# Annual Report 1984/85

# Baker Medical Research Institute

Affiliated with Alfred Hospital and Monash University

**Fifth Annual Report of the Baker Medical Research Institute.**

**Thirty-sixth Annual Report of Clinical Research Unit, Alfred Hospital.**

**Twenty-eighth Annual Report of Ewen Downie Metabolic Unit, Alfred Hospital.**

*Cover:*
*Arterial smooth muscle cell in culture loaded with lipid*

# Research Aims

In Australia one in six persons have high blood pressure and one in four have a cholesterol problem that predisposes to atherosclerosis (arterial disease). Together these account for over 50% of all deaths and much serious illness due to stroke, heart attack, heart failure and kidney failure.

The Institute seeks to increase knowledge of the causes of hypertension and of atherosclerosis. Our aim is to use this knowledge to improve treatment and reduce the incidence of these disorders and, if possible to prevent them.

# Board of Management

**President**
SIR LAURENCE MUIR
V.R.D., L.L.B., F.S.I.A., F.A.I.M.

**Vice President**
J.D. MOIR

**Members**
Dr. R. CUMMING, M.B., B.S.

J.C. HABERSBERGER,
A.O., B.Comm.

D.F. HOGARTH, B.Sc.

Dr. H.B. KAY, A.M., M.D. (Melb.),
F.R.C.P., F.R.A.C.P.

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

W.D. McPHERSON, A.O., A.A.S.A.

Professor G.C. SCHOFIELD, O.B.E.,
M.D.(N.Z.) D.Phil, (Oxon), F.R.A.C.P.,
F.R.A.C.M.A.

Emeritus Professor R.R. ANDREW,
A.O., M.D., B.S.(Melb), F.R.C.P.,
F.R.A.C.P., Hon. M.D.(Monash)

Professor G. RYAN, M.D., B.S.,
Ph.D.(Melb), F.R.C.P.A., F.R.A.C.P.

R.J. BARCHAM

**Secretary**
Dr. T.J. WOOD,
M.B., B.S., M.H.A., F.R.A.C.P.,
F.R.A.C.M.A., F.H.A., F.A.I.M.

**Honorary Treasurer**
W.J. BAILEY

# Development Council

**Chairman**
SIR LAURENCE MUIR,
V.R.D., L.L.B., F.S.I.A., F.A.I.M.

**Members**
R.J. BARCHAM

Dr. C.M. DEELEY

M.G. DOWNES

E.N.M. FINNIE

J.C. HABERSBERGER, A.O., B.Comm.

I.T. PERKINS, F.C.A.

K.O. WILKS, B.Comm.

F.I. MENZIES, B.Ec(Hons), M.Sc.(Econ)

B. PASCOE

R. MILLER, LL.B., LL.M.

# "Before 2000 Heart Appeal" Committee

**Chairman**
Sir LAURENCE MUIR

R. PRATT

R.G. ANSETT

B. PASCOE

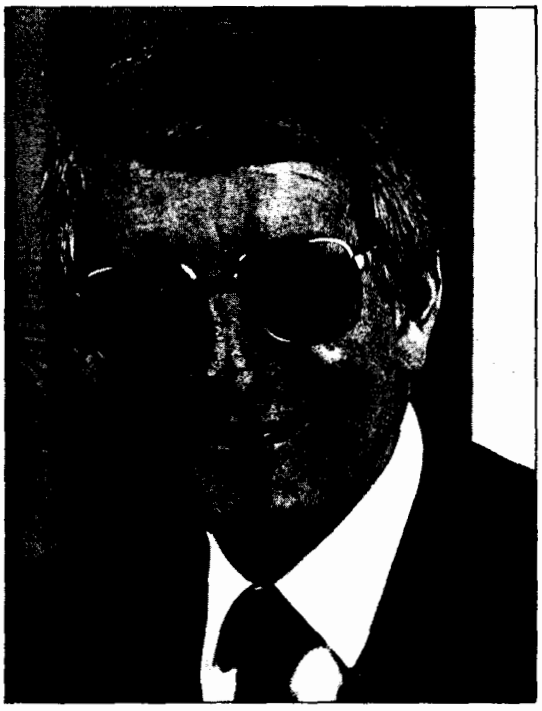
M.G. DOWNES

F. VERLINDEN
(Appeal Co-ordinator)

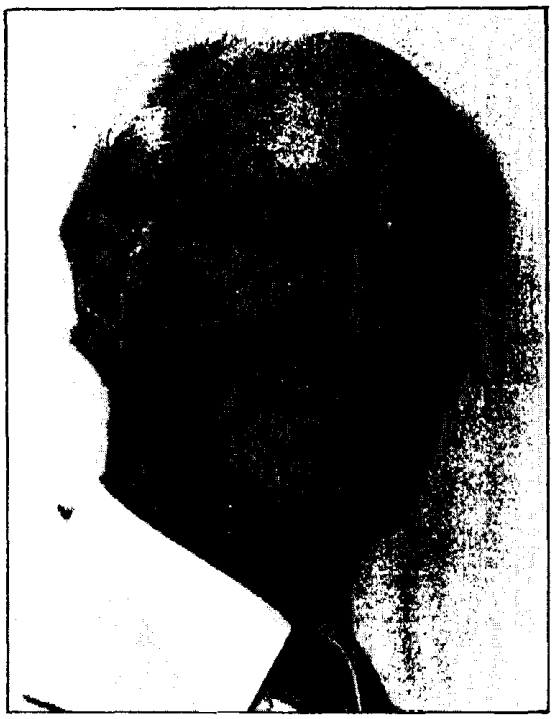
# Board of Management

**Sir Laurence Muir** President, was senior partner of Potter Partners until 1980. He is Chairman of the Canberra Development Board and a member of the Federal Parliament House Construction Authority.

**Mr. J.D. Moir** Vice-President, is a Senior Partner in the legal firm, Gillotts.



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

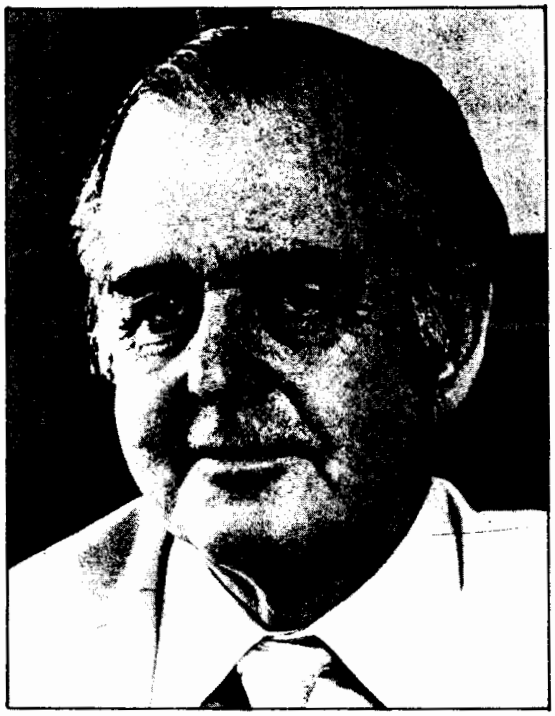


**Dr. R. Cumming** is Secretary of the National Health and Medical Research Council.

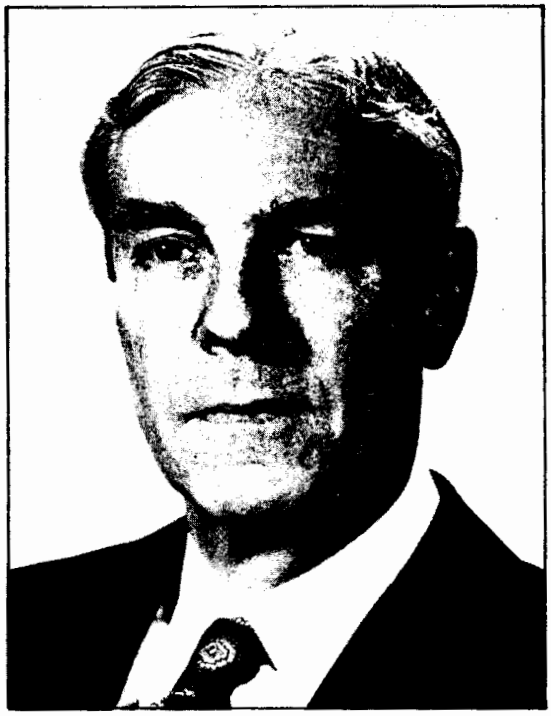
**Professor P.I. Korner** Director of the Baker Medical Research Institute.

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

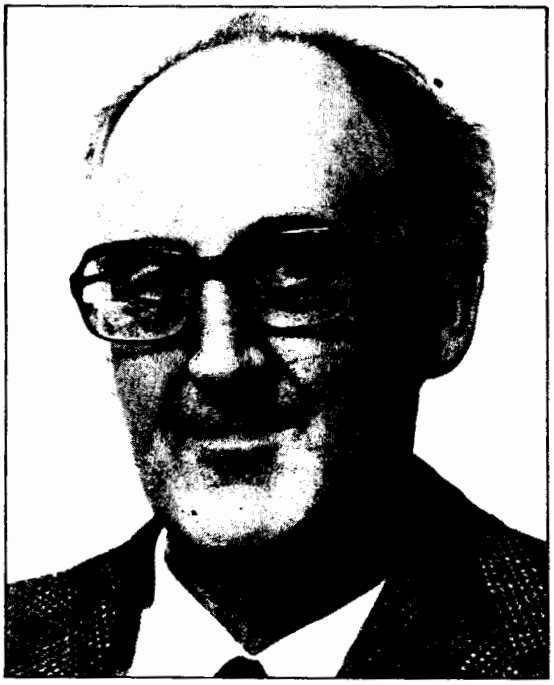
**Mr. D.F. Hogarth** is Chairman of Directors of Kodak (Australasia) Pty. Ltd.



**Emeritus Professor R.R. Andrew** was Foundation Dean of Monash Medical School and is President of the Baker Institute Alumni Association and a Past Vice-President of the Institute.

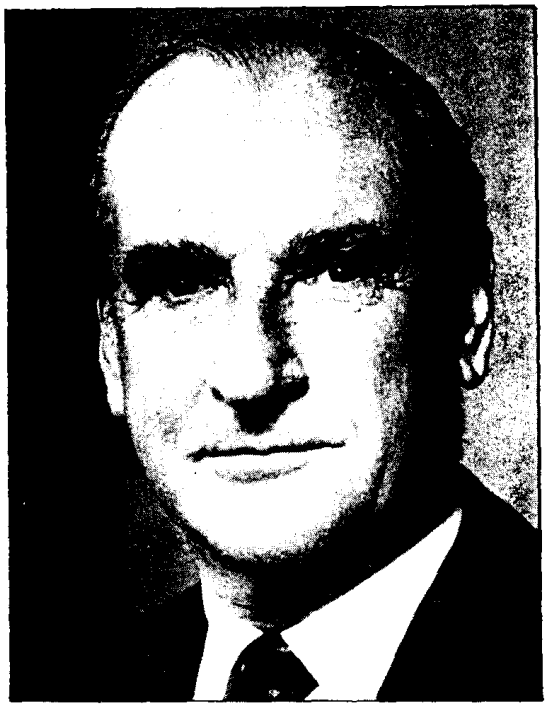


**Mr. J.C. Habersberger** is a Past President of the Institute and of the Alfred Hospital. Until his retirement in 1976, he was Managing Director of Kodak (Australasia) Pty. Ltd.

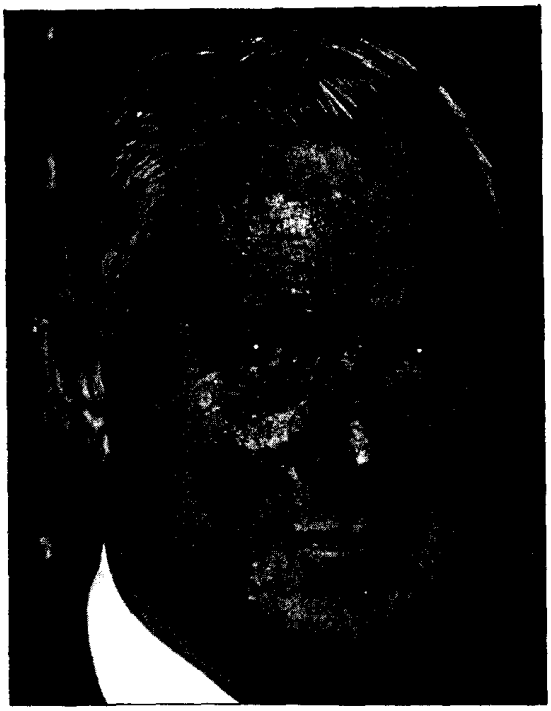


**Professor G.C. Schofield** is Dean of the Faculty of Medicine of Monash University.

**Mr. W.D. McPherson** is Chairman of McPhersons Ltd. and a Director of BHP Ltd.



**Dr. H.B. Kay** is a member of the Alfred Hospital Board of Management.



**Mr. R.J. Barcham** is a retired Australian Trade Commissioner.

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

**Footnote:**
Membership of the Board of Management and the Development Council is as at the time of publication. The Board gratefully acknowledges the contributions to the work of the Institute during the period under review from:

Professor R. Porter
The late J.D. Milne

# Staff

**Director**
PROFESSOR P.I. KORNER, M.D., B.S., M.Sc.(Syd), F.R.A.C.P., F.A.A.
**Deputy Director**
Dr. P.J. NESTEL, M.D.,B.S.(Syd), F.R.A.C.P.
**Associate Director**
PROFESSOR J. LUDBROOK, M.D., Ch.M., D.Sc., F.R.A.C.S., F.R.C.S.

## HYPERTENSION AND CIRCULATORY CONTROL RESEARCH UNIT

**Head**
PROFESSOR P.I. KORNER

*CIRCULATORY CONTROL AND NEUROPHARMACOLOGY LABORATORY*

**Scientific Staff**
PROFESSOR P.I. KORNER, M.D., M.Sc.(Syd). F.R.A.C.P., F.A.A.
Dr. P.K. DORWARD, M.A.(Cantab.), Ph.D.,(Mon.), Senior Research Officer
Ms. J. ABERDEEN, B.Sc.(Hons.)(Melb.) Research Assistant
Mrs. S.L. BURKE, B.Sc.(Hons.)(Syd.), Research Assistant
Ms. J. OLIVER, B.Sc.(NSW), Senior Professional Officer
Dr. B.K. EVANS, B.Sc.(Melb.), Ph.D.(Melb.), Senior Research Associate
Dr. A.W. QUAIL, M.B., B.S.(Syd.), M.D.(Newcastle), F.F.A.R.A.C.S., Research Fellow
Dr. G. HEAD, B.Sc., Ph.D. (from February 1985)
**Technical Staff**
Ms. R. SMITH
Ms. C. DUCH
**Research Student**
Mr. E. BADOER, B.Sc., (Hons.) (Melb.)

*NEUROPHYSIOLOGY LABORATORY*

**Scientific Staff**
Dr. E.M. McLACHLAN, B.Sc., Ph.D.(Syd.)
Dr. G.D.S. HIRST, B.Sc.(Pharm.), B.Sc.(Pharmacol.), Ph.D.(Leeds) (Visiting Scientist)
Ms. S.E. LUFF, B.Sc.(Hull), M.Sc.(Birm.), National Heart Foundation Research Officer (from January 1985)
Dr. B. GRIERSMITH, B.Sc.,(Mon.), Ph.D.(ANU) (Part-time)
Mr. J.F. CASSELL, B.Sc., B.E.(Syd.)
**Research Students**
Mrs. T. SITTIRACHA, B.Sc., M.Sc.(Thailand)
Mr. D. LEWIS (from March 1985)
**Technical Staff**
Ms. K. WALLS

*MORPHOLOGY LABORATORY*

Dr. B.J. OLDFIELD, B.Sc., Ph.D.(Mon.) (until February 1985)
Mr. C. ANDERSON, B.Sc.(Hons.)(Melb.), (from April 1985)
Ms. O. CHRISTIE, B.Sc., M.Sc.(Mon.) (from January 1985)

*HUMAN HYPERTENSION LABORATORY*

**Scientific Staff**
Dr. M.D. ESLER, M.B., B.S., B.Med.Sci. (Melb.), Ph.D.(ANU), F.R.A.C.P., Senior Research Fellow.
Dr. P.A. BLOMBERY, M.B., B.S., B.Sc.(Med.)(Syd.), Ph.D.(Mon.), F.R.A.C.P., Senior Research Fellow.
Dr. G.L. JENNINGS, M.D., B.S., M.R.C.P.(UK), F.R.A.C.P., Staff Physician.

Dr. E. LAUFER, Senior Research Associate.

**Technical Staff**
P. LEONARD, B.Sc. (until December 1984)
Ms. P. SCOTT
Sr. S. SCEALY
Ms. L. ROWLANDS
Ms. J. HILLIARD
Ms. D. BURTON, B.Sc.(Hons.)
Ms. L. NELSON, B.Sc.
Mr. B. BIVIANO, B.Sc. (until May 1985)
Mr. G. LAMBERT, B.Sc.

*RENAL LABORATORY*

**Scientific Staff**
Dr. W.P. ANDERSON, B.Sc., (U.N.E.), Ph.D.(Adel.), Senior Research Fellow
Dr. R.L. WOODS, B.Sc.(Qld), Ph.D.(Mon.)
Ms. K. DENTON, B.Sc., M.Sc.(Mon.)
Dr. R.L. KLINE, Ph.D., Visiting Fellow (till June 1984)

**Technical Staff**
Ms. D. RAMSEY
Ms. J. THOMSON
Ms. J. MAWSON (till November 1984)
Mrs. A. MacPHERSON (from November 1984)
Mr. S. WOOD

*PHARMACOLOGY LABORATORY*

**Scientific Staff**
Dr. J.A. ANGUS, B.Sc., Ph.D.(Syd.), Senior Research Fellow
Dr. T. COCKS, B.Sc. (NSW), Ph.D.(Lond,), Senior Research Officer
Dr. K. SATOH, M.D., M.D.Sci. (Tohuku, Japan) Visiting Scientist

**Technical Staff**
M. LEUHMAN, B.Sc.(Monash) (till July 1984)
R. MacDONALD, B.Sc.(Monash) (till July 1984)
Miss D. KAROMOSHOS, B.Sc. (from October 1984)
Miss E. WRIGHT, Ass. Dip. O. Ed (from July 1984)

**Research Students**
M.J. LEW, B.Sc. (Hons.)(Mon.)
Miss C. WRIGHT, B.Sc. (Hons.)(Mon.)

*BASIC CARDIOLOGY LABORATORY*

**Scientific Staff**
Dr. A. BROUGHTON, M.B., B.S., Ph.D., F.R.A.C.P.
Dr. J. SMOLICH, M.B., B.S., B.Med.Sci., (from January 1985)

**Technical Staff**
Mr. M. ROSS-SMITH, B.Sc.(Hons.)(from October 1984)
Ms. K. PEMPEL (from March 1985)

## CARDIOVASCULAR METABOLISM AND NUTRITION RESEARCH UNIT

**Head**
Dr. P.J. NESTEL

**Scientific Staff**
Dr. P.J. NESTEL, M.D., B.S.(Syd.), F.R.A.C.P.
Dr. N.H. FIDGE, B.Sc., Ph.D.(Adel), Principal Research Fellow
Dr. M.F. REARDON, B.Sc., Ph.D.(ANU), Senior Research Officer (1984)
Dr. T. BILLINGTON, B.Sc., Ph.D.(Mon.), Senior Research Officer
Dr. S. TURLEY, B.Sc.(UNE), Ph.D.(ANU), Post Doctoral Fellow, National Health & Medical Research Council
Dr. A. KAGAMI, M.D.,(Jikei University, Tokyo) Visiting Scientist (till November 1984)
Dr. T. OHTA, M.D., (Kumamoto University, Japan) Visiting Scientist (till November 1984)
Ms. M.G. O'CONNOR, B.Sc.(Melb.), Research Officer
Mrs. E. MONGER, M.Sc.
Ms. P. NUGENT, B.Sc.
Mrs. J. BACHMAIER, B.Sc.
Ms. A. LEONARD-KANEVSKY, B.Sc.
H. EDELSBACHER
Ms. E. FAEHSE

**Research Students**
J. BAZELMANS, B.App.Sc., B.Sc.(Hons.)
J. COHN, B.Sc.(Hons.)
S. WONG, B.Sc.(Hons.)
H. BARRETT, B.Agr.Sc.(Hons.)
B. MEYER, B.Sc.

## RISK EVALUATION CLINIC

**Staff**
Mrs. R. CHUANG, Dietitian
Mr. M. O'CALLAGHAN (till December 1984)
Ms. J. PEARSON (from February 1985)

## CARDIOVASCULAR SURGICAL RESEARCH UNIT

**Head**
PROFESSOR J. LUDBROOK

*VASCULAR LABORATORY*
**Scientific Staff**
PROFESSOR J. LUDBROOK, M.D., D.Sc., Ch.M., B.Med.Sc., F.R.A.C.S., F.R.C.S., Senior Principal Research Fellow
Mr. P.C. RUTTER, M.B., B.S., F.R.C.S.
Mr. W.F. GRAHAM, M.Sc., Research Assistant
Mr. S.J. POTOCNIK, B.Sc.(Hons.) Research Assistant

**Technical Staff**
Ms. J. ANDERSON

*CARDIAC SURGICAL RESEARCH LABORATORY*
**Scientific Staff**
Dr. F.L. ROSENFELDT, M.B., B.S., M.D.(Adel.), F.R.C.S.E., F.R.A.C.S., Senior Research Fellow
Dr. M. RABINOV, M.B.B.S.
Dr. XU WEI GUANG, Dip.Med. (ANHWI) B.App.Sci., Visiting Scientist from February 1984
Dr. CHEN XIAU ZHONG Visiting Scientist from February 1985

**Technical Staff**
J. BOARDMAN
C. BOYES

## CELL BIOLOGY LABORATORY

**Scientific Staff**
Dr. J.H. CAMPBELL, B.Sc.(NSW), Ph.D.(Melb.), Senior Research Fellow
Dr. G.R. CAMPBELL, B.Sc., Ph.D.(Melb.), Senior Research Associate
Ms. L.C. POPADYNEC, B.Sc.(Mon.), Research Assistant
Ms. J.D. ROGERS, Research Assistant, Life Insurance Medical Research Fund
Ms. N. PUGLISI, Technical Assistant, National Heart Foundation

**Research Students**
Mr. ANG AIK HOOI, B.Sc.(Mon.)
Ms. M.J. BLACK, B.Sc.(Melb.)
Ms. R. RENNICK, B.Sc.(Melb.)
Ms. S. KOUDOUNAS (from March 1985)
Ms. E. NG (from March 1985)

## ADMINISTRATION, FINANCE AND SERVICES

**Administration and Finance**
Mr. L.J. HAYES, A.A.I.M., Senior Executive Officer.
Mr. K. SAMPSON, Finance Officer
Mrs. M. NICHOLSON, Senior Secretary
Ms. B. SMITH, Senior Secretary
Ms. SEAH LIAN-KEE, Senior Secretary (till October 1984)
Ms. C. HARRIS, Senior Secretary (from October 1984)
Ms. J. WONG, Secretary (from October 1984)
Ms. K. REYNOLDS, Fundraising Clerk/Receptionist
Mrs. M. SUTCLIFFE, Secretary (till July 1984)
Mrs. M. MIJAK, Secretary (from August 1984)

Ms. K. MOORE, Secretary/Typist (till August 1984)
Ms. M. O'LOUGHLIN, Secretary/Typist (from May 1985)
Ms. F. BAUM, Receptionist/Typist (till April 1985)

*COMPUTER PROGRAMMER*
Mrs. J. GIPPS, B.Sc., G.C.E.

*PHOTOGRAPHER*
Mrs. K. ISLEY, Photographer
Ms. F. HAMMOND, Assistant (till March 1985)
Mrs. T. SMYTH, Assistant (from March 1985)

*THEATRE*
Sr. R. DUMPYS, B.Sc. (till March 1985)
Mr. L. COLSON, B.V.Sc. (from May 1985)

*THE ROUSE FAMILY LIBRARY*
Ms. M. DELAFIELD, Librarian
Mrs. L. LIVINGSTON (till March 1985)

*LABORATORIES SERVICES*
Mr. C.E. LEWIS, Laboratory Supervisor
Mr. A. O'KEEFE, Laboratory Assistant
Ms. J. PRITCHARD, Laboratory Assistant

*BIOLOGY RESEARCH UNIT*
Dr. N.J. WALDEN, B.V.Sc., M.R.C.V.S., Consultant Veterinarian (till April 1985)
Mr. A. BONS, Officer in Charge

**Technical Staff**
Mr. E. TURNBULL, Technical Officer
Mr. P. MARTIN, Assistant Technical Officer
Mr. G. McMAHON, Animal Assistant
Mr. J. KING, Trainee Animal Assistant (from March 1985)
Ms. K. HAUSER, Trainee Animal Assistant (from March 1985)
Mr. S. DURIE, Trainee Animal Assistant (from March 1985)

## ELECTRONICS LABORATORY AND WORKSHOP

Mr. F. HANNEMANN, B.E., Electronics Design Engineer, M.I.R.E.E., Officer-in-Charge
Mr. K.R. HARVEY, B.E. Electronics Engineer
Mr. J.F. BAIRD, Instrument Maker
Mr. P. WESTGARTH, Electronics Technician (till September 1984)
Mr. S.J. CARTER, Electronics Technician (from December 1984)
Mr. G. HILL, Tool Maker (till August 1984)
Mr. C. G. LAWSON, Instrument Maker (from October 1984)
Mr. D. BELL, Electronics Technician
Mr. A. FRY, Apprentice (till February 1985)

## ALFRED HOSPITAL CLINICAL RESEARCH UNIT

**Staff List**

**Director**
P.I. Korner, M.D., B.S., M.Sc., F.R.A.C.P., F.A.A.

**Deputy Director**
P.J. Nestel, M.D., B.S., F.R.A.C.P.

**Associate Director**
J. Ludbrook, M.D., Ch.M., D.Sc., B.Med.Sc., F.R.A.C.S., F.R.C.S.

**Medical Staff**
P.A. Blombery, M.B., B.S., B.Sc.(Med.), Ph.D., F.R.A.C.P.
Vascular Physician
A. Broughton, M.B., B.S., Ph.D., F.R.A.C.P.
Assistant Physician
R.F. Cook, M.B., B.Sc., M.R.C.P.(UK), Assistant Physician

# Contents

## Baker Institute



## Alfred Hospital Clinical Research Unit



## Ewen Downie Metabolic Unit

# History

The Baker Medical Research Institute was founded in 1926. Thomas Baker died in 1928. With his wife Alice and sister-in-law Eleanor Shaw, their estate provided the funds to establish the Institute. Statutory and discretionary grants from the estate continue to be a significant component of the Institute's income.

Thomas Baker and his partner J.J. Rouse were pioneers in the production of photographic materials. Their firm subsequently became Kodak (Australasia) Pty. Ltd.

From its beginning as a laboratory administered by Trustees providing biochemical and clinical pathology services to the Alfred Hospital, the Institute has evolved into a major Australia medical research facility specialising in research into cardiovascular disorders.

Since 1980, the Institute has been constituted under its own Act of Parliament and managed by a Board of twelve members, representing the former trustees, representatives of the Alfred Hospital and Monash University, members of the community and the Institute's Director (ex officio).

The Institute is affiliated with Monash University and participates in the University's higher degree training programmes.

*An early photograph of Thomas Baker's birthplace 11 Bishopston, Montacute, Somerset looking south to St. Catherine's Church. No. 11 is 50 yards north on the right side.*

# 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 has a staff of 130 people. The research is carried out in 14 laboratories and in the Alfred Hospital's Clinical Research Unit.

# President's Report

*Sir Lawrence Muir*

1984 was a challenging year and one in which decisions vital to the future of the Institute were taken. It was a year in which Government support for the Institute faltered somewhat and private support was stronger than ever.

In my report last year, I made mention of the quickening pace of medical research and the increasing dependence on the availability of high technology equipment to maintain the momentum. During 1984 it was evident that the Institute was at a crossroads. Ten years of research into the major cardiovascular disorders had set the scene for the clinical application of the research results. In order to provide this next stage in the Institute's development, the Board recognised the need to invest in new equipment. Almost $200,000 was spent on two items — an amino-acid analyser as the first stage in the establishment of a protein chemistry laboratory and an echocardiograph for use in hypertension studies.

