rat nephroblastoma on the other hand has not been studied but the universal view ascribes the derivation of this neoplasm in mammals to the metanephrogenic blastema. The separate existence of rat mesenchymal tumour and rat nephroblastoma transgresses the traditional concept (in this species at least) that the two malignant tissue forms arise simultaneously by divergent differentiation from the bipotential metanephrogenic blastoma. The rat tumour histogenesis studies infer that the mesenchymal tumour arises from a cell line distinct from the renal blastema.

The 32 human tumours examined were from children ranging in age from 2 days to 13 years. In very broad terms, the histological forms of the tumours included (a) those consistent with the classification of congenital mesoblastic nephroma or leiomyomatous hamartoma, composed entirely of connective tissue mesenchyme incorporating occasional but distinct tubule profiles (b), tumours consisting predominantly of clumps or sheets of basophilic, nephrogenous cells displaying varying degrees of tubular differentiation and dissected by interconnecting stromal bands of mature reticular tissue with prominent intercellular matrix, fibroblasts, or smooth muscle fibres associated with blood vessel walls and (c), biphasic tumours consisting of both tissue forms, a nephrogenous epithelial component set in copious neoplastic connective tissue composed of embryonic mesenchyme, fibroblast-like spindle cells, smooth muscle, and on occasion, rhabdomyoblasts, mature striated muscle and islands of cartilage or bone.

It is apparent that the neoplastic tissue in rat nephroblastoma is identical in nature to the epithelial component of those childhood tumours classified as Wilms' tumour or nephroblastoma (b and c). Excluding those human Wilms' tumours in which an obvious neoplastic mesenchymal component is also present, the range of stromal morphology seen in the majority of the human tumours in this series (as in b) is similar to the range typifying rat nephroblastoma. This comparative observation suggests that the stroma in many human nephroblastomas might represent a benign, supportive reaction too, implying that the neoplasm is essentially epithelial in nature.

Notwithstanding certain differences, rat mesenchymal tumour appears to have its counterpart in the neoplastic mesenchymal tissue of congenital mesoblastic nephroma of infancy and those Wilms' tumours (as in c) which are truly biphasic in nature. In congenital mesoblastic nephroma and in post-infancy neoplasms consisting almost entirely of mesenchymal tissue but often designated as Wilms' tumours, occasional mature tubules may be found scattered through the outer areas of tumour sheets. The form of these epithelial profiles is identical to the tubules seen in rat mesenchymal tumour, where their derivation from preexisting parenchymal has been demonstrated.

It is likely that the tubule profiles in these particular human neoplasms may also arise by the same process. This is further supported by the fact that such tracts of tubule-containing tissue in the human tumours sometimes can be traced back to the junctional zone between neoplasm and unaffected renal tissue. It would seem that encapsulation precludes the sequestration or survival of such preexisting renal elements within the

biphasic or purely epithelial Wilms' tumours, except as engulfed inclusions in the fibrous reaction of the capsule itself.

In view of the seemingly unnecessary role for bipotential differentiation in explaining the character of certain rat kidney rumours which have cellular counterparts in the human, the possibility that the mixed forms of Wilms' tumour in man (those comprising neoplastic epithelial and connective tissue components as in c) represent composite tumours with the different components arising from two separate cell precursors, may be worthy of consideration. In possible conflict with this idea however, is the fact that a few Wilms' tumours show little delineation between the two forms of tissue, nephronoblast-like cells merging and apparently intermingling with fibroblast-like spindle cells in some areas. The issue is not likely to be easily resolved for special histological techniques such as perfusion fixation have proved essential in determining the nature and histogenesis of renal tumours in the rat. Studies analysing further the nature of childhood kidney neoplasms are continuing utilising electronmicroscopy and tissue culture.

Studies in progress
G. C. Hard

A series of additional studies incorporated within the general programme of activity of the Chemical Carcinogenesis group are currently in progress. Some of these have just commenced while others near completion and await data analysis.

1. The modulating effect of immunodepression and immunostimulation on the DMN rat kidney tumour model is under study. Immunodepression is achieved by neonatal thymectomy, anti-lymphocyte serum, or a combination thereof, whereas the effect of immunostimulation is provided by levamisole (Ethnor) administration. The investigation is not directed solely towards analysis of tumour incidence but to appraisal of the sequential events which occur in the kidney during the carcinogenic process as well.

2. A long-term experiment designed to determine the dependence of kidney tumour induction by DMN on age of administration and gender, is nearing completion. Rats of both sexes have been dosed at monthly intervals from birth, with a single high dose of the carcinogen.

3. In collaboration with Dr. B. W. Stewart of Department of Pathology, University of N.S.W., Kensington, assessment of the occurence and persistence of structural alterations, such as single-strand breakage in the DNA of kidney cells isolated from DMN-treated rats has just commenced. The *in vivo/in vitro* system provides an opportunity to observe such consequences of the carcinogenic treatment relative to the emergence of a population of morphologically-transformed cells.

4. Following the demonstration that kidney cells derived from rats treated with a single carcinogenic dose of DMN express morphological transformation *in vitro,* similar experiments with alternative renal carcinogens and renal toxins are presently in progress. As in the DMN system, the chemical is administered to the living rat and the kidneys removed at a later stage for preparation for cell culture. Such *in vitro* experiments are being conducted with (a) nitrosomethylurea, ethyl methanesulphonate and methylazoxymethanol, agents which are associated with renal mesenchymal tumour induction (b), diethylnitrosamine, an inducer of epithelial kidney tumours (c), the renal toxins, mercuric chloride and glycerol which have no known carcinogenic association and (d), the non toxic, non carcinogenic nitrosocopound, diphenylnitrosamine.

5. The conditions of cell culture currently employed in our studies based on the use of Waymouths medium MB 752/1, favour the survival of rat mesenchyme but not renal epithelium. The latter is regarded as notoriously difficult to propagate. Various modifications to the culture conditions are being tested in an attempt to prolong the survival of rat epithelium *in vitro* and to enable continuous culture of cells from renal epithelial tumours. Achievement of this aim is regarded as essential if a broad spectrum of events in renal carcinogenesis is to be explored.

6. A long-term, in-progress experiment involving diethylnitrosamine administration to rats under various conditions has the intent of developing a high incidence system which will permit a meaningful and in depth sequential analysis of the development of epithelial tumours of the kidney.

7. With the support of the Marine Pollution Studies Group from the fisheries and

Wildlife Division, a study is being carried out to assess the possible occurrence of and seasonal fluctuation in neoplasms in species of bottom-feeding fish found in Port Phillip Bay. Overseas experience suggests that ecological variation in skin tumour incidence in fish may prove to be a useful monitoring system for chemical contamination of the marine environment. Port Phillip Bay possesses certain unusual hydrological features that could ideally suit the requirements of a study examining the applicability of fish as an indicator system for chemical or other hazards associated with pollution.

Electronics Laboratory and Mechanical Workshop

The Electronics Laboratory and Mechanical Workshop play an absolutely indispensable role in the work of the Institute. The maintenance of the great variety of electronic and mechanical equipment, while at the same time designing new pieces of apparatus that cannot readily be purchased requires excellent organisation. Ron Wall, Engineer-in-Charge, and Kevin Harvey, John Baird and Frank Forgione manage to do the next to impossible with the very great range of problems that have arisen. The design of an all purpose modular power supply unit for use with locally designed equipment and improvements in printed circuit techniques has facilitated the manufacture of equipment in the Institute. The Electronics Workshop is run jointly by the Institute and Alfred Hospital and contributes to the work of the latter particularly in relation to the work of Clinical Research Unit.

New projects completed during the year include an improved design of the Doppler flowmeter assembly, a recorder for measuring ambulant blood pressure in man, an analogue computer for measuring myocardial contractility in the heart, a gauge for measuring changes in the dimensions of the heart, particularly for use in foetal animals. In addition, there have been many important improvements to countless items of equipment. Ron Wall is organising a group of seminars with other hospital and medical research engineers to provide a larger forum for increased communication between this important section of the medical research effort.

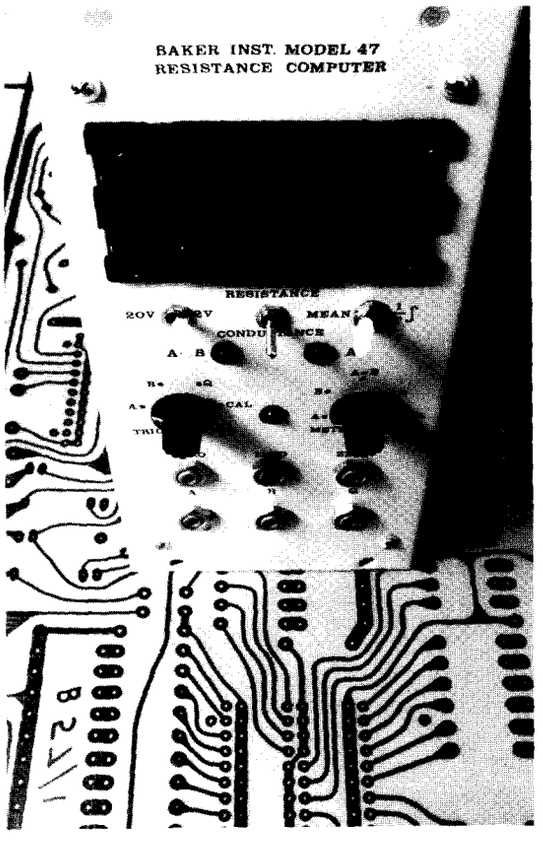


Fig M
***Workshop** — Analogue computer for determining resistance to blood flow designed at the Baker Institute, placed on diagram of a printed circuit board.*

Rouse Library

With the arrival of the Cardiovascular Metabolism and Nutrition Research area some new journals and monographs have been purchased to cater for this important area of cardiovascular medicine. The overall cover in our fields of interest is now satisfactory, and Monash University and Alfred Hospital Library provide excellent co-operation. The library, though small, is absolutely invaluable and is being used to an ever increasing extent.

Operating Theatre

The number of people in the Institute using chronic instrumented preparations has increased greatly for both large and small animals. It is still well within the operating capacity of our theatres. However, it is clear that our holding facilities will require expansion in the very near future. This will be particularly necessary in relation to the possible foundation of an Experimental Cardiac Surgery Laboratory in collaboration with the C. J. Officer Brown Cardiothoracic Surgical Unit.

Secretarial Staff (left to right): Aina Martin, Judith Dods (C.R.U.), Rose Baldi, Antonia Lopez.

Publications

CIRCULATORY CONTROL & HYPERTENSION RESEARCH UNIT

Published or accepted for publication:

W. P. ANDERSON, P. I. KORNER, A. BOBIK and J. P. CHALMERS.
Leakage of dl-propranolol from cerebrospinal fluid to the bloodstream in the rabbit. *J. Pharmacol. Exp. Therapeutics* (in press)

J. A. ANGUS, P. I. KORNER and M. J. WEST.
Assessment of autonomic components of resting hindlimb vascular resistance and reactivity to pressor substances in renal hypertensive rabbits. *Clin. Sci. & Mol. Med.* 51: 57s-59s, 1976.

J. A. ANGUS, A. BOBIK and P. I. KORNER.
Effects of histamine bolus injections and continuous infusions on the H_1-and H_2-receptors in the hindlimb vessels of the rabbit. *Clin. & Exp. Pharmacol. & Physiol.* (in press).

J. A. ANGUS and P. I. KORNER.
Regional vascular resistance and heart rate responses mediated through H_1-and H_2-histamine receptors in the unanaesthetized rabbit. *European J. Pharmacol.* (in press).

J. A. ANGUS, A. BOBIK, P. I. KORNER and M. T. STONEHAM.
Guanethidine vasodilation in the rabbit mediated by endogenous histamine. *Brit. J. Pharmacol.* (in press).

P. J. FLETCHER, P. I. KORNER, J. A. ANGUS and J. R. OLIVER.
Changes in cardiac output and total peripheral resistance during development of renal hypertension in the rabbit: lack of conformity with the autoregulation theory. *Circulation Research,* 39: 633-639, 1976.

P. J. FLETCHER, P. I. KORNER, J. A. ANGUS and J. R. OLIVER.
Cardiac output changes during experimental renal hypertension in the rabbit. *Clin. Sci. & Mol. Med.* 51: 137s-139s, 1976.

D. GANTEN, J. S. HUTCHINSON, H. HAEBARA, P. SCHELLING, M. FISCHER and V. GANTEN.
'Tissue iso-renins'. *Clin. Sci & Mol. Med.* 51: 117s-120s, 1976.

D. GANTEN, J. S. HUTCHINSON, V. GANTEN and P. SCHELLING.
'The intrinsic iso-renin angiotensin system in brain and its relationship to the classical kidney renin angiotensin system'. *In:* Central Nervous Control of Na Balance. George Thieme Verlag, Stuttgart, 1976.

D. GANTEN, J. S. HUTCHINSON, P. SCHELLING, V. GANTEN and M. FISCHER. The iso-tenin angiotensin in extrarental tissue. *Clin. & Exp. Pharmacol. & Physiol.* 3: 103-126, 1976.

J. S. HUTCHINSON, P. SCHELLING, J. MOHRING and D. GANTEN.
Pressor action of centrally perfused angiotensin II in rats with hereditary hypothalamic diabetes insipidus. *Endocrinology* 99: 819-323, 1976.

J. S. HUTCHINSON, P. SCHELLING, J. MOHRING and D. GANTEN. Effect of intraventricular perfusion of angiotensin II in conscious normal rats and in rats with hereditary hypothalamic diabetes insipidus. *Clin. Sci. & Mol. Med.* 51: 391s-394s, 1976.

J. S. HUTCHINSON, D. GANTEN, P. SCHELLING, P. YLITALO, J. MOHRING and M. KALINA.
Central pressor actions of Angiotensin II. *Acta Medica Academiae Scientiarum Hungaricae* 33 (in press).

M. IRIKI, PATRICIA K. DORWARD and P. I. KORNER.
Baroreflex 'resetting' by arterial hypoxia in the renal and cardiac sympathetic nerves of the rabbit. *Pflugers Archiv.* (in press).

