research

baker institute

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annual report

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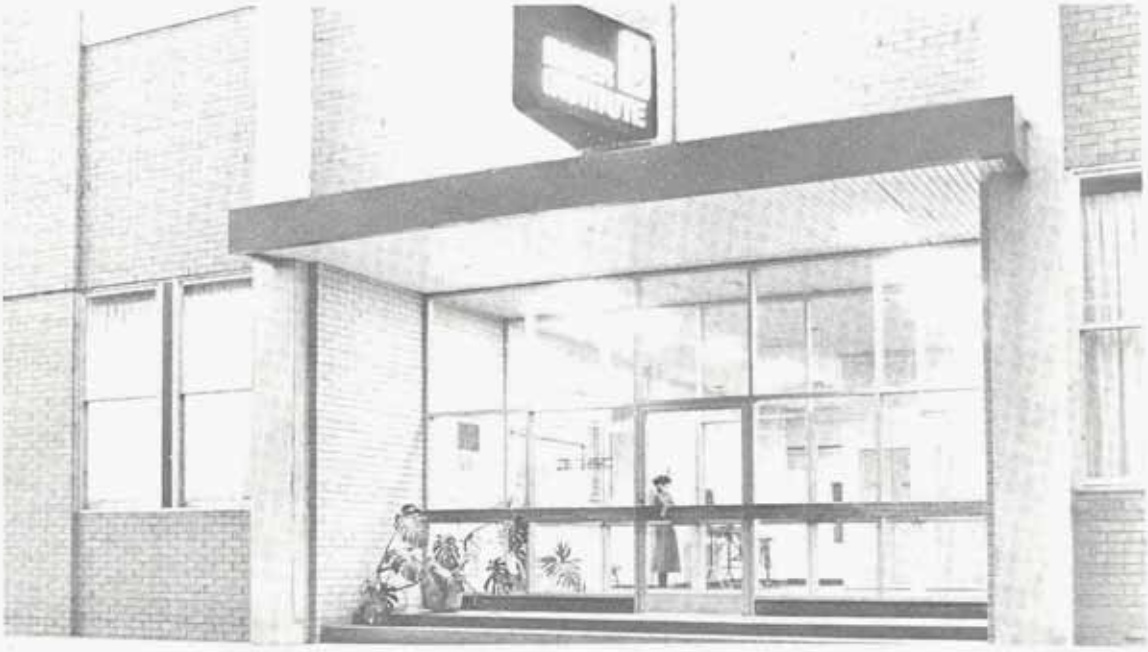
The Clinical Research Unit is a department of Alfred Hospital which conducts investigations in hypertension and coronary heart disease.

The Ewen Downie Metabolic Research Unit is a department of Alfred Hospital which conducts research in endocrinology.



Forty-ninth
Annual Report of

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

Twenty-seventh
Annual report of

THE ALFRED HOSPITAL CLINICAL RESEARCH UNIT

Nineteenth
Annual Report of

THE EWEN DOWNIE METABOLIC UNIT

Reports of

ALFRED HOSPITAL RESEARCH FELLOWS

Baker Medical Research Institute

affiliated with
Monash University

ANNUAL REPORT 1975

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History of the Baker Institute — 1926 · 1974

The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute was founded in 1st April 1926. The initial function of the Institute was to provide a biochemical laboratory service for Alfred Hospital and also to provide facilities for medical research. The idea of forming a medical research institute in close proximity to the Hospital was conceived by Dr. John F. Mackeddie, Physician to In-patients at Alfred Hospital, and Chairman of the Medical Staff Committee, and later first Chairman of Trustees of the Institute. Mackeddie was concerned that Alfred Hospital should keep abreast with the exciting advances in the field of diabetes and other metabolic disorders. For this purpose it was imperative to provide a modern clinical biochemistry laboratory. This required trained staff and a building and the finance for both these were eventually provided by Mackeddie's friend, Thomas Baker.

Thomas Baker was born in Montacute, Somerset, in 1854 and was educated in South Australia where his father, Charles, had established a coach building business. Thomas Baker became a pharmaceutical chemist and practised in Maryborough, Queensland, where in 1877 he married Alice Shaw. He and his wife and her sister, Eleanor Shaw, moved to Melbourne some time between 1877 and 1881. He began to manufacture photographic materials in partnership with J. J. Rouse and the business flourished. In 1908, Baker and Rouse amalgamated with the London Kodak Company and established Kodak (Australasia) Pty. Ltd. and became the new company's joint Managing Directors. It is not certain how Thomas Baker's interest in medicine was first aroused — it may have been through his experience as a pharmaceutical chemist or because he had done more than one year of the medical course in Melbourne during 1882-83. Whatever the exact reason, he became attracted to Mackeddie's idea of providing a modern medical laboratory service for the Hospital. In 1925 Thomas Baker, his wife Alice, and Eleanor Shaw, announced that they would take over the responsibility for the staff and building of a new biochemistry laboratory at Alfred Hospital and it was for this function that in 1926 the Baker Institute was officially opened. The agreement with the Board of Management of the Hospital stipulated that the new laboratory should also function as a medical research department. The generosity of the Baker and Shaw families continues to this day through the charitable trust, Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions which they established in their wills. To date the Trust has contributed nearly $4.5 million towards the various research activities of the Institute.

Thomas Baker died in 1928. His business associate, Mr. J. J. Rouse, took a direct interest in the Institute, and helped the Institute on many critical occasions during the financial stringencies of the depression years. His son, Edgar Rouse, who succeeded his father as Chairman of Kodak, became a Trustee of the Benefactions and from 1942 a Trustee of the Institute. He was Chairman of Trustees of the Institute from the time of Mackeddie's death in 1944 until 1971 (when he was succeeded by the present Chairman, Mr. J. C. Habersberger) though he remained a Trustee until his death in 1974. His financial and managerial skills were an enormous asset in the rebuilding of the Institute in 1967. The long and happy association of the Rouse family with the Institute is commemorated by "The Rouse Library" in the new building.

The Institute began its scientific activities under its first Director, Dr. W. J. Penfold. The chief aim at the time was to improve the quality of the hospital laboratory services. Dr. Penfold had two principal assistants, Dr. A. B. Corkill (later to become the second director of the Baker Institute), and Dr. John Fiddes. Corkill supervised the biochemistry laboratory whilst Fiddes looked after pathology. The stimulus provided by Corkill's work on carbohydrate metabolism led to the establishment of a Diabetic Clinic, first under Corkill then later under Dr. Ewen T. Downie. Penfold retired from the Institute in 1938 because of ill health and was succeeded by Corkill. During the war Corkill served with the Defence Department and the business of the Institute was run by the Acting Director, Dr. E. Singer. Corkill returned to full duty in 1944 and was responsible for separating the research function of the Institute from the service required by the

Mrs. Alice Baker, Miss Eleanor Shaw and Mr. Thomas Baker in the United Kingdom — early 1900's.

Hospital. From 1946 onwards, the Hospital took over the management of the routine clinical, pathology and biochemistry services and the Institute now had a predominantly research function. Unfortunately, Dr. Corkill suffered from increasing ill health and retired as Director of the Institute in 1948. During Penfold's and Corkill's tenure, research had taken second place to the routine hospital service duties. Nonetheless important research was performed with some of the most important contributions at the time coming from Charlotte Anderson, Ewen T. Downie, and A. H. Ennor, all destined to become distinguished clinicians or scientists.

In the meantime a new Clinical Research Unit had been formed at Alfred Hospital at about the time of Corkill's retirement. The newly appointed Director of Clinical Research, Dr. Thomas E. Lowe, was also appointed as Director of the Baker Medical Research Institute in 1949 and since that time the two Units have worked in very close association. Over the next 25 years, under Dr. Lowe's tenure, the Institute saw an enormous increase in the range, volume and quality of basic clinical research activities in many diverse fields. Dr. Lowe's own field was in cardiovascular medicine and he made important contributions on the mechanisms involved in fluid volume control in heart failure and other circulatory disorders.

In the early fifties many of the modern techniques of clinical and cardiological investigations were introduced to Alfred Hospital (and often to Australia) through studies performed in the Baker Institute and Clinical Research Unit by Dr. H. B. Kay, Dr. H. A. Luke, Dr. J. M. Gardiner and Dr. A. J. Goble. An Experimental Surgery Theatre was developed in conjunction with the Alfred Hospital which helped in the introduction of modern open heart surgery through the work of C. J. Officer-Brown, K. Morris and G. R. Stirling. The early 1950's also saw the establishment of the first Hypertension Clinic in Australia through the efforts of A. J. Barnett. He made important contributions in the treatment of severe hypertension and also pioneered many important investigations on the mode of action of anti-hypertensive drugs.

Another distinguished contributor in the Institute in basic cardiovascular pharmacology and physiology was Dr. Winifred G. Nayler who, during her period at the Institute from 1955-1972, performed pioneer studies on the fundamental importance of calcium in heart muscles and the effect of different drugs on calcium metabolism. In addition, she performed many other studies on myocardial metabolism and on the pharmacology of cardiovascular drugs.

The research work of the Institute extended over many different fields. The interest in metabolic and endocrine disorders which had started with Corkill was carried on by Dr. Ewen Downie, Dr. B. Hudson, and Dr. J. Bornstein. Paul Fantl, later to become Associate Director of the Institute, made important contributions in the field of blood coagulation and on the management of coagulation disorders in haemophilia. Work on tumor pathology had been performed at the Institute from its earliest days, notably by R. A. Willis and L. B. Cox, but under Dr. Lowe a Cancer Research Laboratory was established with the aid of the Anti-Cancer Council of Victoria. It has been active in studying biochemical aspects of carcinogenesis by Chev Kidson. More recently mechanisms involved in chemical carcinogenesis have been studied by Gordon Hard. An active research programme on the developing cardio-respiratory apparatus of the foetus was also established in 1972 by J. E. Maloney.

Even in the 1950's it was clear that the laboratory facilities of the Institute were becoming over taxed, and fortunately in 1965 it became possible (through the generosity of the Benefactions), to plan the complete rebuilding of the Institute. Stage 1 was occupied in 1967 and the new building which had cost about $1.4 million, was officially opened in 1969 by Sir Henry Bolte, the then Premier of Victoria, whose Government had contributed $200,000 towards the cost of the building. The building has about 2,250 sq. m. of usable laboratory space. Its facilities include laboratories for physiological and pharmacological work, biochemical laboratories, histological and electronmicroscope facilities, a laminar flowroom for tissue culture, and an experimental surgery theatre.

The Institute has been fortunate in having attracted first class young people from its very earliest days. Many of its 'old boys' have moved to important academic and clinical posts. Those that have become professors in a variety of disciplines all over the world are Charlotte M. Anderson, R. R. Andrew, J. Bornstein, A. J. Ennor, J. Fiddes, B. Hudson, C. Kidson, P. Kincaid-Smith. B. Mac.A. Sayers, F. O. Simpson, R. A. Willis and O. A. Tiegs; five have become directors of important scientific laboratories, fourteen have become readers or associate professors in universities and over twenty-six have become consultants on the honorary staff of many hospitals, particularly of Alfred Hospital.

Dr. Lowe retired as Director of the Clinical Research Unit in 1973 and as Director of the Baker Medical Research Institute in 1974. The work done in both of these Units under his guidance showed how profitable an association between basic and clinical research could be. It must be emphasized that what often seem difficult technical feats in the laboratory become in fact the routine investigations of tomorrow. It is interesting that both the Baker Institute and Clinical Research Unit helped a great deal to establish new clinical service facilities such as the cardiac catheterisation laboratory and the metabolic laboratory in much the same way as routine biochemistry and pathological laboratories had first been established in the hospital through the help of the Institute. The cardiac catheter laboratory is now an autonomous department of the Hospital — The Cardiac Diagnostic Service — and the metabolic laboratory is also autonomous as the Ewen Downie Metabolic Unit. The Institute also played a smaller but important role in the establishment of a gastroenterology service in the Hospital.

Dr. Lowe's successor as Director of the Baker Institute and of the Clinical Research Unit was Professor P. I. Korner who came from the Scandrett Chair of Cardiology at the University of Sydney. He started work in 1975 and the changes since that time are summarised in this year's Director's Report.

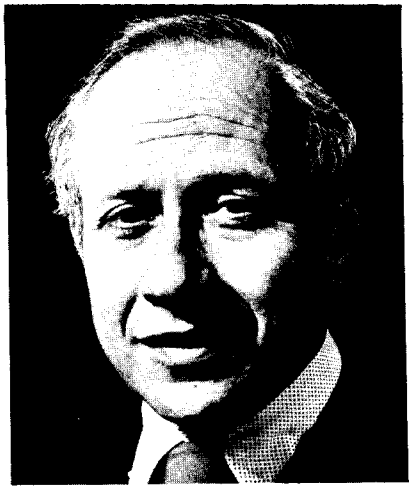
Director's Report for 1975

This report marks the end of my first year as Director of the Baker Medical Research Institute. It is appropriate to pay particular attribute to my predecessor, Dr. Thomas Lowe, whose remarkable achievements over the past twenty-five years have been summarised briefly in the preceding historical sketch. A more detailed account is in preparation.

Dr. Lowe was responsible for introducing close links with the basic scientists and clinicians at the Alfred Hospital at a time when Australia was lagging far behind other countries in this kind of collaboration. In addition to the many scientific contributions made by the Institute staff under Dr. Lowe, the close collaboration between laboratory and clinic which was forged through the Baker-Alfred Hospital axis, was unique in Australia and led to rapid application of new methods of clinical investigation with readily apparent improvements in diagnosis and patient care in many important areas of medicine. During the year the Trustees named the Thomas E. Lowe Seminar Room to commemorate his very distinguished services to the Institute and to Australian medicine.

The main change in research emphasis that has occurred since my arrival in Melbourne has been the decision to develop the Baker Institute (in conjunction with the Clinical Research Unit) as an exclusively Cardiovascular Research Centre. I am convinced that we can obtain the best value for our money and personnel resources by concentrating on one important general field of medical research from a number of different directions. The contribution which the multidisciplinary Cardiovascular Research Centre will make to new knowledge, improved patient care, community health and biomedical training is likely to be far greater than if we spread our efforts on several smaller projects in a whole range of unrelated fields.

I hope that the new Centre will become an internationally important research training and teaching institution in cardiovascular medicine. The present plan of development is to establish three research units in the Institute, each active in both laboratory and clinical fields. Each unit will have a Head and a small nucleus of biomedical scientists to provide continuity for our research and training programme.

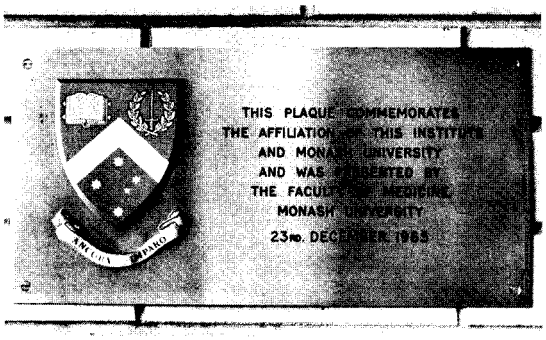


Professor Paul Korner.

Two research units have been operating in 1975: (1) *The Circulatory Control and Hypertension Research Unit*, working under my direction; and (2) *The Developmental Biology Research Unit* with Dr. John Maloney as its Head. Towards the end of 1975 the Trustees of the Institute took a most important step by establishing the *Cardiovascular Metabolism and Nutrition Research Unit* which will work on the problems of coronary artery disease under the Headship of Dr. Paul J. Nestel, a distinguished clinical scientist currently working at the John Curtin School of Medical Research in Canberra. Dr. Nestel will become Deputy Director of the Baker Institute and he and his group will take up duties during 1976.

The direction of our research and the training facilities in these particular fields are particularly appropriate in relation to the health problems of the Australian community since hypertension and coronary disease are the major causes of death, chronic illness and economic loss in Australia today. It is important to emphasise that there are several advantages in having a number of research groups working together under a single roof in a research institute such as the Baker Institute. Firstly, the opportunities of novel attacks through collaborative programmes become greatly enhanced. The opportunities of developing such programmes are greater than in universities because the 'departmentalisation' of our research institute is much less rigid. The second advantage is in the field of biomedical education. The Baker Institute offers particularly good opportunities for postgraduate research training in cardiovascular medicine, on areas not adequately catered for in Australia at the present time by either the Royal Colleges or the Universities. Indeed the training provided to equip the clinical staff in our hospitals with the capacity for evaluating the value of the many new diagnostic and treatment

procedures in cardiovascular medicine is at present inadequate and needs to be remedied by some formal exposure to research methods and problem solving. This sort of training will be required by the staff specialists of the future if our system of medical care is to flourish. This type of postgraduate research training has been much neglected in Australia. It contrasts with the position in the United States, where cardiovascular research centres such as the Heart and Lung Institute in Bethesda, or the San Francisco Cardiovascular Research Institute have made an enormous impact on the general standard of diagnostic training and treatment evaluation.



The Baker Institute, because of its affiliation with Monash University, will provide research training through higher degrees and through programmes for undergraduate research and honours students. We will have post-doctoral programmes in the three research units. We hope to attract hospital residents doing an elective year of Advanced Training in Cardiology of the Royal Australasian College of Physicians. At present, members of our staff also contribute to the teaching programme for undergraduate students in medicine, pharmacology and physiology at Monash and Melbourne Universities.

Science is an international endeavour and we are particularly anxious to have visiting scientists contributing to our research activities. These visitors provide a great stimulus and I was particularly pleased to welcome Dr. Jennifer Angell-James in September 1975, who became the first visiting scientist since my arrival. She comes from St. Bartholomew's Medical College and joined us to establish a Cardiovascular Neurosciences Laboratory. More visitors are scheduled to come to the Institute during 1976.

I have emphasised that a research institute, such as the Baker Institute, has some advantages over university departments from the point of view of providing postgraduate research training. However, I must emphasise that we regard our role as complementary and not in competition with universities. Indeed, the Baker Institute is a medical postgraduate department of Monash University under its affiliation agreement with the University. I have very much appreciated the honour the University bestowed on me by appointing me to a Personal Chair in Medicine. There is also much scientific collaboration between us and the University, e.g. with Professor Colin Johnston's research group. This collaboration has been beneficial for each group in view of our complementary expertise in endocrine and neural aspects of blood pressure control in hypertension.

I want to conclude my report by outlining some of the financial implications of our new development and reorganisation. It has already become clear that whilst our laboratories are excellent from the point of view of buildings and services, a great deal of new equipment will be required over the next two to three years to allow the various units to function effectively. Furthermore, the escalating salary costs have placed an enormous burden on our resources at a time of high inflation; an ever-increasing fraction of our relatively fixed income from the Baker Benefactions — our major source of funds — is going into salaries and our capacity for the purchase of modern equipment, and for educational training posts is becoming severely limited. Expensive though it is to run an institution such as the Baker Institute, it must be emphasised that it is perhaps the most economic way of utilising equipment and capitalising on the excellent physical facilities of the Institute. The different research units *share* equipment, the utilisation rate is high, and there are obvious benefits to the Australian community in connection with new knowledge gained and the research training of clinical and basic scientists in cardiovascular medicine. However, we will need to have a substantial increase in our funds than we have at the present time through the Baker Benefactions and the conventional sources of project grants. At present we get good support from the National Health and Medical Research Council, the National Heart Foundation, the Life Insurance Medical Research Fund, and Alfred Hospital Research Grants. I hope that we will get increased financial support from Federal and State Government sources in the form of block research and training grants. I hope that even though the economy is in a very difficult state they will recognise that such support will bring worthwhile benefits to the health-care of the Australian community.