In order to finance these acquisitions, the Institute had to dig deep into its reserves. In doing so there was recognition that equally costly and necessary equipment could not be financed internally. After a good deal of deliberation, the Board approved the launching of an appeal under the title "Before 2000 Heart Appeal" to raise $2.0 million. The appeal is intended to provide the funds for the remaining equipment for the protein chemistry unit, the replacement of our 15 year old electron microscope, a number of backlogged smaller equipment purchases and the establishment of fellowships to encourage collabora-

tion between our scientists and others, both within Australia and from overseas. It is an ambitious project and one which could not succeed without the enthusiastic support of the Development Council and Board. We have recruited Mr. Bob Ansett and Mr. Richard Pratt, Mr. Bruce Pascoe and Mr. Michael Downes to help us. We are especially grateful to them for joining our Appeal Committee.

1984 and the first days of 1985 brought sadness with the deaths of Sir John Reid, a former Board Member, Mr. John Milne our Honorary Treasurer and Dr. Michael Reardon, Head of the Lipoprotein Biochemistry Unit. Each of them, in different ways, had made a tremendous personal contribution to the Institute and they will be missed.

The 1984 Baker Oration was delivered by Professor Priscilla Kincaid-Smith — a former member of the Baker Institute's staff and one of Australia's most distinguished physicians. Her account of the work she undertook in finding that abuse of analgesics led to a serious form of kidney disease was an enthralling detective story enjoyed by an audience of over 100 scientists and laymen.

1985 heralds the International Atherosclerosis Congress which takes place in Melbourne in October under the Chairmanship of Dr. Paul Nestel, our Deputy Director. Attendance at the Congress looks set to exceed all expectations. This is a tribute to the planning and the programme which he and his colleagues in the Congress organisation have brought together.

Mr. Darren Baillieu was presented with the Baker Institute Award at a small function at his home attended by his family, former Trustees, Board Members, the Director and other Institute staff and the former Director, Dr. T. Lowe. The Award, which acknowledges distinguished service to the Institute, was a fitting tribute to Mr. Baillieu's association with the Institute which extends over almost 25 years. This was only the second time the Award has been presented.

The Baker Institute survives and thrives through the appreciation in the community of the value of its research and what it stands for — prevention of the two most common and serious diseases in the community.

The financial support provided through our Block Institute Grant from the Federal Government did not grow at the rate anticipated during 1984 and we were unable to secure the level of State support we felt justified in seeking. Support from both quarters is a large and essential component of our overall income for which we are grateful. It is important to us that support from Government is constant and paradoxical that while income from them has not matched our expectations, non Government grants from the Baker Benefactions, the Alfred Hospital, the National Heart Foundation, the Ramaciotti Foundation and the many Trusts, Foundations, Companies and private donors have been excellent. I am indebted to members of both the Board and the Development Council for the efforts and influence they exert on our behalf which results in such widespread support from the community. I should like to express particular thanks to those individuals who have recently joined our Board; Mr. W.J. Bailey, Mr. R.J. Barcham and Professor G. Ryan — and our Development Council; Mr. F.I. Menzies, Mr. R. Miller and Mr. B. Pascoe for their part.

I conclude by congratulating our Director and his staff on a further year of achievement. In doing so I recognise that the success of the Institute's work results from team effort and I thank all those who have participated in whatever capacity.

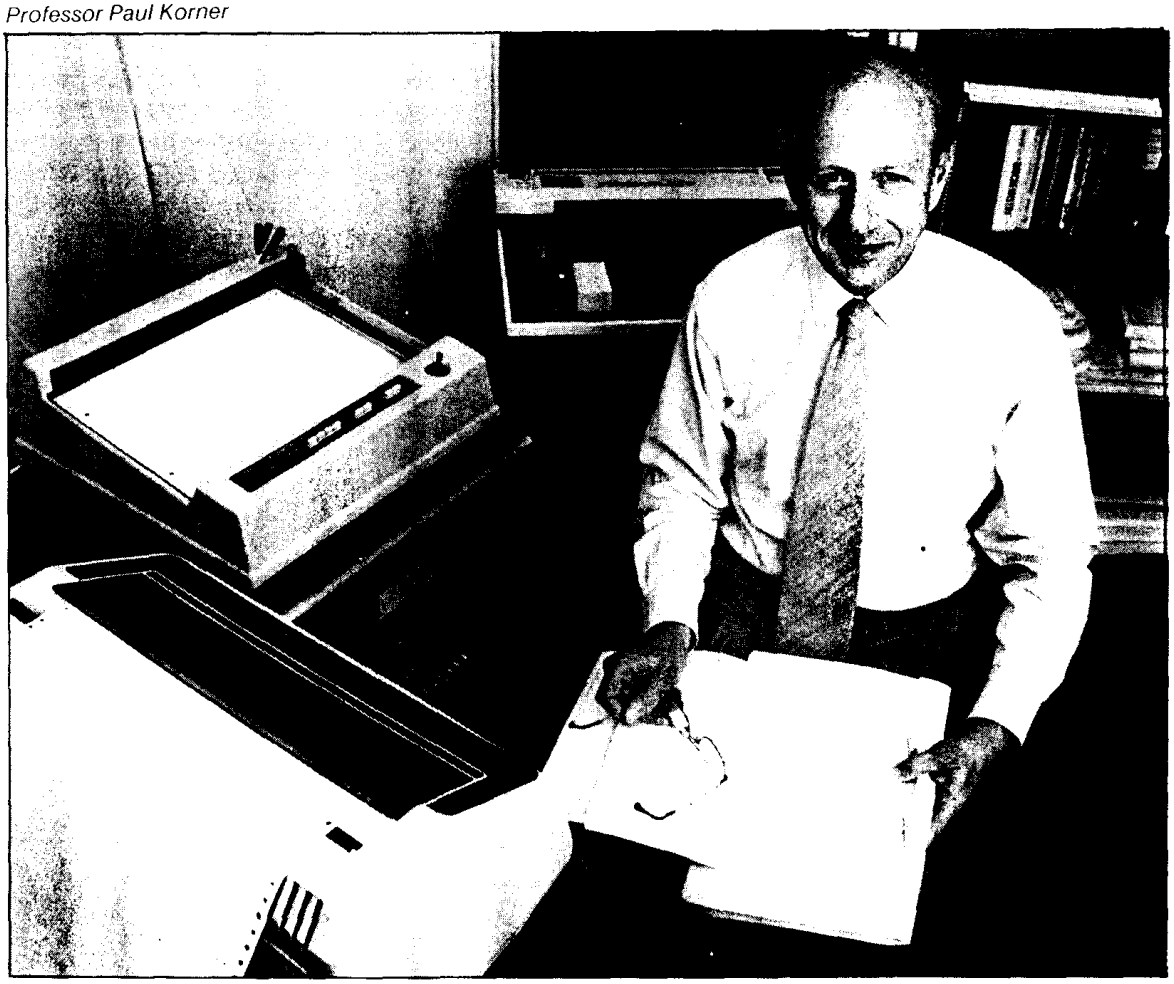
# Director's Report

During 1984 Dr. Richard L. Havel, Director of the University of California Cardiovascular Research Institute in San Francisco and one of our Overseas Advisers returned after an absence of about two years. In his report to the Board, Professor Havel remarked on the great strength and originality of our clinical research programme. It is gratifying to hear this from the head of one of the world's most prestigious cardiovascular research centres. I am sure that the programme owes much to the close integration with the basic research of the various basic disciplines. This has allowed for rapid transfer of information from laboratory to bedside. This collaborative approach has greatly accelerated the evolution of our current concepts related to primary human hypertension.

Last year I attempted to present "highlights" of our research programme in plainer English than we have managed in the Scientific Research Reports. This appears to have found favour with our supporters and is therefore included in this year's report.

The Institute's fourteen research laboratories are currently engaged in over 70 projects. Some of these seek to obtain new information with established methods, but each is engaged in 1-2 projects which require the development of new methods for

*Professor Paul Korner*



their solution. This may take several years' work before any results emerge. Such developmental projects are the scientific equivalent of high risk ventures in industrial enterprises. Mostly only the larger research groups have the resources for a significant number of such projects, without jeopardising their current research productivity. However, their successful development is usually of great benefit to many other groups.

The chairman of the Medical Research Committee of the National Health and Medical Research Council (NH&MRC) has recently referred to the Baker Institute and to the two other medical research institutes that it finances as the "flagships" of Australian medical research enterprise. They should certainly be regarded as a major resource in view of their outstanding record of achievements in original research and development. Yet in last year's Federal Budget across-the- board cuts were made to *all* NH&MRC grants including the Block Grants of the Institutes, to allow funding of additional new projects in universities and teaching hospitals. I sympathize with the hard decisions that had to be made by NH&MRC. The new project grants did provide new opportunities, particularly for up-and-coming researchers. But although the cut in the Baker Institute's budget was relatively small it had a "last straw" effect and led to draconian economies on the purchase of new equipment in 1985, and to some reduction in staff. We can stand this on a once-off basis. It is important to remember the productivity multiplier effect of a collaborative research group and our unique capacity for embarking on developmental projects. Institutes such as ours provide unique opportunities for the relatively rapid solution of important national health problems. For these reasons I very much hope that NH&MRC will leave the Block Institute Grants alone in future budgets.

It is in the above context that the success of our *"Before 2000 Appeal"* becomes of major importance. The name epitomizes our conviction that by the year 2000 AD the work of our scientists will have made a significant contribution to the international research effort in hypertension and atherosclerosis. We believe that this will result in major improvements in the health expectations of most Australians. The funds will help purchase new equipment, replace obsolete equipment and promote collaborative research with other Australian and international centres. The recent comments made by the OECD (Organisation for Economic Cooperation and Development) Examiners (Report to the Australian Government, March 1985) are relevant. *"We urge a sustained effort to improve equipment in universities, institutes of technology and other educational institutions."* Success of the appeal will enhance our effectiveness in Australia's contribution to the international research effort.

# Director's Review of Scientific Highlights

## New strategy for Human Hypertension

The extra pressure load of hypertension leads to enlargement of the muscles and heart and small arteries. As a result, the effect of every stimulus to the heart and vessels becomes exaggerated when compared with responses in the normal circulation. For example, a given level of sympathetic nerve activity leads to much greater narrowing of the small arteries in hypertensive individuals. Studies on experimental renal hypertension in dogs and rabbits by Warwick Anderson, Robyn Woods and myself showed that these "amplifier" effects contributed almost three times as much to the maintenance of chronic hypertension as the basic cause that initiated the pressure rise. This disproportionate contribution of the cardiovascular amplifiers affects every type of hypertension, including chronic primary (essential) human hypertension. Here too the amplifier effect overshadows the contribution to the raised blood pressure made by the basic cause. This explains why detection of the latter has been so elusive.

Knowledge of the role of the enlarged muscles of the heart and arteries led Garry Jennings and his collaborators in our clinical research team to ask whether prolonged anti-hypertensive drug treatment would lead to reversal of the muscle enlargement and at least temporary removal of the "amplifier" effects. We also wondered whether reversal of the muscle enlargement would affect redevelopment of the hypertension once drug treatment was stopped.

It soon became apparent that reversal of the enlargement of the arteries by anti-hypertensive drug treatment was easy. But reversal of the heart muscle enlargement required more prolonged and somewhat greater reduction of blood pressure. It was important to achieve adequate reversal of both heart and arterial muscle enlargement and we then found that when drug treatment was stopped blood pressure remained low and only returned gradually to the pre-treatment level.

To date significant reversal of both heart and vessels has been obtained in 15 patients. In these, after 20 weeks without drugs the blood pressure obtained with the patient lying down had climbed only one quarter of the way to the pre-treatment level. Blood pressure obtained while standing recovered somewhat more rapidly, i.e. to about one third to one half of the way to levels before treatment. Probably the enhanced recovery of the blood pressure on standing is due to an increase in sympathetic nervous activity in the majority of subjects, which may be causally related to the development of this high blood pressure. In 3 other patients we could not take them off anti-hypertensive drugs without fluid retention and rapid return of the hypertension. Possibly these are a subset of "salt sensitive" patients.

*Our new treatment strategy* envisages an initial period of about 2-4 years of drug treatment to reverse the muscle enlargement and to abolish the amplifier effects. This is followed by a period where we attempt to maintain blood pressure indefinitely at normal levels by non-drug methods directed at the basic underlying cause of hypertension in that individual.

We have found that regular moderate exercise, performed 3-4 times per week is one of the most effective ways of lowering blood pressure. In 6 patients treated for over 2 years with drugs to reverse the muscle enlargement, the blood pressure has remained entirely normal for the 40-50 weeks of the study after stopping drugs.

We do not yet know in what proportion of the hypertensive population the new treatment strategy will be effective. Possibly it may be as high as 30-50% of patients. There is now a rational basis for the hope that the use of drugs in high blood pressure need not be a lifelong affair. This could be a matter of great benefit to patients and taxpayers.

## Experimental Renal Hypertension

Stenosis (narrowing) of the renal artery occurs in about 5% of patients with hypertension. Its diagnosis is important because surgical repair of the renal artery narrowing can produce permanent cure of the hypertension. This type of hypertension can be induced in experimental animals and has, as already mentioned, provided us with valuable insight about mechanisms that are common to all types of hypertension, such as the cardiovascular amplifiers.

Warwick Anderson, Robyn Woods and Kate Denton worked out how the action of the hormone angiotensin II (AII) within the kidney helps to maintain its filtering function. When the renal artery is narrowed the pressure beyond the stenosis would fall to too low values for normal filtration of the blood plasma by microscopic filters called glomeruli, which is the first step in the production of urine. Local production of the hormone AII and its constrictor actions on the vessels draining blood from the glomeruli and from a related tissue called mesangium helps to raise the glomerular pressure so that the kidney can produce urine. This elevation of pressure in the kidney also helps reduce the resistance to blood flow offered by the renal artery stenosis. In turn this prevents the development of hypertension when the degree of narrowing of the renal artery is only moderate to 50-60% of its diameter.

Surprisingly AII is one of the most powerful constrictor substances known. But by confining its action locally within the kidney, it acts paradoxically as an anti-hypertensive mechanism in moderate stenosis. Only when the stenosis is extreme, with reduction of the diameter of the artery by more than 80%, does the general blood pressure go up. In severe stenosis the local actions of AII no longer suffice to maintain filtration of plasma and urine formation. AII is now formed in very large amounts, which spill over into the general circulation where it constricts blood vessels outside those of the affected kidney. The development of hypertension really occurs to compensate for the stenosis, since by sending the blood pressure high enough the kidney's filtration function can be maintained. Development of hypertension is good for the kidney but not for the body as a whole.

## Nervous System and Circulatory Regulation

Regulation of the circulation by the nervous system is through the so-called autonomic nervous system — the sympathetic and vagus nerves and the adrenal catecholamines produced by the adrenal gland. These regulate the rate and force of the heart beat and the calibre of the blood vessels. Over half of our laboratories have projects relating to circulatory regulation by the autonomic nervous system. Increased activity of the sympathetic nerves has long been suspected to be a possible cause of primary human hypertension. This is certainly consistent with our recent work on redevelopment of hypertension.

One reason for the large effort is that there are enormous gaps in our understanding of the physiology of circulatory regulation or the exact mechanisms by which drugs influence autonomic function to alter blood pressure. This knowledge is essential to focus research on autonomic nervous system function in humans where the depth of analysis is more limited than in animal studies or in vitro preparations.

We are studying the properties of different sympathetic nerve cells in the brain and the chemical and physical mechanisms that lead to the excitation of one cell by another. Elspeth McLachlan and David Hirst have recently shown that preferential excitation by one group of cells involves opening up by the released transmitter of very localized membrane channels in the adjoining cells, that allow the rapid entry of calcium. In another series of experiments David Hirst, Michael Lew and James Angus have confirmed David Hirst's original observation that transmission of excitation from the sympathetic nerve endings to the arterial muscles after release of noradrenaline, is through a new type of membrane receptor called the gamma receptor. This receptor may become an important target for new drug development and its discovery has far reaching implications for autonomic neuropharmacology.

Alex Bobik and colleagues have studied how pathophysiological enlargement of the heart in hypertension affects sympathetic nerve function. Sympathetic nerve activity increases in this condition at rest, as has also been found in man. This seems to be close to the limit and a further chronic increase in sympathetic nerve activity produces a gradual running down of the catecholamine transmitter stores at the nerve endings. Alex Bobik has found that chronic sympathetic overactivity in longstanding hypertension damages the transport pump that normally recycles transmitter back into the ending after its release. Clearly chronic sympathetic overactivity is something to be avoided in chronic hypertension and may explain the relative ease of developing heart failure.

We are trying to identify the exact site of action in the brain of centrally acting antihypertensive drugs, in order to obtain clues about the brain mechanisms important in longterm regulation of blood pressure. Geoff Head, Alex Bobik, Emilio Badoer, Judy Aberdeen, Gordon Mill, Kay Oddie and I have found that the drugs alpha-methyldopa and clonidine lower blood pressure through their action on nerve cells of the hindbrain that release catecholamines and serotonin as transmitters from their nerve endings. After destroying the endings of catecholamine-releasing nerve cells the lowering of blood pressure due to the central action of alpha-methyldopa is completely abolished. We have pinpointed three groups of nerve cells that are involved in this action and have worked out the effects on the circulation of some of the other catecholamine releasing cell groups.

In addition to the work on molecular properties of the nervous system we are also interested in finding out the gross components important in the integration of incoming information by the brain and how as a result there is adjustment to the levels of sympathetic and vagal nerve activity in the intact animal. Pat Dorward has developed a method where small electrodes are implanted on the renal sympathetic nerve, allowing subsequent recording of its activity over many days in the conscious rabbit. She, Sandra Burke and Walter Riedel have examined the role of various receptors signalling pressure changes in the heart and in the arteries on renal nerve activity. This work has shown that the signals from the heart are much more important than was thought. They act as an inhibitory break which limits excessive rises in sympathetic activity particularly during large falls in return of blood to the heart. Judy Oliver and I have found that this switching off of sympathetic nerve activity is important during haemorrhage in preventing excessive constriction not only in the kidney, but also in the blood vessels of the gut and limbs. In both projects computer programmes developed by Judy Gipps have been essential for the work.

John Ludbrook and Simon Potocnik have begun to examine the effects of exercise on the body's short term nervous regulator of blood pressure — the arterial baroreceptor reflex. They have found that with moderate and even very mild activity the brain suppresses the information arising from the arterial baroreceptors. This allows the blood pressure to increase a little during exercise, possibly owing to compensatory constriction of beds other than the active muscles.

The autonomic nervous system has long been regarded as the major mechanism in short-term circulatory regulation. Recently there has been much controversy whether circulating hormones such as angiotensin II or the pituitary hormone vasopressin also play a role. Tony Quail, Robyn Woods, Judy Oliver and I have found that in haemorrhage there is a significant increase in the secretion of vasopressin only when the blood loss is beyond the performance limits of the nervous system. Although at this time the hormone is secreted in large amounts it makes a negligible contribution to the maintenance of blood pressure. It is of far greater importance in its action on the tubules of the kidney, which assists in the conservation of water by the body.

Murray Esler, Alex Bobik and Graham Jackman, have pioneered over several years the development of new biochemical methods for the accurate analysis of sympathetic nerve function in humans. This allows assessment of tonic levels of sympathetic nerve activity and of the rate of

reuptake of transmitter following release by a membrane pump at the nerve endings. Not only can the average levels of sympathetic nerve activity and reuptake be assessed, but the latest extension of these methods has been to assess these functions in the individual outflows to different organs. We have learned from animal studies that the sympathetic nervous system operates in a patterned manner, with activity increasing in the nerves to some organs and decreasing in those of others. Murray Esler, Greg Hasking and Garry Jennings have found that in patients with heart failure sympathetic nerve activity is particularly high in nerves supplying the heart which may account for their great susceptibility to rhythm disturbances.

Most emphasis has been on the investigation of patients with primary hypertension. In these the sympathetic nerve activity in the kidney is raised in over 50% of young patients which may be important in the causation of hypertension. Patients who are excessively fat also have disproportionately high renal sympathetic nerve activity. Murray Esler has identified a subset of 10-15% of patients in which the reuptake pump of the sympathetic nerve ending fails to return transmitter at an adequate rate, resulting in sympathetic overactivity. All the above studies have discovered a variety of abnormalities of sympathetic nerve function that are of potential significance in the development of hypertension.

Another study may lead to improvements of patients with severe liver disease due to cirrhosis. Ian Willett, Frank Dudley, Murray Esler and Garry Jennings have found that average sympathetic nerve activity to the gastrointestinal tract is particularly high in these patients. They have found that this contributes significantly to the elevation of the portal vein pressure. Portal vein hypertension increases the risk of torrential and often fatal bleeding from the gastrointestinal tract. The new finding is that clonidine, which lowers sympathetic nerve activity significantly reduces portal vein pressure. This may be a valuable approach to risk reduction from bleeding in portal hypertension.

## Spasm of Coronary Arteries

Some patients with angina (pain from the heart) have coronary arteries that are not obstructed by fatty plaques. Cine x-ray studies have shown that the angina is due to spasm of the large coronary arteries supplying the heart. The mechanism of how this comes about has been studied by James Angus, Tom Cocks and Julie Campbell. It turns out that the responsiveness of large arteries to dilator and constrictor stimuli depends on the integrity of the single lining of cells inside the vessels — the endothelial cells. These cells release a local hormone called "endothelium-derived relaxing factor", EDRF. When the endothelium has been damaged and EDRF can no longer be produced, certain circulating substances in the blood, which normally dilate the vessels by increasing production of EDRF, now constrict them through their direct action on the muscle coat. A particularly potent substance in this regard is serotonin which is released from damaged blood platelets. When the endothelium is intact this substance leads to production of EDRF and dilatation, but after damage serotonin through its direct action on arterial muscle can bring about spasm of the vessels. We are seeking to determine the chemical composition of EDRF, which appears a worthwhile target for new drug development.

## Atherosclerosis

High blood fats increase the chances of developing atherosclerosis of the coronary artery, which is responsible for heart attacks. It has been clear for a long time that one of the blood fats, cholesterol, causes coronary artery disease. Recent experiments by Paul Nestel, Julie Campbell, Michael Reardon and Tim Billington have shown that another group of blood fats, the triglycerides, are also an important cause of coronary artery disease. Coronary atherosclerosis occurs when triglycerides accumulate in the blood in association with specific protein particles called lipoproteins. The important findings are that high plasma triglycerides produce coronary disease more readily in women than in men,

probably because female sex hormones strongly influence the fate of blood triglycerides. When triglyceride-rich particles were incubated in tissue culture with different kinds of normal artery cells, the cells changed into the sort of cells found in atherosclerosis. The experiments also revealed the chemical structure in the particles that attracted the triglycerides into the arterial cells. Fortunately it is possible to lower blood triglycerides through dietary interventions. One dramatic way of reducing plasma triglycerides is by eating certain polyunsaturated fish oils, where Paul Nestel and Sue Wong have worked out the mechanism of why this reduction occurs.

A most important question in reducing disease in coronary arteries is how the surplus cholesterol in these arteries is removed. There is a substance in the blood called high density lipoproteins (HDL) which prevents a build-up of cholesterol in the arteries of healthy people. The HDL must first come into contact with the arterial cell so that the cholesterol can be moved out of the artery. Noel Fidge, Paul Nestel and their collaborators in the Cardiovascular Metabolism Research Unit have just discovered how this comes about. They have isolated a special protein on the surface of the cell which acts as a recognition signal for the protein in the HDL. The two proteins attract each other, so that HDL binds firmly to the cell. The specialised proteins in the HDL then pull cholesterol out of the cell. The question whether the receptors can be activated to attract more HDL and thus transfer more cholesterol out of the cell is now under intensive investigation.

Julie Campbell and her colleagues have defined some of the cellular factors that maintain arterial muscle in its normal contractile state. It turns out that here too the endothelial cell plays an important regulatory role. After damage to these cells the arterial muscle cells in response to blood borne substances may lose the capacity to contract. Associated changes in metabolism result in an increased accumulation of cholesterol. Understanding the cellular mechanisms will provide us with new insights about the various factors determining resistance to atherosclerosis and why some individuals with similar cholesterol levels are more resistant than others.

## Cardiovascular Surgical Research

In surgical shock after haemorrhage the body's natural opioid (morphine-like) substances may contribute to the circulatory depression. Peter Rutter and John Ludbrook have discovered that the low blood pressure following haemorrhage can be rapidly reversed by the drug naloxone, which is an antagonist to the actions of morphine and related opioids.

Frank Rosenfeldt and colleagues have continued with their studies on defining the optimum conditions for cooling and reperfusing the heart during open heart surgery. He has developed a pump for uniformly cooling the heart, which has been most valuable in avoiding damage during the procedures and is now regularly used at Alfred Hospital and other cardiac surgical units. More recently he has developed a microprocessor unit to regulate the post-operative infusion of drugs to maintain blood pressure near normal levels during intensive care following open heart surgery.

# The "Before 2000 Appeal"

During 1984 the Board approved a proposal of the Development Council to launch an appeal during 1985 to raise additional funds for the replacement and acquisition of capital equipment and other significant objectives which could not be attained through operating funds.

The building now occupied by the Institute in the grounds of the Alfred Hospital was completed in 1969 and much of its plant and some major equipment was installed soon after that date.

The major purpose of the appeal is to purchase high technology equipment that has become essential for the current direction of our research programme. Amongst these are biotechnology instruments that have become available with the rapid advances in molecular biology such as an amino-acid sequencer, peptide synthetizer and equipment for immuno- histochemistry. There is also a great need to expand our computer facilities. Some existing equipment no longer performs in accordance with requirements (e.g., electron microscope).

'State of the art' equipment is critical to modern research. In this connection it is pertinent to quote from the recently published 1985 report of the OECD (Organisation for Economic Co-operation and Development): *"We urge sustained effort to improve equipment in universities, institutes of technology and other tertiary education institutions".*

Another important emphasis of the Before 2000 Appeal is to establish a Research Fellowship Programme. It has two aims:— Firstly, to make available the Institute's resources to other Australian scientists, by providing stipends to allow development of short-term collaborative programmes with Baker Institute scientists. The second aim is to provide a small number of International Research Fellowships, that will allow outstanding overseas young scientists from other countries to spend a period of collaborative research at the Institute. *We envisage that these programmes will result in bilateral transfer of technological and scientific information within Australia as well as between Australia and other countries. It is likely to have considerable impact in promoting Victoria's and Australia's role in basic and clinical cardiovascular research.*

The last aim of our appeal is to allow a number of building additions and alterations to provide improved use of laboratories.

*The Appeal is being directed towards government and corporate sectors, to individual private donors and to the current financial supporters of the Baker Institute.*

# The Third Thomas Baker Oration*

## A Bex, a cup of tea . . .

**Orator:**
**Professor Priscilla Kincaid-Smith**
**Professor of Medicine, University of Melbourne and Director, Department of Nephrology, Royal Melbourne Hospital**

Through the whole story of the form of kidney disease associated with "the headache powder", which is largely a disease of women (as was predicted by the Lone Hand in 1907), runs a thread of an association with alcohol, perhaps tenuous at times and difficult to unravel and yet sufficient association to make one wonder if alcohol has a role in this disease.

Perhaps it's just an association. People who habitually use excessive quantities of one "drug" or mood-altering substance tend to abuse others. Patients with analgesic nephropathy — the long name used to describe the effects of pain killers on the kidney — are often also alcoholics. Incidentally they are also heavy smokers — another "mood-altering" drug.

Years ago, when visiting the village at the Folk Museum at Swan Hill, which dates back to the last century, I was particularly interested in the pharmacy. In the window of the little old chemist shop was a medical text book open at a page which showed clearcut lesions of renal papillary necrosis, the type of damage which results from pain killers and about which you'll be hearing more this evening. Underneath this picture was the inscription "The evil effects of alcoholic liquor on the kidney". Perhaps they knew something about alcohol and the kidney which we have not yet been able to uncover.

Inside the little old chemist shop, the first thing that caught the eye were old fashioned painted advertisements for Bex and Vincents. A further suggestion to support the claim in "The Lone Hand" that the "headache powder habit" may date back a very long time in Australia.

There's at least one story, on the other hand, which suggests that alcohol may protect the kidney from damage due to pain killers. A colleague of mine had a waterside worker as a patient and when he read in the press in 1964 that pain killers could damage the kidney he went to his doctor and demanded a kidney x-ray, which turned out to be quite normal. He had been in the habit of taking large quantities of Ascotin — a form of APC — for 10 years or more and because his x-ray was normal decided to continue this practice. In keeping with the Australian male image, he also drank 10 pints of beer daily. A few months later he saw the error of his ways and stopped drinking but, as he had been reassured about his kidneys, he continued to take Ascotin and within a year he had developed extensive kidney damage typical of that attributed to analgesics. This story strongly suggests that over the 10 years while he took his 10 pints of beer a day with Ascotin his kidneys were protected but, as soon as he stopped drinking beer, he quickly developed papillary necrosis, or death of the inner part of the kidney, which is typical of the damage caused by pain killers. There is good evidence in animals to show that a big fluid load protects the kidney from analgesics. These drugs (and others) tend to accumulate in the inner part of the kidney and high fluid intake helps to prevent this.