P. I. KORNER.
Central control of blood pressure: implications in the pathophysiology of hypertension. *In:* Regulation of blood pressure by the central nervous system. ed. G. Onesti, M. Fernandes, K. E. Kim. New York, Grune & *Stratton, 1976, pp 3-20.*

P. I. KORNER.
Problems of integrative cardiovascular control. Proc. Aust. Physiol. and Pharmacol. Soc. 7: 35-48, 1976.

P. I. KORNER and P. J. FLETCHER.
The role of the heart in causing and maintaining hypertension. *Cardiovascular Medicine,* 2: 139-155, 1977.

P. I. KORNER, A. M. TONKIN and J. B. UTHER.
Reflex and mechanical circulatory effects of graded Valsalva maneuvers in normal man. *J. Appl. Physiol.* 40: 434-440, 1976.

P. I. KORNER, P. A. BLOMBERY, A. BOBIK, A. M. TONKIN and J. B. UTHER.
Valsalva constrictor reflex in human hypertension and after beta adrenoreceptor blockade in conscious rabbits. *Clin Sci. & Mol. Med.* 51: 365s-368s, 1976.

P. SCHELLING, J. S. HUTCHINSON, V. GANTEN, G. SPONER and D. GANTEN.
Permeability of the blood cerebrospinal fluid barrier for angiotension II in rats. *Clin. Sci. & Mol. Med.* 51: 399s-402s, 1976.

Submitted

W. P. ANDERSON, J. A. ANGUS, P. I. KORNER and C. I. JOHNSTON.
The role of cardiac output during graded renin-dependent one kidney hypertension.

W. P. ANDERSON, P. I. KORNER, J. A. ANGUS, C. I. JOHNSTON and D. CASLEY
Effects of graded renal artery constriction and systemic and renal haemodynamics in different experimental models.

W. P. ANDERSON, P. I. KORNER, J. A. ANGUS, C. I. JOHNSTON and D. CASLEY.
Relation between renin angiotensin system and systemic and renal haemodynamics in graded renin dependent hypertension.

J. A. ANGUS, P. J. FLETCHER and P. I. KORNER.
Guanethidine vasodilation in different regional beds of autonomically blocked rabbits.

M. de DALY, P. I. KORNER, J. E. ANGELL-JAMES and J. R. OLIVER.
Cardiovascular and respiratory effects of carotid body stimulation in the monkey and interactions with some upper respiratory tract reflexes.

DEVELOPMENTAL BIOLOGY RESEARCH UNIT

Published or accepted for publication

D. ALCORN, T. M. ADAMSON, J. E. MALONEY, B. C. RITCHIE, T. F. LAMBERT and P. M. ROBINSON.
Morphological effects of chronic tracheal ligation and drainage in the foetal lamb lung. *Journal of Anatomy* (London).

R. EHRENKRANZ, A. M. WALKER, G. OAKES, M. McLAUGHLIN and R. A. CHEZ.
Effects of ritodrine infusion on uterine and umbilical blood flow in pregnant sheep. *Amer. J. of Obst. & Gynecol.* 126: 343-349, 1976.

J. E. MALONEY, J. CANNATA and B. C. RITCHIE.
The influence of transpulmonary pressure on the diameter of small arterial blood vessels in the lung. *Microvascular Research.* 11: 57-66, 1976.

G. OAKES, A. M. WALKER, R. EHRENKRANZ, R. CEFALO and R. A. CHEZ.
Uteroplacental blood flow during hyperthermia with and without respiratory alkalosis. *J. Appl. Physiol.* 41: 197-201, 1976.

G. OAKES, A. M. WALKER, R. EHRENKRANZ and R. A. CHEZ.
Effect of propranolol infusion on the uterine and umbilical circulations of pregnant sheep. *Amer. J. of Obst. & Gynecol.* (in press).

J. J. SMOLICH, BERNICE F. STRATTFORD, J. E. MALONEY and B. C. RITCHIE.
The development of the upper respiratory epithelium of the rat. *Journal of Anatomy.* (London).

A. M. WALKER, G. OAKES, M. McLAUGHLIN, R. EHRENKRANZ and R. A. CHEZ.
Effects of hypercapnia on uterine and umbilical circulations in conscious pregnant sheep. *J. Appl. Physiol.* (in press).

Submitted for publication

R. A. CHEZ, R. EHRENKRANZ, G. OAKES, A. M. WALKER, L. HAMILTON, S. BRENNAN and M. McLAUGHLIN.
Effects of adrenergic agents on ovine umbilical and uterine blood flows.

R. EHRENKRANZ, L. HAMILTON, S. BRENNAN, G. OAKES, A. M. WALKER and R. A. CHEZ.
Effects of salbutamol and isoxuprine on uterine and umbilical blood flow in pregnant sheep.

R. EHRENKRANZ, A. M. WALKER, G. OAKES, L. HAMILTON and R. A. CHEZ.
Effect of Fenoterol (Th1165a) infusion on uterine and umbilical blood flow in pregnant sheep.

J. E. MALONEY, J. CANNATA, MARGARET H. DOWLING, WENDY ELSE and B. C. RITCHIE.
Baroreflex activity in conscious foetal and newborn lambs.

A. M. WALKER, J. CANNATA, MARGARET H. DOWLING, B. C. RITCHIE and R. E. MALONEY.
Autonomic components of the heart rate response to hypoxia in conscious foetal and newborn lambs.

A. M. WALKER, J. CANNATA, MARGARET H. DOWLING, B. C. RITCHIE and J. E. MALONEY.
Cardiac response to hypotension in conscious foetal and newborn lambs.

A. M. WALKER, G. OAKES, M. McLAUGHLIN, R. EHRENKRANZ, D. ALLING and R. A. CHEZ.
Twenty-four hour rhythms in uterine and umbilical blood flows of conscious pregnant sheep.

Abstracts

D. ALCORN, T. M. ADAMSON, J. E. MALONEY, B. C. RITCHIE and P. M. ROBINSON.
Morphological effects of phrenectomy in the foetal lamb lung. *Journal of Anatomy* (London). (in press).

D. ALCORN, T. M. ADAMSON, T. F. LAMBERT, J. E. MALONEY, B. C. RITCHIE and P. M. ROBINSON.
The effects of tracheal *ligation* on the morphology of developing foetal lamb lung. *Journal of Anatomy* (London). 121:403, 1976.

D. ALCORN, T. M. ADAMSON, T. F. LAMBERT, J. E. MALONEY, B. C. RITCHIE and P. M. ROBINSON
The effects of *tracheal ligation and tracheal drainage* on the morphology of the developing foetal lamb lung. *Australian Paediatric Journal.* 11: II, 124, 1975.

J. E. MALONEY, T. M. ADAMSON, V. BRODECKY, MARGARET H. DOWLING and B. C. RITCHIE.
Modification of breathing movements of the foetus 'in utero'. *Australian Paediatric Journal.* 11: II 124, 1975.

J. E. MALONEY, V. BRODECKY, J. CANNATA, MARGARET H. DOWLING, WENDY ELSE and B. C. RITCHIE.
Control of arterial blood pressure in unanaesthetised foetal and newborn lambs. Australian Paediatric Journal. (in press).

CANCER RESEARCH UNIT

Published or accepted for publication

R. BORLAND, S. M. METCALFE and G. C. HARD.
A combined in vivo-in vitro approach to studies of nitrosamine-induced carcinogenesis. *In:* Screening Tests In Chemical Cardinogenesis. Ed. R. Montesano, H. Bartsch and L. Tomatis. LARC Scientific Publications, Lyon. 12: 433-444, 1976.

G. C. HARD and P. GRASSO.
Nephroblastoma in the rat: histology of a spontaneous tumor, identity with respect to renal mesenchymal neoplasms, and a review of previously recorded cases. *J. Natl. Cancer Institute.* 57: 323-329, 1976.

G. C. HARD.
Proliferating cell populations in the early phase of dimethylnitrosamine-induced renal carcinogenesis. *In:* Detection and Prevention of Cancer. Ed. H. E. Nieburgs, 3: (in press).

G. C. HARD.
The nature of experimentally induced renal tumors of the rat, and possible implications for human renal cancer. *In:* Detection and Prevention of Cancer. Ed. H. E. Nieburgs, 3: 1976. (in press).

G. C. HARD and R. BORLAND.
Morphological character of transforming renal cell cultures derived from rats dosed with dimethylnitrosamine. *J. Natl. Cancer Institute.* 1977. (in press).

G. C. HARD, H. KING, R. BORLAND, B. W. STEWART and B. DOBROSTANSKI.
Length of *in vivo* exposure to a carcinogenic dose of dimethylnitrosamine necessary for subsequent expression of morphological transformation by rat kidney cells *in vitro. Oncology.* 1977. (in press).

G. C. HARD, D. N. SKILLETER and E. REINER.
Correlation of pathology with distribution of Be following administration of beryllium sulfate and beryllium sulfosalicylate complexes to the rat. *Exp. Molec. Path.* 1977. (in press).

G. C. HARD.
Experimental tumours. In: *Scientific Foundations* in Urology. Ed. D. I. Williams and G. C. Chisholm. William Heinemann Medical Books Ltd., Vol. 2: 255-264, 1976.

G. C. HARD and B. H. TOH.
Immunofluorescent characterization of rat kidney tumors according to the distribution of actin as revealed by specific anti-actin antibody. *Cancer Research.* 1977. (in press).

G. J. HOPKINS, C. E. WEST and G. C. HARD.
Effect of dietary fats on the incidence of 7, 12-dimethylbenz — (a) anthracene-induced tumors in rats. *Lipids.* 11: 328-333, 1976.

B. W. STEWART and G. C. HARD.
The biochemistry of morphological transformation. Proc. Clin. Oncol. Soc. Austr. *Cancer Forum.* 7: 56,1976.

B. W. STEWART and G. C. HARD.
Distinctive patterns of proliferative activity in kidney cell cultures derived from normal, dimethylnitrosamine-treated and renal tumor-bearing rats. *J. Natl. Cancer Inst.* 1977. (in press).

B. H. TOH, G. C. HARD, M. N. CAUCHI and H. K. MULLER.
Smooth muscle-associated contractile protein in renal mesenchymal tumour cells and in transformed cells derived from dimethylnitrosamine-injected rats. *Brit. J. Cancer.* 34: 533-545, 1976.

B. H. TOH, M. N. CAUCHI, H. K. MULLER and G. C. HARD.
The relevance of smooth muscle-associated antigens in cancer. *In:* Detection and prevention of Cancer. Ed. H. E. Nieburgs, 3: 1976. (in press).

SEMINAR PROGRAMME — 1976

Date	Title	Lecturer
20 February	Somatostatin	Dr. Y. Patel Prince Henry's Hospital
2 March	Some observations on Substance P.	Dr. M. L. Mashford St. Vincent's Hospital
19 March	Cold paws — warm heart (Vasodilator nerves to the dog paw)	Dr. C. Bell Department of Physiology University of Melbourne
2 April	Balloon counter-pulsation in experimental myocardial ischaemia	Dr John Shaw Royal Melbourne Hospital
23 April	The role of prostaglandins in hypertensive disease	Professor A. L. Boura Department of Pharmacology Monash University
7 May	Some factors involved in the pathogenesis of perinephritic hypertension in rabbits	Dr. P. Fletcher Baker Institute
21 May	Cutaneous sensation	Professor I. Darian-Smith Department of Physiology University of Melbourne
4 June	Regional differentiation of sympathetic efferents	Professor M. Iriki S. A. Smith Visiting Life Insurance Fellow Baker Institute
18 June	Organization of the primate motor cortex for movement performance	Professor R. Porter Department of Physiology Monash University
2 July	Renal hypertension following graded renal artery stenosis in conscious dogs	Dr. W. Anderson and Dr. J. Angus Baker Institute.
16 July	Prejunctional adrenergic mechanisms	Dr. M. McCulloch Department of Pharmacology University of Melbourne
30 July	Comparison of actions of 6-hydroxydopamine and guanethidine on the rat brain	Professor G. Singer Department of Psychology La Trobe University
20 August	Some integrative mechanisms in cardiovascular control	Professor M. de. B. Daly Department of Physiology, St. Bartholomew's Hospital
3 September	Pharmacodynamics and pharmacokinetics of prindolol	Dr. A. Bobik *C.R.U.*
10 September	Control of utero-placental circulation in the pregnant sheep	Dr. A. Walker Baker Institute
1 October	Inactive forms of renin and their relevance to hypertension	Dr G. Boyd Department of Medicine Austin Hospital
14 October	Applied history of lung ultra structure	Professor Lynne Reid Harvard Medical School
15 October	Intra-renal angiotensin II	Dr. F. Mendelsohn Department of Medicine Austin Hospital
29 October	Studies in the development foetal heart	Dr. J. Maloney Baker Institute
12 November	Coronary blood flow and the hypertrophic heart	Professor David Kelly Hallstrom Institute of Cardiology, Royal Prince Alfred Hospital, Sydney.
3 December	Endogenous and exogenous histamine — new facts about an old autacoid	Dr J. Angus Baker Institute
10 December	Central control of the circulation	Professor P. I. Korner Baker Institute

OVERSEAS VISITS

Dr. Maloney presented a paper entitled 'Control of the Foetal Respiratory System' at the International Symposium, VTH European Congress of Perinatal Medicine, in June 1976 in Malmo, Sweden.

Dr. Hard visited the Third International Symposium on Detection and Prevention of Cancer in New York in April 1976. He delivered the following papers:

1. The nature of experimentally induced renal tumors of the rat, and possible implications for human renal cancer.
2. Proliferating cell populations in the early phase of dimethylnitrosamine-induced renal carcinogenesis.
3. The relevance of smooth muscle-associated antigens in cancer.

LECTURES AND MEETINGS

Professor Korner, Dr. Angus, Dr. Anderson, Dr. Angell-James, Dr. Blombery and Dr. Fletcher gave papers at the Fourth Meeting of the International Society of Hypertension in Sydney in February.

The 50th Anniversary of the Institute was celebrated at the Baker International Hypertension Workshop, as discussed in detail elsewhere in this report. The meeting was assisted by Ciba-Geigy Australia Limited.

