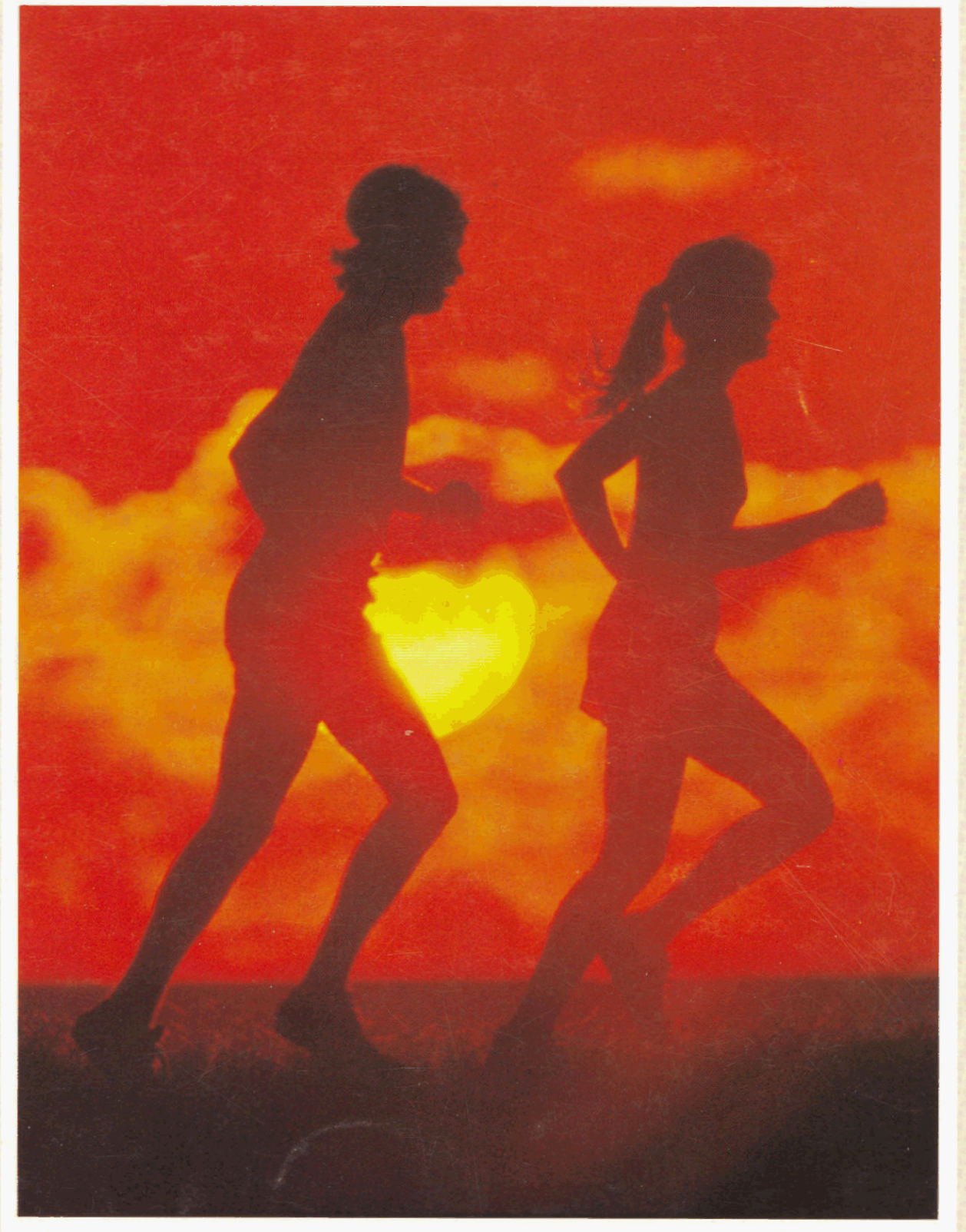
Annual Report 1987/88

Baker Medical Research Institute



Heart Research — The Quest for Life

Baker Medical Research Institute
Commercial Road
P.O. Box 348
Prahran, Victoria 3181
Australia
Telephone: (03) 522 4333
Telex: ALFHOSP AA 31371
Fax No. (03) 521 1362

Annual Report 1987/88

Baker Medical Research Institute

Affiliated with Alfred Hospital
and Monash University

Eighth Annual Report of the Baker Medical Research Institute

Thirty-ninth Annual Report of the Clinical Research Unit, Alfred Hospital

How to support medical research at the Baker Medical Research Institute

The Baker Medical Research Institute is funded from many sources including Federal and State Governments, the Baker Benefactions, investment income, research grants, corporate sponsorship and private gifts.

The field of research in which the Institute is engaged touches the lives of the entire Australian community. If research into heart disease is to succeed it will not be because of the efforts of one sector of the community, either scientists, governments, companies or individuals, but by the combined effort of all Australians in the alleviation of a common problem and the achievement of a common goal.

The Institute needs your help to finance its research.

There are many ways in which you can help. These include making annual or more frequent gifts; placing capital in our account on which the Institute receives interest (but not capital); making provision in your will; making a donation in lieu of a birthday gift or in memory of a loved one.

Donations are tax deductible

For further information contact:-
Public Relations Officer
Baker Medical Research Institute
P.O. Box 348
Prahran Victoria, 3181
Telephone (03) 522 4333

Persons contemplating bequests are most welcome to visit the Institute by appointment. Advice from an Accountant or a Solicitor is always advisable, but the following is a suggested form of a bequest in Wills.

"I ..... bequeath to the Baker Medical Research Institute, Commercial Road, Prahran, in the State of Victoria, to advance the work of the Institute the sum of $...../..... per cent of my estate/the residue of my estate, free of all duties for which the written acknowledgement of the Associate Director shall be sufficient discharge."

Our Target

In Australia 50% of all deaths and serious illness are due to diseases of the heart and circulation.

Most of them are due to hypertension (high blood pressure) and atherosclerosis (clogging up of arteries with fatty cholesterol-laden plaques) which cause stroke, heart attack, heart failure and kidney failure.

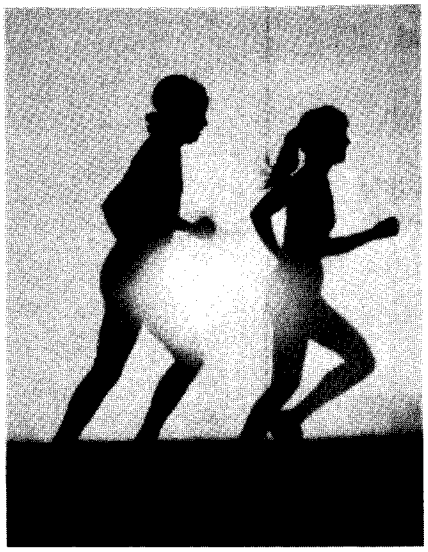
Aims of our Research

To increase understanding of the basic causes of hypertension and atherosclerosis.

To use this knowledge to improve medical and surgical treatment.

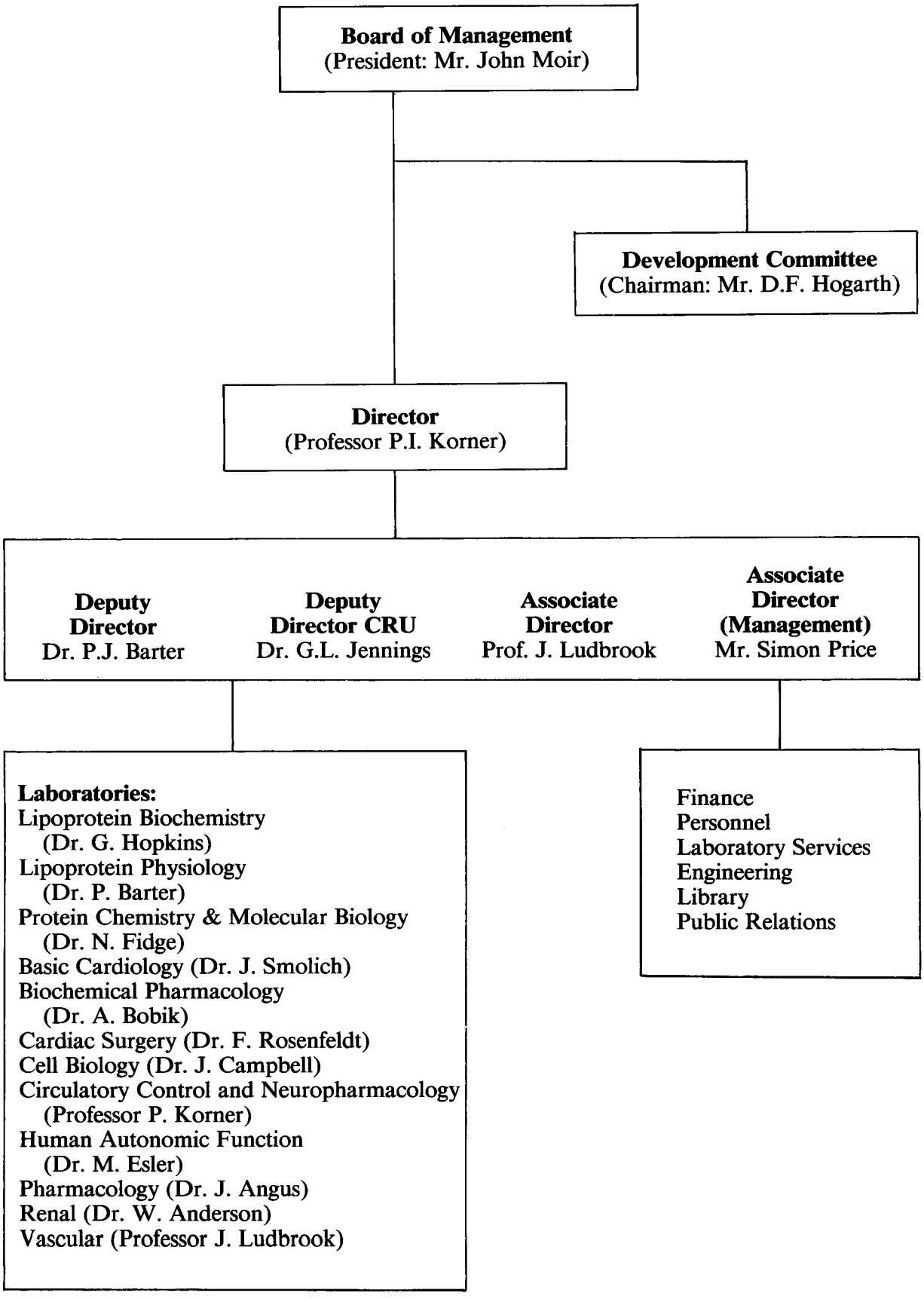
To prevent heart and vascular disease in the community particularly in the younger age groups.

Contents



Heart Research – The Quest for Life

Organisation Chart



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D.F. HOGARTH, B.Sc.

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D. WITTNER

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D.F. HOGARTH, B.Sc.

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J.C. HABERSBERGER, A.O., B.Comm.
Professor P.I. KORNER, M.D., B.S., M.Sc. (Syd), Hon. D.Sc. (NSW), F.R.A.C.P., F.A.A.
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W.D. McPHERSON, A.O., A.A.S.A.
W.G. PHILIP, A.M., B.Comm., F.C.A.
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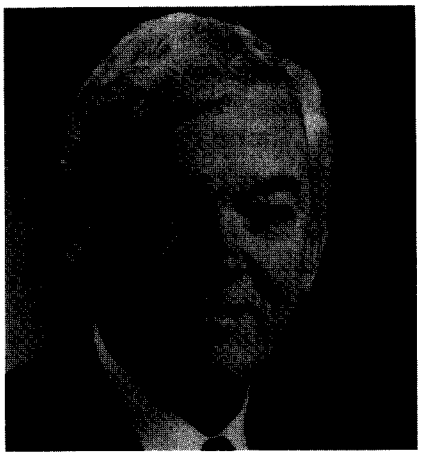


***Mr. J.D. Moir** President, is a Consultant to the legal firm Minter Ellison.*

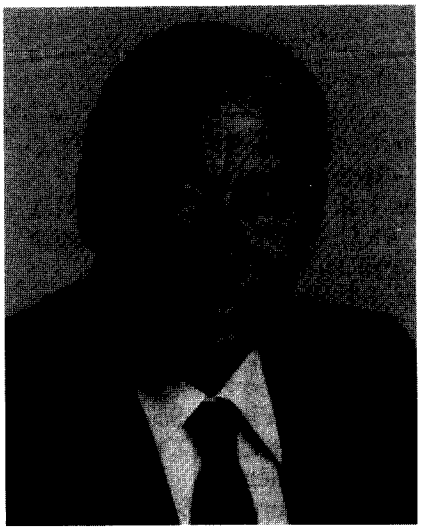
***Mr. D.F. Hogarth** Vice-President, is Chairman of Directors of Kodak (Australasia) Pty. Ltd.*

***Dr. T.J. Wood** Secretary to the Board, is Chief Executive Officer of the Alfred Hospital.*

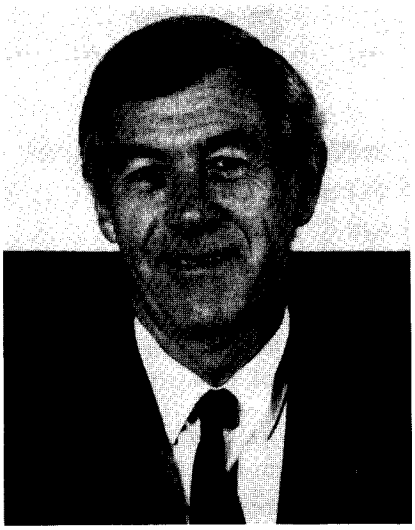
***Mr. W.J. Bailey** Honorary Treasurer, is Managing Director of the ANZ Banking Group.*

***Mr. R.J. Barcham** is a Company Director.*

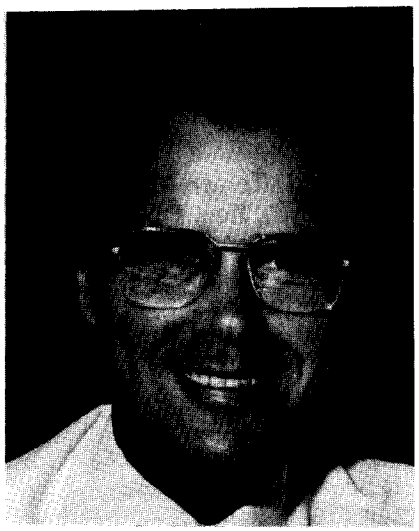
***Professor P.I. Korner** is Director of the Baker Medical Research Institute.*



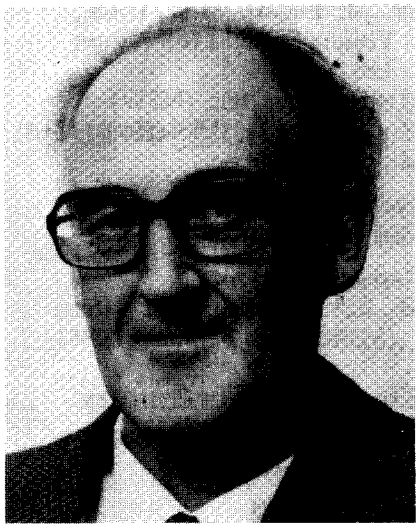
Mr. W.D. McPherson *is Chairman of the Chamber of Manufactures Insurance Ltd. and a Director of BHP Ltd.*



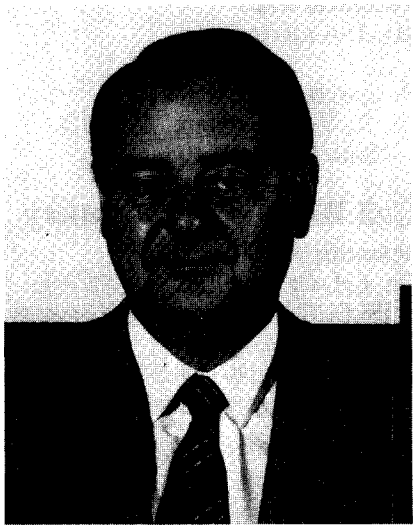
Mr. W.G. Philip *partner in Price Waterhouse, Vice-President of the Amalgamated Alfred, Caulfield and Royal Southern Memorial Hospital.*



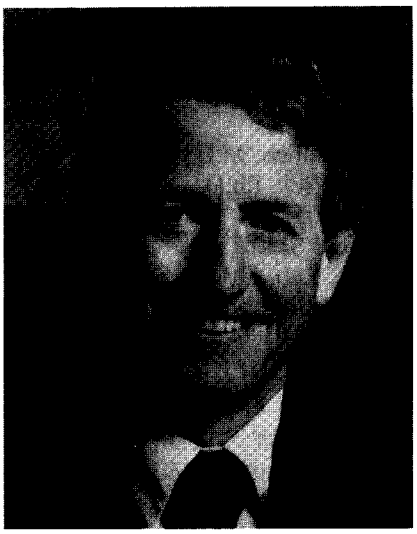
Professor G.B. Ryan *is Dean of the Faculty of Medicine, University of Melbourne.*



Professor G.C. Schofield *is Dean of the Faculty of Medicine of Monash University.*



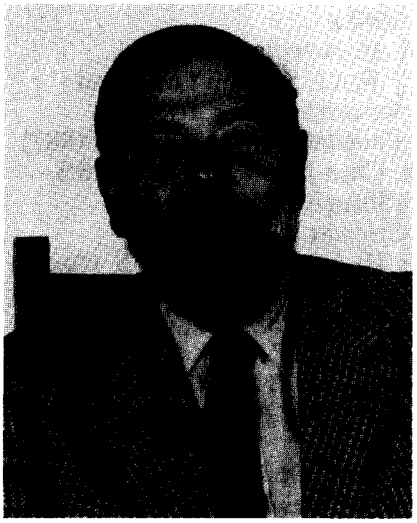
Mr. D. Wittner *Chairman and Managing Director, Wittners Australia Ltd.*



Sir Laurence Muir *was senior partner of Potter Partners until 1980. He is a Member of the Federal Parliament House Construction Authority and a member of the Board of Australian and International Companies.*

Mr. J.C. Habersberger *is a Past President of the Institute and of the Alfred Hospital. Until his retirement in 1976, he was Managing Director of Kodak (Australasia) Pty. Ltd.*

Mrs. F.S. (Joan) Grimwade *O.B.E.*



Professor John Funder *Deputy Director, Medical Research Centre, Prince Henry's Hospital.*

Staff

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Professor P.I. Korner, M.D., B.S., M.Sc. (Syd), Hon. D.Sc. (NSW), F.R.A.C.P., F.A.A.

Deputy Director
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Associate Director (Management)
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Dr. M. Newman, M.B., B.S., F.R.A.C.S., John Lowenthal Research Fellow, RACS Foundation (till December 1987)
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Ms. K. O'Halloran (from March 1988)

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Ms. S. Janevski, B.Sc. (from January 1988)

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Ms. R. Rennick, B.Sc. (Hons)
Ms. S. Horrigan, B.Sc. (Hons)
Ms. G. Cockerill, B.Sc. (Hons)

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Dr. M. Van den Buuse, Ph.D. (Utrecht), Visiting Scientist
Dr. W.A. Wolf, Ph.D. (GWU), Visiting Scientist
Dr. C.A. Courneya, B.Sc. (Guelph), M.Sc. (UWO), Ph.D. (UBC), Visiting Scientist (from September 1987)
Dr. J-L. Elghozi, M.D., D.Bh., Visiting Scientist (till August 1987)
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Mrs. S.L. Burke, B.Sc. (Hons) (Syd)
Mr. C. Rudd, B.Sc.

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Miss S. Godwin

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Ms. S.E. Luff, B.Sc. (Hons) (Hull), M.Sc. (Birm)
Ms. K. Moser, B.Sc. (ANU) (from August 1987)

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Dr. J. Reid, Ph.D. (joint appointment with Pharmacology) (till February 1988)

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Dr. I. Meredith, B.Sc. (Hons), M.B., B.S.

Visiting Scientist
Dr. P. Friberg, M.D., Ph.D. (from July 1987)

Collaborating Scientists
Dr. F. Dudley, F.R.A.C.P., Director, Gastroenterology Service, Alfred Hospital
Dr. M. Horne, F.R.A.C.P., Director, Clinical Neurophysiology, Alfred Hospital
Dr. D. Copolov, F.R.A.C.P., R.A.N.Z.C.P., Director, Mental Health Research Institute of Victoria

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Dr. C. Ehnholm, M.D., Visiting Scientist (from September 1987)
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Dr. B. Grego, B.Sc. (Hons), Ph.D. (Otago)
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Dr. A. Mitchell, B.Sc. (Hons) (Melb), Ph.D. (from September 1987)
Dr. O.V. Rajaram, B.Sc. (Hons) M.Sc. (Bombay), Ph.D. (Flind)
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Mr. T. Tetaz, B.Sc., M.Sc. (from April 1987)
Dr. M. Tozuka, Ph.D. (Shinshu), Visiting Scientist

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Ms. S. Devlin, B.Sc. (Mon) (from March 1987)
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Mr. E. Gruner, B.Sc.
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Ms. S. Unnithan (from April 1987)
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Ms. D. Mathai, B.Sc. (from February 1988)
Ms. B. Meyer, B.Sc. (Hons) (from February 1988)
Mr. J. Morrison, B.Sc. (Hons)
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Mr. P. Vadiveloo, B.Sc. (Hons)

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Dr. C.E. Wright, B.Sc., Ph.D. (Mon), Research Officer (till January 1988)
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Ms. S. Rainone, B.Sc. (RMIT) (till April 1988)
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Ms. M. Dib (from January 1988)

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Dr. D. Alcorn, University of Melbourne
Prof. G. Ryan, University of Melbourne

Risk Reduction Clinic

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Sr. S. Kay, S.R.N.
Sr. L. Watson, S.R.N.
Sr. M. Tress, S.R.N.
Sr. D. Wilson, S.R.N.
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Mrs. J. Broughton (from August 1987)
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Mrs. J. Segal, B.A., M.A., C.B.A. (from July 1987)
Ms. B. Smith

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Mrs. C. Chan, B.App.Sc., Grad.Dip. Secretarial Studies (from May 1987)
Ms. D. Glanz
Ms. L. Henderson (from August 1987)
Mrs. M. Mijak
Mrs. J. Hoskins, B.Sc.
Mr. D. Espinoza

Computer Programmer
Mr. M. Creek, B.Sc. (Hons), B.App.Sc. (Dist), Dip.Ed. (till March 1988)

Photographer
Mr. T. Zylstra, B.Sc.

Theatre
Ms. A. Hulse

The Rouse Family Library
Mrs. T. Morton, B.Sc. (Hons), M.A. (Lib)
Dr. F.A. Cribbin, B.Sc., Ph.D. (December 1987 to May 1988)

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Mr. C.E. Lewis
Mr. A. Ali
Ms. J. Pritchard

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Dr. R. Stanley, B.Vet.Sc. (Hons), Veterinarian
Mr. A. Bons, Senior Technical Officer
Mr. E. Turnbull, Technical Officer

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Ms. S. Morley
Ms. J. Gmehling
Mr. D. Murray (from August 1987)
Ms. E. Langskaill (from December 1987)
Mr. N. Bell (November 1987 to March 1988)
Mr. C. Lekos (from February 1988)

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Mr. K.R. Harvey, B.E. (till September 1987)
Mr. C.G. Lawson
Mr. D. Bell (till September 1987)
Mr. A. O'Keefe (till August 1987)
Mr. G. Northern (till November 1987)
Mr. J. Kirkas
Mr. I. Taylor, Dip.E.E. (from November 1987)

Alfred Hospital — Baker Institute

Clinical Research Unit

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Professor P.I. Korner, M.D., B.S., M.Sc. (Syd), Hon. D.Sc. (NSW), F.R.A.C.P., F.A.A.

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Deputy Director
Dr. G.L. Jennings, M.D., B.S., F.R.C.P., F.R.A.C.P.

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Dr. P.A. Blombery, M.B., B.S., B.Sc. (Med), Ph.D., F.R.A.C.P., Vascular Physician, Senior Associate
Dr. A. Broughton, M.B., B.S., Ph.D., F.R.A.C.P., Assistant Physician
Dr. P. Friberg, M.D., Ph.D., Honorary Assistant Physician
Dr. P.J. Jenkins, M.B., B.S., F.R.A.C.P., Senior Associate
Dr. E. Laufer, M.B., B.S., F.R.A.C.P., Senior Associate
Dr. A. Lim, M.B., B.S., F.R.A.C.P., Assistant Physician
Dr. I.T. Meredith, M.B., B.S., B.Sc. (Hons), Assistant Physician
Dr. H. Newnham, M.B., B.S., F.R.A.C.P., Honorary Assistant Physician
Dr. K.K. Sudhir, M.B., B.S., Honorary Assistant Physician

Attending Physicians
Dr. P.A. Blombery
Dr. M. Esler
Dr. G. Jennings

Biochemical Pharmacology Laboratory

Dr. A. Bobik, Ph.D. (Syd), M.Sc., B.Pharm., Associate Director (Laboratories)
Dr. M.A. Adams, M.Sc. (UWO), Ph.D. (UWO), Post doctoral fellow (till April 1988)
Dr. G.J. Jackman, Ph.D. (Lond), B.Sc. A.R.A.C.I., A.R.C.S.
Dr. P.J. Little, Ph.D. (Syd), M.Sc., B.Pharm.
Dr. C.B. Neylon, B.Sc. (Hons), Ph.D., Post doctoral research fellow (from January 1988)
Dr. P.L. Weissberg, M.B., B.S., M.R.C.P. (UK), Post doctoral fellow (till September 1987)

Dietitians
Ms. V. Fazio
Ms. H. Pluck

Registrars
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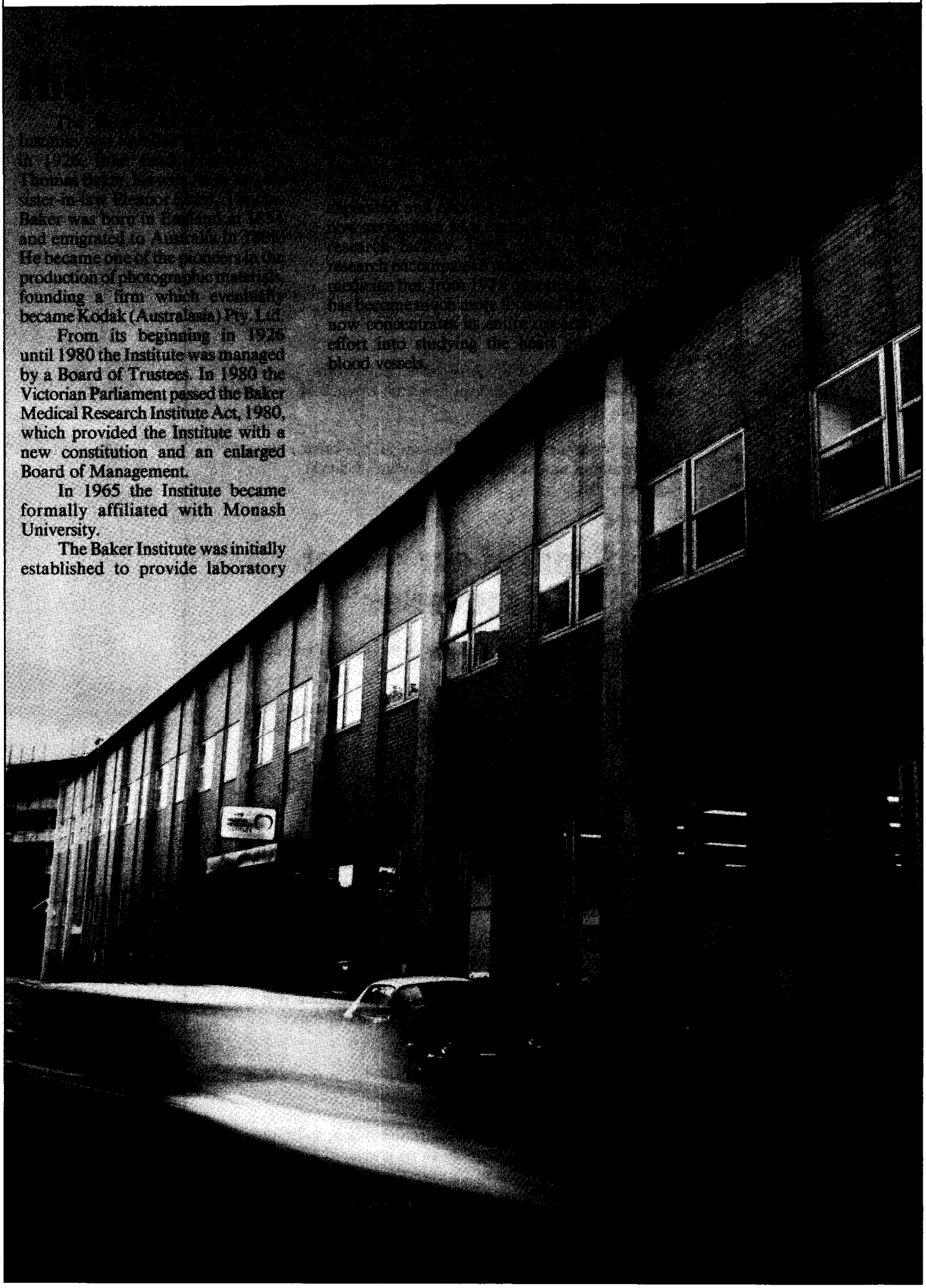
Dr. H. Debinski, M.B., B.S.
Dr. F. Thien, M.B., B.S.
Dr. M. Tuck, M.B., B.S.
Dr. B. Hickey, M.B., B.S.

Scientific and Technical Staff
Ms. E. Dewar, B.Sc.
Mrs. M. Frigo, B.Sc. (till January 1988)
Mrs. S.A. Grinpukel, M.Sc.
Ms. A.N. Groomes, B.Sc.
Mr. G. Mill, B.Sc. (Hons)
Ms. L. Nelson, B.Sc.
Ms. C.J. Oddie, M.Sc.
Ms. L. Russell, S.E.N. (till November 1987)
Ms. P.J. Scott, B.App.Sc. (App Biol)
Ms. S.J. Timmins, B.Sc. (till March 1988)
Sr. S. Scealy, S.R.N.
Sr. A. Bruce, S.R.N.
Sr. D. Carter, S.R.N. (November 1987 to March 1988)

Interns
Dr. S. Lim, M.B., B.S.
Dr. C. Thevathasan, M.B., B.S.
Dr. A. Bennett, M.B., B.S.
Dr. H. Tang, M.B., B.S.

Secretary
Ms. C. Harwood



From its beginning in 1926 until 1980 the Institute was managed by a Board of Trustees. In 1980 the Victorian Parliament passed the Baker Medical Research Institute Act, 1980, which provided the Institute with a new constitution and an enlarged Board of Management.

In 1965 the Institute became formally affiliated with Monash University.

The Baker Institute was initially established to provide laboratory

The Baker Institute at a glance

1. Founded in 1926, through funds provided by Thomas Baker, Alice Baker and Eleanor Shaw.

2. It is Australia's chief research centre working on diseases of the heart and blood vessels.

3. It is an independent research institute with its affairs run by a Board of Management. It is affiliated with Alfred Hospital and Monash University.

4. It is a recipient of a Block Institute Grant from the National Health & Medical Research Council of Australia.

5. It has a staff of 154 people. The research is carried out in 12 laboratories and in the Alfred Hospital's Clinical Research Unit.

Some of our recent discoveries

1. A probable cause and treatment of the spasm that occurs during coronary bypass surgery.

2. That early treatment of genetic hypertension can prevent the otherwise inevitable enlargement of the heart and blood vessels.

3. Identification of the processes dependent on calcium which regulate the movement of sodium into the cells lining blood vessels.

4. That an enzyme released by cells in the arterial wall may be a significant initiating event in the development of atherosclerosis.

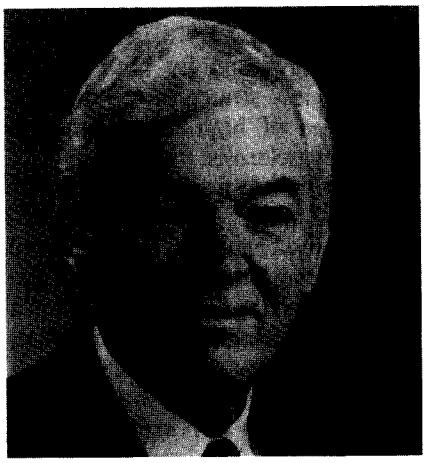
5. That conventional drug treatment of high blood pressure may adversely affect other risk factors.

6. Identification and purification of two cell proteins that bind high density lipoproteins, the fraction that protects against coronary heart disease.

7. Definition of the differing functions of two enzymes that break down plasma triglyceride.

8. That noradrenaline, a brain neurotransmitter, spills into veins leaving the brain where it can be measured to provide one index of brain function.

President's Report



It is a great privilege to address you for the first time as your President. It is not an easy task to follow in the footsteps of Sir Laurence Muir, but I will do my very best as will Don Hogarth, who has become our Vice President. The Institute will strive as always to remain in the vanguard of cardiovascular research and our Board, in the management of the Institute, will do its utmost to continue support of our scientists in achieving this aim.

You will be pleased to know that Sir Laurence remains with us, as the Institute's first Patron and has assured me that he will continue to labour hard on our behalf. The Board has undergone great changes during the year. We welcome our new Board members Mrs. Joan Grimwade, Professor John Funder, Mr. Bill Philip and Mr. David Wittner. They will undoubtedly serve the Institute with distinction.

This is also the occasion to note the retirements of the two longest serving members of the Board, Professor R.R. Andrew and Dr. H.B. Kay. Professor Andrew was a research worker at the Institute in 1947 and became a Trustee in 1960 and in 1980 became a founding member of the present Board. He served as Vice President from 1981-3. His interest in the Institute remains strong and he continues as Deputy Editor of the Baker Institute News and our Honorary Archivist.

Dr. H.B. Kay's association also began in 1947 and he became a Trustee in 1975 and a founding member of our board in 1980. He was of course also most active on the Board of Alfred Hospital and has been a great source of strength to our Clinical Research Unit.

We also said farewell to Dr. Trevor Wood, who retired as Secretary of the Board and has taken up an appointment as Medical Superintendent at Townsville Hospital in Queensland.

The Institute's basic scientific activities remain strong. Following the establishment of the Protein Chemistry Laboratory in 1986, a new Molecular Biology Laboratory is now functioning. Its main work has so far been in collaborations relating to atherosclerosis. It will have much to contribute to other projects in the future.

In April we signed a collaborative agreement with Glaxo, one of the largest pharmaceutical companies in the world. Glaxo will support the work of Dr. James Angus' Pharmacology Laboratory by providing a minimum of $750,000 over a period of five years.