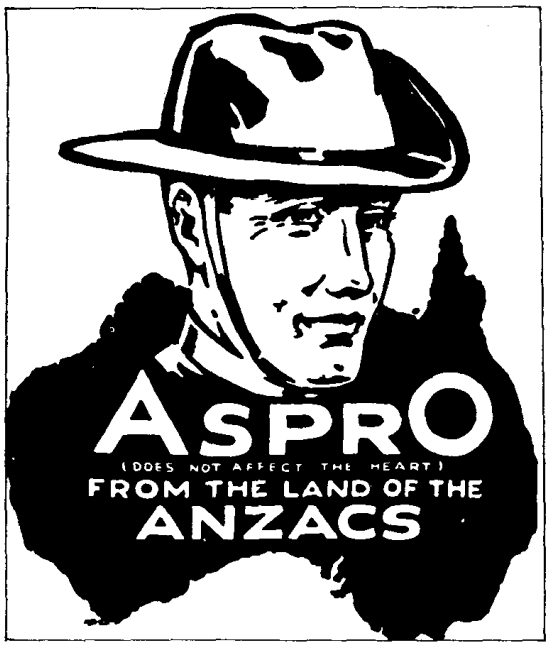
I mentioned these tenuous associations with alcohol partly to emphasise how difficult it can be to establish a causal relationship between any substance and chronic

*Delivered on 20 February 1985*

disease. I think there is now overwhelming evidence that analgesics are the cause of this type of kidney damage. However, that was not always the case. In their campaign against analgesic abuse in our community, organisations such as the Australian Kidney Foundation were bitterly opposed by those sections of the pharmaceutical industry who make and market analgesics. It was interesting to discover how many fortunes were founded on Aspirin. We all know about some of them but, in almost half of the big pharmaceutical companies, there was evidence of a substantial stake in over-the-counter analgesics. Some, like the Aspirin Foundation, were more militant than others and would have been very ready to transfer the blame to alcohol if they could. As the evidence against mixed analgesics such as Bex and Vincents mounted, industry in Australia changed its stance. One drug ingredient present in analgesic mixtures was singled out for blame, the implication being that the other ingredients were harmless. This substance was, of course, phenacetin. As early as 1962 when, as a result of work in this hospital and others, the story first surfaced in the public press, several companies in Australia removed phenacetin from over-the-counter mixtures. They then advertised their own particular brand as "safe — contains no phenacetin". Many companies substituted paracetamol for phenacetin, although they were well aware that paracetamol is the major breakdown product of phenacetin. Some 90 per cent of a dose of phenacetin is converted to paracetamol soon after ingestion and it is paracetamol, not phenacetin, that accumulates in the inner part of the kidney, the part where major damage occurs.

At an experimental level, the evidence against both phenacetin and paracetamol is similar — slender against both, but the same. Aspirin, orginally believed to be free from risk, will on its own produce much more damage in animal experiments than either phenacetin or paracetamol. The APC combination, however, is the one which most readily produces this type of damage to the inner part of the kidney in animals and it is this APC combination which has been associated with abuse of pain killers in man.

It is interesting to try and trace how




phenacetin got its bad name. Nowhere in the world has phenacetin been available as a single substance; therefore, we have no evidence in man that it is harmful. Phenacetin was always combined with other substances in analgesic mixtures and these differed in different countries. Phenacetin really got blamed on the "common denominator" basis and many of you here tonight will know the flaws in such reasoning. A famous pharmacologist, Alfred Gilman, pointed out that, using similar logic, one could well conclude that soda water produced inebriation because Scotch and soda, Vermouth and soda, and brandy and soda all produce the same effect.

I would have to admit to you tonight that the last chapter in this story has not yet been written. We still don't know exactly what contribution is made by the various ingredients of pain killers in different medications around the world. We have had to settle for a "consensus view" adopted recently at an International Meeting in Washington that all mixed analgesics are harmful when taken in excessive amounts and that restriction should aim at control of abuse and control of mixtures. As usual, this international consensus view was about ten years behind the consensus view in Australia which they adopted.

Whatever the reason, analgesic nephropathy appears to be far more frequent in

Australia than it is in the United Kingdom. In February 1959 I came to work at the Baker Institute with Dr. Alf Barnett and Dr. Tom Lowe. As part of my routine, I visited the autopsy room at the Alfred Hospital every day and almost at once became aware of a form of kidney disease which was very frequent at this hospital and which I could not recall having seen in 6 years of daily autopsy attendances at the Royal Postgraduate Medical School in London. I would like to show you what this type of kidney looks like because an important part of the story relates to this. This kidney change was attributed at that time, both in Melbourne and elsewhere in the world, to infection. What I couldn't understand was how kidney infection in Melbourne could produce this change, whereas kidney infection in London did not. I should say that no-one at that time had associated this particular condition, namely papillary necrosis with analgesics. Quite a different kidney lesion has been described in Switzerland and Scandinavia, with a suggestion that phenacetin may be the cause of it. This was involvement of the outer part of the kidney, whilst any involvement of the inner part, with its black papillae, was assumed to be secondary. This created a further stumbling block and diversion, from which our opponents in industry made much capital, namely there was confusion about the type of lesion. I was howled down by European pathologists in 1963 in Prague when I showed some of our cases and suggested that the primary involvement was in the inner part of the kidney. The following year, at a meeting in Boston, I was told very firmly by some of the world's most famous pathologists that papillary necrosis was due to analgesics and the primary lesion in analgesic nephropathy.

The next piece of this puzzle fell into place when my huband, Dr. Ken Fairley, who happened to work with Dr. Bill King, a well-known physician and gastroenterologist in this city, saw a whole series of patients for Bill King, in whom kidney failure developed after operations for peptic ulcer. Indeed, our first description of this kidney condition was in association with peptic ulcer. In taking the history of these patients, several gave a story of renal colic — the type of kidney pain which results from a stone — or other tissue passing down the ureter — the tube which connects the kidney to the bladder. Some of these patients also gave a story of passing little dark fleshy things in their urine. Eventually Ken got hold of one of these and it proved to be one of those black dead papillae. There was thus no doubt the kidney condition in this group of ulcer patients was papillary necrosis — the same lesion as had puzzled me in the autopsy room. All patients gave a story of taking large quantities of Bex or Vincents and so the final piece of the jigsaw puzzle had fallen into place.

Thus, by late 1959, we were convinced that Bex, Vincents and other mixed analgesics caused both peptic ulcers and kidney failure due to papillary necrosis. It took some years to convince others and indeed it was only last year — 25 years later — that the whole concept was accepted by an international group. One of the first three publications in 1962 came from this Hospital and several of the patients were ones whom I had seen in Ward 8 — the ward attached to the Baker Institute — in 1959.

Let me tell you some of the stories about this strange disease and the extraordinary quantities of pain killers taken by some patients. I'd like particularly to emphasise that these patients were taking analgesics not as pain killers, but for their "pick me up" mood altering properties. Four of every five patients are women. They suffer not only from kidney trouble but also have peptic ulcers, psychological disturbance, increased coronary artery disease and other vascular disease, anaemia and various other problems, including premature ageing.

The kidney damage resulting from pain killers itself causes kidney infection, blood in the urine, severe kidney pain, kidney stones and high blood pressure.

Most patients were middle-aged women in lower income groups. Many had acquired the habit as teenagers when first entering the workforce. Whether they worked in factories or as "clippies" on the buses, they told how their workmates introduced them to the habit of having two or three powders with their morning cup of tea for their stimulant effect. Powders — which are ab-

sorbed more rapidly than tablets — were much more popular because they had a much bigger "kick". Headache soon became part of the pattern, probably caffeine withdrawal headache which occurs an hour or two later and reminds the patients to take another dose.

Certainly these patients when "hooked" on analgesics were always fiddling around finding the next dose in much the same way as a smoker does. Let me tell the stories of some of the exceptions that prove the rule — the men.

A doctor — once a well-known surgeon in this city — presented with kidney failure after an operation for peptic ulcer. When questioned about his analgesic intake, he said he took about 30 doses of APC a day. He was a very co-operative patient and very helpful to us. When he actually counted the number he took, it was nearer to 100 than 30 — a dose which would be fatal to anyone unaccustomed to these drugs. This doctor was one of the patients who first made us aware of the stimulant or mood altering effects of analgesics — he took a variety of dummy tablets for us and the ingredient which he missed most because of this mood altering effect was surprisingly not caffeine, but phenacetin. He also noticed when caffeine was missing, but particularly wanted the effect of the phenacetin. He died not of renal failure but of severe coronary artery disease, commonly seen in these patients.

A pharmacist told us that, as he passed through his shop he helped himself at regular intervals to a few APC tablets, which were then kept in barrels because of large demand. At night he took a paper bag home and claimed that a Scotch had much more punch when taken with a handful of APC's.

A taxi driver was admitted with severe kidney failure in 1962:— He took a powder each time he pulled up at the traffic lights without any fluids — an almost impossible task! The main lesson learnt from him was how well kidneys can recover when patients stop taking APC's. Although he required dialysis when we first saw him, his kidney function recovered and he remained well for 5 years. We also learnt from him about the effects of aspirin. Five years after his recovery he suddenly deteriorated again when he started to take, not APC's, but aspirin on its own in a dose of about 10 tablets a day.

Well — what is the story about aspirin? Some of you may be taking aspirin every day to help prevent clotting of your blood. Aspirin is a remarkably effective drug with at least two important pharmacological effects. We all know how effective it is for a headache, or what the advertisers call "rheumatic pains", and more recently it has been shown that it also helps to stop blood clotting. It produces both these effects by inhibiting certain prostanoid substances, one of which causes blood clotting and another inflammation. The damage which aspirin causes in the kidney may also be caused by a similar mechanism; another prostanoid regulates blood flow of blood in the inner part of the kidney. Aspirin, by inhibiting this substance, indeed by causing death of the cells which make it, reduces the blood flow to that part of the kidney and eventually death of the kidney tissue itself. Like many things, aspirin in moderation causes no harm but, in large amounts, it can cause kidney damage.

Those of you who regularly hear about research in this famous Baker Institute will know of a connection between blood clotting and the common disease of the arteries called atheroma. You may already wish to ask why patients who take large quantities of aspirin every day get *more* coronary artery disease rather than less? A very good question. We know that a small dose of aspirin inhibits the formation of a "bad" prostanoid thromboxane, which causes clotting. In large doses, it also prevents the formation of a "good" prostanoid — prostacyclin — and this may be the reason why these patients get more than their share of coronary disease.

In animals, it is much easier to cause papillary necrosis with aspirin than with phenacetin. Indeed it is impossible to cause more than a very minor change with phenacetin. In humans, aspirin on its own rarely causes problems unless taken in very large doses. This is probably why we rarely see this form of kidney damage in patients who take aspirin to control arthritis.

Finally, why are we such a nation of pill-poppers? Various community surveys have confirmed that 3 to 15% of the Australian

population take analgesics every day — 3% in Victoria, more (15%) in Queensland — and, as I told you, kidney failure from this cause is much more frequent in Queensland than in Victoria. The most startling figure revealed in a survey in a country town in New South Wales was that 45% of Aboriginal women took analgesics every day.

I've tried to indicate how peer pressure has introduced people to this habit in factories and this occurred even in schools. The pattern is similar to that of other peer pressure habits like smoking.

The most serious effect of analgesics is kidney failure. This accounts for some 20% of all patients in Australia who require dialysis. This costs the taxpayer $30,000 per year per patient, millions of dollars every year across Australia. All of it could be prevented, which would not only save money but also avoid all the patient suffering which occurs in this disease.

What has happened over the 25 years encompassed by my story? I believe that a major reduction occurred many years ago. Between 1959 and 1962, we collected 100 patients with analgesic nephropathy in one hospital. Now, 25 years later, in the same hospital we see 5 to 6 new patients each year. As I told you, however, official records show no real reduction in those presenting with kidney failure but factors such as acceptance of older patients may influence this.

In attempting to answer the question why we have such a problem in Australia, I wonder if the man who instituted a type of advertising of over the counter analgesics bears some responsibility. It was highly effective and enormously increased their sales. This type of advertising persists today.

# Visiting Scientists

*Dr. Elspeth McLachlan and Dr. David Hirst*

## Dr. David Hirst

The Neurophysiology Laboratory is privileged to expand during 1985 with the arrival of Dr. David Hirst who is visiting from The Department of Pharmacology, John Curtin School of Medical Research, Australian National University, Canberra. David is internationally known for two fields in which his electrophysiological studies have made major contributions. Some years ago his studies of the behaviour of enteric neurones and smooth muscle made great advances in our understanding of the physiology of peristalsis. More recently, he has been investigating the process of transmission between sympathetic axons and arteries. These experiments have revealed the presence of sub-synaptic receptors on arterial smooth muscle cells that differ from those of the conventional *a* and *b* adrenoreceptor classification. This has important implications for the development of better anti-hypertensive drugs. During his stay he has already infiltrated the Pharmacology Laboratory, and has persuaded our pharmacologists to collaborate in experiments designed to establish the functional role of the sub-synaptic receptors.

## Peter Rutter

After graduating M.B., B.S., in London in 1975, Peter has trained as a surgeon in the U.K. obtaining his FRCS in 1980. After some initial work at St. Mary's Hospital in London, he has come to the Institute for a period of 18 months to work in the cardiovascular surgical research unit with Professor John Ludbrook.

He is studying the actions of narcotic antagonists on the sympathetic nervous system in haemorrhage.

His other interests include sailing, skiing and the opera.

# Organisation Chart

# Some of Our Achievements

1. A new strategy for treatment of hypertension and for determining its causes.

2. Discovery that exercise is one of the best non-drug methods for lowering blood pressure and analysis of its nervous control.

3. Sympathetic nervous system abnormalties in patients with primary (essential) hypertension and their possible role in causing high blood pressure.

4. Discovery of mechanisms in renal artery stenosis (narrowing) that help maintain kidney function and eventually lead to hypertension.

5. Discovery of where in the central nervous system anti-hypertensive drugs act to lower blood pressure.

6. Role of the lining cells of coronary arteries on their reactivity and how damage to these cells leads to heart attacks.

7. Functions of specialised plasma and cell proteins called lipoproteins in the transport of cholesterol and how excessive amounts of some of them predispose to heart attacks.

8. Discovery that women with high plasma triglycerides are at special risk from heart attacks.

9. Dietary methods for reducing risk from heart attacks by diminishing production in the body of less cholesterol rich lipoproteins and by methods that lower plasma triglycerides.

10. Specific receptor mechanisms by which special density lipoproteins help to remove cholesterol from the cells.

11. Devices for use in operating theatre and intensive care for preserving and improving heart function during and after open heart surgery.

# Scientific Report

**Hypertension and Circulatory Regulation Unit**
Human Hypertension Laboratory
Basic Cardiology Laboratory
Emily Stewart Renal Laboratory
Neurophysiology Laboratory
Circulatory Control and Neuropharmacology Laboratory
Biochemical Pharmacology Laboratory
Pharmacology Laboratory

**Cardiovascular Metabolism & Nutrition Research Unit**
Lipoprotein Metabolism & Nutrition Laboratory
Lipoprotein Biochemistry Laboratory
Lipoprotein Structure & Function Laboratory
Cholesterol Metabolism Laboratory

**Cardiovascular Surgical Research Unit**
Vascular Laboratory
Cardiac Surgical Research Laboratory

**Cell Biology Group**

# Hypertension and Circulatory Regulation Unit

## Human Hypertension Laboratory

**Heads:** Dr. M. Esler, Dr. G.L. Jennings

### Projects

Measurement of regional sympathetic nervous "tone" in essential hypertension.

Sympathetic nervous system function in obesity-related hypertension.

Sympathetic nervous system in heart failure.

Abnormalities of the circulation in cirrhosis of the liver.

Release of noradrenaline by the human heart.

Flow-dependence of noradrenaline kinetics.

*SEE ALSO CLINICAL RESEARCH UNIT REPORT*

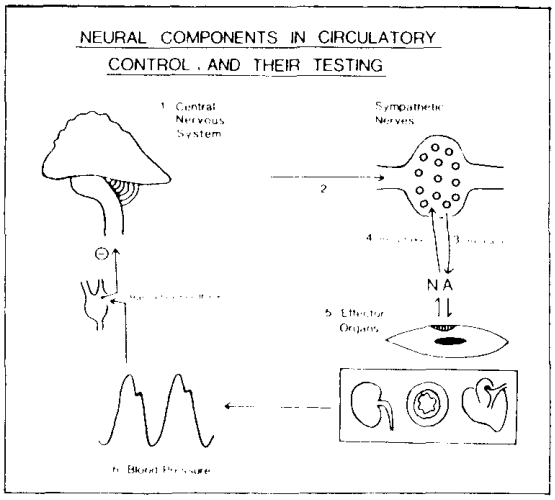
### Summary

Methods for measuring the activity of the sympathetic nervous system include measurements of sympathetic nerve firing rate by electrophysiological methods, measurements of neurotransmitter release, and simple measurements of blood pressure before and after autonomic blockade (Fig. 1). Each has its limitations. With methods for estimating the "neurogenic component in hypertension", based on responses of blood pressure and other haemodynamic variables to pharmacological blockade of the nerves passing to the heart and blood vessels, the measured responses depend on more than the level of sympathetic nerve firing. An accentuated fall in blood pressure in essential hypertension, occurs for example, due to changes in the thickness of the blood vessel wall of the small arteries, quite apart from any increase in sympathetic nerve activity. Electrophysiological methods have much to offer, but one limitation is that the sympathetic nerves to internal organs are not accessible, so that nerve activity can only be measured in sympathetic nerves to skin and muscle.

Biochemical measures of sympathetic transmitter release provide a more practical approach to quantifying sympathetic nerve activity in patients. Noradrenaline is the transmitter at the nerve endings; it is stored in vesicles and released in small packets with each nerve impulse. With few exceptions, the rate at which sympathetic nerve fibres discharge is proportional to the rate of noradrenaline release from the nerve endings. The simplest measure of neural activity is to determine the plasma noradrenaline concentration. But this is less reliable than measurement of noradrenaline (NA) release rate, which we have developed.

Measurement of the NA release or "spillover" rate has advantages over

*Individual elements in the sympathetic nervous control of the cardiovascular system, and their clinical testing. (1) CNS Control Of BP: baroreflex testing/psychometrics/mental stress responses. (2) Sympathetic Nerve Traffic: electrophysiology of peripheral sympathetic nerves. (3) Transmitter Release: plasma noradrenaline concentration/ radiotracer measurement of noradrenaline spillover to plasma. (4) Transmitter Reuptake: clinical studies of the noradrenaline pump. (5) Receptor-Effector Response: adrenergic receptor binding of noradrenaline/pressor responsiveness. (6) Overall Maintenance Of 'Adrenergic Cardiovascular Tone': haemodynamic response to pharmacological autonomic blockade.*



measurement of plasma concentration, which is also affected by the clearance of NA from the circulation. For infused radioactively labelled noradrenaline (NA) then at equilibrium:—

Total NA spillover rate = ($^3$H) NA infusion rate/specific radioactivity in plasma NA, where ($^3$H) NA is noradrenaline labelled with tritium.

NA "spillover" rate into plasma is far smaller than the amount of transmitter released at the sympathetic nerve endings. But animal studies have shown a good correlation between "spillover" and "true" release rate.

Because the sympathetic nerves to the various organs are non-uniform, so total noradrenaline spillover measurements only average the level of sympathetic nerve activity. We have therefore developed methods for measuring NA spillover in the sympathetic nerves of individual organs.

This is given by:

Organ NA spillover = $[(C_V-C_A) + C_A (NA_E)]$ x PF, where $C_V$ = plasma NA concentration in the vein, $C_A$ = arterial plasma NA concentration, $NA_E$ = fractional extraction of tritiated NA, PF = organ plasma flow.

We have determined which sympathetic nerves are most active in essential hypertension, in heart failure, and in cirrhosis of the liver.

## Projects

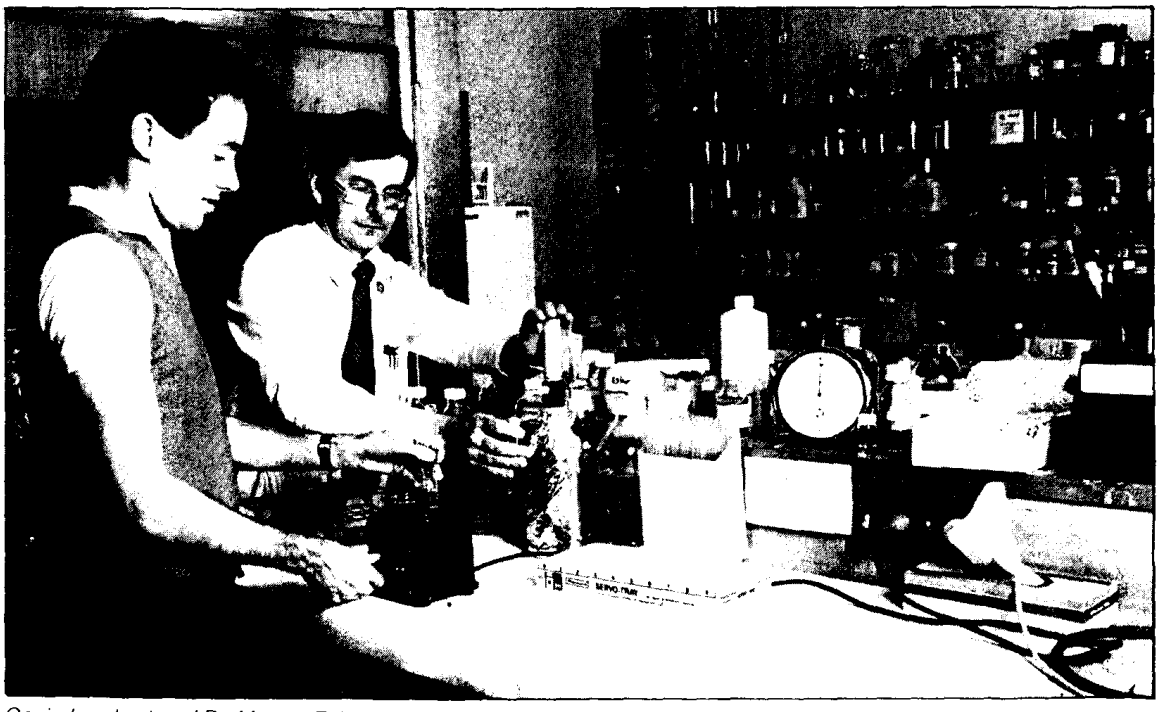
### The Regional Pattern of Sympathetic Nervous System Activation in Essential Hypertension.

M. Esler, G. Jennings, P. Leonard, J. Johns* and F. Burke**

In some patients with essential hypertension the total rate of spillover of noradrenaline (NA) to plasma is increased. We have measured the pattern of NA spillover in different organs, in 33 untreated patients with essential hypertension. Results were compared with those from 21 subjects with normal blood pressure.

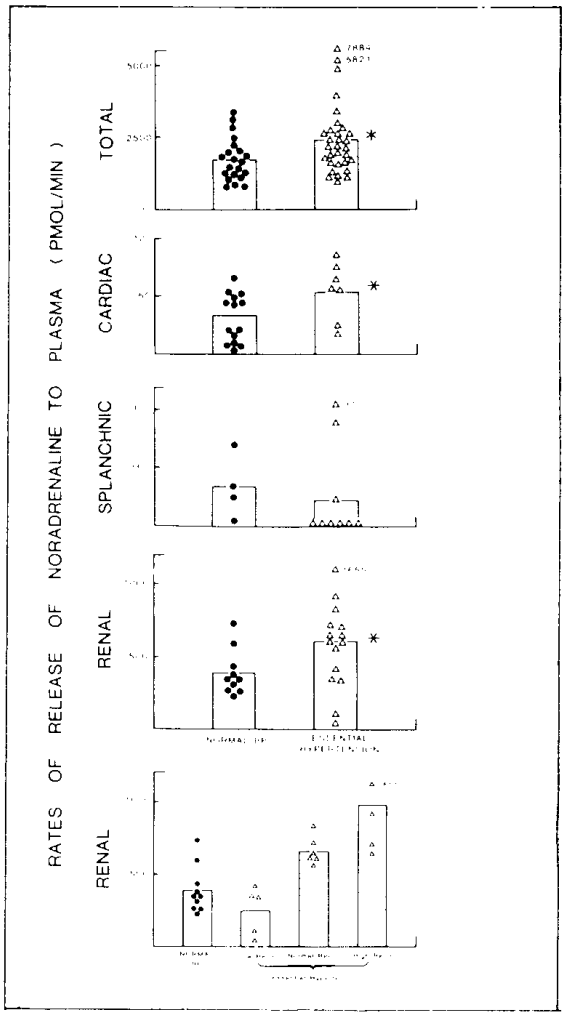
The total rate of NA spillover in the hypertensive patients (median value of 2218 pmol/min) was 35% higher than in subjects with normal blood; $P < 0.05$. Increased NA spillover was due to increased sympathetic activity from the heart and kidneys. Median Krenal NA spillover in hypertensive patients was 628 pmol/min, compared with 349 pmol/min in control subjects. Corresponding

* *Cardiology Service*
** *Department of Radiology*



*Gavin Lambert and Dr. Murray Esler (right) conduct chemical measurement of tracer amounts of the sympathetic nervous system chemical messenger, noradrenaline, tagged with a small amount of radioactivity (tritium).*

cardiac NA spillover values were 68 pmol/min and 28 pmol/min (both $P < 0.05$). The increased sympathetic nerve activity to the kidney and the heart was particularly striking in younger patients in whom levels of the kidney hormone renin are also high (Figure 2). The underlying mechanism is probably not body salt depletion, since increased sympathetic tone from sodium loss seems to be confined to the renal sympathetic nerves (not involving the nerves of the heart). Obesity seems to be one possible factor — renal sympathetic nerve activity is increased in obesity-related hypertension (see below).




*Plasma noradrenaline kinetics in essential hypertension. Renal and cardiac noradrenaline spillover to plasma was elevated in some patients, presumptive evidence of increased sympathetic nervous tone in the kidneys and heart. Renal noradrenaline release to plasma was related to a diagnostic subgrouping of hypertension patients, clinical 'renin status'.*

## Sympathetic Nervous Tone in Obesity-Related Hypertension

M. Esler, G. Jennings, P. Leonard

High blood pressure is common in obesity (excess fat).

Total NA spillover rates (arterial sampling) were similar in 22 lean and 11 obese (weight/height$^2$ > 2.7 g/cm$^2$) hypertensive patients, 208 ± 85 ng/min/m$^2$ (mean ± SD) and 237 ± 151 ng/min/m$^2$, somewhat higher than in 21 healthy subjects with normal BP, 161 ± 68 ng/min/m$^2$ ($P = 0.1$). However, renal sympathetic nervous tone was *selectively* increased in the obese hypertensives — renal NA spillover was 75 ± 44 ng/min/m$^2$, compared with 47 ± 19 ng/min/m$^2$ in lean hypertensives and 36 ± 12 ng/min/m$^2$ in healthy subjects. Age was a second, independent determinant of increased renal NA spillover (higher in patients < 45 yr).

This degree of increase in renal sympathetic nerve activity could cause body salt retention and elevate blood pressure.

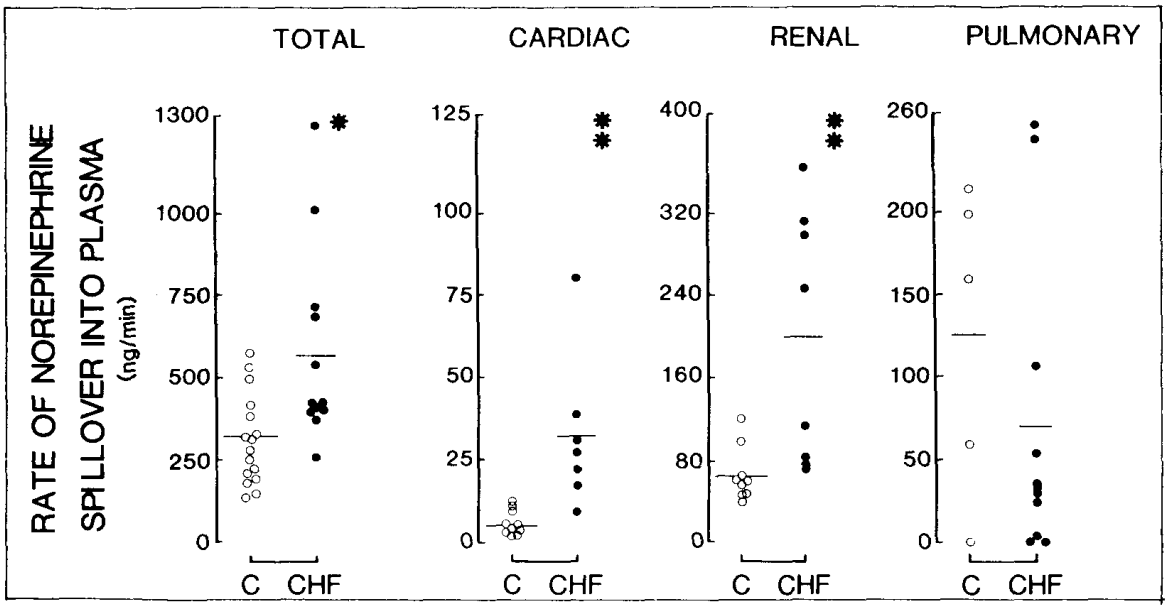
## Sympathetic Nervous Activity in Heart Failure

G. Hasking, M. Esler, G. Jennings, D. Burton, J. Johns*, P. Korner

We have measured total and regional NA spillover and clearance in 12 patients with congestive heart failure and in 16 subjects without heart failure. Mean arterial plasma NA concentration was higher in patients with heart failure (557 pg/ml) than in control subjects (211 pg/ml) ($P < 0.002$). Total NA spillover was increased by 84%, which is considerably less than suggested by the plasma concentration difference. This was partly due to the reduction in clearance in the patients with heart failure. The organs with the greatest NA spillover rates in heart failure were the heart and kidneys which averaged respectively 540% and 206% of corresponding values in control subjects. However, there was no difference between the groups in pulmonary NA spillover (Fig. 3).