Professor Korner participated in an International Conference by Satellite on 8th August 1976 sponsored by the National Heart Foundation and Merck, Sharpe and Dohme, involving participants in Australia (Sydney and Melbourne), New Zealand and London, U.K. He discussed 'Potassium and the Heart'.

Dr. J. E. Angell-James and Professor Michael Daly gave a paper on 'Carotid sinus baroreceptor reflex in the seal and its modification during diving' at the August meeting of the Australian Physiological and Pharmacological Society, based on their work in Alaska done in collaboration with Dr. Robert Elsner in the summer of 1975. At the same meeting Professor M. Iriki, Dr. Patricia Dorward and Professor Korner discussed 'Baroreflex "resetting" in the renal and cardiac sympathetic nerves during arterial hypoxia in the rabbit'.

On 21st September Dr. Paul Nestel talked at a symposium sponsored by the National Heart Foundation on 'The heart risk patient — overcoming the problems of motivation and lipid management'. His talk was entitled 'Diagnosis and when to start lowering lipids'.

On 11th November the Directors of the National Heart Foundation of Australia (Victorian Division) visited the Institute and saw displays of the work of all three units and the work of Clinical Research Unit.

Dr. Maloney gave seminars in April at the Howard Florey Institute of Medical Research on 'Development of the respiratory system'; to the Paediatric Research Society of Australia in Canberra on 'Control of arterial blood pressure in the foetus and newborn'; at the Austin Hospital on 'Development of the lung'. In August he delivered a seminar on 'Breathing before birth' at the Section of Thoracic Medicine, Alfred Hospital; and participated in a symposium on neonatal breathing in the Department of Medicine, University of Sydney.

TEACHING

Members of the staff participated in the special hospital F.R.A.C.P. (Part 1) training programme and the Advanced Training Programme in Cardiology (F.R.A.C.P., Part II) by giving six lectures on Clinical Cardiovascular Pharmacology. Topics considered were (1) Drug Kinetics — Beta Blocking drugs; (2) Hypertension; (3) Cardiac Stimulants; (4) Anti-arrhythmias; (5) Diuretics and oedema; (6) Coronary Disease. Professor Korner also contributed to 6th year medical undergraduate teaching in Medicine at the Alfred Hospital, and gave some correlation seminars in the Departments of Physiology and Pharmacology. Dr. Angus and Dr. Anderson participated in the Science Honours course in Pharmacology, Monash University, while Dr. Fletcher and Dr. Blombery took part in 4th year teaching in Medicine. Dr. Maloney gave lectures on respiration and on foetal physiology in the Department of Physiology, University of Melbourne. Dr. Maloney has participated in undergraduate teaching in both Monash and Melbourne University. At Monash he gave lectures on respiratory failure and respiratory disease, whilst at Melbourne University he gave a course of 7 lectures on respiratory physiology. He has participated in postgraduate teaching at the Royal Australasian College of Surgeons where he gave two lectures on respiratory function.

APPOINTMENTS

Professor Korner is a Member, National Health and Medical Research Council (representing the Royal Australasian College of Physicians); Chairman, National Committee of Physiology, Australian Academy of Science; Member, National Committee of Pharmacology, Australian Academy of Science; Member, Commission on Cardiovascular Physiology, International Union of Physiological Sciences. He is Chairman, Advisory Committee on Advanced Training in Cardiology, Royal Australasian College of Physicians and a member of the committee of management National Blood Pressure Study.

Dr. Nestel is Chairman, Diet and Heart Committee, National Heart Foundation of Australia; Member, Medical and Scientific Advisory Committee, National Health and Medical Research Council; Member, Education Committee, National Heart Foundation of Australia (Victorian Division).

New Arrivals 1977

A welcome arrival for the Circulatory Control and Hypertension Research Unit at the beginning of 1977 is Dr. Murray Esler who will be joining us as N. H. and M. R. C. Research Fellow. He is a graduate from the University of Melbourne (M.B.B.S., B. Med. Sci.) and the Australian National University (Ph.D.). He has had extensive clinical research experience in the field of hypertension. For the last three years he was Assistant Professor in Internal Medicine, University of Michigan, Ann Arbor, U.S.A.

An overseas visitor expected in the latter half of 1977 is Professor Ishio Ninomiya from Hiroshima University, Japan. He is an expert in autonomic neurophysiology.

GRANTS AND DONATIONS

The Baker Medical Research Institute receives its largest donation from the Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions. This donation covers approximately one-third of our current budget and has been an essential element in our survival. However, support from other sources has been most necessary. We are most grateful for the support that we have received from the National Health and Medical Research Council, the State Government of Victoria, the Life Insurance Medical Research Fund of Australia and New Zealand, the National Heart Foundation of Australia, and from the United States National Institutes of Child Health and Human Development. The support of the Alfred Hospital Research Fund is gratefully acknowledged. The Cancer Research Unit was supported by the Anti-Cancer Council of Victoria.

The Institute is particularly indebted to the donors who have generously supported the research effort of the Institute, who are listed below.

Victorian State Government	37,500.00
Boehringer Ingelhein Pty. Ltd.	26,500.00
Estate D. A. Parker	17,950.00
Ciba-Geigy Australia Ltd.	11,000.00
Merck Sharp and Dohme (Aust) Pty. Ltd.	10,000.00
Sandoz (Aust) Pty. Ltd.	10,000.00
Felton Bequest	7,000.00
Ian Potter Foundation	6,000.00
The James and Elsie Borrowman Research Trust	5,600.00
William Anglis (Vic) Charitable Trust	4,000.00
The George Thomas Lockyer Potter Charitable Trust	3,800.00
H. & L. Hecht Trust	3,000.00
Estate Edward Wilson	2,500.00
I.C.I. Australia Ltd.	2,000.00
Appel Family Bequest	1,800.00
Anonymous	1,500.00
Australian Cancer Society	1,500.00
Bell Charitable Trust	1,250.00
Estate Marian and E. H. Flack	1,100.00
Estate Percy Baxter	1,000.00
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Alan F. Drayton	25.00
Geo. Turner Pty. Ltd.	20.00
Peter Shaw	10.00
	$161,956.63

Further contributions were received from —
Australian Eagle Insurance Co. Ltd., 2/14 Australian Field Regiment Association, Mrs E. M. Bambery, Mr. R. L. Blakemore, Mr. & Mrs. M. A. Cuming, Mr. & Mrs. J. Dickie, Mr. J. C. Habersberger, Mrs. Hazel Hills, J. A. Kemp, Kodak (Aust) Pty. Ltd., Geo. E. Knox, Miss H. McPherson, Mrs. T. G. Martin, N. H. Payne, Mrs. M. Rumble, J. E. Thomas, Marian, Ian and Geoffrey Woodside.

In Memory of —
Sir Kenneth Adamson, R. L. Archer, H. Bambery, Bruce Blackburn, Dennis Bowden, Mrs Stella Cheadle, W. Gendall, Richard Gibbs, Arthur Guthrie, Mrs Joan D. Harper, Mrs Irene Hogan, Professor Ronald Francis Jackson, Mrs. N. Jenkins, Jim Jeffrey, John Stott Kirkham, K. M. Kilner, Mrs. E. B. Laycock, A. G. McArthur, Lady Gladys McConnan, Arnold Molloy, Brigadier A. D. Molloy, Frank L. Norris, A. Pullman, Douglas Robinson, H. C. Rowlands, Harold Salter, L. Wiedsmith, Mrs. Myrtle Wight.
Total: $277.00

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

Revenue account for the year ended 31st December 1976

EXPENDITURE	
Salaries and wages	461,227
Laboratory supplies and isotopes	73,060
Additional equipment and building costs	81,343
Library maintenance	8,723
Postage and telephone	5,365
Printing and stationery	5,627
Light and power	31,669
Insurance	19,655
Repairs and renewals	16,248
Animal house contribution	7,000
Sundries	6,040
Travelling expenses	12,468
Public relations	1,462
Stanhope Court	2,097
	$731,984

INCOME		
Donations from Baker Benefactions		
Statutory amount	11,569	
Transfers from Endowment Fund	212,800	224,369
Donations other		32,935
Grants-in-Aid of Research Projects		
Anti-Cancer Council	28,633	
Life Insurance Medical Research Fund of Australia and New Zealand	44,621	
National Health and Medical Research Council	106,666	
National Heart Foundation of Australia	18,534	
Department of Health, Education and Welfare (USA)	14,676	213,130
Other Grants		
The Laura Nyulasy Research Scholarship Fund	619	
The James and Elsie Borrowman Research Trust	5,600	
The William Buckland Research Fund	5,006	
Victorian State Government	37,500	48,725
Interest from Investments		
Held by Trustees of The Baker Institute Grant Trust	4,419	
Other investment income	115,529	119,948
Other Income		
Rentals	24,703	
Sundry sales, recoveries and refunds	66,915	91,618
Deficit for the year		1,259
		$731,984

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

The Trustees, Executors & Agency Co. Ltd. is the custodian and investment manager of some of the investments of the Institute. These investments included in the balance sheet of the Institute are in accordance with statements provided by the custodian company giving details of the Institute's entitlement in securities held by the custodian company in its own name but it has not been practicable for us to carry out normal audit procedures to confirm those investments or the income arising therefrom.

Subject to the above reservations in our opinion, the balance sheet together with the notes thereto, as set out on schedules 2 to 8 is properly drawn up to show a true and fair view of the state of the Institute's affairs at 31 December 1976.

PRICE WATERHOUSE & Co.
M. J. McNULTY
A member of the firm,
Chartered Accountants.

Melbourne
2nd March 1977

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

Balance Sheet as at 31st December, 1976

ACCUMULATED FUNDS AND LIABILITIES

Maintenance Fund		
	Accumulated deficit brought forward (10,691)	
	Deficit for year ..... (1,259)	
	Accumulated deficit	(11,950)
	Bank overdraft	4,023
	Sundry creditors and Accrued expenses	37,242
		29,315
Endowment Fund		
1,269,868	Accumulated fund	1,357,012
1,269,868		1,357,012
Research and Scholarship Funds		
	Restricted fund	121,126
	Edgar Rouse Memorial Fellowship Fund	35,620
	Laura Nyulasy Scholarship Fund	2,863
	William Buckland Research Fund	21,946
	Lang Research Scholarship Fund	4,852
120,928		186,407
$1,398,426		$1,572,734

MAINTENANCE FUND ASSETS

	Cash on hand	100
	Sundry debtors	14,215
	Short term deposits (at cost) held by Trustees of the Institute	15,000
		29,315

Endowment Fund Assets

	Investments (at cost):	
	Held by Trustees of the Institute Government and semi-government stock 88,296	
	Shares and debentures in companies 115,636	
	Short term deposits . 113,788	
	Mortgage loans 491,500	
		809,220
	Held by The Trustees, Executors & Agency Co. Ltd.	
	Shares in companies 62,413	
	Trust units 473,719	
	Short term deposits. 7,200	
		543,332
	Cash at Bank 4,460	
		1,357,012

Research and Scholarship Fund Assets

	Investments (at cost):	
	Held by Trustees of the Institute	
	Shares in companies . . 4,852	
	Short term deposits . . 50,900	
		55,752
	Held by The Trustees, Executors & Agency Co. Ltd. 27,610	
	Cash at bank 103,045	
		186,407
		1,572,734

Notes to the Balance Sheet at 31st December 1976

1. Expenditure included in present or past periods on fixed assets including laboratory equipment, motor vehicles, buildings, improvements and furniture and fittings has been charged against appropriate funds, grants or revenue accounts.
   The insured value of all assets at 31 December 1976, including the building, totalled $3,500,000.
2. There is a commitment amounting to $691 for the balance of the purchase price of investment in quoted ordinary shares.
3. The Laura Nyulasy Research Scholarship Fund and the William Buckland Scholarship Fund are both managed by the Trustees, Executors & Agency Co. Ltd.
4. Investments in shares held by the Trustees of the Institute include amounts of $20,023 for shares and $934 for debenture stocks at probate value being a bequest from the Estate of W.H. Wylie (deceased).
5. The market value of shares in companies listed on the Australian Stock Exchanges at 31 December 1976 was $1,975 below the amount at which they are stated in the accounts.
6. Income is accounted for on a cash basis while expenditure is accounted for on an accrual basis.

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

Year Ended 31st December 1976

RESTRICTED FUND			
Balance at 31st December, 1975		60,031	
National Health and Research Council Grant 1977	75,955		
Baker Benefactions Statutory Amount 1977	11,569		
Donations	97,325		
Investment and bank interest	1,745		
Stanhope Court Bond (repayable)	300		
		186,895	
		246,926	
Transfer to Maintenance 'Statutory Amount' 1976	11,569		
Transfer to Maintenance National Health and Medical Research Council Grant 1976	38,392		
Transfer to Maintenance Specific Donations	75,839		
		125,800	121,126
OTHER FUNDS			
Held by Trustees, Executors & Agency Co. Ltd.			
William Buckland Research Fund	21,946		
Laura Nyulasy Research Scholarship Fund	2,863		
		24,809	
Held by Trustees of the Institute			
Lang Scholarship Research Fund (shares at cost)	4,852		
Edgar Rouse Memorial Fellowship Fund			
Short erm deposit — Euro-Pacific	30,900		
Short term deposit — T.E. & A.	2,800		
Cash at bank	1,920		
	35,620		
		40,472	65,281
ENDOWMENT FUND			
Balance at 31 December 1975		1,269,868	
Donations	328,170		
Shares (Wylie Estate) at probate value	20,957		
Bank interest	271		
Accretion inscribed stock	135		
Interest on discharge of mortgage re-invested	788		
		350,321	
		1,620,189	
Transfer to Maintenance Fund	212,800		
National Trustees (re: Wylie Estate)	50,377		
		263,177	1,357,012
			1,543,419

CLINICAL RESEARCH UNIT

STAFF

Director:	P. I. KORNER, M.D.,F.R.A.C.P.,F.A.A.
Deputy Director:	P. J. NESTEL, M.D.,F.R.A.C.P.
Staff Physician:	G. L. JENNINGS, M.B., B.S., M.R.C.P. (U.K.), F.R.A.C.P.
Clinical Assistants:	P. A. BLOMBERY, M.B.,B.S.,B.Sc.(Med), M.R.A.C.P. P. J. FLETCHER, M.B.,B.S.,B.Sc. (Med), F.R.A.C.P.
Biochemical Pharmacology:	A. BOBIK, B. Pharm.,M.Sc., Ph.D. V. CARSON, M.Sc.
Technical Staff:	P. ASHLEY, B.Sc. M. BANGAH, B.Sc. U. GREGOREK, B.Sc. H. SKEWS, B.Sc. M. STONEHAM, B. App. Biol. J. NEALE

WARD STAFF

Registrar:	E. T. FAGAN, M.B., B.S., M.R.C.P. (U.K.)
Resident M.O.:	A. WU, M.B., B.S. M. MILLER, M.B., B.S. J. KUSTIN, M.B., B.S. D. BLAKE, M.B., B.S.
Ward Sisters:	Sr. ANN GRIFFITHS Sr. ANN HEWETT Sr. JOAN WILDBERGER
Hypertension Clinic:	Sr. D. CHAMBERS (BAKER INSTITUTE)

Director's Report

The Clinical Research Unit continues to provide special diagnostic investigations for the Alfred Hospital and also performs clinical research in cardiovascular medicine that is closely integrated with the programme of the Baker Institute. The main services provided during 1976 were through the *C.R.U. Hypertension Evaluation Clinic* and through quantitative exercise testing in patients with ischaemic heart disease. In addition a special *Hyperlipidaemia Clinic* will be operating from the beginning of 1977, under the direction of the new Deputy Director of C.R.U., Dr. Paul Nestel. Its role will be to provide a diagnostic and consultative service for patients with disorders of lipid metabolism. The clinic will have the services of a dietitian on the staff of the hospital, Miss Denise Winters, who will also assist with the work of the *Risk Evaluation Clinic* at the Baker Institute.