Circulatory Control and Hypertension Research Unit

Main Topics:

- Central Nervous Control of the Circulation
- Pharmacology of Anti-hypertensive Drugs
- Causes of Hypertension

Establishment of Unit

I was particularly fortunate that five members of my research group in Sydney came with me to the Baker Institute — Warwick Anderson, Jim Angus, Peter Blombery, Peter Fletcher and Judy Oliver. Accordingly, there has been a fairly minimal disruption of our research. In addition we have developed new projects in biochemical pharmacology in conjunction with Alex Bobik and Val Carson. In June 1975, Jeffrey Hutchinson joined us after two years of postgraduate study in Heidelberg in the German Federal Republic. He has helped set up a radioimmunoassay laboratory and his main project is to study how angiotensin (a hormone secreted by the kidney in salt deprivation and certain types of hypertension) works in the brain. In September 1975 Dr. Jennifer Angell- James came from the St. Bartholomew's Medical College, London, as the Edward Wilson Visiting Research Fellow to establish a Cardiovascular Neuroscience Laboratory. All these activities have meant setting up several new laboratories all of which required a great deal of new electronic equipment. We were fortunate to appoint Ron Wall as Electronics Engineer-in-Charge of a joint C.R.U.-Baker Institute Electronics Laboratory and Workshop. Major achievements of the Electronics Laboratory have been the complete redesign of the Doppler ultrasonic flowmeter assembly used to monitor blood flow in many of our studies and the building of new equipment for the Neurosciences Laboratory.

General Summary:

Circulatory Control

My laboratory has studied during the last ten years how the central nervous system regulates the circulation. The central nervous system alters the activity of several groups of autonomic nerves thereby changing the force

Members of the Circulatory Control and Hypertension Research Unit. (From left) Peter Fletcher, Peter Blombery, Warwick Anderson, Jennifer Angell-James, Jim Angus and Jeff Hutchinson. Seated are Paul Korner and Judy Oliver.

Dr. Jennifer Angell-James — Edward Wilson Visiting Research Fellow.

of contraction of the heart beat and the diameter of the blood vessels. These effects can raise or lower blood pressure. We have been studying how different types of stress influence brain mechanisms concerned with autonomic function. Up till recently we have concentrated on acute types of environmental stress such as reducing the inspired oxygen concentration or studying the effects of blood loss. Briefly, each stimulus usually activates a number of different groups of peripheral receptors which provide the brain with information about changes in pressure in the arteries and the different chambers of the heart, about respiratory movements and oxygenation of the blood, and many other functions besides. Different environmental stresses produce distinctive changes in activity of each set of receptors that are characteristic of the type and intensity of the particular stress. In turn this results in a characteristic pattern of activation of the different circulatory centres of the brain which result in patterns of peripheral autonomic activity which can alter the distribution of the blood flow to the different organs of the body. Our results have shown that the higher centres of the brain in the cerebral cortex and hypothalamus contribute to these distinctive reflex patterns in addition to the circulatory centres of the lower brains stem. The latter were until recently thought to be the exclusive mechanisms involved in the integration of reflex stimuli.

At present we are beginning to examine autonomic responses to more chronic types of stress. This is highly relevant to the possible role which emotional factors and long acting environmental disturbances may play in the development of high blood pressure in man. We are currently analysing the role of brain amines such as noradrenaline, which are known to function as chemical transmitters between different nerve cells in the central nervous system. This occurs in addition to their well known actions as transmitters in peripheral sympathetic nerve endings and in the adrenal glands. It has been thought that these central noradrenergic neurons play an important role in blood pressure control. However, some of our preliminary data on the effects of selective destruction of these pathways on reflex autonomic function suggests that they play an even more important role in relation to behaviour than in relation to autonomic control of the circulation

Hypertension

Much of our work during the year has been in the field of hypertension. The studies in man are summarised in the report of the Clinical Research Unit. Some of our animal experiments are closely linked to these studies in man and are concerned with analysing the mechanisms of action on the beta-blocking drugs in hypertension. We are also studying whether the beta-blocking drugs are capable of altering the information reaching the central nervous system through the arterial baroreceptors.

Apart from the work on control of the circulation by the central nervous system and different hormones we have also studied several aspects of local chemical control of blood vessels. One important vasoactive substance which is of importance in inflammation and allergies is histamine and we have been examining its effects on blood vessels after release from the tissue stores of histamine.

Another group of projects has been concerned with the working out of the exact mechanisms whereby blood pressure becomes elevated in renal hypertension. It has long been known that lowering the renal artery pressure or impairing the kidneys' blood supply will lead to a rise in blood pressure but the exact steps involved in the process are still understood very imperfectly. It reflects our general ignorance of the fundamental causes of high blood pressure. Probably the reason why the mechanisms are still so poorly understood is that the initial renal disturbance sets off a large number of secondary effects which alter the function of the body's hormonal and autonomic nervous control system with some exceedingly complex interactions between them.

The projects started during the year include a quantitative study of the effects of grading the degree of renal artery constriction and a study on the effects of chronic changes in the intake of dietary salt. We are also examining the way in which angiotensin may act on the autonomic mechanisms of the central nervous system.

PROJECTS

A. Mechanism of Action of Beta-Adrenergic Blocking Drugs in Hypertension

1. *Leakage of Propranolol from Cerebrospinal Fluid*
W. P. Anderson, A. Bobik, J. P. Chalmers*,& P. I. Korner

It has been suggested that the action of beta-blocking drugs in lowering blood pressure results from direct effects within the central nervous system. This hypothesis is based on studies showing that blood pressure falls after intracerebroventricular (i.c.v.) injections of propranolol. However, in all previous studies the doses of drug injected into the cerebrospinal fluid (C.S.F.) have been large. With molecules of the size of propranolol it seems unlikely that its action would be confined to the brain. We wished to examine whether some of it leaked into the blood stream and reached a concentration in plasma sufficient to exert significant 'peripheral' beta-blockade on the heart and blood vessels. Studies in unanaesthetised rabbits showed that 2-4 hours after i.c.v. injection of 400µg dl-propranolol there was a small reduction of blood pressure averaging 3.2 ± 1.4 mmHg. This was statistically significant and was similar to the fall observed after intravenous (i.v.) injection. After i.c.v. injection there was a rapid rise in plasma propranolol concentration and 10 minutes after injection the plasma concentration was 80% of the level observed after giving the same dose intravenously. After i.c.v. injection there was significant blockade of cardiac beta-receptors for at least 2 hours. This was determined from the degree of attenuation of isoprenaline-induced tachycardia in areflexic rabbits. The results show that the technique of i.c.v. injection of propranolol does not enable one to differentiate whether central nervous or peripheral mechanisms are involved in the action of the drug in lowering blood pressure.

2. *Effects of Clonidine on the Autonomic Constrictor Pathways*
P. A. Blombery and P. I Korner

The cardiovascular responses to forced expiration (Valsalva-manoeuvre) have been widely used as qualitative tests of autonomic function in man. We have recently found in man that a rise in total peripheral resistance (TPR) occurs during the manoeuvre in man which is quantitatively related to the level of forced expiratory pressure. A similar model has been developed in rabbits for eliciting stimulus-related constrictor responses. The study is performed in rabbits in which Doppler ultrasonic flowmeters have been implanted at a previous operation. Graded Valsalva-like manoeuvres were induced by applying expiratory pressure ranging from 2.5- 25 mmHg to the inlet and outlet tubes of a respiratory valve and to a sphygmomanometer cuff placed around their thorax and abdomen. There was a linear relationship between expiratory pressure (EP) and rise in TPR above resting. After atropine and after atropine + propranolol at moderate plasma concentrations (72 ± 12 ngml) the slope of the relationship was little altered but after blocking the sympathetic constrictor nerves the rise in resistance was completely abolished. The rise in TPR is thus reflex in nature and is mediated through the sympathetic constrictor nerves.
This reflex has proved very susceptible to the anti- hypertensive drug clonidine which is believed to lower blood pressure through stimulation of alpha-adrenoreceptors located within the central nervous system. For a given expiratory pressure clonidine produced a dose-dependent attenuation of the rise in TPR and at infusion rates greater than 1.0 µg/kg min-'min. the EP-related rise in TPR was completely abolished.

Footnote: Asterisk denotes not a member of the Baker Institute or C.R.U.

3. *Effects of Propranolol on Blood Pressure and Autonomic Constrictor Responses.*
P. A. Blombery, A. Bobik & P. I. Korner

As indicated in the preceding study administration of propranolol to produce plasma concentrations of 72 ng/ml had little effect on the blood pressure and on the rise in TPR elicited by the Valsalva-like manoeuvre in the rabbit. Accordingly we studied the effects of two higher blood concentrations (P_1 = 168 ± 38 ng/ml, and P_2 = 240 ± 33 ng/ml). Rabbits were given appropriate bolus injections and continuous infusions to maintain these blood concentrations for at least one hour before the start of the test. The hypothesis examined was that if propranolol acted to reduce blood pressure by blocking central beta-receptors it should alter the properties of the Valsalva-TPR reflex in a manner analogous to clonidine. In other words, central beta-blockade should unmask central alpha-agonist effects.

At blood concentration P_1 the reflex properties were not altered, and the small reduction in blood pressure was the same as in control animals infused with saline over the same time period. However, at concentration P_2 the mean blood pressure fell significantly further by 7 mmHg. The fall was entirely accounted for by reduction in cardiac output and there was no effect on the Valsalva constrictor reflex. These results suggest strongly that in the rabbit the anti- hypertensive effect of propranolol is mediated through peripheral cardiac beta-blockade and not through any central actions on the autonomic cardiovascular pathways.

4. *Effects of Propranolol on the Baroreceptor Heart Rate Reflex.*

W. P. Anderson, P. I. Korner, A. Bobik and J. P. Chalmers*

dl-propranolol in doses of 500 µg was injected into the CSF of normal rabbits and rabbits with chronic renal hypertension induced by cellophane wrapping 5-8 weeks previously. Properties of the baroreceptor-heart rate reflex were investigated with mean arterial pressures varied by inflating perivascular balloons placed at a previous operation around the inferior vena cava and thoracic aorta. Following i.c.v. injections of propranolol significant changes in MAP- HP curves from control were obtained at 20 minutes and at 1 hour after injection in both hypertensive and normotensive animals. At 20 minutes there was a rise in threshold and a reduction in sensitivity and in the heart period range; at 1 hour heart period range (HPR) alone was still reduced and at 2 and 4 hours the MAP-HP curve was similar to control. The reduction in HPR after cerebrospinal fluid injections could be explained by a rapid escape of propranolol from the cerebrospinal fluid to blood producing high levels of cardiac beta-blockade. The changes in the curves 20 minutes after injection are consistent with a non-specific central nervous action due to the 'local anaesthetic' properties of the drug. At 1 hour the effect was entirely consistent with peripheral beta-blockade with no evidence of residual central nervous action.

5. *Effects of Beta-Blocking Drugs on Arterial Baroreceptor Function.*

J. E. Angell-James.

The laboratory has facilities for perfusing isolated organs of vascular territories and for the continuous monitoring of ECG arterial venous and respiratory pressures and blood flow. The data is collected on a 7 channel tape recorder and can be displayed on a storage oscilloscope and on a 16 channel ultra violet light recorder.

No studies of the effects of beta-blocking drugs on the arterial baroreceptor activity have been previously carried out. In the present study activity of single aortic baroreceptor

fibres is being recorded before and after administration of propranolol. The preparation used is the completely controlled aortic arch preparation previously developed by Angell-James. Studies are being performed in normal rabbits and in hypertensive animals before and during treatment with beta-blockers.

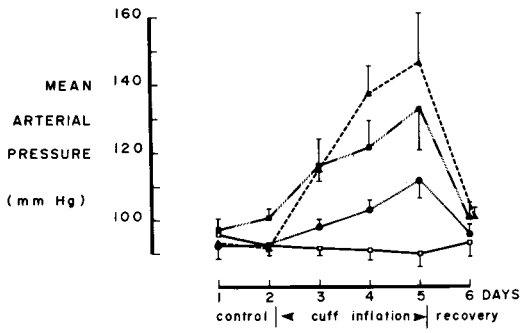
B. Experimental Hypertension

6. *Effects of Graded Renal Artery Constriction in Renal Hypertension.*

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

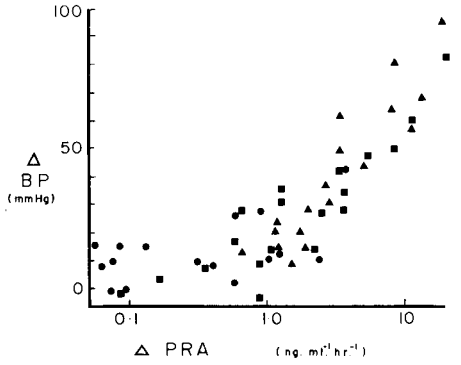
Questions we have attempted to answer are the following:— What were the effects of grading the degree of renal artery constriction on systemic and renal haemodynamics, on blood volume and on the angiotensin II (AII) blood concentration? The study has been undertaken in conscious dogs trained to lie quietly in the laboratory. At a preliminary operation performed several weeks earlier under anaesthesia one kidney is removed and an electromagnetic flowmeter is placed around the aortic root for cardiac output measurement. In addition a Doppler flowmeter is placed around the left renal artery, together with a special inflatable silastic cuff for subsequently producing graded renal artery constriction. Pressure lines are implanted to measure distal renal artery and systemic blood pressures.

Measurements were made on two control days, followed by renal artery constriction to lower renal artery blood pressure to 60, 40 or 20 mmHg for three days followed by measurements one day after the release of pressure. In addition to the haemodynamic measurements blood samples were obtained for plasma renin activity and AII assays and for blood volume determinations. After a week's rest following release of the cuff a further set of observations was made in the same animal at another level of renal artery constriction. In some animals serial observations were made following a sham procedure where the cuff was inflated to lower renal artery pressure to 60 mmHg for 30 seconds twice every day.



Effect of graded renal artery constriction on systemic mean arterial pressure in 'one kidney' experimental hypertension in the dog. Renal artery constricted to 60 mmHg (circles); 40 mmHg (filled squares); 20 mmHg (triangles); control (open squares).

We found the rise in systemic blood pressure was a direct function of the degree of renal artery constriction. The renal blood flow did not alter with a renal artery pressure of 60 mmHg but at lower pressures there was some reduction. On day 1 and day 2 following renal artery constriction the cardiac output tended to increase as did plasma volume, whilst on day 2 and 3 total peripheral resistance (TPR) rose. We observed a close relationship between plasma AII concentration and a rise in systemic blood pressure. With concentrations of AII of 50 pgml or less, the rise in pressure was largely due to a rise in cardiac output but at higher levels there was a rise in TPR. Immediately after the induction of renal artery constriction the acute reduction in perfusion pressure was associated with an initial fall in renovascular resistance which was presumed to be due to autoregulatory mechanisms. This was followed by restoration in resistance suggesting a possible local renal humoral effect. Our findings at present indicate that graded renal artery constriction evokes graded elevation of blood pressure and the time course of the changes suggest that several



Relationship between plasma renin activity (PRA) and change in blood pressure (ABP) during graded renal artery constriction in the dog.

mechanisms may contribute to the hypertension. We are in the process of studying how completely the haemodynamic changes can be reversed by pharmacological inhibitors of AII, and whether impairment of the blood pressure and body fluid homeostatic mechanisms, (e.g. adrenalectomy + autonomic block) may alter the haemodynamic and humoral responses to graded renal artery constriction.

7. *Role of Cardiac Output in the Development of Hypertension*

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

Several recent studies have reported a phase of high cardiac output early in the development of renal and other types of experimental hypertension. At first the high cardiac output accounted for most of the rise in blood pressure, but with time, vascular resistance increased and later on when hypertension was fully established TPR accounted for all the rise in blood pressure, and cardiac output was restored to normal levels.

Ledingham and colleagues first suggested that the rise in TPR might be a compensatory mechanism due to excess tissue perfusion that was inappropriately high in relation to the tissue's metabolic requirements. The matter has been of particular interest since in man with mild (so-called 'borderline' hypertension) cardiac output is also relatively high. It was therefore thought that cardiac hyperactivity might be a primary mechanism leading to the development of hypertension. We have studied the problem experimentally in rabbits using an experimental model of hypertension of relatively slow onset.

After two days of control measurements of cardiac output, blood pressure, TPR and heart rate, the animals were subjected alternatively to bilateral cellophane wrapping of their kidneys or to a sham operation and further serial measurements were made over the next 32 days. During the first week after operation, cardiac output increased identically in the wrap and sham operated group. Thereafter cardiac output fell gradually in the hypertensive animals but remained at control levels in the sham operated group. Blood pressure and TPR of the renal wrap animals already exceeded the values of sham operated rabbits during the first post-operative week. The results suggested that the cardiac output changes during the first week were a non-specific consequence of the preceding wrap or sham operation. They

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bore no apparent relationship to the subsequent development of the hypertension which was resistance-mediated from the earliest stages. The findings in the rabbits differ from those reported in other species and do not conform to the changes predicted by the auto-regulation theory of hypertension.

8. *Role of Dietary Salt Intake in the Production of Renal Hypertension.*

P. J. Fletcher, P. I. Korner C. I. Johnston*

The role of sodium balance has been of particular interest in connection with many types of clinical and experimental hypertension. Epidemiological data has suggested that there is an association between the incidence of essential hypertension and the level of sodium in the diet of different

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countries. Salt balance is an important factor in many types of human renal hypertension and the blood pressure of patients with this disorder can be readily raised through fluid overload. Much previous work on experimental renal hypertension has been performed against an unknown background of dietary and sodium potassium intake.

In this investigation we have examined the effects of different intakes of dietary sodium on the haemodynamic electrolyte and catecholamine excretion and plasma renin levels before and after induction of renal hypertension (bilateral renal cellophane wrapping) or sham operation. The range of sodium in the diet was about 25 fold. Surprisingly, the resting blood pressure increased by the same amount quite independently of the dietary sodium intake. Thus the groups on high sodium diet, normal sodium diet and low sodium diets all showed a rise in blood pressure by about 40-50 mmHg. In all groups the rise in blood pressure was due to a rise in TPR (i.e. vascular narrowing) and the cardiac output remained the same in all groups. In the renal hypertensive rabbits on low sodium diets the rise in TPR was sustained to a significantly greater degree through the renin-angiotensin system than in the other groups. The autonomic nervous system apparently played little role in the maintenance of the raised TPR. It remains to be seen what the effect of impairment of neuroendocrine homeostasis is on the height of the blood pressure with different levels of dietary sodium intake.

9. *Cerebral Renin-Angiotensin System — Possible Role in Hypertension.*

J. Hutchinson and C. I. Johnston*

Angiotensin is known to be involved at least in certain stages of renovascular hypertension. During renal artery constriction it is secreted from the kidney. It has not been thought to play a significant role in *essential* hypertension. However, in 1972 Goldstein and colleagues found that there was a close positive correlation between an angiotensin-like material in the cerebral spinal fluid and the level of blood pressure in patients with essential hypertension. Since that time there has been much interest in the cerebral angiotensin-like material. This was further increased when Ganten, Hutchinson and Schelling demonstrated that the blood pressure can be restored close to normal in rats with genetic hypertension, by injecting an angiotensin antagonist into the cerebrospinal fluid (CSF).

This angiotensin-like material in CSF does not come from the blood but is generated in brain tissue. The object of the present investigation has been to define the nature of the CSF — brain angiotensin system and to examine some of the physiological conditions which lead to alterations in its activity, i.e., changes in dietary salt. Using a combined polyacrylamide gel radio-immunoassay system it was found that immunoreactive angiotensins I and II (AI, AII) are both present in CSF of rats. The AI-like material is the same as intact molecular AI with the presence occasionally of some additional as yet unidentified immunoreactive material. To-date all the AII-like material has been found to consist of immunoreactive fragments of the parent molecule. Whilst AI and AII fragments appear also to be present in the brain in much higher concentration than in CSF, it has not so far been possible to overcome a number of technical problems associated with the tissue assay. Preliminary studies suggest that there is a reciprocal relationship between CSF angiotensin-like material and blood concentration of angiotensin.

We are currently studying the effects of changes in dietary sodium on the cerebral angiotensin system. In addition the pharmacological effects of perfusing angiotensin through the brain ventricle are also being analysed. Under these conditions blood pressure increases and the rise appears to be mediated in the rat through the release of the pituitary hormone vasopressin into the blood stream.

* Not a member of the Baker Institute or C.R.U.