The high sympathetic activity in cardiac

*Cardiology Service




*Total, and regional release of noradrenaline (norepinephrine) to plasma, indices of overall and organ-specific sympathetic nervous tone, in heart failure patients (CHF) and control subjects.*

failure may not necessarily be beneficial. An increase in renal sympathetic activity (1) promotes salt and water retention. Increased vascular resistance to blood flow can increase the work of the heart. Moreover, the extraordinarily high level of cardiac sympathetic activity may contribute to the high incidence of heart rhythm disturbances in these patients with heart failure.

### Abnormalities of the Circulation in Cirrhosis of the Liver

I. Willett*, M. Esler, G. Jennings and F. Dudley

In patients with cirrhosis of the liver cardiac output is markedly increased. In addition, the pressure in the vein from the intestine to the liver (portal vein) is often very high, which can lead to torrential and sometimes lethal bleeding from the upper gastrointestinal tract.

Total NA spillover rate was significantly higher in patients with cirrhosis than in healthy people, with an average fourfold increase. Cardiac NA spillover increased sixfold and may have contributed to the elevation in cardiac output. In addition, there was striking elevation of NA spillover rate from the liver and gut. In this disorder there is more pronounced enhancement of sympathetic tone than in other disorders we have studied.

*Gastroenterology Service

Administration of clonidine is used to lower sympathetic activity in patients with essential hypertension. In patients with cirrhosis, after i.v. administration, the drug lowered NA spillover rate and reduced corrected wedged hepatic vein pressure (a measure of portal vein pressure) by 25%, while maintaining blood flow to the liver normal. If the same effect is observed with chronic oral administration, it may be possible to lower portal vein pressure and reduce the risk of bleeding.

### Release of Noradrenaline by the Human Heart

M. Esler, G. Hasking, J. Johns*, P. Leonard, I. Willett** and G. Jennings

In 7 control subjects, cardiac noradrenaline spillover rate was 6 ± 4 ng/min (mean ± SD). Cardiac NA spillover was elevated (12 ± 5 ng/min, $P < 0.05$) in 8 patients with essential hypertension. Cardiac NA spillover rate was normal in 6 patients with coronary artery disease (8 ± 4 ng/min) while pain-free at rest, but rose with angina during atrial pacing to 18 ± 9 ng/min. Such an increase in cardiac sympathetic tone could lead to the development of abnormal heart rhythms when cardiac blood supply is inadequate.

* *Cardiology Service*
** *Gastroenterology Service*

# Basic Cardiology Laboratory

**Head:** Dr. Archer Broughton

## Projects

The Proton-Sensitive Microelectrode

Computer-averaged surface electrocardiogram

Assessment of cardiac contractile performance

## Summary

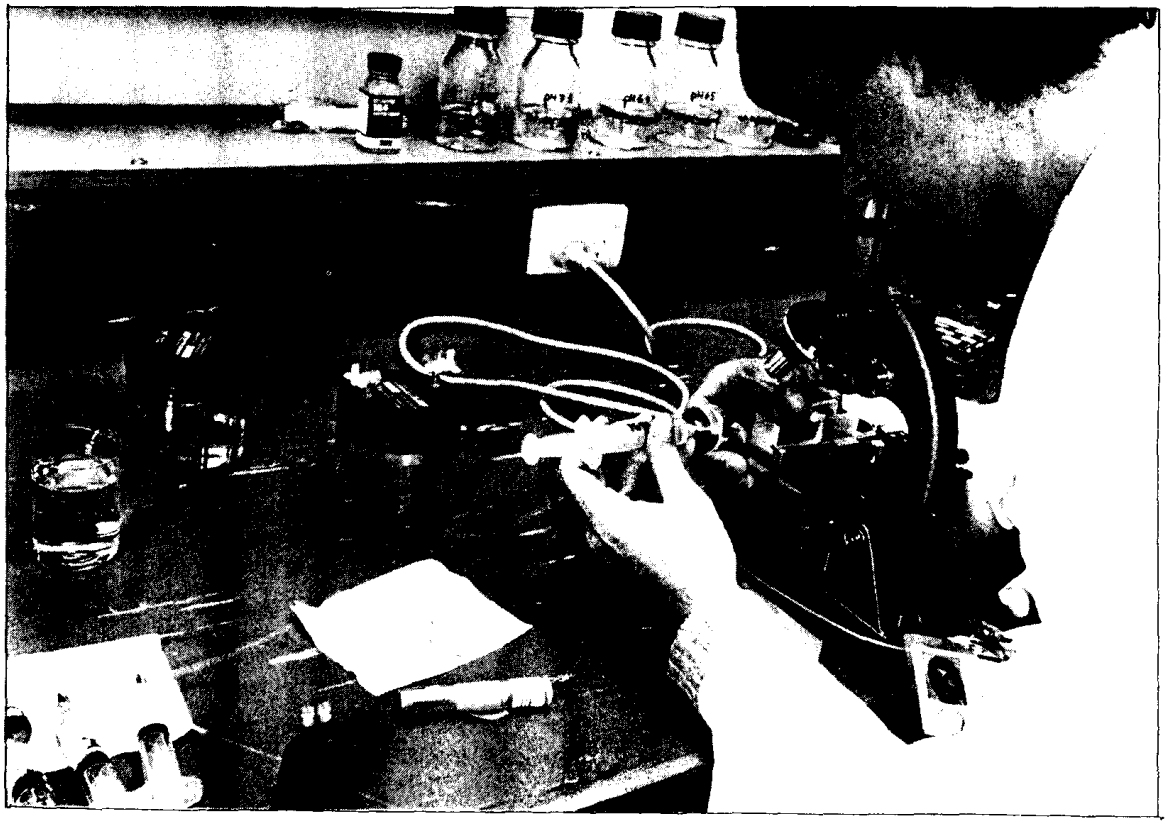
Our work is directed to increasing our understanding of the electrical and mechanical derangements within abnormal heart muscle. We are studying the changes in electrical stability and in mechanical performance within heart muscle once coronary blood flow has become inadequate in advanced hypertension or coronary disease. This has been the first year of operation of the new laboratory and we have established a number of new techniques which will allow us to solve a number of important problems related to disturbances of cardiac rhythm and pumping capacity.

## Projects

### The Proton-sensitive microelectrode

A. Broughton, M. Ross-Smith

We wish to determine the effect of intracellular ionic abnormalities which develop in patients with ischaemic heart disease on the actions of drugs which are used clinically to stabilize the heart's rhythm. Critical reduction in coronary blood flow leads to a marked accumulation of hydrogen and other ions within the cardiac cell. These ionic disturbances are responsible for many lethal arrhythmias in the setting of acute myocardial infarction. Potentially life-saving antiarrhythmic drugs are believed to act from the inside aspect of the cardiac cell surface membrane where they are influenced by changes in ionic environment. We still know little about the consequences of such changes for drug action. Advances in this



*Mark Ross-Smith preparing sensitive microelectrodes*

field will bring clinical benefits by stimulating the development of drugs which approach their best performance under the abnormal ionic conditions of myocardial infarction. To characterise the effects of hydrogen ion accumulation we have constructed a special glass microelectrode to record intracellular pH. The electrode is based on the neutral ion carrier dodecylamine, which is highly selective for hydrogen ions. Despite the very high electrode resistance (100,000 megohms) we have obtained stable recordings of intracellular hydrogen ion activity 30-60 seconds after introduction of the electrode into a single living cardiac cell isolated under highly controlled conditions. It has been known for several years that extracellular acidosis appears to potentiate the actions of antiarrhythmic drugs. We now hope to clarify the effects of the more relevant intracellular accumulation of hydrogen ions.

### Computer-averaged surface electrocardiograph

A. Broughton, J. Cassell, M. Ross-Smith

Up to 15-20% of patients surviving a heart attack subsequently die in the next year, many of them suddenly. One important challenge has been to identify the subset of patients prone to ventricular arrhythmias before their discharge from hospital and to improve their outlook by appropriate treatment. But the logistics of screening all patients with acute myocardial infarction for electrical instability are daunting. Several investigators have advocated the use of the signal-averaged surface ECG to detect small deflections in the ST segment in patients at risk. Averaging of several hundred heart beats is necessary to improve the signal to noise ratio of the recording. The microvolt-sized "late potentials" represent delayed activation of portion of the scarred ventricular heart muscle, which is responsible for the rhythm disturbances.

This approach has not been much used clinically because of the many hours of computer time that are necessary to precisely synchronise all QRS complexes from each patient prior to averaging. By the combination of Fortran and machine language programming, we have reduced this time to about 20 minutes, so that "least squares" alignment of 250 beats can be accomplished. Our present technology features 12-bit resolution and a sampling rate of 600 Hz. We plan to screen patients with myocardial infarction for late potentials and to compare the results of signal averaging with other more invasive assessments requiring cardiac catheterization. Because delayed potentials have only been detected in 25-50% of patients with ventricular electrical instability we will signal average the ECG obtained during atrial pacing. Delayed potentials can then be sought at a variety of paced heart rates above the spontaneous rate. Since conduction velocity through diseased myocardium is liable to be further depressed at high rates, there is a good chance that late potentials will be accentuated by atrial pacing, or even develop for the first time. This can be done by inserting a special pacing catheter into the oesophagus (gullet), which will allow control of the atrial rate without the use of intravascular catheters.

### Two-dimensional (2-D) echocardiographic assessment of cardiac function in the experimental laboratory

J. Smolich, G. Hasking, A. Broughton

Previously our assessment of cardiac mechanical performance has been based on measuring the rate of pressure development within the ventricular cavity and from the ventricular pumping capacity under carefully controlled loading conditions. These approaches have provided information about the overall capability of the ventricle, but they leave open the question of the integrity or otherwise of the individual contractile subunits within the chamber wall. Knowledge about the latter is important because functional deterioration of each subunit can be compensated for by adaptations, which reduce the loading stress. In the hypertensive heart this occurs owing to an increase in total muscle mass and also by a

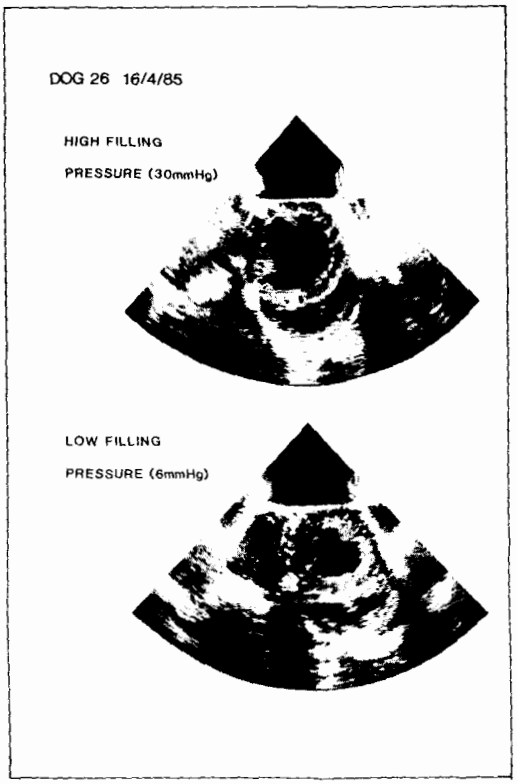



*Figure 1*

reduction in the size of the chamber cavity. The 2-D echocardiograph has proven an ideal instrument for obtaining information about muscle thickness and cavity dimensions in anaesthetised dogs studied under open-chest conditions. The cross-sectional cuts shown in Figure 1 demonstrate the marked changes in left ventricular cavity size and wall thickness which occur when cardiac filling pressure is reduced. Knowledge of these changes in ventricular dimensions obtained with the echocardiograph during reduction in filling pressure have shown that ventricular performance is preserved through the dimensional adjustments, even though contractile performance of individual muscle units is reduced. For example, reduction in filling pressure from 30 to 6 mmHg was associated with a 30% decrease in the maximal rate of cavity pressure development. From the echocardiographic measurements the changes in cavity pressure were converted to changes in force (stress) within the cardiac muscular wall. The rate of wall stress development decreased by 50% i.e. much more than cavity pressure development. We are currently examining the interrelationship between the patterns of regional coronary perfusion and ventricular and myocardial performance in the hypertensive heart.

# Emily Stewart Renal Laboratory

**Head:** Dr. W.P. Anderson

## Projects

Mechanisms causing hypertension during stenosis of one renal artery

Glomerular morphological changes in renal artery stenosis

Atrial natriuretic factor

Sympathetic nervous system in renal hypertension

Renal ultrastructure in renal wrap hypertension

Morphological analysis of renin release by the macula densa

Role of arterial and cardiac baroreceptors in vasopressin secretion during haemorrhage.

## Summary

We have shown previously that narrowing of the renal artery (stenosis) when there is only one kidney needs to be extreme before hypertension develops. This is because of the local intrarenal action of the hormone angiotension II which has the effect of reducing the resistance to blood flow of the stenosis. With extreme narrowing the local renal effects of the hormone cannot adequately maintain renal function and hypertension develops, as a compensatory mechanism. This year we have studied the mechanisms of so-called "two-kidney" hypertension where the artery to one kidney is narrowed but the other kidney is completely normal. This resembles human renal hypertension. Experiments were performed in chronically instrumented unanaesthetized dogs and have shown that renin release from the stenotic kidney was responsible for

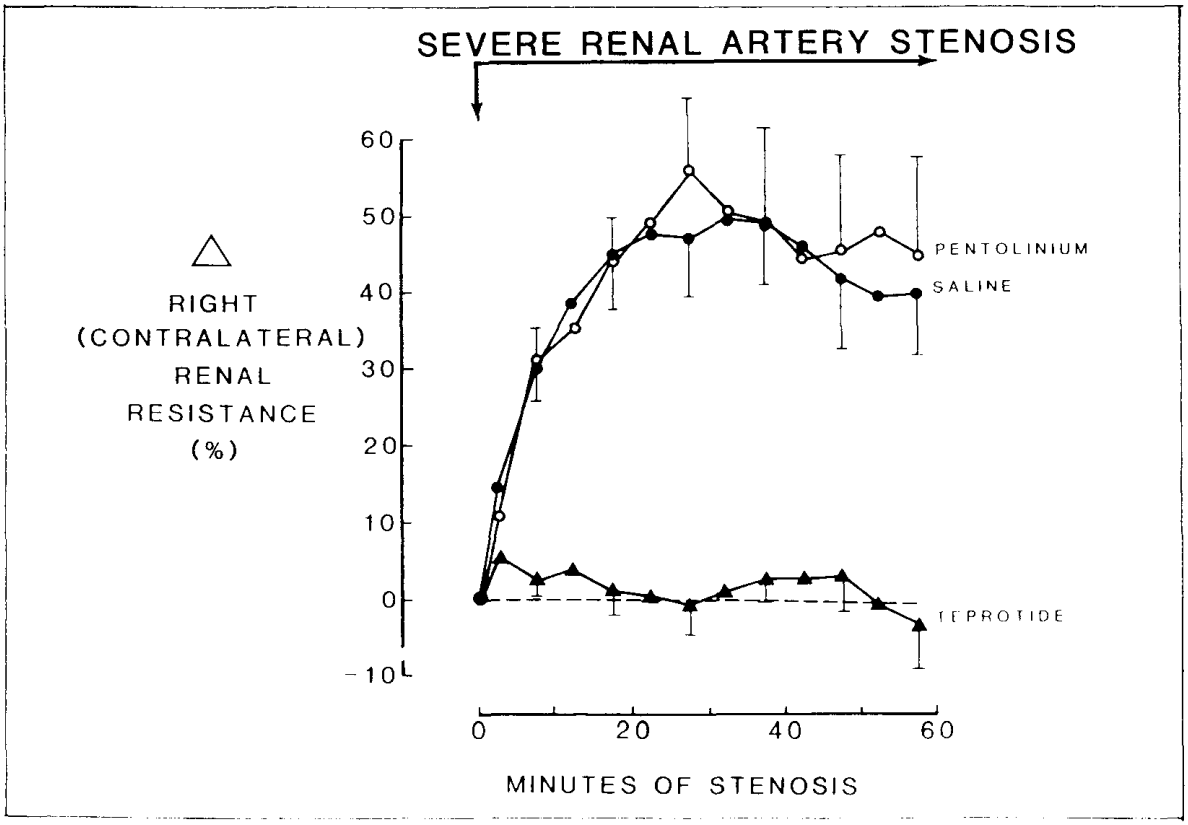
most of the circulatory changes in the "good" kidney and for the constriction of the blood vessels in many other organs. Again the stenosis of the "bad" kidney has to be very tight before hypertension develops. The constriction of the blood vessels of the "good" kidney appears to be critical for the development of high blood pressure.

We also examined the ultrastructural changes in glomerulus during several manoeuvres designed to alter angiotensin II concentration. This hormone has previously been thought to act predominantly on the renal blood vessels rather than on the glomerulus, or kidney filter (the first step in the production of urine). Our preliminary findings suggest that angiotensin II alters glomerular structure in a manner likely to influence both the circulatory and the filtering function of the kidney.

We also examined an hypothesis that has recently become popular, that an increase in plasma circulating adrenaline concentration boosts the activity of sympathetic nerves and thereby leads to chronic hypertension. This did not occur with prolonged infusions of adrenaline in trained dogs, but we did see a modest rise in blood pressure if cortisone was administered at the same time. Since both ACTH and adrenaline concentrations can rise markedly in various types of stress, we tested whether such potentiation occurred. Infusions of ACTH produced marked hypertension which were accompanied by dramatic changes in renal function, but even quite high levels of adrenaline did not affect the magnitude of the elevation of blood pressure.

Vasopressin is another hormone, normally important in the reabsorption of water by the kidney, but also capable of constricting blood vessels. It is released from the hypothalamus and posterior part of the pituitary gland. We examined the role of the arterial and cardiac baroreceptors on its secretion during haemorrhage. Interestingly, with this disturbance the receptors from the heart provided almost the entire "drive" to release of this hormone, with almost no involvement of the arterial baroreceptors.

*Angotension II is responsible for construction of the normal kidney.*



## Projects

### Hypertension in renal artery stenosis of only one kidney ("two-kidney" hypertension)

W.P. Anderson, R.L. Woods, R.L. Kline, J.T. Thompson, and D.E. Ramsey

Stenosis of one renal artery may cause hypertension despite the presence of a "normal" contralateral kidney. This suggests that the stenotic kidney is affecting the function of the normal kidney. We have shown that the link is through the renin-angiotensin system, which is responsible for the initial circulatory responses of the normal kidney (see Figure). In chronically instrumented dogs graded stenosis of one renal artery produced an immediate increase in blood pressure and vasoconstriction of the contralateral normal kidney. Both effects were proportional to the severity of stenosis and no longer occurred if the dogs were pretreated with converting enzyme inhibitor. Hence, renin released from the stenotic kidney produces angiotensin II which constricts the blood vessels of the normal kidney (see Figure1) and of other organs. The afferent nerves from the kidney were without effect on these responses, which were unaffected by ganglionic blockade of the autonomic nervous system (pentolinium). After stopping angiotensin II formation renal artery stenosis was no longer associated with any other systemic cardiovascular changes suggesting that hormones such as kinins, prostaglandins, and medullary lipids) were probably not released from the kidney even under conditions of very severe renal artery stenosis. We do not yet know whether this would also apply to chronic experiments, over periods of days or weeks.

The rise in blood pressure following unilateral renal artery stenosis was entirely due to increased total peripheral resistance with no changes in cardiac output. Almost half of the rise in resistance was due to an increase in resistance of both kidneys, including the kidney subjected to experimental narrowing of the renal artery and the vessels of the opposite kidney, where the constriction was mediated through blood-borne angiotensin II.

### Effects of ACTH and adrenaline on blood pressure

W.P. Anderson and D.E. Ramsey

We studied whether adrenaline and ACTH potentiate each other's actions in increasing blood pressure. With ACTH infusion mean arterial pressure increased reproducibly by about 15 mmHg, whereas adrenaline infusions had no effect. When infused together, the rise in blood pressure produced by the two hormones was no greater than with ACTH alone.

### Changes in the renal macula densa during urinary sodium loss

W.P. Anderson, D. Alcorn, G.B. Ryan and D.E. Ramsey

The macula densa is thought to sense the composition of distal tubular fluid and then send a signal to the arterioles, possibly through the release of renin. We have examined changes in its structure during experimentally induced alterations in renal

*Ms. K.M. Denton, Dr. W. Anderson*

sodium excretion. Normally, large basolateral spaces exist between the cells of the macula densa. But, we found few or no spaces between the macula densa cells after administration of diuretic drugs to promote salt excretion. This apparent closure of the spaces occurred with the osmotic diuretic mannitol as well as with frusemide and ethacrynic acid. The closure of the spaces suggests alterations in fluid fluxes across the macula densa in response to electrolyte changes in distal tubular fluid. Plasma renin levels did not correlate well with closure of the spaces, casting some doubt whether these effects were due to renin. These levels may not be reliable guides to local juxtaglomerular renin concentration.

### Renal nerves in renal hypertension

R.L. Kline and K.M. Denton

Nerve signals travelling both to and from the kidney have been implicated as important factors contributing to the development of some types of renal hypertension. Efferent sympathetic renal nerves regulate renal blood flow, renin release and possibly sodium transport. Afferent nerves from the kidney can respond to chemical and physical changes and reflexly influence arterial pressure through the central nervous system.

We have studied the role of these nerves in the development of hypertension due to cellophane wrapping of the kidneys. We studied the effects of renal wrapping in intact rabbits and in another group with both kidneys denervated. Four weeks after wrapping, there was similar rise in blood pressure in both groups. Effectiveness of renal denervation was assessed from the large decrease in renal noradrenaline content which was less than 5% of control. Renal nerves are not apparently involved in this form of hypertension.

### Role of arterial and cardiac baroreceptors in vasopressin secretion during haemorrhage

A.W. Quail, R.L. Woods, A.M. Macpherson
J. Kreibich, C.J. Duch

The neural regulation of vasopressin (AVP) release was investigated by constructing blood volume-hormone response curves during graded haemorrhage. We assessed the role of each baroreceptor input on the evoked hormone response and whether the response to one was assessed by the presence or otherwise of the other input.

Conscious rabbits were bled at 2% of blood volume per minute. Blood samples for AVP measurement were taken before haemorrhage and at 15, 20, 25, 30 and 35% blood volume loss.

| | | PLASMA AVP ($pg.ml^{-1}$) | |
|---|---|---|---|
| PREPARATION | n | CONTROL | 35% BLOOD VOLUME LOSS |
| AC | 12 | 3.2 ± 0.3 | 34.8 ± 9.2* |
| C | 6 | 3.1 ± 0.6 | 29.1 ± 8.3* |
| A | 6 | 4.1 ± 0.6 | 6.3 ± 1.7 |
| O | 6 | 3.2 ± 0.7 | 4.7 ± 0.7 |

Values are mean ± S.E.
*Level of significance $P < 0.05$

The threshold for release of AVP in rabbits with all baroreceptors intact (AC) occurred between 25 and 30% blood volume loss and was followed by a dramatic rise in plasma AVP with more pronounced blood volume loss (Table). When the cardiac receptors were intact but the arterial baroreceptors removed by sino-aortic denervation (HC), the rise in AVP concentration was closely similar to that observed in intact rabbits (AC). By contrast, in rabbits with cardiac receptors blocked with intrapericardial procaine but with arterial baroreceptors either intact (A) or removed (O) in AVP concentrations did not increase.

We conclude that the drive for increased AVP secretion in haemorrhages comes from the cardiac baroreceptors alone. From the blood volume-mean arterial pressure relationships studied under the various conditions of afferent drive, the increase in AVP occurred only after "failure" of the sympathetic neural response during haemorrhage (see Circulatory Control and Neuropharmacology Laboratory).

# Neurophysiology Laboratory

**Head:** Dr. E.M. McLachlan

## Projects

Functional neuroanatomy of lumbar sympathetic neurons.

Evaluation of techniques for retrograde labelling in the peripheral nervous system.

Electrophysiological characterization of vasomotor neurons.

Computer modelling of sympathetic neurons.

Development of vasomotor ganglion cells in the lumbar sympathetic chain.

Functional innervation of arterioles.

The distribution of descending peptide containing pathways to sympathetic neurons in the spinal cord.

Evaluation of retrograde transsynaptic tracers to identify sympathetic preganglionic neurons of known function.

## Summary

Our work aims to understand the basic cellular mechanisms by which the activity of nerve cells (neurons) of the sympathetic nervous system modify the contraction of the heart and the degree of constriction of the small peripheral blood vessels.

The sympathetic pathways that carry outgoing (efferent) signals from the central nervous system (CNS) arise from nerve cell bodies in the thoracic and lumbar segments of the spinal cord. The nerve fibres travel in peripheral nerve trunks and their endings make connections (synapses) with other neurons that lie in the sympathetic ganglia. The spinal nerve cells are called preganglionic neurons, whilst the axons of the ganglionic cells project to the organs, where they end around smooth muscle (e.g. in the walls of arteries) or near gland cells. These ganglion cells are called postganglionic neurons. The sympathetic nerves regulate many other body functions apart from the cardiovascular system. One of our current aims is to find out how to identify cardiovascular neurons from those that act on the lungs, intestine or bladder.

Sensory (afferent) pathways carry signals to the CNS about the state of the organs in which their endings lie. Little is known of the function of the sensory endings near blood vessels, although they are widely believed to be responsible for sensations of cardiac and vascular pain. This is because they can be shown to discharge when the tissue around them lacks oxygen, or if there is accumulation of the products of metabolism. Reflexes arising from stimulation of these endings may produce nerve-mediated vasoconstriction without necessarily involving the conscious sensation of pain. We are trying to define the distribution of these sensory endings and their pathways, before examining their function.

We are trying to distinguish vascular from non-vascular sympathetic neurons by anatomical and electrophysiological methods. The first project involves identifying the distribution of nerve cells with endings primarily on blood vessels and to compare this with the distribution of nerve cells with endings involved with motility or secretion in the viscera. These studies have been carried out in collaboration with Dr. W. Jänig, from the University of Kiel, West Germany. We have used the small protein enzyme, horseradish peroxidase (HRP) to label the nerve fibres that travel predominantly to the vessels of skeletal muscle or skin and have compared the distribution of these neurons with those obtained after HRP labelling of the sympathetic nerves to the pelvic organs. Another anatomical technique involves the application of immunochemical methods to demonstrate the presence of peptides inside neurons. A newly discovered peptide, Neuropeptide Y, is present in nerve endings on blood vessels in most parts of the body, and also in CNS nerve pathways involved with cardiovascular regulation. Immunocytochemical demonstration of this peptide, in conjunction with the neurotransmitter noradrenaline (NA), is thought to be characteristic of vascular neurons. This technique is being developed in association with Dr. I. Llewellyn-Smith, Department of Human Morphology, Flinders Medical Centre, Adelaide.

We have recorded the transmembrane potential in single neurons, by inserting fine glass capillary micropipettes (less than 0.1 um in diameter) into their cell bodies. The neurons are stimulated by passing electric current through the recording microelectrode, and recording the resulting nerve impulses (action potentials). In addition, the ionic currents that flow across the membrane can be recorded while holding (clamping) the membrane potential at a particular voltage level. Currents are measured under controlled conditions in vitro during changes in the concentration of ions and drugs in the solution bathing the neurons. This powerful technique (using a single microelectrode to both pass current and record potential) has enabled us to identify different distributions of ion channels in vascular and non-vascular sympathetic ganglion cells. Three channels specific for potassium ions account for the characteristically different discharge patterns of vascular and visceral neurons (see below).

We are also studying the responses of the neurons to neurotransmitters released from presynaptic nerve endings. These substances interact with receptors in the neuron's membrane and briefly open synaptic channels through which cations move. The synaptic currents that are recorded tell us about the molecular properties of these channels, and the effects of different drugs. We have found that vascular pathways always have one "strong" or powerful preganglionic to postganglionic connection.