The *C.R.U. Hypertension Evaluation Clinic* has been expanding its activities under the direction of Dr. G. L. Jennings. There is close collaboration with Dr. J. Stockigt of the Ewen Downie Metabolic Unit and Dr. J. Sabto of the renal unit so that an integrated diagnostic service and a consultative service for difficult therapeutic problems is provided through the clinic. Members of the staff working in the clinic included Dr. Peter Blombery, Dr. Peter Fletcher and Dr. John O'Sullivan from the Department of Social and Preventive Medicine. Dr. O'Sullivan provided the clinic with much needed background of the problems faced by general practitioners in the management of hypertension. A number of new tests are now available through our Biochemical Pharmacology Laboratory which works under Dr. Alex Bobik and is located in the Baker Institute. These include a radioenzymatic assay for total plasma catecholamines, measurements of urinary adrenaline, noradrenaline, metanephrine and VMA concentrations. The laboratory also determines acetylator status in patients on hydrallazine therapy, which is an important factor in assessing the maximum 'safe' dose of this anti-hypertensive drug. Other assays currently available for special problems include the measurement of plasma concentrations of several beta-blocking drugs including propranolol, prindolol and timolol. The unit is also investigating the use of frequent ambulant blood pressure measurements by the patient in the course of ordinary daily activity using the Remler semi-automatic blood pressure recorder.

From 1977 special biochemical investigations of lipoprotein disorders will be available through the Biochemical Laboratory of the *Cardiovascular*

Staff of C.R.U. ***Left to right*** *(sitting) Denise Winters, Ursula Gregorek; (standing) Professor Korner, Sr. Anne Hewett, Dr. Alex Bobik, Sr. Denise Chambers, Sr. Sue Seally, Peter Ashley, Dr. Garry Jennings, Dr. Peter Jenkins, Moham Bangah, Dr. P. Nestel, Helen Skews.*

Metabolism and Nutrition Research Unit at the Baker Institute. This unit operates under the direction of Dr. Paul Nestel and Dr. Noel Fidge.

The research activities of the unit have involved studies on the pharmacokinetics of prindolol in man, and also on the pharmacodynamics of this beta-blocking drug. Of particular interest is the apparently long duration of action of beta-blocking drugs. Thus on the work-related heart rate response during exercise the drug acts for at least 24 hours, i.e. beyond the level of measurable plasma concentration. This long duration of action suggests either strong binding to the cardiac beta-receptor or action through an effect in a 'deep' compartment possibly at an intracellular site. It provides the basis for clinical studies on the less frequent administration of beta-blocking drugs in the treatment of hypertension. A clinical trial to investigate the anti-hypertensive efficacy of once daily as against twice daily administration of drugs is at present in progress using timolol.
Other studies on hypertension relate to the assessment of acute withdrawal of various anti-hypertensive drugs on autonomic function. A long term study is under way to evaluate the reversibility of vascular resistance changes in well treated patients with chronic hypertension. Studies are also in progress to assess the likelihood of a central nervous action of beta-receptor blocking drugs in lowering of peripheral resistance in patients with hypertension.

Studies in ischaemic heart disease have mainly involved quantitative multi-level exercise and measurement of the associated electrocardiographic changes. In patients with angina there appears to be a relationship between dose of beta-blocking drug and the degree of amelioration of myocardial ischaemia above concentrations that produce maximum attenuation of heart rate changes. With any of the beta-blocking drugs so far tested maximum work capacity is unaltered. This differs from the effects on exercise capacity after successful aorto-coronary bypass grafting in the surgical treatment of patients with disabling angina. This study performed by Dr. G. L. Jennings has shown a striking increase in maximum work capacity following successful cardiac surgery, in addition to amelioration or disappearance of ischaemic changes. Tests such as

Portable blood pressure recording apparatus used during patient's normal daily activities.

these are a beginning in providing *objective* evaluation of the effects of coronary artery surgery on myocardial function. Considerable stepping up of these and more sophisticated assessment of myocardial function is much needed to evaluate the real role of this form of treatment of established coronary artery disease.

1. Clinical Effects of Beta-Blockers and their Relationship to Plasma Concentrations
G. L. Jennings, A. Bobik, E. Fagan and P.I. Korner

The effects on heart rate and blood pressure of several doses within the clinical range of the beta-blocking agents prindolol and timolol has been studied in normal volunteers after single oral or in administration. With prindolol there was significant reduction of resting, and exercise heart rate 1, 3, 5, 7 and 24 hours after 3 mg i.v., 5 mg orally and 20 mg orally. The effect at 24 hours which is not associated with measurable plasma levels of prindolol was confirmed in one patient by a ten fold shift in the isoprenaline dose-response curve at that time after 10 mg of prindolol and represents strong receptor binding.

However, despite a good relationship between plasma concentration and effect on

exercise heart rate in individual patients no clear dose response relationship occurred when the results of 5 patients were pooled. This was because of great variation between patients in response to prindolol, and to the very small increase in the magnitude of the heart rate changes with increasing plasma concentrations between 10 and 100 ng/ml. This suggests that the clinically used doses of prindolol are on top of the dose response curve for this drug in terms of its effect on heart rate. With timolol, however, a clearer dose response relationship has been demonstrated following 5 mg and 20 mg orally.

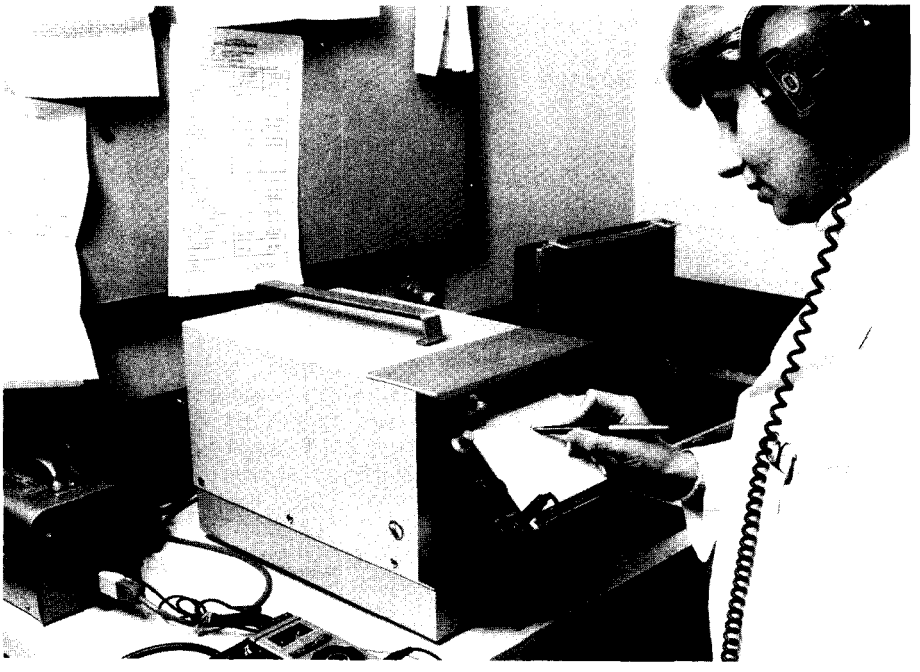
2. Prindolol in Angina
G. L. Jennings, E. Fagan, B. Bobik and P.I. Korner

Comparable groups of patients with ischaemic heart disease were given either 2 mg or 3 mg of prindolol intravenously. On quantitative exercise testing as described in the previous annual report there was similar inhibition of exercise heart rate and of blood pressure in the two groups. However, significantly less amelioration of ischaemia evidenced by ST depression occurred with 3 mg, than with 2 mg suggesting a dose related effect on ischaemia. Studies within patients are under way in order to confirm this finding.

3. Exercise Testing Before and After Coronary Artery Surgery
G. L. Jennings and G. R. Stirling

Quantitative exercise tests have been performed on patients before and after coronary bypass surgery. The tests involved *sprint* tests where exercise is increased at 1 minute intervals from zero load by 100 k.p.m. up to the maximum work capacity of the patient (Wmax). At each level of exercise the degree of ST-segment depression is determined using the Avionics Exerstress machine. The sprint test is followed by *'steady-state'* exercise at rates of 0.25, 0.50 and 0.75 Wmax, and both the *sprint* and *'steady-state'* tests are subsequently repeated after beta-receptor blockade. An identical protocol has been performed 3 months and 12 months following coronary bypass surgery and also in a number of patients on medical treatment, who were considered unsuitable for surgery on technical grounds.

Before coronary surgery beta-blockade decreased the degree of ST-segment depression at a given workload, but had no effect on Wmax. After coronary surgery there was a significant increase in the majority of patients Wmax (to 24% above presurgery level) and ST-segment was



Decoding from the Ambulatory Blood Pressure Recorder.

either abolished or greatly attenuated. In one patient where there was no relief in pain Wmax was less than before operation. In the patients on chronic medical treatment there was no alteration in Wmax or in the degree of ST-segment depression on a given workload.

4. Clinical Pharmokinetics of Prindolol in Man

A. Bobik, G. Jennings and P. I. Korner

In recent years the efficacy of prindolol in the treatment of hypertension has been well established. However, the doses required to lower blood pressure vary greatly between patients. Such variation in doses for other beta adrenergic antagonists such as propranolol or metoprolol have at least been partially explained by variations in first pass metabolism through the liver and its subsequent elimination either by metabolism or excretion. Thus the present study was aimed at examining the pharmokinetics of prindolol following both intravenous and oral administration.

Using a fluorimetric method for analysis of prindolol in plasma we have shown that the decline in plasma concentration of prindolol follows the bioexponential equation $C = A^{-\alpha t} + Be^{-\beta t}$, where C is plasma concentration, A,α, B,β are constants, t is time. The experimental data indicates that prindolol was rapidly and extensively distributed to extravascular tissues, the half-life of the distribution process being 8.4 min. The apparent volume of distribution (Vd,β) was 86 L and the half-life for the elimination process 2.9 h.

Following oral administration of 5, 10 and 20 mg prindolol, it was well absorbed by all patients. Maximal plasma levels occurred between 0.5 and 2 hours after administration and varied approximately two fold between subjects administered equivalent doses. The elimination half-life determined from the post-absorptive phase was 2.9 h and was independent of dose. The systemic bioavailability of prindolol was directly proportional to the orally administered dose and ranged from approximately 60 to 80%. In whole blood the partition coefficient of prindolol between erythrocytes and plasma was 0.69 and approximately 40% or plasma prindolol is bound to plasma protein over the concentration range 8 to 260 ng/ml.

In summary, the overall study indicates that clinically used doses of prindolol are well absorbed from the gastrointestinal tract with relatively small variations in resulting plasma concentrations of the drug. These variations could be accounted for by differences in absorption and plasma clearance of prindolol in subjects. Plasma protein binding is not important in determining variations in sensitivity of subjects to the drug.

5. Identification and Characterization of Cardiac Beta Adrenergic Receptors

E. A. Woodcock*, A. Bobik, J. W. Funder* and C. I. Johnston*

* Monash University, Department of Medicine

It has recently been suggested that altered sensitivity of the heart to catecholamines in several pathological states such as congestive heart failure or hyperthyroidism may possibly reflect changes in the number or binding affinity of substrates to beta adrenergic receptors. In order to study the properties of beta-receptors in such conditions we have developed a beta adrenergic antagonist 1-isopropylamino -3- (4-iodophenoxy) propan -2- ol (IIP) labelled with ^{125}I to high specific activity (see 1975 report). The present report deals with the binding characteristics of this beta adrenergic antagonist to rat myocardial membranes.

Rat myocardial membranes (10-30 x 10^3g pellet) were prepared by differential centrifugation of rat ventricle homogenates. IIP bound to myocardial membranes in a dose related fashion. Saturation of specific binding sites occurred at 0.06 picomole IIP per mg membrane protein. The binding process was found to be rapid, bimolecular in nature, with IIP binding to a single type of membrane site. The dissociation constant (K_D) for the specific binding of IIP to myocardial membrans was 5 nM and this value agrees with the dissociation constant determined from the IIP inhibition of isoprenaline stimulated adenyl cyclase (4 nM). Competition experiments with *d-* and *l-* propranolol indicates that specific binding of IIP was sterio-specific with 50% inhibition of IIP binding occurring in the presence of 10^{-8}M *l-* propranolol and zero inhibition in the presence of 10^{-7} *d-* propranolol. Specific binding of IIP is rapidly displaceable (◄ 5 min) by other beta adrenergic agonists and antagonists.