10. *Brain Amines and Reflex Circulatory Control.*

P. I. Korner, J. R. Oliver, V. Carson.

It has recently been discovered that noradrenaline, a hormone long known to be liberated from the adrenal gland during excitement, is also an important chemical transmitter in the brain. It is located in those brain regions involved in circulatory control and evidence has been produced that it plays a role in the control of blood pressure. It has recently become possible following the discoveries of Thoenen and colleagues, to effect destruction of the central terminals containing this transmitter by injecting small quantities of 6-hydroxydopamine (6-OHDA). To-date much of the work in other laboratories has concentrated on whether injection of 6-OHDA lowers blood pressure in different types of experimental hypertension. There has been surprisingly little study of the role of the catecholaminergic pathways in *reflex* cardiovascular control. We have developed a preparation where a number of different reflexes can be studied at one time in the unanaesthetized rabbit. At a preliminary operation a Doppler ultrasonic flowmeter is implanted on the aortic root; in addition, aortic and vena caval perivascular balloons are implanted for raising and lowering the blood pressure.

We have studied different groups of rabbits:— (1) animals injected with 6-OHDA into the cisterna magna, and (2) control animals injected only with vehicles. The following reflexes have been examined:— (i) the arterial baroreceptor—heart rate reflex; (ii) the Valsalva constrictor reflex; (iii) the effect of different grades of hypoxia, which are mainly evoked through the arterial chemoreceptors; (iv) the 'smoke' nasopharyngeal reflex which is primarily evoked through trigeminal afferents. With each reflex several stimulus levels have been examined allowing the construction of stimulus circulatory response curves. The method is sensitive for detecting relatively small changes in reflex function for any particular reflex.

Preliminary studies have shown that injections of 6-OHDA acutely raise blood pressure, but have only slight effects on the baroreceptor heart rate and 'smoke' reflexes. However, after one week when the spinal cord catecholamines have fallen by 80% normal, there is surprisingly little effect on all the autonomic reflexes tested. Effects are minimal on the arterial baroreceptor-heart rate reflex, on the reflex effects of hypoxia and on the 'smoke' reflex. However, there are significant effects on the circulatory response to the Valsalva reflex after 6-OHDA. A given rise in expiratory pressure is now associated with a much more marked reduction in cardiac output than in control animals though the rise in TPR appears if anything to be greater. The abnormal Valsalva response appears to be related to somatic factors rather than to autonomic disturbances of the catecholaminergic pathways. The animals do not tense their limb muscles during the rise in expiratory pressure and the loss of muscle pump action may lead to greater translocation of blood to the limb. Moreover, the animals tolerate the large reductions in cardiac output to a degree which would not be observed on control animals. There are other profound behavioural accompaniments associated with 6-OHDA. The animals lose their appetite and require artificial

feeding. Studies are in progress to investigate further why depletion of brain catecholamines have so little effect on reflex autonomic function. The studies include the use of higher doses of 6- OHDA, anatomical ablation methods combined with the chemical ablation produced by 6-OHDA, and the use of 5, 6- dihydroxytryptamine to deplete the serotonergic pathways.

11. *Local Control of the Peripheral Blood Vessels:— Characterisation of Exogenous and Endogenous Histamine Vascular Receptors in the Rabbit.*

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

High levels of tissue histomine are found in skin and muscle and peripheral nerves, but its role in local control of blood flow is poorly understood. Histamine has been known to stimulate at least two types of vascular receptors in the small arterial blood vessels. Until recently only drugs that block one of these receptors have been available making it difficult to analyse the method through which tissue histamine acts when it is released. We have investigated the effects of injected histamine on the hindlimb vasculature of the rabbit and have also discovered that the anti-hypertensive drug, guanethidine, causes an easily reversible release of tissue histamine through one of its side-effects.

We have studied the effects of exogenous and endogenous histamine on the hindlimb vasculature of rabbits in which the autonomic nervous system has been blocked to avoid complicating effects of cardiovascular reflexes. When histamine is given by i.v. bolus injections there is a biphasic response of the resistance vessels of the limb (i.e. small arteries and arterioles):— there is an initial vasodilation mediated through the H_2 receptors and this is followed by a larger and longer lasting vasoconstriction mediated through the H_1 receptors. The H_2 response is blocked equally well by the three specific antagonists now available — burimamide, metiamide and cimetidine. The H_1 response is blocked by the specific antagonist, mepyramine. The different vascular responses evoked through the H_1 and H_2 receptors in the rabbit distinguish this species from most others in which the H_1 – and H_2 – mediated circulatory effects both produce vasodilatation. This makes it easier to asses the mechanism of action of endogenous histamine in the rabbit. In contrast to the effects of bolus injections when histamine is given by continuous infusion the H_1 constriction at equilibrium is of the same magnitude as the H_2 dilation, with little net effect on vessel tone. The results obtained with both bolus and infusion suggest that the number of H_1 – and H_2 – receptors in the hindlimb resistance vessels are about the same.

Guanethidine produces a dose-dependent vasodilatation. The evidence that this is due to endogenous release of histamine is as follows: (i) pre-treatment with burimamide or simultaneous infusion of endogenous histamine competitively blocks the guanethidine induced vasodilator response; (ii) when radioactive histamine is infused to equilibrate with the tissue histamine stores, injection of guanethidine causes release of labelled histamine, as do other histamine release agents; (iii) guanethidine releases histamine in vitro from rat peritoneal Mast cell suspensions. Other experiments have shown that the vasodilatation was not mediated through the autonomic nervous system or by other transmitters or metabolic chemicals, e.g. 5-hydroxy-tryptamine, bradykinin, dopamine, adenosine or prostaglandins. Metiamide exerted no blocking action at all on the guanethidine-induced vasodilatation. We conclude that the endogenous histamine released by guanethidine may stimulate a vascular histamine receptor different from the H_2 receptors since it is blocked by burimamide but not by metoamide or cimetidine.

Developmental Biology Research Unit

MAIN TOPICS:

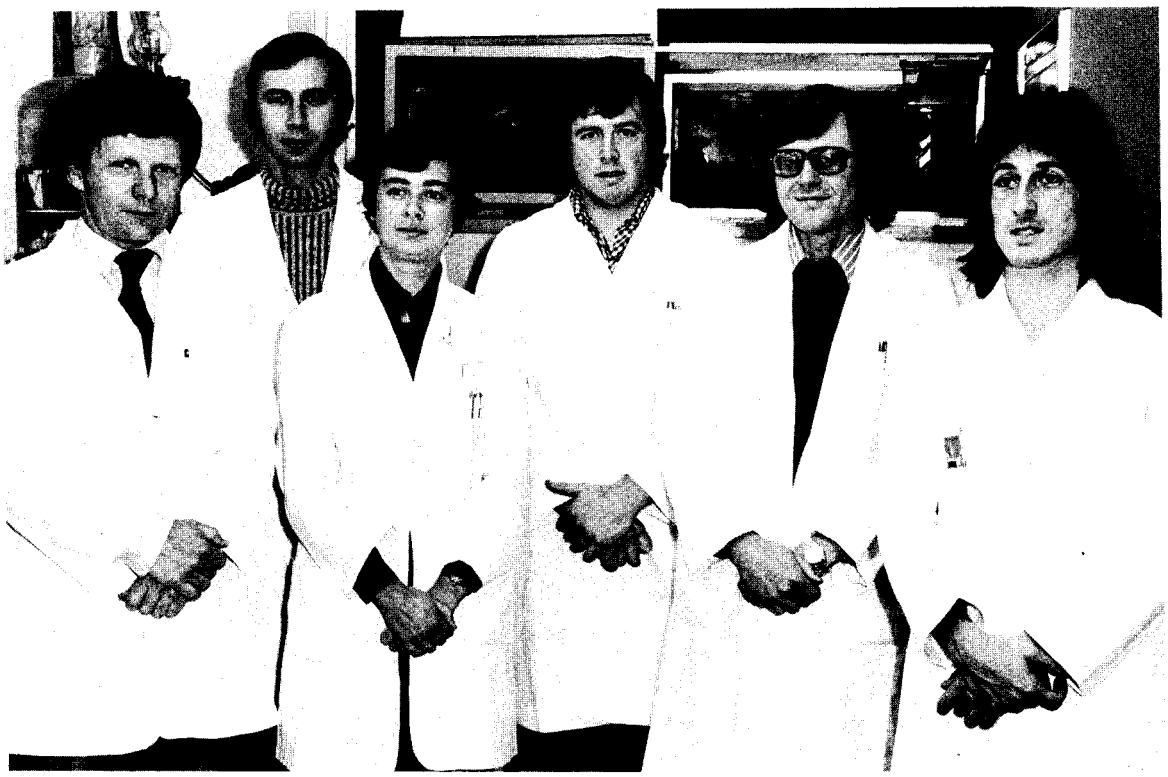
- Studies of Development of respiration in the foetus
- Development of heart and the autonomic nervous system in the foetus
- Management of cardio-respiratory disorders in the newborn
- Control of pulmonary circulation

General Outline of Work

The central aim of the research programme is to investigate the structural, physiological and biochemical development of the cardiovascular and respiratory systems of the foetus during life 'in utero', at birth, and in the newborn period. Such studies provide a background for examining some important problems of the infant round the time of birth. These include apneoa of the premature newborn; the early diagnoses of cardio-respiratory malfunction in the foetal and preterm infant and the role that impaired development of cardio-respiratory system plays in the sudden infant death syndrome.

Our Unit is investigating these problems in laboratory studies on animals and also in the clinical situation of the Neonatal Intensive Care Unit. In the laboratory, during the last five years, we have developed a preparation in conscious sheep which permits study of the circulatory and respiratory systems over the last third of gestation, during birth and in the immediate neonatal period. In the Hospital we have recently started to monitor cardiovascular and respiratory function in premature infants and in babies at term, concentrating particularly on those with respiratory and cardiovascular problems. We hope that these approaches will help synthetize a clearer understanding of the biology of development of the cardio-respiratory systems and that it may provide clues for the better management of some of the health problems of the human foetus, newborn, and perhaps even the adult.

The animal studies have been mainly concerned with subjecting the circulatory and respiratory systems of the foetus 'in utero' to a variety of stimuli over a period of about 50 days during the last one-third of gestation (which is 150 days in the sheep). These studies can be



Members of the Developmental Biology Research Unit. (From left) John Maloney, Vojta Brodecky, Margaret Dowling, Robert Smith, Adrian Walker and John Cannata.

performed on the foetus developing in its natural maternal environment without requiring removal from the mother and without anaesthesia. In contradistinction to the concept held by earlier workers that the respiratory system is not active 'in utero', evidence has now been obtained that rapid respiratory movements do occur under normal conditions in the foetus. However, in striking contrast to the adult, reduction of the foetal oxygen supply produces suppression of the foetal respiratory movements. In contrast to this paradoxical response to hypoxia of the foetus, increasing carbon dioxide concentration stimulates the foetal respiration in the same way that it does during the newborn period and in the adult.

Autonomic control of the cardiovascular systems has also developed by the last third of gestation. Low oxygen and high carbon dioxide concentrations in the foetus cause a decrease in heart rate, which is again the opposite to that seen in the newborn. Furthermore, reflex function to changes in blood pressure is also established by the last third of term and cardiac showing can be evoked in response to a rise in blood pressure. The sensitivity of the baroreceptor reflex arc does not change during development 'in utero' and is similar to that observed in the newborn period.

The morphology of both the heart and the lung changes considerably in the course of gestation. By 100 days (two-thirds of gestation) the myocardium is still incompletely developed with the cells containing large nuclei, small mitochondria and a considerable proportion of glycogen. A relatively large fraction of the lung volume contains potential 'spaces' by this time. Cell differentiation has occured in the upper airways but the development of the remainder of the lung is less well advanced. Disturbance of the natural environment of growth by continuous drainage or occlusion of the trachea or by section of the phrenic nerves produces profound alteration in lung development.

In the clinical studies of the human newborn we are particularly concerned with the consequences and causes of apnoea in the premature baby and the mechanisms whereby it controls cardio-respiratory function. An area in need of immediate investigation in this field relates to the problem of the interaction of respiratory and cardiovascular function under pathological circumstances at a time when each system is undergoing rapid changes consequent upon air breathing after birth. Non-invasive techniques are being developed to allow better monitoring of cardiovascular and respiratory function in very young sick infants. At present we are correlating these with the changes in systemic vascular pressures and blood gas tension in clinical situations where catheters are already in place for patient management.

Projects

A. **Development of the Foetal Respiratory System**

1. *Diaphragmatic Activity and Lung Liquid Flow in Unanaesthetised Foetal Sheep:*

J. E. Maloney, B. C. Ritchie, T. M. Adamson*, V. Brodecky and M. Dowling

A chronic foetal sheep preparation was developed in which catheters were implanted into the trachea, amniotic fluid compartment and transthoracic E.C.G. leads placed on the foetus. Fine electromyographic wires were sewn into the diaphragm and catheters placed in the carotid artery and/or jugular vein (Fig. 10). This preparation forms the basis for studies of the maturation of both the respiratory and cardiovascular systems.

For some time it has been suggested that breathing movements are made 'in utero' and recently measurements of tracheal pressure and lung liquid flow in chronic foetal preparations have led to the hypothesis that rapid changes in these parameters are the result of respiratory muscle activity. To test this hypothesis diaphragmatic electrical activity was measured in unanaesthetised foetal sheep and correlated with lung liquid flow and tracheal pressure. Diaphragmatic activity led to a fall of tracheal pressure and movement of a small volume of lung liquid into the lung. After the activity ceased, tracheal pressure returned to normal and flow diminished to zero or was directed out of the lung. The breathing pattern was unassociated with the net movement of lung liquid out of the lung. A histogram of the interval between breaths revealed a changing pattern of activity throughout gestation. The pattern was significantly altered after premature delivery of an animal with a respiratory problem. These observations provide direct evidence that respiratory muscles are active 'in utero' and that the pattern of activity changes throughout gestation.

2. *Modification of Respiratory Centre Output in Unanaesthetised Foetal Sheep 'in utero':*

J. E. Maloney, B. C. Ritchie, T. M. Adamson*, V. Brodecky and M. Dowling

Diaphragmatic electromyographic activity, tracheal and amniotic fluid pressures, lung liquid flow (Fig. 10) and carotid and jugular venous pressures were measured in foetal sheep who survived for periods of 9-43 days post-operatively. Neuromuscular transmission to the diaphragm was blocked with d-tubocurarine. The foetal gestational age ranged from 98 to 113 days at operation. Respiratory centre output of the foetus as indicated by electromyographic activity was (i) suppressed by anaesthesia and foetal hypoxia; (ii) tonically reduced by lung inflation; (iii) stimulated by cyanide injections into the foetal jugular vein. These experiments indicate that central and motor pathways to the diaphragm are sufficiently mature by 101 days in the foetal sheep to alter their output in response to chemical and mechanical stimuli.

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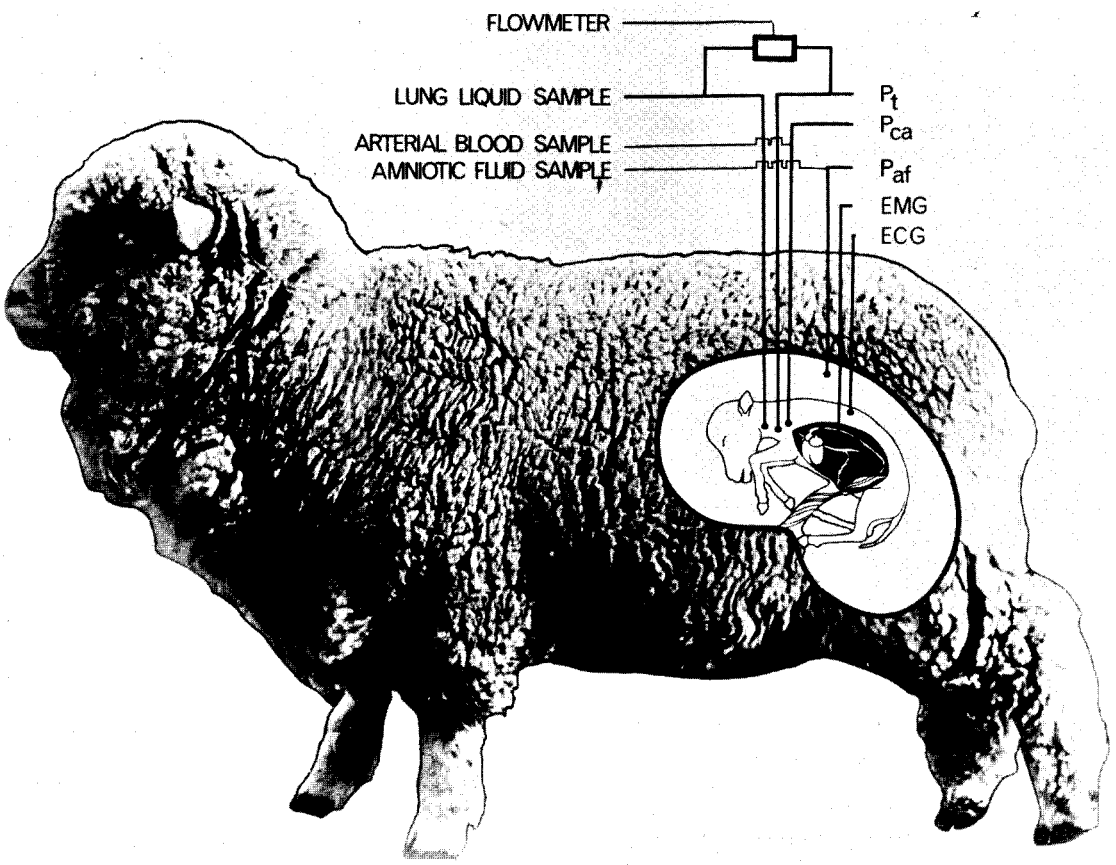


A diagram of the 'un utero' foetal sheep preparation used in the study of the future respiratory system before birth.

3. *Morphology of the Developing Lung:*

J. E. Maloney, B. C. Ritchie, T. M. Adamson* and D. Alcorn*

(i) *The influence of tracheal ligation and drainage on the morphology of the developing lung.*

The ultrastructure of the developing lung was examined in foetal sheep following the continuous drainage of lung liquid or tracheal ligation for a period of 20-23 days from day 110 gestation. The morphology obtained from these groups was compared with that from control animals (130-133 days old) whose lungs were prepared and fixed for transmission and scanning electron microscopy, and for light microscopy and with two other foetal control animals 110 days old. Continuous removal of lung liquid results in a decreased cell mass, thick potential air space sets septa, and enhanced cellular maturation with a significant increase in the number of type II cells present. By contrast tracheal ligation results a grossly enlarged lung, with increased tissue mass and impaired cellular maturation.

(ii) *Development of the Tracheal Epithelial Surface in the Newborn Rat*

The development of the epithelium of the trachea has been examined in 18 Porton Albino Wistar stock rats using scanning electron microscopy and light microscopy. Tracheas were prepared daily from 11 littermates from birth and at weekly intervals for 5 weeks from a second litter. The cellular ography was contrasted with that from a further 3 littermates at 5 months. In the laryngeal region two cell types predominate, a small microvillus cell of medium to high electron responsiveness and dimensions approximately 10 μm × 5m and a larger 20 μ × 7 μ cell of low electron responsiveness. With age the majority of cells in this region became smaller (5 μm × 5 μm), with moderate brightness (electron responsiveness) and a topography similar to that in the neighbouring cartilaginous and intercartilaginous zones. After birth the commonest cell in the cartilaginous and intercartilaginous zones of the trachea is non-ciliated with flattened surfaces from which protrude numerous microvilli. These cells become domed and bright with age. Ciliated cells commence to develop and in the later mature period may almost completely cover these zones being more dense in the distal regions. By comparison with other regions of the trachea the early membranous portion shows a significant increase in the number of immature and mature ciliated cells the density of which reduce initially with age and may progressively increase in the late mature period.