In other experiments we are investigating the changes in the peripheral sympathetic pathways that occur in the course of development. These experiments have been conducted in collaboration with Dr. G.D.S. Hirst of the Department of Pharmacology, John Curtin School of Medical Research, A.N.U., Canberra. To date we have correlated particular features, such as the outgrowth of the dendrites of sympathetic ganglion cells, or the presence of specific ion channels in their membranes, with the development of specific functions in these neurons. This helps our understanding of their role in the adult animal. For example, calcium channels are opened and carry some of the current during the action potential in sympathetic ganglion cells. At newly-established synapses in vascular neurons, these calcium channels appear to be associated both structually and functionally with the "strong" synaptic connection on only one dendrite.

## Projects

### Functional neuroanatomy of lumbar sympathetic neurones.

E. McLachlan, T. Sittiracha, W. Jänig, R. Baron

The neurons involved in sympathetic and sensory outflow to the colon and pelvic organs have been compared with corresponding nerves involved with hindlimb vasoconstriction. In the cat most sympathetic neurons in the caudal lumbar paravertebral chain project to the skeletal muscle and skin vasculature of the hindlimb, and this has now been confirmed in guinea pigs. In addition, we have applied HRP to the caudal sympathetic trunk just proximal to these ganglia in order to identify a discrete population of vascular preganglionic neurons. The arrangement of these cell bodies has been found to differ from that of neurons labelled from the lumbar splanchnic nerves in the same animal. The vascular population lies rostral and lateral to the neurons that project to the viscera, with a small region of overlap at the lateral border of the grey matter. The latter may correspond to the vascular component of the innervation of the visceral organs.

There are some differences in the anatomical organisation between rats and rabbits; for example, in the tail which is a major thermoregulatory organ in the rat but not in the rabbit. We plan to exploit these differences to find out whether vascular neurons can be subdivided according to the role of the sympathetic innervation in different organs.

### Evaluation of techniques of retrograde labelling in the peripheral nervous system.

T. Sittiracha

The HRP method can provide reasonable quantitative estimates of the components of peripheral nerve trunks, in that

the highest numbers of labelled cells are close to the number of axons counted from electronmicrographs. However, in some experiments, we label lower numbers. This appears to be related to poor uptake of HRP by fine diameter axons. We have now systematically tested several detergent and penetrant substances to improve uptake of HRP but so far with little success.

**Electrophysiological characterization of vascular and non-vascular neurons.**

E. McLachlan, J. Cassell

We are using intracellular recording techniques to study the properties of the cell membranes of vascular and non-vascular sympathetic ganglion cells. Lumbar sympathetic ganglia (containing vascular neurons) and inferior mesenteric ganglia (containing mostly non-vascular neurons) are isolated in vitro from guinea pigs or rats.

The most remarkable finding has been that vascular and non-vascular neurons show quite different patterns of discharge when action potentials are initiated in them by passage of current. Almost all neurons (97%) in the lumbar sympathetic chain fire action potentials in a high-frequency burst which then ceases, while action potentials in non-vascular neurons in the inferior mesenteric ganglion fire slowly and rhythmically for as long as they are stimulated. These neurons have been classified as "phasic" and "tonic" respectively. The distribution of neurons with these firing patterns reported by others in several different sympathetic ganglia is consistent with our interpretation that phasic neurons have vascular functions. This is the first functional difference ever described between vascular and non-vascular neurons.

Analysis of the currents recorded in voltage clamp has shown that there are three potassium channels which become active during depolarization. These are present to different extents in the two types of neuron and we are studying their contribution to the differences in discharge pattern.

**Computer modelling of sympathetic neurons.**

J. Cassell, E. McLachlan

In order to interpret the electrical events recorded with a microelectrode in the cell body of a neuron, it is necessary to describe the electrical behaviour of the neuron precisely. This is complicated by the fact that the cell is composed of heterogenous elements. For example, in addition to its axon it bears a number of dendrites on which lie many of the synaptic contacts made with other cells. It is possible to compute the electrical responses of a model circuit that resembles the nerve cell, if the electrical and structural properties of the components are known.

A computer program that simulates the responses of a nerve cell body with an attached dendritic tree has been devised by Mr. J. Clements and Dr. S.J. Redman of the Experimental Neurology Unit, John Curtin School of Medical Research, A.N.U. We have used their approach to simulate the synaptic responses of vascular and non-vascular neurons. The results obtained are not consistent with any differences in the channels activated by the neuro-transmitter substance in sympathetic ganglia. But the differences in synaptic currents can be explained by the electrical properties of the nerve cell membrane, and differences in location of the synaptic contacts on the nerve cell.

**Development of vasomotor ganglion cells**

D. Hirst, E. McLachlan, B. Griersmith

We have studied the structure and function in the lumbar sympathetic chain of the rat during the first three weeks after birth. The distribution of calcium ion channels changes as the dendrites grow out from the cell body with the channels appearing on one dendrite and moving distally as the dendrite grows. The association of the most powerful of several synaptic inputs with these channels ensures transmission in one of the pathways to peripheral blood vessels.

Another finding has come from analysis of the electrical responses of the neurons after the addition of barium ions (which block some potassium channels). Dr. S.J. Redman of the A.N.U. has tested a range of theoretical solutions to explain our experimental findings. The modification of normal electrical properties can be mimicked in a model neuron only if it is assumed that resting membrane conductance to

potassium is not uniform. Preliminary experiments suggest that this unprecedented observation also applies in guinea pig sympathetic neurons, and may be a widespread property of nerve cells.

### Functional innervation of arterioles

S. Luff, D. Hirst, E. McLachlan

A current scientific controversy concerns the type of receptor activated by noradrenaline (NA) when it is released from sympathetic nerve terminals around small blood vessels. Recent electrophysiological experiments have failed to demonstrate an effect of alpha-adrenoceptor antagonists on the potentials evoked in small arteries by stimulation of the sympathetic nerve. David Hirst has shown that very localized application of NA activates alpha-receptors without change in membrane potential. However when NA is applied close to the sites of some nerve terminals, the response mimicks the potentials evoked by nerve stimulation, but is again not blocked by alpha-blocking drugs. He has proposed the existence of a new type of receptor called the gamma-receptor.

We are investigating whether the above sites correspond to structurally-specialized regions like other nerve-muscle junctions or synapses between neurons. We are now studying the regions of contact between sympathetic nerve terminals and smooth muscle cells of arterioles with the electron-microscope.

### The distribution of descending peptide-containing pathways to sympathetic neurons in the spinal cord

B.Oldfield

The projection of NA fibres to spinal sympathetic neurons are distributed heterogeneously. Some groups of sympathetic neurons receive a dense NA innervation and others are hardly innervated at all. These "gaps" in NA innervation may be filled by other types of fibre, such as peptide-containing fibres.

Immunological techniques were used to map the distribution of neurophysin (a

*Dr. E. McLachlan (left), Sue Luff discussing structure of nerve/muscle junction in a guinea pig arterial preparation.*

precursor/carrier molecule for oxytocin and vasopressin) and Substance P-containing fibres arising, respectively, from cells in the paraventricular nucleus of the hypothalamus and the ventrolateral medulla, which may participate in cardiovascular regulation. Antisera to neurophysin (NP) and Substance P (SP) were applied to sections of the spinal cord. Thereafter bound antibodies could be localised utilising the extremely strong affinity of the B vitamin biotin for avidin, a glycoprotein-derived from egg white. In this technique both residues are carried to the site of the bound antisera by a second antibody. An avidin-biotin-peroxidase complex is produced which reacts to form a light or electron dense precipitate.

Peptide-containing fibres of both types were found throughout the thoracolumbar regions of the spinal cord; however, they are clearly aggregated at specific spinal levels and are preferentially directed to specific sympathetic subgroups. The upper thoracic segments are poorly innervated by NA fibres but are rich in nerves containing Substance P. Conversely NP fibres are sparse in the upper thoracic cord but go preferentially to the lower thoracic segments. The close association of SP fibres with sympathetic neurons largely concerned with the innervation of the heart and vasculature is in accord with the recently reported increase in blood pressure resulting from localised spinal application of this substance. These data and the origin of SP fibres in a powerful vasopressor region of the medulla suggest that a SP-containing pathway may contribute to the sympathetic outflow to the heart.

# Circulatory Control and Neuropharmacology Laboratory

**Head:** Professor P.I. Korner

## Projects

Renal sympathetic baroreflex — effects of resetting and anaesthesia. Quantification of constrictor responses to haemorrhage.

Afferent mechanisms concerned in vasopressin release in haemorrhage.

Properties of renal sympathetic nerve units in the rabbit.

Circulatory function of noradrenergic pontomedullary cell groups.

Role of different central noradrenergic cell groups in circulatory actions of alpha-methyl-dopa and clonidine.

## Summary

Our aim is to work out how the central nervous system regulates the circulation in intact animals and to identify "faults" in regulation in hypertension. The first step in the analysis usually requires definition of the different sensory receptors activated by the disturbance, particularly those which sense changes in arterial and intracardiac blood pressures. Information from the different sensory receptors, reaches the central nervous system (CNS) where they project to different "centres" in the brain. Changes in the activity of the latter leads in turn, to changes in the activity of the vagus and sympathetic nerves to the heart and in the sympathetic nerves to the different organs and to the adrenal medulla.

Using a new technique for directly recording sympathetic activity in conscious rabbits, we have completed an investigation of the role of the pressure-sensitive receptors (baroreceptors) from the heart and arteries on renal sympathetic nerve activity. Both sets of baroreceptors contribute significantly to the changes in renal sympathetic nerve discharge.

We have developed a method in conscious rabbits of continuously recording blood pressure and blood flow changes in the arteries supplying the kidney, gastrointestinal tract and hindlimb during haemorrhage. This provides an indirect means of assessing the role of the various autonomic outflows in this disturbance. We are particularly interested in whether catecholaminergic (CA) neurons in the brain are normally part of the CNS network of nerve cells that participates in the autonomic responses to haemorrhage. Much of our re-

cent effort has been to characterize the cardiovascular functions of individual groups of CA neurons and to see which ones are involved in the central actions of alpha-methyldopa and clonidine — two commonly used anti-hypertensive drugs.

## Projects

### Quantification of constrictor responses to haemorrhage in the rabbit.

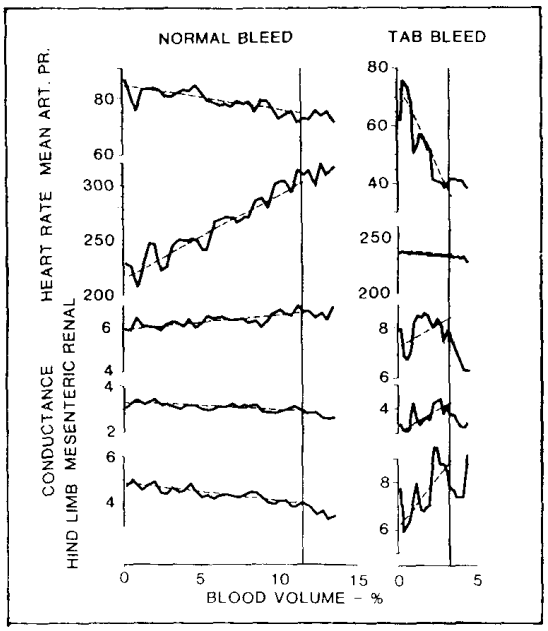
P.I. Korner, J.R. Oliver, J. Gipps and F. Hannemann.

Blood is withdrawn at a constant rate through a previously implanted catheter, whilst continuously measuring mean arterial pressure (MAP), heart rate and blood flows (pulsed Doppler method) in the renal and superior mesenteric arteries and in the lower aorta (= hindquarter). The conductances of the regional beds (flow/ pressure) are computed continuously as an index of changes in vessel calibre; a rise in conductance indicates dilatation and a fall indicates constriction.

An on-line computer programme processes the pressure and flow signals and samples their average values every third of a minute. During haemorrhage some of the blood withdrawn is immediately replaced by an "autotransfusion" from the animal's own tissues. which can be assessed by measuring the haemotocrit ratio. The computer programme subsequently calculates the true blood volume remaining in the circulation throughout the experiment. This is related to changes in (1) MAP; (2) heart rate; (3) blood flows in kidney, gut and limb and to (4) corresponding vascular conductances.

The role of the autonomic nervous system is assessed from comparisons of the responses of normal rabbits with those obtained after pharmacological blockade of autonomic function. After the latter blockade the circulatory response is determined by local factors, such as tissue oxygenation, and by circulating hormones. After autonomic blockade MAP falls linearly with reduction in blood volume; there is little change in heart rate; regional conductances are increased indicative of local dilatation of blood vessels.

When autonomic function is normal, blood pressure is well maintained until blood




*Circulatory responses in a normal rabbit (left) and after total autonmic block (right).*

volume has been reduced by 15-20%. Thereafter, it fall precipitously, indicating a limit of effective regulation by the autonomic nervous system (Fig. 1). Reduction in blood volume is associated with a linear increase in heart rate and falls in regional conductance, indicating vasoconstriction. Other studies performed by Dr. Tony Quail and Dr. Robyn Woods have shown that major increases in the pressor hormone vasopressin only occur beyond the limit of compensation through the sympathetic nervous system.

### Role of arterial and cardiac baroreceptors in vasopressin secretion during haemorrhage

A.W. Quail, R.L. Woods, A.M. Macpherson, J. Kreibich, C.J. Duch

(See Emily E.E. Stewart Renal Laboratory)

### Renal sympathetic baroreflex properties and effects of resetting and anaesthesia.

P.K. Dorward, W. Riedel*, S. Burke, J. Gipps, P.I. Korner

Curves relating renal sympathetic nerve activity (RSNA) and mean arterial pressure

*W.G. Kerckhof Institute, Bad Nauheim, West Germany.

*Dr. Patricia Dorward*

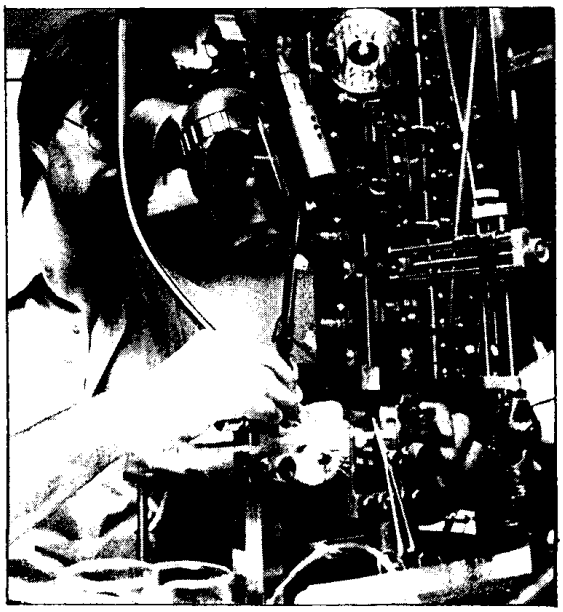
(MAP) were derived in conscious rabbits during ramp changes in MAP and other intravascular pressures, elicited by perivascular balloon inflation. The RSNA-MAP relationship consisted of a high gain sigmoidal region about resting, where RSNA rose or fell in response to moderate falls and rises of MAP. With larger pressure rises RSNA first fell to a lower plateau and then reversed at even higher MAP. When MAP was lowered below resting, RSNA rose to an upper plateau and then reversed abruptly towards resting at low MAP. Both arterial and cardiac baroreceptors exerted substantial inhibitory influences on RSNA at all pressure levels. These effects appeared additive over the central high gain region of the curve, but beyond this region there were non-additive interactions. The latter were affected considerably by alfathesin anaesthesia. In other experiments, we studied the effects of sustained alterations in resting MAP produced by infusing nitroprusside and phenylephrine. These produced rapid resetting of the renal baroreflex which could be partly accounted for by resetting of the threshold of the arterial baroreceptors and partly by contributions from other afferents, probably the cardiac receptors. During nitroprusside-induced hypotensive resetting, high gain reflex adjustments in RSNA to moderate changes in MAP were preserved. But during phenylephrine-induced hypertensive resetting, resting RSNA lay on the lower curve plateau so that the apparent gain of the reflex RSNA response to moderate changes in MAP was considerably reduced.

**Circulatory function of different groups of central noradrenergic (NA) nuclei.**

P.I. Korner, E. Badoer, G.A. Head and J. Aberdeen

In these experiments we have studied the circulatory function of the $A_1$, $A_2$, ($A_1$ + $A_2$), $A_5$ and ($A_6$ + $A_2$) NA nuclei in the medulla and pons of the rabbit. We used the

*Dr. Geoff Head using rat sterotectic procedures for studying the brain stem vasomotor areas, in particular C1 adrenaline.*



circulatory changes elicited by intracisternal (ic) administration of 6-OHDA as the reference response and determined to what extent this was altered by bilateral electrolytic lesions in each of the above nuclei. We have previously found that the effects on blood pressure and heart rate that occur in the first few hours after 6-OHDA are due to central release of catecholamines. From the responses of intact rabbits, pontine animals and sino-aortically denervated (SAD) rabbits we found that release of catecholamines activated (1) a short latency bulbo-spinal depressor pathway; (2) a long latency suprapontine pressor pathway; (3) and (4) short latency bulbo-spinal pathways which slowed heart rate, respectively, by increasing vagal tone and by inhibiting cardiac sympathetic nerve activity; (5) a long latency bulbospinal vagal pathway that produced reflex slowing of the heart; (6) a long latency suprapontine pathway that increases heart rate through the cardiac sympathetic nerves.

Information about all these pathways can be obtained from the responses of intact and SAD rabbits. Intact rabbits provide information about pathways (2), (3) and (5) listed above, whilst SAD rabbits provide information about pathways (1), (4) and (6). Two subgroups of lesioned animals were studied, one with intact arterial baroreceptors and one subjected to SAD. The effects of ic 6-OHDA were compared with the reference responses in the appropriate groups of sham-operated rabbits. At least two weeks were allowed after each set of lesions for degeneration of nerve terminals. On average 85% of NA cells were destroyed in the different nuclei and never less than 75%. The rabbits were in good condition and ate and drank normally, with no signs of neurological disturbances.

Our results suggest that in normal rabbits the $A_1$ nucleus contributes to the bulbospinal depressor pathway, whilst the ($A_6$ + $A_7$) nuclei contribute to the suprapontine pressor pathway. The $A_1$ nucleus contributes to the short-latency cardio-inhibitory pathway. The $A_2$, $A_5$ and ($A_6$ + $A_7$) nuclei contribute to the reflexly activated vagal pathway. Combined lesions ($A_1$ + $A_2$) completely abolish reflex heart rate responses through the latter pathway.

# Biochemical Pharmacology Laboratory

**Head**
Dr. A. Bobik

## Projects*

Physiological regulation of neuronal uptake in cardiac sympathetic nerves.

Central sites of antihypertensive actions of alpha-methyldopa.

Vascular smooth muscle cell adrenoceptors in hypertension.

Sodium transport in vascular smooth muscle.

"Non-receptor" mechanisms for stimulating the heart.

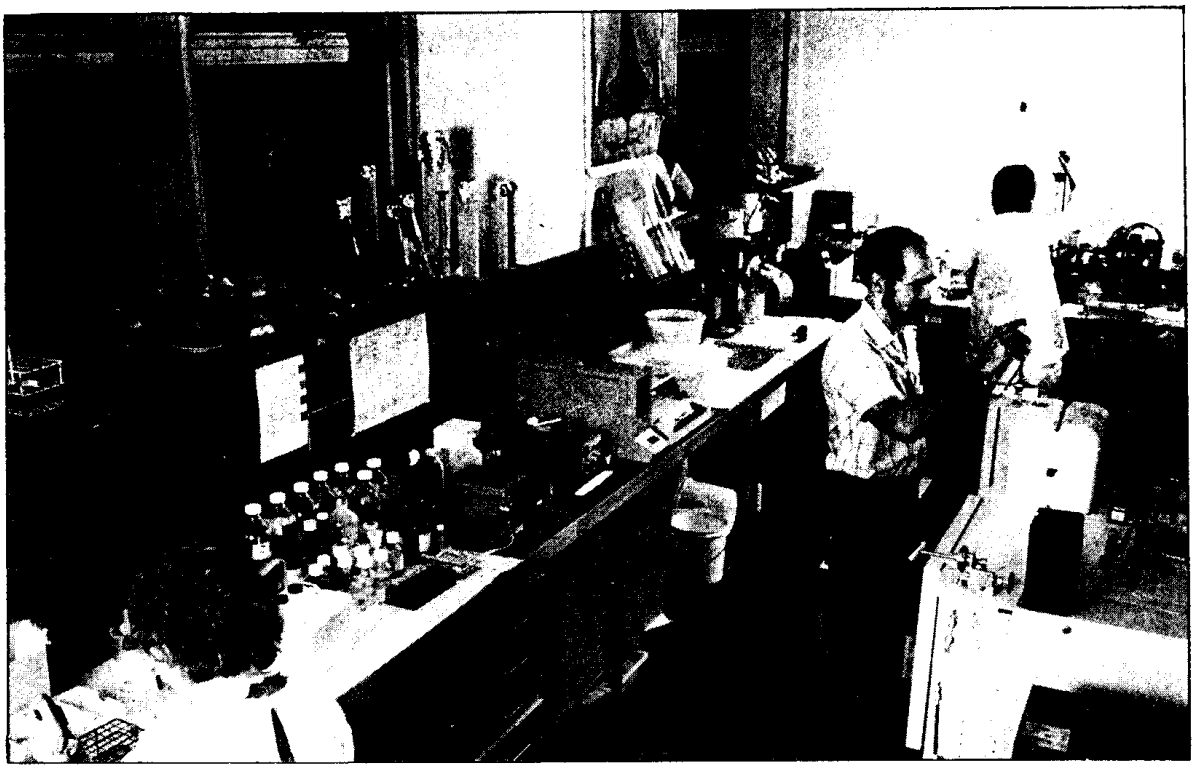
## Summary

Our research has concentrated on basic cellular mechanisms which affect cardiac and vascular smooth muscle responses to changes in activity of the autonomic nervous system. The amount of noradrenaline interacting with specific receptors in the heart and blood vessels as well as the activities of various cellular ion transport systems are the prime determinants of cell responsiveness.

We are also involved in studies on the basic and clinical pharmacology of drugs that stimulate the heart. The clinical studies are described in the Clinical Research Unit Report.

We have found that in experimental neural hypertension sympathetic overactivity, induced by sino-aortic denervation (SAD), is associated with severe reduction in cardiac catecholamine stores in the sympathetic nerve endings. The effect is similar to findings in human heart failure even though this was not in evidence. The main mechanism appears to be the development of a "fault" in the re-uptake mechanism of the nerve terminal of the hypertrophied hypertensive heart subjected to chronic sympathetic over-

*see also Clinical Research Unit report.



*The Biochemical Pharmacology Laboratory*

activity. This does not occur after SAD in the normal heart. Other basic work related to hypertension has been on alpha-adrenoceptor properties in arteries and analysis of the determinants of ionic "pumps" in vascular smooth muscle, which affect contractile performance.

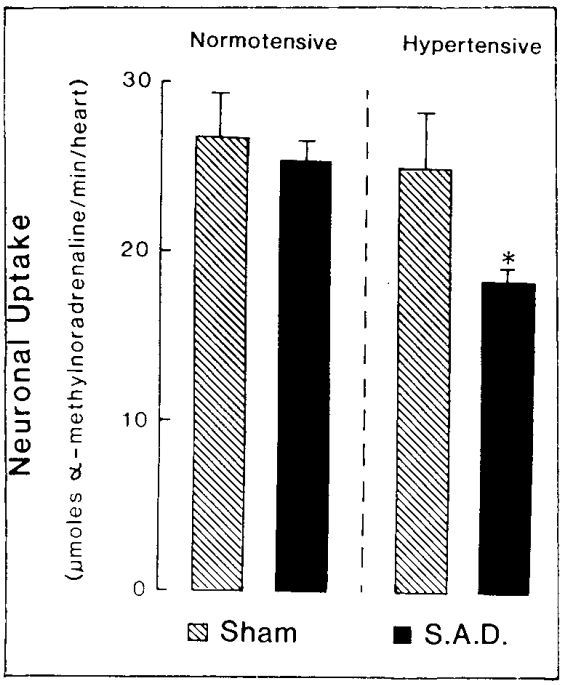
Last year we reported findings on several new beta-adrenoceptor stimulants, which were developed with a view of providing better longterm support for the failing heart than is possible with existing drugs (eg. digitalis). Our findings suggested that these new drugs were unlikely to be successful because of "down-regulation" of beta-receptors with chronic use (i.e. reduction in beta-receptor numbers). We have begun looking at analogues of a naturally occurring substance — forskolin — to try to produce drugs suitable for longterm stimulation of the heart, that bypass the beta receptors on the cell membrane.

Another new approach has been to extend our neurochemical horizons in relation to work on the function on central neurons that release brain amines. In the first instance this work is directed to investigate brain mechanisms contributing to the action of the anti-hypertensive drug alpha-methyldopa (MD). We have developed new microdissection procedures of the brain and have linked them to a series of sensitive assays involving high pressure liquid chromatography. To date our studies suggest that of the noradrenergic nuclei $A_1$, $A_2$ and $A_5$ are involved in the central actions of MD, but not $A_6$ and $A_7$.

### Physiological Regulation of Neuronal Uptake in Cardiac Sympathetic Nerves

J. Snell, P. Scott, A. Bobik

We have now completed a study to determine why cardiac noradrenaline (NA) stores are reduced following sino-aortic denervation (SAD) in renal hypertensive rabbits, but not in normotensive animals. Reduction in left ventricular NA content in the former was primarily due to increased cardiac sympathetic activity associated with increased turnover of labelled NA. We examined whether the major pathway for inactivating NA release from nerves ("neuronal uptake") was impaired under these circumstances. We compared neuronal uptake rates in hearts of normotensive and renal hypertensive rabbits after "sham operation" and SAD. Experiments on isolated perfused




*Maximal rates for alpha methylnoradrenaline uptake into isolated perfused hearts of autonomically intact (sham) and 'sino-aortically denervated' (S.A.D.) normotensive and renal hypertensive rabbits.*

hearts showed that the maximum rate of uptake for alpha-methylnoradrenaline into sympathetic nerves was reduced by 33 percent in SAD hypertensive rabbits compared to either hypertensive rabbits or to the normotensive subgroups (figure). The reduction in neuronal re-uptake appeared to be closely associated to the reduction in left ventricular NA turnover time. Neuronal uptake in the left ventricle of hypertensive rabbits subjected to chronic increased sympathetic stimulation appears to be reduced, probably due to damage of the nerve ending.

## Vascular Alpha$_1$-adrenoceptors in Renal Hypertension

A. Bobik, P. Scott

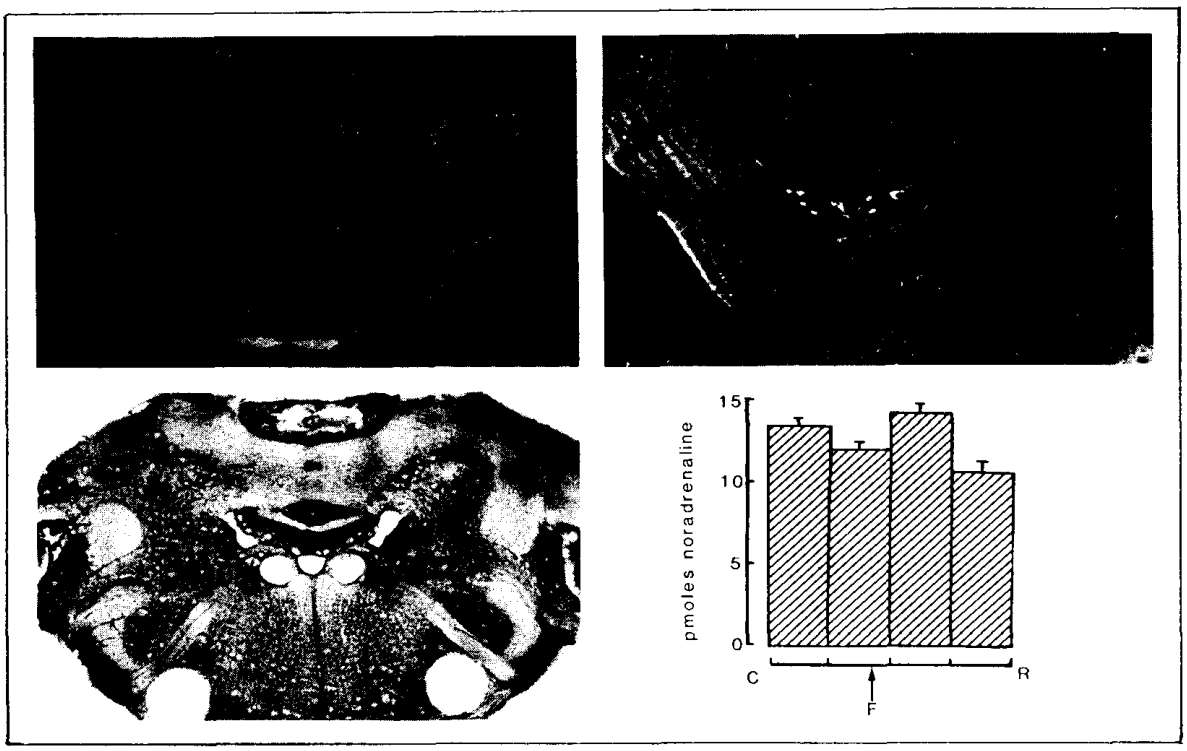
It has been suggested that the increased effect of noradrenaline (NA) on the constriction of blood vessels is due to an increase in number or affinity of alpha$_1$-adrenoceptors on vascular smooth muscle cells. We compared the number of alpha$_1$-adrenoceptors in the aorta, mesenteric and renal artery of renal hypertensive and normotensive rabbits. There was some reduction in number of alpha$_1$-adrenoceptors in cultured vascular smooth muscle when subjected to prolonged stimulation with alpha$_1$-adrenoceptor agonists. We also investigated in vivo whether vascular alpha$_1$-adrenoceptor numbers could be influenced by chronic alterations in sympathetic activity. Reduction in sympathetic activity was achieved by constant i.v. infusion of guanethidine and an increase in activity by sino-aortic denervation (SAD). Neither manoeuvre altered the number of alpha$_1$-adrenoceptors in the blood vessels of hypertensive rabbits. The increased responsiveness of hypertensive blood vessels to noradrenaline is mainly due to structural changes associated with muscle hypertrophy and not due to alterations in alpha receptor number.