The K_D values for isoprenaline, adrenaline and noradrenaline calculated from com-

petitive IIP binding studies were 2 x 10^{-7}, 10^{-5} and 5 x 10^{-5}M respectively.

In summary, IIP binding studies have detected binding sites on myocardial membranes which mimic the known properties of -adrenergic receptors.

6. Effect of Clonidine Withdrawal and that of other Anti-hypertensive drugs on Blood Pressure, Urinary Catecholamine Excretion and Plasma Catecholamines

G. L. Jennings, V. Carson and E. Fagan

We have continued studies on the effects of withdrawal of clonidine on blood pressure and catecholamine metabolism. To-date 9 patients have been studied. In the majority serial blood pressure measurements and daily urinary catecholamine excretion have been obtained, and in a small number (2) we have also measured plasma catecholamine concentrations. In all patients there has been a significant increase in blood pressure averaging 28.5 ± 9.0 mmHg mean arterial pressure. The very large rises in blood pressure described in the classic clonidine 'rebound' have not commonly been observed. However, urinary catecholamine excretion virtually doubled in the first 24 hours after withdrawing the drug. The rise involved mainly noradrenaline, suggesting that it was predominantly due to neuronal release.

To date only a small number of patients involving withdrawal of antihypertensive drugs have been studied. These include prindolol (2), propranolol (1), bethanidine (2) and debrisoquine (1). The results suggest higher catecholamine excretion on these drugs with no rebound effect in urinary catecholamines on abrupt cessation of -blockers. However, in the patients studied on withdrawal of post-ganglionic sympathetic neuron blockers preliminary results suggest an increase occurring 1-2 days later than with clonidine.

7. Evaluation of the Antihypertensive Effect of Timolol and Moduretic once daily in Patients with Essential Hypertension.

G. L. Jennings and staff of Hypertension Clinic

One problem in the long term management of hypertension is drug adherence by the patient. It seems reasonable to suppose that reduction in the frequency of medication and a lowering in the number of tablets required will improve patient adherence. Preliminary studies in our laboratory and by others have suggested that the bonding between beta-adrenoreceptors and beta-receptor antagonists is fairly prolonged. Hence the relatively long lasting effect on blood pressure might be expected during the 'steady-state' phase of chronic treatment with beta-receptor blocking drugs.

A clinical trial has been designed to compare the effects of timolol given 1/day with timolol given 2/day. The study is on volunteers with moderately severe essential hypertension. During an open period the patient's initial blood pressure is established in the course of several visits to the Hypertension Clinic. In addition ambulant blood pressure is recorded by means of the Remler portable semi-automatic apparatus. The doses of timolol (at a standard dose of moduretic = hydrochloro-thiazide + amiloride) are increased weekly from a dose of 5 mg bd up to a maximum of 15 mg bd or where the blood pressure falls to less than 85 mmHg dialostic pressure. The patient's adherence to the beta-blocking drug therapy is monitored during this phase by measuring blood concentrations of timolol. The open phase is followed by a double blind part of the clinical trial involving the following six week treatment periods for each patient.

A = moduretic placebo + timolol placebo
B = moduretic 1/day + timolol 1/day
C = moduretic 1/day + timolol 2/day
D = moduretic 1/day + timolol placebo

These results allow assessment of the absolute changes in blood pressure in each patient during the latter part of each treatment phase. Allocation to each phase is by means of a 4 x 4 Latin Square design. Patients visit the Clinic alternate weeks while the clinic sister makes one domiciliary visit to measure the patient's blood pressure on alternate weeks. During the second half of each phase there is a recording over 1-2 days with the Remler semi-automatic blood pressure apparatus to evaluate its potential use in clinical trial. All 16 patients are now in the trial and the result will be available towards the end of 1977.

8. Effects of Treatment of the Non-Autonomic Component of Peripheral Vascular Resistance in Hypertension

G. L. Jennings and P. I. Korner

In patients with established hypertension the elevation of blood pressure is due to elevation of peripheral vascular resistance. Even after 'total' autonomic block there is still an elevation in peripheral resistance in hypertensive compared with normotensive subjects. This elevation could be due to differences in level of vasoactive substances but more probably it reflects structural differences in the circulation. The latter statement is based on studies in man by others where the hand vessels were fully dilated to remove all muscle tone, and on studies by our own group in animals with experimental hypertension. The structural changes result from medical hypertrophy which encroach on the intima and lead to vascular narrowing. The question is whether these changes are reversible after satisfactory treatment of hypertension.

The study is in volunteers with mild, moderate or severe hypertension. The patients are for the most part patients not on previous therapy. Resting haemodynamics are determined and the non-autonomic component of vascular resistance is estimated after 'total' autonomic block. Patients will be followed for a period of 12 months in the first instance and the haemodynamic assessment then repeated. Criteria will include severity of hypertension, and quality of treatment. The study is important to determine at what stage the structural vascular changes are reversible.

PUBLICATIONS

Published or Accepted for Publication

I. K. BAILEY, S. D. ANDERSON, P. J. ROZEA, L. BERNSTEIN, G. NYBERG and P. I. KORNER.
Effect of beta-adrenergic blockade with alprenolol on ST-segment depression and circulatory dynamics in patients with effort angina. *Am. Heart J.* 92: 416-426, 1976.

A. J. BARNETT, A. BOBIK, V. CARSON, J. S. KORMAN and A. J. MCLEAN.
Pharmokinetics of Methyldopa. Plasma Levels following Single Intravenous, Oral and Multiple Oral Dosage in Normotensive and Hypertensive Subjects. *Clin. Exptl. Pharmacol & Physiol.* (In Press).

A. BOBIK and A. J. McLEAN.
Cardiovascular Complications due to Pheniramine Overdosage. *Aust. N.Z. J. Med.* 6: 65-67, 1975.

A. BOBIK and A. J. MCLEAN.
Drug Analysis in the Overdose Patient, its Application to Clinical Toxocology. *Med. J. Aust.* (In Press).

A. BOBIK, E. A. WOODCOCK, C. I. JOHNSTON and J. W. FUNDER.
The Preparation and Purification of (^{125}I) 1-Isopropymamine-3- (4-Iodophenoxy) - 2 - propranolol a Beta Adrenergic Antagonist. *J. Labelled Compounds and Radiopharmaceuticals.* (In Press).

P. I. KORNER, A. M. TONKIN and J. B. UTHER.
Reflex and Mechanical Circulatory effects of Graded Valsalva Maneuvers in Normal Man. *J. Appl. Physiol.* 40: 434-440, 1976.

Submitted for Publication

P. I. KORNER, A. M. TONKIN and J. B. UTHER.
Valsalva Constrictor and Heart Rate Reflexes in Subjects with Essential Hypertension and with Normal Blood Pressure.

LECTURES AND MEETINGS

A. BOBIK:
Paper to Australian Society for Clinical and Experimental Pharmacology: 'Pharmacokinetics Studies of Prindolol in Man'.

G. L. JENNINGS:
Paper to Australian Society for Clinical and Experimental Pharmacology: 'Clinical Effects of Prindolol in Man and their Relationship to Plasma Concentration'.

Ewen Downie Metabolic Unit — Annual Report

STAFF

Physician in Charge	PINCUS TAFT, M.D.,F.R.A.C.P.
Deputy Physician	J. R. STOCKIGT, M.D.,F.R.A.C.P.
Visiting Physician	H. D. BREIDAHL, M.D.,F.R.C.P.,F.R.A.C.P.
Honorary Assistant Physician	P. Z. ZIMMET, M.B.,B.S.,Ph.D.,F.R.A.C.P.
Honorary Consulting Physicians	EWEN DOWNIE, M.D.,F.R.A.C.P. BRYAN HUDSON, M.D.,F.R.A.C.P.
Honorary Consulting	JOSEPH BORNSTEIN, D.Sc.,M.D.,F.R.A.C.P.
Biochemist	
Clinical **Assistants Registrar**	D. LORDING, M.B.,B.S. MARGARET SANDERS, M.B.,B.S. D. M. ENGLER, M.B.,B.S.,M.R.A.C.P.
Resident Medical Officers	R. I. HOLMES, M.B.,B.S. BETTY C. T. KONG, M.B.,B.S. P. J. ELLIOTT, M.B.,B.S. P. L. BYTH, M.B.,B.S.
Biochemists	M. J. MARTIN, PH.D. (LOND) MARJORIE BROWNE, B.A. (Hons), until Nov. '76. S. PETROU, B. Appl.Sc.
Nurse Technologist	ELAINE BRENNAN, (nee Higgs)
Technical Staff	IDA EKKEL, A.A.I.M.T. LOUISE SHIEL, B.Sc. W. H. HUDSON FREDERICKE RABOLD JULIE-ANN ROY (until April, 1976) JENNY WELLS
Secretary	LYNNE PRING (until October, 1976) W. VAN DER LOOS
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Chiropodist	V. DE VERE M.A.,Ch.A. (Deceased Sept. '76)
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General Summary

Laboratory based endocrine diagnosis and research received a great impetus in the 1960's with the introduction of radioimmunoassay as a technique for the measurement of hormone levels in blood, as few hormones could then be estimated directly. These measurements initially complemented the existing methods of assessment of endocrine function. At that time estimation of function of organs and tissues responsive to the action of the hormone under study and measurement of hormone metabolite excretion were the methods usually used to derive an index of endocrine activity. With extension of radioimmunoassay methods to measurement of most hormones, this principle has almost completely supplanted older techniques.

The application of this new method to the measurement of a hormone metabolite, reverse T_3, is a synthesis of old and new approaches which has been applied to the study of thyroid physiology and pathophysiology. Unit activities in this regard are reported in the account of research activities.

New ways of looking at old problems often provide new information. The detailed study of the course of events in the natural history of disease is such a technique. With its wide experience in clinical endocrinology and with clinical and laboratory aspects of unit function so closely interrelated the opportunity for the design of such studies is apparent and accounts of some are included in this report.

In the course of the hospital service-commitment in clinical and laboratory endocrinology, the unit works collaboratively with Endocrine Units and services in hospitals throughout the Metropolitan area, sharing specialised skills and resources. The close liaison which has been built up with the Clinical Research Unit and the Baker Institute has been maintained and we continue alternate weekly Endocrine Seminars with our Sister Unit at Prince Henry's Hospital. We also continue to enjoy a close collaboration with the Howard Florey Institute, University of Melbourne in our work on Endocrine Hypertension.

It has been our privilege to enjoy financial support and gifts in kind from generous benefactors. Our gratitude is acknowledged to —

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Estate of the late Vincenza Acton
Estate of the late G. E. Gillet
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Victorian Diabetes Association — Alan Docking Memorial

Overseas Visits

Dr. Stockigt attended the American Endocrine Society Meeting in San Francisco in June 1976 and the Fifth International Congress of Endocrinology in Hamburg in July 1976, where he presented the paper "Aldosterone regulation in glucocorticoid-remediable mineralocorticoid hypertension". During two weeks in San Francisco he renewed his association with the groups with whom he had worked in 1968-1971. He also visited Dr. John Laragh and Dr. Maria New at Cornell Medical Center, New York.

Dr. Taft and Dr. Zimmet attended the Annual Meeting of the European Association for the Study of Diabetes in Helsinki, Finland. A joint paper on "The Incidence of Diabetes in a Central Pacific Island" was presented and a further communication was read by title. Dr. Breidahl attended the Ninth Congress of the International Diabetes Federation in New Delhi in November 1976, where he presented an invited paper on "Hyperosmolar Coma". At that meeting he was elected as a Vice-President of this Federation.

Lectures and Meetings

Dr. Stockigt and Dr. Taft conducted workshops at the Alfred Hospital during the meeting of the Royal Australasian College of Physicians in Melbourne in October 1976 on the topics "Endocrine Problems for the Physician" and "Complications of Diabetes". Dr. Stockigt also gave a symposium paper on "Diagnosis of primary aldosteronism and renin-secreting tumour".

The Unit was well represented on the programme at the meeting of the Endocrine Society of Australia in Brisbane in August 1976. Those presenting work were Mr. E. Donaldson, Ms Ida Ekkel, Ms Elaine Higgs and Drs. D. Engler and J. Stockigt.

Teaching

Drs. Breidahl, Stockigt and Taft taught Clinical Medicine to the fourth, fifth and final years of Monash University Students at Alfred Hospital during 1976. They also gave lectures in the third year preclinical

course and in the fourth, fifth and sixth year lecture programmes.

Seminar Programme

In addition to our regular postgraduate teaching programme, Monday lunchtime Seminars have been conducted in rotation with the Endocrine Unit at Prince Henry's Hospital. The following topics were presented at the Alfred Hospital:

Lactic Acidosis
17 Hydroxylase Deficiency
Carcinoma of the Thyroid
Treatment of Acromegaly
Hypothyroidism and Surgical Stress
Pituitary Apoplexy with a normal fossa
Familial Adrenal Insufficiency
Genotype-Phenotype non-concordance
Hyperplasia of the Parathyroid Glands
Mineralocorticoid Hypertension — Medical Treatment
Thyroid function tests after ^{131}I therapy
Long term follow-up of untreated Pituitary Tumours
Antithyroid Drug Intolerence
Hypocalcaemia of uncertain cause
Natural history of "Chemical Diabetes"
Management of Malignant Exophthalmos

Renin, Aldosterone and Hypertension

In about 5% of patients with high blood pressure, a correctable renal or adrenal cause can be found which allows effective surgical or medical treatment. In such cases routine antihyptertensive therapy is often unsatisfactory and effective treatment may only be achieved when the underlying cause is recognised. Simple, readily-available screening tests, such as measurement of plasma potassium or intravenous pyelography, are the usual means by which such causes are initially suspected, but aldosterone and renin determinations are essential for precise diagnosis.

The entity of primary aldosteronism, due to a unilateral benign aldosterone-producing adenoma, has now been known for 20 years, but in the past decade it has become apparent that numerous patients with hypertension, hypokalaemia, supressed renin and non-suppressible aldosterone excess do not have a distinct unilateral aldosterone source. Diagnosis and management of such cases remains controversial and difficult, and indiscriminate adrenalectomy is best avoided. Lateralizing procedures such as adrenal vein catheterization, ^{131}I iodocholesterol scanning and therapeutic trial of glucocorticoid response are some of the procedures which we use in defining this difficult group.