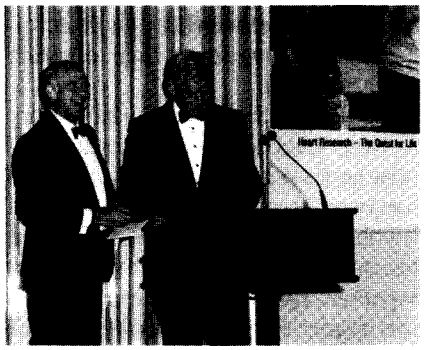
We hope to develop similar associations in the future as we are well placed to provide valuable expertise in solving problems of interest to the pharmaceutical industry grappling, as it is, with the production of satisfactory products relative to the prevention and cure and the relief from the suffering of cardiovascular diseases.

Our capacity to attract overseas visiting scientists remains high and we have been most fortunate to have up to 20 visitors collaborating with us over the past 12 months, many with their own funding for periods of up to 2 years.

I am happy to report the completion of the Rotary Club of Melbourne Heart Risk Reduction Clinic at the Institute. This has been developed through the generosity and hard work of the Rotary Club of Melbourne. Through its close association with the basic science laboratories of the Institute it will become a unique type of community health laboratory. One of its objectives will be to provide good diagnostic assessment of the risk of having heart and vascular disease and to work out the best way of reducing this risk. Needless to say this should be done without loss of the enjoyment of life. Our more ambitious long term objective will be to see to what degree high blood pressure and atherosclerosis can be prevented, by focussing on the children of those who have the disorder. Animal work done here suggests this may be possible and we hope that this will one day be translated into a new preventive strategy for man.

As always I want to thank all the Institute staff for their tremendous contribution. Professor Korner has had a complicated year performing his normal work here, as well as serving as President of the new Amalgamated Alfred, Caulfield and Royal Southern Memorial Hospital. It is an important task, since in the increasingly complex task of delivering health care with limited resources, medical research should not be regarded as a luxury but as a necessity. Hence, there is a tremendous need for increased funding from both the government and the private sectors to increase the impact of medical research on patient care.

Last, I should like to thank our various supporters, both those who have been loyal to us for many years and those who have just joined the team. In particular I would like to acknowledge the substantial and most generous increases in contributions from the NH & MRC and the Baker Benefactions. I also thank other supporters including the Victorian State Government, trusts, foundations and companies and private donors who are listed later in this Annual Report.



Tony Charlton and John Moir at the Annual Dinner at the Hyatt on Collins.

Director's Report and Overview

In the 1987 quinquennial review of the Institute's work and future programme by the National Health & Medical Research Council (N.H. & M.R.C.), we received fulsome praise and a modest 20% increase in the level of our Block Institute Grant. It has not taken long to come back to earth and face the future with some anxiety, lest our science should become too constrained by problems of funding. It is not entirely unlike 'Alice Through the Looking Glass', where the Red Queen says: *"Now, here, you see it takes all the running you can do to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that!"*

During the year we established a molecular biology laboratory and completed the Heart Risk Reduction Clinic. Both will be invaluable in our quest to determine the causes of hypertension (high blood pressure) and atherosclerosis (fatty cholesterol-containing plaques, which obstruct large arteries).

The molecular biology laboratory is long overdue. The questions of how genes regulate cellular processes has been responsible for the greatest revolution in biological thinking and has brought diverse fields much closer together. Quite often the body uses the same chemical molecules or membrane receptors to control different body functions. For example, progress in the cancer field may give new insights into how blood vessels grow and develop, whilst advances in basic neurochemistry and neurophysiology may provide us with clues about blood pressure regulation, but at the same time throw light on the regulation of behaviour. The Baker Institute owes much of its international scientific standing to our capacity to unravel how complex regulating systems work and the introduction of the tools of molecular genetics and protein and peptide chemistry could not have come at a more opportune time.

The Heart Risk Reduction Clinic will provide a new type of community health laboratory. At one level of its operation it will be like clinics elsewhere: the potential susceptibility of an individual to develop cardiovascular disease will be assessed, by measuring blood pressure, plasma cholesterol and triglycerides, body weight and noting the person's family history and life style. However, from the viewpoint of community health research it has two main objectives: (1) to work out the best treatment to reduce the existing risk factors in a given individual by drugs and life style changes and by both; (2) to develop new strategies for primary prevention of high blood pressure and atherosclerosis.

The first objective will be met through a novel type of 'clinical trial' that uses fewer subjects than the large scale epidemiological studies of the past. This will be offset by the use of a range of physiological and biochemical methods to assess the degree of reduction of risk factors much more accurately than is possible in large scale studies. We can determine whether the treatment has reversed heart muscle thickening in hypertension, or whether the size of an obstructive plaque has been reduced.

The second objective is to improve the diagnosis and detection of the genetic component of risk in young individuals with a family history of cardiovascular disease. Some of our laboratory studies encourage us to believe that primary prevention in hypertension may be possible by preventing some of the early structural changes.

Prof. Paul Korner accepting a cheque for $5,000 for the Risk Reduction Clinic, from the President of South Melbourne Rotary.

Almost every Australian visitor to the Institute asks about the criteria by which we judge the Institute's international standing. They include invitations to prestigious international scientific meetings and excellent 'ratings' of our scientific publications in various bibliometric indices. Our current record holders for citations are James Angus and Tom Cocks, whose work on the pharmacology and physiology of EDRF (endothelium-derived relaxing factor) published in Nature about two years ago has been the most widely quoted paper. However, all our other major projects have also received a great deal of interest and attention.

Probably the best indicator of our standing is the number of visiting scientists who elect to come to the Institute for study leave or for post-doctoral training. Over the last 10 years we have had 77 such visitors who have worked here from periods of several months up to 2-3 years. They have come from Canada, China, Denmark, Finland, France, India, Israel, Japan, the Netherlands, New Zealand, Sweden, Thailand, the U.K. and the U.S.A. They have contributed greatly to our work and have, I am sure, also learned something from us. One of the truly wonderful features of international science is the establishment of networks of scientific information exchange and collaboration that last for many years.

Some Scientific Highlights

One of the most spectacular advances we have made has been in our work on the causes of hypertension. Through a collaborative programme that has at times involved about 80% of the laboratories in the Institute, it has become clear that the cause of hypertension is excessive growth of the muscles of the blood vessels, which occurs early in development. This was originally suggested by the clinical studies of Garry Jennings and colleagues and this hypothesis has been confirmed in spontaneously hypertensive rats (SHR) where the cause is genetically determined. In a study performed by Alex Bobik, Mike Adams and Peter Friberg we found that enlargement of the muscle cost is virtually present at birth, making the vessels liable to contract more vigorously than in animals with normal blood pressure. The most interesting finding of all — which is serving as the role model for the Heart Risk Reduction Clinic's programme on primary prevention of hypertension — is that if the early enlargement of the muscle cost is prevented by a brief period of treatment early in life, the SHR are practically cured of their hypertension for the rest of their life. The genetic stimulus to muscle growth seems to involve the sympathetic nervous system and probably several other growth factors.

In other areas of hypertension Drs. Jennings, Sudhir and Blombery have performed a collaborative study with Dr. Judith Whitworth of the Howard Florey Institute on the mechanism of steroid hypertension. Dr. Ian Meredith has determined how long it takes for regular exercise training to lower blood pressure (about two weeks). Drs. Eljas Laufer and Jennings and I, have developed a discriminant analysis technique for picking structural changes in the heart in patients with very mild hypertension. This is a technique using multiple variables and may be of value for our community health programme in picking individuals who will eventually develop hypertension.

The atherosclerosis programme has flourished tremendously and has, to date, been the chief beneficiary of the new resources in molecular genetics and protein and peptide chemistry. Drs. Philip Barter, Noel Fidge, Boris Grego, Garry Hopkins and their colleagues seek to know why individuals in whom there is an increased concentration of high density lipoproteins (HDL) are 'protected' from developing atherosclerosis. The group has isolated two proteins (i) the HDL binding protein and (ii) the HDL conversion protein, which play a role in regulating the size of the fat particles in the plasma. The smaller the particles the more dangerous they appear to be from the viewpoint of plaque formation.

In order to determine the exact functions of the lipid transfer protein and of the enzyme hepatic lipase which is also involved, the group is using 'transgenic' animals. This is a study involving Noel Fidge, Alana Mitchell and colleagues working in collaboration with Drs. Tim Adams and Mal Brandon of the Veterinary School of the University of Melbourne. Genes for the above proteins have been obtained from colleagues in the U.S.A. and will be incorporated into the eggs of rabbits and rats. The rabbit is normally deficient in hepatic lipase and is also extremely susceptible to develop atherosclerosis from a high dietary intake of cholesterol. We will test the hypothesis that the susceptibility will be reduced by the insertion of the gene which can manufacture the enzyme. Rats are normally deficient in lipid transfer protein and very resistant to atherosclerosis, and our hypothesis is that their susceptibility will become greater when the gene is introduced.

The regulating systems of the arterial wall also influence an individual's resistance to atherosclerosis. Julie and Gordon Campbell and their colleagues have been examining these regulating systems which cause the muscle cells in the wall to switch from their normal contractile state to one of increased metabolic activity, which is often associated with increased accumulation of cholesterol. The switching mechanism involves complex interactions between a number of growth factors in plasma, substances released from macrophages (scavenger cells, better known for their role in inflammation) and the matrix substance of the arterial wall.

The Biochemical Pharmacology group with Dr. Alex Bobik has been studying basic cellular processes that may link salt to the development of hypertension. They have been studying the transport mechanisms involved in the movement of sodium, hydrogen ions and potassium in and out of muscle cells and their role in the regulation of intracellular acidity, which is another potential growth factor that could stimulate muscle growth early in development.

The Pharmacology Laboratory under Dr. James Angus obtained major support for some of its programme from Glaxo, one of the world's major pharmaceutical companies (see President's Report). Their programme is focussed around the factors that determine the 'reactivity' of blood vessels and the heart. Their

analysis of EDRF, a local dilator hormone released from the endothelial cells lining the arterial wall, has been mentioned earlier. One major project performed in collaboration with Dr. Michael Mulvany from Denmark has been the demonstration that the sympathetic nerves to vessels release two transmitters simultaneously (i) noradrenaline that is coupled to produce muscular contraction; (ii) adenosine triphosphate that is coupled to produce electrical changes in the cell. One important project currently undertaken relates to the physiological and pharmacological properties of the vessels in human hypertension and in SHR in the Mulvany apparatus. The human studies are currently obtained from small skin biopsies.

Dr. Joe Smolich in the Basic Cardiology Laboratory is examining how coronary blood flow is regulated when the muscle has enlarged. This work shows that the heart becomes much more vulnerable than the normal heart, to brief periods of lowering the blood pressure. This is another good reason why reduction of the enlarged muscle mass is an important parameter in the treatment of hypertension, and not just reduction in the patient's blood pressure.

Several laboratories are working on the circulatory regulation by the autonomic nervous system. Geoff Head, Pat Dorward and I have studied the properties of baroreceptor reflexes in a genetic mutant strain of rabbits bred by Professor Marta Weinstock in Israel (who came to work with us on this project). In these rabbits the role of pressure influences from the heart can be easily assessed because they lead to the release of an endogenous opiate transmitter in the brain; the action of the latter can be reversed by naloxone. From other studies in rabbits we have become much clearer about the role of the brain's noradrenergic nerve cells in various reflexes. John Ludbrook has continued his analysis on the central opiate mechanisms that turn off the sympathetic nervous system in very severe haemorrhage. One speculation at present is that these might be the same mechanisms that lower sympathetic activity in man indulging in regular exercise.

Murray Esler continues to explore human autonomic function with his elegant technique. He has shown that in salt reduction in the diet sympathetic function increases predominantly in the kidney which, in turn, helps to conserve salt. His studies have also made it clear how close to the regulatory limit is sympathetic function in patients with heart failure.

In the Renal Laboratory, Warwick Anderson and Kate Denton have perfected the rabbit micropuncture techniques. Some of the predictions about the role of angiotensin II in renal hypertension have been verified. Robyn Woods continues to explore whether the 'heart hormone' atrial natriuretic peptide, which was cloned many years ago, really has a physiological function.

The Cardiac Surgery Research Unit under Frank Rosenfeldt is doing some 'developmental' research with a new type of laser designed to remove plaques inside coronary arteries without open heart surgery. An interesting basic project has been the demonstration in dogs that the nucleic acid precursor orotic acid makes the heart after myocardial infarction much less vulnerable to injury during open heart surgery. This regime has been used in patients, so far to good effect.

Is Australia spending enough on medical research?

Australia's annual expenditure on health care is currently $16.4 billion, which is 8% of G.D.P., or $1,000 p.a. for every man, woman and child. Health care is an enormous business and it is becoming clearer and clearer that only strategies aimed at the *prevention* of disease, offer real hope of limiting these costs. In turn, preventive strategies require deeper insights into the causes of disease and not just the application of existing knowledge.

Australia is currently spending only $65 million p.a. through NH & MRC (0.4% of health costs) and about the same sum from all other sources. As the national proprietors of the health care business we should feel uneasy about the low level of research and development in this particular industry. This is particularly the case when we consider that (1) Australian medical research workers such as those working at the Baker Institute have shown particular talents in biomedical research and (2) that a major effort in this field provides a stimulus to innovative biotechnology, which is the white hope for Australia's industrial future.

Government, both Federal and State, should not regard biomedical research as merely a 'cultural' pursuit, otherwise we *will* become the proverbial banana republic. Greater support from the private sector, both philanthropic and commercial, is also needed. It is imprudent not to expend at least 4% of the cost of the health business on R & D. If we wish to have a better health system an increased Australian research effort is not an option but a necessity.

Dr. Philip Barter and Prof. Paul Korner showing the Minister for Health, David White, some research results.

Visiting Scientists

Dr. Carol-Ann Courneya from the Department of Physiology, University of British Columbia, arrived in September 1987. She will be working for two years with Professor Korner in the Circulatory Control/ Neuropharmacology Laboratory on baroreceptors and vasopressin release.

Dr. Michael Adams returned to Canada in April, 1988, after a stay of just over two years. He had been working with Dr. Alex Bobik, Dr. Julie Campbell and Professor Paul Korner on the development of cardiovascular hypertrophy in relation to autonomic function in the spontaneously hypertensive rat (SHR), a Japanese strain with genetic hypertension.

Dr. Christian Ehnholm from the National Public Health Institute, Helsinki arrived in September 1987 for a one year sabbatical. He is working with Drs. Fidge, Barter and Hopkins in the Lipoprotein Biology Laboratory on problems related to the regulation of high density lipoproteins.

Dr. Jean Luc Elghozi returned to France in September 1987 after spending 14 months with Dr. Geoff Head and Professor Korner.

Dr. Maarten Van den Buuse from the Rudolf Magnus Institute for Pharmacology in the Netherlands has been working with Dr. Geoff Head and Professor Paul Korner on problems related to the central control of blood pressure in the Circulatory Control/Neuropharmacology Laboratory.

Dr. William Wolf, National Institutes of Health, USA, is in his third year at the Baker working with Dr. Alex Bobik and Dr. Geoff Head on biochemical aspects of brain function.

Dr. Peter Friberg from the University of Göteborg, Sweden is working with Dr. Garry Jennings and Dr. Murray Esler in the Clinical Research Unit and with Dr. Joe Smolich (Basic Cardiology) on the mechanisms of cardiovascular hypertrophy; he is also performing studies of the sympathetic nervous system response to restriction of dietary sodium intake and of the associated problems related to the regulation of coronary blood flow.

Dr. K. Sudhir from Madras has also been working with Dr. Jennings, Dr. Esler and Dr. Angus on the haemodynamics of human hypertension and on sympathetic nervous function in steroid hypertension and essential hypertension.

Dr. He Guo Wei from the Fu Wai Hospital in Peking has spent his second year at the Institute working with Dr. Jim Angus and Dr. Frank Rosenfeldt on internal mammary artery and saphenous vein graft preservation for coronary bypass surgery.

Dr. Minoru Tozuka from Shinshu University Hospital in Matsumoto, Japan is also into his second year at the Institute, working with Dr. Noel Fidge and Dr. Boris Grego on the isolation and characterisation of the high density lipoprotein binding protein.

Dr. Kazuo Kondo from the National Defence Medical College in Japan left the Institute in April 1988 after working for just over one year with Dr. Noel Fidge on the regulation of apolipoprotein A-IV metabolism.

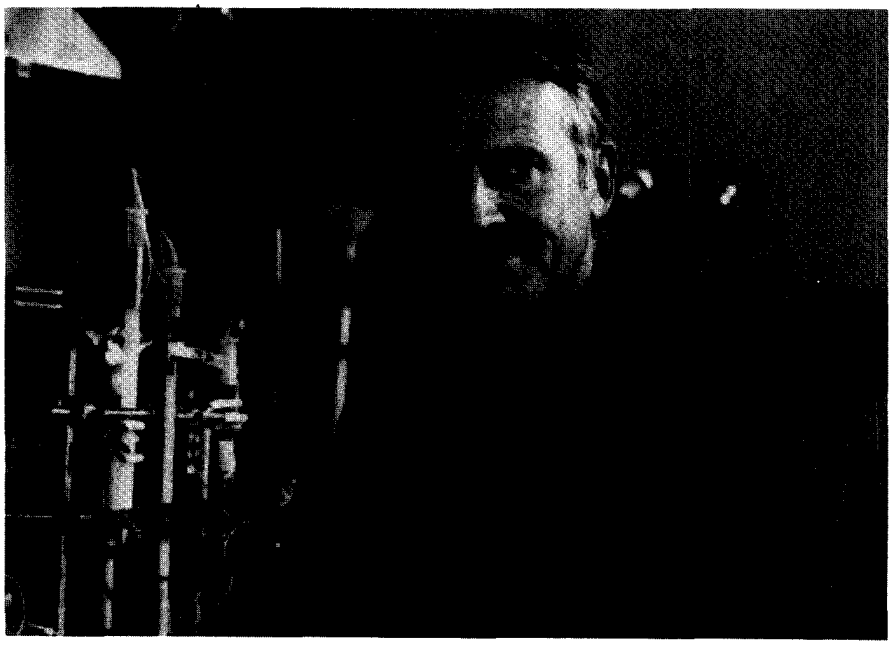
Dr. Masanobu Takata from the Toyama Medical and Pharmaceutical University, Toyama, Japan, has been working with Dr. Warwick Anderson in the Renal Laboratory on the pathogenesis of renal hypertension.

Dr. Christopher Munsch from the Wessex Cardiothoracic Centre in the United Kingdom is working with Dr. Frank Rosenfeldt in the Cardiac Surgical Research Unit to investigate the effects of orotic acid and ribose on the damaged myocardium. Also, a clinical trial is envisaged on the effects of ribose in combination with orotic acid in patients with recent heart attacks.

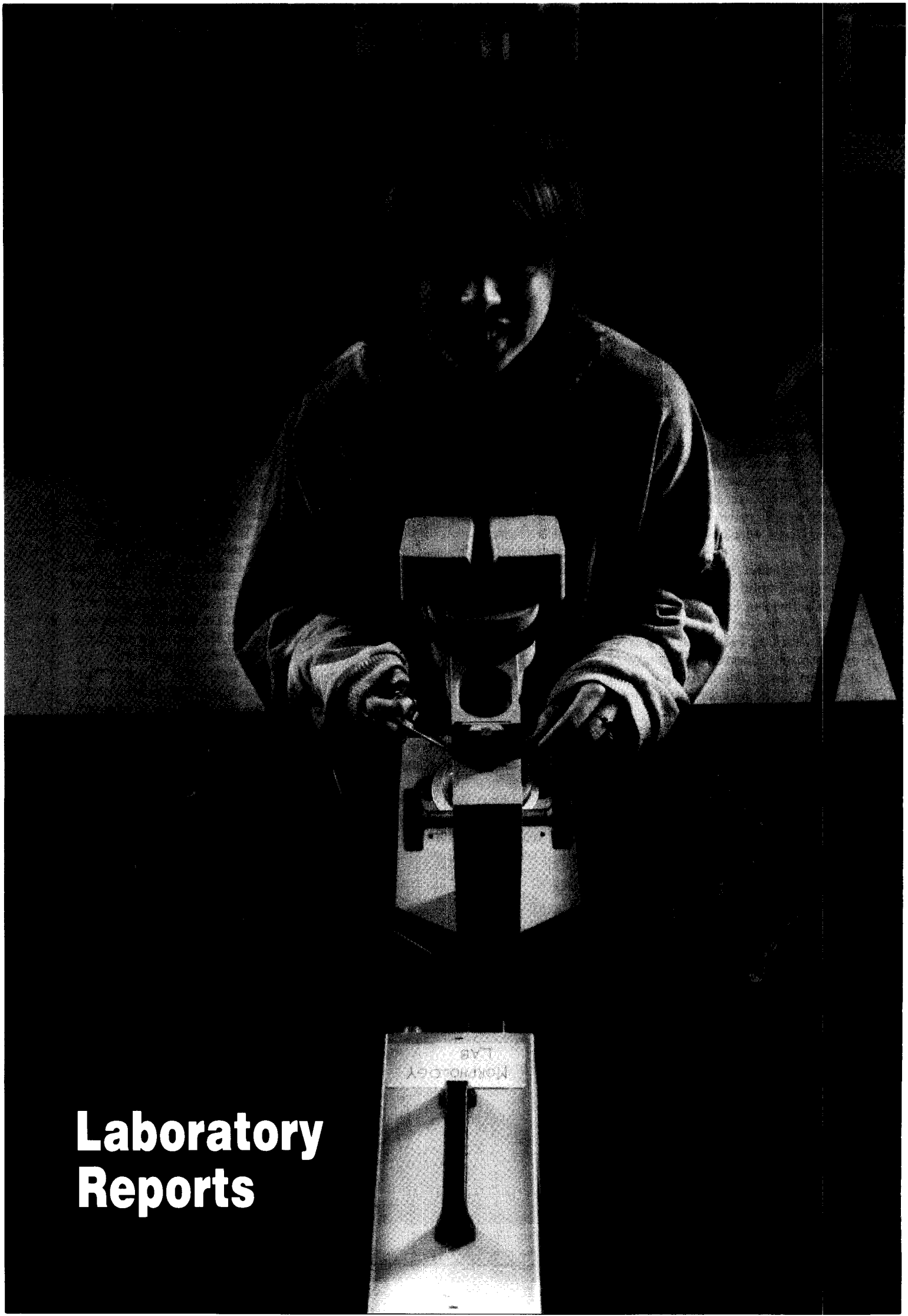
Dr. Merv Merrilees from the University of Auckland was an Esso Fellow in the Cell Biology Laboratory for four weeks during September/ October 1987, working with Dr. Julie Campbell on endothelial influences on smooth muscle glycosaminoglycan synthesis. He returned in April 1988 for 10 days to complete the project.

Dr. Frances Cribbin from University College, London has been assisting Institute staff in the preparation of manuscripts for international publications including journals and a book. Dr. Cribbin has been training other Baker staff in scientific editing.

Dr. Steve Hunyor, Head of the Hypertension Unit, Royal North Shore Hospital, Sydney spent a 'mini-sabbatical' here in September-October 1987 with CRU and Pharmacology.



Dr. Christian Ehnholm, a visiting professor on sabbatical leave from Helsinki, beside the immunoaffinity columns he uses to separate the A-I (HDL) binding protein from human placenta.



Laboratory Reports

Basic Cardiology Laboratory

Head: Dr. J. Smolich

Projects

Effect of arterial pressure reduction on left and right ventricular blood flow in normal hearts.

Left and right ventricular blood flow in hearts with hypertensive left ventricular hypertrophy.

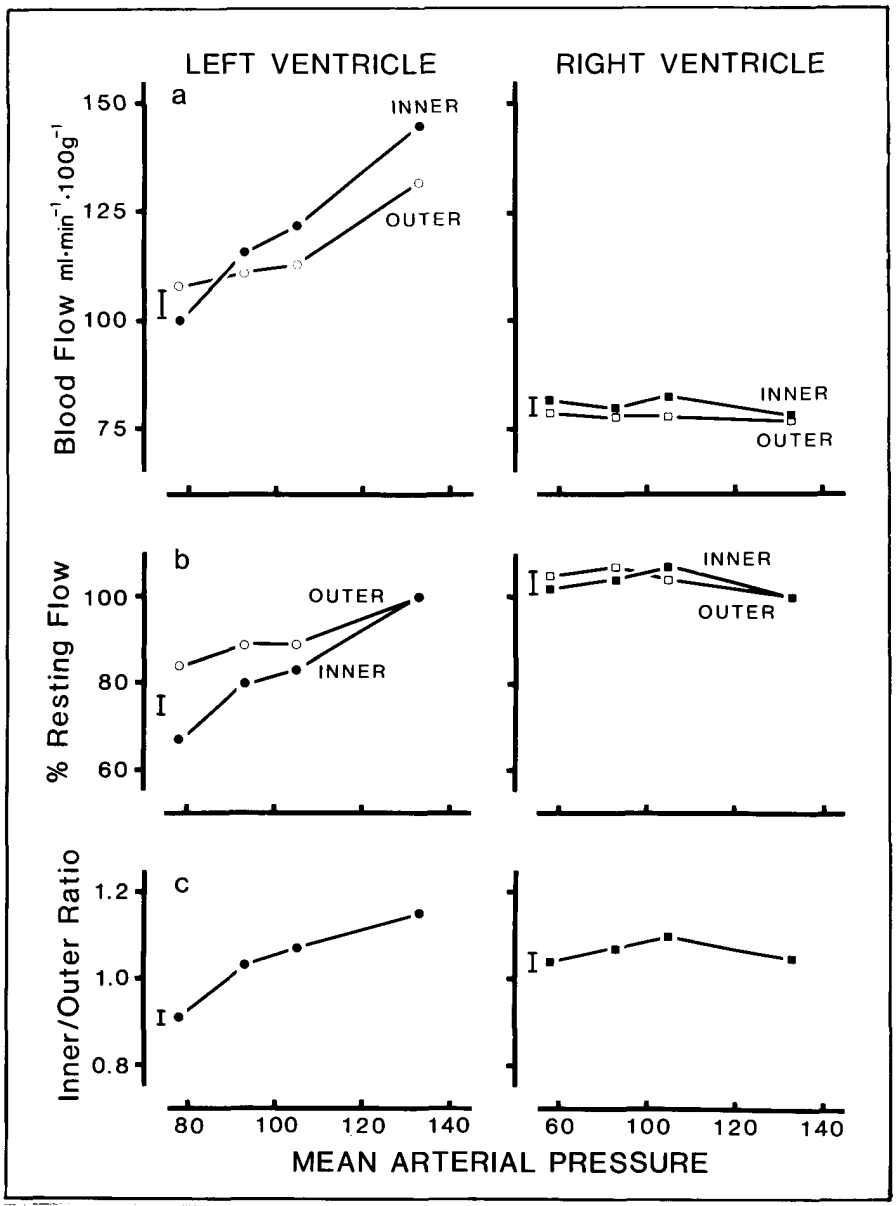
Influence of changes in heart size and geometry on two-dimensional echocardiographic estimates of left ventricular mass.

Summary

We have continued our studies on the effect of reducing arterial pressure on blood flow in normal hearts and those with left ventricular hypertrophy secondary to chronic hypertension. In normal hearts, lowering arterial pressure caused a substantial redistribution of left ventricular blood flow towards outer muscle layers. However, unlike the redistribution reported with falls in coronary pressure alone, this redistribution appeared to be a normal physiological phenomenon because it was not accompanied by electrical or mechanical evidence of impaired cardiac function. The decrease in arterial pressure had little effect on global and regional blood flow in the right ventricle, indicating that this portion of the heart was capable of 'autoregulating' its blood flow.

Reducing arterial pressure also caused a redistribution of left ventricular blood flow towards outer muscle layers in hypertrophied hearts. However, the changes were more pronounced than in normal hearts at moderately low pressures and were then associated with an impaired mechanical performance of the left ventricle. We evaluated the contribution of hypertension-induced vascular changes to these left ventricular flow abnormalities by studying blood flow in the non-hypertrophied right ventricle of the same animals. We found that right ventricular blood flow was initially maintained during the decrease in arterial pressure. However, right ventricular flow fell significantly with further pressure reduction, implying that chronic hypertension impaired autoregulation at moderately low pressures.

In a separate project we examined the reliability of two-dimensional echocardiographic (2-D echo) formulae for predicting left ventricular mass over a range of cardiac loading conditions. We derived systolic and diastolic formulae under control conditions by relating 2—D echo dimensions measured in these parts of the cardiac cycle to true left ventricular mass. We then evaluated these formulae over a range of loading conditions which altered heart size and geometry. We found both formulae to be reliable over a wide range of loading conditions. However the systolic formula overestimated true mass with large reductions in left ventricular cavity size.



In normal hearts, reducing arterial blood pressure causes a decrease in blood flow to the inner and outer halves of the left ventricle but not the right ventricle (panels a and b). There is an associated redistribution of flow towards outer muscle layers in the left ventricle, manifested as a decrease in the inner/outer flow ratio, but no significant change in the right ventricle (panel c).

Effect of arterial pressure reduction on left and right ventricular blood flow in normal hearts

J.J. Smolich, P. Weissberg, A. Broughton, P.I. Korner

Diurnal fluctuations in arterial blood pressure are common in normal individuals. However, until recently, little was known about the effect of moderate reductions in arterial pressure on regional blood flow to the left and right ventricles. We addressed this question in anaesthetized dogs by lowering arterial pressure with an arteriovenous shunt and measuring blood flow to the heart with radioactive microspheres.

Total blood flow to the left ventricle declined with the decrease in arterial pressure, reflecting the diminished workload of this chamber. Regionally, a redistribution of blood flow towards outer muscle layers was also evident because the flow reduction was most marked in the innermost layers (Figure). Importantly, the absence of any electrical or mechanical evidence of impaired cardiac function indicated that this redistribution was a normal physiological phenomenon which occurred without myocardial ischaemia. Our finding thus pointed to an important difference between the effect of a fall in arterial pressure generally and that of a decrease in coronary artery pressure alone (e.g. caused by vessel narrowing) because the latter only produces a redistribution of left ventricular blood flow **after** the development of myocardial ischaemia and a deterioration of cardiac function.

Decreasing arterial pressure had little effect on the metabolic demands of the right ventricle. We were thus able to assess the ability of the right ventricle to 'autoregulate', i.e. to maintain its blood flow in the face of a changing perfusion pressure. Although autoregulation is well-established in the left ventricle, previous studies had not clearly demonstrated its existence in the right ventricle. In our study, right ventricular blood flow and its distribution remained constant over a broad range of arterial pressures (Figure). We therefore concluded that the right ventricle was capable of effective autoregulation.

Left and right ventricular blood flow in hearts with hypertensive left ventricular hypertrophy

J.J. Smolich, P.L. Weissberg, P. Friberg, A. Broughton, P.I. Korner

In the left ventricle exposed to chronic hypertension, structural changes in blood vessels (e.g. wall thickening) coexist with enlargement (hypertrophy) of muscle cells. As a result, the contributions of the vascular changes and the muscle hypertrophy to blood flow abnormalities in the hypertrophied left ventricle are not easily dissociated.

We had previously examined left ventricular blood flow during decreases in arterial pressure in anaesthetized, renal hypertensive dogs with left ventricular hypertrophy. As in normal dogs (see preceding section), lowering arterial pressure decreased total blood flow to the hypertrophied left ventricle and caused a substantial redistribution of flow towards outer muscle layers. However, at the lowest pressure studied, both the reduction in total flow and the extent of the redistribution was much more pronounced than in normal hearts and was then associated with a falloff in left ventricular performance.

To separate the effects of the vascular changes induced by hypertension from those induced by muscle hypertrophy, we examined blood flow responses in the non-hypertrophied right ventricle of the same animals. Total and regional right ventricular blood flow at rest was similar to that in normal hearts. Moreover, consistent with intact autoregulation, right ventricular blood flow and its distribution was initially preserved as arterial pressure was lowered with the arteriovenous shunt. However, in contrast to normal hearts, right ventricular blood flow fell by about 20% and was redistributed towards outer muscle layers at the lowest pressure. These findings imply that hypertensive vascular changes impair autoregulation at moderately low perfusion pressures. This appears to be at least partly responsible for the flow abnormalities observed in the hypertrophied left ventricle.

Influence of changes in heart size and geometry on two-dimensional echocardiographic estimates of left ventricular mass

J.J. Smolich, E. Laufer, P.L. Weissberg, A. Broughton, P.I. Korner

Two-dimensional echocardiography (2-D echo) is established as an important non-invasive means of estimating left ventricular mass. However, the accuracy of commonly used 2-D echo mass formulae has not been validated under conditions of altered left ventricular dimensions and geometry. This point is relevant to the assessment of sequential changes in left ventricular mass in conditions where heart size and shape change (e.g. hypertension, cardiac failure).

We used 2-D echo to measure left ventricular short and long axis dimensions in the contraction (systole) and relaxation phase of the cardiac cycle (diastole) in anaesthetized normal dogs under control conditions. We derived formulae relating the product of these measurements to true mass obtained later at post-mortem. We tested the ability of these formulae to predict left ventricular mass during two types of intervention. Firstly, we altered left ventricular filling pressure to produce elongated, thick-walled hearts with small cavities at low pressures, and globular, thin-walled hearts with large cavities at high pressures. Secondly, we kept left ventricular filling pressure constant but varied the extent of cardiac contraction by changing arterial pressure.

We found that left ventricular mass calculated with the diastolic formula varied by 4% or less during both manipulations. The systolic formula was similarly reliable under most loading conditions but overestimated true mass by 7% at the lowest arterial pressure and 11% at the lowest left ventricular filling pressure. We therefore concluded that systolic and diastolic 2-D echo formulae provide reliable estimates of left ventricular mass over a wide range of cardiac loading conditions but that a systolic formula may become inaccurate with large reductions in left ventricular cavity size.

Biochemical Pharmacology Laboratory

Head: Dr. A. Bobik

Projects

Dependence of sodium-hydrogen antiport activity in vascular smooth muscle on calmodulin-dependent kinases.

Effects of alterations in the sodium-potassium pump and the sodium-hydrogen antiport activity on the kinetics of vascular smooth muscle cell proliferation.

Analysis of the processes involved in the initiation of spontaneous oscillations in cytoplasmic free calcium concentration in vascular smooth muscle.

The effects of prevention of cardiovascular hypertrophy during early development on blood pressure in spontaneously hypertensive rats.

Interaction between α-methyldopa and central serotonergic neurons.

Administration of α-methyldopa enhances catecholamine release from isolated noradrenergic nerve terminals.

6-Hydroxydopamine induces noradrenaline release from central noradrenergic neurons.

Differential sensitivity of rat heart and lung adenylate cyclase to forskolin: dependency on specific membrane-protein interactions.

Summary

The main interest of the Biochemical Pharmacology Laboratory during 1987 has been the regulation of cell membrane sodium and calcium ion permeability and its effects on vascular smooth muscle and cardiac function. Alterations in the permeability of sodium and calcium ions are involved in modulating vessel contraction in response to nerve stimulation and circulating hormones. They also have the potential to regulate the basal tone of vessels and this may be involved in the development of vascular hypertrophy (thickening of blood vessel walls). Our studies on genetic hypertension in spontaneously hypertensive rats indicate that vascular hypertrophy is a prime event which occurs prior to the development of hypertension and is probably the initial component of a complex series of events which ultimately lead to sustained hypertension. One of our aims is to understand how alterations in membrane ion transport processes influence the development of vascular hypertrophy in hypertension.