## Distribution and Effects of Alpha-methyldopa on Central Noradrenaline Neurons

C. Oddie, A. Bobik, P. Scott, G. Mill, G. Jackman, P. Korner

The hypotensive effect of alpha-methyldopa (MD) in patients with essential hypertension or in rabbits is mainly due to its action in the central nervous system (CNS). In rabbits the role of catecholaminergic (CA) neurons is critical. We have developed new microdissection procedures and highly sensitive high pressure liquid chromatographic assays to measure the endogenous catecholamines noradrenaline and dopamine, as well as alpha-methyldopa and its metabolite alpha-methylnoradrenaline in brain regions close to the main noradrenergic (NA) cell groups of the pons and medulla. The brain stem is cut into 200 *u* sections and the regions close to the NA cells is dissected with circular punches (see figure). We have used this technique to quantitate the distribution of noradrenaline with the $A_1$, $A_2$, $A_5$, $A_6$ and $A_7$ cell groups. This correlates closely with the noradrenergic cell distribution.

We have compared the effects of intracisternal (ic) and intravenous (iv) administration on the distribution of MD and its metabolites. Eight hours after ic MD, its effects on blood pressure are maximal. At this time catecholamine concentrations within the $A_1$, $A_2$ and $A_5$ noradrenergic cell groups are greatly increased due to accumulation of al-




*Localisation and distribution of noradrenaline within the $A_5$ cell group of the rabbit brain.*

*Left top panel: 30μm section of unstained brain stem at the level of the facial nerve (F) and superior olivary nucleus (SO).*

*Right top panel: Noradrenaline fluorescent cells (shown by arrows) of the $A_5$ cell group along the ventrolateral border of the superior olivary nucleus.*

*Left bottom panel: 200μm cresyl violet stained section following microdissection of the $A_5$ cell group.*

*Right bottom panel: Caudal (C) to rostral (R) distribution of noradrenaline within the $A_5$ cell group. F represents the location of the facial nerve within the cell group. One division represents 1000μm.*

pha methylnoradrenaline, which accounts for 86 percent of total catecholamines. In the $A_6$ and $A_7$ cell groups only small quantities of alpha methylnoradrenaline can be detected and NA is near normal. Following iv MD, alpha methylnoradrenaline accumulates in the $A_6$ and $A_7$ cell groups to a much greater extent than when given by the ic route, though the effects on blood pressure are similar. Thus $A_1$, $A_2$ and $A_5$ NA cell groups probably contribute to the circulatory actions of MD, but $A_6$ and $A_7$ cell groups do not (see also Circulatory Control and Neuropharmacology Laboratory).

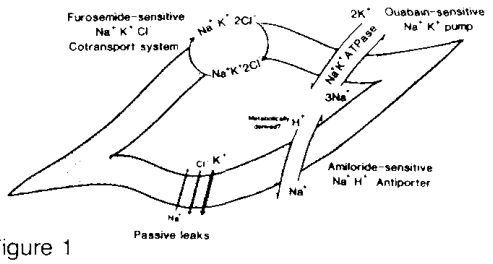
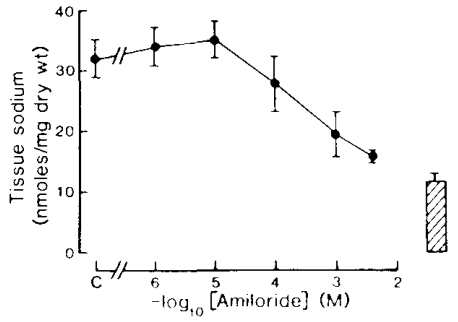



Figure 1

*Top panel depicts some of the more important ion transport systems which transport sodium, potassium and hydrogen ions across vascular smooth muscle cell membranes.*

*Bottom panel demonstrates the effects of inhibiting the sodium hydrogen exchange (antiporter) system by amiloride on the accumulation of sodium by vascular smooth muscle cells whose sodium potassium pump has been inhibited by ouabain (C); normal sodium levels (□) are also depicted.*



## Sodium Potassium Pump in Vascular Smooth Muscle

P.J. Little, L. Rowlands, P. Scott, A. Bobik

In some populations sensitivity to salt appears to be genetically inherited and is associated with a high incidence of hypertension. However, we still do not know which sodium transport pathways in cells of such patients are implicated in salt "sensitivity". There has been much interest in various cellular transport systems in red and white blood cells. Our interest has been in transport processes of vascular smooth muscle cells.

We have studied the sodium-potassium "pump" of vascular smooth muscle cells grown in tissue culture and have examined the effects of a prolonged period of inhibition by hypokalaemia. Subsequent to a 24 hour period of 50 percent inhibition of normal pump activity, sodium-potassium pump activity is increased by about 20 percent. This increase in cell pump activity appears to be due to the production of more pump units (induction). But another sodium-potassium transport system inhibited by low potassium, the frusemide sensitive sodium-potassium chloride cotransport system does not undergo induction. Reports on inhibition of the sodium-potassium pump in blood vessels by either endogenous inhibitors in blood or hypokalaemia have been reported in a number of experimental models of hypertension. Induction of sodium-potassium pump activity may be the reason why some of these experimental models have elevated sodium-potassium pump activity in their vessels.

## Sodium-hydrogen Exchange in Vascular Smooth Muscle

P.J. Little, L. Rowlands, A. Bobik

Vascular smooth muscle possesses a mechanism that exchanges sodium ions for hydrogen ions. This has been described in several other cell types and is inhibited by the diuretic amiloride. We have found that the sodium-hydrogen exchange system is the major entry pathway for sodium into cells. In experiments where sodium extrusion is reduced by inhibiting the sodium-potassium pump with ouabain, accumulation of sodium in the cell may be inhibited by up to 80 percent with amiloride both in segments of rat aorta and in cultured smooth muscle cells. In cultured vascular smooth muscle cells, the activity of this exchange system is increased when internal pH falls, or when extracellular pH rises. The level of activity of the $NaH^+$ exchange system directly affects the activity of the sodium-potassium pump (figure 1). When its activity is high (influx of sodium is rapid), sodium-potassium pump activity is high and when its activity is reduced, pump activity is also reduced.

## Non-receptor Mechanisms for Stimulating the Heart

G. Jackman, A. Bobik

Elevating intracellular cyclic AMP concentrations in the failing heart increases its performance. Short term increases in myocardial performance through this mechanism, may be obtained with drugs which stimulate beta adrenoceptors. However, such drugs lose their ability to maintain the performance of the heart during long term therapy. This is due to "down-regulation" of beta adrenoceptors. If we are to increase cyclic AMP production over prolonged periods, it is desirable to by-pass the betareceptors of the heart muscle membrane.

The naturally occurring diterpene, forskolin, increases cyclic AMP production in a variety of cells by activating the adenylate cyclase enzyme system at sites distal to membrane receptors. Our initial structure-activity studies in heart, liver and lung adenylate cyclase indicate that forskolin and several of its derivatives are equipotent in stimulating adenylate cyclase in heart and liver. However, the 1-acetoxy derivative of forskolin is more selective in activating liver than cardiac adenylate cyclase. All derivatives including forskolin are poor activators of lung adenylate cyclase. Our work suggests that the site at which forskolin and its derivates act in the adenylate cyclase system differs sufficiently from tissue to tissue so as to make it a potential target for developing a new class of adenylate cyclase stimulant and possibly cardiac ionotrope. Binding studies carried out with ($^3H$) forskolin indicate that it most probably does not interact with the G protein of the adenylate cyclase system. We are

studying whether forskolin and its analogues interact with the catalytic unit or with some other unknown protein.

# Pharmacology Laboratory

**Head:** Dr. J. Angus

## Projects

The isolation of endothelium derived relaxing factor (EDRF).

Large artery spasm and loss of endothelium.

Vascular reactivity in hypertension.

Vascular depression and the response of alpha$_2$-adrenoceptor agonists.

Mulvany technique for studying small resistance vessel reactivity *in vitro.*

Vasoconstrictor responses to alpha-adrenoceptor agonists in large and small hindlimb vessels.

## Summary

Our efforts have been directed to the mechanisms that alter the "reactivity" of large and small arteries. One area concerns the mechanisms involved in spasm of large arteries. Some patients suffer from angina at rest and cine x-rays have shown that a segment of a large coronary artery goes into spasm, with complete obstruction to blood flow. In between attacks the arteries appear to be unobstructed by the usual fatty plaques. We have attempted to answer what is the signal that makes the large artery constrict and why it constricts to such an extent. Spasm of a coronary artery is a very serious complication that may cause some types of heart attacks and even sudden death.

We and others have found that the endothelial lining is a very important determinant of the reactivity of large arteries. When a piece of rabbit aorta is suspended in an organ bath administration of drugs such as acetylcholine, which are generally considered to be "dilator" agents, will in fact dilate (relax) the vessel only if the endothelial cells, that line the inside of blood vessels, are intact. After removal of these cells the artery wall no longer responds to acetylcholine by dilating, but may now constrict. Endothelial cell integrity also appears important for arterial reactivity to many other drugs and to many substances produced by the body. These act on the endothelial cells through specific receptors (recognition sites) which causes the release of a powerful "hormone" which relaxes the muscle cells in the vessel wall, thereby opposing the *direct* constrictor actions of the substance. Endothelial cells can be grown in tissue

*Dr. K. Satoh (left), Dr. Jim Angus (right)*

culture, so that we can study the effects of various substances on the release of the endothelium derived relaxing factor (EDRF). We have developed an assay for measuring release of EDRF and to obtain clues about its chemical nature. This work has been performed in close collaboration with the Cell Biology Laboratory.

Concurrently, we are studying the *in vivo* responses of large arteries after damage or removal of the endothelial cells. We have developed a preparation for studying reactivity of the carotid artery in the conscious greyhound for up to four weeks after surgical removal of the endothelium. We measure arterial diameter, blood flow and the pressure in the artery. Direct infusion of serotonin (a substance released from damaged blood platelets) into the carotid artery induces spasm in the side from which the endothelium has been removed. This is the first time that spasm of such a large artery has been experimentally induced. We have also investigated the responses of *isolated* ring segments of carotid arteries from dogs and rabbits four weeks after removing endothelium, when there has been re-growth of endothelium. This tells us whether there still are changes in vascular reactivity.

We studied whether the reactivity of the small blood vessels of rabbits were altered in renal hypertension, or when this was combined with an increase in plasma cholesterol. Hypertension causes an increase in blood vessel wall thickness which makes them more sensitive to vasoconstrictor or dilator agents but it is still controversial whether additional mechanisms are also involved. Our experiments in instrumented conscious rabbits are examining whether cholesterol and hypertension alter the release of EDRF by the endothelial cells of the whole vascular tree and not just of the large arteries.

A new method for measuring the reactivity of the resistance vessels has recently been developed by M.J. Mulvany at the Biophysics Institute, University of Aahrus, Denmark. With his help, we have begun to learn about the reactivity of very small arteries (smaller than a hair thickness) under controlled conditions. So far we have explored the differences in response of mesenteric, muscle, skin and myocardial vessels (150-250 *u*) from human, rabbit and guinea pig organs.

**Isolation and properties of endothelium derived relaxing factor (EDRF)**

T. Cocks, J. Angus, J. Campbell, D. Karamoshos, N. Puglisi.

We have used a technique for growing endothelial cells in pure culture on plastic beads on a relatively large scale. About 50 million cells are placed in a small tube and perfused with a physiological, oxygenated solution in a temperature-controlled chamber. After administering the test substance, the cells release EDRF into the perfusing solution. We have developed a bioassay for quantifying the amount released:— a ring of dog or pig coronary artery with endothelium removed is bathed by the solution from the endothelial cell column. The force developed by this strip is measured continuously and the release of EDRF is associated with relaxation, in proportion to the amount released.

With this bioassay we have detected release of EDRF from endothelial cells from bovine aorta, vena cava and coronary artery, human umbilical vein, pig aorta and dog coronary artery. Other endothelium-

*Dr. T. Cocks (left), Diane Karamoshos*

related cells examined included bovine endocardium, pig epicardium and pig gut mesothelium. All cells released EDRF in response to bradykinin, trypsin and the $Ca^{2}+$ ionophore A23187. To date all have failed to respond to acetylcholine and substance P which may be due to the exact method of preparation of the cell culture.

EDRF is a very potent dilator substance, which appears to be inactivated relatively rapidly upon release from the endothelial cell. It is negatively charged at pH 7.4 and is hydrophilic. It binds or is inactivated by large protein molecules, including haemoglobin and albumin.

## Endothelium and spasm of the carotid artery

J. Angus, T. Cocks, D. Prior, K. Satoh, E. Wright, R. Dumpys, G. Campbell.

Isolated ring segments of large coronary artery contracted more powerfully to noradrenaline and serotonin, if endothelial cells had been removed. This was due to the loss of EDRF mechanism as discussed above. We have now studied, in conscious dogs, the reactivity of a large artery over four weeks after the loss of endothelium.

At surgery endothelium was removed from one carotid artery by drawing a partially inflated 3F Fogarty balloon catheter through the lumen of an 8 cm length of artery (E–). In one carotid artery endothelium remained intact (+E) and served as a control. On each vessel we implanted Doppler flowmeters and ultrasonic crystals to measure changes in artery wall diameter. In addition, we implanted catheters to measure blood pressure and to infuse drugs. For the first 2-7 days after surgery local infusion of serotonin (1-1000 ng/kg/min) produced similar constriction of the large artery, with reduction in diameter of 20-30%. At the same time blood flow increased by nearly 100% as the small resistance vessels dilated. Beyond 7 days after operation the carotid artery without endothelium constricted more than the artery with intact endothelium, with a more marked reduction of its diameter. We found that in three out of six dogs the –E artery went into spasm, with complete cessation of blood flow. This was rapidly reversed by stopping the serotonin infusion. Ketanserin, a new selective serotonin blocking drug completely abolished the vasoconstrictor response and spasm of the large vessel but did not alter the small vessel dilatation. The $alpha_1$-selective agonist methoxamine, caused only small falls in diameter in both arteries. Acetylcholine dilated only the +E artery throughout the four week period. At autopsy electronmicroscopy showed that the lumen of the rubbed artery was mostly covered with endothelium but there was considerable thickening of the intima. This geometrical factor of lumen encroachment from the intimal thickening may also have contributed to the enhanced reactivity. This thickened intima was composed of a layer 3-15 smooth muscle cells thick in the "synthetic state" (no longer contractile) and also contained large amounts of extracellular material.

## Alpha-adrenoceptor mediated pressor responses: are they differentiated by calcium antagonists or by functional antagonism

M.J. Lew, J.A. Angus

Drugs of diverse chemicals classified as calcium antagonists are widely used in the treatment of hypertension and other disorders. They block the pressor action of $alpha_2$-adrenoceptor agonists only poorly. Hence, it has been thought that $alpha_2$- but not $alpha_1$-adrenoceptors are coupled with calcium channels on vascular smooth muscle. Since all calcium antagonists cause cardiovascular depression we wondered whether this could be responsible for this alleged selectivity to the two types of alpha receptors. Theoretically, a partial agonist is more susceptible to functional antagonism than is a full agonist.

Pressor dose-response curves were constructed in ganglion blocked anaesthetized rats to the full $alpha_1$-adrenoceptor agonist B-HT 920. The calcium channel blocking drug nifedipine preferentially inhibited the effects of B-HT 920, in agreement with the findings of others. Lowering the starting pressure by haemorrhage or by nitroprusside infusion or by deeper pentobarbitone anaesthesia also induced preferential inhibition of the pressor effect of

B-HT 920. Moreover, when the maximum effect of methoxamine was reduced by the irreversible alpha-adrenoceptor antagonist phenoxybenzamine, the pressor responses were now sensitive to inhibition by nitroprusside infusion. Thus functional antagonism by vascular depression, rather than a mechanism involving calcium channels, may explain the apparent selectivity of calcium antagonists for alpha$_2$-selective pressor agent.

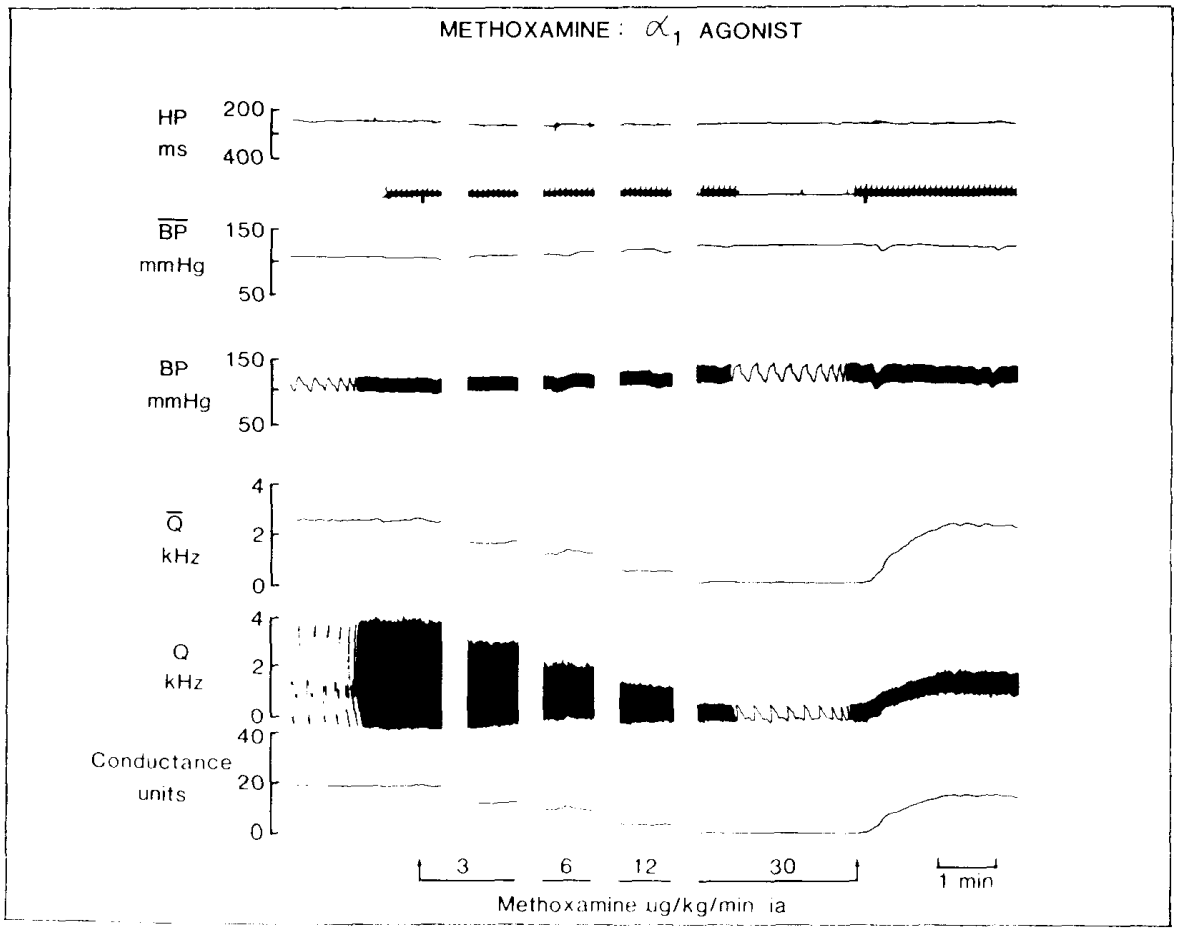
### Hypertension and hypercholesterolaemia on vascular reactivity of small vessel

C. Wright, J. Angus

One of the major determinants of organ blood flow is the calibre of the small "resistance" arteries, which are 100-500 *u* in diameter. We have studied the reactivity of the hindquarter vascular bed of conscious rabbits to a number of vasodilator and vasoconstrictor agents. Hypertension causes medial thickening of blood vessel walls, leading to lumen encroachment which amplifies the responses to vasoconstrictor and dilator stimuli. It is still controversial whether additional factors also contribute to the changes in vascular responsiveness.

We studied instrumented rabbits subjected either to cellophane wrapping of their kidneys or to sham operation 5 weeks after surgery. Half the rabbits received 1% cholesterol in their diet for 4 weeks before the experiments. After pharmacological autonomic blockade, dose-response curves were constructed for intravenous infusion of acetylcholine, adenosine, serotonin, noradrenaline, methoxamine and angiotensin II. We found that neither hypertension nor cholesterol diet altered the sensitivity (location of $ED_{50}$) of dilator action of acetylcholine, suggesting that EDRF release was unaffected by these treatments. Similar results occurred with adenosine

*Chart records from a conscious rabbit showing the intense vasoconstriction to methoxamine in the hindquarter vascular bed.*



and serotonin infusions or to reactive hyperaemia.

Similarly, we could not demonstrate any changes in hypertensive rabbits in the sensitivity to intra-arterially administered alpha-adrenoceptor agonists. These rabbits had elevated hindlimb vascular resistance (HVR) at rest and responded with larger changes in HVR to dilators or constrictors. This is in accord with the expected changes associated with medial hypertrophy. Our experiments could not demonstrate any effect of cholesterol, even for those agents known to cause release of EDRF in vitro.

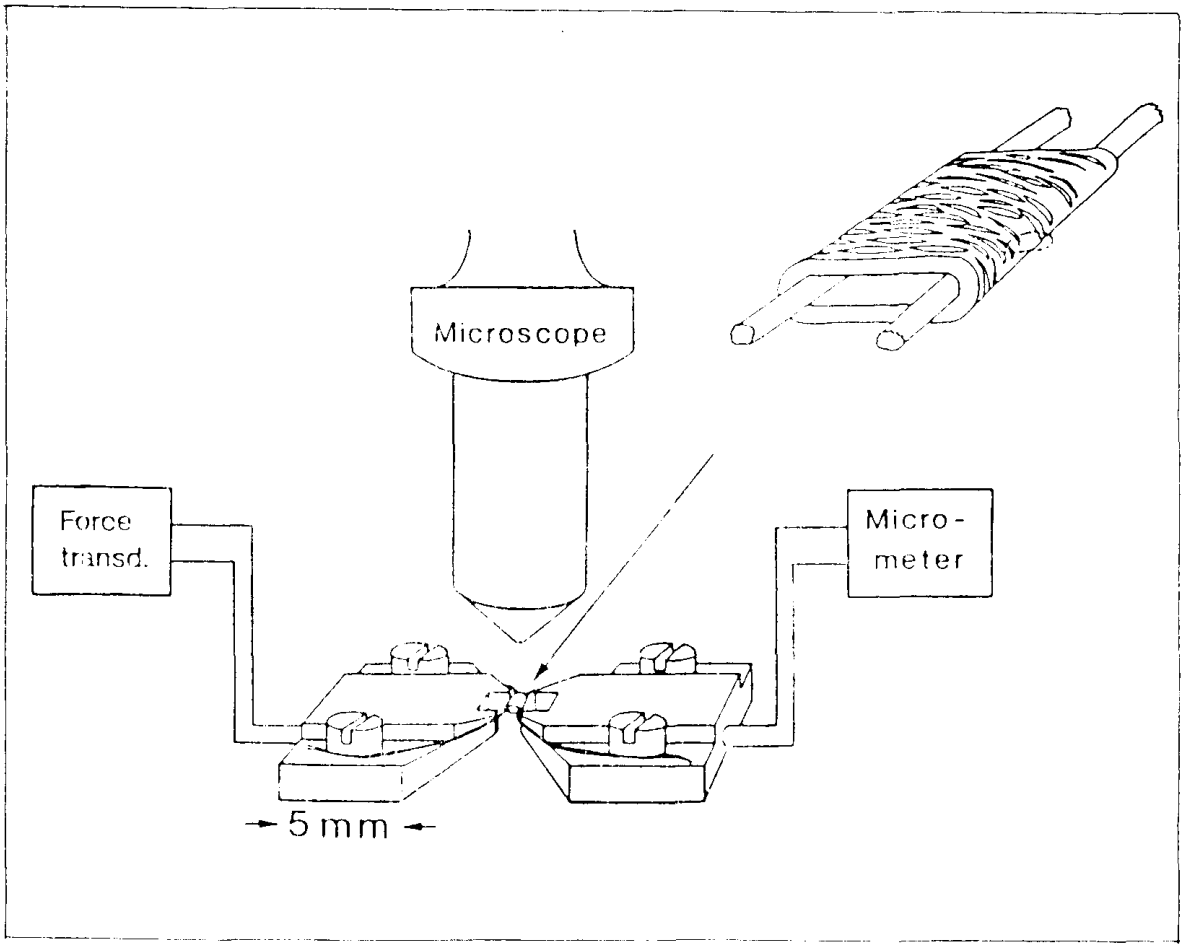
**Pharmacology of small arteries in vitro — Mulvany technique**

J.A. Angus

Recently, it has become possible to study very small vessels, in a manner analogous to that employed for studying large vessels in an organ bath. We set up 2 mm long segments of microvessels of 150-300 *u* in diameter on 40 *u* diameter wires. These wires are attached to a strain gauge (to measure the force developed as the blood vessel contracts), and also to a micrometer. Two vessels can be measured simultaneously in a single, temperature controlled, oxygenated chamber. As well as measuring force of contraction, wall dimensions can be accurately determined by microscopy.

We have shown that even in microvessel the endothelial cells are obligatory for the relaxation to acetylcholine. To our surprise, the selective $alpha_2$-adrenoceptor agonist B-HT 920 caused a poor contractile response in human, rabbit and guinea pig micro-vessels. In these vessels the predominant receptor is clearly the $alpha_1$-adrenoceptor. The drug-receptor compartment comes into rapid equilibrium, so that data for full dose-response curves can be obtained in a few minutes.

*Diagrammatic representation of the myograph and preparation of the microvessel.*



# Cardiovascular Metabolism and Nutrition Research Unit

**Unit Director: Dr. Paul Nestel**

**Lipid Metabolism and Nutrition Laboratory**
Head: Dr. P. Nestel

**Lipoprotein Structure and Function Laboratory**
Head: Dr. N. Fidge

**Lipoprotein Biochemistry Laboratory**

**Cholesterol Metabolism Laboratory**
Head: Dr. S. Turley

## Summary

This year saw the publication of several intervention studies from the United States and Europe involving patients with coronary atherosclerosis. The conclusions from all these studies were consistent and as unequivocal as clinical trials into a disorder of multifactorial causation can ever be. They indicate that lowering serum cholesterol levels of apparently healthy subjects results in substantially less clinical coronary heart disease.

The United States Coronary Primary Prevention Trial studied about 3800 men, randomly allocated into two groups. Half took a cholesterol-lowering drug and half were given a placebo in addition to a cholesterol-lowering diet. After 7 years those who took the drug in full dosage lowered their serum cholesterol levels by 25% and experienced only half the number of coronary events (deaths plus heart attacks) that were observed among the controls. Further analysis showed that the benefit came about from a simultaneous reduction in low density lipoprotein cholesterol and a rise in high density lipoprotein cholesterol.

The results in the control group (placebo + diet), though not yet published, are equally interesting. Although the diet did not lower the cholesterol as much as the drug, there was a similarly significant correlation between the degree of cholesterol reduction and the prevention of coronary disease.