During 1976, fourteen patients from the Alfred Hospital and other centres have been investigated for primary aldosteronism with positive results in five. In cases which fail to show lateralization, medical treatment, with either spironolactone or amiloride, has been monitored with serial measurements of renin activity to avoid over-correction of mineralocorticoid excess, characterized by deterioration of renal function and hyperkalaemia. Preliminary findings indicate that measurement of the blood pressure response is a poor index of this complication.

In the past year we have investigated two non-hypertensive conditions which show marked renin excess, Addison's disease and Bartter's Syndrome. In Addison's disease, renin excess is a clear indication of diminished effective plasma volume and the renin level during treatment is a very sensitive index of effective treatment. In Bartter's syndrome it remains uncertain whether the renin excess is a primary abnormality or whether it is the consequence of abnormal renal prostaglandin production. The recent accounts of successful treatment of Bartter's syndrome with anti-inflammatory drugs which inhibit prostaglandin production offer a novel therapeutic approach, but the mechanism of this response remains uncertain. Our recent investigations have attempted to determine whether the response is a direct drug-effect, or whether it is the result of drug-induced sodium retention.

Renal vein renin studies, performed by the Department of Radiology, have now become widely used in the search for correctable unilateral renal hypertension. During 1976, 46 patients (31 from Alfred Hospital, 15 from other centres) have been studied by this technique and intravenous diazoxide has been used as an acute stimulus to renin secretion in 29 of these studies. Only 11 of these cases have shown definite lateralization, emphasizing the need for detailed investigation of hypertensive patients with renal asymmetry before surgery is considered.

The blood pressure response to infusion of the competitive angiotensin antagonist, Saralasin ^{R} (P113) has been advocated as a simple screening test for renin-dependent hypertension which might reduce the

number of negative renal vein renin studies. However, our preliminary findings with this investigative drug suggest that the response is extremely dependent on the state of sodium balance, thus limiting its effectiveness as a screening test.

There have been few changes during 1976 in the laboratory methodology used for investigation of hypertension. The assay of plasma aldosterone is now performed after isolation of aldosterone by affinity chromatography, and assay of renin substrate and plasma renin concentration have been added to the routine assay of renin activity. We continue to benefit from the collaboration of the Howard Florey Institute and are grateful to them for assay of adrenal vein samples, for detailed steroid studies in glucocorticoid-responsive mineralocorticoid hypertension and for providing the samples on which our plasma aldosterone method was evaluated.

Projects:

1. **Affinity Chromatography Purification in Radioimmunoassay of Plasma Aldosterone**
   Ida Ekkel and J. R. Stockigt.

Because of the 1000-fold excess of cortisol, preliminary separation is usually necessary prior to plasma aldosterone radioimmunoassay even with highly specific antibodies. Antibodies linked to solid phase have been extensively used to facilitate separation of bound from free hormone during radioimmunoassay, but immobilized antibodies can also be used to isolate aldosterone from biological fluids. Our preliminary findings indicate that reusable aldosterone-antibody columns can be used to prepare plasma for radioimmunoassay.

Antibodies were isolated from immune serum using AH Sepharose 4B*-aldosterone hemisuccinate prepared by carbodiimide conjugation. To remove non-covalently-bound steroid the Sepharose was alternately washed with large volumes of Tris HC1 pH 9.0 and Glycine HC1 pH 2.4 each containing 1M NaCl. After washing with Phosphate buffer, pH 7.4 immune serum was added to the aldosterone-Sepharose. Sodium chloride (2M) removed 85-90% of attached protein with loss of less than 10% of antibody. Antibody was eluted with 0.1M acetic acid with 50-60% recovery and after freeze-drying was reacted with cyanogen bromide-activated Sepharose* at pH 8.0. After washing with 1M Ethanolamine and alternate low and high pH buffers, followed by neutral buffer, the Sepharose-antibody was placed in small columns.

Unextracted plasma (2 ml), containing ^{3}H aldosterone (500 CPM) was cycled through these columns at 4°C four times and the columns washed with water and then eluted with 2M propionic acid. The eluate was extracted with dichloromethane, washed, dried and reconstituted for assay and determination of recovery correction. Studies with ^{3}H cortisol added to plasma indicated that less than 0.2% of cortisol was present in the final eluate. Recovery of ^{3}H aldosterone was initially 70-80% falling to 40-60% after columns had been used 8 times. Corrected recovery of unlabelled aldosterone added to plasma was $107 \pm 7\%$ (SD, $n = 9$).

Specificity was examined by assay of plasma from an adrenalectomized subject on routine maintenance therapy ($<$ 2ng/100ml, $n = 8$). Assay of plasma to which cortisol had been added showed aldosterone values of 24.9, 25.6, 27.4 and 27.4 ng/100ml for cortisol concentrations of 8.6, 58, 104 and 156 µg/100ml. Samples previously assayed by the double isotope dilution derivative assay showed a correlation of $r = 0.97$ ($n = 24$).

Plasma aldosterone was 8.6 ± 2.9 (SD) ng/100ml in 9 consecutive assays of a normal plasma pool. Following infusion of 2L isotonic saline, plasma aldosterone was suppressed to $<$5ng/100ml in essential hypertensives and normal subjects, while patients with primary aldosteronism showed persistence of aldosterone excess after saline infusion (Fig.1). This procedure offers a rapid and definitive test for primary aldosteronism.

The preparation of Sepharose-antibody is laborious, but repeated use of columns makes the procedure feasible. Affinity chromatography prior to extraction with organic solvents may have wider application for isolation of steroids which show weak binding to plasma proteins.

*Pharmacia.

2. **Plasma Renin in Primary and Secondary Adrenal Insufficiency**
   Elaine J. Higgs, P. Taft and J. R. Stockigt

Renin activity was determined in untreated Addison's disease and during replacement therapy in Addisonian and

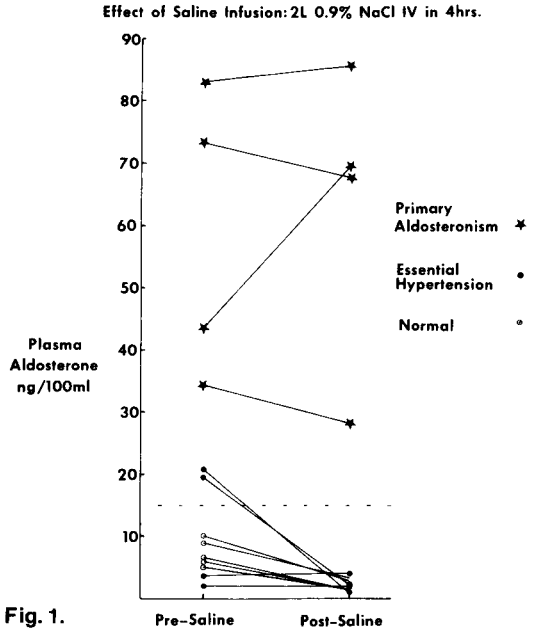


Fig. 1.

Effect of acute sodium chloride infusion (2L 0.9% Na C1 in 4 hours) on plasma aldosterone in primary aldosteronism, essential hypertension and normal subjects.

adrenalectomized subjects, to establish whether renin can serve as an index of appropriate treatment, and to study Addisonian patients requiring unusual doses of 9α Fluorohydrocortisone (9αF). Ambulant renin activity was also measured, on ad libitum sodium intake, in hypopituitary patients receiving steroid replacement with cortisone acetate. Renin activity was measured by radioimmunoassay of generated angiotensin I (Normal ranges: 2-8ng/ml/3hr recumbent (R); 4-15ng/ml/3hr ambulant (A) on unrestricted sodium intake).

As previously described, renin was greatly increased in three untreated cases of Addison's disease with values falling to normal after treatment (Fig.2). Renin was high in one untreated case before development of any plasma electrolyte abnormality or postural hypotension (R 60, A 135ng/ml/3hr). Renin substrate was subnormal prior to treatment, but the substrate deficiency was not severe enough to eliminate the in vitro increase in renin activity (Fig.2).

In 20 bilaterally adrenalectomized or Addisonian subjects on long-term replacement with cortisone acetate 37.5 — 50 mg/day and 9αF 0.1 mg/day, ambulant renin was 6.8 ± 1.3 (SEM) ng/ml/3hr, corresponding closely to normal values. In one patient inadvertently treated only with Prednisolone 5mg b.d., renin activity was 180 ng/ml/3hr.

In 16 hypopituitary patients receiving cortisone acetate 25-37.5mg/day as their only steroid replacement, ambulant renin activity was 4.4 ± 0.8 (SEM) ng/ml/3hr, suggesting that this regimen was not associated with mineralocorticoid deficiency.

Two patients with Addison's disease associated with diabetes and mild renal insufficiency required abnormally high doses of 9 F (0.2mg, 0.5mg) to prevent hypotension and hyperkalaemia. Treatment with 9 F 0.1mg/day was associated with elevated renin levels (96, 46 ng/ml/3hr) which decreased to normal with appropriate higher dosage.

In a single Addisonian subject hypertension developed on standard replacement therapy, associated with undetectable renin. Hypertension resolved when 9 F was reduced to 0.05mg/day.

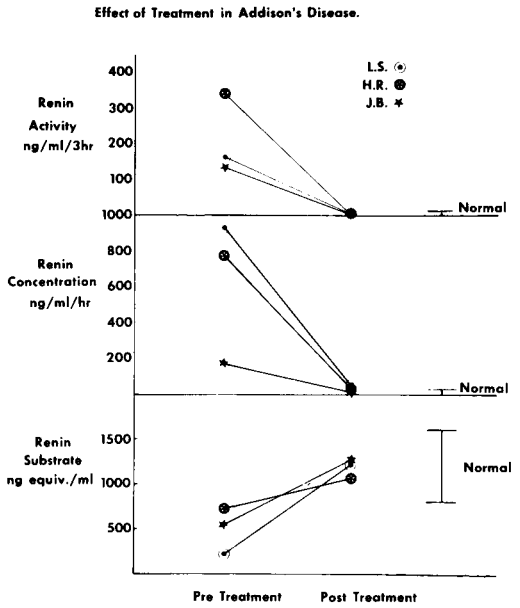


Fig. 2.

In untreated Addison's disease both plasma renin activity and concentration are extremely high, while renin substrate is subnormal. The levels return to normal with combined Cortisone and 9 Fluorohydrocortisone treatment and measurement of renin activity is then a sensitive index of adequate treatment.

These findings suggest:—

1. That renin activity is normal in most patients with primary adrenal insufficiency on standard replacement therapy with cortisone acetate and 9α-F.
2. That renin may be used to determine optimal mineralocorticoid therapy, especially when associated renal disease or hypertension require unusual dosage.
3. That elevation of plasma renin may be a sensitive index of early adrenal insufficiency.
4. That cortisone Acetate alone is effective steroid replacement in hypopituitary patients.
5. That a hyperactive renin-angiotensin system may be an important defence against hypotension in adrenal insufficiency.

3. Studies in Glucocorticoid-Remediable Mineralocorticoid Hypertension.

J. R. Stockigt, E. J. Higgs, B. A. Scoggins*, J. P. Coghlan* and C. J. Oddie*

*Howard Florey Institute, University of Melbourne.

This uncommon disease superficially resembles primary aldosteronism, with which it shares the features of hypertension, hypokalaemia, suppressed renin and aldosterone excess. However, it differs, because all of these features are correctable with glucocorticoid which results in effective longterm medical treatment. Investigation of a patient with this problem has continued along three lines:
(i) To further define the anomalous regulation of aldosterone, which falls to undetectable levels during dexamethasone treatment, in the face of marked increases in renin and potassium.

(ii) To define the optimum dose of dexamethasone for longterm treatment, because 2mg/day leads to deterioration of renal function, with excessive sodium loss and marked renin elevation (Fig.3).

(iii) To establish whether an unknown steroid contributes to the features of mineralocorticoid excess.

Studies of aldosterone regulation indicate that ACTH may be the dominant regulator of aldosterone secretion in this condition. Infusion of ACTH at the very low dose of 10mU/hour resulted in an increase in plasma aldosterone without an increase in cortisol. While plasma aldosterone responses to potassium and angiotensin infusion are clearly detectable, the rise in potassium and renin during dexamethasone-treatment fails to stimulate aldosterone.

(ii) The condition can be controlled with dexamethasone 0.25mg twice daily which allows plasma aldosterone to return to pre-treatment levels, but does not lead to return of hypertension, hypokalaemia or renin suppression (Fig.3). Following cessation of Dexamethasone treatment the features of the disease take at least three weeks to return.

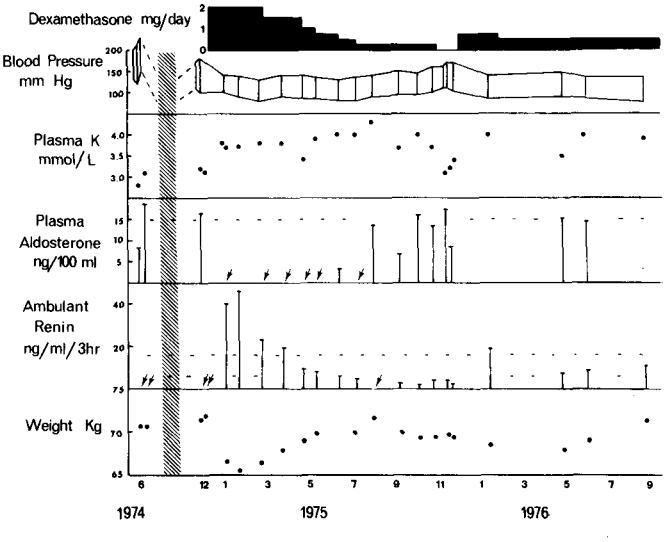


Fig. 3.

Effect of longterm treatment with low-dose Dexamethasone in glucocorticoid-remediable mineralocorticoid hypertension. Only partial adrenal suppression with Dexamethasone is necessary to sustain normotension, normal potassium and normal renin. Dexamethasone 2 mg/day was associated with supranormal renin and excessive weight loss, suggesting overtreatment. During the shaded period the patient was treated with Spironolactone.