Our previous studies have demonstrated that the sodium-hydrogen antiport system is the major mechanism for sodium entry into vascular smooth muscle cells. The antiport also controls intracellular pH. Since the antiport can be activated by growth factors, we have investigated its importance in growth control. We found that its activation increases the frequency at which smooth muscle cells divide. Relating this finding to cardiovascular hypertrophy and hypertension, we concluded that an elevation in the level of antiport activity contributed to the mechanism by which vessels thicken in hypertension. In parallel experiments, we have found that an elevation in the activity of the sodium-potassium pump also increases the frequency of smooth muscle cell division when the cells are exposed to growth factors. Reductions in activity of the sodium-potassium pump were found to reduce the rate of cell division by reducing protein synthesis. This resulted in a prolongation of the interval before the cells commence to replicate DNA.

Since the sodium-hydrogen antiport plays an important role in influencing many functions in vascular smooth muscle, we are also investigating the mechanism by which this membrane protein transports sodium ions into the cell in exchange for intracellular hydrogen ions. Our results implicate chemical modification by the process of phosphorylation, with at least two sites being modified. One site appears to control sensitivity of the protein to changes in intracellular pH, whilst the other site is involved in actually transporting the sodium and hydrogen ions.

We have also continued our studies designed to define more clearly the sequence of cardiovascular events which lead to the development of hypertension. Recent experiments in pre-weanling rats have shown that vascular hypertrophy is already present very early in development, which provides powerful support to our idea that vessel wall thickening is a vital component of genetic hypertension. During the past year we have set up a variety of new biochemical and histological procedures which will enable us to identify the growth factors and hormones responsible for the development of vascular hypertrophy.

In separate but related studies, we have continued to monitor the blood pressure and re-development of cardiac and vascular hypertrophy in rats in which cardiovascular hypertrophy had been reversed by vigorous antihypertensive treatment. We have found that the re-development of vascular hypertrophy occurs only very slowly in these animals, although there is a greater tendency for the cardiac hypertrophy to re-develop. However, the blood pressure of these animals remains well below that observed in the untreated hypertensive animals. These experiments suggest that a life time of antihypertensive treatment may not be required in human subjects if the cardiovascular hypertrophy can be either prevented or successfully reversed in the early stages of the disease. In order to achieve such a goal, we need to understand the precise mechanisms by which reductions in the thickness of blood vessel walls can be achieved.

In our neurochemical studies we have continued to use synaptosomes (pinched off nerve endings) to study the properties of noradrenaline- and serotonin-containing nerves in the central nervous system. We have demonstrated that the acute cardiovascular effects of the neurotoxin 6-hydroxydopamine, extensively studied by Professor Korner and his group, are due to the release of noradrenaline.

We have also demonstrated that the ability of the antihypertensive drug, α-methyldopa, to lower blood pressure is due to its capacity to increase the basal level of catecholamine release from noradrenergic nerves. This increase in basal catecholamine release mimics the effects of nerve activation in causing a fall in blood pressure.

Dependence of sodium-hydrogen antiport activity in vascular smooth muscle on calmodulin-dependent kinases

P. Little, P. Weissberg, A. Bobik

We have previously shown that the sodium (Na^+)-hydrogen (H^+) antiport system in vascular smooth muscle is a major mechanism for Na^+ entry into the cell and is also an important regulator of intracellular ph (pHi). We now demonstrate that activation of this antiport requires the presence of calmodulin-dependent kinases. Activation of the antiport following either exposure of the cells to serum or by the induction of intracellular acidosis, could be markedly attenuated by pre-incubating the cells with calmodulin antagonists. The calcium/calmodulin dependency of antiport activation was also evaluated by examining the effects of elevating intracellular calcium with the divalent ionophore ionomycin. Ionomycin transiently elevates intracellular pH via the Na^+-H^+ antiport. Under these conditions the accelerated $^{22}Na^+$ influx into the cells could be markedly attenuated by pre-incubating the cells with either calmodulin antagonists or removal of extracellular calcium. Pre-incubation of the cells with 2-deoxy-D-glucose, a compound which interferes with glucose utilisation, thereby reducing cellular ATP by at least 80%, greatly attenuates the activity of the antiport. Under these conditions the ability of cells to recover from acidosis is reduced by approximately 95%. Likewise, $^{22}Na^+$ uptake in these cells is also reduced to a similar extent. Examination of the mechanism by which the reduction in cellular ATP content reduced Na^+-H^+ antiport activity indicated that calcium/calmodulin-dependent phosphorylation processes were involved. Two phosphorylation sites on the antiport protein have been postulated — one which modifies the intracellular proton-dependent regulatory mechanism and another which affects the maximum activity of the antiport.

Effects of alterations in the sodium-potassium pump and the sodium-hydrogen antiport activity on the kinetics of vascular smooth muscle cell proliferation

A. Bobik, P. Little, S. Grinpukel, A. Grooms

The univalent cations, sodium, potassium and hydrogen ions, play a critical role in regulating the growth of a number of mammalian cell types. We have used tissue culture to investigate how two cation transport processes, the sodium-potassium (Na^+-K^+) pump and the sodium-hydrogen (Na^+-H^+) antiport, affect the kinetics of replication of vascular smooth muscle. In these experiments cell growth associated with elevations in the activities of the two transport processes was initiated with growth media containing 10% serum. The times taken for cells to progress through the G_1 phase of the cell cycle and into the DNA synthetic (S) phase were assessed using thymidine [^{3}H]. Generation times were assessed by cell counting. Reductions in Na^+-K^+ pump activity greatly prolonged cell generation times. Exposure of cells to 100 and 200 μM ouabain prolonged the normal generation time (approximately 21 h) by 109 and 174% respectively. The lack of a direct effect of ouabain on mRNA translation and protein synthesis *in vitro* suggested that the effects of ouabain were due to its ability to inhibit the Na^+-K^+ pump with a consequent prolongation in the time taken for cells both to exit from the G_1 phase and to enter into the S phase. Attenuation of Na^+-H^+ antiport activity in vascular smooth muscle cells also prolonged their generation time. In this instance prolongation of the generation time was due to a reduction in the rate at which the cells entered the S phase. The overall results suggest that elevations in Na^+-K^+ pump and Na^+-H^+ antiport activity facilitate vascular smooth muscle proliferation in response to growth factors.

Dr. Peter Little and Ms. Annette Grooms preparing human internal mammary artery explants for the study of ion transport processes in cultured vascular smooth muscle cells.

Analysis of the processes involved in the initiation of spontaneous oscillations in cytoplasmic free calcium concentration in vascular smooth muscle

P. Weissberg, P. Little, A. Bobik

Vascular smooth muscle maintains a constant basal level of activity or resting tone which dictates vascular resistance. In addition, a number of vessels undergo spontaneous cyclic contractions. Although both of these processes are known to be critically dependent on calcium, the underlying cellular mechanisms are obscure. We have recently discovered that confluent quiescent vascular smooth muscle cells exhibit transient elevations in cytosolic calcium which could account for the ability of smooth muscle to maintain tone and undergo spontaneous cyclic contractions. Cells labelled with the fluorescent calcium indicators Fura 2 and Quin 2 exhibit temperature dependent cyclic elevations in cytosolic calcium which average 60 nM and last approximately 30 s. The process is accompanied by intracellular calcium release followed by an influx of extracellular calcium via membrane calcium channels. Both the removal of extracellular calcium and blockade of calcium channels

with verapamil and diltiazem attenuated the duration and amplitude of the cyclical changes. By contrast, the calcium channel agonist (—) BAY K8644 increased their duration. The transients were abolished when the cells were exposed to agents (e.g. caffeine and 8-(N, N-diethylamino)-octyl 3,4,5-trimethoxybenzoate) which interfere with the release of calcium from the sarcoplasmic reticulum. These results suggest that the sarcoplasmic reticulum of vascular smooth muscle cells can spontaneously release calcium into the cytoplasm, even in the absence of receptor stimulation. In turn, this activates specific membrane calcium channels which result in calcium influx.

The effects of prevention of cardiovascular hypertrophy during early development on blood pressure in spontaneously hypertensive rats

M. Adams, P. Korner, A. Bobik

We have previously used the converting enzyme inhibitor enalapril (25 mg/kg/day) to treat rats with genetically inherited hypertension (SHR). Not only did this treatment reduce their blood pressure but it also caused a regression of the associated cardiovascular hypertrophy. We have now extended these investigations into the effects on blood pressure and cardiovascular hypertrophy, by commencing the treatment in very early life. We have found that antihypertensive treatment of SHR from 4 to 14 weeks of age kept blood pressure at normal levels and prevented any development of cardiac or vascular hypertrophy (assessed from consistent flow perfusion of hindlimb vasculature). In fact, the vascular hypertrophy which is present before the rise in systolic blood pressure in the 4 week old animals, regressed completely during the treatment interval. After discontinuing the antihypertensive therapy at 14 weeks of age, blood pressure rose slightly (~ 15 mmHg) as did left ventricular weight. Vascular hypertrophy, however, showed no tendency to re-develop. In these animals blood pressure remained close to normotensive levels throughout the subsequent monitoring period (up to 40 weeks of age). The results of the study suggest that the early abnormal development of vascular hypertrophy plays a critical role in the hypertension which develops later in life. Whether this represents an abnormal interaction between growth factors in the vasculature and the autonomic nervous system requires further investigation.

Dr. Alex Bobik (L) and Dr. Peter Friberg with Dr. Michael Adams at the microscope examining the hypertrophied microvessels from rats with genetic hypertension.

Interaction between α-methyldopa and central serotonergic neurons

W. Wolf, A. Bobik

The ability of the antihypertensive agent, α-methyldopa to lower blood pressure is believed to involve an interaction with central vasopressor serotonin-containing neurons. While the precise nature of this interaction is unknown, it has been suggested that α-methyldopa directly reduces serotonin synthesis and release from nerve terminals. This hypothesis was directly tested *in vitro* using nerve terminal preparations known as synaptosomes. In these studies synaptosomes were prepared from rats given either a hypotensive dose of α-methyldopa or an injection of saline. α-Methyldopa was found to have minimal effects on synaptosomal serotonin synthesis and levels. Furthermore, depolarisation-induced release of serotonin from the nerve terminals was also unchanged, suggesting that α-methyldopa may not significantly alter serotonergic transmission through a direct interaction with serotonin-containing neurons. On the other hand, we demonstrated that one α-methyldopa metabolite, α-methyldopamine, was produced, stored and released from serotonergic nerve terminals. These results suggest that an accumulation of α-methyldopamine in serotonergic neurons during chronic therapy with α-methyldopa may contribute to its pharmacological effects.

Administration of α-methyldopa enhances catecholamine release from isolated noradrenergic nerve terminals

W. Wolf, A. Bobik

We have previously postulated that α-methyldopa lowers blood pressure by enhancing the activity of central depressor catecholaminergic pathways. In the present studies we utilised synaptosomes to demonstrate that an enhancement in the synthesis of the α-methylcatecholamines results in an increase in catecholamine release from the nerve terminals. Following administration of α-methyldopa to rats, both α-methyldopamine and α-methylnoradrenaline were rapidly synthesised and stored in central nerve terminals. Release from the nerve terminals of these α-methylcatecholamines was demonstrated even in the absence of nerve depolarisation. Moreover, the magnitude of this release was similar to what is seen when synaptosomes from control saline-treated rats are depolarised to release dopamine and noradrenaline. The overall results suggest that antihypertensive doses of α-methyldopa induce a constant enhancement of α-methylcatecholamine efflux from central catecholaminergic neurons, which is of sufficient magnitude to activate postsynaptic α_2-adrenoceptors, which, in turn, reduce blood pressure.

6-Hydroxydopamine induces noradrenaline release from central noradrenergic neurons

C.J. Oddie, A. Bobik

Whilst much is known about the ability of the neurotoxic agent 6-hydroxydopamine to destroy nora-

drenergic nerve terminals in the central nervous system, virtually nothing is known about how it alters noradrenergic neurotransmission in the short term. This question is important, since following central administration of 6-hydroxydopamine to rabbits, its cardiovascular effects have been attributed to the enhanced release of noradrenaline. We tested this hypothesis by examining the effects of 6-hydroxydopamine on a noradrenergic-containing synaptosome preparation. We found that 6-hydroxydopamine induced a concentration-dependent depletion of noradrenaline from synaptosomes. Depletion by 6-hydroxydopamine was due to release of noradrenaline from intraneuronal storage vesicles and subsequent release into the extracellular medium. Little if any of the noradrenaline released by 6-hydroxydopamine was metabolised intraneuronally by type A monoamine oxidase. Release of noradrenaline did not depend on extracellular calcium, nor was it prevented by desmethylimipramine. It was also found that 6-hydroxydopamine had no effect on the release of noradrenaline induced by depolarisation of the nerve terminals with potassium. The overall results from the study indicate that the predominant short term effect of 6-hydroxydopamine is to release noradrenaline from nerves in the brain, a property that can more than account for its acute cardiovascular effects.

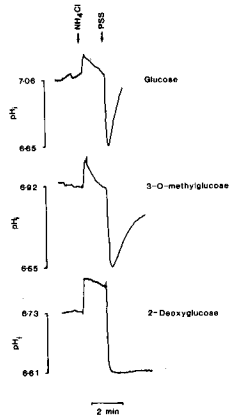


FIGURE 1: The effects of ATP depletion on the activity of the Na^+-H^+ antiport system in vascular smooth muscle. As ATP levels are progressively lowered (3-0-methylglucose and deoxyglucose), the intracellular pH of the cells falls and their ability to recover from an acid load is either greatly attenuated or abolished.

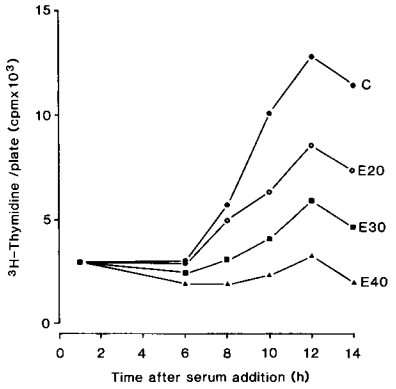


FIGURE 2: The effects of the Na^+-H^+ antiport inhibitor ethylisopropylamiloride on mitogenesis in rat aortic smooth muscle. Simultaneous exposure of quiescent rat aortic smooth muscle to 10% foetal calf serum and various concentrations of ethylisopropylamiloride (E, μm) attenuates their rate of entry into the S phase of the mitotic cell cycle.

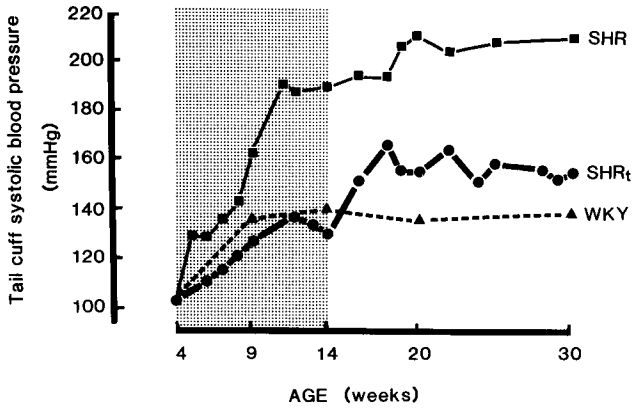


FIGURE 3: The effects of reversing and subsequently preventing cardiovascular hypertrophy on the development of hypertension in rats with genetically inherited hypertension. Treatment of the rats from 4 to 14 weeks of age with the converting enzyme inhibitor enalapril largely prevented these animals from becoming hypertensive in later life (SHR). SHR and WKY represent untreated animals with genetic hypertension and a control group of rats with normal blood pressures studied at the same time. The shaded area represents the antihypertensive treatment interval.

Differential sensitivity of rat heart and lung adenylate cyclase to forskolin: dependency on specific membrane-protein interactions

G. Jackman, A. Bobik

We have previously reported that the capacity of forskolin to activate rat heart adenylate cyclase is about 10-fold greater than that achieved with the lung enzyme. In the present study we have investigated whether this difference in tissue responsiveness to forskolin can be attributed to an alteration in the interaction between the catalytic unit and G proteins in the two membranes, to different isoforms (types) of catalytic units or to specific membrane-protein interactions. The studies were carried out on purified membrane preparations as well as on solubilised preparations of adenylate cyclase from the two tissues. We discovered that there were differences in the manner in which the Gs proteins interacted with the catalytic unit of adenylate cyclase in the two membrane preparations. Under conditions (4 mM manganese chloride) known normally to cause dissociation of the Gs protein from the catalytic unit, only the ability of the cardiac membrane-bound adenylate cyclase system to respond to Gs stimulation was abolished and the stimulation by forskolin attenuated. However, after disruption of membrane integrity by n-octyl-β-D-glucopyranoside, the behaviour of the adenylate cyclase systems from both tissues was similar to that observed in the cardiac membrane preparations. The overall results confirm our previous finding that forskolin stimulates heart adenylate cyclase to a much greater extent than the enzyme bound to lung membranes. This difference in responsiveness can be attributed to the manner in which the Gs protein and the catalytic units interact with other as yet unidentified membrane components. Whether slight modifications in the Gs and/or catalytic unit protein structure contribute to this difference remains to be determined.

Cardiac Surgical Research Unit

Head: Dr. F.L. Rosenfeldt

Projects

Metabolic supplementation of the heart with orotic acid.

Hypothermic cardioplegia and reperfusion in the hypertrophic heart.

Pharmacology of the saphenous vein and internal mammary artery grafts.

Ablation of atherosclerosis by excimer laser.

Preservation of the atrium during hypothermic cardioplegia.

Effect on the coronary circulation of particulate contamination of cardioplegic solutions.

Summary

The work of the Cardiac Surgical Research Unit includes both laboratory and clinical research. In the laboratory, the techniques of the basic medical sciences are applied to the challenges and problems of cardiac surgery. In the clinical area, the findings of the laboratory research are applied in the cardiac surgical operating theatres and wards. The clinical and laboratory components complement each other. The clinical component initially provides the questions for laboratory investigation and after these have been completed, it provides the means for evaluating the relevance and efficacy of the laboratory findings in improving surgical technique and patient care.

The decision taken in 1985 to place a greater emphasis on myocardial metabolism has borne fruit with the completion of the first project concerning the use of orotic acid in the setting of cardiac surgery following acute myocardial infarction. The primary findings in this study were that orotic acid can produce a modest improvement in cardiac function after myocardial infarction and that this improvement becomes more marked when the acute infarction is followed by surgery. An unexpected finding was the extremely low tolerance of hearts with recent myocardial infarcts to the cardioplegic arrest which is induced during cardiac surgery.

A two-part study has been completed investigating the effect of left ventricular hypertrophy on the response of the heart to cardioplegic arrest and reperfusion during cardiac surgery. This has resulted in a greater understanding of the particular response of the hypertrophic heart to cardioplegia. It has enabled us to specify optimal conditions for cardioplegic arrest in hypertrophic hearts in terms of volume of the cardioplegic solution, the myocardial temperature and the coronary artery pressure during reperfusion.

A fruitful collaboration with the Pharmacology Laboratory has continued in our studies of the reactivity of the internal mammary artery and saphenous vein which are used as coronary bypass grafts. The purpose of this research is to devise a means of preventing spasm of these vascular conduits during and after bypass surgery. The findings from this research have led to the development of new techniques to prevent spasm (see report from Pharmacology Laboratory). The efficacy of these techniques is currently being tested during bypass surgery at the Alfred and Epworth Hospitals.

A new treatment for coronary atherosclerosis is coronary excimer laser angioplasty. In this technique, an ultraviolet laser beam is directed into the patient's heart via an optical fibre. The laser is directed into an atheromatous plug obstructing a coronary artery, after which the atheroma is vaporised and the artery unblocked. At the time of writing, this has been possible only during open heart surgery. The challenge is to find ways of directing the laser accurately via a catheter threaded into the heart from an artery in the arm or leg. With the financial support of a Melbourne businessman, Mr. Hilton Nicholas, we have now commenced the testing of an advanced type of laser generator which has been loaned to the Institute by a Californian firm, Acculase Inc. Using this generator we are seeking to develop a technique for delivering laser energy directly into the heart.

Metabolic supplementation of the heart with orotic acid

M. Newman, C. Munsch, X.Z. Chen, M. Rabinov, F.L. Rosenfeldt, J. Williams*

In 1967 the Russian physiologist, Meerson, proposed the 'relative deficiency' hypothesis. He postulated that when the heart is placed under stress and begins to hypertrophy, despite a normal dietary intake by the body, it may become relatively deficient in certain building blocks needed for protein synthesis. Meerson reasoned that if substances such as orotic acid were added to the diet, the rate of protein synthesis might be enhanced. Orotic acid is a precursor for nucleic acids which, in turn, are essential for the production of proteins. It is a non-toxic substance found in high concentration in cow's milk. Meerson demonstrated in rabbits given an acute pressure overload on the heart, that contractility could be improved if orotic acid was given for 4 days. The finding was confirmed in rats by Williams. Also, clinical investigators in Russia have treated patients suffering from cardiac failure or recent myocardial infarction with orotic acid and have claimed dramatic benefits.

In patients with a recent myocardial infarction, the non-infarcted portion of the heart is stressed and undergoes hypertrophy. We reasoned that the heart might respond poorly to the further stress of cardiac surgery and that this could be an ideal setting for orotic acid therapy. To investigate this question we produced a myocardial infarction in greyhounds. The dogs were then divided into two groups, one was fed orotic acid, the other was untreated. Four days later the greyhounds underwent simulated cardiac surgery including 1 h of hypothermic cardioplegic arrest. A third group of greyhounds without infarcts also underwent the same period of simulated surgery.

* (Australian National University)

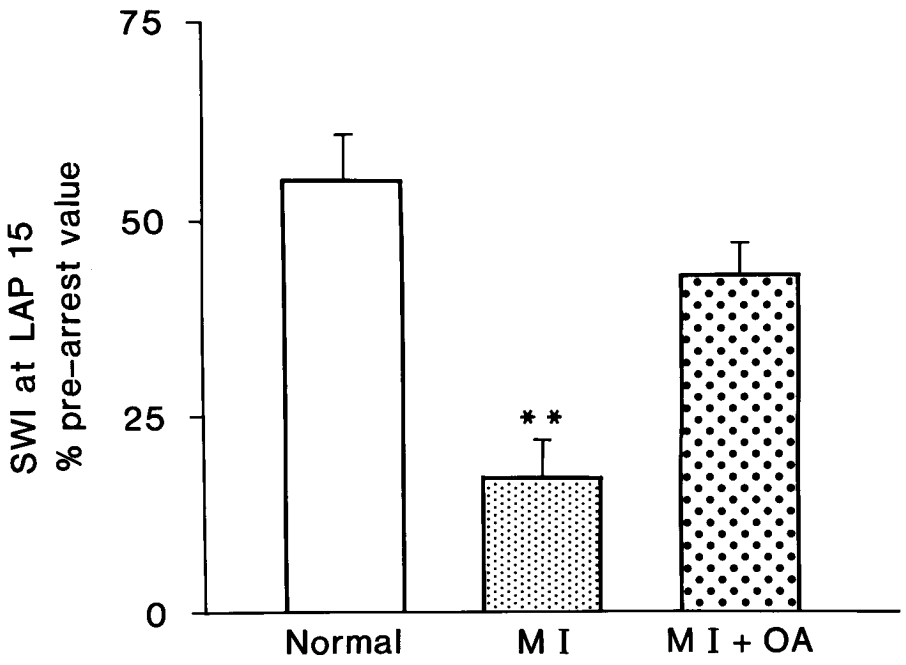


Fig. 1: Recovery of cardiac contraction after simulated cardiac surgery for normal, untreated infarcted (MI) and orotic acid treated infarcted hearts (MI & OA). Stroke work index (SWI) at a left atrial pressure (LAP) of 15mmHg after surgery is expressed as a percentage of the corresponding pre-arrest value.
***P < 0.01 : untreated infarct vs other two groups.*

The results showed that after myocardial infarction the heart responds poorly to hypothermic cardioplegia. This had not been documented before. Orotic acid therapy produced a doubling in the recovery of contractility after surgery (Fig. 1). Orotic acid also produced significant improvements in oxygen consumption and myocardial levels of ATP and glycogen. On the basis of these findings, the cardiac surgeons and cardiologists at the Alfred Hospital and the Royal Children's Hospital have begun to treat some of their high risk patients with orotic acid. The initial results in 11 patients are encouraging. However before embarking on a formal clinical trial of metabolic support for the stressed myocardium, we wished to perform laboratory tests of another metabolic supplement, namely ribose. Theoretical considerations and the results of studies in France led us to believe that ribose might have a synergistic effect with orotic acid. We felt that the best model in which to study the additive and the separate effects of ribose and orotic acid is the isolated rat heart. We were fortunate in being able to obtain the necessary perfusion apparatus (Taegtmeyer) on loan from Professor John Williams of the Australian National University. These studies in rat hearts are now proceeding and we hope will assist us to design a clinical trial to commence in 1989.

Hypothermic cardioplegia and reperfusion in the hypertrophic heart

M. Rabinov, M. Newman, F.L. Rosenfeldt

Patients with severe left ventricular hypertrophy undergoing cardiac surgery have an increased risk of death or complications compared to patients with non-hypertrophied ventricles. This could be due to events occurring during the phase of cardioplegic arrest or reperfusion. We have now completed studies of both these phases. We found that in the arrest phase, compared to normal hearts, the induction of arrest in hypertrophic hearts is slower, requires more cardioplegic solution to achieve asystole and is accompanied by a greater depletion of high energy phosphates. These differences can be minimised by doubling the dose of cardioplegic solution.

The second part of the study concerned the reperfusion phase and looked at the effect of different levels of coronary reperfusion pressure on recovery. With a Melbourne firm, Titron Ltd., we developed a miniature glass electrode which can give a continuous readout of myocardial pH during arrest and reperfusion. We then used myocardial pH to monitor cardiac recovery after cardioplegic arrest.

In the reperfusion phase, the empty beating hypertrophic heart required a higher coronary pressure than the normal heart to perfuse the myocardium adequately. At a coronary pressure of 40 mmHg in the hypertrophic heart, reperfusion was unable to reverse the myocardial acidosis that developed during ischaemia and frequently exacerbated it. This effect was even more marked if the ventricle was fibrillating. The end result of this low pressure reperfusion was a depression of contractility and depletion of high energy phosphates. These adverse effects were not seen in the hypertrophic heart if coronary pressure during reperfusion was maintained at 80 mmHg. The normal heart was much more tolerant to low pressure reperfusion. These findings enabled us to formulate recommendations to enable surgeons to conduct reperfusion so as to maximise myocardial recovery.

Ablation of atherosclerosis by excimer laser

F.L. Rosenfeldt, J. Angus

Many laser techniques have found an application in medicine. The various laser wavelengths used range from the infra-red end, to the ultraviolet end of the spectrum. However, relatively few of these lasers have been used in the cardiovascular system. Most experience has been gained to date with the argon laser. This generates radiation in the green area of the visible spectrum. It can penetrate atherosclerotic obstructions purely by the heat it generates. However heat can damage the surrounding vessel wall which becomes macroscopically charred and microscopically vacuolated. The damaged intimal surface predisposes to re-occlusion. To prevent re-occlusion

and improve the luminal diameter, it is standard practice to combine thermal laser angioplasty with a subsequent balloon angioplasty. This increases the complexity and cost of the procedure.

The pulsed excimer (ultraviolet) laser ablates atheroma by a different mechanism. The energy of the radiation is such that it can vaporise tissue or atheroma without heating it. The craters made by the excimer laser are clearly punched out and the surrounding tissue is normal. Like the argon laser, the excimer laser is directed into the arterial system through a flexible quartz fibre. One of the challenges of the excimer laser is to channel a large amount of energy down the fibre without destroying it.

A Melbourne entrepreneur, Mr. Hilton Nicholas, has a financial interest in a Californian based company, Acculase Inc. which has developed and produced an advanced excimer laser generator. A co-operative effort has been mounted between Acculase and the Baker Institute to test the laser generator and to develop catheter systems to deliver the radiation into the arterial system, particularly the coronary arteries. A laser generator has been delivered to the Baker Institute (Fig. 2) and its potential to ablate atherosclerotic obstruction is being evaluated in cholesterol-fed rabbits. A direct comparison is being made with the results of conventional balloon angioplasty in the same animals. In parallel with this study, new fibreoptic delivery systems will be tested in dogs. When these investigations have been completed it is planned that clinical application will commence, at first during open heart surgery and later in the cardiac catheterisation laboratory.

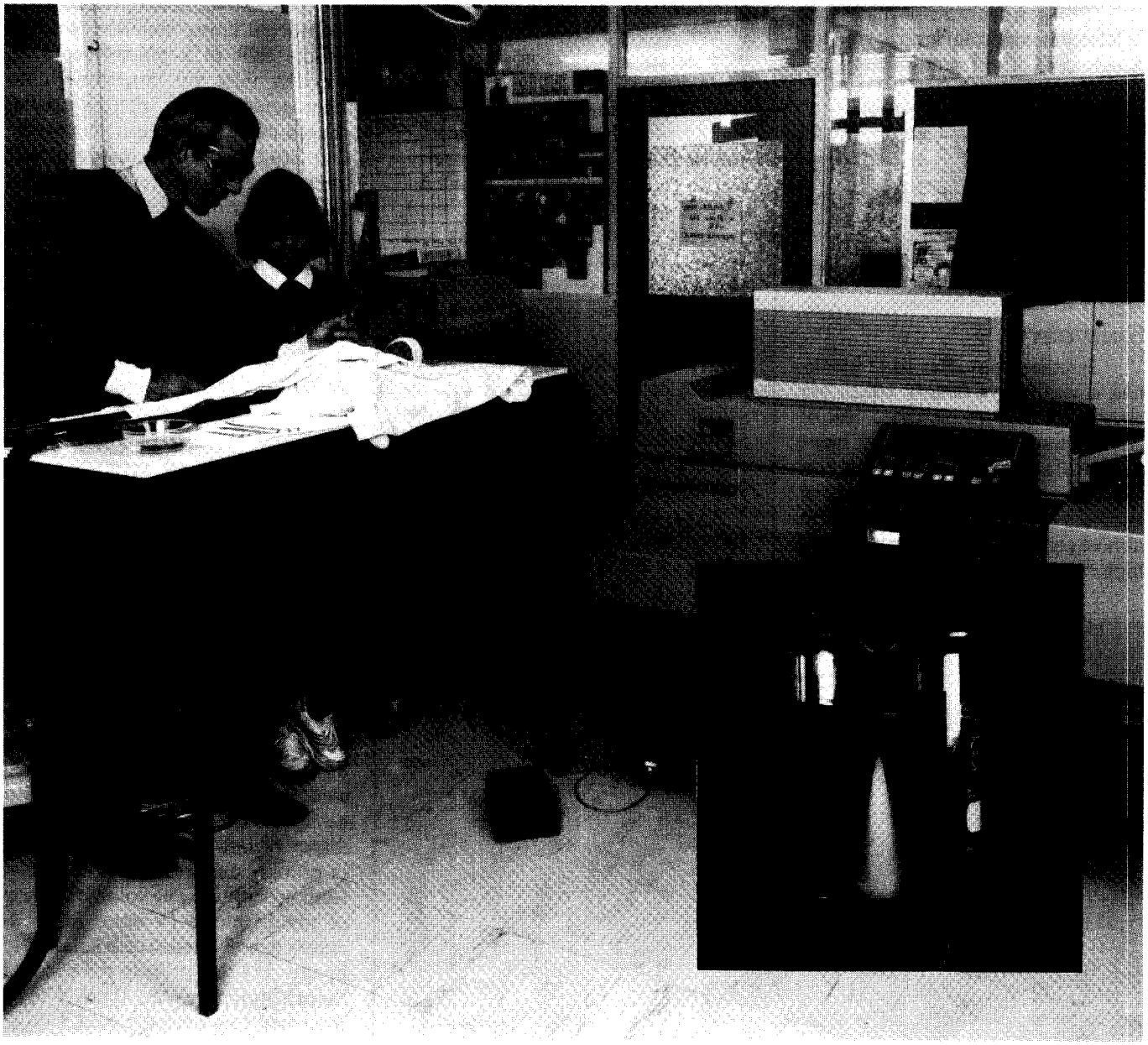


Fig. 2: Dr. Frank Rosenfeldt and Christine Boyes performing an experiment with the medical excimer laser generator.

Cell Biology Laboratory

Head: Dr. J. Campbell

Projects

Expression of smooth muscle α-actin mRNA as a marker for smooth muscle phenotype.

Macrophage-smooth muscle interactions.

Glycosaminoglycan synthesis by smooth muscle cells of different phenotype: effect of endothelium.

Collagen synthesis at the tissue, cellular and molecular level in smooth muscle cells of different phenotype.

Smooth muscle polyploidy in the development and regression of hypertension.

Modification of β-VLDL by macrophages.

Molecular biology of smooth muscle phenotypic change.

Summary

Population studies have identified a number of factors which are associated with an increased risk of atherosclerosis and coronary heart disease. Factors such as hypertension and elevated concentrations of low and very low density lipoproteins are almost certainly causative. Yet some high risk subjects may have minimal vascular disease, while others whose identified risk is apparently low may have extensive atherosclerosis. Furthermore, in a given high risk individual the lesions do not occupy every arterial surface; rather, they are patchy with areas of marked involvement alternating with areas of virtually normal artery.

To the unaided eye the atherosclerotic plaque appears as a well-circumscribed, elevated thickening, grey to pearly white in colour, which protrudes into the lumen of the artery. In histological section it appears as a base or core of lipid covered by a fibrous cap of connective tissue and cells. The cellular composition is mainly smooth muscle with smaller numbers of monocyte-derived macrophages and T-lymphocytes. Proliferation of smooth muscle cells, their synthesis of extracellular matrix and accumulation of lipid are characteristics of this disease; however, it is not known why they occur in focal and specific regions.

We have shown that the smooth muscle cells in regions of arterial intima adjacent to atherosclerotic plaques have an altered phenotypic expression; that is, have a reduced volume fraction of myofilaments (low $V_v$myo). Our studies in culture have shown that associated with a low $V_v$myo is the ability of the smooth muscle cells to proliferate in response to mitogens, to synthesise extracellular matrix and to accumulate lipid. Furthermore, smooth muscle cells with a high $V_v$myo, such as those which occur in normal regions of artery, are unresponsive to atherogenic stimuli. Thus, we believe that alteration in smooth muscle phenotype is critical to the atherosclerotic process and our studies are principally aimed at elucidating the factors controlling smooth muscle phenotypic expression. We have shown that an undamaged endothelial lining maintains smooth muscle cells in a phenotypic state whereby they are unresponsive to atherogenic stimuli, whereas macrophages induce a change in phenotype. Our studies further indicate that smooth muscle phenotype is controlled via interactions between smooth muscle and extracellular matrix and that cells such as macrophages and endothelial cells exert their effect on smooth muscle via the extracellular matrix. Recently, we have also begun to map the changes in gene expression which accompany or cause phenotypic change of smooth muscle cells.

From L to R — Elpis Spanidis, Julie Campbell, Silvia Janevski and Sophie Horrigan, preparing tissue for cell culture.