B. Development of the Foetal Cardiovascular System

4. *The Development of Baroreflex Activity in the Foetus and Newborn:*

J. E. Maloney, B. C. Ritchie, J. Cannata and M. Dowling

Baroreflex activity was measured in unanaesthetised foetal sheep over the last third of gestation and in unanaesthetised newborn lambs 3-7 days old. Two methods of measuring baroreflex sensitivity were used: (i) a transient method which was expressed as the ratio of the slowing of the heart per unit time divided by the rate of increase in peak systolic pressure following an injection of phenylephrine; (ii) a 'steady-state' method, the pulse interval was related to blood pressure approximately 45 seconds after injection of phenylephrine or nitroprusside. The results of this study indicate that baroreflex pathways are functioning by day 100 in foetus and that the sensitivity of the baroreflex loop is unchanged over the last third of gestation and during the first 35 days of life. The average sensitivity of the baroreflex by the transient method is 7.8 ± 0.9 msec/cm H_2O. The results under steady-state conditions indicate that systemic vascular pressure influences heart period over a range of pressures from 40 cm H_2O to approximately 130 cm H_2O whilst in the adult this range extended from 50 cm H_2O to 170 cm H_2O.

5. *Cardiovascular Effects in the Foetus and Newborn of Changes in Oxygen and Carbon Dioxide Concentrations:*

J. E. Maloney, B. C. Ritchie, J. Cannata and M. Dowling

The cardiovascular response to hypoxia and hypercapnia was studied in unanaesthetised foetal sheep over the last third of gestation and in newborn lambs for up to 52 days following birth. In each animal catheters were chronically implanted into the carotid artery and jugular vein and transthoracic E.C.G. leads placed about the chest. In two foetal animals an electromagnetic flow probe was placed on the ascending aorta for measurement of left heart output. The foetus was made hypoxic for 30 minutes on 13 occasions over a range of gestational ages from 105-140 days. During the hypoxic period the heart rate fell significantly whilst carotid artery and jugular venous pressure and cardiac output remained unchanged. On the other hand during hypoxia of the newborn there was a large rise in heart rate, whilst intravascular pressures did not change significantly. The influence of carbon dioxide on the foetal circulation was examined over a gestational age range of 103-139 days. In the foetus there was no significant change in systemic vascular pressure, but a transient bradycardia and no change in cardiac output in 3 experiments in which it was measured. In the newborn there is a marked tachycardia and a significant increase in carotid artery pressure.

6. *Ultrastructure of the developing heart*

J. E. Maloney and D. Seward

Pilot studies have been undertaken in adult sheep and foetal lambs designed to develop techniques which would enable a satisfactory examination of the ultrastructure of the developing heart. Successful techniques were developed for perfusion fixation of the coronary circulation in the foetal and adult lamb and the initial results indicate that by day 100 in the foetal lamb the myocardium is still incompletely developed. Many small mitochondria, large nuclei, incomplete fibrillar development and large glycogen filled spaces characterise the myocardial cell at this stage.

C. Control of the Pulmonary Circulation

7. *The Influence of Transpulmonary Pressure on the Diameter of Small Arterial Blood Vessels in the Lung*

B. C. Ritchie, J. E. Maloney * R. J. Smith

The diameters of pulmonary arterial blood vessels from approximately 800μ - 5000μ were measured in a double heart by-pass preparation at known intravascular and transpulmonary pressures. Measurements were made from lateral radiographs of the lung on the deflation limb of the pressure volume curve at transpulmonary pressures of 30, 20, 10 and 0 cm H_2O. Mean intravascular pressure decreased with decreasing transpulmonary pressure (TPP), while pulmonary vascular resistance showed no significant change. During deflation from a TPP of 30-0 cm H_2O, the smallest arteries (800μ) increased their diameter by 40% and the larger arteries (1600-2000μ) by 7%. No systematic changes in diameter occurred in vessels with diameters ranging from 2800μ to 5000μ: In two lungs, changes in lengths were measured during deflation for vessels with diameters greater than 2000μ. Vessel lengths decreased by an average of 38% and 23% and 23% as TPP changed from 30 to 0 cm H_2O. Thus it appears that lung inflation causes a narrowing of the smaller pulmonary blood vessels. The length changes of the larger vessels, however, contribute to the increase in pulmonary vascular volume with lung inflation.

* Not a member of the Baker Institute or C.R.U.

8. *The Effects of Aortic and Carotid Chemoreceptor Stimulation by NaCN on Pulmonary Vascular Resistance in the Anaesthetised sheep:*
B. C. Ritchie, J. E. Maloney * R. J. Smith

The effects of aortic and carotid chemoreceptor stimulation on the systemic circulation are well documented in various species. Reports of chemoreceptor control of the pulmonary circulation have been few and conflicting. The changes in pulmonary vascular resistance (PVR) produced by NaCN injected into the aortic root and common carotid arteries were studied in anaesthetised open-chest lambs. PVR was calculated from measurements of pulmonary artery pressure, left atrial pressure, and pulmonary artery flow (electromagnetic flow-meter). Spatial separation of the aortic and carotid chemoreceptors was obtained by insertion of loops of 150 cm length in the common carotid arteries which increased the transit time from the root of the aorta to the carotid sinus of a bolus injection by more than 15 sec.

The effects of mid-cervical vagotomy and intravenous atropine (0.5 mg/kg) on the pulmonary vascular response to NaCN were examined as were the effects of alpha- and beta-blockade. The effect of denervation of the carotid chemoreceptors on the pulmonary vascular response to NaCN was studied in three closed chest anaesthetised animals where the appropriate catheters and flowmeter had been inserted one week prior to the experiment.

After aortic injections of NaCN no significant change in pulmonary vascular resistance (PVR) occurred and this was not altered by vagotomy and atropine. By contrast direct carotid artery injection distal to the coil produced a significant rise in PVR which was attenuated after alpha-blockade. Beta-blockade with propranolol had no significant effect on this response. Denervation of the carotid chemoreceptors failed to attenuate the rise in PVR after injection of NaCN into the carotid artery.

Cancer Research Unit

Main Topics:

- Experimental production of kidney cancer
- Tissue culture analysis of structure, biochemistry and immunology of cancer cells.

General Summary:

Utilising a model system in which one dose of the chemical carcinogen, dimethylnitrosamine (DMN) results in an exceedingly high incidence (up to 100%) of kidney cancer in rats, we are continuing to identify and analyse the phenomena which occur during the process of tumour development. The approach involves co-ordinated studies relating to light microscopic, electronmicroscopic, immunologic, tissue culture and biochemical aspects of the developmental stages of kidney cancer.

By electronmicroscopy, structural changes in various cell types in the kidney have been identified shortly after the injection of the carcinogen. These findings have been correlated with the results obtained from studies using a radioactive isotope label which localises the subsequent proliferative response in the kidney specifically elicited by the carcinogen. In this way the cell types which are acutely injured by the carcinogen can be identified as well as those (known as the target cells) which will be altered so as to have the potential to proliferate in an unconstrained fashion to form a cancerous growth.

Tissue culture has provided an important technique for purifying the populations of target cells from kidney. When cells from kidney cancers are isolated and maintained in tissue culture, they grow continuously, demonstrating a virtually unlimited ability for survival. In contrast, cells from normal kidneys survive for only very short periods of 2 to 3 months. Significantly, cells taken from kidneys of rats shortly after injection with the carcinogen behave in a similar fashion to the tumour cells in culture, and exhibit a potential for virtually unlimited survival. Even within 4 hours of the *in vivo* carcinogenic treatment, the cells upon which the chemical exerts its action are committed to this process of sustained, abnormal behaviour known as "transformation".

Because the kidney is composed of a diversity of cell types, the information gleaned from biochemical studies performed on the whole organ is somewhat limited. The tissue culture system yields purified populations of target cells and thus provides the opportunity for

Members of the Cancer Research Unit (from left) Gordon Hard, Marilyn Perry, John Lee, Helen King and Ted Turnbull.

better resolution for the study of the cellular and molecular changes involved in the cancer process. Using the same culture system a number of biochemical parameters are being investigated in an attempt to characterise the properties of the normal cells, the cancer cells, and the cells from DMN-treated rats before and after they have displayed the phase of "transformation". This aspect of the programme is conducted in collaboration with Dr. B. W. Stewart of the Department of Pathology, University of New South Wales. It has been found that the "transformation" point can be accurately identified by a sudden, significantly increased ability of the cultured cells to synthesise DNA. In this respect, the DMN-treated cells match the properties of the cancer cells themselves, and stand in marked contrast to the normal cells. This *in vivo-in vitro* culture system qualified by such a highly reproducible biochemical parameter provides the basic ingredients for the development of a useful short-term test for predicting the long-term carcinogenic hazards of certain chemicals.

Investigations also continue into the structural composition of kidney tumours in laboratory animals. The classification and interpretation of these tumours, in particular the nephroblastoma, which is often confused with the purely mesenchymal neoplasm induced by DMN, is being reviewed. Critical study of the renal tumour series found in laboratory animals provides some perspective to an understanding of human kidney tumours and to the extrapolation to man of data obtained from animal carcinogenesis studies.

Estimates of the proportion of human cancer that may have possible causal relationship to chemicals in the environment, either natural or synthetic, range from 60 to 90 per cent. An understanding therefore of the sequence of events which chemicals initiate in a particular organ and which lead inevitably to the development of a malignant tumour, is essential to comprehension of the disease process. The basic research investigation pursued in this unit therefore aims to assemble information concerning the sequence of events essential to and preceding the clinical manifestation of renal cancer. Such data is prerequisite for the planning of strategies needed to interrupt, prevent or reverse cancer development in general, and for the interpretation of related kidney tumours in man.

Projects

1. **Autoradiographic Analysis of Renal Carcinogenesis**

   G.C.Hard.

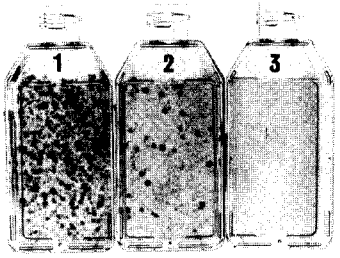
The proliferative activity of renal epithelial and mesenchymal cell sub-populations following a single carcinogenic dose of DMN has been traced by autoradiographic analysis of tritiated thymidine uptake during the 3 weeks immediately post-treatment. The initial response to DMN was a depression in DNA synthesis and mitosis to near zero levels in all segments of the nephron and in attendant mesenchymal cells for a period of 1 to 3 days. Following the initial inhibition, increased DNA synthetic activity was observed in certain sub-populations of both epithelium and mesenchyme and these patterns were matched by equivalent mitotic activity. A stimulation of DNA synthesis was observed in cells of the proximal and distal tubules of zones 1 and 2, but in no other epithelial segments. The increased activity was most intense in zone 1 epithelium reaching a peak at the tenth day after DMN injection, 4 days after epithelial cell necrosis had commenced. In renal mesenchyme, the major response involved only the interstitial cells of zones 1 and 2. At day 3, there was a wave of increased DNA synthetic and mitotic activity in the free interstitial cells of the cortex, followed by a second, more intense peak of activity at day 6. The cells responding at day 3 appeared to involve the resident population of cortical fibrocytes while the major contribution to the day 6 peak came from infiltrating mononuclear inflammatory cells, although resident fibrocytes and capillary endothelium also contributed. A significant wave of increased activity involved the interstitial cells of zone 2, but the peak, although of equivalent intensity to the response in zone 1, was single and occurred 3 days later at day 9. Apart from a small, brief and variable wave of activity in interstitial cells of zone 3 from days 8 to 10, no other mesenchymal cell populations in the kidney were stimulated by the injection of DMN. The carcinogen therefore exerts its most significant effect on those epithelial and mesenchymal cell sub-populations from which the respective neoplasms are believed to be derived.

2. **Morphological Transformation in Target Kidney Cells**

   G. C. Hard.

Earlier studies have shown that kidney cortex cells isolated from DMN-treated rats 1 to 7 days after carcinogen administration give rise to clones of mesenchymal cells with altered behaviour patterns. At subculture 5, the cells grow as piled-up, dense colonies possessing the morphological features of mesenchymal tumour cells *in vitro* and such additional tumour cell features of increased growth-plating and cloning efficiencies and growth in semi-solid media. The time-sequence of the acquisition of this transformation behaviour with respect to carcinogen exposure has been determined by isolating kidney cortex cells *in vitro* from DMN-treated rats at 1 to 8 hours after dosing. The morphological and behavioural features of these cells were monitored through successive subcultures, in direct comparison with kidney isolates from untreated rats. All cultures derived from rats treated 1 to 8 hours previously with DMN exhibited an increased life span in contrast to cells from normal rats which usually reached senescence by subculture 3 or 4. At subculture 5, cells from the 4 to 8 hour isolates demonstrated morphological transformation (piled-up colonies) whereas this change did not become manifest in the 3-hour isolate until subculture 6, or subculture 7 in the case of the 1 and 2-hour isolates. The time of appearance of transformed colonies coincided with an increase in growth-plating efficiency and an acquired ability for the cells to form clones at very low seeding rates (positive cloning efficiency). The capacity for forming colonies in semi-solid media, carboxymethyl cellulose gel, agar or agarose did not appear until a number of subcultures later, usually after subculture 10. The study has indicated therefore that the commitment to

the transformation process occurs very rapidly, within one hour, after the exposure of the target organ *in vivo* to the carcinogen. However, an initial delay of 4 to 5 subcultures seems prerequisite before this altered behaviour becomes manifest. It is unknown yet whether this period represents an adaptive process to the conditions of tissue culture or is a necessary lag required for expression of the altered characteristics.



Morphological transformation of kidney cells cultured in vitro *after isolation rats treated with a carcinogenic dose of DMN (flasks 1 and 2) is manifest as dense plagues, representing colonies of piled-up cells, which stand out prominently from the surounding cell monolayer. For contrast, flask 3 illustrates the monolayer growth pattern of normal rat kidney cells.*

3. **DNA Synthesis in Renal Cell Cultures.**

G. C. Hard and B. W. Stewart*

Using the tissue culture system described above, the growth properties in terms of radioactive thymidine incorporation, of normal kidney cell populations, renal mesenchymal tumor cells, and "pre" and "post-transformed"cells have been quantitated. It was established first that monitoring the cells at hourly intervals over a period of 3 hours, 24 hours after seeding into replicate microtest cell-culture wells, guaranteed a reproducible analysis of the optimum proliferative properties of the populations under study. Lines of cells derived from DMN-induced renal mesenchymal tumors were typified by rates of DNA synthesis many times those which characterised normal cells in culture. Kidney cells derived from DMN-treated rats possessed rates of synthesis equivalent to those of normal kidney cells until subculture 5. At this point, coincident with the manifestation of morphologically transformed colonies, rates of synthesis increased by up to 50 fold over normal levels. Once acquired, this markedly increased propensity for proliferation was retained, and consistently demonstrable in this system, in all subsequent subcultures. The precise definition of growth rates of this variety of cell populations provides a basis for comparison of the respective cells following addition of an exogenous chemical. The cell population most responsive to addition of a known carcinogen, and hence potentially useful in 'rapid screening test', may be identified.

4. **Metabolism of DMN by Rat Kidney Cells In Vitro**

G. C. Hard and B. W. Stewart*

A study has been made of the metabolism of DMN by the various renal cell populations described in previous sections. In this particular situation, as distinct from most other experimental models, the various biological parameters, such as dose, time of exposure, etc., can be related directly to the whole organism; the sensitivity of the kidney cells *in vivo* to the toxin is known. Cultures of renal cells were exposed to physiological concentrations of radioactively-labelled DMN and metabolism assayed by incorporation of radioactivity into cellular nucleic acids. Following the detection of low levels of radioactivity incorporated, the nucleic acids were further degraded and analysed for the presence of methylated bases found when the nitrosamine was administered to intact animals. Such modified bases were not detected. In rats, DMN intoxication is associated with inhibition of protein and RNA synthesis. However, after addition of DMN to renal cells in culture, rates of protein and RNA synthesis remained unchanged. In contrast, inhibition of RNA synthesis by renal cells was observed when the cells were exposed to the carcinogen *in vivo* (i.e. by treatment of the intact animal) and subsequently isolated, grown in culture and assayed. Data from these experiments suggest that specific differences exist between the responses of renal cells in culture to DMN on the one hand and changes detected in intact animals on the other. It would appear that the use of cell cultures for the 'bio-assay' of carcinogens will require extensive fundamental research and the most rigorous definition of all parameters.

*Not at Baker Institute or C.R.U.

5. **Immunological Studies in Renal Carcinogenesis.**

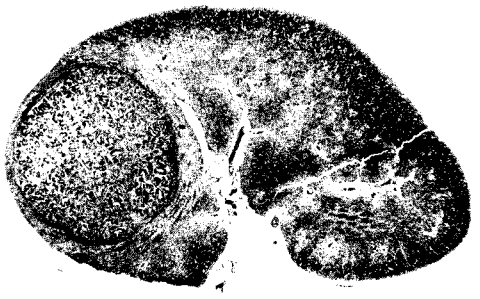
G. C. Hard.

As one measure of cell-mediated immunity, the blastogenic response of enriched spleen lymphocytes to phytohaemagglutin (PHA) was assayed at sequential daily and weekly intervals following the administration of DMN as a single dose sufficient to induce renal tumors in 100% of surviving rats. PHA responsiveness of DMN-treated rats was not suppressed at any stage of tumor induction in contrast to rats which had been intentionally immunodepressed by neonatal thymectomy. It was concluded that DMN is unlikely to facilitate the cancer process by impairing thymus-dependent lymphocyte function. The results of this study are summarised in graphic form in the accompanying figure.

6. **Classification and Interpretation of Laboratory Animal Kidney Tumors.**

G. C. Hard.

Confusion has existed in the literature concerning the interpretation of laboratory animal kidney tumors, particularly in the discrimination between nephroblastoma and neoplasms consisting of both malignant connective tissue mesenchyme and profiles of pre-existing renal tubules. Consequently, the histology of a spontaneously-occurring neoplasm of the rat kidney conforming to a classification of nephroblastoma has been compared with that of DMN-induced renal mesenchymal tumors. Rat nephroblastoma was an encapsulated epithelioid neoplasm of relatively uniform histological pattern. Clumps of densely-crowded, hyperchromatic cells frequently associated with central, well-differentiated ducts were supported by a less cellular, interconnecting stroma of loose areolar or mature fibrous connective tissue. Organisation of neoplastic cells into primitive ill-defined tubular formations was also a



Spontaneously-occurring nephroblastoma in the rat kidney. This neoplasma is a distinct entity from the DMN-induced renal mysenchymal tumour.

feature. The neoplastic cellular component displayed a strong resemblance to metanephrogenic blastema. In contrast, renal mesenchymal tumor was non-encapsulated consisting of a heterogeneous mixture of connective tissue elements including fibroblast-like spindle cells, smooth muscle and embryonic mesenchyme which engulfed and sequestered pre-existing renal tubules and glomeruli. Thus, nephroblastoma and renal mesenchymal tumor in the rat exist separately as unrelated entities. Rat nephroblastoma conforms morphologically with the malignant epithelial component of human Wilm's tumor whereas the counterpart of rat renal mesenchymal tumor resides with the mesenchymal component of Wilm's tumor and with congenital mesoblastic nephroma (leiomyomatous harmartoma) of infancy.

7. **Acute Beryllium Toxicity.**
G. C. Hard.

Exposure to berillium (Be) compounds is associated with a range of disease manifestations in man. Experimentally, Be produces toxic changes in a variety of organs and particularly, as in man, affects immunological mechanisms. In addition, Be is an experimental carcinogen, producing osteogenic sarcomas in rabbits and pulmonary adenocarcinomas in rats. It thus provides a model for the study of metal-induced carcinogenesis. Re-examination of the acute pathology of Be using various salt complexes and perfusion-fixation techniques in the light of the effect on immunological function and induction of carcinogenesis has been completed recently in collaboration with associates from the Toxicology Unit, Medical Research Council Laboratories, Carshalton, England. The distribution of lesions has been correlated with the tissue distribution of the radioactive metal and with the predominant form of the injected salt, soluble or particulate. A tendency for the more soluble complexes to induce more severe lesions was noted and a general correlation demonstrated between tissue sites of pathological change and tissue content of metal. The major site of the initial toxic action in the liver involved the midzonal hepatocytes while in the kidney, the injury commenced in the second segment of the proximal convoluted tubules. The nature and distribution of lesions in the lymphoid organs, particularly the spleen, lymph nodes and thymus suggested that Be has a toxic predilection for lymphocytes in thymus-dependent traffic zones, as well as for macrophages and possibly antigen-trapping reticular cells. In contrast, the lymphocyte population of bone marrow was unaffected, despite a high bone deposition of the metal. However, a previously unrecorded effect on bone cells, namely destruction of osteocytes in lacunae of the metaphysis, was noted consistently. The effect on bone cells provides some basis for known ability of Be to induce rickets and bone cancer in certain experimental animals.