(iii) The marginal excess of aldosterone in the untreated state, the poor correlation of plasma aldosterone with response to treatment and the presence of excessive non-specific urinary steroid metabolites suggest that other steroids may be involved. Collaborative studies of plasma mineralocorticoid activity, using rat kidney tissue for mineralocorticoid radioreceptor assay, are being performed with Dr. J. D. Baxter of University of California, San Francisco. Preliminary findings suggest that this patient's plasma contains more activity than can be accounted for by known steroid hormones.

4. Medical Treatment of Primary Mineralocorticoid Hypertension.
J. R. Stockigt, E. J. Higgs.

Adrenal surgery is now not recommended in patients with primary aldosteronism who do not show evidence of a unilateral source. Longterm medical treatment with competitive aldosterone antagonists or the distal-acting diuretic, amiloride, may result in satisfactory control of plasma potassium and blood pressure, but the pattern of response is unpredictable.

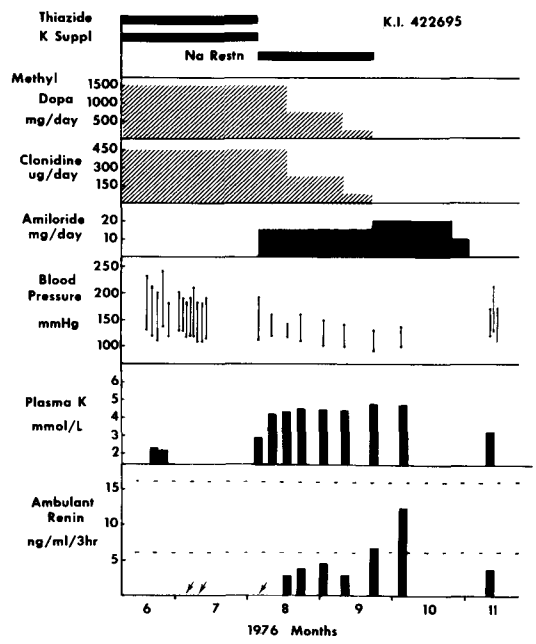


Fig. 4.
Effect of Amiloride in Primary Aldosteronism, showing correction of hypokalaemia and refractory hypertension, associated with reversal of renin-supression, during a 3 month period of treatment.

The response to medical treatment has been followed by repeated measurements of ambulant renin, blood pressure and potassium in seven patients with primary aldosteronism. In all cases there has been a close temporal association of the hypotensive response with reversal of renin suppression, in most cases associated with weight loss. This pattern was seen with spironolactone and also with amiloride (Fig.4).

In one case, where renal function was initially normal, correction of hypertension with Spironolactone was associated with deterioration of renal function, hyperkalaemia and marked renin excess. No dose of Spironolactone could be found which allowed control of blood pressure without evidence of excessive volume depletion, and a combined regimen of treatment with beta blocker, vasodilator and Spironolactone has been necessary.

These findings suggest:

1. That Amiloride is a satisfactory alternative to Spironolactone in the medical treatment of primary aldosteronism.
2. That the response to treatment must be carefully observed to avoid over-correction of mineralocorticoid excess.
3. That elevation of renin into the normal range is a useful index of appropriate longterm treatment.
4. That observation of the renin response to diuretic treatment in low renin hypertension may provide useful information on the pathogenesis of this disease.

5. Effect of Indomethacin and Ibuprofen in Bartter's Syndrome
J. R. Stockigt, E. J. Higgs, I. Ekkel, D. M. Engler and H. G. Standish.

The features of Bartter's Syndrome are lethargy, myalgia, muscle weakness, and retarded growth in children, associated with hypokalaemia and renal potassium loss. The patients have low or normal blood pressure, with renin and aldosterone excess associated with marked insensitivity to the pressor effect of angiotensin II. Treatment of the condition with massive doses of potassium supplements, spironolactone, manipulations of sodium intake and total adrenalectomy has been unsatisfactory. Recent reports indicate a favourable response to the non-steroid anti-inflammatory drugs indomethacin and Ibuprofen, which inhibit synthesis of prostaglandins. It has been suggested

that excessive renal prostaglandin synthesis may be the primary abnormality in this syndrome.

Studies have been performed with these drugs in four patients with Bartter's Syndrome in order to determine whether the effect of these drugs is a direct one, or whether it is mediated by drug-induced sodium retention. During sodium restriction, Ibuprofen 1200mg/day failed to reverse the pressor insensitivity and failed to suppress aldosterone or renin to normal. Sodium loading in the absence of these drugs produced only transient suppression of renin and did not reverse pressor insensitivity. However, sodium loading together with Ibuprofen resulted in suppression of both renin and aldosterone and restoration of pressor sensitivity to normal.

These preliminary findings suggest:

1. That Ibuprofen or Indomethacin act as antinatriuretic agents in Bartter's syndrome and that their effect is dependent on sodium retention.
2. That the emphasis in longterm treatment should be on increased sodium intake rather than sodium restriction, as is customary in other types of mineralocorticoid excess.
3. That a marked improvement in muscular symptoms can occur during combined treatment with increased sodium intake and either Ibuprofen or Indomethacin, without correction of hypokalaemia.

6. Angiotensin Antagonist in Suspected Renal Hypertension

J. R. Stockigt, E. J. Higgs, P. I. Korner*, G. L. Jennings* and E. T. Fagan*.

*Baker Institute and Clinical Research Unit.

The detailed investigation of patients with suspected renal hypertension is laborious and involves renal arteriography and renal vein renin sampling. Results of renin assays are known only after a delay of 1-2 weeks, so that a simple screening test which might give bedside results is most attractive. The development of angiotensin II analogues which will bind competitively to vascular receptors has made such a test feasible.

Interpretation of results is complicated by the fact that these compounds have a weak agonistic action, so that their action resembles angiotensin, with rise in blood pressure, when blood angiotensin is low. A fall in blood pressure, suggesting a high level of circulating angiotensin, may have diagnostic value in defining renal hypertension, although such a response may also be induced in normal subjects by sodium depletion. Authorities currently differ on the diagnostic value of this approach in the investigation of renal hypertension.

The analogue Saralasin R (P113) which has been used in human studies since 1973, became available as an investigative drug in Australia in 1976. Our preliminary findings in seven patients with suspected renal hypertension show an absence of false-positive findings and confirm the marked sodium dependence of the response (Fig.5).

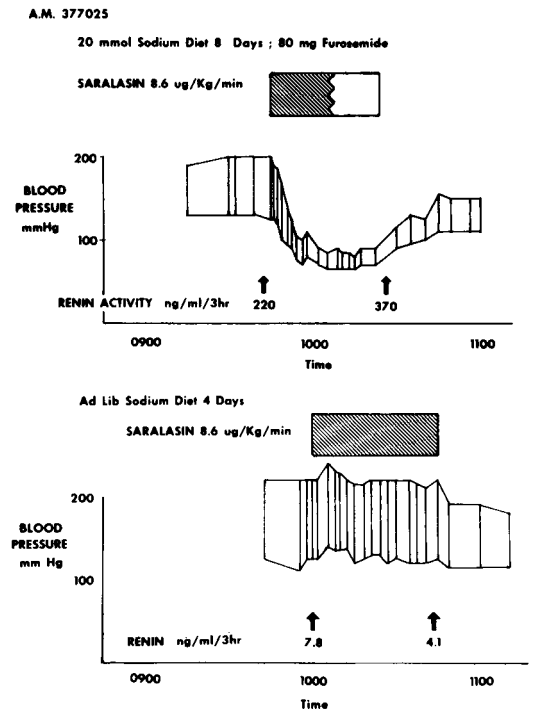


Fig. 5. Contrasting blood pressure response to Saralasin during sodium depletion and repletion in a patient with significant left renal artery stenosis. Renal vein renin activity: Left 330, Right 65, Peripheral 75ng/ml/3hr during sodium restriction.

This variability of response suggests that infusion of this analogue will not supersede other approaches to the investigation of renal hypertension, but the test may have some value in screening, provided the degree of sodium depletion can be standardized.

Thyroid Function and Thyroid Hormone Physiology

During 1976 the Unit has performed 4,900 routine tests of thyroid function, 3,300 from the Alfred Hospital and 1,600 from outside the hospital. Determination of total serum thyroxine and binding index, with calculation of free thyroxine index remains the first-line investigation, but additional assays for triiodothyronine or thyrotrophin were performed on 40% of samples. These additional tests are selected by the laboratory staff on the basis of clinical information given by the referring physician. This amount of work provides a wealth of material for clinical investigation and detailed follow-up studies.

In the past two years it has become clear that extrathyroidal metabolism of thyroxine (T_4) is an important aspect of thyroid hormone physiology. About 75% of 3,3',5 triiodothyronine (T_3), the major metabolically-active thyroid hormone, is produced by peripheral monodeiodination of T_4 and this mechanism may be acutely inhibited during starvation, severe non-thyroidal illness and after surgery. In these circumstances there is increased production of 3,3',5 triiodothyronine (reverse T_3), (rT_3) which is metabolically inactive. The regulation and balance between these alternative activating and deactivating pathways of T_4 metabolism is currently a very productive area of research. An assay for reverse T_3 has been established and is now used to assess peripheral T_4 metabolism. Mr. Eric Donaldson, a Monash University Medical Student, obtained a Bachelor of Medical Science with 1st class Honours, for a thesis which reported the development of this assay and the results of clinical studies. Clinical assessment of thyroid function in patients with an associated severe non-thyroidal illness poses a formidable problem and great reliance is placed on laboratory tests in this situation. While measurement of serum T_3 has become widely accepted as a more sensitive index of hyperthyroidism than serum T_4, our findings suggest that T_3 loses its diagnostic value in the face of severe associated illness, because of a shift in T_4 metabolism towards the inactivating pathway. In such situations the diagnosis of hyperthyroidism is best based on demonstration of T_4 excess and thyroid antomomy. In patients with hyperthyroidism due to isolated T_3 excess it is uncertain whether the biochemical criterion of this diagnosis would disappear with associated illness.

Thyroid hormones show a high degree of binding to plasma proteins so that the metabolically-active free-fraction forms only a minute proportion of the total serum hormone concentration. Changes in the concentration of binding proteins are quite common and may lead to alterations in the total serum hormone concentration which do not reflect thyroid dysfunction. It has therefore become customary to perform tests of protein binding such as the T_3 resin uptake, in order to correct for binding. However, the derivation of such tests is arbitrary and the technique has not been validated for the different thyroid hormones. For this reason we have modified and extended the technique of Sephadex partition to apply this method to calculation of free fraction for T_4, T_3 and reverse T_3.

Projects

1. **Radioimmunoassay of 3, 3, 5 Triiodothyronine (Reverse T_3).**
   E. B. Donaldson, J. R. Stockigt, S. Petrou.

Reverse T_3 is an alternative deiodinative metabolite of thyroxine without intrinsic biological activity. The low triiodothyronine (T_3) level which occurs in systemic illness is accompanied by an increase in the level of reverse T_3, but the factors regulating the two alternative pathways of thyroxine deiodination remain uncertain.

Antibodies to L-reverse T_3 were produced in rabbits by injection of reverse T_3 coupled to rabbit gamma globulin and ^{125}I reverse T_3 was prepared by substitution radioiodination of L-reverse T_3 using Chloramine T. Labelled hormone was purified by chromatography on Sephadex LH 20_R. Initial attempts to establish the assay with unextracted samples, using Merthiolate to inhibit hormone-binding to plasma proteins, were unsuccessful, but a satisfactory assay was developed using standards prepared in hormone-free serum. Antibodies showed 0.11% cross reactivity with T_4 so that each result required correction for the T_4 content of that sample.

The normal range for serum reverse T_3 was $<$ 5-35ng/100ml in 80 normal subjects aged 20-80. Reverse T_3 showed no age-related changes in serum level. As previously described, reverse T_3 was in-

creased in hyperthyroidism and decreased in hypothyroidism. In T_3 toxicosis reverse T_3 was subnormal, suggesting that altered peripheral metabolism of T_4 may contribute to isolated T_3 excess.

During non-thyroidal illness, T_3 and reverse T_3 showed reversible inverse changes. In hyperthyroidism associated with another severe illness, T_3 was normal or subnormal in 9 of 11 cases, associated with a very high level of reverse T_3 which was greater than the reverse T_3 level in uncomplicated hyperthyroidism or sick euthyroid subjects ($p < .05$).

The effect of glucocorticoids on peripheral T_4 metabolism was studied in normal volunteers. Dexamethasone, 2-8 mg, given as a single dose, decreased T_3 and increased reverse T_3 as previously reported, but this effect could not be reproduced by cortisone acetate 200mg/day, suggesting that the effect of dexamethasone may be a pharmacological one which cannot be reproduced by greater relative doses of natural glucocorticoid.

Stimulation of the thyroid gland by endogenous TSH, following injection of thyrotropin-releasing hormone, failed to increase reverse T_3, confirming that the thyroid gland is not a significant direct source of reverse T_3.

Table 1
Ratio of T_3 to rT_3 in Various Diseases.

	T_3* ng/100ml	rT_3* ng/100ml	T_3:rT_3 Ratio
Normal	95	15	6.3
Hyperthyroid	360	88	4.1
Athyreotic + T_4	90	20	4.5
Liver Disease	45	75	0.65
Euthyroid Sick	60	62	0.97
Thyrotoxic Sick	140	180	0.78
T_3 Toxic	225	7.5	30

* Mean Values

The findings summarized in Table 1 indicate that the relative proportions of T_3 and reverse T_3 in serum are not markedly altered from normal in uncomplicated hyperthyroidism, or during replacement therapy with T_4. However, a major associated illness can exert a dominant influence on the relative levels of T_3 and reverse T_3, irrespective of the state of hormone secretion from the thyroid gland.

2. Measurement of Free Fractions of Thyroid Hormones

D. M. Engler and J. R. Stockigt.

Because the quantities of circulating free thyroxine and free triiodothyronine are minute, in the presence of vast excess of bound hormone, it is exceedingly difficult to measure the free concentration directly. Binding is usually evaluated by indirect methods such as T_3 resin uptake, but the free concentration can also be expressed as the product of total hormone concentration and the free fraction in diluted serum, provided binding varies inversely with dilution.