Expression of smooth muscle α-actin mRNA as a marker for smooth muscle phenotype

J.H. Campbell, O. Kocher, O. Skalli, G. Gabbiani, G.R. Campbell

Smooth muscle cells in atherosclerotic lesions have a decreased expression of α-actin mRNA compared with cells of the unaffected media. In order to determine the mechanism by which this occurs, smooth muscle cells from 9 week old rabbit aortic media were enzyme-dispersed into single cells and plated in primary culture at different initial seeding densities. The volume fraction of myofilaments ($V_v$myo) in moderately densely seeded cells fell sharply by day 5, 1 day prior to the onset of logarithmic growth. The $V_v$myo remained low over the next 3 days, then began to rise as the density of cells increased, returning almost to original levels after confluency and 1.84 cumulative population doublings

(CPD). The expression of α-actin mRNA followed a similar time course of change. In very densely seeded cultures, the $V_v$myo fell only slightly by day 5 and rose upon confluency after only 0.33 CPD. Similarly, the expression of α-actin mRNA decreased only slightly by day 5, rising to original levels by day 12. The $V_v$myo of sparsely seeded cells fell on day 5 then remained low throughout the culture period, including 5 days after confluency (day 24) when the cells had undergone 5.37 CPD. The α-actin mRNA, after an initial decrease early in culture, remained low even after confluency. Cells which were maintained subconfluent and quiescent on day 7 in culture had the same low $V_v$myo and low α-actin mRNA expression and α-actin protein content as actively proliferating cells. Also, there was no significant difference in percentage of α-actin protein between cells of G_o/G_1 and S/G_2 phases of the cell cycle when the cells were taken from 5 day cultures in which the moderately seeded cells had a low $V_v$myo but had not begun logarithmic growth.

The results show that change in α-actin mRNA expression as a percentage of total actin mRNA in primary cultured smooth muscle cells occurs concomitantly with change in phenotype (change in $V_v$myo). Thus the low expression of α-actin mRNA in atherosclerotic lesions reflects the altered phenotypic state of the constituent smooth muscle.

Macrophage-smooth muscle interactions

R.E. Rennick, J.H. Campbell, G.R. Campbell

One of the earliest changes observed in the arterial wall of animals fed a high cholesterol diet is the invasion of monocyte-derived macrophages into the subendothelial space. In order to determine whether the macrophages potentially have an influence on smooth muscle in the initial stages of atherosclerosis, we grew the two cell types in co-culture. Ultrastructual morphometry showed that macrophages stimulate smooth muscle cells to modulate from a predominantly 'contractile state' (high $V_v$myo) toward a 'synthetic state' with a significant decrease in $V_v$myo from 39.5 $\pm$ 1.2% on day 0 to 31.2 $\pm$ 0.9% on day 3, after which time the smooth muscle cells proliferate logarithmically in response to serum mitogens and mitogens released from macrophages themselves. In addition, the smooth muscle cells co-cultured with macrophages have a binding capacity (B_{max}) of 60.5 ng β-VLDL protein/10^6 cells compared with 46.0 ng β-VLDL protein/10^6 cells when smooth muscle cells are cultured alone.

These results show that interactions between macrophages and smooth muscle cells play a role in both initiation and progression of atherosclerosis by effecting a change in smooth muscle phenotype, stimulating their proliferation and enhancing their binding of cholesterol-rich lipoproteins.

Modification of β-VLDL by macrophages

R.E. Rennick, J.H. Campbell

We have shown that the macrophage, a cell commonly found in atherosclerotic lesions, enhances the binding of the atherogenic lipoprotein β-VLDL to cultured smooth muscle cells. To examine the mechanism of this process, we (1) incubated macrophages in the presence of β-VLDL for 3 h at 4°C, after which the 'macrophage-conditioned' β-VLDL was added to cultures of smooth muscle; (2) added to cultures of smooth muscle medium which had been conditioned by macrophages for 3 h at 4°C then fresh β-VLDL added, and (3) added to cultures of smooth muscle fresh medium with fresh β-VLDL. It was found that binding to the smooth muscle cells which were incubated with fresh β-VLDL combined with fresh and 'macrophage-conditioned' medium was 35.42 $\pm$ 5.8 ng/10^6 cells and 33.23 $\pm$ 1.9 ng/10^6 cells respectively. By contrast, smooth muscle cells incubated with 'macrophage-conditioned' β-VLDL bound 430.9 $\pm$ 14.4 ng/10^6 cells, a 12-fold increase in binding. The results show that macrophages alter the β-VLDL particle making it more readily bound to smooth muscle cells, rather than directly altering the receptor number or affinity. The nature of the modification is now under investigation.

Molecular biology of smooth muscle phenotypic change

G. Cockerill, G.R. Campbell, J.H. Campbell

We are investigating the factor(s) involved in modulation of vascular smooth muscle phenotype at the level of the gene. On the assumption that the extent of sequence complexity of mRNA species is not significantly different between high $V_v$myo and low $V_v$myo smooth muscle cells, we are using the technique of subtractive hybridization to enrich for specific sequences in the modulation from one phenotype to the other.

Messenger mRNA has been prepared from high $V_v$myo smooth muscle cells at their point of modulation in culture and the ^{32}PcDNA subtracted against the mRNA from low $V_v$myo cells. Exclusive cDNA sequences have been cloned into λgt10. A subtracted probe has also been prepared with which to screen libraries constructed from high $V_v$myo and low $V_v$myo smooth muscle cells. Clones which are differentially represented between the two libraries are currently being examined by Northern blot analysis to determine whether their expression is regulated in synchrony to the events of modulation. Clones of interest will be further investigated by Southern blot analysis and sequencing.

Circulatory Control/ Neuropharmacology Laboratory

Head: Professor P.I. Korner

Projects

Cental Nervous System

Role of spinal monoamine pathways in cardiovascular reflexes.

Contribution of forebrain noradrenaline projections to cardiovascular reflexes.

Mechanisms involved in central pressor action of angiotensin II.

Reflex Regulation

Baroreceptor-heart rate reflex in spontaneously hypertensive rats.

Influence of cardiac baroreceptors and endogenous opiates on renal nerve activity during haemorrhage.

Time-dependent effects on renal baroreflex properties.

Renal sympathetic baroreflex in acute hypertension.

Differentiation of cardiac baroreflex properties using cuff and drug method and naloxone in two strains of rabbits.

Effect of autonomic blockade on threshold for vasopressin and renin release in haemorrhage.

Summary

Circulatory regulation is mediated through the brain and autonomic nervous system and a number of hormones. The individual components of the system include (1) the heart and blood vessels; (2) various groups of sensory receptors which provide the brain with information about blood pressure (= baroreceptors) and other body functions; (3) the brain, which integrates the sensory information from the baroreceptors and other 'cardiovascular' receptors in conjunction with behavioural and somatic stimuli; (4) these integrative processes give rise to changes in the activity of the autonomic nerves (vagus nerve, sympathetic nerves) and in the secretion of various hormones. The autonomic and hormonal responses evoked by changes in sensory inputs are called a set of reflex responses. In the intact organism an environmental disturbance usually stimulates several types of sensory receptors, so that the relationships between the inputs and outputs are relatively complex. For all that, input-output analysis provides a great deal of information on how the 'system' behaves.

One area of our research examines the role of certain regions of the brain in the regulation of blood pressure, heart rate and other circulatory functions. The regions include the source of the pathways through which certain antihypertensive drugs (e.g. α-methyldopa, clonidine) exert their action. We are becoming increasingly interested in whether certain circulating substances and peptide/ hormones can modulate the responsiveness of nerve cells in a number of important integrative regions of the brain. Another area examines the factors that can alter the properties of circulatory reflexes, and to what extent these are altered in hypertension.

In the work on the brain we have focussed on discrete groups of brain cells, where nerve impulses release monoamines (e.g. noradrenaline, serotonin) from their endings. The latter are one group of so-called neurotransmitters because their release alters the activity of the next nerve cell in the pathway. Our earlier work examined the gross effects on blood pressure and heart rate of simultaneous transmitter release from most noradrenergic or serotonergic nerve cells. More recently we have studied the role of some of the individual cell groups on these functions following chronic lesions and examining how this altered transmitter release. In our current work we have developed new techniques for removing the large nerve tracts that go from the nerve cells in the hindbrain (1) to the motor cells in the spinal cord, which are the sources of sympathetic innervation to the heart and vessels; (2) to other integrative sites in the forebrain involved in cardiovascular regulation. These studies have provided very clear evidence of the specific involvement of each set of pathways in distinctive reflexes.

One of our most interesting findings relates to the action of the hormone angiotensin II in the central nervous system. This hormone is present in small amounts in the blood and increases during haemorrhage or on a low salt diet. Its role in the brain has been somewhat uncertain because rather high concentrations were required to increase blood pressure by injecting the hormone intracisternally. By using rabbits in which the arterial baroreceptors had been denervated, we demonstrated a large increase in the potency of the hormone, so that only a few thousand molecules now increase blood pressure.

Our work on reflexes has examined the development of baroreceptor function in the spontaneously hypertensive rat (SHR), where there is gradual inhibition of the vagus starting a few weeks after birth, as the blood pressure rises. The end result resembles that obtained in our earlier findings in experimental renovascular hypertension. In the current work on SHR we have found that treatment of the rats with an ACE-inhibitor (enalapril) restores baroreflex function to normal.

A novel approach to reflex analysis, has been the use of two methods (one of which imposes a much greater load on the heart than the other) to characterise the baroreceptor-heart rate reflex in two genetically related strains of rabbits. These were bred in Israel by Professor Marta Weinstock, who came last year, to collaborate with us on this problem. The reflex of one of the strains is very sensitive to naloxone, indicating that some of the nerve cells

involved in the reflex, release an unusual neurotransmitter from one of the pathways involved in the reflex, which is not released in the genetically related strain or in normal rabbits. The transmitter is an endogenous opiate (i.e. a morphine-like substance) the effects of which can be abolished by the morphine antagonist naloxone. The transmitter thus serves as a marker of activity in a particular pathway involved in the reflex. The study has provided unique insights into how the different pressure sensitive receptors of the arteries and heart alter the properties of baroreceptor regulation of heart rate.

Although endogenous opiates are not involved in the baroreceptor-heart rate reflex of normal rabbits, they occur in the latter's pathways for the regulation of vessel calibre by the sympathetic nerves. In studies in conscious rabbits with electrodes on the sympathetic nerves to the kidney, we have obtained evidence suggesting that in haemorrhage renal nerve activity first increases, but after a blood volume loss of about 30-50%, the pathway that releases the opiates acts to turn off sympathetic nerve activity as a 'last resort' mechanism when haemorrhage has become very severe. At this point the blood flow has become so low that nervous control places greater stresses on the heart than if it is not there at all. This is obviously only a short term measure of coping with haemorrhage and the best way is to restore the blood volume to normal.

We have continued to examine nerve-hormone interrelationships in haemorrhage. Up to a blood loss of almost 30% of the blood volume, nervous control is paramount and the two main pressor hormones of the body (angiotensin II and vasopressin) play a negligible role. After complete autonomic blockade their importance increases dramatically and we have begun to examine the sensory mechanisms that bring this about.

Dr. Geoff Head and a visiting scientist from the Netherlands, Dr. Maarten Van Den Buuse, studying the role of catecholamine pathways in the brain in cardiovascular regulation.

Role of spinal monoamine pathway lesions on cardiovascular reflexes in conscious rabbits

G.A. Head, J-L. Elghozi, P.I. Korner, W.A. Wolf, C. Anderson

The preganglionic sympathetic vasomotor cells in the spinal cord receive a rich innervation from cell bodies in the brainstem that contain neurotransmitter monoamines such as noradrenaline and serotonin. We have examined the effect on cardiovascular reflexes of selectively removing these pathways by injecting small quantities of neurotoxic drugs into the monoamine bundles as they enter the spinal cord. Bilateral intraspinal injections of 6-hydroxydopamine (20 nmol) produced selective depletion of thoracic spinal cord noradrenaline after 3 weeks by 55% with little non-specific damage as assessed by histology. Similarly, 5,6-dihydroxytryptamine (20 nmol) reduced serotonin levels by 70%. We studied the sympathetic component of the MAP-heart rate reflex and found that the spinal serotonin pathways but not the noradrenaline pathways contributed to the reflex. By contrast, both neurotransmitters contributed to the normal pressor responses evoked by nasopharyngeal stimulation. We have also examined the antihypertensive effects of centrally administered clonidine and α-methyldopa in these animals but found no difference in responses to those of vehicle injected rabbits. We conclude that spinal monoamines make very specific contributions to regulation of blood pressure and heart rate.

Central angiotensin II pressor responses in conscious rabbits: involvement of baroreceptor input and spinal noradrenaline pathways

J-L. Elghozi, G.A. Head

The purpose of the present study was to investigate the contribution of spinal noradrenaline pathways and the modulation by the baroreceptor afferent input of the central AII responses. Dose response curves to intracisternal (i.c.) AII were examined in intact and sino-aortically denervated rabbits 3-4 weeks after bilateral 6-hydroxydopamine or vehicle injections into the noradrenaline bundles of the first cervical segment. Graded bolus i.c. injections of AII produced dose dependent sigmoidal increases in MAP (ED50= 5pg). Pressor responses were markedly reduced by i.c. injections of an AII antagonist or by intravenous prazosin. Dose response curves to i.c. AII were similar in vehicle and 6-hydroxydopamine treated animals. SAD rabbits were approximately 1000 times more sensitive to i.c. injections of AII (ED50= 5fg), with the steeper dose response curves and a slightly greater maximum. The increase in sensitivity to AII however was not observed in SAD rabbits with depletion of spinal noradrenaline. The results suggest that spinal catecholamine pathways are only involved in the vasoconstrictor responses following the stimulation of central AII receptors when baroreceptor input has been removed. It illustrates the advantages of using SAD preparation for examining central autonomic pathways.

Contribution of forebrain noradrenaline projections to cardiovascular reflexes and the release of vasopressin during haemorrhage

M. Van Den Buuse, G.A. Head, C. Anderson, C. Oddie, P.I. Korner

The innervation of the forebrain by the brainstem noradrenaline-containing cell bodies travels through the midbrain in two discrete fibre tracts called the dorsal and ventral bundles. We have applied the same approach used in the spinal cord to selectively lesion these bundles with microinjections of 6-hydroxydopamine (20 nmol). After 3 weeks the

hypothalamus and other forebrain regions were depleted of noradrenaline by 70-80%. In these animals we tested the MAP-heart rate reflex using intravenous injections of vasopressor (methoxamine) and vasodepressor (nitroprusside and glyceryltrinitrate) to produce steady state (constant) changes in MAP. We have found that the gain or sensitivity of the reflex is very much greater than normal due to enhanced vagal activity. This suggests that ascending noradrenergic activity normally inhibits cardiac vagal responses, in contrast to brainstem noradrenergic function, which we have previously shown to enhance vagal tone.

Deficit in vagal component of baroreceptor-heart rate reflex in spontaneously hypertensive rats (SHR) can be prevented with enalapril

G.A. Head, M.A. Adams

In many forms of hypertension there is attenuation of the gain (sensitivity) of the reflex. Many of these studies have used adaptations of the 'ramp technique' which correlates pulse interval to a rapidly changing blood pressure. We have developed a drug injection method in conscious rats which uses steady-state changes in blood pressure to assess the properties of both cardiac effectors independently. In this study we have applied this technique to the SHR, an inbred strain of rats that develop high blood pressure. We have examined whether differences in the baroreflex control of heart rate between SHR and an inbred strain of rats that have normal blood pressure (WKY) (1) involved changes to the cardiac vagus or sympathetic and (2) were temporally correlated with the development of hypertension and/or maturation. We studied weight matched SHR and WKY, aged 6, 9, 14 and 20 weeks. Each was implanted with an aortic catheter for mean arterial pressure recording and dual jugular catheters, for injection of vasopressor and depressor drugs. Changes in MAP and reflex heart rate responses were fitted to an S-shaped logistic function and the parameters heart rate range and average gain (slope between the two inflection points) were determined before and after vagal inhibition with methylatropine. Heart rate range and gain decreased with age in SHR, but increased in WKY. After methylatropine, no differences in the cardiac sympathetic baroreflex range or gain parameters were observed between strains or ages. Treating SHR with enalapril attenuated the subsequent elevation of blood pressure by about 50% but abolished the differences in the baroreflex parameters between strains. We conclude, that older SHR have normal sympathetic but reduced vagal capacity to control HR in response to changes in MAP. This deficit is not dependent on the absolute level of blood pressure, but can be prevented by giving antihypertensive drugs at a time when blood pressure is increasing rapidly. Because the differences were confined to the vagus and not the sympathetic, we suggest that SHR have altered central processing rather than arterial baroreceptor properties.

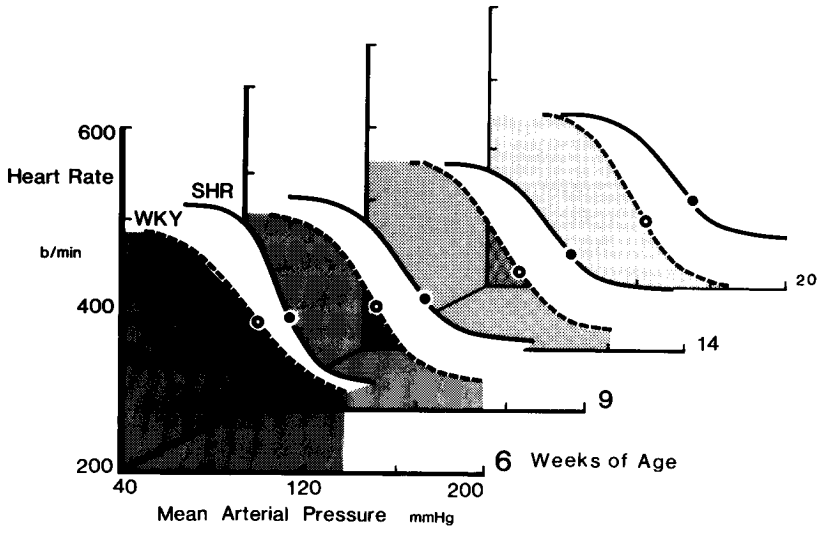


Fig. 1: Baroreceptor-heart rate reflexes during development from 6 to 20 weeks of age in normotensive rats (WKY) and spontaneously hypertensive rats (SHR). Note the reducing slope and range of the SHR curves as they mature and hypertension develops.

Influence of cardiac baroreceptors and endogenous opiates on renal nerve activity during haemorrhage

S.L. Burke, P.K. Dorward

We studied the relationship between blood pressure and renal nerve activity during either moderate or severe haemorrhage and compared the effect of blocking input from cardiac sensory receptors during blood loss, with that of blocking opiate synapses in central nervous system by naloxone.

During moderate haemorrhage we found a rise in renal nerve activity and that the effector response of the renal sympathetic baroreflex was inhibited slightly. When blood loss was severe (more than 30% of the blood volume) we saw an abrupt fall in nerve activity and in blood pressure, which was accompanied by marked attenuation of the effector response range and of alterations in some of the other parameters of the reflex.

After giving intravenous naloxone to block the central opiate synapses or after giving intrapericardial procaine to block the afferent nerves from the heart, the falls in nerve activity and in pressure after severe blood loss were prevented but the increase in renal nerve activity seen during moderate haemorrhage remained unaffected. Naloxone prevented the inhibition of the range of the baroreflex at both levels of haemorrhage, while pericardial procaine prevented about 60% of the inhibition seen during severe haemorrhage without affecting that found during moderate haemorrhage.

We conclude that cardiac, but

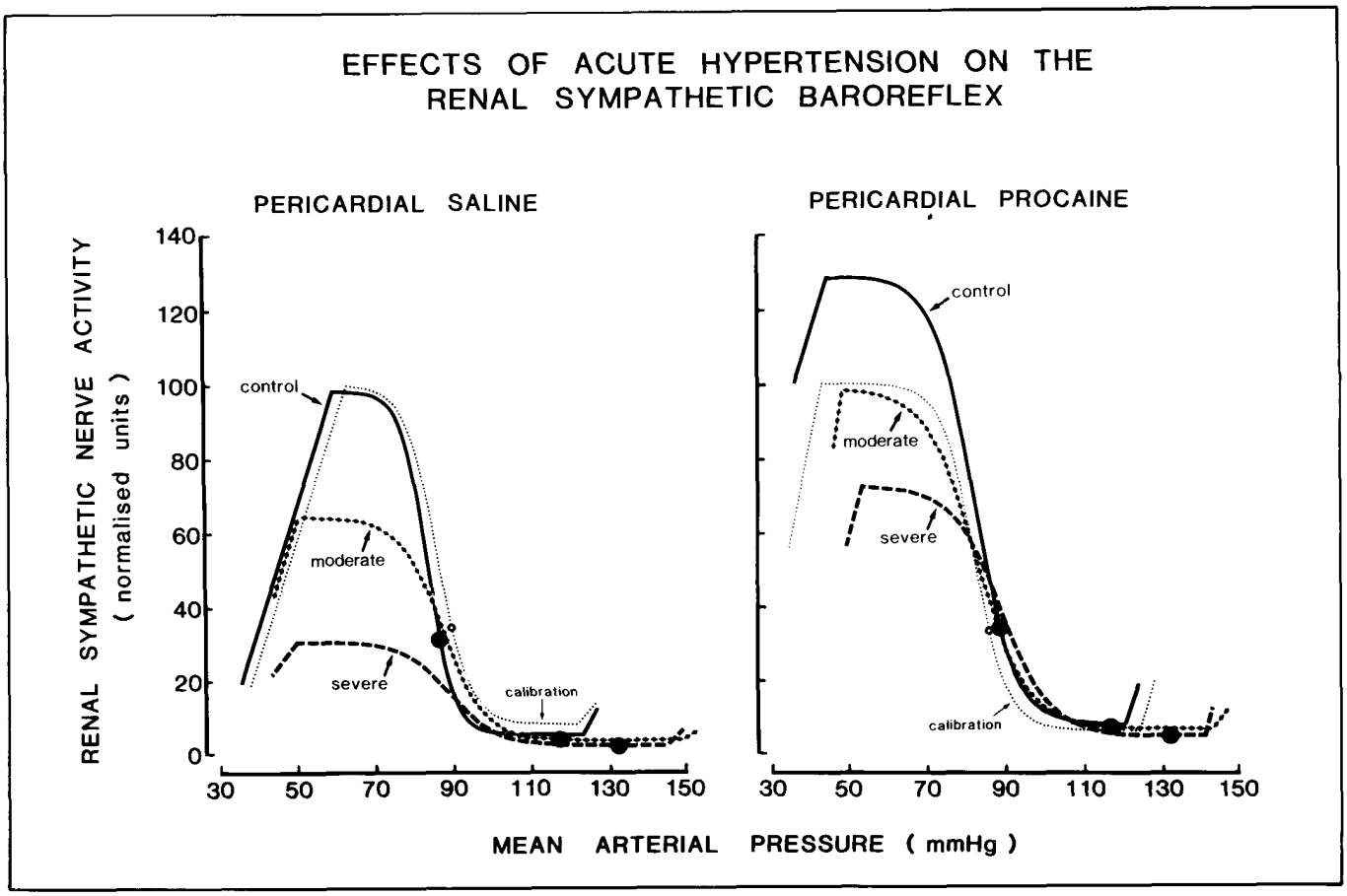


Fig. 2: Graph of renal sympathetic baroreflex function curves, relating mean arterial pressure to renal sympathetic nerve activity in conscious rabbits, with implanted nerve electrode and pericardial catheter. The results show that acutely raising resting blood pressure (large solid symbols on each curve) depresses the upper plateau of the reflex (control = no treatment; moderate = increase of resting pressure 26 mmHg; severe = increase of 41 mmHg). Left panel shows responses with all afferents intact (pericardial saline). Instillation of pericardial procaine (right panel) reduced the depression by about 30%, as discussed in text.

not arterial, baroreceptors have an opiate synapse on the reflex pathways to the renal nerve. A major part of the action of naloxone during haemorrhage can be explained by blockade of this type of synapse on pathways that project to the renal, and probably to other sympathetic vasoconstrictors. The presence of 'cardiac receptor'-resistant but naloxone-sensitive mechanisms during haemorrhage, suggests a role for extra-cardiac baroreceptors with opioid central nervous connections.

Time dependent changes in the renal sympathetic baroreflex

L.B. Bell, P.K. Dorward

Our initial studies of the renal sympathetic baroreflex in conscious rabbits established that the reflex was stable over a period of 3 hours, when a 1 hour episode of cardiac baroreceptor blockade occurred between curve estimates. In recent experiments, we noticed a reduction in the upper RSNA plateau of approximately 15% between morning and afternoon experiments in rabbits with intact cardiac afferents.

We studied a time protocol with triplicate estimates of the reflex during 8 time periods, 4 in the morning and 4 in the afternoon separated by a 1-2 hour rest period. We found that the upper plateau of the reflex curve was reduced by about 20% over the 5 hour experimental period. In other rabbits, given a greater number of balloon inflations, the reduction in upper plateau over the day was greater indicating that both the number of reflex estimates and the duration of the experiment may be influencing the magnitude of the reflex. The attenuation of the baroreflex over the experimental period was prevented by blocking afferent input from cardiac receptors with pericardial procaine.

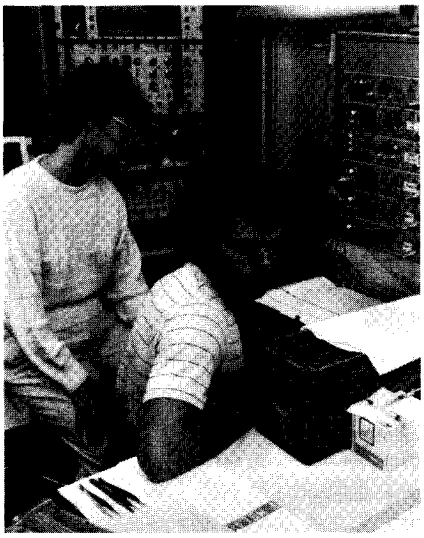
Renal sympathetic baroreflex in acute hypertension

P.K. Dorward, L.B. Bell, C.A. Rudd

We have studied the effect on the renal sympathetic baroreflex of acutely raising blood pressure with vasoactive drugs. In an earlier study, we found that mild phenylephrine-induced hypertension (+8 and +12 mmHg) produced parallel resetting of the reflex curve to higher pressures as expected from the resetting of the arterial baroreceptors, but after a pressure increase of +20 mmHg the BP_{50} (location) of the curve returned to the control location, in spite of the continuing baroreceptor resetting. In the current series, we have produced larger pressure rises with methoxamine infusion in the presence of cardiac efferent nerve blockade. Moderate hypertension (ΔMAP, +26 mmHg) again prevented the expected resetting of the curve to higher pressures and in

addition caused a small reduction in the range and gain of the reflex. Severe hypertension (ΔMAP, +41 mmHg) greatly reduced reflex range and gain and continued to prevent curve resetting.

Blocking input from cardiac afferents did not affect the reflex inhibition or lack of curve resetting during moderate hypertension but attenuated the reduction in reflex range during severe hypertension by about 30%. The cardiac receptor component of hypertension-induced baroreflex inhibition is thus less than that found in haemorrhagic hypotension.



Dr. Pat Dorward and Carl Rudd studying renal sympathetic nerve activity in a conscious rabbit.

Differentiation of cardiac baroreflex properties by cuff and drug methods and naloxone in two strains of rabbits

M. Weinstock*, P.I. Korner, G.A. Head, P.K. Dorward

We obtained sigmoidal mean arterial pressure (MAP)-heart rate (HR) curves in two genetically related strains of rabbits (Groups I and II), bred in Israel. We examined the responses mediated through the vagus and sympathetic nerves, using the cuff and drug method to alter MAP. We also studied the effects of intravenous naloxone, which affected only Group I. With the cuff method the vagal and sympathetic contributions to the gain (sensitivity) of the reflex were both greater in Group I than in Group II, but after naloxone the difference was greatly reduced. With the drug method gain was similar in Groups I and II and was unaffected by naloxone. With both methods the vagal component of the HR range between the tachycardia and bradycardia plateaus was greater in Group I than in Group II, due to more pronounced bradycardia at the lower plateau. The difference between the strains was eliminated by naloxone with the drug method, but was unaffected by the cuff method.

The cuff method produces relatively greater changes in cardiac load than the drug method, so that there is a difference in engagement of the receptor groups involved in the reflex. We have derived an input-output model, which suggests that with the drug method gain is almost entirely determined by the input from the arterial baroreceptors. This accounts for the small difference in gain between Groups I and II with this method, which simulates normal physiological

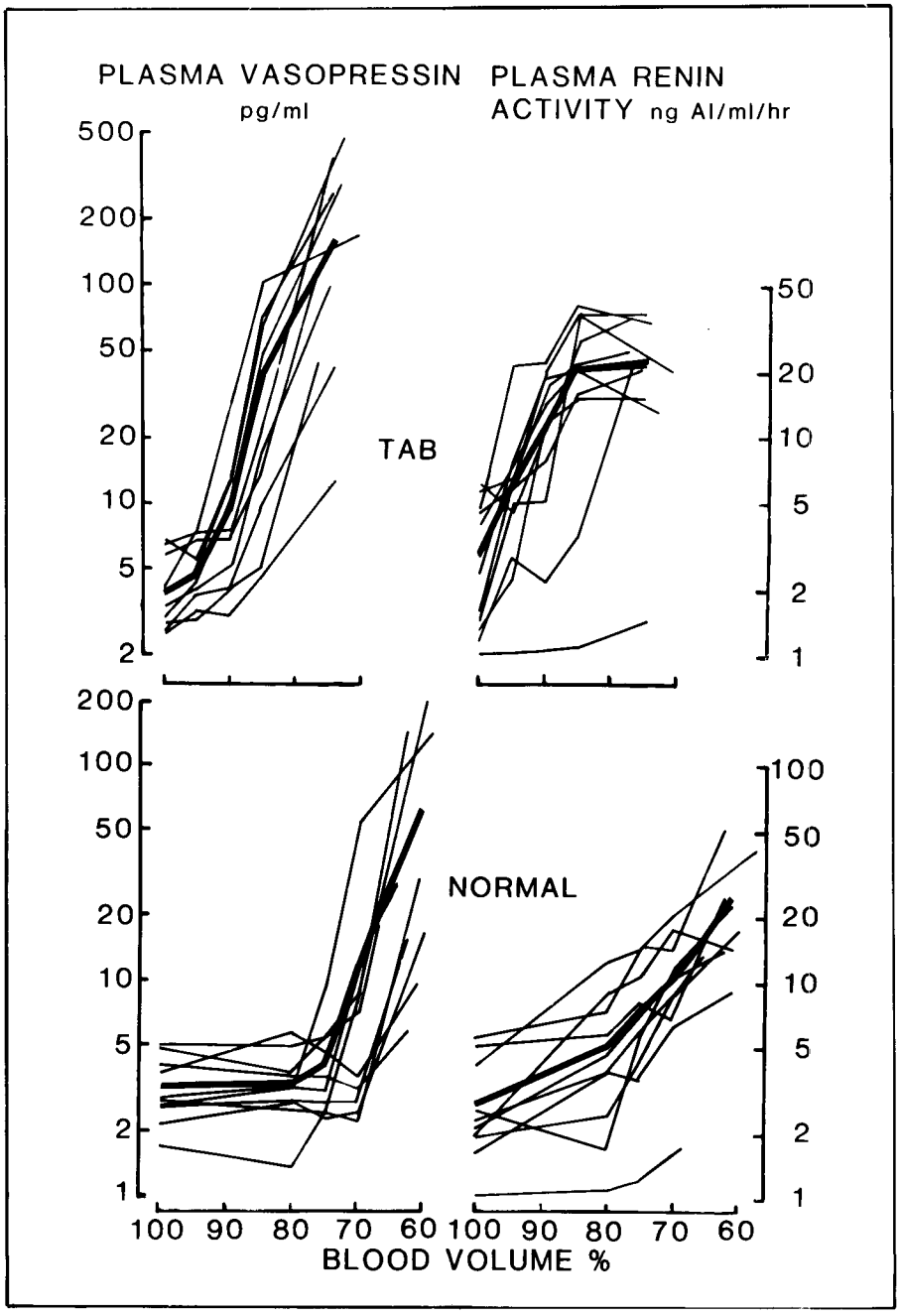


Fig. 3: Secretion of the hormones vasopressin and renin during haemorrhage in normal and total autonomic blocked (TAB) rabbits. Both hormones show reduced blood volume threshold after TAB.

(* Department of Pharmacology, Hadassah Medical School, Jerusalem)

alterations in vascular tone. With the cuff method, the gain is determined by a non-linear interaction in the central nervous system, involving the inputs from the arterial and cardiac baroreceptors, which accentuates the response. The central opiate mechanism is associated with the input from the cardiac baroreceptors and has amplifying properties, which account for the difference in gain between Groups I and II. The two methods provide a novel way of differentiating the effector pattern of the reflex, whilst naloxone serves as a marker of activity in a particular pathway.

Autonomic blockade alters threshold of arginine vasopressin (AVP) and plasma renin activity (PRA) responses to haemorrhage in rabbits

P.I. Korner, J.R. Oliver, R.L. Woods

Our previous haemodynamic studies have suggested that after complete blockade of the autonomic nervous system with mecamylamine there was enhancement of constrictor effects mediated through AVP and through the renin-angiotensin system. In the present study we examined this hypothesis directly in 10 rabbits. In each rabbit we studied the effects of haemorrhage on blood pressure (BP) and heart rate and on AVP and PRA concentrations, by determining blood volume-circulatory response curves and the blood volume-plasma hormone concentration relationship. In each animal we examined the effects of haemorrhage at the rate of 2 ml/min and 4 ml/min under normal conditions and after total autonomic blockade (TAB) with the ganglion blocking drug mecamylamine (10 mg/kg); after TAB the animals received a constant infusion of noradrenaline to maintain BP close to normal. In normal rabbits bleeding was continued to the breakpoint at which there was a sudden fall in BP, whilst after TAB blood was removed until BP fell to 47 mmHg and the blood was then reinfused.

In each rabbit the resting concentrations of AVP and PRA were unaffected by TAB. However, the BV threshold at which those hormones began to increase was markedly affected. There was a parallel shift after TAB in the hormone-concentration curves. With AVP a concentration of 20 μg/ml was normally reached after removal of 36% of the blood volume, with all effectors intact, but after TAB only 16% of blood volume was required to reach the same concentration. There was a similar shift in blood volume-PRA relationship. Current work is in progress to examine the afferent mechanisms that contribute to these shifts.

Judy Oliver and Dr. Carol-Ann Courneya, a visiting scientist from Canada, recording an experiment.

Sir Thomas Ramsey Electron Microscopy Laboratory and the Morphology Laboratory

Heads: S.E. Luff and C.R. Anderson

Projects

Quantitative electron microscopy of autonomic neuromuscular relationships in the cardiovascular system.

The relationship of descending bulbospinal pathways to sympathetic preganglionic neurons.

The neurochemistry of neurons in the C1 nucleus.

Summary

The Sir Thomas Ramsey Laboratory and the Morphology Laboratory have dual roles. They act as service laboratories, offering specialist skills in electron microscopy (in the Sir Thomas Ramsey Laboratory) and a range of histological techniques to other laboratories. They also carry out their own research.