Publications

CIRCULATORY CONTROL AND 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 blood stream of the rabbit. *Journal of Pharmacology and Experimental Therapeutics.* (In Press).

J. A. ANGUS, M. J. WEST and P. I. KORNER:
Estimation of magnitude of autonomic and non-autonomic components of hindlimb vascular resistance in renal hypertension. *Clinical and Experimental Pharmacology and Physiology, 2* (supplement 2), 149-152, 1975.

P. J. FLETCHER, J. A. ANGUS, J. R. OLIVER and P. I. KORNER
Cardiac output changes during the development of experimental renal hypertension. *Clinical and Experimental Pharmacology and Physiology,* 2 (supplement 2), 145-148, 1975.

P. J. FLETCHER, P. I. KORNER, J. A. ANGUS and J. R. OLIVER.
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D. GANTEN, J. S. HUTCHINSON and P. SCHELLING:
The intrinsic brain iso-renin angiotensin system: its possible role in central mechanisms of blood pressure regulation. *Clinical Science and Molecular Medicine,* 48, 265S-268S, 1975.

J. S. HUTCHINSON, J. MOHRING, P. SCHELLING and D. GANTEN:
Differential pressor responses to centrally administered angiotensin II (AII) in conscious rats, homozygous and heterozygous for hypothalmic diabetes insipidus (DI). *Naunyn-Schmiedeberg's Archives of Pharmacology,* Supplement to Vol. 287 R 52, 1975.

P. I. KORNER and J. B. UTHER:
Reflex autonomic control of heart rate and peripheral blood flow. *Brain Research,* 87, 293-303, 1975.

P. I. KORNER and E. SIMON:
Regional organisation of autonomic nervous system. *Brain Research,* 87, 339-340, 1975.

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Assessment of cardiac autonomic excitability in renal hypertensive rabbits using clonidine-induced resetting of the baroreceptor heart rate reflex. *European Journal of Pharmacology,* 33, 353-362, 1975.

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Action of propranolol on arterial pressure and on cardiovascular reflexes, *Drugs.* (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. G. Onesti, M. Fernandes, K. E. Kim, Editors Grune and Stratton: New York. (In Press).

P. SLEIGHT, M. J. WEST, P. I. KORNER, J. R. OLIVER, J. P. CHALMERS and J. L. ROBINSON.
The action of clonidine on the baroreflex control of heart rate in conscious animals and man, and on single aortic baroreceptor discharge in the rabbit. *Archives internationales de pharmacologie et therapie,* 214, 4-11, 1975.

M. J. WEST, J. A. ANGUS and P. I. KORNER.
Estimation of non-autonomic and autonomic components of iliac bed vascular resistance in renal hypertensive rabbits. *Cardiovascular Research,* 9, 697-706, 1975.

Submitted for Publication

J. A. ANGUS, A. BOBIK and P. I. KORNER.
Comparison of histamine bolus injections and continuous influsion on the vascular H_1 and H_2 receptors in the rabbit.

J. A. ANGUS, A. BOBIK, R. O. DAY and P. I. KORNER.
Guanethidine-mediated hindlimb vasodilatation: evidence for mediation through endogenous histamine release.

DEVELOPMENTAL BIOLOGY RESEARCH UNIT

Published or accepted for Publication

T. M. ADAMSON, T. LAMBERT, S. CRANAGE, V. BRODECKY, J. E. MALONEY and B. C. RITCHIE.
Lung liquid production and composition in chronic foetal sheep. *Australian Journal of Experimental Biology and Medical Science, 53;* 65-75, 1975.

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D. ALCORN, I. G. S. ALEXANDER, J. E. MALONEY and B. C. RITCHIE.
The effects of tracheal occlusion and lung liquid drainage on the morphology of the airways of the developing lung.

J. E. MALONEY, J. CANNATA, V. BRODECKY, MARGARET DOWLING and B. C. RITCHIE.
The development of baroreceptor function in the foetal lamb.

J. SMOLICH, B. C. RITCHIE and J. E. MALONEY.
The morphological development of tracheal epithelium in the newborn rat.

Abstracts

D. ALCORN, T. M. ADAMSON, T. F. LAMBERT, J. E. MALONEY, B. C. RITCHIE and P. M. ROBINSON.
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D. ALCORN, T. M. ADAMSON, T. F. LAMBERT, J. E. MALONEY, B. C. RITCHIE and P. M. ROBINSON.
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J. E. MALONEY, T. M. ADAMSON, V. BRODECKY, M. DOWLING and B. C. RITCHIE.
Modification of breathing movements of the foetus 'in utero'. *Australian Paediatric Journal.* (In Press).

CANCER RESEARCH UNIT

Published or Accepted for Publication

R. BORLAND, G. C. HARD and S. M. METCALFE.
A combined *in vivo in vitro* approach to studies of nitrosamine-induced carcinogenesis. In: *Rapid screening tests to predict late toxic effects of environmental chemicals.* Proceedings International Agency for Research on Cancer/Commission of the European Communities Workshop, 1975. (In Press).

W. H. BUTLER and G. C. HARD.
Chemical carcinogenesis: early biological responses in induced carcinogenesis of the kidney. In: *Chronic toxicity as an acute Phenomenon.* Proceedings 17th Meeting, European Society of Toxicology, 1975. (In Press).

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

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Analysis of the early phase of DMN-renal carcinogenesis. Proc. XI International Cancer Congress. Ed. P. Bucalossi, V. Veronesi and N. Cascinelli. *Excerpta Medica,* Amsterdam. In Press 1975.

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G. C. HARD and R. BORLAND.
In vitro culture of cells isolated from dimethylnitrosamine-induced renal mesenchymal tumors of the rat. 1. Qualitative morphology. *Journal of National Cancer Institute,* 54; 1085-1095, 1975.

G. C. HARD and R. BORLAND.
In vitro culture of cells isolated from dimethylnitrosamine-induced renal mesenchymal tumors of the rat. II. Behaviour and morphometry. *Oncology,* 30; 485-492, 1975.

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Thymus-dependent lymphocyte function in dimethylnitrosamine-induced renal carcinogenesis is not depressed. *Oncology,* 31; 139-146, 1975.

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B. W. STEWART and G. C. HARD.
The biochemistry of morphological transformation. *Cancer Forum*, 7; 1976. (In Press).

B. W. STEWART, G. C. HARD, R. BORLAND, D. BRUCE.
Nucleic acid metabolism in renal cell cultures transformed *in vivo* by dimethylnitrosamine. *Proceedings of Australian Biochemical Society*, 8; 108, 1975.

Submitted for Publication

B. W. STEWART and G. C. HARD.
Thymidine incorporation as a criterion of transformation of rat kidney cells by dimethylnitrosamine in an *in vivo-in vitro* culture system.

B. T. TOH, G. C. HARD, M. N. CAUCHI, H. K. MULLER.
Smooth muscle-associated contractile protein in renal mesenchymal tumor cells and in transformed cells derived from dimethylnitrosamine-injected rats.

Support Services

ELECTRONICS LABORATORY AND MECHANICAL WORKSHOP

During the year the facilities of the Electronics and Mechanical Workshop have been improved considerably. The service functions under the direction of Mr. Ron Wall, graduate Electronics Engineer, who is assisted by Kevin Harvey (electronics technician) and by John Baird (mechanical technician). In the Institute there is the problem of maintenance of much diverse electronic equipment usually remote from the country of manufacture and this alone makes a good electronics laboratory essential. However, an additional very important requirement involves the design of new electronics equipment required by the various biomedical groups. The equipment designed and built during the year includes a Doppler ultrasonic flowmeter assembly for blood flow measurements, equipment of the neurosciences laboratory with a variety of equipment including a discriminator pulse height analyser for single nerve fibre recording, the design of a cardiac contractility analogue computer, design of telemetry apparatus for monitoring physiological variables from foetal animals. There are facilities for making printed circuit boards in equipment designed in the Institute. This will lead to easier maintenance and duplication of equipment. We have purchased new equipment for the mechanical workshop and have now the capacity to make a number of high-cost items such as instrument racks which will allow us to capitalize on the cost of the equipment in a relatively short space of time.

LIBRARY

The library of the Baker Institute functions in conjunction with the other (campus) libraries of Alfred Hospital and the Monash Medical School. The main changes this year have been to increase the number of circulatory journals and cardiovascular monographs in line with the new interest of the Institute.

Staff of the Electronics Laboratory and Mechanical Workshop — from left, Frank Forgione, John Baird, Ron Wall and Ken Harvey

OPERATING THEATRE

The key to the success of many of our clinical animal preparations has been the excellent facilities of the Operating Theatre Suite. The service provided by Jan Dixon and her staff for the many different species and specific problems has been particularly good. Without the capacity of implanting catheters and flowmeters under sterile conditions much of our work on hypertension and in foetal sheep would be impossible. The theatre also functions as an experimental cardiac surgical theatre and we are engaged on some collaborative work with the Officer Brown Cardiothoracic Surgical Unit.

Sister Jan Dixon checking the theatre before operations commence.

BAKER INSTITUTE SEMINAR PROGRAMME — 1975

Date	Title	Lecturer
28 February	Histamine Vascular Receptors	Dr. James Angus Baker Institute
14 March	Studies on the Identification of Key Cellular Events in Chemical Carcinogenesis.	Dr. Gordon Hard Baker Institute
4 April	Excitatory Nociceptor Reflexes from the Heart — Role of Prostaglandins	Dr. J.Staszewska-Barczak University of Melbourne
18 April	Renin and Angiotensin in Renal Function	Dr. G. Matthews Prince Henry's Hospital
2 May	Arterial Wall Metabolism and Atherogenesis	Prof. A. Day University of Melbourne
16 May	A Central Nervous System Action for beta-blockers?	Dr. Warwick Anderson Baker Institute
30 May	Conversion of Angiotensin I to Angiotensin II in the Circulation	Dr. Kevin Ng, Austin Hospital
13 June	Polar Human Biology	Dr. Des Lugg Antarctic Division Department of Science
27 June	Central Nervous System and Sodium Excretion	Mr. Michael McKinely Howard Florey Institute
11 July	Studies on Steroid Receptors	Dr. John Funder, Jnr. Prince Henry's Hospital
1 August	Clinical Studies in Hypertension	Dr. J. Stockigt Alfred Hospital
8 August	Cardio-respiratory Development in the Foetus	Dr. John Maloney Baker Institute
22 August	Central Hormonal Mechanisms in Hypertension	Dr. Jeff Hutchinson Baker Institute
5 September	Some Aspects of the Autonomic Nervous System	Professor Mollie Holman Monash University
19 September	Studies of Autonomic Activity in Hypertension	Professor W. J. Louis Austin Hospital
3 October	Modulation of Adrenergic Transmission	Professor M. J. Rand University of Melbourne
17 October	Clinical Studies in Post-transplant Hypertension	Dr. J. Sabto Alfred Hospital
24 October	Studies of Autonomic Activity in Hypertension	Professor W. J. Louis Austin Hospital
14 November	The Arterial Baroreceptors and Hypertension	Dr. Jennifer Angell-James University of London and Baker Institute
28 November	The Influence of Angiotensin Antagonists on Aldosterone Secretion	Dr. John Blair-West Howard Florey Institute
12 December	The Valsalva manoeuvre; a Test of Cardiovascular Reflex Function	Dr. Peter Blombery Baker Institute

OVERSEAS VISITS

Professor Korner attended the Fourth Hahnemann International Symposium on Hypertension in Philadelphia, U.S.A., in March 1975. The theme of the symposium was "Regulation of Blood Pressure by the Central Nervous System" and Professor Korner gave an invited paper entitled "Central Control of Blood Pressure: Implications in the Pathophysiology of Hypertension". He also lectured in the Department of Medicine and Physiology, Harvard Medical School, and in the Department of Physiology, University of Missouri. In July 1975 he attended the International Congress of Pharmacology in Helsinki, Finland, where he delivered a paper on "Histamine Release by Guanethidine in the Hindlimb". In October 1975 he attended a Symposium on Hypertension in Queenstown, New Zealand, and gave a paper on "Action of Propranolol on Arterial Pressure and on Cardiovascular Reflexes".

Dr. Maloney attended the meeting of the American Thoracic Society and Canadian Thoracic Society in Montreal, Canada. He gave a paper entitled "Modification of Respiratory Activity of the Foetus *'in utero'*." He also lectured at the University of Manitoba, Winnipeg, Canada.

Dr. Hard attended the International Agency for Research on Cancer — Commission of the European Communities Workshop, Brussels, Belgium. He gave a paper entitled "A combined in vivo and in vitro approach to studies of nitrosamine-induced carcinogenesis". He attended the 17th Meeting of the European Society of Toxicology, Montpellier, France, and gave a paper "Chemical Carcinogenesis: Early Biological responses in Induced Carcinogenesis of the Kidney".

LECTURES AND MEETINGS

Professor Korner gave a seminar on "Autonomic Performance in Hypertension" at the Alfred Hospital Clinical Research Society. He gave seminars on "Central Nervous Control of the Circulation" at the Howard Florey Institute and on "Haemodynamics of Hypertension" at the Department of Medicine, Royal Melbourne Hospital, University of Melbourne. Drs. Angus and Anderson gave papers at the Australian Physiological and Pharmacological Society. Professor Korner and Dr. Blombery gave papers at the Australian Society for Clinical and Experimental Pharmacology. Professor Korner and Dr. Fletcher attended the meeting of the Australian Cardiac Society and Royal Australasian College of Physicians in Sydney.

Dr. Maloney gave a lecture on "Monitoring of the Circulation and Respiratory System of the Foetus 'in utero'," at ANZAAS, in Canberra, and lectures on "Mechanics of the Respiratory System" and "Gas Exchange in the Lungs" at the Royal Australasian College of Surgeons, Melbourne.

Dr. Hard gave papers at the Annual Meetings of the Australian Society for Experimental Pathology in Canberra, the Clinical Oncological Society of Australia in Sydney and the Australian Society for Immunology in Adelaide. He gave a seminar at the Walter and Eliza Hall Institute on "Cellular Analysis of the Developmental Stages of Chemically Induced Renal Cancer in Rats" and at the Australian Cancer Society on "Investigations into the Identification and Analysis of Key Cellular Phenomena in Chemical Carcinogenesis".

TEACHING

Professor Korner gave lectures on cardiovascular physiology and on heart failure at the Monash Department of Physiology and two lectures on "Cardiovascular Control" in the University of Melbourne. Dr. Angus and Dr. Anderson contributed to the Monash pharmacology programme. Dr. Blombery and Dr. Fletcher participated in the 4th year teaching of Medicine. Dr. Maloney gave lectures on respiratory and neonatal physiology in the Department of Physiology, University of Melbourne.

APPOINTMENTS

Professor Korner was appointed to a Personal Chair in the Department of Medicine, Monash University. His other appointments were as 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.

GRANTS AND DONATIONS

We are most grateful for the continuing project grant support that we have received from the National Health and Medical Research Council, the State Government of Victoria, the National Heart Foundation of Australia, the Life Insurance Medical Research Fund of Australia and New Zealand, and the Anti-Cancer Council of Victoria. We are also greatly indebted for the continuing support of Alfred Hospital Research Fund, which has helped with many of our activities, particularly in connection with the Experimental Surgery Unit and the Biochemical Pharmacology Laboratory.

We are also grateful for the other donations made by our supporters and acknowledgers below:

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

Victorian State Government	$35,000.00
Anonymous	10,000.00
The James and Elsie Borrowman Research Trust	5,650.00
Kodak (Australasia) Pty. Ltd.	5,000.00
Merck Sharp and Dohme (Aust,) Pty. Ltd.	5,000.00
Sandoz Australia Pty. Ltd.	5,000.00
Astra Chemicals	4,000.00
William Angliss Charitable Trust	3,500.00
Estate of H. and L. Hecht	3,000.00
Estate of Edward Wilson	3,000.00
H. V. McKay Charitable Trust	2,000.00
Lions Club, Forest Hill	2,000.00
The George Thomas and Lockyer Potter Charitable Trust	1,825.00
Appel Family Bequest	1,600.00
"B" Group	1,100.00
Bell Charitable Trust	1,000.00
Cuming-Smith & Co.	1,000.00
Estate Marian and E. H.Flack	1,000.00
Estate Edgar Rouse	1,000.00
Truby and Florence Williams Charitable Trust	1,000.00
Estate Alfred Edments	850.00
Rothmans University Endowment Fund	800.00
Carlton and United Breweries Ltd.	500.00
Mrs. Winnifred L. Crane	500.00
J. C. Habersberger	500.00
D. Moynahan	500.00
Myer Emporium Ltd.	500.00
Mrs. L. A. Simpson	500.00
George F. Little Trust	475.00
Sunshine Foundation	465.00
Australian Eagle Insurance Co. Ltd.	300.00
Darren Baillieu	275.00
Oakleigh Leo Club	269.38
M. A. Cuming	250.00
W. J. Ould	250.00
S. M. Kimpton	200.00
R. J. Mitchell	200.00
Pethard Tarax Charitable Trust	200.00
Dr. A. Piper	200.00
Mr. and Mrs. E. Rogers	200.00
Mr. and Mrs. K. Allen	150.00
General Motors Holden	150.00
Dr. James Syme	150.00
M. L. Bell	100.00
Dr. F. A. Dibden	100.00
Dr. G. Donnan	100.00
P. O. Flecker	100.00
J. F. T. Grimwade	100.00
Dr. H. B. Kay	100.00
Prof. P. I. Korner	100.00
Dr. C. Laing	100.00
W. D. McPherson	100.00
Fred Manning	100.00
L. M. Muir	100.00
Oliver J. Nilsen (Aust.) Pty. Ltd.	100.00
Dr. G. Pinner	100.00
Price Forbes, Leslie Sedgman Pty. Ltd.	100.00
Dr. B. A. Roberts	100.00
Dr. J. Rouse	100.00
Dr. T. H. Steel	100.00
G. R. Stirling	100.00
The Specialty Press Ltd.	100.00
John Warlow	100.00
Reginald Blakemore	63.27
Prof. R. R. Andrew	50.00
W. A. J. Baker	50.00
Dr. J. G. Barratt	50.00
Dr. Geoffrey J. Benness	50.00
Miss N. E. Cameron	50.00
G. B. Canham	50.00
F. D. Danglow	50.00
Mrs. Lilian Grace	50.00
A. S. Leslie	50.00
Dr. B. W. McEwan	50.00
Seigfreid Meyer	50.00
F. H. Wright	50.00
Dr. A. K. Chapman	37.50
Miss Nancy McBean	30.00
Alan F. Drayton	25.00
E. J. Hardcastle	25.00
Prof. W. Hare	25.00
A. Wonson	25.00
W. J. King	20.00
J. J. McKernan	20.00
K. J. Mangold	20.00
Noel Monteith	20.00
C. W. Donne	10.00
D. Merry	10.00
K. Patterson	10.00
I.B.M. Ltd.	5.79
N. Grecian	5.00
D. & R.Jeffrey	5.00
C. Stuart Tompkins	5.00
	$104,020.94

Further contributions were received from —

Australian Broadcasting Services, Australian Eagle Insurance Co. Ltd., 2/14th Australian Field Regiment Association, Mr. Darren Ballieu, Mr. W. A. J. Baker, Jean Smith Bish, Miss N. Cameron, G. B. Canham, Will Denham, Mrs. Ivy Dickie, Beryl L. Dowsett, Eastman Kodak Company, Mr. and Mrs. N. Grecian, Mrs. D. Habersberger, Mr. J. C. Habersberger, Miss J. Taylor-Hall, Miss Jessie T. Hall, Misses J. & D. Jeffrey, G. Knox, Kodak (Australia) Pty. Ltd., Alfred Lazer, Miss M. McPherson, Nathan Blight Pty. Ltd., Pam Newton, W. J. Ould, Neil Payne, Mr. and Mrs. Marshall Peter, Mr. H. M. & Mrs. C. E. Petley, Miss J. V. Smith, P. Thomas, Eric Wilkinson.