The other trials dealt with the control of the underlying disease, coronary atherosclerosis, assessed by coronary arteriography repeated over a number of years. In untreated subjects the disease progressed and the extent of progression was most closely correlated with the serum concentration of cholesterol, mainly involving a fraction known as intermediate density lipoprotein. The groups treated with cholesterol-lowering drugs experienced a significant arrest in the progress of the diseases which was related to both a lowering of low density lipoproteins and an increase in high density lipoproteins.

Similar conclusions are now emerging from clinical studies of patients who had undergone coronary bypass surgery. Both the grafts and the adjacent coronary arteries are at risk of developing new and further atherosclerosis. The major determinant appears to be the persistence of high blood lipid levels.

The consistency of all these reports provides strong impetus for pursuing the "lipid hypothesis" of atherosclerosis. There is now a solid basis for planning and mounting campaigns based on reduction of important risk factors, including raised cholesterol and triglyceride levels. At the same time we do not yet know enough about how atherosclerosis begins or the precise manner in which lipids influence the development of the disease. We know that the process is very complex, as is the regulation of the lipoproteins that transport lipids through the circulation and in and out of tissues including the arteries. These factors may eventually allow more specific and effective approaches to the prevention of atherosclerosis in the community-wide interventions.

# Lipoprotein Metabolism and Nutrition Laboratory

**Head:** Dr. P. Nestel
Dr. M. Reardon

## Summary

Our research has focused on the nutritional and metabolic regulation of lipoproteins, especially those triglyceride-rich lipoproteins that may cause premature arterial disease.

The highly polyunsaturated fatty acids of fish oils, the so-called omega-3 fatty acids, comprise about 25% of the fatty acids of fish from cold northern seas. These fatty acids are more potent than any currently used drug for lowering plasma triglyceride, which is becoming recognized as a risk factor for coronary artery disease (see below). We have been the first to demonstrate the metabolic basis for this triglyceride lowering effect. We found that these fatty acids stop the production of other fatty acids in the liver and stimulate the oxidation of fatty acids. This leaves the liver without the store of fatty acids needed to synthesize triglyceride. However these fatty acids exert other potent effects in other biological systems and the margin of safety for their use has not yet been established.

We also demonstrated that high triglyceride levels, especially within certain classes of lipoproteins, were associated with more severe coronary artery disease in women than in men. This work was done in collaboration with Dr. Rick Harper and the Alfred Hospital Cardiology Service. It is an important new finding, since high triglyceride levels have not been regarded as particularly atherogenic (inducing atherosclerosis), unlike high cholesterol levels where association with accelerated coronary atherosclerosis has long been recognised. A recent reappraisal, still unpublished, of a large population study (in Framingham, USA) has reached a similar conclusion.

The Unit has long held this view about the atherogenicity of hypertriglyceridaemia (high blood triglycerides) and two projects relate to this question. The first is an examination at the cellular level of the capacity of various classes of triglyceride-rich lipoproteins to induce lipid accumulation in those cells which form the basis of atherosclerosis in arteries. Two types of cells have been studied, the macrophage or "scavenger" cell and the smooth muscle cell of the artery wall. They become engorged with lipids (both cholesterol and triglyceride), to form the so-called foam cell, one of the earliest abnormalities found in the fatty deposits of arteries.

The findings have been exciting. Both the macrophage and the smooth muscle cells can be stimulated to take up increased amounts of triglyceride and cholesterol enriched lipoproteins. In fact, the fatty particles that circulate in the blood after a fat meal (chylomicron remnants) were taken up very avidly. We have identified specific receptor (recognition) sites on the cells to which the lipoproteins were attracted and bound. In collaboration with Dr. Julie Campbell we observed that only some and not other types of smooth muscle cells accumulated lipid. The susceptible cells which Dr. Campbell calls the "irreversibly synthetic cells" are not normally present throughout the arteries which may help explain the patchiness of atherosclerosis.

Another project dealing with triglycerides is being carried out in normal people and in patients with increases in plasma triglycerides. It is believed that only some forms of this disorder lead to arterial disease. This is probably due to the many different causes for hypertriglyceridaemia, with a distinct pattern of lipoprotein abnormalities for each disease type. Although all these lipoproteins are rich in triglyceride, they come from different organs (liver or intestine) and have different transport functions and are at different stages in the process of dismantling (so-called remnants). It is likely that the remnants are more atherogenic than the parent particle and that those which are atherogenic carry a specific mixture of lipids and proteins which attracts them to those cells that become part of the fatty deposit in an artery.

Since all of the lipoproteins which carry triglycerides are present in the blood, it has been important to devise methods to separate and identify them and then to

"track" them by injecting radioactively labelled lipids or proteins. We have used computer modelling to simulate the manner and speed with which these labelled substances disappear from the circulation. This allows us to estimate the amounts and rates of production and the manner in which one is converted to another and removed from the blood.

Similar studies of lipoprotein turnover have been carried out in rats to determine which organs remove circulating lipoproteins by specific receptor mechanisms. This has been combined with isolated cell studies to show how high density lipoproteins (HDL) are metabolised. This lipoprotein has a very important role in cholesterol transport, regulating the movement of cholesterol in and out of cells. We are studying this key process with isolated cells, using different types of HDL-like particles to determine the optimum conditions for promoting cholesterol efflux from cells.

Another project, which has combined experiments in the whole animal and in isolated cells is related to the effects of dietary proteins on cholesterol metabolism. We have shown that a diet rich in animal proteins impairs the capacity of the liver to recognize and clear certain circulating lipoproteins but that this does not occur in a diet rich in vegetable proteins.

### Marine polyenoic acids — potent triglyceride lowering nutrients

P. Nestel, S. Wong Sau Heng, M. Reardon.

We have previously reported the striking capacity of certain fish oils to lower plasma triglyceride concentrations. In man, this was shown to reflect inhibition of VLDL secretion from the liver; the production rates of both the protein and lipid constituents were greatly suppressed. Studies in rats, with perfused

*Dr. P. Nesel (left). Mrs. E. Monger (centre) and Ph.D Student Miss Wong Sau Heng (right).*

livers and isolated liver cells confirmed these findings.

Experiments with liver cells from normal rats and from rats which are genetically hypertriglyceridaemic, clarified the probable sequence of events. Two distinctive fatty acids, eicosapentaenoic and docosahexaenoic acids (both have an unusually large number of unsaturated "bonds"), suppress the synthesis of new fatty acids in the liver. This leads to a marked increase in oxidation (utilization) of fatty acids reaching the liver from the circulation. More of these fatty acids are also used for cellular phospholipids, in membranes for instance. There is therefore a massive diversion of fatty acids away from triglyceride production in the liver, and so the triglyceride concentration in plasma falls.

We have recently demonstrated a similar mechanism in isolated, cultured human liver cells. While this is good news for treating hypertriglyceridaemic patients, we have not found a similar effect on LDL metabolism. Cholesterol levels are therefore not reduced.

### Casein and soy protein in hypercholesterolaemia

J. Cohn and P. Nestel

One of several factors that lower the serum cholesterol levels in vegetarians is the type of dietary protein. This is best seen in people who have high cholesterol levels. We have therefore carried out studies in the cholesterol fed hypercholesterolaemic rat.

When soy protein was fed instead of casein (a milk protein) the cholesterol concentration was reduced in the plasma VLDL fraction. Studies of cholesterol metabolism showed that a significantly larger fraction of cholesterol was excreted from the body with soy than with casein. This, in turn, was found to reflect a more rapid rate of removal of VLDL from the circulation of soy fed rats. The central role of the liver was demonstrated by in vivo and in vitro experiments. The initial binding of injected VLDL by the liver was 30% greater in soy fed rats. Isolated hepatocytes from soy fed rats also bound a similarly greater amount of VLDL in vitro. Of the two hepatic lipoprotein receptors, the receptor that recognised the E apoprotein of VLDL was probably responsible for this differential response to soy and casein, since the other receptor (the LDL or B/E receptor) was suppressed by the cholesterol in the diet. Since the VLDL of casein and soy fed rats appear similar in their protein and lipid composition (other than the higher concentration of cholesterol-rich VLDL in the casein group), it seems likely that the expression of the apo E receptor can be affected by dietary protein.

### Lipoprotein protein and triglyceride metabolism in man

H. Barrett, M. Reardon, P. Nestel, P. Nugent, N. Baker and R. Boston

High plasma triglyceride levels are very common and have multiple causes, e.g. excessive alcohol or energy intake, or to a variety of metabolic disturbances. The description of triglyceride metabolism through kinetic analysis of labelled triglyceride has in the past been based on simple models. We have previously reported overproduction as the prime cause for some forms of high triglyceride levels in plasma and diminished removal as the cause in other varieties.

However the triglyceride transporting lipoproteins are very heterogeneous and at any given time the plasma will contain a mixture of newly secreted lipoproteins and other particles at varying stages of catabolism. We have developed a technique through which these classes can be separated and studied. We are now carrying out experiments in subjects with normal and high triglycerides in which both the proteins and triglyceride are labelled and traced. The data are being fitted to computer based models for analysis to increase our understanding of the metabolic causes of increased plasma triglycerides. Unlike our earlier models in which the liver appeared to secrete into the circulation a single species of VLDL which then underwent a series of catabolic delipidation steps to yield a single remnant, the current experiments reveal the input of several species of VLDL and the formation of multiple remnants. Since the metabolic fates of these appear to be distinct, it is likely that there will need to be reclassification of the causes of high plasma

triglycerides into several types according to the predominant disturbance of this complex system.

### High density lipoprotein metabolism in the rat

J. Cohn, P. Nestel, N. Fidge

HDL are the major cholesterol transporting lipoproteins in the rat. The major protein, apo $A_1$ is involved in several functions of cholesterol transport: promoting the movement of cholesterol out of cells, esterifying and "stabilising" cholesterol in the circulation and transferring cholesterol to specific organs.

We have studied HDL apo $A_1$ metabolism by several in vivo and in vitro techniques:— measurement of turnover of HDL by 16 hr constant infusions, estimating the initial in vivo binding of HDL after reinjection and quantifying the in vitro binding to isolated hepatocytes. The liver, adrenals and kidneys were the only tissues to recognise and bind, by a high affinity process, in vivo and in vitro, HDL $A_1$ apoprotein. Quantitatively the liver was predominant, probably reflecting the transfer of cholesterol from HDL for excretion. The adrenal which showed the highest affinity for HDL $A_1$, derives sterol for steroid production by this route. The kidney appears to be an important catabolic site for apo $A_1$.

Important physiological differences were also found:— females catabolized less HDL $A_1$ than males, and fasting increased the removal of HDL $A_1$. Aging exerted no effect. The in vivo experiments have correlated well with the findings of in vitro binding.

Similar studies are being carried out with another prominent HDL apolipoprotein, apo $A_4$, which may clarify the as yet unknown function of this protein.

### Lipoprotein metabolism in macrophages

P. Nestel, T. Billington, J. Bazelmans

Macrophages are found in the arterial intima at the very beginning of cholesterol-induced atheroma and constitute the initial foam or fat-laden cell. We have found that macrophages have the capacity to take up and metabolize large amounts of human triglyceride-rich lipoproteins especially those derived during chylomicron catabolism. Furthermore, the receptor involved in the initial binding of these lipoproteins was found to be the *B*-VLDL receptor which is not tightly regulated, thus allowing substantial influx of lipid.

Cultures of human monocytes are now being prepared and tested with a view to a large population study to determine the variability among people in the capacity of their monocytes and macrophages to accumulate lipid.

### Lipoprotein metabolism in smooth muscle cells

M. Reardon, J. Campbell, P. Nestel, P. Nugent, L. Popadynec and E. Monger

We have studied the metabolism of different rabbit lipoproteins by the various phenotypes of rabbit smooth muscle cells. Of greatest relevance to the development of atherosclerosis was the finding that substantial cellular lipid accumulation occurred with only one phenotype, the irreversibly synthetic smooth muscle cell. Furthermore this occurred only when this cell type was incubated with *B*-VLDL, the characteristic lipoprotein of the cholesterol fed rabbit. The nature of the binding interaction was defined by Scatchard plots and competition studies with other lipoproteins. This showed that the *B*-VLDL interacted at the LDL or B/E receptor through its high content of apo E. The binding with *B*-VLDL showed a much higher capacity than for normal VLDL or LDL and the binding capacity of the irreversibly synthetic cell was much higher than shown by the reversibly synthetic or contractile state cells.

### Lipoprotein metabolism with hepatocytes

M. Reardon, E. Faehse, P. Nestel, E. Monger

Our first studies were initially carried out with rat hepatocytes. More recently a strain of human hepatoma cell has been established, which shows most of the characteristics of a normal liver cell. This will permit studies of human cells with human lipoproteins. Current studies are examining the mechanism of uptake of chylomicron remnants

and the subsequent intracellular events. One consequence is the suppression of lipoprotein secretion, as measured by the rates of synthesis of apolipoprotein B and triglyceride. This may explain the in vivo observation of paradoxically low plasma triglyceride levels the day after a very fatty meal.

### Plasma triglyceride levels predict coronary atherosclerosis in women

M. Reardon, P. Nestel, E. Faehse, R. Harper

Last year we were the first to document an independent correlation between the severity of coronary atherosclerosis in women and the triglyceride concentration in plasma and in VLDL, IDL and LDL. By contrast, disease severity in men was related independently to LDL cholesterol.

Since substantial numbers of women were receiving thiazide diuretics for hypertension and/or beta adrenergic blocking drugs for hypertension or angina, further studies were carried out to examine possible confounding effects. Both drugs influence lipoprotein and lipid concentrations. We also re-examined the effects of alcohol consumption and of diabetes mellitus. Exclusion of these variables supported the original conclusion. Indeed, the correlation between disease severity and IDL (the principal remnant of triglyceride catabolism) was strengthened.

### Cholesterol efflux from cells

P. Nestel, B. Meyer, T. Billington, E. Monger

Although HDL is a major acceptor of cellular cholesterol and its major apolipoprotein, (apo A1) promotes this efflux, other plasma constituents may be equally effective. We are carrying out a systematic study using both naturally occurring plasma fractions containing predetermined mixtures of lipids and apolipoproteins.

# Lipoprotein Structure and Function Laboratory

**Head:** Dr. N. Fidge

## Projects

### Studies on the HDL receptor
### Whole cells

#### (a) Adrenocortical cells

N. Fidge and P. Nestel

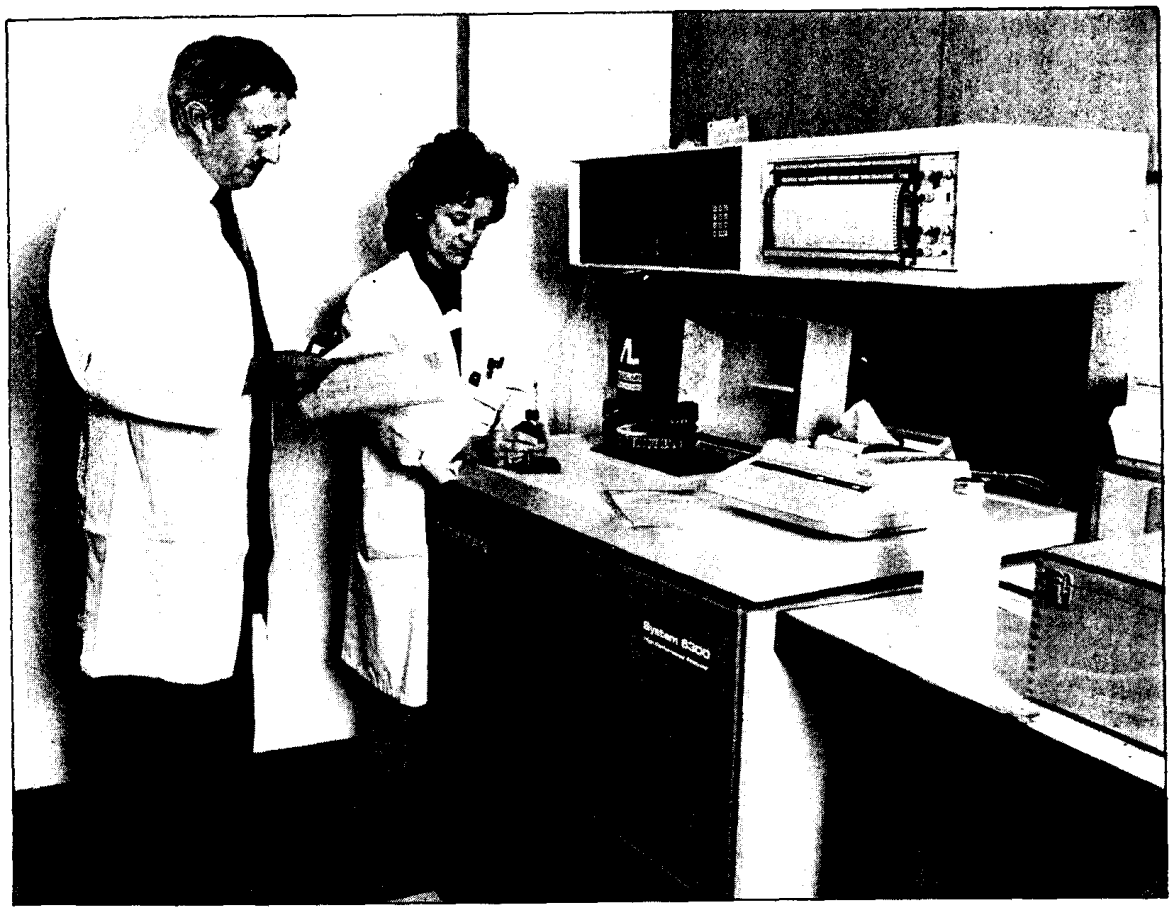
Investigations have continued with adrenocortical cells and intestinal mucosal cells in order to characterise the ligand-receptor interaction in more detail.

Previously we showed that HDL interacted specifically with adrenocortical cells and that this interaction could be regulated by the hormone ACTH. We now wished to determine if specific HDL apoproteins are recognised by the putative receptor. Using specific antibodies, we found that immunosuppression of binding occurred only when Fab fragments against AI or AII were added to incubations, suggesting that those peptides were recognised by the receptor(s). In further experiments, we found that both apo AI and apo AII, when recombined with phospholipid, bound with saturable kinetics to cells whereas another HDL component, apo CIII did not demonstrate specific or saturable binding to adrenocortical cells. Further studies are being carried out to investigate which regions of AI and AII are recognised by the receptor.

#### (b) Intestinal mucosal cells

A. Kagami, N. Fidge and P. Nestel

HDL also binds to a receptor on rat mucosal cells and we investigated the possibility that the binding may be associated with cholesterol metabolism in the intestinal cell. Experiments were performed to relate the specific binding of $^{125}$I-labelled $HDL_3$ to parameters of cholesterol metabolism in mucosal cells obtained from rats which had been treated to alter cholesterol metabolism. Although cholesterol synthesis could be increased up to 5-fold by treating rats with cholestyramine



*Dr. Noel Fidge and Ms. Margaret O'Connor analysing the amino-acid composition of A IV apolipoprotein.*

or surfomer (inhibitor of absorption) or by biliary diversion, the capacity of mucosal cells to bind and degrade $^{125}$I-labelled HDL remained unchanged. The data therefore suggest that HDL binding and sterol synthesis are not related and the interaction of HDL with the intestine may be associated with other metabolic events. However, since the cellular cholesterol content remained unchanged due to compensatory changes in synthesis, we have not ruled out a role for HDL in cholesterol homeostasis in intestinal cells. In fibroblasts cholesterol loading results in increased HDL binding which promotes cholesterol efflux.

### Isolation of the HDL Receptor

N. Fidge, A. Kagami and P. Nestel

We have found that HDL binds to the plasma membrane of mucosal cells with parameters that compare well with binding to the whole cell. Similar binding parameters were also demonstrated using membrane preparations of rat and sheep adrenocortical cells.

The isolation of the HDL receptor has been attempted using sheep adrenocortical membranes which had been solubilised with Triton X-100 or octyl = *B*-D-glucopyranose; approximately 65% of the total binding activity resided in the soluble fraction. This was further fractionated by affinity chromatography on columns packed with HDL coupled to CNBr activated sepharose. After removal of unbound material, a fraction that bound to HDL was obtained; this contained one major protein with a $M_r$ of approximately 50,000. This material was desorbed with 6M guanidine-HCl and may therefore have been altered during chromatography because the specific activity of HDL binding was not higher than with the unbound fraction.

To further identify the membrane fraction which bound to HDL, the solubilised

membrane was first separated by electrophoresis on SDS-polyacrylamide gels (10%) and then transferred to nitrocellulose by the Western blotting technique. When this was incubated with HDL, followed by an antibody preparation to AI and amplification with biotinstreptavidin coupled to HRP, an interaction was demonstrated between HDL and a membrane component of approximately 75,000. This again suggested that a specific protein exists which recognises and binds $HDL_3$. The same binding protein has now been found in kidney cortex membrane fractions and rat liver with kidney showing most intense binding reaction. This is consistent with in vivo parameters of HDL removal in the rat.

### AIV Apolipoprotein metabolism

T. Ohta, N. Fidge and P. Nestel

We have continued to investigate the physiological function of this unusual peptide which is a major component of lymph chylomicrons, but which in human plasma remains largely unassociated with the major lipoproteins. Recently we tentatively described up to eleven isoforms of AIV apolipoprotein, which were present (in minor amounts) in all lipoproteins as well as in the lipoprotein-deficient fraction. We have now confirmed their identity using specific AIV antibodies following immunoblotting techniques. We are studying the transfer of apo-AIV between plasma lipoproteins in relation to physiological events.

### Monoclonal antibodies for apolipoprotein studies

N. Fidge, M. Leonard-Kanevsky and J. Hayes

Monoclonal antibodies (McAb) have successfully been produced for human B apolipoprotein after immunising mice with apo B48. Approximately 12 different IgG have been purified from ascites fluid and partly characterised.

Epitope maps are being constructed following studies on LDL binding to fibroblasts. In these experiments, the ability of each McAb to suppress binding of $^{125}$I-labelled LDL to the cultured cells is measured; those that cause most inhibition are assumed to interact with those sites (epitopes) involved in receptor recognition.

In addition, McAbs to human apoAI have been produced and McAbs to AII apoprotein are also being developed. These antibodies will be useful in determining which regions of the peptides are recognised by the HDL receptor and if the sequences are shared in common by apo AI and AII.

# Cholesterol Metabolism Laboratory

**Head:** Dr. S. Turley

## Projects

Developmental changes in whole body cholesterol production and plasma cholesterol levels in the obese rat.

Regulation of hepatic cholesterol production in the obese rat.

## Summary

Obese individuals are more prone to develop cardiovascular disease partly because they frequently manifest elevated plasma cholesterol and triglyceride levels. The exact cause of the hypercholesterolaemia associated with obesity is not known. On an ideal body weight basis, whole body cholesterol production is much greater in obese subjects than in normal individuals. However, it is not known which particular tissues synthesise excess cholesterol or whether such overproduction has any role in raising blood cholesterol levels in these subjects.

The SHR/N-cp rat spontaneously develops obesity, hyperlipidaemia and diabetes and is an ideal experimental model to obtain more specific information about the changes in cholesterol metabolism that occur in obesity. These studies have focused on measuring plasma cholesterol levels and the rate of cholesterol production in each of the tissues and in the whole animal in obese and lean rats at different stages of development. The results of this work suggest that the increase in plasma cholesterol

levels found with obesity may not be related to the rate of cholesterol production by the tissues but may instead result from an impaired ability of the tissues to clear lipoproteins from the plasma.

### Developmental changes in whole body cholesterol production and plasma cholesterol levels in the obese rat

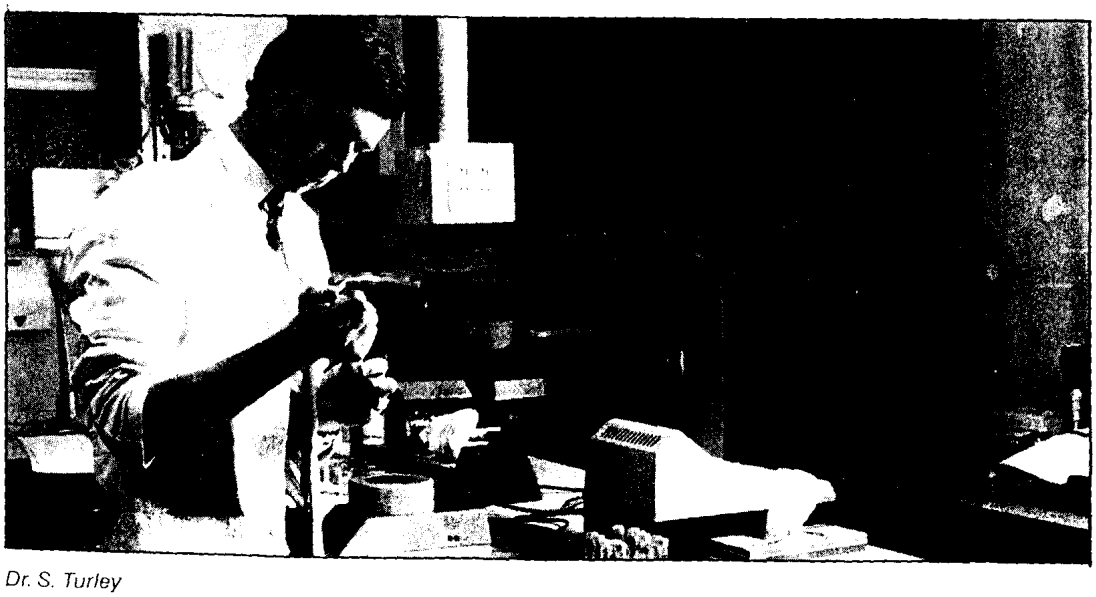
S. Turley

At an early age whole body cholesterol production in obese rats is greater than in their lean littermates. However, as the corpulent animals become older and more obese, cholesterol production by the liver falls dramatically. Although the obese animals produce more cholesterol in adipose tissue than lean animals, this does not fully compensate for the decline in cholesterol production by the liver. Hence whole body cholesterol production in the older obese animals falls to a rate that is similar to that found in age-matched lean animals. Despite the decline in whole body cholesterol production, the degree of hypercholesterolaemia increases as the rats become older and more obese. These findings suggest that the increase in plasma cholesterol levels is not related to the rate of cholesterol production by the tissues. It may instead result from an impaired tissue clearance of lipoproteins from the plasma. This could arise if one of the receptor-mediated processes involved in lipoprotein uptake was either absent or defective in the obese animals.

### Regulation of hepatic cholesterol production in the obese rat

S. Turley

In obese rats maintained on a normal chow diet the rate of cholesterol production in the liver is lower than in age-matched lean animals. The degree of suppression of hepatic cholesterol production becomes greater as the animals become older and more obese and is more pronounced in males than in females. In normal rat strains hepatic cholesterol production can be suppressed if the level of esterified cholesterol in the liver is greatly increased by feeding the animals a high cholesterol diet. However in this strain of obese rat the suppression occurs spontaneously and without a dramatic change in the level of esterified cholesterol in the liver. In addition, when obese rats are fed a diet containing sufomer (a newly developed drug that blocks cholesterol absorption and hence the delivery of cholesterol to the liver) the rate of hepatic cholesterol production remains low. These findings suggest that the reduced production of cholesterol by the liver and the elevation in plasma cholesterol levels in this model are not due to the absorption of excess dietary cholesterol from the intestine.



*Dr. S. Turley*

# Cardiovascular Surgical Research Unit

**Unit Director:**
**Professor J. Ludbrook**

## Vascular Laboratory

**Head:** Professor J. Ludbrook

Reflex effects of arterial baroreceptor and cardiac receptor input during exercise.

Cardiovascular effects of instantaneous arterial baroreceptor denervation in the conscious rabbit.

Studies to confirm that the reflex effects of arterial baroreceptor input are suppressed at the onset of exercise.

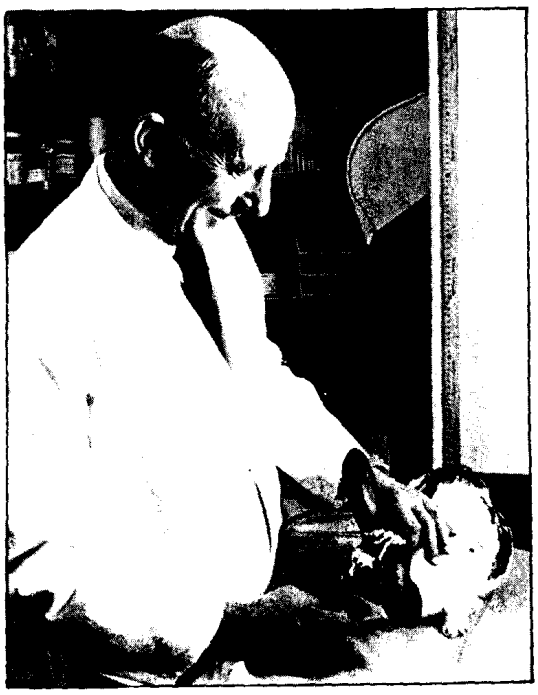
Reflex effects of arterial baroreceptor input during spontaneous behaviour.