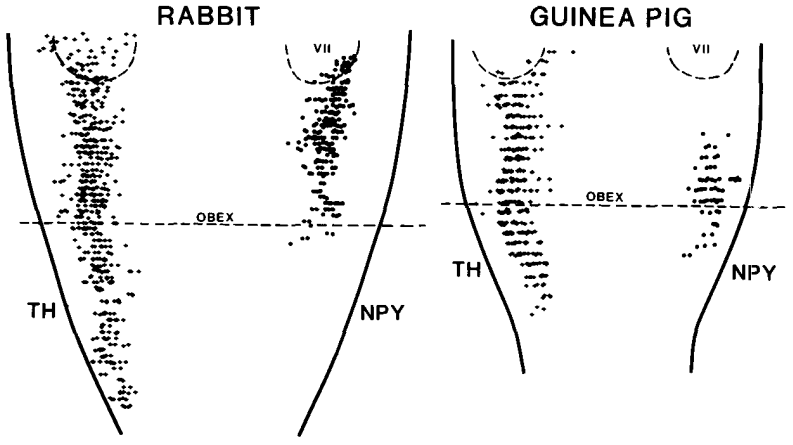
The Sir Thomas Ramsey Electron Microscope Laboratory is a new facility in the Institute, having now been in full operation for 12 months. A very generous donation from Sir Thomas Ramsey has enabled us to establish a new laboratory and purchase a new Jeol electron microscope. In 1987 we have added the necessary ancillary equipment to bring the facility to full operation.

In the Electron Microscope Laboratory we have been studying the relationship of nerve fibres to the muscle cells around blood vessels. We have shown that the fibres make a very close contact with the muscle cells they innervate. This information, which is in marked contrast with earlier beliefs, has important ramifications in interpreting the results of many previous experiments. We have now gone further to show that the close contacts are a common feature of many different vessels.

During this first year of operation the laboratory has also been involved in collaborative studies with other laboratories. With the Protein Chemistry and Molecular Biology Laboratory we have been involved in one project examining the interaction between lipoproteins and cells and another in which electron microscopy has been used to establish the homogeneity of various subcellular fractions. With the Biochemical Pharmacology Laboratory the electron microscope has been used to examine cultured smooth muscle cells to determine the degree of specialisation for contraction or for synthesis when grown under special conditions. In collaboration with the Cardiac Surgery Laboratory we have conducted studies to ascertain whether there are any structural differences in the ultrastructural appearance of the myocardium of dogs with infarcts with and without prior treatment with orotic acid. The preliminary evidence suggests that orotic acid treatment improves recovery of the heart muscle after a cardiac arrest.

In the Morphology Laboratory we have now completed work on a study of nervous pathways in the rat in collaboration with Dr. E. McLachlan. Specifically we have been investigating pathways from the brain to the spinal cord that control blood pressure. We have used a computer to reconstruct the exact location in three dimensions of both the nerves thought to control blood pressure and the neurons they innervate. We have shown that the pathways from the brain to the spinal neurons which control blood pressure are both direct and, via a relay neuron in the nearby spinal cord, indirect. Furthermore, some neurons considered to be involved in blood pressure control were shown not to receive any innervation at all from the pathways we examined, suggesting that some pathways have yet to be discovered. These studies are continuing.

In another series of experiments with Dr. McLachlan we have focussed on the location and chemical characterisation of the blood pressure



Computer reconstruction of the brainstem of rabbit and guinea pig, showing the location of neurons containing the enzyme tyrosine hydroxylase (TH, LHS of medullae) and neuropeptide Y (NPY, RHS of medullae). In the rabbit NPY is in some of the neurons that contain tyrosine hydroxylase where it marks a group of neurons thought to be vital in regulating blood pressure. In guinea pig, while there is a distribution of tyrosine hydroxylase neurons similar to that in the rabbit, it seems that NPY is marking quite a different group of neurons.

regulating neurons in the brainstem that send their projections down the spinal cord. We compared the same neurons in a range of different species and showed that the chemical characteristics of the neurons are different in different species. Such information is important as it is knowledge of the precise chemical nature of a neuron that allows the correct drug to be used to modify neuronal behaviour when blood pressure control goes awry. It now seems that it cannot be assumed that the neurons of all species, including humans, are exactly like those of common experimental animals.

Other laboratories in the Institute have also made use of the facilities of the Morphology Laboratory. In collaboration with the Circulatory Control Laboratory we have used immunohistochemistry and three-dimensional reconstruction techniques to monitor the site and extent of neurotoxin lesions in the ascending catecholamine bundles of the rabbit midbrain. As part of the study on genetic hypertension a technique has been developed for counting the number of dividing muscle cells in the blood vessel walls of new born rats before they develop hypertension. This information will demonstrate the contribution that dividing muscle cells make to the narrowing of the vessel lumen and the thickening of the wall that results in this disorder.

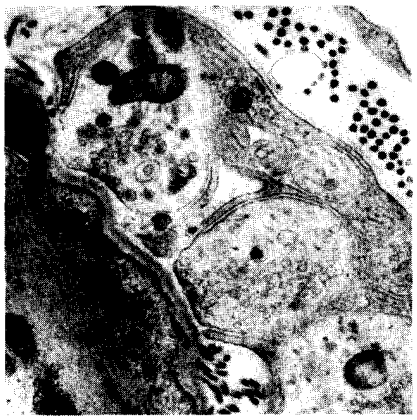
Quantitative electron microscopy of autonomic neuromuscular relationships in the cardiovascular system

S.E. Luff, E.M. McLachlan

Recently we showed that conventional neuromuscular junctions are formed by most of the varicosities in the plexus overlying the submucosal arterioles in the guinea pig. This year, we have examined the innervation of terminal arterioles and also determined the frequency of neuromuscular contacts in larger arteries in several species.

In studies of very small arterioles (10-25 fm) supplying the guinea pig mucosal epithelium, we found that the sympathetic innervation was restricted to clusters of adrenergic varicosities that occur at the branch points. These provided the only innervation to the vessels and formed close neuromuscular contacts. We observed small, presynaptic membrane specialisations, resembling those found between neurons, on approximately 50% of the larger varicosities. There were many small varicosities, some of which formed contacts, but these were generally small in area and had few vesicles. The contacts formed by small varicosities did not have presynaptic membrane specialisations.

In other studies we have identified neuromuscular junctions on larger arterial vessels, both muscular and elastic, from rats, rabbits and guinea pigs. In general, most muscular arteries, which vary in diameter from 150-1500 μm, were found to have junctions similar to those in the submucosal arterioles. However, while some large elastic arteries such as the rat and guinea pig superior mesenteric and the rat renal arteries have close contacts, the majority of large elastic arteries (particularly the aorta of all species) lack such contacts. The frequency of contacts is inversely proportional to the vessels' diameter although there are some differences between vessels supplying cutaneous and deep vascular beds. Taken together, our data provide important new information on the control of blood pressure in different vascular beds and will provide essential basic information for future work in hypertensive animals.



An electron micrograph showing two nerve varicosities forming close specialized junctions with a smooth muscle cell. Both varicosities have a thickened region of the membrane on the presynaptic side with vesicles aggregated towards it. Such neuromuscular junctions can be found in almost all muscular arteries. Most elastic arteries do not appear to have such junctions.

The relationship of descending bulbospinal monoamine pathways to sympathetic preganglionic neurons

C.R. Anderson, E.M. McLachlan

The location within the intermediate zone of the spinal cord of monoaminergic axons was compared to the distribution of retrogradely labelled sympathetic preganglionic neurons (SPN). The monoamine neurons were identified immunohistochemically by their content of tyrosine hydroxylase, phenylethanolamine-N-methyl transferase, 5-hydroxytryptamine or neuropeptide Y or by formaldehyde-induced fluorescence. While all histologically identified fibres were widely distributed throughout the autonomic subnuclei it is apparent that some SPNs, at the most rostral and caudal limits of the intermediolateral column, receive no innervation from the monoaminergic fibres studied. Furthermore, while SPNs are present in the midline over the central canal in T13 and L1, medially located SPNs are very rare at all other segmental levels. The presence of medially located aggregations of monoamine fibres at all segmental levels suggests that bulbospinal pathways may terminate on interneurons in the medial parts of the intermediate zone.

Human Autonomic Function Laboratory

Head: Dr. M.D. Esler

Projects

Release of noradrenaline from the heart during simulated stressful life events.

Pattern of sympathetic nervous activation with exercise.

Effects of physical conditioning on cardiac stress responses.

The sympathetic nervous response to a low salt diet.

Fainting attacks: nature of the sympathetic nervous deficit.

Neuronal uptake of noradrenaline in essential hypertension.

Release of dopamine and its metabolites into the cerebrovascular circulation.

Release of noradrenaline into the cerebrovascular circulation in patients with essential hypertension.

Circulatory and sympathetic nervous system in cirrhosis.

Sympathetic nervous response to mild experimental mental stress.

Study of the neural deficit in autonomic insufficiency syndromes.

Investigation of the contribution of cardiac sympathetic nervous overactivity to cardiac arrhythmias.

Mechanisms of steroid hypertension.

* See also Clinical Research Unit Report

Summary

We have applied our methods for measuring the rate of release of the sympathetic nervous system transmitter noradrenaline, to gauge the firing rate of the sympathetic nerves. Studies have been performed in a variety of new and clinically relevant contexts. These include testing the activity of the sympathetic nerves to the heart during experimental 'mental stress' (where excessive firing may lead to cardiac rhythm abnormalities), during exercise (where cardiac stimulation enhances athletic performance) and during fainting attacks (where the reduction of nerve firing towards zero contributes to the low blood pressure).

A further application of these methods has been in people placed on a low salt diet. Dietary salt restriction is commonly used as a non-drug way of treating high blood pressure. The blood pressure fall, however, is often disappointingly small. Our testing incriminates the sympathetic nerves to the kidney as one of the reasons why low salt diets are not always effective in patients with hypertension; the nerves to the kidney fire at an increased rate such as to make the body conserve sodium and thus largely overcome the effect of the dietary restriction.

New research has commenced to investigate the capacity of the body to inactivate noradrenaline. This will complement our studies of sympathetic nervous activity and noradrenaline release. Our earlier research had implicated a fault in the inactivation of noradrenaline after its release as a possible cause of high blood pressure. We can now perform precise studies of the mechanisms of noradrenaline inactivation on very small (3-5 mm) segments of veins obtained by biopsy from consenting patients. The work is ongoing.

In another new research development, we are investigating the function of the central nervous system (CNS), by measuring the rate at which CNS transmitters overflow into the internal jugular vein. Sampling from the jugular vein is possible during the course of diagnostic catheterization of the kidney veins or heart. One cogent reason for this line of research concerns the intimate links between CNS function and sympathetic nervous activity. It may prove possible to study the central nervous control of the circulation, and the importance of the sympathetic nervous system in circulatory control, by simultaneously measuring transmitter overflow from both central and sympathetic nervous systems. Research techniques of this type may provide insights into psychosomatic ('mind-body') aspects of cardiovascular disease.

Fainting attacks: nature of the sympathetic nervous deficit

M. Esler

The fainting reaction is a circulatory response characterized by low blood pressure and cardiac slowing. It is typically abrupt in onset, and may occur, particularly in young people, with prolonged standing or in response to unpleasant or threatening emotional experiences such as the taking of a blood sample by medical staff. The cardiac slowing during the fainting reaction has been attributed to the influence of the vagus nerve on heart rate. It is clear from our own observations that the reduction in heart rate is also due in part to reduced sympathetic nervous activity in the cardiac sympathetic nerves, and not solely to the action of the vagus. Cardiac sympathetic nerve firing, based on measurements of noradrenaline spillover from the heart, falls to near zero during syncope. In the one patient in whom observations are available, release of noradrenaline by the kidney also fell to zero. It is probable that during the fainting reaction there is abrupt and almost complete withdrawal of sympathetic nervous system tone.

Neuronal uptake of noradrenaline in essential hypertension

M. Elser, K. Sudhir, G. Lambert, G. Jennings

Noradrenaline release from sympathetic nerves is inactivated primarily by an uptake process which transports the transmitter back into the nerves. Faulty noradrenaline uptake could lead to persistently high levels of

transmitter near the receptors on which it acts. This would provide a plausible cause of high blood pressure resulting from over-stimulation of the heart and blood vessels. Some of our earlier findings did, in fact, suggest a defect in noradrenaline uptake in human hypertension.

We have now developed methods to investigate nerve uptake of noradrenaline more precisely using a small (0.3-0.5 cm long) fragment of hand vein obtained by biopsy from consenting patients with high blood pressure. The vein is immersed in a bath containing noradrenaline tagged with radioactivity. This noradrenaline is taken up into the nerves in the vein wall. The nerves are then electrically stimulated to make them release this 'tagged' noradrenaline. This is done both in the presence and absence of a drug which reduces noradrenaline uptake. The results so far suggest that transmitter uptake is faulty in veins from hypertensive patients. Overflow of noradrenaline from the veins of such patients is greater with stimulation, but the uptake-blocking drug has no additional effect on overflow.

Release of dopamine and its metabolites into the cerebrovascular circulation

M. Esler, J. Reid

Dopamine, like noradrenaline, is an amine transmitter distributed in the central nervous system. It is involved principally in locomotion, mood and behaviour. Central nervous system dopamine is, in addition, implicated in the circulatory adjustments in mental stress reactions. We tested, using internal jugular vein sampling, whether overflow of dopamine (and a major metabolite, DOPAC) occurs into the circulatory system. Similar to our finding with noradrenaline, net spillover into the cerebrovascular circulation was demonstrated. The disorder in which abnormal function of dopamine is most clearly implicated is Parkinson's disease (an incapacitating affliction of body movement); here dopaminergic underactivity has been demonstrated. Measurements of cerebral dopamine overflow quite clearly might have application beyond the boundaries of cardiovascular research!

Release of noradrenaline into the cerebrovascular circulation in patients with essential hypertension

M. Esler, G. Jennings, G. Lambert

The conventional view is that noradrenaline, released as a neurotransmitter in the brain, does not pass into the blood stream; a 'blood-brain barrier' is thought to block its overflow. We have tested directly for spillover of noradrenaline from the brain by sampling via high right and left internal jugular venous catheters in 19 untreated patients with essential hypertension. A demonstrable release of noradrenaline into the cerebrovascular circulation was detected. The concentration of noradrenaline was 14.9% higher in right jugular venous than arterial plasma, and 25.7% higher in left jugular venous plasma. Mean cerebral noradrenaline spillover accounted for 12% of total noradrenaline release to plasma in the hypertensive patients. Release of noradrenaline from the brain to the blood stream appeared not to be due to disruption of the blood-brain barrier by hypertension, since radiolabelled noradrenaline did not readily pass in the reverse direction, from blood to brain; mean cerebral extraction of tritiated noradrenaline was only 6%, compared with 40-70% by other organs. The ganglion blocker, Arfonad, was administered to determine whether brain cells or cerebrovascular sympathetic nerves were the source of noradrenaline release. Since whole body noradrenaline spillover (largely derived from sympathetic nerves) fell by 40% with Arfonad, but cerebral spillover was not reduced, the noradrenaline overflow appears to originate from brain neurons. Whether jugular venous noradrenaline measurements can provide a direct 'window' into brain mechanisms in essential hypertension is under investigation.

Sympathetic nervous system response to mild experimental 'mental stress'

M. Esler, G. Jennings, G. Lambert

Measures of plasma noradrenaline concentration and of noradrenaline spillover to plasma were used to estimate the degree of sympathetic nervous system activation during cognitive challenge (forced mental arithmetic) in 12 patients with untreated essential hypertension. The relation which exists between the rate of sympathetic nerve firing in an organ and the overflow of noradrenaline into its venous drainage provides an experimental justification for this use of transmitter measurements to quantify stress responses in the sympathetic nervous system.

When plasma noradrenaline measurements are used to quantify human sympathetic nervous responses to laboratory-induced stress, particular problems arise from the non-homogeneity of the sympathetic nervous system activation which occurs in stress responses. This is especially so if antecubital venous blood sampling is used. Antecubital venous blood is largely representative of the venous drainage from skeletal muscle. Since sympathetic nervous outflow to muscle in humans tends to fall in stress reactions, peripheral venous plasma noradrenaline measurements are not well suited for monitoring the human stress response. The plasma noradrenaline concentration in arterial blood provides a better guide, since it represents a composite of all inputs of noradrenaline into the circulation. The extent of cardiac sympathetic nervous activation, which may often be of central importance in adverse reactions to stress, however, cannot readily be inferred from arterial plasma noradrenaline values, since the heart is responsible for only a small proportion of the total release of noradrenaline to plasma. Radiotracer-derived measures of cardiac noradrenaline overflow provide precise information on the function of the sympathetic nerves of the heart and could provide a useful tool for the study of psychosomatic aspects of diseases of the heart and circulation. Examples of such research would be studies testing the possibility that increased sympathetic nervous outflow to the heart constitutes a causal link between Type A (aggressive, overstriving) behaviour pattern and coronary heart disease, or between life stress and disordered cardiac rhythm.

Lipoprotein Biology Laboratories

Lipoprotein Biochemistry Laboratory
Head: Dr. G.J. Hopkins

Lipoprotein Physiology Laboratory
Head: Dr. P.J. Barter

Protein Chemistry & Molecular Biology Laboratory
Head: Dr. N.H. Fidge

Projects

Effects of hepatic lipase and lipoprotein lipase during *in vitro* incubation of rabbit plasma lipoproteins.

Role of lipid transfer and hepatic lipase on the particle size and composition of LDL and HDL.

Isolation of an HDL conversion factor from human plasma and its activation by apolipoprotein A-IV.

Apolipoprotein A-I inhibits changes in HDL particle size induced by incubation of human plasma.

Isolation of two high density lipoprotein binding proteins from human and rat liver plasma membranes.

Ligand specificity of the HDL receptor.

Distribution of apolipoprotein A-IV in human plasma measured by a new solid phase (ELISA) immunoassay.

Molecular biology studies.

Purification of plasma apolipoproteins by HPLC.

Summary

The Lipoprotein Biology Group has been further strengthened during 1987 by the recruitment of Dr. Alana Mitchell. Alana, with the support of Ha Ying Chea, Noel Fidge and Tim Tetaz, has set up a functioning molecular biology facility which will vastly expand the scope of the questions being addressed by the group. As now constituted, the Lipoprotein Biology Laboratories are equipped to address issues that span a wide spectrum of disciplines. We are able to interrelate and to integrate very clinical studies with more basic studies of physiology and biochemistry; we can also draw on the resources of our immunology laboratory, our state of the art protein chemistry laboratory and now on our molecular biology facility. Over the next 5 years this integrated group of laboratories will make a considerable contribution to understanding the relationship between plasma lipoprotein metabolism and atherosclerosis.

Another high point of 1987 was the arrival of Dr. Christian Ehnholm who has come from Helsinki to spend a 1 year sabbatical with the group. Christian is a talented and experienced lipoprotein researcher and his expertise and enthusiasm has been an enormous boost to the hepatic lipase and the high density lipoprotein binding protein projects.

In 1987 we continued our studies of high density lipoproteins (HDL), the lipoprotein fraction that protects against coronary heart disease. We have also been examining how HDL interrelate with some of the other lipoprotein fractions which are known to cause coronary heart disease and, in the process, have made some observations of potential importance in understanding the metabolism of the atherogenic low density lipoproteins (LDL). This work, performed by Harvey Newnham, Moira Clay, Garry Hopkins and Philip Barter, may develop into one of the principal future projects of the group.

During 1987 we made real advances in the isolation and characterization of HDL binding proteins. The contribution to this project made by Minoru Tozuka, a visiting scientist from Japan, cannot be overstated. His work in collaboration with Noel Fidge and Boris Grego has been most impressive. Similarly impressive has been the progress made by Rajaram, Linus Chang and Boris Grego in the project seeking to isolate the HDL conversion protein.

The past year has seen the beginning of our attempts to bring the techniques of molecular biology to our physiological projects. We have obtained the genes for rat hepatic lipase (provided by Dr. Michael Schotz from Los Angeles) and for human lipid transfer protein (provided by Genentech, San Francisco). Now, in collaboration with Drs. Malcolm Brandon and Tim Adams from the Veterinary School, University of Melbourne, we are planning to introduce the hepatic lipase gene into rabbits (a species naturally deficient in the enzyme) and the lipid transfer protein gene into rats (a species naturally deficient in lipid transfers). These studies will provide definitive information about the roles played by these important proteins in either causing or protecting against coronary heart disease.

Finally, 1987 has seen the merging of several smaller groups into the single large entity we now call the Lipoprotein Biology Laboratories. The success of this venture has depended on many people, not the least of whom are the research assistants and technicians (Deidre De Silva, Shona Devlin, Hubert Edelsbacher, Ernie Gruner, Elaine Kecorius, Arezzo Khorrami, Trish Nugent, Gulweig Reid, Lily Salvatore, Tim Tetaz, and Sheela Unnithan), who have actually done the work.

Effects of hepatic lipase and lipoprotein lipase during *in vitro* incubation of rabbit plasma lipoproteins

M. Clay, G.J. Hopkins, P.J. Barter

Rabbits are very susceptible to the development of diet-induced atherosclerosis. When fed cholesterol, rabbits accumulate very high concentrations of a plasma lipoprotein that deposits cholesterol into arterial walls and causes the disease. Even when

fed normal, low cholesterol diets, rabbits possess a pattern of plasma lipoproteins which differs from that in other species, especially from that in an atherosclerosis resistant species such as the rat. The pattern of lipoproteins in humans is intermediate between that of rabbit and rat.

Rabbit LDL and HDL consist of particles which are larger and contain more triglyceride than the corresponding fractions in human plasma. Compared to humans, rabbits are also deficient in activity of hepatic lipase. Lipoprotein lipase activity in rabbits, however, is comparable to that in humans. We have tested the hypothesis that the enlarged triglyceride-rich HDL and LDL in rabbits are a consequence of the relative deficiency of hepatic lipase.

Rabbit lipoproteins (d < 1.21 g/ml plasma fraction), supplemented with albumin, were either kept at 4°C, or incubated at 37°C for 6 h in: (i) the absence of added lipase, (ii) the presence of hepatic lipase purified from dog post-heparin plasma, and (iii) the presence of lipoprotein lipase purified from bovine milk. Following incubation, samples were subjected to gel-permeation chromatography to define the distributions of protein, cholesterol, phospholipid and triglyceride across the lipoprotein spectrum. Samples were also subjected to gradient-gel electrophoresis on 2-16% and 4-30% polyacrylamide gels to determine the particle size distribution of LDL and HDL, respectively. Incubation at 37°C in the absence of lipase had no effect on the lipoproteins. The presence of hepatic lipase induced marked reductions in HDL triglyceride and to a lesser extent, LDL triglyceride. The effect on VLDL triglyceride was minimal. These changes were associated with reductions in the particle size of both HDL and LDL. Thus, following incubation with hepatic lipase the rabbit HDL and LDL resembled their human counterparts in terms of both composition and particle size. By contrast, incubation of rabbit lipoproteins in the presence of lipoprotein lipase had no effect on HDL and minimal effect on LDL. In this case, the lipase acted primarily on VLDL triglyceride.

It has been concluded that the enlarged triglyceride-enriched LDL and HDL in rabbit plasma reflect the deficiency of hepatic lipase in this species. These studies have also demonstrated a marked difference in substrate specificity of lipoprotein lipase and hepatic lipase; whereas lipoprotein lipase acted preferentially on VLDL, the preferred substrate for hepatic lipase was HDL.

Role of lipid transfer and hepatic lipase on the particle size and composition of LDL and HDL

H. Newnham, G.J. Hopkins, P.J. Barter

We have shown previously that the size of HDL particles in human plasma correlates inversely with the concentration of triglyceride-rich lipoproteins. We established that this is a consequence of lipid transfers between HDL and triglyceride-rich lipoproteins followed by activity of hepatic lipase which hydrolyses the triglyceride which had been transferred to the HDL. The end result is the formation of smaller HDL particles (radius 3.9 nm) which resemble those in the plasma of many subjects with premature atherosclerosis.

It has also been reported that the particle size of LDL in human plasma correlates inversely with the concentration of triglyceride-rich lipoproteins. This is potentially important since smaller LDL appear to be much more atherogenic than are larger ones. It has been postulated by others that the particle size of LDL is a function of the larger particle from which it is derived. We, however, have hypothesized that the mechanism explaining the varying particle size of LDL is the same as that we established for HDL. We have now performed several studies in which the results have been consistent with this hypothesis. We have observed a marked reduction in the size of human LDL particles during incubations performed under the same conditions as shown previously to reduce the size of HDL. These studies are now being extended into an examination of factors regulating the particle size of the atherogenic VLDL remanants.

Isolation of an HDL conversion factor from human plasma and its activation by apolipoprotein A-IV

O.V. Rajaram, L.B.F. Chang, N.H. Fidge, C. Ehnholm, P.J. Barter

The HDL in human plasma are heterogeneous in terms of particle size, density and apolipoprotein composition. There are two major subfractions: HDL_2 which contains particles of larger size and lower density and HDL_3 which contains smaller and more dense particles. The use of gradient gel electrophoresis to separate particles of varying size has revealed further heterogeneity, identifying at least two subpopulations of HDL_2 and three subpopulations of HDL_3. It has been shown *in vitro* that the size and density of HDL particles are influenced by several plasma factors and processes, including lecithin:cholesterol acyl transferase, lipid transfers, lipoprotein lipase and hepatic lipase. Recently, another factor has been identified, a putative HDL conversion factor which has been shown to modify the particle size distribution of human HDL_3. The most characteristic feature of this latter process is the appearance of a population of particles very much smaller than the original HDL_3. These small HDL are of potentially great importance in the process of reverse cholesterol transport by which tissue cholesterol is transported through the plasma to the liver for elimination from the body.

The HDL conversion factor has been partially purified from human plasma by ammonium sulphate precipitation, ultracentrifugation, cation-exchange chromatography, anion-exchange chromatography and chromatography on a column of hydroxy apatite. This resulted in a preparation that appeared on SDS polyacrylamide electrophoresis as one major and at least three minor bands. To date, attempts at further purification have resulted in a loss of conversion activity.

The role of apolipoproteins in the conversion process has been investigated. Apolipoprotein (apo) A-I, apoA-II, a mixture of the C apolipoproteins and apoE were shown

to have no intrinsic conversion activity, nor to influence activity of the conversion factor. ApoA-IV, by contrast, although having no intrinsic conversion activity, had a marked, dose-dependent potentiating effect when added to preparations of the conversion factor. Furthermore, this activation by apoA-IV was apparent at physiological concentrations of the apolipoprotein and may therefore be of major importance in the regulation of HDL subpopulation distribution and reverse cholesterol transport.

Apolipoprotein A-I inhibits changes in HDL particle size induced by incubation of human plasma

G.J. Hopkins, P.J. Barter

Incubation of human plasma *in vitro* results in large changes in the particle size of HDL. We have investigated whether these changes are modulated by apoA-I. Human plasma or VLDL-deficient plasma, either alone or supplemented with graded amounts of purified human apoA-I, was kept at 4°C or incubated at 37°C. Incubations at 37°C were performed (i) in the presence of lecithin: cholesterol acyltransferase (LCAT) activity, (ii) in the presence of a chemical inhibitor of LCAT, and (iii) in the presence of an artificial triglyceride emulsion (Intralipid) and lipid transfer protein activity. Following incubation, the HDL was recovered and subjected to gradient gel electrophoresis to determine the HDL particle size.

Incubation of plasma at 37°C for 7 h in the presence of LCAT activity resulted in increases in HDL particle size and the formation of particles within the HDL_2 size range. This increase was markedly inhibited when the incubated plasma was supplemented with as little as 65 μg apoA-I/ml of plasma. Addition of 259 μg apoA-I/ml of plasma, corresponding to an 18% increase in the concentration of plasma apoA-I, completely abolished the increase in HDL particle size. ApoA-I added to incubations at 37°C did not inhibit nor activate cholesterol esterification. In control incubations, added apoA-I was without effect when the plasma was kept at 4°C or when the apoA-I was present only during the final hour of incubation at 37°C.

Incubation of the VLDL-deficient plasma at 37°C for 10 h in the presence of a chemical inhibitor of LCAT resulted in the appearance of populations of HDL which were both larger and smaller than HDL kept at 4°C. These changes in HDL particle size were even more pronounced when incubations were supplemented with Intralipid. As in the previous experiments, the addition of relatively small amounts of apoA-I markedly inhibited changes in HDL particle size.

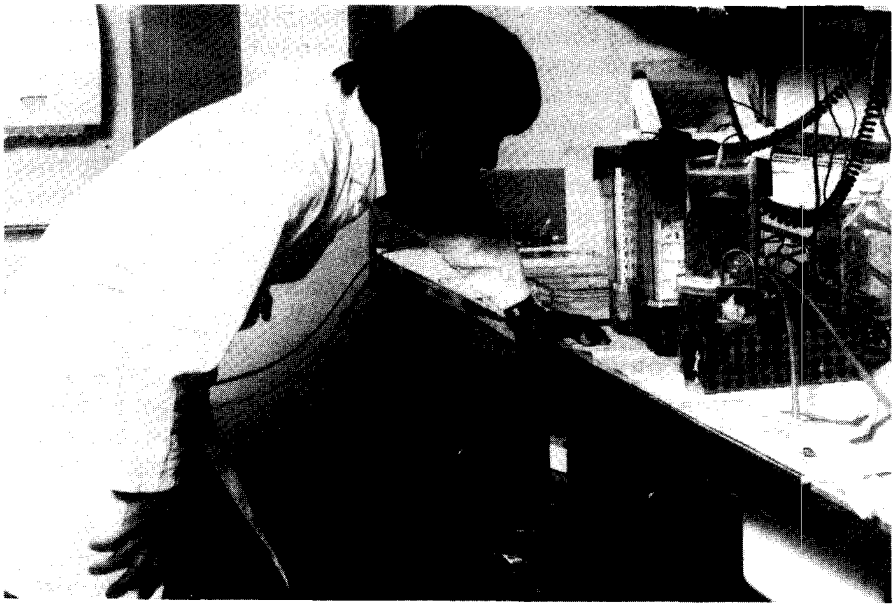
Thus, we have found that apoA-I has a major inhibitory effect on changes in HDL particle size that occur during incubation of plasma or VLDL-deficient plasma. This effect was observed within the reported concentration range of free apoA-I *in vivo* and may, therefore, be of physiological significance.

Isolation of two high density lipoprotein binding proteins from human and rat liver plasma membranes

N. Fidge, M. Tozuka, B. Grego, H. Edelsbacher, C. Ehnholm.

Reports from several laboratories suggest the existence of HDL-binding proteins in various tissues. Despite these observations, evidence is still lacking regarding the specificity and properties of these binding proteins and it is therefore still not possible to define their roles as possible receptors or as regulators of HDL metabolism. Evidence of specificity has been constrained by assay systems which do not permit a full investigation of these properties or enable quantitative assessment of these potential receptors. We have addressed the problem of an adequate assay system, and have developed a ligand blotting technique which enables us to monitor the purification of two HDL binding proteins.

Plasma membranes were isolated from rat or human liver, solubilised with detergent and then fractionated by various separation techniques. Samples were analysed by ligand blotting, using ^{125}I-labelled human HDL_3. Two HDL binding proteins were detected (approx. 100 and 120 K daltons); another 140 kDa was found in human placenta. All three proteins recognised apoA-I. The 120 and 140 kDa bands were sensitive but the 100 kDa band was insensitive to βmercaptoethanol. The placental protein was reduced to an 85 kDa protein which still retained its HDL binding properties. Apparent differences in specificity were noted, with the binding to the higher molecular weight liver protein being the most reduced by unlabelled HDL_3, although binding of none of the proteins to



Dr. Minoru Tozuka, a visiting scientist from Shinshu University, Japan, supervising a critical stage in electrophoresis of the newly discovered HDL binding protein.

HDL was significantly reduced by unlabelled LDL or EDTA. Preincubation of transblotted membranes with detergents or phospholipid but not cholesterol, appreciably influenced the intensity of binding.

Two of the liver membrane proteins have now been purified to apparent homogeneity and both retain the same binding properties seen in unfractionated preparations. Characterization of these proteins is now under way.

Ligand specificity of the HDL receptor

J. Morrison, P. Vadiveloo, B. Grego, N. Fidge

HDL contain two major apolipoproteins, A-I and A-II and current information supports the involvement of apoA-I as a major ligand recognised by the HDL receptor. However, some laboratories have shown that apoA-II may also play a significant role either through direct binding to the receptor or by influencing the spatial configuration of the ligand.

We are addressing these questions using two approaches. Firstly, we are asking whether a specific domain, or sequence of apoA-I is responsible for receptor binding and secondly, we are comparing the binding of two different HDL particles, LpA-I and LpA-II, with the putative receptor.

Four apoA-I fragments, designated CNBr I → IV, have been prepared by incubating human apoA-I with cyanogen bromide, and each has been tested in competitive binding studies for its ability to displace intact apoA-I from the receptor site. Displacement was most pronounced for CNBr IV (COOH terminus) which inhibited binding by 75% at 50 μg/ml. We are currently seeking to confirm these studies and then we propose to perform further fragmentation of CNBr IV to narrow down identification of the binding domain.

Distribution of apolipoprotein A-IV in human plasma measured by a new solid phase (ELISA) immunoassay

K. Kondo, P.J. Barter, N.H. Fidge

A widening of interest in A-IV apolipoprotein is apparent following recent reports about its possible role in cholesterol transport and high density lipoprotein metabolism. However, the unusual relationship between function and the distribution of this peptide still remains a puzzle and estimates of its plasma concentration and proportional distribution vary quite significantly between laboratories. We have attempted to exploit the simplicity, sensitivity and rapidity of the ELISA system for determining apoA-IV concentrations in human plasma with the objective of developing a reliable and accurate measurement of this important apolipoprotein.

After initial comparisons between competitive and 'sandwich' ELISA systems, the former method was chosen for further development, using a polyclonal (rabbit) antibody which was monospecific according to Western blotting against mixtures of apolipoproteins. Using appropriate dilutions of first and second (HRPO conjugated) antibody, a working range of 1.5-12 μg/ml apoA-IV was optimal. Measurement of over 100 samples has so far indicated mean values of 16.4 $\pm$ 5.4 mg % apoA-IV in normal human plasma.

Distribution of apoA-IV was determined after gel permeation of plasma through a column of Superose 12. Most apoA-IV was present either in the lipoprotein free fraction or in the HDL fractions, but some variation in the relative proportions was noted between individuals. After incubation at 37°C for 240 min significant amounts of apoA-IV redistributed into the HDL fraction. Analysis of the HDL fractions and of plasma samples by gradient gel electrophoresis and immunoblotting showed that apoA-IV in HDL exists only in particles of more than 4.1 nm radius whereas apoA-I is distributed across the whole size spectrum of HDL. The physiological significance of these observations and the effect of other plasma factors on the redistribution of apoA-IV are the subject of ongoing studies.