In memory of —

William Alexander, Mr. Ashton, John Campbell Baker, F. S. Bellair, Tom Bellair, E. Boekemann, Sue and David Clarke, R. Clemons, Mrs. Janet Denham, L. Dowsett, Jennifer and John Ellis, Nancy Garland, W. Hille, S. F. Howell, D. Hutton, Bruce Kidd, John Kierce, Wallace Knox, Mrs. Alice McPherson, H. R. Mirams, Mrs. Moser, J. Moss, Margaret Oakley, J. P. Ogge, Lionel A. Pepperell, Sir William Philip, Ross L. Reed, Leonard Reid, Edgar Rouse, John Sexton, Barbara and Graham Shearer, Gilbert and Bronwyn Smith, Bill Stevens, Ivy Sumner, Isaac Walker-Drummond, Herbert Norman Webb, Elsa Williamson, Mr. Fung Mo-Ying.

Total: $615.00

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

Revenue Account for the Year Ended 31st December, 1975

EXPENDITURE	
Salaries and wages	$327,350
Laboratory supplies and isotopes	48,045
Additional equipment and building costs	67,622
Library maintenance	12,713
Postage and telephone	3,968
Printing and stationery	5,162
Light and power	22,226
Insurance	8,431
Repairs and renewals	12,949
Animal house contribution	6,000
Sundries	5,187
Travelling expenses	5,747
Public relations	453
	$525,853

INCOME		
Donations from Baker Benefactions		
Statutory amount	$11,569	
Transfers from Restricted Fund	150,731	
		$162,300
Donations other		8,915
Grants-in-Aid of Research Projects		
Anti-Cancer Council	25,510	
Life Insurance Medical Research Fund of Australia and New Zealand	35,203	
National Health and Medical Research Council	55,061	
National Heart Foundation of Australia	8,051	
		123,825
Other Grants		
The James and Elsie Borrowman Research Trust	5,650	
The William Buckland Research Fund	1,211	
Victorian State Government	35,000	
		41,861
Interest from Investments		
Held by Trustees of The Baker Institute Grant Trust	4,195	
Other investment income	104,265	
		108,460
Other Income		
Antarctic Division Rental	21,919	
Sundry sales, recoveries and refunds	42,633	
Equipment reserve	15,000	79,552
Deficit for the year		940
		$525,853

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

Balance Sheet as at 31st December, 1975

ACCUMULATED FUNDS AND LIABILITIES		
Maintenance Fund		
Accumulated deficit brought forward	($9,751)	
Deficit for year	(940)	
Accumulated deficit		($10,691)
Bank overdraft		10,602
Sundry creditors and accrued expenses		7,719
		7,630
Endowment Fund		
Accumulated fund		1,269,868
		$1,269,868
Research and Scholarship Funds		
Restricted fund	60,031	
Edgar Rouse Memorial Fellowship Fund	31,529	
Laura Nyulasy Scholarship Fund	2,863	
William Buckland Research Fund	21,653	
Lang Research Scholarship Fund	4,852	
		120,928
		$1,398,426

ASSETS			
Cash on hand		100	
Sundry debtors		7,530	
			7,630
Investments: (at cost)			
Held by Trustees of the Institute:			
Government and semi-government stock	$15,361		
Shares in companies	89,334		
Short term deposits	140,000		
Mortgage loans	443,500		
		$688,195	
Held by the Trustees, Executors and Agency Co. Ltd.:			
Shares in companies	62,173		
Trust units	473,719		
		535,892	
Cash at bank		45,781	
			$1,269,868
Investments: (at cost)			
Held by Trustees of the Institute:			
Shares in companies		4,852	
Short-term deposits		30,400	
		35,252	
Held by the Trustees, Executors and Agency Co. Ltd.:		24,516	
Cash at bank		61,160	
			120,928
			$1,393,426

Notes to the Balance Sheet at 31st December, 1975

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 31st December, 1975, including the building, totalled $3,500,000.
2. There is a commitment amounting to $691 for the balance of 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 and Agency Co. Ltd.
4. The market value of shares in companies listed on the Australian Stock Exchanges at 31st December, 1975 was $38,144 below the amount at which they are stated in the accounts.

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

The Trustees, Executors and 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 6 is properly drawn up to show a true and fair view of the state of the Institute's affairs at 31st December, 1975.

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

Melbourne,
9th February, 1976.

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

Year Ended 31st December, 1975

RESTRICTED FUND		
Balance at 31st December, 1974		$26,569
Transfer from Estate of Thomas Baker	$300,228	
Investment income	321	
Donations — other	53,392	
		353,941
		380,510
Transfer to Endowment Fund	149,497	
Transfer to Revenue Account	150,731	
Transfer to Edgar Rouse Memorial Fellowship Fund	5,000	
Equipment costs	15,251	
		320,479
Balance at 31st December, 1975		$60,031
ENDOWMENT FUND		
Balance at 31st December, 1974		1,104,887
Donations	18,609	
Investment income	1,708	
Transfer from Restricted Fund	149,497	
		169,814
		1,274,701
Transfer to Edgar Rouse Memorial Fellowship Fund		4,833
Balance at 31st December, 1975		$1,269,868

THE CLINICAL RESEARCH UNIT

STAFF

(to December 1975)

Director	P. I. KORNER, M.D., F.R.A.C.P., F.A.A.
Associate Director	A. J. BARNETT, M.D., F.R.C.P., F.R.A.C.P. (until October 1975).
Staff Physicians	R. DARGAVILLE, M.B. B.S., F.R.A.C.P. (until March 1975). I. TAUBMAN, M.B., B.S., F.R.A C.P. G. JENNINGS, M.B., B.S., M.R.C.P.(U.K.)
Visiting Physician	A. FRIEDMAN, M.B., B.S., M.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.), M.R.A.C.P.
Biochemical Pharmacology	A. BOBIK, Ph.D., B.Pharm., M.Sc. V. CARSON, M.Sc.
Technical Staff	Miss S. GRAHAM Mr. J. KORMAN Mr. R. LOWE Mrs. R. MUSKETT Miss J. NEALE Mr. M. STRONG Miss H. WADDELL

WARD STAFF

Registrars	B. WILSON, M.B., B.S. F. PANNETTA, M.B., B.S. E. FAGAN, M.B., B.S., M.R.C.P.(U.K.) S. K. POON, M.B., B.S. K. G. TEH, M.B., B.S.
Resident Medical Officers	A. SILCOCK, M.B., B.S. A. Y. T. WU, M.B., B.S.
Ward Sister	Sr. A. GRIFFITHS

Director's Report

During the year we have taken the first steps to integrate the work of the Clinical Research Unit closely with the work of the Baker Institute. Both facilities are being developed as a predominantly Cardiovascular Research Centre. Two of the Research Units active in the Baker Institute will use the facilities of C.R.U. They are the Circulatory Control and Hypertension Research Unit working under my direction, and the recently formed Cardiovascular Metabolism and Nutrition Research Unit, working under the direction of Dr. P. J. Nestel, which will commence late in 1976. Our research effort is thus mainly in the fields of hypertension and coronary artery disease, which are the two cardiovascular diseases responsible for most deaths in our community. Each also accounts for enormous national economic losses since they strike at the middle-aged population in whose skills and education the community has made large investments.

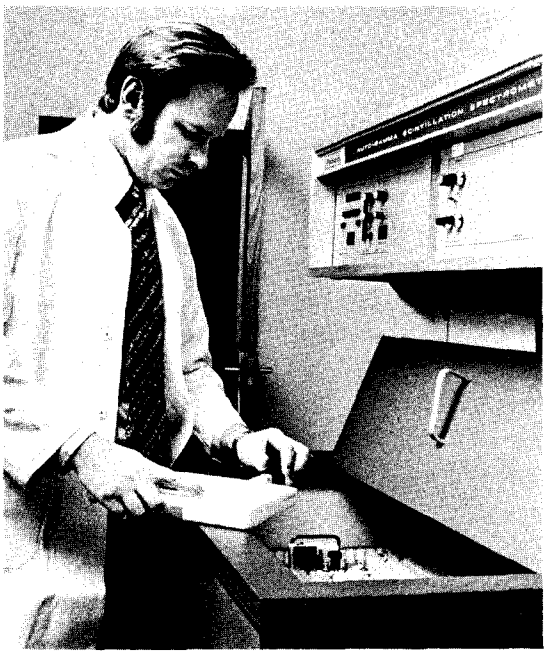
Our Hypertension Clinic was reorganised in 1975. C.R.U. has long been active in this field of medicine in the Hospital through the work of Dr. Barnett but much of this has involved special long continuing follow-up and treatment of patients with severe hypertension. However, in 1976 we established a new out-patient clinic — the *C.R.U Hypertension Evaluation Clinic.* This will provide a routine diagnostic service for hypertensive patients and also a consultative service for difficult therapeutic problems. The clinic is operated by C.R.U. staff working in close collaboration with other groups interested in hypertension in the Hospital, particularly Dr. J. Stockigt and Dr. J. Sabto.

During the year our clinical research work in hypertension has consisted of evaluating the mechanisms of the anti-hypertensive action of the beta-blocking drugs and of assessing the properties of the autonomic nervous system of patients with hypertension. Both approaches have been a direct consequence of our laboratory studies in the Baker Institute. The Biochemical Pharmacology Laboratory can now measure plasma concentrations of several of the beta-blocking drugs. We are using these measurements as an aid for assessing the patient adherence to the drug regime prescribed and to estimate whether the drugs are being taken in therapeutically effective doses. A number of pharmacokinetic studies

Members of Clinical Research Unit (from left), Dr. Alex Bobik, Dr. Elton Fagan, Dr. Peter Fletcher, Professor Korner, Dr. Peter Blombery, Valerie Carson, Sister Pam McCracken, Sister Joan Wildberger and Dr. Garry Jennings.

are being undertaken to help plan optimal oral dosage schedule of beta-blocking drugs. Our overall aim is to make anti-hypertensive therapy as simple and rational as possible and as tailor- made to the needs of the individual patient. I believe this will make it more effective than it often is at the present time. The Biochemical Pharmacology Laboratory is providing a service on the measurement of urinary catecholamine which is used in many of our clinical pharmacological studies and are working on a method for the accurate assessment of plasma catecholamines.

In the field of coronary artery disease we have developed a more discriminating test for evaluating E.C.G. changes during exercise in patients with ischaemic heart disease. This is designed to be both sensitive and highly specific and to reduce the number of false positive results which are associated with many exercise E.C.G. protocols. We are engaged in a joint programme with the other cardiac departments in the Hospital to use this test for objectively evaluating the results of cardiac coronary by-pass surgery. Other studies in ischaemic heart disease include a pharmacokinetic study for evaluating the duration of efficacy of beta-blockers in attenuating the heart rate response to exercise. The Biochemical Pharmacology Laboratory has also performed important studies on the fundamental nature of the beta-receptor and its alteration in cell membranes in different forms of heart disease.



Alex Bobik near autogamma counter (purchased with the assistance of a grant from the Potter Foundation).

A number of projects previously under way have been continued during the year. These include studies on drug overdosage, particularly those involving tricyclic anti-depressants, and studies on scleroderma, and peripheral vascular disease. As in previous years the C.R.U. staff has participated in undergraduate teaching. There have been a number of staff changes during the year. Dr. Barnett has left C.R.U to become Staff Physician to the Peripheral Vascular Unit in the Hospital. It is fitting to pay particular attribute to the valuable contribution he has made to the Hospital and C.R.U. over 25 years and, for his work as Acting Director of C.R.U. during 1974.

I am hoping that in 1976 there will be a considerable expansion of our activities in hypertension and in relation to the clinical pharmacology of anti-anginal drugs. We are looking forward to new activities in the field of coronary artery disease which will follow the arrival of Dr. Nestel's group. Amongst other things this will allow us to introduce a much better lipid evaluation service than is available at the moment anywhere in Melbourne. It will allow us to take a new look at the treatment of lipid disorders in patients with hypertension, since both are very important 'risk' factors for the development of coronary artery disease. Other new ventures envisaged include the establishment of a pilot lipid screening clinic as part of a coronary artery prevention programme.

I am confident that the establishment of a Baker-C.R.U. Cardiovascular Research Centre will make a useful contribution to the work of the Hospital in the future, as indeed it has in the past. I particularly hope that the Centre will help fill the great need to provide training in research methods to the residents likely to become future staff physicians. With the increasingly complex nature of medical sciences required in cardiovascular medicine it is becoming increasingly necessary to obtain formal training from professional research workers in the important areas of data evaluation and planning of clinical investigations. I believe that the newly organised Cardiovascular Centre is in a good position to meet the local and national need for such training, and I hope our programmes for research training in cardiovascular medicine will be attractive to physicians in advanced training.

PROJECTS

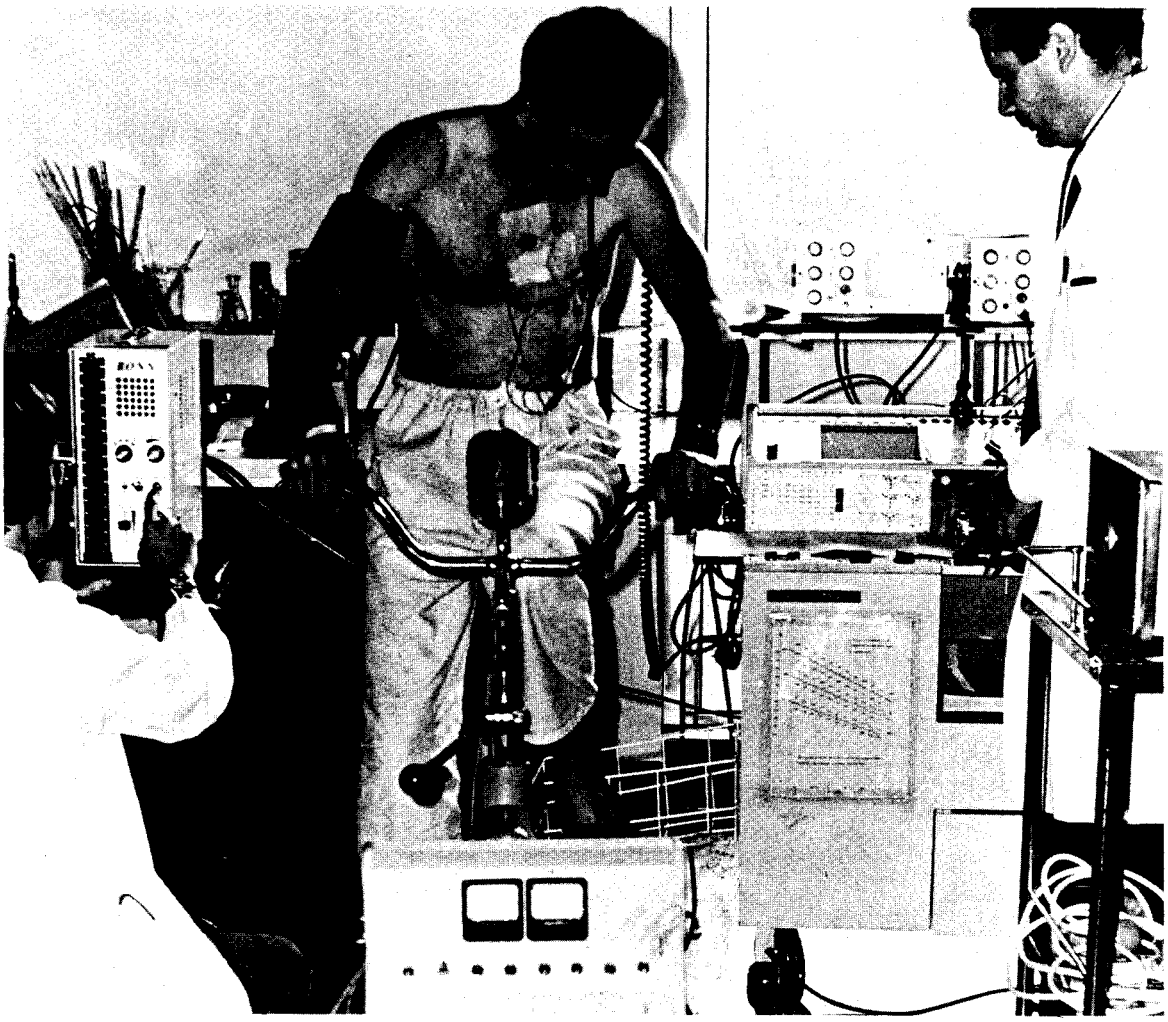
1. Reflex Constrictor Responses in Essential Hypertension

P. I. Korner, A. M. Tonkin* and J. B. Uther*

It has often been considered that hypertensive patients show much greater rises in blood pressure and much more severe constriction of their blood vessels than normal subjects. However, it has been difficult to establish reliably the truth of this assertion. We have developed recently a quantitative test for examining reflex constrictor responses in man. We have found in normal subjects that the Valsalva manoeuvre provides an accurately gradable and rapidly reversible method for testing reflex constrictor and heart rate responses. Changes from resting in total peripheral resistance (TPR) and heart rate which occur during the lesser part of a Valsalva manoeuvre of standard duration are reproducibly related to the magnitude of the applied forced expiratory pressure. Moreover, the magnitude of the reflex and mechanical component of the circulatory responses can be estimated from the expiratory pressure-circulatory response curves obtained before and after autonomic block.

We studied the Valsalva constrictor reflex in three groups of subjects: (i) normal volunteers with resting mean arterial pressures from 75-90 mmHg (i.e., in the lower half of the normal range of blood pressure); (ii) normal volunteers with resting mean arterial pressures from 91-104 mmHg (i.e. in the upper half of the normal blood pressure range); (iii) patients with mean arterial pressures from 150-165 mmHg (i.e. patients with established essential hypertension having minimal complications. The sensitivity of their constrictor response increased by a small but significant amount with rising blood pressure between the groups. The slight enhancement of absolute constrictor activity in the hypertensive subjects could be explained on the basis of elevation of their resting vascular resistance. Chronic structural changes in the wall of the small blood vessels in these subjects was probably responsible for some encroachment upon the vascular lumen as a result of medial hypertrophy as has been suggested by Folkow. This results in an enhanced constrictor response for a given degree of smooth muscle shortening even though the autonomic pathways are normally excited by the reflex stimulus.

* Not a member of the Baker Institute or C.R.U.



Dr. Jennings supervises an exercise test in the Clinical Research Unit.

2. Effect of Beta-blockade on the Valsalva Constrictor Reflex

P. A. Blombery, G. Jennings, P. J. Fletcher, A. Bobik and P. I. Korner

In order to investigate whether beta-blocking drugs lowered blood pressure through their action on the central nervous system we have studied the side effects of acute intravenous administration of propranolol in patients with established essential hypertension. If beta-blocking drugs acted on the central nervous beta-receptors we would expect a dose-dependent attenuation of sensitivity of the Valsalva constrictor reflex as has been found in animal studies with clonidine. We have administered propranolol i.v. to reach blood concentrations between 100 ng/ml to 250 ng/ml; in none of the patients was there an effect on the sensitivity of the Valsalva constrictor reflex even at the highest blood concentrations. This result in man is in agreement with our findings in normal animals (see Baker Medical Research Institute Project Reports). However, at the levels administered the blood pressure was lowered in our hypertensive subjects by an average of 10 mmHg and the findings suggest that propranolol does not exert its effect on the central sympathetic constrictor pathways but probably lowers blood pressure directly through its effects on cardiac output.

3. Electrocardiographic Changes during Exercise

G. Jennings, F. Panetta, E. Fagan, A. Bobik and P. I. Korner

The response to exercise is an established way of testing for the existence of and progress of ischaemic heart disease. However, the multiplicity of methods and the qualitative way in which the results are assessed has led to difficulties of interpretation in a large proportion of exercise tests. In our laboratory a quantitative exercise test is used in the diagnosis of ischaemic heart disease, and in evaluating the effects of interventions such as beta-blocking drugs and coronary by-pass surgery.