The present method, adapted from the work of C.H.G. Irvine, uses Sephadex G25R beads which exclude large proteins and which selectively absorb free iodothyronines. In this system, small molecules such as free T_4 and free T_3 enter the Sephadex beads and are selectively absorbed, whereas protein-bound T_4 or T_3 is excluded. The free hormone distributes into both the supernatant and Sephadex volumes, whereas the protein-bound hormone remains outside the Sephadex beads. Because Sephadex has a large capacity to absorb T_4 and T_3 only a small fraction of the free hormone remains in the supernatant volume, irrespective of the presence or absence of serum.

The Sephadex counts, calculated from the difference between total and supernatant counts, therefore represent a large and constant fraction of free counts which allows calculation of the free fraction for that dilution of serum.

The product of the free fraction and the total hormone concentration gives a value for the free hormone which is valid when different dilutions of serum are studied.

In an adult population without thyroid disease, the normal range for free thyroxine is 1.2 — 3.5ng/100ml and for free T_3, 0.2 — 0.6ng/100ml.

The assay has a between-assay coefficient of variation of 9% and results may be obtained in 4 hours. The method has been extended to determine the amount of free reverse T_3 in serum and may be generally applicable to the measurement of free concentrations of small molecules which show extensive binding to serum proteins and selective absorption to Sephadex.

3. Effect of Propylthiouracil on Reverse T_3 in Hyperthyroidism

E. B. Donaldson, Marjorie E. Browne, P. Taft and J. R. Stockigt.

Propylthiouracil (PTU) has previously been shown to partially inhibit the deiodination of thyroxine (T_4) and PTU treatment results in an abrupt fall in triiodothyronine (T_3) in hyperthyroidism and in hypothyroid subjects maintained on T_4. Malnutrition and severe illness also causes an abrupt fall in T_3 with increase in 3,3',5' — triiodothyronine (reverse T_3, rT_3). The present study examined the effect of PTU treatment on serum rT_3 in hyperthyroidism to determine whether the PTU effect on T_4 deiodination is similar to that reported in malnutrition and severe illness.

Reverse T_3 was measured as described above and values for rT_3 were corrected for T_4 content of each sample. Standards were prepared by adding L-rT_3 to charcoal-treated serum containing undetectable T_4 and samples and standards were extracted with 2 volumes ethanol. Assay was performed in 0.05M barbital buffer, pH 8.6 in a final ethanol concentration of 13.8%. Dextran-charcoal was used for separation and the minimum detectable rT_3 was 0.005 ng/tube. Serum rT_3 concentration was 5-35ng/100ml in 80 non-hospitalized euthyroid subjects.

Five subjects with classical uncomplicated hyperthyroidism were studied on days — 3, 0, 2, 4, 8 or 9, 15 or 16 and 22 or 23 of treatment with PTU 200 mg four times daily. T_4 was measured by Ames Tetralute and T_3 by direct radioimmunoassay, using Merthiolate 2mg/ml to inhibit binding of T_3 to plasma proteins. Mean pretreatment values were: T_4 20.0 ± 1.1ng/100ml, $T_3$510 ± 54ng/100ml and $rT_3$134 ± 15ng/100ml.

Table 2
Serum T_4, T_3 and rT_3 during PTU treatment as percent of pre-treatment values (n = 5)

Days:	-3,0	2	4	8/9	15/16	22/23
T_4	100	108±4 (SEM)	93±8	89±9	78±13	67±11*
T_3	100	64±4**	58±3**	59±4**	54±5**	52±6**
rT_3	100	100±12	101±11	81±7*	67±13*	50±8**

Paired t-test for difference from pre-treatment values (*p 0.1 **p .01)

The absence of a significant change in rT_3 after 2 days treatment indicates that the abrupt fall in T_3 is due to a selective effect on T_4 deiodination. The failure of rT_3 to rise suggests that PTU does not result in preferential removal of the 5 tyrosyl iodine as occurs in malnutrition, while failure of rT_3 to fall abruptly (as T_3 does), suggests that PTU does not block T_4 deiodination to rT_3. The results suggest that an increase in rT_3 is not inevitable when peripheral formation of T_3 is inhibited and that the T_3 — lowering effect of PTU is different from the effect observed in malnutrition, or non-thyroidal illness, where rT_3 increases. This finding suggests that the alternative pathways of T_4 monodeiodination may be independent, rather than inversely linked.

4. Hyperthyroidism without Triiodothyronine Excess

D. M. Engler, E. B. Donaldson, S. Petrou, P. Taft and J. R. Stockigt.

Ther peripheral monodeiodination of thyroxine (T_4) is altered during severe illness, with decreased production of 3,3', 5-triiodothyronine (T_3) and increased formation of 3,3',5'-triiodothyronine (reverse T_3, rT_3). The serum concentration of T_3 may become subnormal during severe illness in euthyroid subjects, associated with an increase in reverse T_3 and it has been suggested that measurement of T_3 may lose its diagnostic value when suspected hyperthyroidism is associated with a severe non-thyroidal illness.

The aim of this study was to follow serial changes in thyroid hormone levels during and after acute illness or surgery in patients suspected of hyperthyroidism.

Serial studies were made in 9 patients, as follows:

1. Two thyrotoxic patients who presented with associated severe non-thyroidal illness (cholecystitis, large bowel obstruction).
2. Two thyrotoxic patients during elective cholecystectomy, while untreated for

mild or partially-controlled thyrotoxicosis.

3. Five euthyroid patients undergoing elective abdominal surgery.

Patients in categories 2 and 3 were studied on days 0, 1, 2, 5, 7 and 11 (relative to surgery) with TRH tests (200 ug TRH) on days 0, 5 and 11. Studies of patients in category 1 extended over 3 to 7 months during periods of acute illness, recovery, relapse and surgery.

Total serum T_4 was measured by Ames Tetralute, and free thyroxine index (FTI) calculated from T_4 and resin uptake measured by Nuclear Medical Laboratories Tri Tab. Absolute free T_4 was calculated from free T_4 fraction, determined by a modification of the method of Irvine. T_3 was measured by radioimmunoassay using unextracted serum, with Merthiolate 2 mg/ml to inhibit endogenous binding; reverse T_3 was measured by radioimmunoassay of ethanol-extracted serum and standards prepared in hormone-free serum.

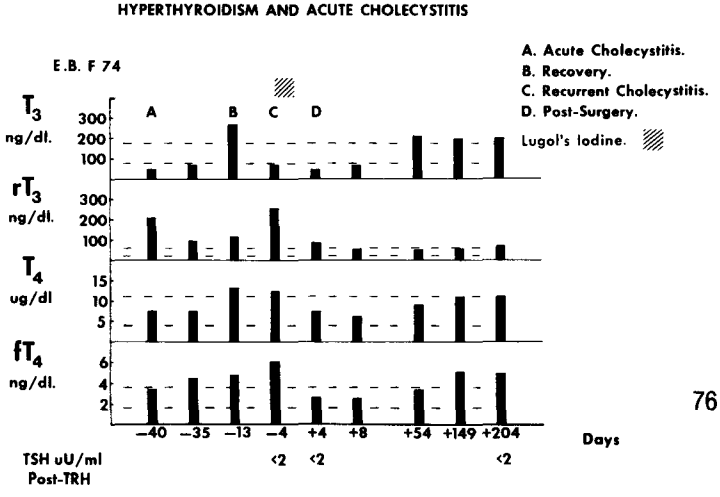
Findings in a 74 year old woman, who presented with recurrent acute cholecystitis and clinical features suggestive of hyperthyroidism, are shown in Fig. 6. The serum T_3 concentration was subnormal during two episodes of cholecystitis, associated with a marked elevation in the concentration of reverse T_3, but typical findings of hyperthyroidism were seen during remission and after convalescence. Hyperthyroidism was further supported by the lack of a TSH response to thyrotopin — releasing hormone on three occasions.

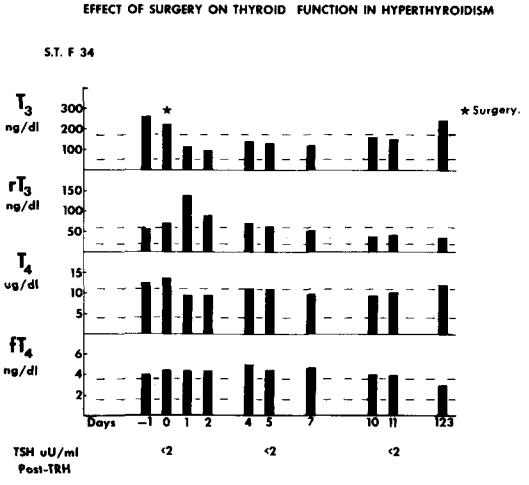
The findings in a 34 year old woman, in whom elective cholecystectomy was performed in the face of mild untreated hyperthyroidism are shown in Fig. 7. Serum T_3 fell to normal levels postoperatively, associated with a marked rise in reverse T_3.

In 5 euthyroid patients, T_3 decreased by 30% on the first postoperative day after elective abdominal surgery, while reverse T_3 rose by 80%.

These findings indicate rapid, reversible, inverse changes in the serum concentrations of T_3 and reverse T_3 during surgery or illness and suggest that T_3 loses its diagnostic value when hyperthyroidism coexists with a non-thyroidal illness. In this circumstance the diagnosis is more firmly based on the demonstration of T_4 excess and evidence of thyroid autonomy. It remains uncertain whether T_4 excess alone can sustain hyperthyroidism because of the slow offset of thyroid hormone action.

Fig. 6. Effect of acute cholecystitis, recovery, recurrence, surgery and convalescence on thyroid hormone levels. Days are referable to day of cholecystectomy. Treatment with Lugol's Iodine was given from day — 4 to 0.fT = free thyroxine.





Serial changes in thyroid hormone levels after elective cholecystectomy in a patient with mild untreated hyperthyroidism. Days referable to day of surgery. fT_4 = free thyroxine.

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C. J. Officer Brown Cardiac Surgery Unit

Director: Mr. George R. Stirling
Cardiac Surgeons: Mr. Eric Cooper
Mr. Bruce B. Davis
Miss E. Anne Shanahan

Coronary Artery Surgery

During 1976 almost 400 cardiac operations, including 330 'open heart' procedures, were carried out in the Unit. The major increase has been in the number of operations carried out for coronary artery disease, amounting to 148 cases during the year. The overall mortality for open heart surgery has been 3.8% and the mortality for elective myocardial revascularisation has been 2.5%.

The availability of an electro-magnetic flow meter has made possible the routine measurement of the rate of blood flow in the by-pass conduit after saphenous vein graft by-pass and it is hoped to improve the criteria for selection and better estimate prognosis by correlating this data with information obtained from coronary angiography.

The acquisition of a cardiac output computer using the thermo-dilution technique has improved post-operative care in patients suspected to have a low cardiac output after cardiac surgery. The cardiac output is correlated with left atrial pressure of pulmonary artery wedge pressure measurements to better determine decisions in post-operative management.

Circulatory support by counter-pulsation using the AVCO Intra-aortic Balloon Pump (I.A.B.P.) has now been available in the Unit for the last two years. The use of this technique has been confined to patients with severely reduced cardiac output due to left ventricular failure usually arising immediately after surgery. In 3 patients, the I.A.B.P. technique has been used preoperatively to allow investigations and emergency operation in critically ill patients with complications of myocardial infarction. The method has resulted in a survival rate of 60% in a group of patients who may well not have survived without it. The availability of the I.A.B.P. has allowed an extension of the indications for operation to patients with extreme left ventricular dysfunction which is appropriate if an underlying mechanical cause, such as ventricular aneurysm, ventricular septal defect or mitral reguritation can be corrected.

Valve Surgery

Although the Bjork-Shiley tilting disc prosthesis has been used for most valve replacements, the necessity for permanent anticoagulation makes this an inadequate choice in some cases. The Hancock Porcine Xenograft has been used in 30 cases in whom anticoagulants were contra-indicated or in the young female intending to have children. Two minor emboli have occurred in the early post-operative period and, in another case where para-valvular leak occurred, the bioprosthesis was removed.

A proportion of cases with Porcine Xenograft mitral valve replacement have a mid-diastolic murmur and minor obstruction has been documented by cardiac catheterisation in one case. We had had no experience with serious malfunction of the prosthesis so far but longterm follow-up is necessary to decide whether this change in policy is wise.

Comparison of Oxygenators

A comparison of the Rygg Bubble Oxygenator and the Teflo Modulung Pseudo-Membrane Oxygenator was carried out during 1975 and 1976. A matched series of 100 cases was used, half the cases being perfused with one and the other half with the other oxygenator. The indices measured included clinical assessment, including cerebral function, pulmonary complications determined by chest radiography, total post-operative drainage, total in-hospital blood usage and platelet count. No significant differences between the two groups were established. There seemed no reason to change the policy of using the Rygg Oxygenator for short term cardio-pulmonary by-pass.

Psycho-Social Correlates of Cardiac Surgery

This study consists in carrying out a very detailed social and psychological survey, correlated with full clinical information on a group of 50 patients undergoing myocardial revascularisation. The patients are then restudied 6 months after surgery to assess the total effectiveness of the operative procedure with regard to social function, psychological sequelae and physical performance. The technique involves a detailed structured interview by a social worker, the administration of three self-rating psychological tests and the compilation of the full clinical information

on each patient. The input of pre-operative patients is almost complete and the earlier patients are now returning for post-operative assessment. The study should be completed in June 1977.

Post-Operative Myocardial Infarction

Dr. Frank Panetta and Dr. Aubrey Pitt, with the Department of Nuclear Medicine, carried out a study on the incidence of post-operative myocardial infarction using Technetium Pyrophosphate Radio-Nucleide Image Scanning. Using standard electrocardiographic criteria, the incidence of post-operative infarction has been 6% whereas using radio-nucleide scanning it appears that the incidence of positive scans lies between 20 and 30% depending on the case group studied. The significance of these findings and the correlation with creatine phosphate M.B. is being pursued and it is hoped to add Thallium scanning to the study in 1977.

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