Molecular biology studies

A. Mitchell, Ha, Y.C., N. Fidge, T. Tetaz

The molecular biology laboratory is now established and several projects have commenced. Two of these projects involve the development of transgenic animals to enable us to study the physiological and pathological significance of two proteins thought to be important in the development of atherosclerosis. Both

Dr. Alana Mitchell and Peter Griffiths programming the DNA synthesiser in the new Molecular Biology Laboratory, to make oligonucleotide probes for cloning experiments.

projects have been hastened by collaborative agreements reached with Genentech and Dr. Michael Schotz. Genentech provided us with the human cholesteryl ester transfer protein gene and Dr. Schotz supplied the cDNA for rat hepatic lipase. Together with Drs. Tim Adams and Mal Brandon of the Veterinary School, University of Melbourne, we are preparing gene constructs suitable for insertion into rat or rabbit ova and the subsequent development of animals expressing either lipid transfer protein or hepatic lipase. Rats are naturally deficient in lipid transfer protein activity and are resistant to atherosclerosis; we postulate that the transgenic rats will produce atherogenic lipoproteins and possibly develop atherosclerosis. By contrast, rabbits are naturally deficient in activity of hepatic lipase and are highly susceptible to atherosclerosis. We postulate that they will produce lipoproteins which are less atherogenic if they are able to express hepatic lipase as a result of the gene introduction.

The unit also possesses clones to most of the apolipoproteins and experiments are planned to study factors which regulate the mRNA expression of several key apolipoproteins. This will be approached using hybridization studies to quantitate changes in tissues or cultured cells which result from drug, hormone or dietary manipulations. We also plan to produce expression systems in prokaryote or eukaryote cell lines for further studies of the synthesis of these important apolipoproteins.

Purification of plasma apolipoproteins by HPLC

B. Grego, T. Tetaz, E. Kecorius, C. Ehnholm, N. Fidge

The Protein Chemistry Laboratory is involved in a number of projects concerned with relating the structure of proteins to their function. The major rate-limiting step in obtaining such structure-function data is that of protein purification. Often, existing purification methods are inadequate and force us into investigating and developing new methods. In particular, we have been investigating high-performance liquid-chromatographic (HPLC) methods which separate proteins on the basis of differences in their hydrophobicity. Specifically, we have been investigating this technique in the purification of plasma apolipoproteins.

Interest in the structure and function of apolipoproteins has followed recent demonstrations of their important roles in lipoprotein metabolism, which include ligands for cell receptors, activators of lipolytic enzymes and possible determinants of lipoprotein particle size and concentrations. Strategies have been developed for isolating apolipoproteins but in many respects present methods are slow and inefficient. We have addressed the role of HPLC technology in improving purification techniques. We have now developed an HPLC method for the rapid purification of human and rat soluble apolipoproteins (A-I, A-II, A-IV, C's and E) in high yield from sources such as delipidated lipoproteins and lymph chylomicrons. Fractionation of apolipoproteins was achieved on a commercially available reversed phase column of very low hydrophobicity (TSK Phenyl 5PW). Delipidated apolipoproteins dissolved in 20mM phosphate, pH 2.3, were loaded onto the reversed phase column equilibrated with 20mM phosphate, pH 2.3. The adsorbed lipoproteins were eluted with an increasing gradient of acetonitrile concentration. Purified apolipoproteins were identified by a combination of SDS-polyacrylamide gel electrophoresis, specific antisera and N-terminal amino acid sequence. Analysis of the fractionated apolipoproteins revealed homogenous apolipoproteins with the following elution order: apoCs, apoA-II, apoA-IV, apoA-I and apoE. The method has several advantages over existing methods, and enables rapid purification in preparative yields by a one step procedure.

From L to R — Dr. Boris Grego, Elaine Kecorius and Tim Tetaz, caught between a crucial phase of HPLC separation and gas phase sequencing of the new HDL binding protein.

Pharmacology Laboratory

Head: Dr. J. Angus

Projects

Stabilization of EDRF released from cultured endothelial cells.

Comparative pharmacology of EDRF and NO on vascular and non-vascular smooth muscle.

Coronary artery reactivity in atherosclerosis.

Sympathetic neurotransmission in blood vessels — no role for the γ receptor.

Endogenous serotonin release causes renal artery spasm and the Bezold-Jarisch like reflex.

Collateral blood vessel reactivity in the rabbit hindlimb.

Effect of maturation and hypertension on the cardiac sympathetic neuroeffector junction in the rat.

Reactivity of small skin arteries and arm veins in untreated patients with hypertension.

Analysis of stuctural changes in the design of arteries and the consequences for reactivity in renovascular and genetic hypertension.

Pharmacology of the human internal mammary artery in relation to perioperative spasm.

Summary

Our main thrust is to determine the underlying causes of changes in vascular reactivity in coronary artery spasm, atherosclerosis and hypertension. The techniques we have developed include: (i) instrumented conscious rabbits to record regional blood flow during local infusion of drugs or during autonomic reflexes, (ii) the measurement of wall contraction of large arteries and veins in organ baths and (iii) the simultaneous measurement of wall force and membrane potential in very small resistance arteries in a myograph. In addition, we culture endothelial cells to provide local vasoactive hormones that are characterised by bioassay and chemical analysis.

One recent finding has been that sympathetic nerves innervating small resistance arteries release noradrenaline and possibly adenosine 5'-triphosphate (ATP) that have quite different actions on the smooth muscle cell. Noradrenaline contracts the cell via α_1-adrenoceptors, while ATP causes membrane depolarisation and a very small contraction. The two actions in concert would amplify the contraction to either substance alone. This project has benefitted greatly from collaboration with Dr. Mike Mulvany, a visiting scientist from Denmark and Dr. Arch Broughton, who has particular skills in electrophysiology. This area of research involves the mounting of 2 mm long segments of small arteries on 40 μm diameter wires in a myograph. Currently, we isolate human small skin arteries from a biopsy sample and coronary small arteries from the right atrial appendage which is discarded after coronary bypass graft operations. These specimens are being used to determine the nature of hormone receptors and the reactivity and electrophysiological properties of human blood vessels.

In other studies, we have been continuing our investigation of endothelium-derived relaxing factor (EDRF), a short-lived, powerful vasodilator which is released from the endothelial cells that line all blood vessels. Recently, EDRF has been identified in England as nitric oxide (NO). Our work with cultured endothelial cells has suggested that NO is released attached to a carrier molecule (i.e. R-NO). Nevertheless, we have confirmed and extended the observations that EDRF and NO behave in a very similar manner and thus support the proposition that EDRF is indeed NO or R-NO.

In further studies of vascular reactivity in hypertension we have combined *in vivo* and *in vitro* approaches. We have devised a new method to analyse the regional resistance changes of both vasoconstrictor and vasodilator stimuli in the hindquarter vascular bed of the conscious rabbit. This method enables calculation of the whole range of effective blood vessel diameter and has clearly demonstrated the importance of the resistance-amplifier in blood vessels with a thickened wall as occurs in hypertension. Careful analysis of the reactivity of isolated aorta and mesenteric small arteries of rats with genetic hypertension are providing insights into the changes that occur in blood vessels as the rats mature and as hypertension develops. The isolated organ chamber (aorta) and myograph (resistance arteries) are ideal techniques to test receptors and nerve-mediated responses. In addition, we have studied the morphology of each artery and directly related functional responses to the structural changes that occur as the wall thickens in response to the hypertension.

Working with human large internal mammary artery in the organ bath, Dr. He has characterised the receptors that may cause spasm of this vessel during coronary bypass graft surgery. Several vasodilator drugs have been assessed to determine the ideal drug for use in preventing this spasm. Serotonin release from platelets may be important in causing regional spasm in migraine, angina and peripheral vascular disease. Thus, Dr. Wright has characterised the regional vascular action of serotonin in conscious rabbits and has shown that when serotonin is released from platelets it can cause renal artery spasm and stimulate a vagal reflex. Dr. Wright has recently left to continue her postdoctoral studies at Sandoz, Switzerland.

Stabilization of EDRF released from cultured endothelial cells

T. Cocks, B. Grego, J. Angus

Cultured endothelial cells release EDRF in response to bradykinin. This EDRF can be assayed biologically by exploiting its capacity to relax a ring of dog coronary artery which has been depleted of its own endothelium. Because EDRF is very unstable, we have investigated ways of concentrating and stabilising the factor. EDRF was assayed either

directly at 37°C or collected via a cooling coil (0-0.5°C) for reassay 3 min later (Fig. 1). To reassay this latter material, the EDRF was re-heated (via a short coil) for 3-5 s just prior to superfusion over the artery ring. We recovered 70-90% of the cooled EDRF activity compared with that measured in the direct assay. The degree of transfer and the rate of loss of relaxing activity on storage at 0°C were increased and decreased respectively by the addition of the O_2—scavenger superoxide dismutase. The respective losses of activity with and without superoxide dismutase at 0°C were 0.07% ± 0.01 (n = 4) and 0.4% ± 0.05 (n = 5) for each minute of storage at 0°C. In other experiments, rapid cooling with liquid N_2 followed by freeze-drying resulted in preservation of EDRF activity for up to 3 weeks (Fig. 1).

These data suggest that EDRF is not released as a gas *per se* since it would not survive the freeze-drying under vacuum. In recent studies from the Wellcome Research Laboratories, Beckenham, Moncada has shown that EDRF is a gas, NO, as measured by chemiluminescence after acid treatment. Our results which show that EDRF activity is retained after freeze-drying, however, suggest that the factor may not be released from cells in a gasseous state. In other experiments, we have shown that NO

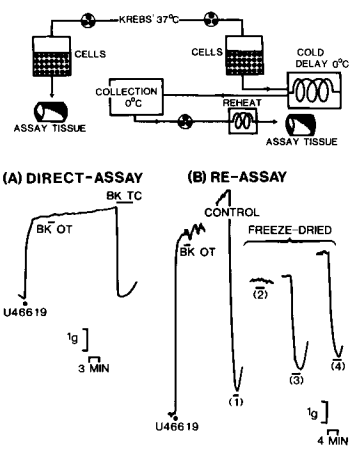


Figure 1
Top: Schema illustrating the direct bioassay (left) of EDRF or cooling in a delay coil before reheating and bioassay.
Bottom: Traces showing the relaxation of dog coronary artery by EDRF either directly or after freeze-drying and reconstitution.

Ms. Silvina Rainone culturing aortic endothelial cells for production of vasoactive hormones.

generated by acidified $NaNO_2$ has very similar biological activity to EDRF released from cultured cells. We postulate that the active moiety of EDRF is probably NO but that it is released from the endothelium coupled to a molecule of unknown structure.

Sympathetic neurotransmission in blood vessels — no role for the γ receptor

J. Angus, M. Mulvany, A. Broughton

Stimulation of the perivascular nerves of small resistance arteries (150-300 μm diameter) suspended in a myograph causes a contraction and membrane depolarisation of the smooth muscle cells. Short trains of stimuli (0.2-0.25 ms pulse width) at 25 Hz for 3 s cause a large contraction (F) and depolarisation of 10-20 mV (control, Fig. 2). In the presence of the α_1-adrenoceptor antagonist prazosin (PRAZ, Fig. 2) the contraction is markedly attenuated but the depolarisation is unaffected. The ATP receptor desensitizing agent, α,β-methylene ATP, completely prevented the initial membrane depolarisation but left the contraction at almost normal levels. Simultaneous addition

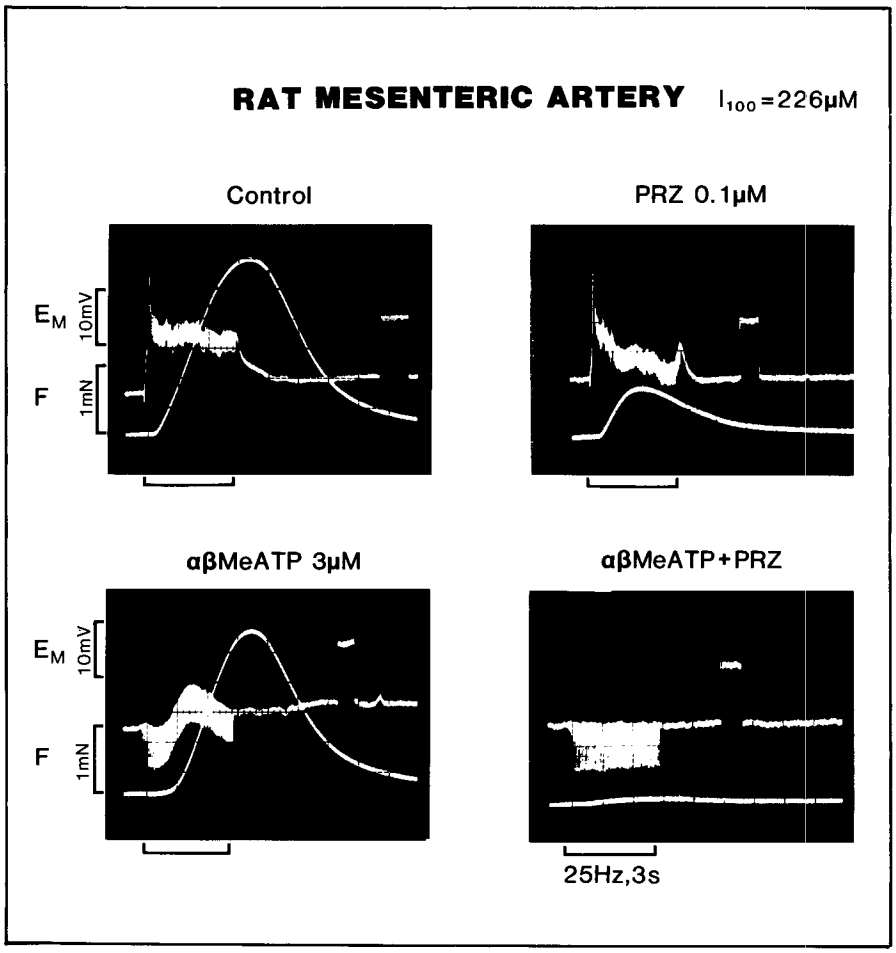


Figure 2
Simultaneous oscilloscope records of membrane potential (E_M) and isometric force (F) of small mesenteric resistance arteries of diameter 226 μm in the myograph. These traces show how the contraction and membrane potential can be dissociated and support the concept of co-transmission.

of both drugs abolished both responses. These results are consistent with the sympathetic nerve terminal releasing both noradrenaline and ATP (as a co-transmitter) and an action post-junctionally on specific receptors. The ATP causes the initial depolarisation and perhaps contributes to a small contraction. Noradrenaline causes a late but small depolarisation and a large contraction. These results were found in rat, guinea-pig and rabbit mesenteric small arteries. Our results do not support the speculation of a novel "γ" adrenoceptor but are consistent with the co-transmission hypothesis.

Endogenous serotonin release causes renal artery spasm and the Bezold-Jarisch like reflex

C. Wright, J. Angus

In previous studies we classified the various cardiovascular actions of serotonin in conscious rabbits. 5-HT_2-receptors mediated renal artery vasospasm while 5-HT_3-receptors mediated the 'Bezold-Jarisch like' (B-J) bradycardia reflex. The classification of 5-HT_2- and 5-HT_3-receptors was supported by specific receptor antagonists. In the case of 5-HT_1-receptors that possibly mediate vasodilatation, no specific antagonist was available. To aid in this classification, we tested the serotonin analogue 5-carboxamidotryptamine (5-CT) which is considered to be 5—HT_1-specific on many isolated tissue assays. However, this agonist caused renal artery spasm (5-HT_2) and elicited the B-J reflex (5-HT_3) at doses 5-10 times lower than serotonin, as well as being a vasodilator of the hindlimb as predicted (5-HT_1) (Fig. 3). One explanation was that 5-CT was causing release of 5-HT from the platelets, thereby losing any specificity for the 5-HT_1 receptor *in vivo*. Reserpine pretreatment for 24 h prior to an experiment lowered the total serum serotonin level by 97.7%. 5-CT was now unable to elicit the B-J reflex nor the renal artery spasm but still caused peripheral vasodilatation. These data show how important platelet serotonin can be in causing vasospasm and suggest that any specific 5-HT_1-agonist should be tested for inducing platelet serotonin release. This work is an example of the importance of *in vivo* preparations in drug research and illustrates that specificity in isolated tissue assays may not translate into the intact animal.

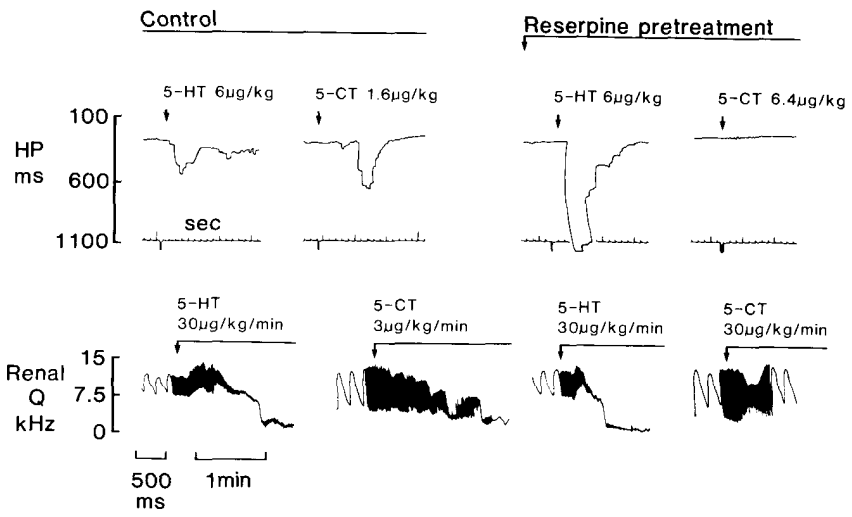


Figure 3
Chart records of heart period (HP) and renal blood flow in conscious rabbits before and after reserpine to deplete serum serotonin. The potent 5-HT_1-receptor-specific agonist 5-carboxamidotryptamine only elicits the 5—HT_2-mediated renal spasm and 5-HT_3-mediated Bezold-Jarisch reflex by releasing platelet serotonin.

Effect of maturation and hypertension on the cardiac sympathetic neuroeffector junction in the rat

A. Dyke, J.A. Angus, P.I. Korner

The greater sympathetic nervous system activity in young spontaneously hypertensive rats (SHR) by comparison with normotensive (WKY) rats may contribute to the development of hypertension in these animals. This may indicate an increase in nerve traffic, but may also reflect defects in neuronal uptake or reduced autoinhibitory feedback with a consequent increase in junctional concentrations of transmitter and post-junctional receptor stimulation. We have used the technique of tachycardia in the rat isolated right atrium to measure transmitter release during sympathetic nerve stimulation of 1-16 field pulses. Right atria were removed from male SHR and WKY rats of 4, 9, 14, 20 and 50 weeks of age.

The post-junctional β-adrenoceptor was apparently fully coupled by 4 weeks of age and there was no difference in the range of the tachycardia nor in the sensitivity (EC_{50}) between SHR and WKY atria. This differs from the slower maturation of the α-adrenoceptor in the blood vessels over the first 9 weeks. Neuronal uptake was assessed by the increase in the half-time (t 1/2) of disappearance of the tachycardia in response to field stimulation. The increase in t 1/2 caused by blockade of neuronal uptake (desipramine) was similar in both strains and again did not alter with age. Finally, the pre-junctional α_2-adrenoceptor that reduces noradrenaline release was tested by the α_2-adrenoceptor agonist, clonidine. Clonidine inhibited the tachycardia response to field stimulation to a greater extent in the older rats and also in WKY rats compared with SHR at matched ages.

These studies indicate that β-adrenoceptors and neuronal uptake mature early in the rat heart and the only abnormality at the nerve terminal that may enhance sympathetic transmission in SHR rats is a reduced autoinhibitory feedback via α_2-adrenoceptors.

Renal Laboratory

Head: Dr. W.P. Anderson

Projects

Regulation of glomerular filtration rate — micropuncture analysis.

Metabolic clearance of atrial natriuretic peptide.

Atrial natriuretic peptide in the renal failure of renal wrap hypertension.

Pathogenesis of two-kidney renovascular hypertension.

Glomerular response to reduced renal artery pressure — morphometric analysis.

Renal interstitial pressure in renal wrap hypertension.

Systemic and renal conductance changes in renal wrap hypertension.

Summary

The discovery of atrial natriuretic peptide (ANP) earlier this decade has led to renewed interest in the humoral control of body fluid balance and blood pressure. We have used our chronically instrumented dog and conscious rabbit preparations to study this hormone, including its metabolism, the mechanism by which it acts in the kidney and on the peripheral circulation, and its potential as a therapeutic agent in renal failure.

In both dogs and rabbits, ANP lowers blood pressure by reducing the cardiac output and it is not a vasodilator. Part of this fall in cardiac output is due to a rapid, non-renal fall in plasma volume. We are investigating whether this is due to movement of fluid into the gut.

Careful measurements of the clearance of ANP have shown that the renal clearance is a relatively minor component of its removal from the blood. It also appears that its rate of clearance from plasma is lower when angiotensin II levels are high, indicating yet another interaction between these two hormones.

We were disappointed to show that ANP did not increase glomerular filtration rate (GFR) in the impaired kidneys of renal wrap rabbits. Unlike other clinically used diuretic agents, ANP can raise GFR in normal kidneys, and it was hoped that it would turn out to be the ideal diuretic for use in cases of renal impairment.

Another major area of research has been the pathogenesis of experimental renal hypertension. This year we have performed a detailed study of the development of hypertension from stenosis of one renal artery with the other artery normal. Angiotensin II is clearly a dominant factor in hypertension and also in the increased resistances of both the stenotic and the contralateral kidneys. By contrast, the autonomic nervous system appears to play a negligible role, even in the renal responses. Studies of renal wrap hypertension also failed to show any pathogenic role for the sympathetic nervous system.

The regulation of glomerular filtration rate has been studied by both electron microscopy and physiological techniques. For the latter studies, we have been exploiting our colony of rabbits with superficial glomeruli. This colony has been developed at the Institute over the last two years. Previously, almost all direct measurements of glomerular function have been made in a single strain of rats. Not only do our rabbits afford an opportunity to verify these observations in another species, but these larger animals enable us to make measurements of the physiological regulation of glomerular function which are not possible in small animals. Much of our effort this year has been expended on ensuring optimal renal and cardiovascular function in these rabbits, and we are now beginning studies on hormonal influences on glomerular function.

Regulation of glomerular filtration rate — micropuncture analysis

K.M. Denton, A.I. Gilchrist, W.P. Anderson

Recent evidence has changed the long-held view that the glomerulus is a passive filter. Micropuncture experiments have indicated that various hormones, including angiotensin II and vasopressin, change the filtration properties of the glomerulus. To date, this evidence comes almost exclusively from a single species of rat which have glomeruli on the surface of their kidneys. These glomeruli are thus accessible to study *in vivo*. However, there is debate on how generally applicable these findings are for mammalian kidneys and the study of the physiological regulation of circulatory and renal function is difficult in small rodents.

We have now begun studies in rabbits with surface glomeruli. This colony has been bred at the Institute over the last two years, following the chance discovery of a single animal with this renal feature during routine histological examinations.

Work during the year has established conditions in the anaesthetized rabbits for achieving renal function close to that measured in conscious animals. This involves careful regulation of fluid balance of the animals and, crucially, their posture. Under the conditions now used, average GFR is 4 ml/min in the anaesthetized animals, close to that in conscious animals. Single nephron GFR averages about 30 ml/min, similar to that measured in rats. Preliminary measurements of glomerular capillary pressures, however, differ considerably from those measured in rats.

Experiments are now in progress to study the effects of endogenous angiotensin II, atrial natriuretic hormone and vasopressin on the glomerulus.

Metabolic clearance of atrial natriuretic peptide

R.L. Woods, J. Lineham

A great deal of recent research has been devoted to unravelling the biological actions and effects of the newly discovered peptide, ANP, that is released from the heart into the circulation. Nevertheless, very little is known of the way the body metabolizes and removes the hormone

from the circulation. An important role for the kidney in the clearance of ANP has been proposed.

We performed a series of clearance experiments in conscious dogs to assess the contribution of the kidney. We have developed a sensitive and specific radioimmunoassay for plasma ANP and have used it to show that the whole body metabolic clearance of ANP is extremely high, at 1736 ± 145 ml/min, approaching the cardiac output in these dogs. Surprisingly, the contribution of the kidney is only about 9% of the total metabolic clearance, which contrasts with the suggestions of other workers that the kidney contributes as much as 59% to the overall clearance. Of this renal clearance, the GFR contributed approximately 30%. Plasma t(1/2) was normally 59.6 ± 7.9 s and was increased to 96.1 ± 7.1 s when GFR was reduced to zero by acute renal artery narrowing.

These results show that the non-renal tissues of the body are the major sites for removal of ANP, while glomerular filtration contributes significantly to the relatively small clearance by the kidney.

Atrial natriuretic peptide in the renal failure of renal wrap hypertension

M. Takata, K.M. Denton, R.L. Woods, W.P. Anderson

Unlike other diuretic agents, atrial natriuretic peptide increases glomerular filtration rate, at least in normal kidneys. It has therefore been suggested that it might be an ideal diuretic agent for renal insufficiency. We have tested its effectiveness in the renal failure of renal wrap hypertension where GFR is about 50% reduced. Infusion of 2μg/min of ANP raised GFR and caused a significant natriuresis in normal rabbits but was without significant effects in renal wrap animals. ANP also lowered the arterial pressure and when the effects of an equihypotensive dose of nitroprusside was compared, Na^+ excretion rate fell significantly. Thus, ANP maintained Na^+ excretion compared to nitroprusside. It was, however, ineffective in raising GFR and increasing Na^+ excretion in the impaired, renal-wrap kidneys.

Pathogenesis of two-kidney renovascular hypertension

M. Takata, D. Ramsey, W.P. Anderson

The progressive haemodynamic and hormonal changes during the development of 'two-kidney, one clip' hypertension have been studied in chronically instrumented dogs. Marked narrowing of the left renal artery caused a hypertension of 15-20 mmHg, due entirely to a fall in total peripheral conductance. Preliminary results indicate that the fall in peripheral conductance was dependent on angiotensin II, both initially when the circulating levels of renin were high and, to a lesser but still significant extent, after three weeks when renin levels had returned close to pre-constriction levels. The resistances of both the stenotic and the contralateral kidneys were elevated throughout. Captopril administration lowered resistance in both the contralateral kidneys and the stenotic kidney. The fall in renal artery pressure distal to the stenosis after captopril administration was a particularly striking finding.

The role of the autonomic nervous system in the hypertension and the renal haemodynamic changes was studied by giving pentolinium after three weeks of hypertension. None of the systemic or renal haemodynamic changes appeared to be significantly dependent on the autonomic nervous system.

Thus, preliminary results suggest that the renal and systemic changes in this form of hypertension remain very dependent on the renin-angiotensin system even when circulating levels are near normal; they also suggest that the autonomic nervous system is not involved.

Glomerular response to reduced renal artery pressure — morphometric analysis

D.A. Alcorn, A.I. Gilchrist, K.M. Denton, G.B. Ryan, W.P. Anderson

We recently found that angiotensin II caused marked contraction of the glomerular mesangium following acute renal artery stenosis. Other investigators have suggested that mesangial cells may alter their degree of contraction in response to glomerular pressure alone, i.e. even without the action of angiotensin II. We have investigated this by comparing the state of contraction of the mesangium in kidneys with acute stenosis of the renal artery and in contralateral kidneys being perfused at normal aortic pressure, following blockade of angiotensin II formation.

Detailed morphometric comparison of these kidneys revealed no differences in the extent of mesangial contraction. It is therefore concluded that the mesangium does not contract in response to reduced renal perfusion pressure if angiotensin II formation is blocked. The mesangium therefore does not 'autoregulate' its state of contraction in direct response to changes in glomerular capillary pressure.

In other studies, the stenotic and contralateral 'normal' kidneys of dogs with chronic unilateral renal artery stenosis are being compared.

Dr. Warwick Anderson observing superficial glomeruli in a kidney.

Vascular Laboratory

Head: Prof. J. Ludbrook

Projects

A technique for stimulating the haemodynamic and humoral responses to acute blood loss in unanaesthetized rabbits.

Effects of intracisternal naloxone and cardiac afferent nerve blockade on the haemodynamic response to simulated haemorrhage in unanaesthetized rabbits.

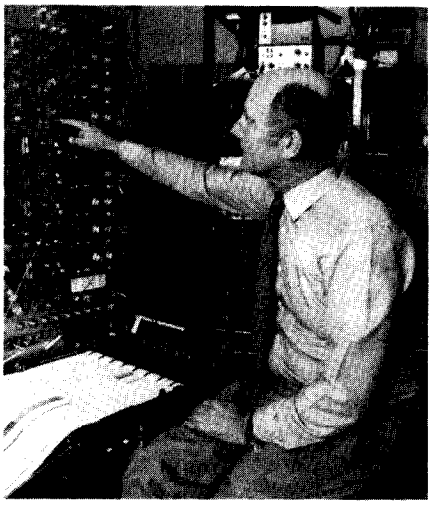
The circulatory responses to exercise during progressive heart failure induced by adriamycin.

Summary

During the past year the greater part of our work has again been concerned with the role of endogenous opiate mechanisms in the response of the circulation to acute blood loss. The background to this goes back more than 40 years, when it was shown that as blood was withdrawn from human volunteers there was at first widespread constriction of blood vessels, which maintained blood pressure at an almost normal level. Then, when about one-quarter of the blood volume had been removed, the vasoconstriction suddenly failed and there was a profound fall of blood pressure. A similar sequence of events has been observed many times in clinical practice, and in some cases the sudden fall of blood pressure has caused death.

Similar phenomena have been observed in unanaesthetized rabbits, in our laboratory and in other laboratories at the Baker Institute and overseas. As blood is withdrawn from a rabbit there is at first progressive vasoconstriction, and blood pressure is well maintained. The vasoconstriction is due to a reflex (the arterial baroreflex), in which a fall of blood pressure is signalled to the brain and results in increased activity in the sympathetic vasoconstrictor nerves and the release of vasoconstrictor hormones from the adrenal glands. However, when more than about one-third of the blood volume is withdrawn the reflex constriction of blood vessels fails, and there is a profound fall of blood pressure. This failure of vasoconstriction is triggered by a signal which is sent to the brain from the heart, probably because its chambers have become almost empty of blood. Dr. Patricia Dorward has found that the failure of sympathetic drive, and we have found that the failure of vasoconstriction, can be prevented by injecting into the blood stream a large dose of naloxone, a drug which antagonises the action of opiate drugs such as morphine. Morphine-like substances are normally present in many of the body tissues, and the action of these endogenous opiates is also prevented by naloxone. We presume, therefore, that the sudden failure of the arterial baroreflex to maintain blood vessels constricted is due to the action of endogenous opiates at some unknown site.

During 1987 we set out to determine where the endogenous opiate mechanisms which cause the circulatory collapse are located. In order to do this we have developed a technique for simulating haemorrhage in conscious rabbits, so that the role of endogenous opiates can be investigated without having to withdraw blood. We can now mimic the effects of haemorrhage on the circulation by gradually inflating a cuff on one of the large veins that carries blood back to the heart (the inferior vena cava).



John Ludbrook in the course of an experiment on endogenous opiate mechanisms in haemorrhage.

We have shown that the profound fall of blood pressure which occurs during simulated haemorrhage can be prevented by injecting a minute dose of naloxone into the cerebrospinal fluid, and that this is as effective as injecting into the bloodstream a dose that is 100 times greater. It appears, therefore, that the endogenous opiate mechanisms which cause the circulation to fail during haemorrhage are located in the brain.

Our research is directed not only towards understanding the role of endogenous opiates in circulatory shock, but also to the possibility that an opiate antagonist drug could be used in man as an emergency treatment in cases of severe haemorrhage. The drug naloxone would be far from ideal, since it blocks the action of all classes of opiates, including morphine which is so often necessary for pain relief. We are currently testing whether more selective drugs, which do not antagonise the action of morphine, will prevent circulatory collapse during haemorrhage.

We have also continued to work with Dr. Barry McGrath of the Monash University Department of Medicine on the effects of heart failure on the responses of the heart and circulation to exercise. It has been impossible to study these in a precise fashion in patients, so that we have made use of the fact that the drug adriamycin causes the slow development of heart failure in rabbits. Normally, when a rabbit exercises on a treadmill the output of its heart increases, and the blood vessels in the exercising muscles dilate so that more oxygen-containing blood is directed to them. We have found that a rabbit with incipient heart failure responds to exercise in a quite abnormal fashion. At the onset of exercise the output of its heart falls, rather than rises. At the same time the blood vessels in many parts of the body constrict rather than dilate, so that the heart has to work against a greater resistance. As a result the supply of oxygen to the exercising muscles is inadequate, and the rabbit is unable to run at a normal speed or for a normal distance. We are currently engaged in determining

whether treatment of the heart or treatment of the blood vessels is the more beneficial in terms of tolerance to exercise, with a view to developing new strategies for treating humans with congestive heart failure.

A technique for simulating the haemodynamic and humoral responses to acute blood loss in unanaesthetized rabbits

J. Ludbrook, S.J. Potocnik

Though there are methods for simulating haemorrhage in human volunteers, there has been no corresponding way of doing this in conscious animals.

In conscious rabbits, a cuff which has been placed round the inferior vena cava can be inflated so as to cause a progressive restriction of venous return and a progressive fall of central blood volume. By this means the linear rate of fall of cardiac output that occurred when blood was withdrawn at 2.7 ml kg^{-1} min^{-1} for ~8 min could be matched exactly. The changes in dependent haemodynamic variables such as systemic vascular resistance, arterial pressure and heart rate were mimicked closely. These changes were reproducible when simulated haemorrhage was performed three times at 90 min intervals, and when it was repeated four times over 12 days. Inferior vena caval pressure below the cuff rose by only 6 mmHg in the course of simulated haemorrhage.