The test is based on the relationship between work-level and ischaemia expressed by the measured deviation of the ST-segment of the electrocardiogram during a graded series of exercise tests employed in our laboratory. The ST-segment responses are examined over a large range of workloads before and after administration of a beta-blocking drug during both 'sprint' and 'steady state' exercise. In patients with ischaemic heart disease there is normally a curvilinear relationship between workload and ST- segment depression which is shifted to the right after giving beta-blocking drugs. In other words after beta-blockade there is less ST-segment depression for a given workload than before block. The test is designed to provide a high degree of sensitivity and specificity and to avoid false positives. Acute administration of beta-blocking drugs does not significantly increase the maximum workload attained. Testing three months after coronary by-pass surgery there is less ST-segment depression for a given workload compared to the pre-operative test in subjects in which the grafts are presumed to have remained patent. The work level at which the patient stops exercising is also increased, however in patients where the graft appears to have closed there is little change from pre-operative workload ST-segment curve.

The tests have also been used for assessing how long beta-blocking drugs inhibit the normal exercise-induced rise in heart rate. We have performed this in relation to pharmacokinetic studies where the blood concentration of various beta-blockers has been measured in relation to the degree of inhibition of exercise tachycardia. We have found with prindolol that there was good correlation between inhibition of the exercise-response and plasma concentration of drug during the first eight hours after injecting an intravenous bolus. However the inhibition persisted for longer than the time at which a measurable amount of drug could be detected in plasma.

4. Effect of Clonidine Withdrawal on Urinary Catecholamine Excretion

G. Jennings, V. Carson and P. I. Korner

The occurrence of a marked rebound rise in blood pressure following sudden stopping of clonidine in the treatment of hypertension is relatively uncommon. However, when it occurs it resembles an acute episode of hypertension observed in patients with phaechromocytoma and is dramatic and dangerous for the patient. As a result many physicians have become increasingly cautious about prescribing clonidine even though the latter is an effective anti-hypertensive drug. We have done studies to determine the frequency with which abrupt cessation of clonidine is associated with evidence of autonomic hyperactivity. Patients have been studied under careful supervision and monitoring of their blood pressures whilst in-patients of C.R.U., and their urinary catecholamine excretion has been determined for several days before and after stopping of the drug. To date six patients have been studied and all have shown a large rise in urinary catecholamine excretion on the first day after stopping treatment to about twice the pre-withdrawal value. In all these patients there was a small statistically significant rise in blood pressure associated with the high levels of catecholamine excretion. The results suggest that after stopping clonidine there is a larger rise in autonimic activity than is apparent from the rise in blood pressure. Studies are in progress to examine what extent stopping other anti-hypertensive drugs may have similar effects on urinary catecholamine excretion.

5. Biochemistry of the Beta-Adrenergic Receptor

A. Bobik, J. Funder*, C. I. Johnstone* and Elizabeth Woodcock*

To date most studies aimed at identification and characterisation of beta-adrenergic receptors have utilised tritium-labelled agonists such as isoprenaline or adrenaline in binding studies to membranes, e.g. heart microsomes or turkey erythrocyte ghosts. Although the binding sites identified by these studies are of a uniform nature, their binding characteristics are not what would be expected of intact physiological beta-adrenergic receptors. For example, they show no stereospecificty for catecholamines which may be readily displaced by non-biologically active catechols. Furthermore, beta-adrenergic antagonists only compete with catecholamines for these binding sites at high concentrations. In view of these differences between the binding characteristics of these sites and physiological properties of beta-receptors, we and others have been searching for a suitable radioactively labelled adrenergic ligand, the binding characteristics of which might reflect all of the properties to be expected of beta receptors.

Isopropylamino-3-(4-iodophenoxy)-2-propanol is a new beta-blocker recently synthesised in our laboratories which is proving to be a useful tool for probing the molecular characteristics of beta-adrenergic receptors. The synthesis of this compound involves diazotisation of isopropylamino-3-(4-aminophenoxy)-2-propanol and incorporation of ^{125}Iodine via the Gattermann reaction. Radiochemical and chemical purity is achieved by selective solvent extraction and chromatographic procedures. The use of ^{125}Iodine means that it is possible to achieve high specific activities up to 1000 c/mmole. This is necessary to characterise beta receptors in tissues where they are present in relatively low concentrations on the cell membranes.

In vivo isopropylamino-3-(4-iodophenoxy)-2-propanol inhibits isoprenaline induced tachycardia. *In vitro* it binds to cardiac membranes with approximately 85-90% of the binding being specific. The binding process is rapid and reversible with

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half maximal binding occurring at approximately 5 nM. The membrane-bound radio-label is readily displaced by beta-adrenergic agonist and antagonists.

Further studies are in progress in the characterisation of beta receptors using this compound in normal and abnormal hearts.

6. Pharmacokinetics of Methyldopa in Hypertensive and Normotensive Subjects

A. J. Barnett, A. Bobik, V. Carson, J. Korman and A. J. McLean

Anti-hypertensive therapy with methyldopa may be unsuccessful in up to one-third of patients due to poor hypotensive response or side effects. Possible factors contributing to the poor hypotensive action of methyldopa may be the attainment of sub-therapeutic concentrations of its active metabolites at their sites of action. This may be due to poor absorption, distribution or metabolism of methyldopa to its active metabolites. The importance of these factors in anti-hypertensive therapy with methyldopa has been studied.

Using a fluorimetric assay for methyldopa we have studied its pharmacokinetics in normotensive and hypertensive subjects following intravenous dosage. The decline in plasma levels were best described by the biexpotential equation, $C = Ae^{-\alpha t} + Be^{-\beta t}$ where C is the plasma concentration, A and α are constants governing the initial rapid decay observed and B and β are constants governing the slow terminal decay. This fits the theoretical two-compartment open model of drug distribution and elimination from the body. There were no significant differences in these parameters between normotensive and hypertensive subjects with normal renal function. Following similar dosages orally, maximal plasma concentrations occurred after 2 to 3 hours and were approximately 1/5th of those achieved intravenously. The bioavailability of methyldopa after an oral dose varied between 10 and 20%. In a relatively large study in hypertensive patients, plasma methyldopa of patients taking similar doses varied greatly and showed no correlation with its observed anti-hypertensive action.

In summary the overall results suggest that variations in plasma methyldopa concentrations following oral dosage are mainly due to differences in absorption rates and bioavailability. Only small differences were observed in the rate of plasma methyldopa clearance from patients with normal renal function. Plasma methyldopa levels do not seem to be useful in explaining the poor hypotensive action of methyldopa observed in some patients.

7. Metabolic and Excretory Studies with Amitryptyline following overdosage.

A. Bobik and A. J. McLean

In spite of the widespread involvement of amitryptyline in overdosage, there is scant quantitative data on its metabolic detoxification following overdosage. For this reason the metabolic and excretory patterns of six hospitalised amitryptyline overdosed patients were studied. Four basic constituents were identified in urine by selective solvent extraction, TLC, GLC, UV spectroscopy and mass spectrometry. The major metabolites identified were glucuronide derivatives of the 10-hydroxy derivatives of amitryptyline and the monodesmethyl derivative of amitriptyline. These accounted for approximately 25 and 50% of total urinary metabolites excreted with the remainder being due to amitriptyline its 10-hydroxy derivative, the monodesmethyl derivative and its 10-hydroxy derivative.

Following overdosage the concentration of amitryptyline metabolites in urine varied greatly and were inversely related to urinary volume voided. In all cases 50% of total urinary metabolites were excreted between 30 and 60 hours following hospitalisation. For tertiary amine metabolites of amitriptyline the rate of urinary excretion declined exponentially with time according to the equation $\log \frac{dME}{dt} = Ae^{-\alpha t} + Be^{-\beta t}$ where A and α are constants relating to a rapid phase of decline and B and β are constants relating to the slower terminal decay phase. For secondary amine metabolites of amitriptyline A and α are constants relating to their initial rate of increase in excretion and B and β to the decline in their excretion rates.

8. Long-term Follow Up of Hypertension

A. J. Barnett and I. Taubman

A special clinic for the treatment of severe hypertension was commenced by the Clinical Research Unit in 1950, with the aim of studying the effect on prognosis of the recently introduced ganglion blocking drugs. At first entry was restricted to patients with malignant hypertension but later was extended to other patients with severe complicated hypertension. An early report (Barnett 1956) demonstrated the beneficial effect of treatment on the hypertensive disease and improved prognosis in malignant hypertension. Other drugs were used as they became available and the long-term results were reported by Barnett and Silverburg (1973).

The prognosis was improved by treatment but was still worse than for the corresponding age and sex groups from the general population. We decided to assess the present position of treatment in 160 current patients in the clinic, mainly survivors of the long term study described above. Blood pressure at entry was usually over 200 mmHg systolic and 120 mmHg diastolic. The patients had been treated for various times up to 25 years, most for more than 5 years. They were generally treated with a combination of drugs, one of which was a thiazide (thiazide and one other 51, thiazide and two others 34, thiazide and three others 13). Blood pressure control was assessed as "good" (diastolic generally less than 100 mmHg) in 59, "fair" (diastolic 100 to 110 mmHg) in 32 and "poor" (diastolic over 110 mmHg) in 9. The patients' general condition was classified as "good" (no significant symptoms) in 69, "fair" (minor symptoms) in 29, and "poor" (major symptoms or invalidism) in 11. It is apparent that modern treatment with a variety of drugs produces good or fair blood pressure control with good or fair symptomatic state in the majority of severely hypertensive patients, but there remains a small proportion (10%) inadequately controlled.

9. Treatment of Scleroderma.

A. J. Barnett.

Over the past 25 years I have had the opportunity of studying a large series of over 100 patients with scleroderma (progressive systemic sclerosis). Although much data has been accumulated on the clinical and pathological features and cause of this disease (Barnett, 1974) the aetiology remains unknown and there is no specific cure. Recently trials have been conducted of the effect of drugs reputed (by overseas workers) to have a specific effect on the connective tissue disturbance. Preliminary results with two of these (norethisterone and salazopyrin) were described in the Research Report of the Baker Institute and Alfred Hospital in 1974. These trials have now been completed and the results published (Barnett and Marks, 1975). Neither drug proved of specific value and the use of norethisterone acetate was associated with undesirable side effects. D-penicillamine has been reported to have an effect on the solubility of collagen and a recent report indicates that this occurs only in the active stage of the disease. A trial is therefore being conducted on the effect of this drug in patients with diffuse (type 3) scleroderma in the actively progressive stage, using objective criteria of assessment similar to those in the norethisterone acetate and salazopyrin trials. As yet only four patients have been entered in the trial. One has completed the course, with no significant benefit; the others have been treated for a short time only.

Publications

Published or accepted:

I. K. **BAILEY, S. D. ANDERSON, P. J. ROSEA, L. BERNSTEIN, G. NYBERG and P. I. KORNER.**
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Pharmacokinetics of Methyldopa: Plasma levels following single intravenous, oral and multiple oral dosage in normotensive and hypertensive subjects. *Clinical and Experimental Pharmacology and Physiology.* In Press.

A. J. BARNETT and R. MARKS.
Norethisterone acetate in the treatment of scleroderma. *Australasian Journal of Dermatology,* 16: 45-54, 1975.

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Salazopyrin in the treatment of scleroderma. *Australasian Journal of Dermatology,* 16: 55-59, 1975.

A. J. BARNETT, E. TWIST and A. BALFE.
A review of lower limb amputation in a general hospital in the years 1970-1973. *Medical Journal of Australia.* In Press.

A. BOBIK and A. J. McLEAN.
Cardiovascular complications due to pheniramine overdosage. *Australian and New Zealand Journal of Medicine.* In Press.

P. I. KORNER, A. M. TONKIN and J. B. UTHER.
Reflex and mechanical circulatory effects of graded valsalva manoeuvres in normal man. *Journal of Applied Physiology.* In Press.

Submitted for Publication:

A. J. BARNETT and I. TAUBMAN.
Current status of treatment of severe hypertension: drugs used and results.

A. J. BARNETT, I. TAUBMAN and S. K. POON.
Scleroderma (progressive systemic sclerosis) with severe bowel involvements. Treatment by extensive resection of the small intestine.

LECTURES:

Dr. A. J. Barnett visited Japan and China during September and October, 1975. In Japan and China he lectured on "Scleroderma" and "Long-term management of severe hypertension". Dr. A. Bobik gave a lecture on "Drug analysis and its application to clinical toxicology" to the Australian Association of Clinical Biochemists, and on "Some aspects of Drug Metabolism" to the Victorian College of Pharmacy.

C. J. Officer Brown Cardiothoracic Surgical Unit

Director: G. R. STIRLING
Cardiothoracic Surgeon: BRUCE B. DAVIS

The C. J. Officer Brown Cardiac Surgery Unit serves those hospitals associated with the Monash University — Alfred, Prince Henry and Queen Victoria. During 1975, over 350 cardiac operations were performed including 290 'open heart' procedures.

Though the Unit is essentially a service unit, it has had a continuing involvement in the development and evaluation of new techniques of surgery since the early 1950's. A major theme of the Unit's laboratory interests over the years has been in studying the influence of surgical interference on the function (contractility) of the heart muscle. Past studies have included the examination of cardiopulmonary by-pass, ischaemic arrest, potassium induced asystole and hypothermia on myocardial performance.

A second major theme of continuing study has been in the development of satisfactory techniques and in the evaluation of new apparatus for cardiopulmonary by-pass. Such evaluations have always preceded the introduction of new apparatus or new techniques into the operating room and the Unit regards it as a continuing responsibility to evaluate such changes in the laboratory before translating them to the human situation.

Protection of the Hypertrophied Myocardium during Aortic Valve Surgery

In cases of severe aortic stenosis marked left ventricular hypertrophy occurs. Whilst most operations for aortic stenosis can be carried out with a very low risk, the risk rises to a high level in a small number of cases with severe concentric left ventricular hypertrophy because of the sporadic occurrence of intro-operative myocardial infarction in the deeper layers of the myocardium. It is probable that this occurs because of interference with the normal mechanism of coronary perfusion during the procedure.

Two quite opposite attempts have been made to overcome this problem. In the first it has been the object to continue coronary perfusion during the total period of interference in the aortic valve area by the use of individual cannulae placed into the coronary arteries. In the second instance, no coronary perfusion is used at all but the heart is 'protected' by immersion and lavage in ice cold electrolyte solution. By cooling the myocardium the oxygen requirements are reduced to very low levels so that the effects of ischaemia are greatly reduced.

A comparative study is in progress in a series of dogs who have had constriction bands passed around the proximal aorta to induce severe left ventricular hypertrophy. Two separate sets of animals from this series are then submitted to simulated aortic valve operation of one hour's duration. In the first instance the coronaries are continuously perfused at normal temperature and in the second group no perfusion is used but the heart is cooled by immersion. After completion of the mock procedure, estimates are made of myocardial contractility by standard techniques, biopsies are taken for histo-chemical and electron-microscopic study and comparisons are made. After sacrifice of the animals some days later, the hearts will be further studied by histological techniques. It is hoped that these studies on the hypertrophied heart will lead to decisions as to the comparative effectiveness of the two techniques of myocardial 'protection'. It is further hoped that in the course of such experiments, further leads to technical improvement may be observed.

Comparison of Oxygenators

During the last five years, the major clinical experience has been with the use of the Rygg Bubble Oxygenator. During 1975, the Teflo-Modulung Membrane Oxygenator became available for clinical evaluation. A comparative study is in progress to compare two matched series of clinical cases using alternatively the Rygg Oxygenator system and the Teflo-Modulung Membrane system.

Comparisons will be made on the clinical course of the patients, with special reference to cerebral function, the incidence of pulmonary complications, the amount of post-operative drainage and the adequacy of the coagulation mechanisms. Further studies on platelet preservation and function will also be carried out to determine the relative efficacy of the two systems.

Psycho-Social Assessment of Results of Coronary Artery Surgery

Over 300 cases have now been submitted to coronary artery surgery and 120 operations were carried out in 1975. Whilst many studies both here and elsewhere have indicated a high degree of success in achieving relief of angina and also a lesser degree of success in achieving continued patency of the venous by-pass conduit, it has been found that there is a disappointing correlation between physically successful operations and the overall results of surgery when looked at from a broader point of view including psycho-social readjustment. It is planned to carry out a detailed prospective study on patients submitted to coronary artery surgery in 1976 to fully evaluate the effects of operation from psychological, social and physical parameters with the object of determining the overall success of surgery and defining the causes of failure. It is hoped that this study might point to improved techniques of selection and management and help to define the facilities required for proper post-operative management of this enlarging case group. The patients included in this study will also be studied by in-exercise electrocardiography before and after surgery to provide better physical correlation. (See C.R.U. report.)

The incidence of Myocardial Infarction after Coronary Artery Surgery

Patients submitted to coronary artery surgery will be studied by the techniques of myocardial isotope infarct image scanning to determine the incidence of myocardial infarction after coronary artery surgery in collaboration with Dr. Aubrey Pitt.

The Ewen Downie Metabolic Unit, 1975

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Annual Report

During 1975 the Unit has continued its dual role as a Service and Investigative Department within Alfred Hospital. The care of Endocrine patients poses many unanswered questions and provides opportunity for Clinical Investigation using the Unit's Laboratory and Patient-care facilities. The major Laboratory emphasis has moved towards investigation of Thyroid disease and Endocrine Hypertension with our laboratory facilities now directed very largely towards these two areas, despite a very diverse Clinical load. The trend towards rationalization of Endocrine laboratory facilities among the Melbourne teaching hospitals continues and we are grateful to colleagues at Prince Henry's Hospital for assistance in the laboratory investigation of patients with problems in Reproductive Endocrinology, to our counterparts at St. Vincent's for laboratory assistance with problems of Carbohydrate Metabolism, and to the Endocrinology Section at the Royal Melbourne Hospital for aid with problems of Calcium Metabolism. The Unit has provided laboratory data in the investigation of Endocrine Hypertension for the Royal Melbourne, Prince Henry's, Queen Victoria and St. Vincent's Hospitals, and has provided Thyroid Hormone data for patients from the Royal Children's and supplementary thyroid assays for Prince Henry's, Queen Victoria, Repatriation and Southern Memorial Hospitals.

In addition to the Undergraduate teaching done by members of the Unit, the Postgraduate programme in Endocrinology, in the form of weekly or twice weekly Seminars, is designed to provide candidates with basic and advanced training for the F.R.A.C.P. qualification. The Unit is now staffed by a Registrar as well as by four rotating second year basic trainees in Internal Medicine, with consequent expansion of the in-service training previously available in Endocrinology.

During 1975 members of the Unit have continued to visit Hospitals in the Gippsland area on a monthly basis to provide an Outpatient Consultant service which is becoming increasingly successful. In addition, the Unit enjoys a continuing association with numerous Country Consultative Physicians in the management of patients with complex Endocrine problems.

In the Investigative work of the Unit the collaboration of colleagues at the Howard Florey Institute, University of Melbourne, Medical Research Centre, Prince Henry's Hospital and Baker Medical Research Institute, Alfred Hospital has continued to be a source of challenge, stimulus and assistance, which is most gratefully acknowledged. We continue to enjoy the help and co-operation of the Clinical and Diagnostic departments of Alfred Hospital; in particular the work of the Biochemistry, Radiology and Nuclear Medicine departments has been of great assistance to us.

Grateful acknowledgement is made of grants in aid and in kind by — Estate of the late Vincenza E. M. Acton, Difrex (Aust.) Pty. Ltd., Hoechst Australia Ltd., Miles Laboratories Aust. Pty. Ltd., Mr. C. Rotstein, Sandoz Aust. Pty. Ltd.

PINCUS TAFT,
Physician-in-Charge.

Thyroid Hormone Physiology

Introduction

During 1975 the Unit has performed thyroid hormone assays on 3,600 samples from patients from Alfred Hospital and other centres. In about 85% of these a clear-cut diagnosis of thyroid status was made with routine assays, but in about 15% additional laboratory assays were required, and in 2-3% it was necessary to perform further investigation. This core of more difficult cases had formed the basis for several worthwhile lines of investigation.