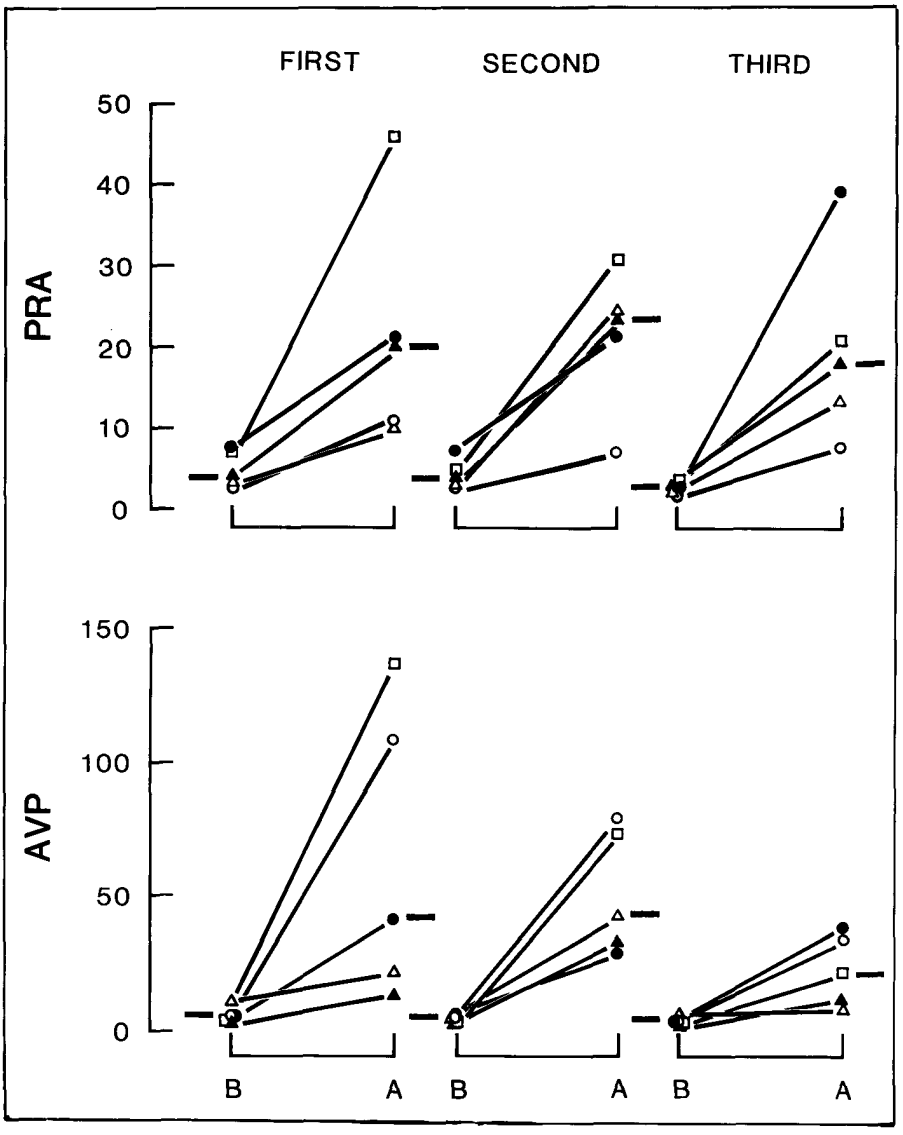
Simulated haemorrhage caused rises in plasma renin activity (PRA) and plasma arginine vasopressin concentration (AVP) that were similar to those reported after haemorrhage. The response of PRA was unaltered when simulated haemorrhage was repeated three times at 90 min intervals, but the response of AVP was blunted on the third occasion (Fig. 1).

When the shed blood was reinfused after haemorrhage, cardiac output remained low, and systemic vascular resistance high, for at least 10 min. When the caval cuff was deflated after simulated haemorrhage, the recovery was much faster and all haemodynamic variables returned to normal within 2 min.

Haematocrit fell from 37.6 to 31.4% during haemorrhage, and remained low for at least five days after replacement of the shed blood. It was unaffected by simulated haemorrhage.

We conclude that the haemodynamic effects of acute haemorrhage can be closely and reproducibly simulated by inflating a cuff on the inferior vena cava. This provides a useful technique for repeatedly studying the effects of acute reduction of central blood volume in conscious animals, without the need to withdraw blood.

Figure 1: Levels of plasma renin activity and vasopressin concentration before (B) and 30 s after (A) three simulated haemorrhages at 90 min intervals. PRA, plasma renin activity (ng AI/ml/hr). AVP, plasma arginine vasopressin concentration (pg/ml). Symbols indicate individual rabbits. Bars indicate median levels.



Effects of intracisternal naloxone and cardiac afferent nerve blockade on the haemodynamic response to simulated haemorrhage in unanaesthetized rabbits

J. Ludbrook, S.J. Potocnik

We have shown previously that naloxone HCl, given in a dose of ~10 mg intravenously, will prevent the abrupt failure of vasconstriction and the profound fall of blood pressure that occur when >30% of blood volume is rapidly withdrawn from unanaesthetized rabbits. This strongly suggests that an endogenous opiate mechanism is involved in the failure of vasoconstriction, but does not indicate whether the mechanism is located in the central nervous system or peripherally. There is indirect

evidence that the mechanism is central, in that the fall of renal sympathetic nerve activity that occurs during profound haemorrhage is also prevented by large intravenous doses of naloxone (Dorward, personal communication). We have set out to test directly whether the mechanism resides in the central nervous system.

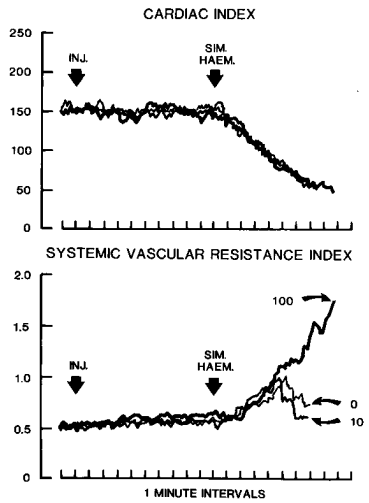


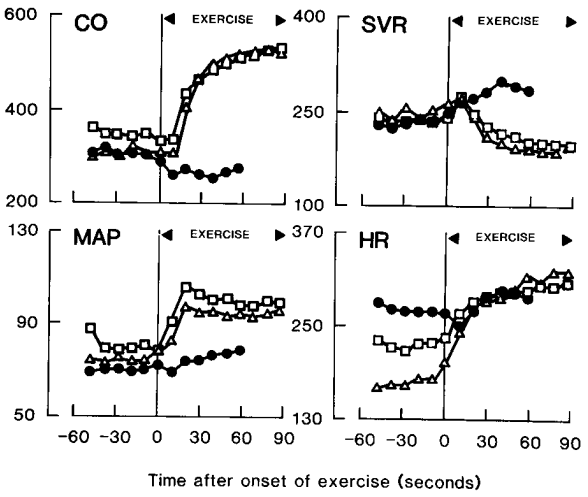
Figure 2: Effect of intracisternal naloxone on the responses to simulated haemorrhage in a conscious rabbit. Cardiac index, ml/kg/min. Systemic vascular resistance index, 10^3 mmHg per ml/kg/min. Doses of naloxone given at INJ were 0 (saline), 10 μg and 100 μg. Note that 100 μg naloxone prevented the abrupt fall of systemic resistance.

Figure 3: Haemodynamic responses of a rabbit to treadmill exercise at 16 m/min. CO = cardiac output (ml/min.). MAP = mean arterial pressure (mmHg). SVR = systemic vascular resistance (10^3 MAP/CO). HR = heart rate (beats/min). □ = control. Δ = after 4 weeks of adriamycin 1 mg twice-weekly. ● = after 8 weeks adriamycin treatment.

Four experiments were done on separate days, 5-7 days apart, on unanaesthetized rabbits in which cardiac index (CI), mean arterial pressure (MAP), systemic vascular resistance index (SVRI = MAP/CI) and heart rate (HR) were measured. During each experiment haemorrhage was repeatedly simulated by inflating a cuff on the inferior vena cava. The protocols for the experiments consisted of giving the following treatments prior to simulated haemorrhage: (1) Intracisternal saline (control), naloxone HCl 0.5, 5 and 50 μg. (2) Intracisternal saline (control), naloxone HCl 1.0, 10 and 100 μg. (3) Intravenous saline (control), naloxone 0.1, 1 and 10 mg. (4) Intrapericardial saline (control), 5% procaine HCl (to block conduction in cardiac afferent and efferent nerves) and atenolol 125 μg plus hyoscine methyl bromide 25 μg (to block neurohumoral effects on the cardiac pacemaker). The order of the protocols was randomized.

During simulated haemorrhage under control conditions SVR and HR steadily rose, and MAP fell only slightly, until CI had fallen by ~50%. Then SVRI and MAP fell precipitately until the caval cuff was deflated. These same changes occurred after all other treatments except intravenous naloxone (10 mg), intracisternal naloxone (10-200 μg) and intrapericardial procaine. After each of these three treatments SVRI continued to rise, and MAP fell only slightly, throughout the simulated haemorrhage (Fig. 2).

We conclude that when cardiac output is reduced by ~50% a signal is transmitted from the heart to the brain, where it activates an endogenous opiate mechanism. This blunts the arterial baroreceptor reflex, so that systemic vascular resistance and arterial blood pressure fall abruptly.



The circulatory responses to exercise during progressive heart failure induced by adriamycin

B. Jover, B.P. McGrath, J. Ludbrook

Rabbits were matched in pairs for their ability to perform treadmill exercise. They were equipped so that the following cardiovascular variables could be measured: cardiac output (CO), arterial blood pressure (BP), systemic vascular resistance (SVR) and heart rate (HR). One rabbit of each pair was treated with intravenous (i.v.) adriamycin, 1 mg twice weekly (a regimen that causes global heart failure to develop after 6-8 weeks). The other member of the pair was treated with twice weekly i.v. injections of saline.

The cardiovascular responses of the rabbits to treadmill exercise at 8 and 16 m min^{-1} were measured before treatment, and at two-weekly and later one-weekly intervals during treatment. The resting levels of the cardiovascular variables and their responses to exercise were consistently normal throughout sham treatment, and during the first six weeks of adriamycin treatment. That is, exercise caused an asymptotic rise in HR, CO and BP, and an asymptotic fall in SVR. In the adriamycin-treated rabbits the onset of heart failure was indicated by a high resting HR and SVR, and a low resting CO and BP. The responses to exercise were grossly abnormal, and the rabbits could not complete the exercise schedule (Fig. 3). The rises of HR and BP were attenuated, CO fell instead of rising, and SVR rose instead of falling.

It is not yet clear whether the abnormal HR response is due to a direct action of adriamycin on the cardiac pacemaker. Neither is it yet clear whether CO falls during exercise because left ventricular afterload (SVR) rises, or whether SVR rises during exercise because CO and BP remain low.

Clinical Research Unit

Director: P.I. Korner
Deputy Director: G.L. Jennings

Projects

Influence of low and normal sodium diets on cardiovascular function and noradrenaline turnover.

Prevalence of left ventricular hypertrophy and other structural and functional abnormalities in untreated primary hypertension.

Relationship of short- and long-term changes in blood pressure to transmitral diastolic flow velocities and left ventricular hypertrophy in hypertension.

Dipyridamole stress testing: Why thallium-201 scans and echocardiographic findings may differ.

Simple dietary advice and its effect on cardiovascular risk factors.

Coronary risk scores: how reliable is risk assessment?

Early detection of carotid artery atherosclerosis and its prediction by cardiovascular risk assessment.

A comparison of felodipine and diuretic for initial therapy of hypertension.

Exaggerated atrial natriuretic peptide release during acute exercise in essential hypertension.

Hydrocortisone-induced hypertension in man: studies of pressor responsiveness and sympathetic nervous system function.

Reversal of cardiac and vascular hypertrophy in essential hypertension.

The venous system in essential hypertension.

Time course study of the cardiovascular effects of exercise.

Training effects on regional cardiac and renal noradrenaline spillover at rest and during manipulation of central blood volume.

The role of cardiac sympathetic activity in the genesis of myocardial ischaemia during daily events and their simulation in the laboratory.

The role of cardiac sympathetic nerves in the genesis of ventricular arrhythmias (VAST Study).

Regional cardiac noradrenaline spillover and extraction in autonomic neuropathy.

Simvastatin in patients with hypercholesterolaemia.

Resting and exercise cardiac and renal sympathetic activity in heart failure.

Effect of exercise on cardiovascular risk factors at different levels of background activity.

Effect of acute and chronic alcohol ingestion on ambulatory blood pressure.

Effect of fish oil on alcohol-induced hypertriglyceridaemia.

Summary

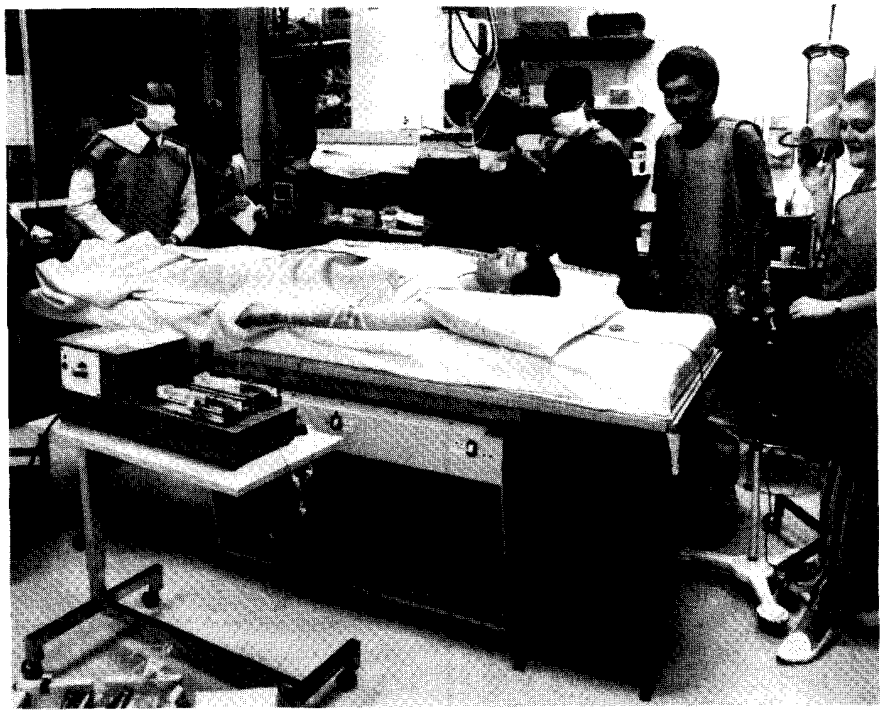
It is 40 years since the Board of Management of the Alfred Hospital determined that the Clinical Research Unit should be established, a decision which led to the founding of the Clinical Research Unit in 1949. Dr. Thomas Lowe, Director of the Baker Medical Research Institute in his 1949 Director's report explained that the purpose was 'to make any basic discoveries available for treatment of patients'. There had always been good co-operation between Hospital and Institute, but 'the time which busy honoraries can devote to research is limited'. 'The Institute and the Clinical Research Unit should in a short time become indispensable to each other . . . Further, to make the utmost use of the facilities of both it is desirable that there should be a core of research in a field common to both. To provide this core it has been planned to carry on studies in cardiovascular diseases as a common line of research.' Despite the enormous changes in medical practice and technology, and the comings and goings of very many distinguished medical scientists who have worked in the Clinical Research Unit in the intervening 40 years, these guidelines still apply and thankfully the Clinical Research Unit is very much a part of *both* the Alfred Hospital and the Baker Medical Research Institute. The two have become indispensable to one another. The past year has been the busiest yet. To the Hospital the Clinical Research Unit provides a number of clinical services, including the specialist Hypertension and Lipid Clinics, a general medical unit and a range of (mainly) cardiovascular diagnostic services. There is also collaboration and consultation in clinical research. To the Institute the Clinical Research Unit is a place to examine the clinical application of new findings, and also increasingly a source of new questions which have arisen in clinical studies, but can be carried further only in the controlled environment of the basic laboratory. To the general public we provide a 'state of the art' cardiovascular risk reduction service which we expect to make substantial contributions to the field of heart disease prevention, particularly after the Rotary Club of Melbourne facility is fully established in the coming year.

The number of project titles listed above is higher than last year and reflects the increased scientific activity. The latter largely represents the influx of young medical and science graduates who are now working towards higher degrees in our Unit. The demands on space, facilities and technical support have been heavy, particularly in the laboratory area. We are very short of senior scientific manpower and look forward to expanding our senior medical staff establishment as recommended by the NH & MRC to assist in the supervision of projects.

The four largest fields of clinical research at present relate to hypertension, hyperlipidaemia, sympathetic nervous activity and exercise. In hypertension we have continued our interest in studying the pathophysiology in man and particularly the role of changes in structure of the heart and vasculature. For some time now we

have been using echocardiography and Doppler to study the heart. A major advance in the past year has been the application of new techniques of studying the microvasculature in patients with hypertension. Small arterioles are isolated from skin biopsy material and their structure, function and pharmacology studied by Drs. Angus and Sudhir using techniques developed initially by Dr. Michael Mulvany, a recent visitor working at the Baker Institute. Veins are also studied in the same subjects. A unique feature of this work has been the parallel use of *in vitro* and *in vivo* techniques in the same subjects. The same subjects have been studied before and after long periods of treatment to try to separate primary 'causal' abnormalities from secondary consequences of hypertension. Our interest in sympathetic nervous activity has been carried forward by Dr. Esler, not only in hypertension, but in diverse clinical conditions, all of which are affected by sympathetic responses such as heart failure, cirrhosis, autonomic insufficiency and ischaemic heart disease. In the hyperlipidaemia field, we have been studying the effects of a major new group of drugs, the so-called HMG CoA reductase inhibitors. Our work represents one of the first studies in Australia with this class of drugs. These drugs lower blood cholesterol by reducing cholesterol synthesis in the body and we have participated in studies to determine the proper dosage, and their effects in combination with other lipid lowering agents. In exercise studies we have shown that in the heart of patients with hypertension, who participate in an exercise programme, there is beneficial long-term remodelling of the heart.

As always, we are grateful to our many collaborators who have contributed to the diversity of our work. During the year we welcomed to the Unit Dr. Peter Friberg from Göteborg, Sweden, and Dr. Ian Meredith and Dr. Harvey Newnham, both Alfred Hospital graduates. Drs. Peter Blombery and Peter Jenkins were made Senior Associates to the Institute in recognition of their clinical work within the Clinical Research Unit. We farewelled Dr. Peter Weissberg who was appointed at Cambridge (U.K.) and Dr. Gillian Deakin who has submitted her MD thesis.



Catheter study investigating sympathetic nervous function in the heart and kidney.

Influence of low and normal sodium diets on cardiovascular function and noradrenaline turnover

P. Friberg, I. Meredith, G. Jennings, M. Esler, V. Fazio, P. Korner

The body is able to regulate its total amount of salt in the face of large changes in dietary intake. During a low salt intake, several regulatory mechanisms are activated. The sympathetic nervous system and the renin angiotensin system measured elevations of plasma noradrenaline (stress hormone) and plasma renin activity (PRA) (kidney hormone) respectively. The increased level of noradrenaline during salt restriction reflects an integrated response from the whole body. However, we do not know from which organ it emanates. Similarly, the interaction between the sympathetic and the renin angiotensin systems regarding the maintenance of cardiovascular balance is not known.

Preliminary results show that short-term salt restriction increases markedly the noradrenaline released from the kidney, whereas the release from the heart remains unaltered. It is therefore likely that the previously observed elevation of noradrenaline originates from the kidneys and that this regional increased sympathetic activation may allow the organism to keep the urine losses of salt at a minimum.

Prevalence of left ventricular hypertrophy and other structural and functional abnormalities in untreated primary hypertension

E. Laufer, G. Jennings, P. Korner

The reported prevalence of left ventricular hypertrophy (LVH) in human primary hypertension is less than in animal models. This may be due to the inclusion of previously treated patients in some earlier studies or to the large variance of LV mass index in the normal population. We examined the role of these factors in the incidence of LV abnormalities by performing two-dimensional and M-mode echocardiography in 89 normal volunteers, 57 patients with established hypertension and 38 patients with mild hypertension. Because of small, but significant differences in BSA between the three groups, we used covariance analysis

to correct the anatomical variables — LV mass (LVM), wall thickness (WT) and LV internal diameter (LVID) — to a common value of 1.8 m^2. No adjustment was used for wall thickness to cavity radius ratio (WT/R). For the diastolic and systolic functional variables we determined the Doppler transmitral early/late flow velocity ratio (E/A) and the fractional systolic shortening (FS) respectively. The former was standardized for age by analysis of covariance.

With LVM the prevalence of LVH was 30% in established and 15% in mild hypertension. Corresponding figures were 65 and 32% for WT, and 60 and 40% for WT/R. With single functional variables the prevalence of abnormalities was 28% (established) and 11% (mild) by E/A, with no useful separation provided by FS. With a multivariate discriminant function the best results were obtained with WT, E/A and FS when there was correct classification of 82% of normal subjects, 65% of patients with established hypertension and 61% of patients with mild hypertension.

We conclude that the majority of patients with hypertension have a structural and/or a functional abnormality. The prevalence of LVH as determined by LVM was similar to previously reported studies implying that previous treatment is not a major contributing factor. Of the single variables, WT and WT/R provided the best echocardiographic separation, suggesting that the differences in estimates with the different variables was related to the relative magnitude of variances in their respective normotensive groups. For the most reliable detection of mild hypertension, discriminant function analysis using combined anatomical and functional criteria is necessary.

Dipyridamole-stress testing: why thallium-201 scans and echocardiographic findings may differ

R.J. Hicks, E. Laufer, V. Kalff, M.I. Kelly

Dipyridamole is used to provoke ischaemic changes in patients with angina during cardiac scanning in the diagnosis of coronary artery disease. The effect of intravenous dipyridamole on regional myocardial perfusion was assessed by thallium scintography and regional wall motion (by two-dimensional echocardiography) in 26 patients. The development of angina during the tests was used to indicate true ischaemia induced by dipyridamole. Planar thallium and echocardiographic findings were compared in 22 patients who had technically adequate echocardiographic studies.

Both echocardiographic and thallium studies suggested prior infarcts in 12/22 patients. Angina during the test occurred in 10/12 patients with prior infarction, but did not occur in any of the 10 patients without prior infarction ($P < 0.01$).

	Angina	No Angina
Reversible Perfusion Defects (P=NS)	8/10	6/12
Reversible Wall Motion Abnorm. ($P < 0.05$)	7/10	0/12

The results suggest that positive dipyridamole tests may have different mechanisms in patients with and without angina during the test. When there is no angina or deterioration in regional wall motion, reversible perfusion defects presumably result from an abnormal coronary blood flow reserve in stenosed vessels. In patients with angina during the test and reversible perfusion defects, the association with reversible wall motion abnormalities suggests true ischaemia is provoked. Further, the association with prior myocardial infarction in this group suggests that ischaemia may be due to coronary artery steal affecting collateral-dependent peri-infarct myocardium.

Simple dietary advice and its effects on cardiovascular risk factors

C. Reid

Simple dietary advice may be the most cost effective method of reducing the risk of cardiovascular disease. The purpose of this data analysis is to determine whether risk factor profiles of individuals with elevated lipid levels differ when tested after giving advice (a) through a group dietary seminar, or (b) through giving the subject appropriate literature on diet, and without attending a seminar.

We analysed data from 134 subjects whose initial risk factor evaluation revealed a cholesterol level of >/= 6.5 mmol/l or a triglyceride level of >/= 2.0 mmol/1. Dietary information alone (pamphlet) was received by 80 subjects (group 1), while 54 attended a group dietary session (group 2). Retests were carried out 6-8 weeks after the first test.

Significant reductions ($P < 0.05$) were seen in both groups in systolic blood pressure, body mass index and in cholesterol levels, whilst the small changes in other variables were not significant. The extent of reduction, expressed as % change in Table 1, was greater for the group attending the diet seminar (group 2) in all variables except triglyceride.

TABLE 1: Amount of change in 8 weeks following initial testing and intervention. Values represent % change of group means from initial to final test result.

Variable	Group 1	Group 2
Cholesterol	— 8%	—13%
Triglyceride	—11%	— 5%
Weight	— 1%	— 3%
Body Mass Index	— 2%	— 4%
Systolic Blood Pressure	— 4%	— 3%
Diastolic Blood Pressure	— 1%	— 3%
Mean Arterial Pressure	— 2%	— 3%

Body Mass Index = weight/(height x height) x 1000
Mean Arterial Pressure = diastolic + (systolic — diastolic)/3

These results indicate that attendance at a group dietary session appears to have a greater effect on reducing the risk factor profile than can be achieved through literature information and 'going it alone'. However, there appears to be some benefit even in the latter approach.

Coronary risk scores — how reliable is risk assessment?

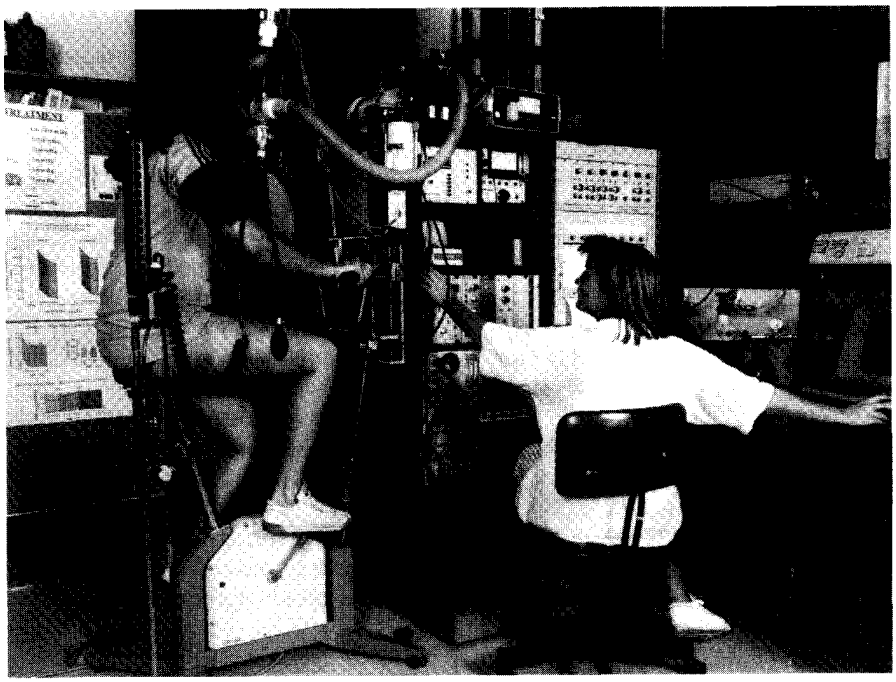
C. Reid

Coronary risk is normally determined by one of two methods. The first is to determine the individual level of major coronary risk factors, for example blood pressure, cigarette smoking and cholesterol levels. However, this method fails to incorporate the additive effects of multiple risk factors in a given individual. The Framingham Study has shown that the additive effects are important. A coronary risk score was developed to interpret the multiple risk factor effect.

More recently, a second coronary risk score has been developed as a result of follow-up data collected in the U.S.-based MRFIT (Multiple Risk Factor Intervention) Study. We set out to determine whether coronary risk assessment was more reliable based on the measurement of individual risk factors or on coronary risk scores, which took account of additive effects.

The subjects were initially screened for cardiovascular risk factors through the risk clinic. They were randomly allocated to one of three groups and were asked to return for a repeat test either 1, 2 or 4 weeks following the initial screening. All subjects were asked to maintain their initial diet and activity for the duration of the follow-up period.

The results showed that single risk factors showed considerable variability between successive measurements but tests based on multiple measurements were much more stable.



Lisa Nelson conducting an exercise test in Clinical Research Unit.

A comparison of felodipine and diuretic for initial therapy of hypertension

G.L. Jennings, K. Sudhir

The present study was designed to examine the effects of two different antihypertensive regimens on left ventricular hypertrophy over 1 year. Previously untreated patients were randomly allocated to single blind treatment with either a diuretic (Moduretic; one tablet daily) or felodipine (Plendil; 5 mg b.d.). Dosage was adjusted fortnightly according to a fixed protocol until a target supine diastolic blood pressure of 85 mmHg was achieved. If necessary, to achieve this target prazosin (Minipress) was added to the Moduretic and metoprolol (Betaloc) to the felodipine regimens.

Twenty patients have entered the study (10 Moduretic ± prazosin, 10 felodipine ± metoprolol). Results over the first 6 months of therapy are shown below:

			Supine Blood Pressure				Number on
	n	Age	Entry	1 mth	3 mths	6 mths	Monotherapy
F	10	52.6	152/102	128/85	121/81	128/78	9
M	10	43.7	151/101	128/85	126/86	124/80	5

F = felodipine, M = Moduretic

Both regimens achieved the target level of blood pressure control within 1 month in most patients. In 9/10 patients, felodipine alone was sufficient at the initial dose of 5 mg b.d. Five patients required the addition of prazosin to Moduretic. The most common adverse reaction on felodipine was mild ankle swelling (five patients), whereas Moduretic + prazosin patients experienced postural hypertension and/or eye irritation (four patients). None of these symptoms warranted cessation of therapy.

Felodipine is a satisfactory agent for initial therapy of hypertension and is less likely to require the addition of a second agent than Moduretic.

Early detection of carotid atherosclerosis and its prediction by cardiovascular risk assessment

C. Reid

With the aid of carotid duplex scanning, it is now possible to make pre-clinical assessment of atherogenesis, by showing the presence of intimal thickening (IT) and plaque formation (PF) in persons who have no clinical manifestations of atherosclerosis. Preliminary results (n=28) have shown that, through discriminant analysis, in 92% of subjects there is good correlation between the demonstration of pathology or otherwise, from duplex scanning with the results of multivariate analysis of risk factors in the same subjects (Table 1).

TABLE 1: Classification Results: Two-way discriminant function — Disease (IT and/or PF) or No Disease

Actual Group	No. of Cases	Predicted Group Members Group 1	Group 2
Group 1 No Disease	8	7	1
		87.5%	12.5%
Group 2 Disease	20	1	19
		5.0%	95.0%

Percent of grouped cases correctly classified = 92.86%

Exaggerated atrial natriuretic peptide release during acute exercise in essential hypertension

K. Sudhir, R.L. Woods, G.L. Jennings, L. Nelson, E. Laufer, P.I. Korner

Atrial natriuretic peptide (ANP) is a hormone released from the heart, which has diuretic properties. We studied the effect of acute graded

exercise on plasma concentrations of ANP in hypertensive patients and normal subjects. The exercise was performed on a bicycle ergometer with workload increased each minute until exhaustion (Wmax). Normal subjects could reach a greater Wmax than hypertensive patients. Blood pressure and heart rate rose more steeply in hypertensive patients than in normals. Plasma ANP was the same at rest in both groups and increased during exercise to a greater degree in hypertensive patients than in normal subjects. Moreover, the increase in ANP during exercise was greater in hypertensives with left ventricular (LV) hypertrophy, with a positive correlation between LV mass and the percentage rise in ANP during exercise. The enhancement of ANP release during exercise in hypertension may reflect both cardiac structural changes and high central blood volumes.

Hydrocortisone-induced hypertension in man: studies of pressor responsiveness and sympathetic nervous system function

K. Sudhir, G.L. Jennings, M.D. Esler, P.I. Korner, P. Blombery, G. Lambert, B. Scoggins, J.A. Whitworth (Howard Florey Institute and Department of Nephrology, Royal Melbourne Hospital)

Hypertension is a common complication of steroid administration in man, but the reason for this is poorly understood. Oral hydrocortisone (HC) has been shown previously to increase blood pressure (BP) and enhance pressor responsiveness to intravenous phenylephrine. To further elucidate the mechanisms involved, we studied the effects of 1 week's oral HC on BP, cardiac output, forearm vascular resistance and noradrenaline spillover to plasma in eight healthy male volunteers. Body weight, systolic BP and cardiac output rose significantly. Resting forearm vascular resistance remained unchanged, but the vascular response to the cold pressor test as well as to intra-arterial infusion of noradrenaline was markedly accentuated after a week of HC. Measurements of noradrenaline spillover to plasma (in the forearm and for the body as a whole) and in noradrenaline uptake under resting conditions showed that there was no increase in sympathetic nervous activity, but that local responsiveness of the vessels was increased. The reason why HC raises pressure is due to an increased cardiac output and increased responsiveness of the peripheral vasculature to pressor stimuli, but not to a tonic increase in sympathetic nervous activity.

Time course study of the cardiovascular effects of exercise

I. Meredith, E. Dewar, G. Jennings, M. Esler, A. Bruce

We have previously shown that at the end of one month's regular exercise there is significant lowering of blood pressure and sympathetic nervous activity. The precise rate of onset of the effects of training and detraining are not known and we have determined the time course of the antihypertensive effects of regular exercise in 10 young healthy subjects. Alternate training and detraining periods were performed over 3 months. Cardiac structural and functional changes were examined using two-dimensional and Doppler echocardiography. Plasma catecholamines and haemodynamic variables were also measured.

The results showed that bicycle training (40 min, three times per week at 70% Wmax) for 1 month increased resting and maximal oxygen consumption (an index of fitness) by approximately 7-8%. Supine blood pressure fell by on average 6/8 mmHg ($P < 0.05$) and erect blood pressure fell by 10/6 mmHg. All blood pressure variables had fallen significantly by the second week of training with no further reduction during the following two weeks. Detraining resulted in blood pressure returning to pre-exercise values after two weeks. Cardiac structural and functional changes were not apparent during training by echocardiographic techniques over this period.

Thus, regular endurance exercise lowers blood pressure in normal healthy young subjects within 2 weeks, before there are demonstrable changes in the structure or compliance of the heart.

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Staff Activities

During 1987 Professor Korner completed his term as President of the Australian Physiological and Pharmacological Society and continued as Chairman of the High Blood Pressure Research Council of Australia. In 1987 he was elected President and Chairman of the Board of the Amalgamated, Alfred, Caulfied & Royal Southern Memorial Hospital, which is now the second largest hospital in Australia. He was invited to participate at the Centenary Celebrations of the American Physiological Society in Washington in March 1987 as an Invited Speaker and, in conjunction with this, spent a week at the Harvard Medical School (Massachusetts General Hospital) and in the Physiology Department at the University of Buffalo. In June 1987, Professor Korner visited Poland as a Guest Lecturer of the University of Warsaw. He visited Göteborg in June 1987 as an Invited Speaker at a symposium to honour Professor Björn Folkow. He participated at the Catecholamine Symposium in Jerusalem, as an Invited Speaker on the 'Circulatory Effects of the Brain Amines'. He also participated at a satellite symposium of the International Symposium of Hypertension in Athens. Professor Korner was invited to visit Brisbane as part of the State's Queensland Medical Research Week activities in October 1987. He gave a plenary lecture on Circulatory Control at the conjoint meeting of the Australian Societies of Experimental Biology (ASEB), held in Canberra to celebrate the Australian Bicentennial. Drs. Geoffrey Head and Patricia Dorward participated at the International Pharmacologial Congress in Sydney in August/September 1987, and in the ASEB meeting in Canberra.

Dr. Philip Barter was an invited speaker at the annual GERLI meeting on Lipids & Lipoproteins held at Dijon, France in June 1987. In August 1987 he gave the Plenary Lecture at the 7th Senri Seminar on Lipid Metabolism at Osaka in Japan. Also in August he gave an invited talk in Melbourne at the annual meeting of the Australian Cardiac Society. In November 1987 Dr. Barter attended the American Heart Association meetings in Anaheim, California. In February 1988 he visited Moscow and Leningrad in Russia as a member of a government delegation investigating possible areas of collaboration in medical research between Australia and the Soviet Union. He was also chairman of the committee organizing the National Heart Foundation's Bicentennial Research Symposium held in Canberra in February 1988, and an invited speaker at the Royal Australasian College of Physicians Golden Jubilee Meeting in Sydney in May 1988. Dr. Barter continued as Chairman of the National Heart Foundation's National Medical & Scientific Advisory Committee and concluded his term as the Chairman of the Foundation's Research Grants Committee. He also concluded a six year term as a member of the Scholarships Committee of the National Health & Medical Research Council of Australia. He continued during 1987 as Chairman of the Australian Atherosclerosis Society. Dr. Barter has recently been appointed to the Board of Governors of the new Sydney Heart Research Institute.

Dr. Noel Fidge attended a conference on Probucol in Los Angeles in November 1987 and then attended the American Heart Association meeting in Anaheim, California. He also visited the Genentech laboratories to discuss collaborative projects between a Genentech division and the Lipoprotein Laboratory at the Baker Institute. Dr. Fidge gave an invited talk at the National Heart Foundation's Bicentennial Research Symposium held in Canberra in February 1988, and presented two papers at the Australian Atherosclerosis Society meeting at Batemans Bay in February 1988. Dr. Fidge became a Senior Research Associate of the Biochemistry Department, Melbourne University and was appointed as part time lecturer of the Physiology Department, Melbourne University. Occasional lectures were given to Biochemistry Departments of Melbourne and Monash Universities.

Dr. John Ludbrook gave the inaugural R.P. Jepson Lecture on 'Cardiovascular Responses to Acute Blood Loss' at the Silver Jubilee Meeting of the Surgical Research Society of Australasia held in Dunedin, New Zealand. He continued to be a member of the Scientific Advisory Committees of the Clive and Vera Ramaciotti Foundations and the Royal Australasian College of Surgeons Foundation. He joined the Medical Liaison Committee of CSIRO, and the Scientific Advisory Board of Pacific Biotechnology. He is co-editor of the Australian and NZ Journal of Surgery.

Dr. Garry Jennings presented a paper entitled 'Long term effects of exercise on blood pressure, sympathetic activity, and left ventricular hypertrophy in essential hypertension' at the U.S. High Blood Pressure Research Council in New Orleans in October 1987. He was an invited speaker at the International Union of Pharmacology meeting in Sydney, presenting a paper on 'Inotropes and vasodilators for heart failure in man'. Dr. Jennings was an invited speaker at the International Symposium on Comparative Studies in Hypertension held at Titisce-Neustadt, Germany, lecturing on 'The place of exercise in the management of essential hypertension'. He also presented at the International Society of Hypertension meeting in Heidelberg, Germany. Dr. Jennings was appointed to the editorial boards of the Journal of Human Hypertension and the Journal of Clinical and Experimental Hypertension. Dr. Jennings is a member of the Hypertension Committee and the Heart Week Committee of the National Heart Foundation of Australia. He is also a member of the 'Prospects for Prevention' Organising Committee of the Royal Australasian College of Physicians. Dr. Jennings is Chairman of the Hypertension Working Group of the Cardiac Society of Australia & New Zealand and serves on the Medical Advisory Committee of the National Heart Foundation (Victorian Division).

Dr. Frank Rosenfeldt attended the American Heart Association Annual Meeting in Anaheim, California, in November 1987 and the Asian Pacific Congress of Cardiology in Auckland, New Zealand in February 1987. Dr. Rosenfeldt visited the United States in November to learn about the use of lasers in the cardiovascular system. He visited

Cedars Sinai Hospital and also two manufacturing plants for medical laser generators. The Cardiac Surgical Research Unit collaborated with Drs. Mark O'Brien and David McGiffen from Brisbane to conduct at the Baker Institute the first workshop in Australia on homograft heart valves. Dr. Marc Rabinov won the Alfred Hospital Surgical Research Prize for a presentation of his work on 'The response of the hypertrophic heart to hypothermic cardioplegia'. Dr. Guo Xia Jiang, the head of the Fu Wai Hospital and the Cardiovascular Research Institute of the Chinese Academy of Science, visited the Institute to discuss the setting up of a programme to enable cardiac surgeons from China to learn research and to obtain higher degrees through Peking Union Medical College and Monash University. The Cardiac Surgical Research Unit also hosted visits by Professor P. Olsson, Professor of Experimental Surgery at the Karolinska Hospital in Stockholm and Professor Charles R.H. Welhevur from the Research Division of the Department of Cardiopulmonary Surgery at the University Hospital at Gronigen in Holland.

Dr. Julie Campbell was an invited speaker at the Princess Lilian Cardiology Foundation symposium 'Blood cells and arteries in hypertension and atherosclerosis', held at Brussels, Belgium in November 1987. On the same trip she spoke at the French Atherosclerosis Society meeting in Paris, and in the departments of Professor J. Larrue, Bordeaux, Professor L. Robert in Paris and Dr. J.V. Small in Salzburg. Dr. Campbell was an invited speaker at the National Heart Foundation Bicentennial Meeting in Canberra in February 1988 and at the Royal Australasian College of Physicians Golden Jubilee Meeting in Sydney in May 1988. Dr. Campbell won an Achiever's Award from the national women's magazine, 'New Idea', which consisted of a 10-day trip for two persons to London. Dr. Campbell continues to serve on the editorial board of the American Heart Association journal, Arteriosclerosis, and to lecture to Science III students at Melbourne University. Ms. M.J. Black won the Australian and New Zealand Society for Cell Biology Young Scientist Award for 1988, and was invited to present her research findings at the Annual Meeting in Canberra in February 1988. Ms. Sophie Horrigan was a runner-up in the same competition and also won an encouragement award from Commonwealth Serum Laboratories in their Young Cell Culturist Competition.

Dr. Murray Esler was a member of the Organizing Committee of the Fourth Asian Symposium on Hypertension in Sydney in February 1987. He was also an invited speaker on 'Catecholamines' at the Annual Scientific meeting of the Endocrine Society of Australia, held in Bowral, NSW in March 1987. In June 1987 he was an invited speaker at the Sixth International Catecholamine Conference in Jerusalem where he presented a paper entitled 'Clinical study of sympathetic nervous function using measurements of regional noradrenaline release' Dr. Esler also presented an invited paper on 'Autonomic nervous system regulation of the circulation in cardiovascular health and disease' at the International Congress of Psychosomatic Medicine held in Sydney in August 1987. Dr. Esler was appointed as a Member of the Medical Research Ethics Committee (a national committee of NHMRC). He was also an on-site advisor to the University of Michigan Federal Hypertension Grant Application in March 1988. Dr. Esler was an invited speaker at the American Society of Hypertension Annual Meeting in New York City in June 1988 and presented a paper titled 'Noradrenaline release and the pathophysiology of primary hypertension'. He was also an invited speaker on 'Regional noradrenaline turnover in human hypertension' at a satellite meeting to the International Society of Hypertension meeting, held in Sapporo, Japan in May 1988.

Dr. Warwick Anderson attended the International Congress of Nephrology in London in July 1987. Dr. Anderson was also appointed Editor of Clinical and Experimental Pharmacology and Physiology. He was reappointed Chairman of the Animal Experimentation Ethics Committee of NHMRC and invited to speak at many meetings on the use of animals in medical research. Dr. Anderson was co-opted to the Medical Research Committee of NHMRC in January 1988.

Dr. Jim Angus was an invited speaker in a symposium on Mechanisms of Hypertension at the 9th Asian-Pacific Congress of Cardiology held in Auckland in February 1987. He was a member of the Scientific Programme Committee for the Xth International Union of Pharmacology meeting, Sydney, August 1987. He presented papers at and was a member of the organising committees for the IUPHAR satellite meetings in Newcastle (Neurochemical control of the coronary circulation); in Melbourne (Vascular neuroeffector mechanisms) and on Heron Island (Serotonin). He continued to serve the NHMRC as Chairman RGIC, Adelaide B; on the Scholarship and Research Evaluation Committee and on the Assigners Panel. He was invited to join the editorial board of the Journal of Hypertension. He was an occasional lecturer to the medical and science students at Monash University and addressed the Careers Forum in December 1987. Dr. Tom Cocks gave an invited paper in the IUPHAR Congress symposium on EDRF.

Dr. Alex Bobik presented a paper on Na^+/H^+ exchange in vascular smooth muscle at the 3rd Scientific Meeting of the European Society of Hypertension in Milan, Italy in June 1987. He also presented a paper on 'Ionic mechanisms involved in regulating the intracellular pH of vascular smooth muscle', by invitation, at the IUPHAR Satellite Meeting on 'Neuroeffector mechanisms regulating smooth muscle cell function', in Melbourne in October 1987. Drs. Bobik, Adams, Little, Wolf, Jackman and Ms. C. Oddie presented papers at the Xth International Congress of Pharmacology held in Sydney in August 1987. Dr. Adams also presented papers at the IUPHAR Satellite Meetings on 'Mechanisms in hypertension' and 'Neuroeffector mechanisms regulating smooth muscle cell function', in Melbourne in October 1987. Dr. Bobik has joined the Editorial Board of the Journal of Cardiovascular Pharmacology.

Ms. Susan Luff was an invited speaker at the 6th International Symposium on Vascular Neuroeffector Mechanism held in Melbourne in September 1987. The title of her talk was 'Neurovascular junctions in arterial vessels'.

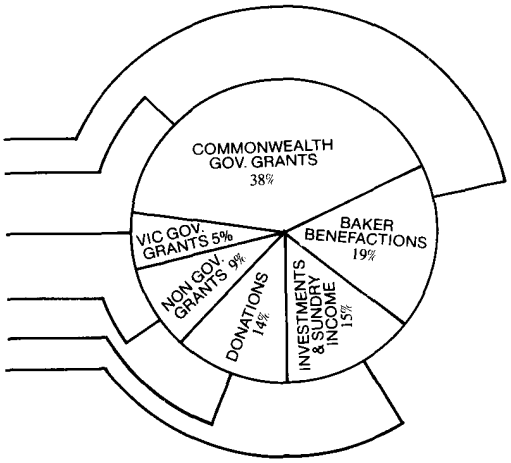
Financial Report

BAKER MEDICAL RESEARCH INSTITUTE
Year ended 31 December 1987

Income and Expenditure at a glance

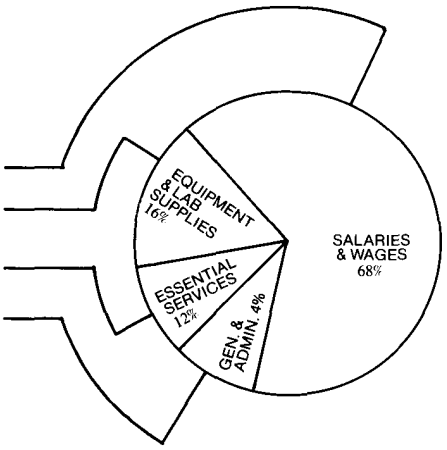
Income Derived from the Following Sources:

1986 000s	1986 %		1987 000s	1987 %
727	18	Baker Benefactions	880	19
1740	42	Government Grants - Commonwealth	1743	38
214	5	Government Grants — Victorian	228	5
329	8	Non-Government Grants	431	9
620	15	Donations	645	14
515	12	Interest from Investments and Sundry Income	681	15
4145	100		4608	100



Expenditure Distributed as Follows:

1986 000s	1986 %		1987 000s	1987 %
2764	66	Salaries & Wages	3190	68
732	17	Scientific Equipment and Laboratory Supplies	742	16
525	13	Essential Services	579	12
170	4	General & Administration	196	4
4191	100		4707	100



Balance Sheets at 31 December 1987 — Schedule 2

1986 $		$	1987 $
	Operating Fund		
	Accumulated Funds and Liabilities		
(428,272)	Accumulated (deficit) — Schedule 4		(527,523)
215,541	Bank overdraft		288,830
57,093	Sundry Creditors & Accrued Expenses		59,813
	Provision for leave entitlement		
192,266	— Annual Leave	194,829	
87,370	— Long Service Leave	164,540	
279,636			359,369
$123,998			$180,489
	Represented by:		
	ASSETS		
450	Cash on hand		450
104,971	Sundry debtors & prepayments		170,039
13,438	Monies due from other funds		—
	Short term deposits held by the		
5,139	Institute		10,000
$123,998			$180,489

1986 $		$	1987 $
	Endowment Fund		
	Accumulated Funds and Liabilities		
$1,588,448	Accumulated fund — Schedule 5		$1,768,826
	Represented by:		
	ASSETS		
	Investments (at cost)		
	Held by the Institute		
77,550	Government and semi-government stock	77,550	
245,382	Shares & debentures in companies	305,537	
—	Trust Units	265,033	
523,679	Short term deposits	402,147	
49,400	Mortgage loans	34,400	
896,011			1,084,667
	Held by ANZ Executors & Trustee Co. Ltd.		
8,410	Government & semi-government stock	—	
62,815	Shares & debentures in companies	43,794	
360,485	Trust units	357,972	
248,451	Short term deposits	280,000	
680,161			681,766
98,512	Cash at Bank		2,393
1,674,684			1,768,826
	LIABILITIES		
72,798	Monies due to Before 2000 Fund		—
13,438	Monies due to Operating Fund		—
86,236			—
$1,588,448			$1,768,826

1986 $		$	1987 $
	Research Scholarship and Other Funds Accumulated Funds and Liabilities		
	Accumulated Funds		
948,900	Restricted Fund — Schedule 6	160,335	
148,766	Edgar Rouse Memorial Fellowship Fund — Schedule 6	89,058	
107	Research Fund — Schedule 6	270	
—	Before 2000 Heart Appeal Fund — Schedule 2	22,990	
2,915	Laura Nyulasy Scholarship Fund — Schedule 6	2,892	
39,724	William Buckland Research Fund — Schedule 6	92,564	
4,852	Lang Research Scholarship Fund — Schedule 6	40,300	
101,488	Bertalli Family Fund — Schedule 6	106,014	
74,586	Ruby Wallace Travel Scholarship Fund — Schedule 6	77,789	
—	Ethel Mary Baillieu Fund — Schedule 6	125,227	
1,321,338			717,439
	Liabilities		
141,785	Equipment Creditor		—
	Sundry creditors — monies due to		
$1,463,123			$717,439
	Represented by:		
	Assets		
	Investments (at cost)		
	Held by the Institute		
4,852	Shares in companies	—	
1,322,328	Short term deposits	667,448	
1,327,180			667,448
	Held by ANZ Executors & Trustee Co. Ltd.		
8,253	Shares in companies	8,665	
33,959	Trust units	34,577	
426	Short term deposits	(50)	
42,638			43,192
20,507	Cash at Bank		6,799
72,798	Monies due from Endowment Fund		—
$1,463,123			$717,439
	Before 2000 Fund Accumulated Funds and Liabilities		
$ —	Accumulated fund — Schedule 6		$22,990
	Represented by:		
	Assets		
67,355	Short term deposits held by the Institute	22,895	
72,798	Monies due from Endowment Fund	—	
1,632	Cash at Bank	95	
141,785			22,99
	Liabilities		
—	Monies due to Operating Fund	—	
141,785	Equipment creditor	—	
141,785			—
$ —			$22,990

Statement of Movement in Accumulated Funds — Schedule 4

1986 $		$	1987 $
	Operating Fund		
4,144,845	Income		4,608,481
	Less		
4,190,817	Expenditure		4,707,732
(45,972)	Deficit for year		(99,251)
(382,300)	Accumulated deficit opening balance		(428,272)
$(428,272)	Accumulated deficit closing balance		$(527,523)
	Schedule 2		
	Operating Fund		
	Income		
	Donations from Baker Benefactions		
11,569	Statutory amount	11,569	
715,000	Transfers from Endowment Fund	868,787	
726,569			880,356
619,746	**Other Donations (Net of transfers)**		644,792
	Grants-in-aid of Research Projects		
1,740,001	National Health & Medical Research Council	1,743,078	
202,564	National Heart Foundation of Australia	240,057	
45,070	Alfred Hospital	98,442	
12,000	Royal Australian College of Surgeons	50,000	
8,601	U.S. Dept. of Health and Human Services	15,725	
—	ALCOA Foundation	27,081	
2,008,236			2,174,383
	Other Grants		
9,500	The James & Elsie Borrowman Research Trust	38,671	
5,876	The William Buckland Research Fund	5,351	
213,600	Victorian State Government	228,400	
2,120	The Laura Nyulasy Research Scholarship Fund	449	
15,000	Clive & Vera Ramaciotti Foundation	—	
7,183	Ruby Wallace Travel Scholarship	6,540	
20,183	E.E. Stewart Estate	49,663	
1,417	Bertalli Family Research Fund	9,152	
—	E.M. Baillieu Fund	43,154	
—	Lang Scholarship Research Fund	1,486	
274,879			382,866
	Income from Investments		
5,098	Held by the Trustees of the Baker Institute Grant Trust	5,098	
312,911	Other investment income	269,467	
318,009			274,565
	Other Income		
139,844	Sundry sales, recoveries & refunds	171,826	
57,562	Clinical services	79,692	
197,406			251,518
4,144,845	**Total Income**		$4,608,481

1986 $		$	1987 $
	Operating Fund		
	Expenditure		
2,764,095	Salaries & Wages		3,190,204
403,363	Laboratory supplies & isotopes		541,632
328,823	Additional equipment & building costs		200,140
63,510	Library maintenance		52,904
16,021	Postage		19,188
20,418	Telephone		33,859
50,769	Printing & Stationery		58,227
99,152	Light & Power		103,026
53,783	Insurance		56,200
110,720	Repairs & Renewals		128,779
10,000	Animal house contribution		—
12,500	Collaborative grant — NH & MRC programme		—
92,326	Travelling expenses		60,513
17,702	Public Relations		39,499
81,449	Fundraising expenses		134,054
41,543	Sundries		6,291
14,113	Clinical Services		17,000
5,392	Data Processing		—
5,138	Freight & Cartage		6,166
—	Legal & Accounting Expenses		9,202
—	Bank & Government Charges		6,835
—	Staff Amenities & Advertisements		26,091
—	Volunteers		17,922
$4,190,817	**Total Expenditure**		$4,707,732

Statement of Movement in Accumulated Funds — Schedule 5

1986 $		$	1987 $
	Endowment Fund		
1,566,586	Opening Balance		1,588,448
	Income		
715,930	Donations — Baker Benefactions	868,787	
—	T.E.&A. recovery	735	
53,975	Interest — Investment	49,333	
	— Bank	19	
256,206	Profit on sale of shares & redemption of stocks	141,303	
9,550	Sale of motor vehicles	14,000	
950	Sale of Port bottle	—	
24,407	ANZ E & T	1,605	
—	Loan Repayments from B/2000 Fund	75,000	
1,061,018			1,150,782
2,627,604			2,739,230
	Expenditure		
82	Federal tax & Bank fees	82	
845,092	Transfer to Operating Fund:		
	— Baker Benefactions	868,787	
	— Interest	49,333	
	— Payments	50,000	
25	Brokerage fees	—	
100	Recognition — Softwoods Products fully paid out in 1977	—	
121,059	Dr. Barter's Laboratory establishment expenses	—	
72,798	Transfer to Before 2000 Fund	2,202	
1,039,156			970,404
$1,588,448	Closing Balance — Schedule 2		$1,768,826

Income received from Endowment Fund investments is transferred to the Operating Fund upon receipt.

Statement of Movement in Accumulated Funds — Schedule 6

Research, Scholarship & Other Funds

1986 $		$	1987 $
	Restricted Fund		
49,847	Opening Balance		948,900
	Income		
11,569	Baker Benefactions — Statutory amount	11,569	
950,992	Grants & Donations	174,504	
16,197	Investment income & bank interest	14,107	
730	Transfer from Operating Fund	—	
979,488			200,180
1,029,335			1,149,080
	Expenditure		
	Transfers to Operating Fund		
11,569	Baker Benefactions Statutory amount	11,569	
17,104	N.H. & M.R.C.	854,170	
51,660	Payments — other	122,903	
102	Bank charges & Federal Tax	103	
80,435			988,745
$948,900	Closing Balance — Schedule 2		$160,335
	Edgar Rouse Memorial Scholarship Fund		
122,093	Opening Balance		148,766
	Income		
3,949	Donations	4,550	
22,728	Investment income & bank interest	15,951	
26,677			20,501
148,770			169,267
	Expenditure		
4	Federal tax		9
—	Payments		80,200
$148,766	Closing Balance — Schedule 2		$89,058
	Research Fund		
74,356	Opening Balance		107
	Income		
499,799	Donations	651,624	
6,095	Investment Income	2,617	
505,894			654,241
	Expenditure		
502,062	Transfer to Operating Fund	588,651	
640	Payments — other	18,394	
209	Federal Tax & Bank Fees	508	
77,232	Transfer to Other Funds	46,525	
580,143			654,078
$107	Closing Balance — Schedule 2		$270

1986 $		$	1987 $
	Before 2000 Heart Appeal Fund		
424,875	Opening Balance		—
	Income		
133,645	Donations	95,760	
37,955	Investment Income	4,468	
72,798	Transfer from Endowment Fund	2,202	
244,398			102,430
	Expenditure		
81,345	Transfer to Operating Fund	4,401	
69	Federal Tax & Bank Fees	39	
	Payments		
216,207	Electron-microscope	—	
363,493	Protein Chemistry Sequencer	—	
8,159	Other	—	
—	Transfer to Endowment Fund	75,000	
669,273			79,440
$ —	Closing Balance — Schedule 2		$22,990
	Lady Nyulasy Scholarship Fund		
4,344	Opening Balance		2,915
	Income		
727	Investment income		446
	Expenditure		
2,120	Transfer to Operating Fund	449	
36	Payments — other	20	
2,156			469
$2,915	Closing Balance — Schedule 2		$2,892
	William Buckland Research Fund		
39,241	Opening Balance		39,724
	Income		
6,707	Investment income		6,218
	Expenditure		
5,876	Transfer to Operating Fund	5,351	
348	Payments — other	291	
6,224			5,642
$39,724	Closing Balance — Schedule 2		$40,300
	Bertalli Family Research Fund		
—	Opening Balance		101,488
	Income		
100,000	Donation	—	
2,905	Investment Income	13,677	
102,905			13,677
	Expenditure		
1,417	Transfer to Operating Fund		9,151
$101,488	Closing Balance — Schedule 2		$106,014

1986 $		$	1987 $
	Ruby Wallace Travel Scholarship Fund		
—	Opening Balance		74,586
	Income		
71,000	Donation	—	
10,843	Investment Income	9,810	
81,843			9,810
	Expenditure		
7,183	Transfer to Operating Fund	6,540	
74	Federal Tax & Bank Fees	67	
7,257			6,607
$74,586	Closing Balance — Schedule 2		$77,789
	Lang Research Scholarship Fund		
4,852	Opening Balance		4,852
	Income		
—	Profit on Sale of Shares	86,980	
—	Investment Interest	2,218	
—			89,198
	Expenditure		
—	Transfer to Operating Fund	1,486	
			1,486
$4,852	Closing Balance — Schedule 2		$92,564
	Ethel Mary Baillieu Fund		
	Opening Balance		154,048
	Income		
	Investment Interest	14,406	
			14,406
	Expenditure		
	Transfer to Operating Fund	43,154	
	Federal Tax & Bank Fees	73	
			43,227
	Closing Balance — Schedule 2		$125,227

Statement of Investments (at cost) — Schedule 7

Endowment Fund
Held by the Trustees of the Institute:

	$	1987 $
Government & semi-government stock:		
Commonwealth inscribed stock	75,000	
MMBW stock	2,550	
		77,550
Other stock:		
AGC debentures		100,000
Shares in companies:		
Argo Investments Co. Ltd.	70,235	
Australian Consolidated Industries Ltd.	11,394	
Westpac Banking Corporation	13,831	
BHP	5,976	
CRA Ltd.	5,876	
CSR Ltd.	18,415	
CSR Convertible Notes	1,650	
Coles Myer	6,507	
Nat. Australia Bank	2,834	
BHP Gold Mines	516	
Templeton Global Growth Fund	50,000	
Norgold Ltd.	475	
North Broken Hill Ltd.	11,028	
Woolworths Convertible Notes	6,800	
		205,537
Cash Management Trust Units:		
PP Cash Management Trust		265,033
Short term deposits:		
Toronto Dominion Australia Ltd.		402,147
Mortgage loans		34,400
		$1,084,667

Baker Medical Research Institute
Notes to and Forming Part of the Accounts for the Year Ended 31 December 1987 — Schedule 3

1. Incorporation

On 1 August 1980, The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute was incorporated as the "Baker Medical Research Institute" under the Baker Medical Research Institute Act 1980. At this date the assets and liabilities of the original Institute were vested in the new Baker Medical Research Institute at book value.

2. Statement of Accounting Policies

The accounting policies of the Institute, which are consistent with those applied in previous years, are as follows:

(a) Historical Cost

The accounts of the Institute are prepared on the basis of historical cost and unless otherwise stated do not take into account the effect of changing money values or current valuations of non-current assets.

(b) Institute Funds, Income and Expenditure

The work of the Institute is financed from grants, endowments, donations and bequests of both general and specific natures. Income is taken to Restricted Funds where the terms of any relevant covenants apply to that income.

Income from investments is accounted for on an accrual basis.

Income from donations is accounted for on a cash basis.

Other income and expenditure is accounted for on an accrual basis. Any deficiency arising therefrom is carried forward in the Operating Fund.

(c) Capital Expenditure and Depreciation

Capital expenditure made by the Institute in respect of buildings, furniture and equipment in present and past periods has been charged against appropriate funds, grants or revenue accounts and expensed in the period in which it was incurred. Accordingly, no depreciation charge appears in the Institute's accounts.

The insurance cover of such accumulated capital expenditure, including buildings, to 31 December 1987 was approximately $10,000,000.

3. Investments — Endowment Fund

The market value of shares in companies listed on the Australian Stock Exchange at 31 December 1987 was $620,930 (1986 $778,935).

Investments managed by the ANZ Executors & Trustee Company Limited are included in the balance sheet of the Institute in accordance with statements provided by the custodian company, giving details of the Institute's entitlements in securities held by the custodian company in its own name.

4. Contingent Liability

A contingent liability exists where the Institute has indemnified a former staff member in a libel action brought against him in circumstances where he was representing the Institute. The action is presently pending and it is the opinion of the solicitors of the Institute and the Board of Management that the result of this action cannot be assessed at this time.

Auditors' Report to the Board of Management of Baker Medical Research Institute

We have audited the attached accounts as set out on Schedules 2 to 7 in accordance with Australian Accounting Standards.

As indicated in note 2(c), it is the Institute's policy to write off all capital expenditure as incurred.

In our opinion, with the exception of the effect of the omission of these assets, estimated to have a replacement cost of $10 million, the attached accounts are drawn up so as to give a true and fair view of the accumulated funds of the Institute and the net assets representing those funds at 31 December 1987, and the movements in those funds for the year to 31 December 1987, and have been made out in accordance with Australian Accounting Standards applicable to non-business entities.

Price Waterhouse
E.A. Alexander
A member of the firm
Chartered Accountants

Melbourne
8 June 1988

Donations

Received to 31st December, 1987

AMP Society Medical Research Fund	$50,000
Brockhoff Foundation	$43,500
Alcoa Foundation	US$38,000
The Ian Potter Foundation	$20,000
Clive & Vera Ramaciotti Foundations	$20,000
H.M Schutt Trust	$15,000
Smorgon Family Charitable Foundation	$15,000
Windermere Hospital Foundation	$15,000
Allan Williams Trust Fund	$11,000
BHP Limited	$10,000
Dame Elisabeth Murdoch	$10,000
Emily E.E Stewart Estate	$ 8,877.27
William Angliss Charitable Fund	$ 8,000
The Felton Bequest's Committee	$ 8,000
H & L Hecht Trust	$ 8,000
William Buckland Foundation	$ 7,700
Telectronics Pty Ltd	$ 6,000
Bell Charitable Trust	$ 5,000
Jack Bernard Hollole	$ 5,000
Besen Charitable Foundation	$ 5,000
Miss M.L Dillon	$ 5,000
OTC — Courtesy of R.G Ansett	$ 5,000
Trustee of Late Edward Wilson	$ 4,000
Marian & E.H Flack Trust	$ 3,800
Eric Arthur Ormond Baker Charitable Fund	$ 3,000
The George Hicks Foundation	$ 2,800
BP Australia Ltd	$ 2,500
Glaxo Australia Pty Ltd	$ 2,500
National Australia Bank	$ 2,500
Leopold Klein Charitable Foundation	$ 2,100
Werge Batters Trust	$ 2,000
Coles Myer Ltd	$ 2,000
ICI Australia	$ 2,000
Mr R.E Nelson	$ 2,000
Peat Marwick Hungerford	$ 2,000
Mrs P.S Row	$ 2,000
Union Fidelity Trustee Co. of Australia	$ 2,000
Mr & Mrs P Arnhold	$ 1,840
M.K.A Bell Memorial Fund	$ 1,500
Mr L.J Fitzgerald	$ 1,500
Vernier	$ 1,500
Miss E.W Wortley	$ 1,270
McPhersons Ltd	$ 1,250
Lady McGrath	$ 1,200
Mr & Mrs A Marks	$ 1,200
Mr & Mrs M Downes	$ 1,050
Mr K Eisner	$ 1,050
Mr & Mrs H.A Webster	$ 1,020
Mr F.K Alfredson	$ 1,000
R.G Ansett	$ 1,000
Atlas Steel (Australia) Pty Ltd	$ 1,000
E.L & C Baillieu	$ 1,000
Comalco	$ 1,000
CRA Ltd	$ 1,000
Mrs F Danglow	$ 1,000
Dr & Mrs A.E Dickman	$ 1,000
Mrs N.A Edwards	$ 1,000
Anonymous	$ 1,000
H & R Properties Pty Ltd	$ 1,000
S.B Myer	$ 1,000
Pacific Dunlop Ltd	$ 1,000
Mr G.F Pugsley	$ 1,000
George & Edith Ramsay Charitable Trust	$ 1,000
Scientific Glass Engineering	$ 1,000
Stadiums Pty Ltd	$ 1,000
Tattersalls Sweep Consultation	$ 1,000
The Age — David Syme & Co Ltd	$ 1,000
Mr & Mrs H.F Wakefield	$ 1,000
J.B Were & Son	$ 1,000
Lady Gladys Reid	$ 800
A.A Assurance Company Ltd	$ 750
ANZ Bank	$ 750
Bongiorno & Co Pty Ltd	$ 750
Commercial Union Insurance	$ 750
Mrs L.C Dickson	$ 750
Rev. F.S Imray	$ 750
Mayne Nickless Ltd	$ 750
Sir Arvi Parbo	$ 750
Mrs Beth Smith	$ 750
Mrs A Bult	$ 700
Flower Drum Market Lane	$ 700
Miss E Gaylard	$ 700
Mrs K Kapahi	$ 700
Mrs D Martin	$ 700
Mr A Muir	$ 700
Mr R.M Reid	$ 700
Miss K.M Bishop	$ 695
Ms P.A Milne	$ 665
Mr J.D Moir	$ 620
Dr V.V Bower	$ 600
Mrs B Freshwater	$ 600
Miss E.C Hansen	$ 600
Mr D.C Vollmerhause	$ 560
Mrs W Keir	$ 550
Mrs C.Y Sullivan	$ 550
Mr A.L Abrahams	$ 500
Amcor	$ 500
Mrs A.L Bottomley	$ 500
Brambles Industries Ltd	$ 500
Bryon Moore Day & Journeau	$ 500
Mrs M.A Burne	$ 500
Commonwealth Industrial Gases	$ 500
Construction Engineering (Australia)	$ 500
Containers Packaging	$ 500
Dalgety Australia Holdings Ltd	$ 500
Mr J.L Davis	$ 500
Mr A Douglas	$ 500
Mr D Drewin	$ 500
Mr E.R Forrest	$ 500
P & M Harbig Holdings Pty Ltd	$ 500
John Holland Holdings Ltd	$ 500
Hooker Corporation Ltd	$ 500
Mr & Mrs I Hunter	$ 500
Misses D & J Jeffrey	$ 500
Leighton Holdings Ltd	$ 500
Miss B.E McAuley	$ 500

Moore Business Systems Ltd	$	500
Sir Laurence Muir	$	500
Nestle Australia Ltd	$	500
Nilsen Electrical Sales	$	500
Nicholas Kiwi Australia Ltd	$	500
Mr H Penaluna	$	500
Mr M.I Pierce	$	500
Wormald International Ltd	$	500
Mrs D.H Behrend	$	450
Mr P Brett	$	450
Mr L Janover	$	450
Mr L Long — Leonard Long Studios	$	450
Miss H.D Glascodine	$	420
Mr E.J Collings	$	400
Mr F.D Culley	$	400
Mr H.H Gillbank	$	400
Mr A Goode	$	400
Mr N Jackson	$	400
Miss A.P Kennedy	$	400
Mrs J Mackay	$	400
Pilkington ACI	$	400
Mr & Mrs H Schreiber	$	400
Mrs R.H Cunningham	$	395
Mr A & Miss E McDougall	$	395
Mr A.W McDonald, QC	$	380
Mrs N Drummond	$	370
Mr R Croft	$	370
Mr L.A Armstrong	$	350
Mr H.T Cadwallader	$	350
Mr B.A Counsel	$	350
Mr J.F Edmonds	$	350
Mr J Fuller	$	350
Mr W.G Philip	$	350
Mr D Wittner	$	350
Mrs N.L Duncan	$	320
Mrs M Grose	$	320
Cadbury Schweppes Pty Ltd	$	300
Contract Drafting Services Pty Ltd	$	300
Mr A.G Coulthard	$	300
Daytex Fabrics Pty Ltd	$	300
Don A Morgan Dental Supplies	$	300
Mr Fusspots Dry Cleaners	$	300
Mr J.S Grey	$	300
Mr F Hannemann	$	300
Mr R.B Johnstone	$	300
Mr L.J King	$	300
Mr. N.R Korner	$	300
Kraft Foods Ltd	$	300
Mr Norman Lees	$	300
Mr & Mrs T.G Pickford	$	300
Mrs E Pitman	$	300
Randalls & Company	$	300
Mr & Mrs E Rogers	$	300
Mr D.F Smith	$	300
Mrs A.K Stewart	$	300
Mrs M.A Simpson	$	300
Mr A Stickland	$	300
Mr M Gordon	$	275
Dr D.N Madill	$	275
Mr W.J Bailey	$	250
Mr M.D Bridgland	$	250
Brunckhorst Investments — Mr J Brunckhorst	$	250
Bunge (Australia) Pty Ltd	$	250
Mr G.E Carrington	$	250
Cheetham Limited	$	250
Concorde Engineering	$	250
Mrs J.F Donges	$	250
N.S Eckersley Pty Ltd	$	250
Mr & Mrs A Edwards	$	250
Engelhard Pty Ltd	$	250
J Gadsden Australia Ltd	$	250
Glo-Weave Consolidated Pty Ltd	$	250
W.R Grace Australia Ltd	$	250
Mr A.W Hall	$	250
Mrs B Hewitt-Lees	$	250
Sir John Holland	$	250
The Hoyts Corp Pty Ltd	$	250
Joubert & Joubert Pty Ltd	$	250
Mr J.B Levingston	$	250
Mr R.K Lourey	$	250
Mr B.M Mckay	$	250
Minett Partners	$	250
E.E Muir & Sons Pty Ltd	$	250
Miss M Murdoch	$	250
G & K O'Connor Pty Ltd	$	250
Mr R.G Pitcher	$	250
Portland House Group	$	250
Mrs J.C Price — Price Family Trust	$	250
Miss E.M Reedy	$	250
Professor G.C Schofield	$	250
Siemens Ltd	$	250
E.R Squibb & Sons Pty Ltd	$	250
Miss C.M Teague	$	250
Tubemakers of Australia Ltd	$	250
Unitus Industries Pty Ltd	$	250
Mr E.A Wade	$	250
Wardley Investments Management	$	250
Wenzel Distributors Pty Ltd	$	250
Mr N.A Wigmore	$	250

Another 179 donations were received between $200 and $250.

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