It is now clear that there are two important circulating thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3). In most cases of thyroid overactivity both hormones are in excess and diagnosis is clear-cut, but it is now apparent that in some situations thyroid overactivity can be clinically significant with excess of either hormone alone. Our findings over the past 2 years suggest that the diagnosis of thyroid overactivity may be missed in about 10% of cases if T_4 alone is used as the diagnostic index — such cases have an isolated excess of T_3. However, the diagnosis of hyperthyroidism with isolated excess of T_3 is complex because normal values for T_3 vary with age and are altered in severe associated illness or malnutrition. For this reason we have come to rely on the thyrotrophin (TSH) response to intravenous thyrotrophin releasing hormone (TRH) in doubtful cases.

It is now clear that the majority of T_3, the more potent thyroid hormone, is produced outside the thyroid by removal of one iodine atom from T_4. This process of T_4 to T_3 conversion is currently poorly understood, but has become an important aspect of thyroid physiology both in health and disease. The circumstances where there is dissociation between T_3 and T_4 levels will become increasingly important in defining the conversion step, and will also throw light on the current controversy regarding the relative roles of T_3 and T_4 in health and disease.

In order to assess the peripheral effects of thyroid hormone action we have returned to earlier measurements such as basal metabolic rate, ankle-jerk relaxation time, red-cell sodium content and red cell 2-3-diphosphoglycerate concentration. While of limited diagnostic value, these measurements are well suited to serial measurements in the same patient to assess changes in hormone effects. They have been of particular interest where there is dissociation between T_3 and T_4 levels, either induced by Propylthiouracil or during severe associated illness.

The routine use of T_3 radioimmunoassay has enabled the diagnosis of thyroid overactivity to be made at an earlier stage. In the elderly this allows prompt treatment, but in the younger age group, where symptoms may be mild it presents the opportunity to follow the clinical and biochemical course without treatment. This provides a valuable opportunity to examine both the pathogenesis and natural history of thyrotoxicosis, two areas where there is current uncertainty.

Triiodothyronine (T_3) Radioimmunoassay in the Diagnosis of Thyrotoxicosis

D. M. Engler, J. R. Stockigt, V. Feller, J. A. Roy, M. Browne, S. Petrou and H. P. Taft.

Since September, 1973, radioimmunoassay of T_3 has been performed in all patients with suspected thyrotoxicosis. The prevalence of isolated excess of T_3 (T_3 toxicosis) appears to be 10-15% that of classical thyrotoxicosis and over 40 cases of T_3 toxicosis have been defined. In elderly patients with cardiac presentations determination of T_3 is especially critical because the free thyroxine index is often normal or only marginally elevated. In younger patients with T_3 toxicosis symptoms are often mild and in 18 cases the course of the disease has been followed without antithyroid drugs. Patterns of progression to classical thyrotoxicosis, persistence of isolated T_3 excess and remission have emerged but at present the natural history is not predictable.

The thyrotropin (TSH) response to thyrotropin-releasing hormone (TRH), 200 µg intravenously, has been examined in 19 patients with marginal isolated T_3 excess. In 10 there has been no detectable TSH rise — a response characteristic of thyrotoxicosis. A subnormal TSH response has been seen in 6, while 3 showed a normal TSH response which indicates a normal thyroid-pituitary axis and rules out thyrotoxicosis. These findings suggest that while measurement of T_3 is essential for sensitive diagnosis of thyrotoxicosis, isolated T_3 excess may in some cases be a false-positive finding where the TSH response to TRH offers better discrimination. It has recently been demonstrated that serum T_3 falls with advancing age and application of age-corrected normal values may limit the false-positive findings. Prolonged follow up of patients with persistent isolated T_3 excess is in progress to further define the natural history. Preliminary findings in several cases suggest that remission and exacerbation may occur with rise and fall of T_3 showing good correlation with TSH response to TRH. The serum level of T_3 falls in states of starvation or with severe associated illness; when thyrotoxicosis coexists the level of T_3 may be "normal", and in 3 patients an absent or subnormal TSH response to TRH has been found with isolated thyroxine excess. These findings indicate that while T_3 is of great value in the diagnosis of thyrotoxicosis, the T_3 level cannot always be used as the sole discriminant. Further, our findings suggest that thyrotoxicosis may result from selective excess of either T_3 or T_4.

Propylthiouracil-induced Dissociation of Thyroxine (T_4) and Triiodothyronine (T_3) in the Treatment of Thyrotoxicosis

H. P. Taft, J. R. Stockigt, E. J. Higgs, M. Browne.

The anti-thyroid drug Propylthiouracil (PTU) rapidly inhibits the peripheral formation of T_3 from T_4 in addition to its

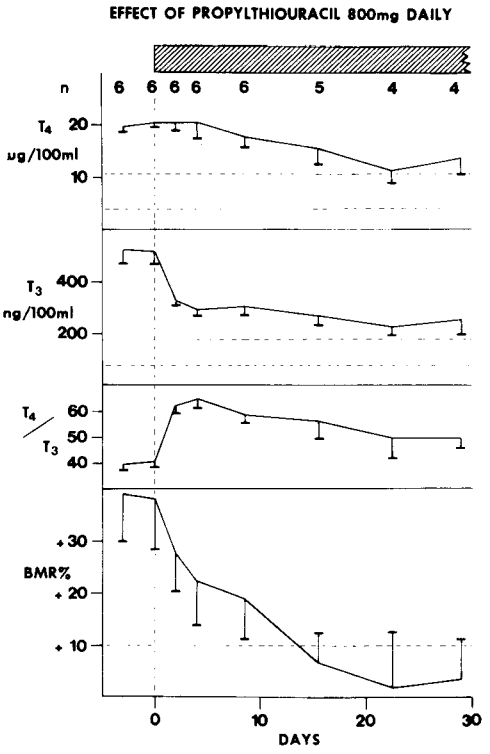

Fig. 1. Effect of Propylthiouracil 800 mg daily on T_4, T_3 and BMR in 6 subjects with classical thyrotoxicosis, showing fall in BMR during isolated fall in T_3, with normalization of BMR in the face of persistent excess of both hormones.

slower direct inhibitory effect on production of both hormones from the thyroid. When Propylthiouracil is used in thyrotoxicosis there is an immediate fall in T_3 level, without change in blood T_4 which falls much more slowly over 10-15 days. Basal metabolic rate (BMR) was used as an index of thyroid hormone action to determine the relative significance of changes in T_3 and T_4 during the phase of PTU-induced dissociation. BMR, T_3 and T_4 were measured on days — 3, 0, 2, 4, 8 and weekly thereafter in 6 volunteers with classical uncomplicated thyrotoxicosis. Serum T_3 fell to 63% of mean pre-treatment level after 2 days treatment, 57% after 4 days, and 58% after 8 days without significant change in T_4, and with a marked rise in T_4/T_3 ratio. BMR fell significantly with the isolated fall in T_3 and became normal in the face of persistent excess of both hormones (Fig. 1). These findings indicate that T_3 is the dominant thyroid agonist in thyrotoxicosis, and suggest that blood levels of T_3 and T_4 do not accurately reflect metabolic status during treatment with PTU. The normalization of BMR in the face of persistent hormone excess suggests the possibility that the PTU-induced alteration of peripheral T_4 metabolism might result in production of a thyroid hormone antagonist, or that there is relative resistance to thyroid hormone effects in thyrotoxicosis, or that PTU antagonizes thyroid hormone action. Previous *in vitro* data argue against the latter two possibilities.

Endocrine Hypertension

Introduction

The control of normal blood pressure is diverse, involving multiple neural, vascular, cardiac, renal and hormonal mechanisms, which normally show complex interactions which become more tangled as hypertension develops. Numerous hormonal abnormalities are described in hypertension but the majority of these may be the result rather than the cause of hypertension. However, in about 5% of patients with hypertension a hormonal abnormality may be causative and in such cases a cure of hypertension can often be achieved by removal of abnormal tissue, surgical restoration of normal renal blood supply, suppression of abnormal secretion or use of hormone antagonists.

Such cases are usually first detected by routine investigations such as plasma electrolyte measurements or intravenous pyelography, but these screening tests do not define the conditions with sufficient accuracy to justify specific treatment. The techniques of Endocrinology, notably radioimmunoassay of renin activity and aldosterone are an important component of the detailed investigations required in this minority of hypertensives.

The typical finding in primary aldosteronism due to a benign adrenal tumor is non-suppressible aldosterone excess with marked suppression of renin, associated with evidence of asymmetrical adrenal function either on adrenal scanning or adrenal vein catheter. In renal hypertension the typical finding which suggests surgical curability is predominant renin secretion from the abnormal kidney with suppression of secretion in the normal tissue of the non-involved kidney.

In the past two years over 150 patients have been studied with renin or aldosterone measurements and twenty-nine have had specific treatment for positive results — either renal or adrenal surgery, suppressive treatment with glucocorticoids or use of aldosterone antagonists.

Hormone measurements may also have a place in defining the mechanisms of essential hypertension, but their exact application is at present controversial. For example, some authorities believe that the renin status of any patient with essential hypertension is an important factor in choosing the most effective therapy. This proposition requires further testing and in order to do this one needs to perform hormone measurements on substantial numbers of patients prior to treatment or the development of complications.

Glucocorticoid-Remediable Mineralocorticoid Hypertension

J. R. Stockigt, E. J. Higgs, E. R. Cukier, I. Ekkel.

An uncommon type of mineralocorticoid hypertension, which is completely correctable by suppressive doses of glucocorticoid, has been described in several previous reports. The condition resembles primary aldosteronism in all basic features with hypokalaemia, elevated aldosterone and suppressed renin and can currently only be distinguished by therapeutic trial of glucocorticoid. A 17-year-old male, who initially presented with a blood pressure of 230/120, has been studied since June 1974 in collaboration with B. A. Scoggins, J. P. Coghlan and C. J. Oddie of the Howard Florey Institute. His hypertension can be maintained in complete remission with Dexamethasone 0.75 mg daily and our findings indicate that aldosterone is abnormally regulated in a way which is unique to this condition, being completely suppressible with glucocorticoid and inversely related to renin and potassium, while being non-suppressible with exogenous mineralocorticoid. Recent acute studies of aldosterone regulation suggest that aldosterone is also unresponsive to sodium restriction and potassium infusion. Low dose ACTH infusion has been given to compare the cortisol and aldosterone threshold responses; the results do not indicate abnormal sensitivity of aldosterone to ACTH. Basal levels of aldosterone have not been sufficiently increased to alone account for the degree of mineralocorticoid excess, suggesting that an additional mineralocorticoid may be present. While no other specific steroid is in excess, urinary metabolites show excess 17 hydroxy corticosteroids and androsterone suggesting a novel abnormality of adrenal steroid biosynthesis. During progressive reduction of Dexamethasone to 0.25 mg daily there is further presumptive evidence of an abnormal steroid, because aldosterone and deoxycorticosterone return to pre-treatment levels without recurrence of hypertension and hypokalaemia.

Current studies are aimed at correlating total plasma mineralocorticoid activity with levels of known mineralocorticoids and at further fractionation of abnormal metabolites. The identification of a novel ACTH-dependent mineralocorticoid would be of great interest in the further delineation of low renin hypertension.

Diazoxide-induced Stimulation of Renin Release in Renal Vein Renin Sampling

J. R. Stockigt, E. J. Higgs, N. Sacharias-Saarelinn*

Renal vein renin sampling can define a unilateral renal abnormality causally-related to hypertension by demonstrating predominant renin secretion from the affected side, with suppression of contralateral secretion, as shown by comparable renin levels in caudal inferior vena cava and contralateral renal vein. However, basal recumbent samples may give inconclusive results if renin secretion is low at the time of sampling. Renal vein renin samples were collected in the basal state and 8-10 minutes after I.V. bolus injection of 150-300 mg Diazoxide in 41 patients with suspected renal hypertension. Plasma renin activity was measured by radioimmunoassay of Angiotension I. In 25 studies which showed no lateralization, Diazoxide raised the mean peripheral renin to 198% of control, while the renal vein renin ratio showed no significant change (1.16 to 1.19, $p > 0.5$). In 11 of these negative studies the gradient between the renal vein samples and peripheral renin in basal samples was insufficient to account for the peripheral level at the time of sampling, but active symmetrical renin secretion was demonstrated after Diazoxide. In 16 studies which showed lateralization, Diazoxide raised the mean peripheral renin to 256% of control and the mean renal vein renin ratio increased from 1.89 to 3.58 ($p > .05$). In 4 of these 16 cases definite lateralization of renin secretion was

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apparent only after Diazoxide; three of these 4 cases had been treated with Propranolol just prior to study. The ratio of contralateral renal vein to peripheral renin showed no change following Diazoxide (1.06 before, 1.07 after) indicating a persistence of contralateral renin suppression after stimulation with Diazoxide. These findings suggest that Diazoxide stimulates symmetrical secretion of renin in patients without a unilateral renin source, while increasing the asymmetry of secretion in those with a significant unilateral renal abnormality. An acute pharmacological stimulus to renin secretion is simpler than low sodium diet or upright posture and may be especially useful in patients in whom a significant lateralization may be missed because antihypertensive therapy cannot be safely stopped.

Adrenal scanning with C19 ^{131}I Cholesterol

L. M. Dugdale* and J. R. Stockigt

Cholesterol labelled with ^{131}I at the C19 position is now available for adrenal scanning and has been used in 2 mCi doses in 11 patients with suspected primary aldosteronism or Cushing's syndrome, to compare the function of the two adrenal glands. It has been widely presumed that ^{131}I Cholesterol functions as a steroid precursor and that adrenal uptake may reflect steroid biosynthesis. Our findings suggest that this is not so for three reasons:

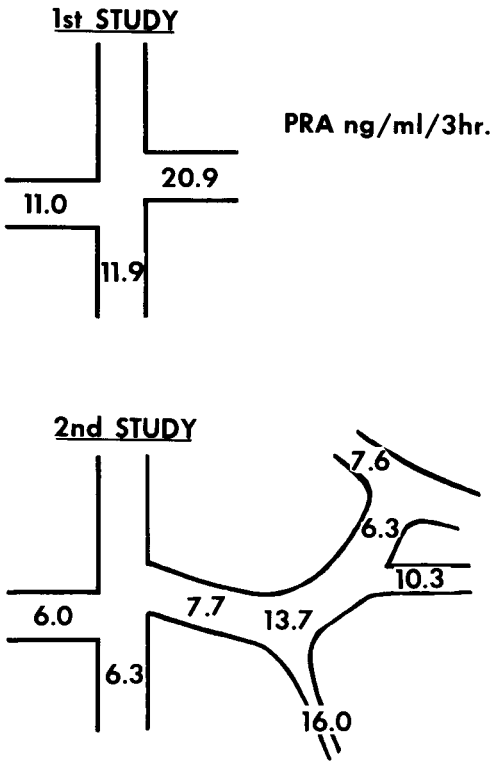

Fig. 2. Initial common renal vein study and subsequent selective study in a patient with localized ischaemia of the lower pole of the left kidney. Left lower partial nephrectomy has corrected hypertension from 160/110 to 130/80. PRA = Plasma Renin Activity.

(1) In primary aldosteronism two separate doses given before and during Dexamethasone 2 mg daily showed a minimal alteration in the adrenal images 7-10 days after dosage, suggesting that suppression of glucocorticoid production had little effect on isotope uptake or distribution.
(2) Examination of excised adrenal tissue in primary aldosteronism shows no significant difference in isotope accumulation between adenoma, normal cortex and medullary tissue.
(3) In a case of Cushing's syndrome due to bilateral adrenal hyperplasia, 0.26% of the dose was seen in the right adrenal and 0.11% in the left adrenal. Adrenal exploration showed a haematoma in the right adrenal gland.

These findings suggest that scanning with C19 ^{131}I Cholesterol does not accurately reflect steroid biosynthesis. Rather, the technique demonstrates adrenal configuration and must be interpreted with knowledge of this limitation.

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Segmental Renal Vein Renin and Partial Nephrectomy for localized Renal Ischaemia causing Hypertension

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Localized renal ischaemia can cause hypertension and is characteristically associated with predominant renin secretion from the ischaemic zone with suppression of renin secretion in normal renal tissue. Ischaemnia usually affects one whole kidney, as in main vessel renal artery stenosis, but segmental ischaemia may also cause hypertension. The localized high renin effluent from segmental ischaemia may be missed with common renal vein sampling because the low volume of high renin effluent is diluted by blood from normal tissue. The technique of segmental vein sampling has been developed for such cases. The demonstration of a localized renin source in the lower pole of the left kidney has been followed by correction of hypertension by partial nephrectomy in a 16-year-old youth who developed gradual onset of persistent hypertension (160/110) after traumatic partial rupture of the left kidney. Intravenous pyelography showed a non-functioning lower pole, but major vessels were patent on arteriography. Segmental renal vein renin sampling showed that renin secretion was confined to the left lower pole, with suppression of secretion from right kidney and left upper pole (Fig. 2). Excision of 45 gram of tissue from the left lower pole has resulted in a blood pressure of 125/80 one year after surgery. This finding suggests that segmental sampling can be used to define the role of partial nephrectomy for segmental renal ischaemia.

Radioimmunoassay for Plasma Aldosterone: Affinity Purification

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Although specific antibodies are now available for aldosterone radioimmunoassay, most plasma methods require preliminary purification to remove the 1000-fold excess of cortisol which is a significant cross reactant even with the most specific antibodies. A novel purification system has been developed using aldosterone antibody linked to Sepharose. Aldosterone antibody is first purified in high yield from the serum of immunized rabbits using aldosterone hemisuccinate coupled to Sepharose. The purified antibody is then coupled to activated Sepharose and is used in small columns. Unextracted plasma (in which cortisol remains predominantly protein-bound) is then

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cycled through the column, the column washed with water, and the aldosterone eluted with acid and extracted for assay. Recovery of aldosterone is 60-80% and cortisol less than 1%. Preliminary results show good correlation with samples assayed by double isotope dilution. The method is rapid and may become straightforward. Further applications of this type of technique are attractive because it allows sensitive measurement in a non-specific system after specific preliminary purification.

Epidemiology of Diabetes Mellitus — Prevalence Survey on a Pacific Island

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Population studies of the incidence and distribution within populations and the cause of disease — epidemiology — are being used increasingly in attempts to understand more of the aetiology of diabetes and of the causes of its complications.

Previous epidemiological studies have demonstrated a high diabetes prevalence rate (4 to 9%) in Polynesians who have moved from a native environment to an urban society, e.g. in Honolulu and Auckland such Polynesian communities have been identified in which the prevalence figures are as high as seven times that for a comparable Caucasian population in those cities.

The island of Nauru provides a unique opportunity to study a Polynesian society which has become urbanized (or westernized) in its own environment. Nauru is a small island situated 3,000 miles north-east of Melbourne, and 26 miles south of the equator. The Nauruan population is 3,500 and the people have the highest per capita income in the world.

In May 1975, a prevalence survey was undertaken on 15% of the population aged 15 years and over. The prevalence of diabetes (as defined by a 2-hour plasma glucose level of 150 mgm% or more after 75 Gm glucose orally) was 35%. This is equal to the highest incidence this far recorded — that of the Pima Indians in Phoenix, Arizona. The study has also incorporated analysis of weight, height, blood pressure and skin fold thickness. Additionally, measurements of urea, creatinine, uric acid, cholesterol, triglycerides, liver function tests, plasma insulin, organ specific antibodies and HL-A typing have been made for later correlations relating to aetiology and complications of diabetes. From a clinical point of view, it was noted that the diabetes is almost entirely maturity-onset in type, keto-acidosis not occurring. There is early onset and rapid progression of diabetic microvascular complications in the face of absent or negligible control of diabetes. Noteworthy is the low incidence of large vessel disease (e.g. coronary, peripheral arteries) amongst Nauruan diabetics.

Plans have been made to proceed with more detailed clinical and biochemical documentation of the newly discovered diabetics to prepare for prospective studies relating to diabetic complications, and the low incidence of large vessel disease.

Apart from this, diabetes presents a major current and future health problem in Nauru, and by inference in other urbanized Pacific Islands. As the result of these studies it is hoped that programmes of education and plans for treatment might be devised using conventional methods modified to local use so that this public health problem might be managed.

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