Why does naloxone restore blood pressure in hypovolaemic hypotension?

### Summary

Our work is in the field of circulatory regulation relevant to surgery or anaesthesia. In addition, we are working on the neural mechanisms contributing to circulatory regulation in exercise and in other everyday activities. We have found that one of the important systems that has been regarded as of major importance in maintaining the status quo of the circulation, appears to do just the opposite in some circumstances! The arterial baroreceptors, which sense the pressure in some of the larger arteries, usually act through the central nervous system to maintain blood pressure at a normal level. Work in Paul Korner's and Pat Dorward's laboratories, as well as overseas, has indicated that the baroreceptor reflexes do not invariably play this role. We have found in rabbits that at the onset of exercise the way in which these reflexes work is drastically altered, so that rather than maintaining blood pressure constant they actually drive it upwards. Thus the way in which the brain processes the signals that it receives from the baroreceptors is dramatically altered, by the act of will to do exercise and by signals from the exercising muscles. We found that the reflex properties become altered with different forms of behaviour, such as grooming or eating. Here too the normal influence of the baroreceptor reflex is transiently suppressed and the reflex now appears to drive the blood pressure upwards. It seems likely that many of the variations in blood pressure that take place in man during everday activity are a direct result of this type of modulation of the baroreceptor reflex. Probably complex disturbances that attend acute blood loss in man, including emotional factors also affect the capacity of the baroreceptor reflex to sustain blood pressure.

We have just commenced to study some interesting mechanisms that come into play during blood loss. When blood loss is profound there is a failure of blood vessels to constrict and thus maintain blood pressure leading to "shock". Recent reports suggest that the drug naloxone, an antagonist to morphine, reverses this effect. These two



*Professor John Ludbrook*

observations are of great importance and may lead to better understanding and treatment of states of shock in man. We are currently investigating the cause(s) of the failure of blood vessel constriction, and the mode of action of naloxone and the possible sites in the brain and peripheral circulation.

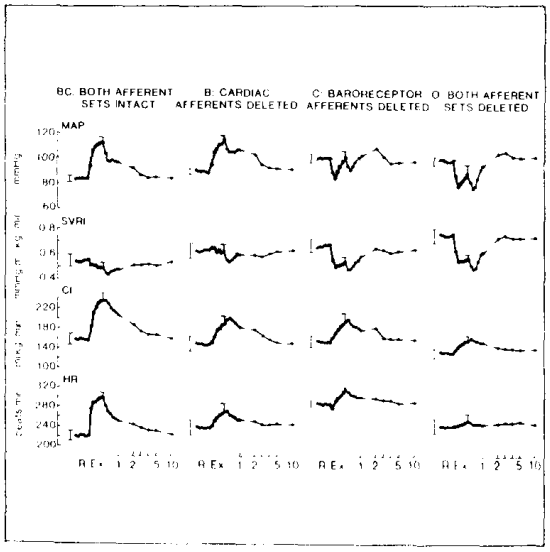
## Projects

### Reflex effects of arterial baroreceptor and cardiac receptor input during exercise

J. Ludbrook.

Six rabbits were exercised on a moving belt at 13 m/min for 60 s. Heart rate (HR), mean arterial pressure (MAP), cardiac index (CI) and systemic vascular resistance index (SVRI) were measured. Exercise was done with all four possible permutations of input from the arterial baroreceptors (B) and cardiac receptors (C), i.e. BC, both inputs present; B, only baroreceptor input (intrapericardial procaine); C, only cardiac receptor input (surgical barodenervation); O, both inputs deleted. The reflex effects on SVRI of the two inputs were calculated as (B — O) and (C — O), and their interaction as [BC — O] — [(B — O] + (C — O)]. The effects of baroreceptor input plus interaction on all cardiovascular variables were also calculated, as (BC — C). At rest, (B — O) and (C — O) each tonically depressed SVRI, without interacting, and (BC — C) tonically depressed SVRI, MAP and HR. Within 10 s of commencing exercise these tonic effects were abolished, though a small, SVRI-lowering, interaction appeared. Suppression of the tonic reflex effects of arterial baroreceptor and cardiac receptor input supported systemic vascular resistance at the onset of exercise and contributed to the rise of arterial pressure.

*Fig. 1. The effects of altering input to the control nervous system from arterial baroreceptors on the cardiovascular responses to exercise. Note particularly the dramatic effect of deleting baroreceptor afferents.*

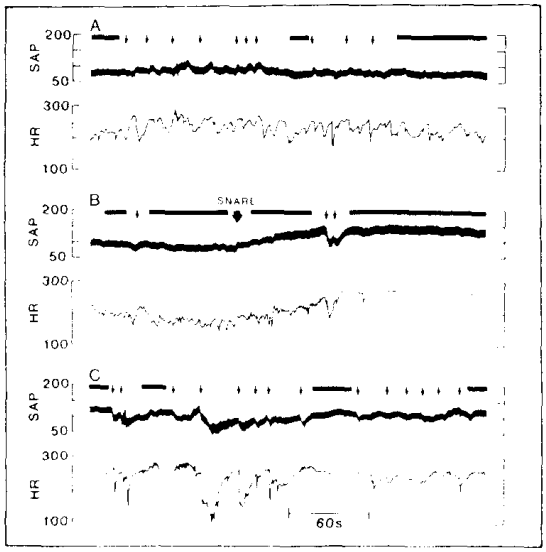



### Cardiovascular effects of instantaneous arterial baroreceptor denervation in the conscious rabbit

J. Ludbrook, S. Potocnik

A snare was used to denervate the carotid sinus in conscious rabbits whose other arterial baroreceptors had been denervated 8-14 days previously by surgical operation. Within 2 min of tightening the snare arterial blood pressure had risen from 81 ± 2 to 119 ± 5 mmHg, and heart rate

*Fig. 2. Effects of deleting baroreceptor input on behavioural modifications of blood pressure and heart rate in a conscious rabbit. -, 'basal' conditions when rabbit spontaneously quiet or being petted., spontaneous activities such as exploring box, standing or grooming. Symbols and units as in A, 20 min. prior to tightening snare on sinus nerve. Note that activity elevates SAP and HR above 'basal' levels. B, period during which snare was tightened. C, 20 min. after tightening snare. Note that activity now depresses SAP and HR below 'basal' levels.*



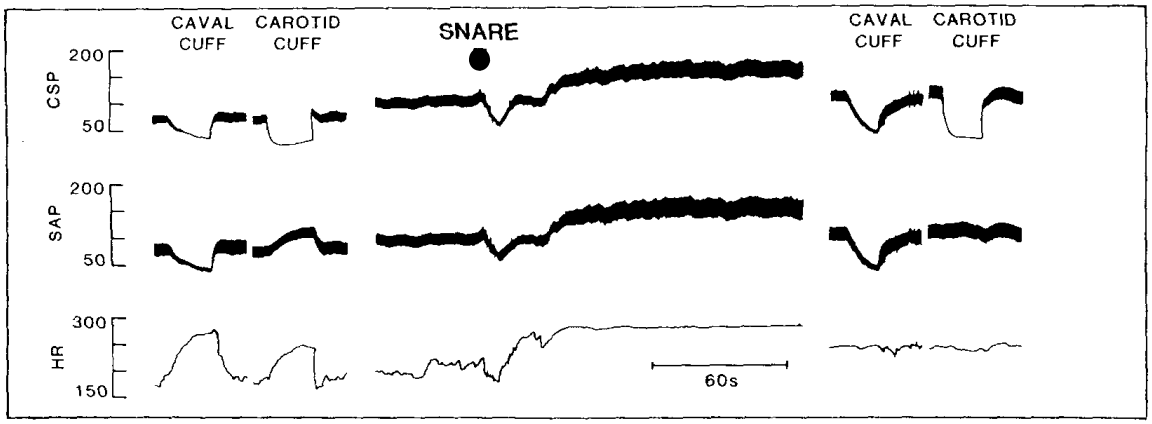



*Fig. 2. Immediate effects of deleting the sole source of arterial baroreceptor input in a conscious rabbit, by tightening a snare on the carotid sinus nerve. CSP, SAP: carotid sinus, and systemic arterial, pressures (mm Hg); HR: heart rate (beats/min). Note that reflex responses (left) to inflating caval or common carotid cuffs are abolished (right). Note also that when the snare is tightened there is a transient bradycardia and hypotension, presumably due to sinus nerve discharge.*

had risen from 214 ± 9 to 280 ± 9 beats $min^{-1}$. They remained at these high levels for 15 min but this was not sustained and after 30 min blood pressure and heart rate were 103 ± 9 mmHg and 265 ± 8 beats $min^{-1}$ respectively. They declined towards control at a somewhat irregular rate over the succeeding 7 days. The previously brisk baroreceptor-heart rate and carotid sinus reflexes were completely abolished by ensnaring the carotid sinus nerve, and remained absent over the succeeding 7 days. The snare method is not confounded by general anaesthesia or surgical injury, and allows observations to be made within minutes after removing this input. The rapid decline in blood pressure and heart rate after 15 min, and the subsequent slow downward trend, are unexplained and may be important. In intact rabbits spontaneous activity in their cage was usually associated with transient rises of blood pressure and heart rate, but after removing the baroreceptors the same activity resulted in transient falls in these variables.

## Studies to confirm that the reflex effects of arterial baroreceptor input are suppressed at the onset of exercise

J. Ludbrook

We wished to confirm that tonic reflex depressor effects of input to the central nervous system from arterial baroreceptors is abolished at the onset of treadmill exercise, thus contributing to the rise of systemic arterial pressure (SAP) and heart rate (HR). Rabbits were instrumented with an inflatable cuff around one common carotid artery, after surgical denervation of the remaining baroreceptors. Transient inflation of the cuff caused reflex rises of SAP and HR, which were much reduced during the first minute

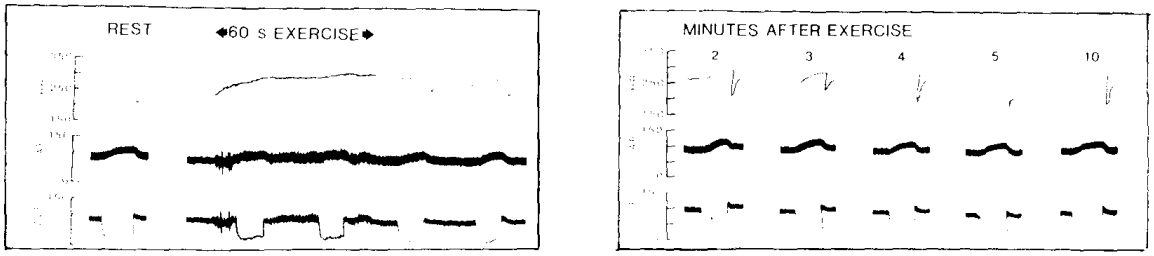



*Fig. 3. Effects of unloading the arterial baroreceptors during exercise on heart rate (HR, beats/min) and blood pressure (SAP, mm Hg). CSP: carotid sinus pressure above an inflatable cuff. All baroreceptors except those in one carotid sinus denervated. Note that the reflex response of HR is nearly abolished, and that of SAP much reduced, during 60 s treadmill exercise.*

of exercise. Deflation of the cuff caused a brisk fall of HR, which was completely abolished by exercise. A snare was placed around one carotid sinus nerve, after the remaining arterial baroreceptors had been surgically denervated. When the snare was tightened all arterial baroreceptor reflexes were immediately and permanently abolished. The difference provided a measure of the previous role of the baroreceptor input. The magnitude of the calculated effects, at rest and during exercise, diminished according to how long after barodenervation the observations were made.

We conclude from the above experiments that the resting tonic reflex depression of SAP and HR caused by baroreceptor input is much reduced at the onset of exercise but not completely abolished. In addition, from the effects of partial surgical barodenervation and of unloading the carotid baroreceptors prior to exercise that resting input from the arterial baroreceptors must be near-zero before the cardiovascular response to exercise is qualitatively altered.

## Reflex effects of arterial baroreceptor input during spontaneous behaviour

S. Potocnik, J. Ludbrook

At the onset of treadmill exercise in the rabbit the reflex effects of arterial baroreceptor input to the central nervous system are suppressed. We have tested the hypothesis that this also occurs with minor levels of spontaneous everyday activity and related behaviour patterns.

Six rabbits were observed for 2 hours under each of 3 conditions: $B_4$, all sets of baroreceptor afferents intact; $B_1$, afferents from only one carotid sinus intact; $B_0$, all baroreceptor afferents deleted. We obtained consecutive 10s mean values of blood pressure (BP) and heart rate (HR) during different types of spontaneous behaviour such as standing or lying, exploring their enclosure, grooming, and eating. There were interposed periods of inactivity during which BP and HR were defined as basal. The results were analysed as the proportion of time during activity that BP or HR were above or below the basal levels.

In condition $B_4$, spontaneous activity caused transient rises of BP and HR, so that they were above "basal" levels 82% of the time. In condition $B_1$, BP and HR were above "basal" levels 64% and 76% of the time. In condition $B_0$, spontaneous activity caused transient falls of BP and HR, so that they were above basal levels only 21% and 28% of the time. Supplementary experiments showed that in condition $B_0$ the transient falls of BP were not prevented by blockade of cardiac afferents or efferents.

We conclude that the tonic reflex effects of baroreceptor input are centrally suppressed during this type of spontaneous behaviour, and that this contributes to the concomitant rises of BP and HR. If a similar phenomenon occurs in man it would explain the direct, rather than inverse, relation between BP and HR during long-term recordings.

## Why does naloxone restore blood pressure in hypovolaemic hypotension?

P. Rutter, J. Ludbrook

Preliminary studies have been made in conscious rabbits subjected to haemorrhage over 5 min to a level of mean systemic arterial pressure (MSAP) of 40 mmHg.

*Mr. Peter Rutter and Simon Potocnik measuring the response to naloxone after haemorrhage.*

These have confirmed that intravenous naloxone, 5 mg/kg, raises MSAP by 30-40 mmHg, and lowers heart rate (HR). The effect is similar whether naloxone is given 5 min or 30 min after the haemorrhage. The effect is not diminished by prior surgical sinoaortic barodenervation, nor by cardiac nerve blockade with intrapericardial procaine (IPP), so that it is not mediated via baroreceptor or cardiac receptor afferents. Fixation of HR by IPP did not diminish the effect, so that it is likely to be caused by increase in peripheral resistance. Studies are in progress to determine whether the pressor effect is mediated by sympathetic vasoconstrictor nerves, and hence is central in origin.

# Cardiac Surgery Laboratory

**Head:** Frank Rosenfeldt

## Projects

Response of the hypertrophic heart to hypothermic cardioplegia

Optimal reperfusion pressure after hypothermic cardioplegia

Long term results of cardiac surgery

Microprocessor control of drug infusion after cardiac surgery

## Summary

We have continued our search for new ways of preventing unwanted damage to the heart muscle during cardiac surgery, and of improving recovery of the patient afterwards.

Some of the initial work in this field has been carried out on the normal heart of the experimental animals. However, the threshold for damage in patients whose heart muscle has been altered by disease may be less than that of the normal heart. The diseased heart may have thickening of the muscles (hypertrophy), or scarring due to coronary artery disease. We have therefore examined the optimum operating conditions in animals with experimentally created states of heart disease. Various surgical techniques are then applied to the diseased hearts, and the response compared to the effects of similar procedures in dogs with normal hearts. This knowledge is then applied for strategies for optimum operating in cardiac surgical patients and for critically evaluating the results.

In the early days of cardiac surgery, operative results were evaluated mainly in terms of the technical procedure being completed successfully, the patient surviving the operation, and then leaving hospital. However, we are now recognising that technical success and hospital survival are not sufficiently discriminatory to detect small improvements or shortcomings in surgical techniques. We now place increasing reliance for our quality control on the late results of surgery, such as five to ten year survival, late recurrence of symptoms and continuing ability to work and take part in physical recreations. Over the last four years in the Cardiac Surgery Unit of the Alfred Hospital there has been a painstaking effort to collect data on the long term results of cardiac operations. In the Cardiac Surgery Unit's microcomputer we have information on 7000 patients who have had heart surgery over the last twenty years. During the last year we have begun to analyse the late results of coronary bypass surgery with the assistance of mainframe computers at Monash University.

All too often have we heard it said that in Australia we make great advances in basic research but fall down when it comes to applying the results, especially when this application involves collaboration with commerce or industry. In recent years the Cardiac Surgery Laboratory in collaboration with the Royal Melbourne Institute of Technology has developed a special microprocessor unit to regulate delivery of drugs to control blood pressure after heart surgery. In addition, we are working with a Melbourne electronics firm, Newtronics Limited, to assess the commercial potential of this device. In 1984 we secured a $10,000 grant from the Victorian Government Department of Industry, Commerce and Technology to to build two commercial prototypes for further clinical testing.

*(l-r) Ms. J. Boardman, Ms. C. Boyes, Dr. Chen Xiao Zhong, Dr. F. Rosenfeldt engaged in open heart surgical procedures.*

## Projects

### Response of hypertrophied heart to hypothermic cardioplegia

F.D. Rosenfeldt, M. Rabinov

We wished to answer the following questions:— Firstly, is there a biochemical difference in the pre-arrest state of the hypertrophic ventricle? Secondly, is there a different biochemical response to hypothermic cardioplegia? Thirdly, can any differences between the response of hypertrophied and normal hearts be compensated for by cooling? Subcoronary stenosis of the aortic valve was created in dogs by oversewing the non-coronary cusp of the aortic valve in 6 week old pups. Six animals were studied 6-9 months later, when they had developed marked left ventricular hypertrophy. Each heart was arrested by infusion of cold cardioplegic solution. The heart was then excised and the left ventricle was divided into four segments, each of which was stored for 6 hours at one of the following temperatures 4°C, 12°C, 20°C and 37°C. Biopsy samples were taken before and immediately after arrest and then at 2 hourly intervals for 6 hours. Biopsies were assayed for levels of high energy phosphates and lactate. For comparison, the same study was carried out on six normal dog hearts. The results showed no difference between the hypertrophic hearts and the normals in baseline ATP, creatine phosphate and lactate. The rate of rise of lactate in the hypertrophied hearts during hypothermic cardioplegic arrest was greater than in the normals. The preliminary results also showed a tendency for ATP and creatine phosphate to fall at a greater rate than normal. Cooling to 4-12°C caused a marked attenuation in the response of ATP, creatine phosphate and lactate to cardioplegia in all hearts, and an abolition of differences in response between hypertrophied and normal hearts.

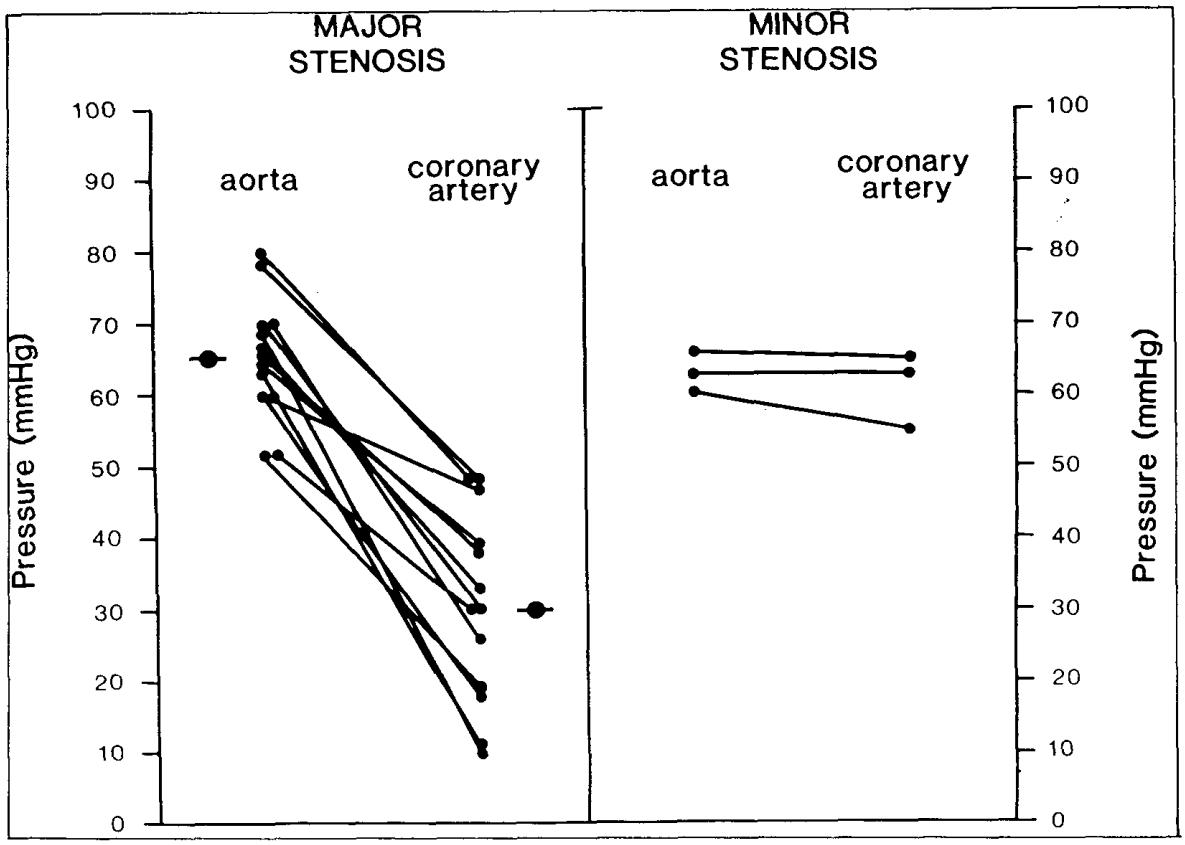



*Fig. 1. Simultaneously measured mean pressure during reperfusion in the aorta and in coronary arteries distal to a stenosis, in 10 patients undergoing coronary bypass grafting. The small dots represent the individual values and the large dots the means. Major stenosis: 70-100% diameter narrowing; minor stenosis: less than 70% diameter narrowing.*

## Measurement of Coronary Pressure During Reperfusion in Patients

F.D. Rosenfeldt

In a previous project we studied the reperfusion phase following hypothermic cardioplegic arrest and determined the coronary pressure range which was optimal for myocardial recovery. We found that the lower cutoff point lay between 20 and 40 mmHg. Coronary pressures below this point during reperfusion delayed myocardial recovery. This study was performed in dogs with normal coronary arteries. However, in patients with coronary artery disease, during reperfusion the myocardium is perfused via arteries with severe stenoses or via small diameter collaterals. Thus the only pressure reading available to the surgeon comes from the aorta proximal to these high resistance pathways and this may differ greatly from the pressure distally. If the resistance in the coronary arteriolar bed is low compared to the stenosis resistance, which is usually the case during reperfusion, the best estimate of coronary reperfusion pressure is the pressure measured just beyond the stenosis.

The pressure distal to coronary stenoses is difficult to measure and hence is usually ignored in clinical practice. In patients undergoing coronary artery bypass grafting, we measured the pressure beyond the stenosis during reperfusion by passing a fine catheter down the vein graft before it was anastomosed to the aorta.

In 13 arteries with major stenoses, distal mean coronary pressure averaged 31 mmHg while the simultaneously measured mean aortic or radial artery pressure averaged 66 mmHg. Thus the average gradient across the stenosis was 35 mmHg (range 15-60 mmHg). We concluded that during reperfusion in patients with severe coronary artery disease, mean coronary pressure should be kept in the range 80-100 mmHg.

# Cell Biology Laboratory

**Head:** Dr. J. Campbell

## Projects

Influence of endothelial cells on smooth muscle lipoprotein metabolism.

The extracellular matrix and atherosclerosis.

Macrophage influences on smooth muscle phenotype and proliferation.

Smooth muscle polyploidy and hypertension.

Morphometric analysis of the cells of human diffuse intimal thickenings.

## Summary

The cells of the artery wall are in functional equilibrium, with any alteration in one group influencing others, resulting in either normal repair mechanisms or producing pathological processes. For example, in the normal blood vessel under normal steady-state conditions the endothelial cells form a continuous single cell layer overlying smooth muscle. However, if the endothelium is damaged medial smooth muscle cells migrate into the intima and proliferate causing myointimal thickening. The smooth muscle ceases to proliferate when re-endothelialization occurs. While contact with substances from the blood is important in this process, recent research by ourselves and others has shown that endothelial cells themselves play a role in the regulation of smooth muscle proliferation. We have found that undamaged layers of endothelial cells maintain smooth muscle in the contractile phenotypic state where it is incapable of proliferation, and that they inhibit synthetic state cells from proliferating in response to blood factors. Proliferating (regenerating) endothelial cells stimulate the growth of synthetic state smooth muscle as do monocyte-derived macrophages which have migrated from the blood through an intact endothelium.

Since proliferation of smooth muscle is a characteristic feature of atherosclerosis, the study of how cells in the artery wall interact is important for understanding the causation of this disorder. We have shown that the endothelium not only influences smooth muscle proliferation, but has a profound influence on the metabolism of atherogenic lipoproteins by the smooth muscle cells. We found that monocyte-derived macrophages control the biology of smooth muscle cells, and that as well as producing a growth stimulator for smooth muscle cells they can under certain conditions produce a growth inhibitor.

We have examined the type and amount of extracellular matrix components (collagen and glycosaminoglycans) that is produced by smooth muscle cells of different biological states in relation to the development of atherosclerosis. In addition, we have studied the smooth muscle phenotypes associated with diffuse intimal thickenings (which are sites of predilection for atherosclerosis) of human carotid arteries, and whether change in ploidy (multiples of normal chromosome numbers) of smooth muscle cells plays a role in aetiology of hypertension.

## Projects

### Influence of endothelial cells on smooth muscle lipoprotein metabolism

J.H. Campbell, L. Popadynec

Using cell culture procedures we previously determined the manner in which smooth muscle cells of different biological states metabolize lipoproteins in the absence of endothelium. The cells interacted more avidly with lipoproteins of the triglyceride-rich spectrum (*B*-VLDL from cholesterol fed rabbits and normal VLDL) than of the cholesterol-rich spectrum (LDL). Smooth muscle phenotype markedly influenced the degree of cellular interaction with lipoproteins. This was reflected in the lipid synthesis and accumulation and in the maximum binding capacities of the lipoproteins and rates of degradation which were considerably greater for cells in the irrevers-

*Dr. Julie Campbell (right) and Ms. N. Puglisi measuring the amount of DNA in cells by measuring fluorescence intensity after staining.*

ible synthetic state than in the reversible synthetic state which in turn were greater than with cells in the contractile state.

We then carried out a series of studies to determine whether the presence of endothelial cells influenced the metabolism of *B*-VLDL by irreversible synthetic state cells. For these studies we grew a feeder layer of either confluent, quiescent (equivalent to normal artery) or proliferating (regenerating) endothelial cells on one coverslip of a modified Rose chamber. Irreversible synthetic state cells were grown on the opposite coverslip for 3 days co-culture. The cells were then incubated in increasing concentrations of $I^{125}$-labelled *B*-VLDL for 3 hours at 37°C to determine the amount of lipoprotein cell-associated (bound + internalized), or for 24 hours in 75 *u*g/ml unlabelled *B*-VLDL to determine cholesterol/cholesteryl ester accumulation by gas chromatography and Fettrot staining for cellular lipids. It was found that smooth muscle cells grown in the presence of endothelium (even when the endothelium was removed during the actual incubation with lipoprotein), had a significantly higher lipid accumulation than smooth muscle cells grown alone. This was true for both confluent quiescent and proliferating endothelial cell feeder layers. In all cases, the endothelium itself bound a considerable amount of the lipoprotein, but lipid was not accumulated.

These studies suggest that the lipid accumulation by smooth muscle cells of atherosclerotic plaques is a result of (1) the presence of an atherogenic lipoprotein (*B*-VLDL) in the blood; (2) the presence of smooth muscle cells in the irreversible synthetic state, and is exacerbated if the endothelium is intact or is regenerating. This latter finding is consistent with reports that lipid preferentially accumulates in re-endothelialized as compared with de-endothelialized areas of a lesion.

### Smooth muscle phenotypic expression in human carotid arteries

P.J.L. Mosse, G.R. Campbell, Z.L. Wang and J.H.Campbell

In man, certain arteries have a diffuse intimal thickening (DIT) which forms over the first two or three decades of life from smooth muscle cells which have migrated from the media and proliferated. DIT's occur in those arteries which are particularly susceptible to atherosclerotic plaque formation, such as coronary and carotid arteries.

We carried out ultrastructural morphometry of the cells of DIT's from human carotid arteries obtained during surgery and found that 87% were of smooth muscle origin and that many of these cells expressed the synthetic phenotype. Overall there was a 100% increase in the amount of smooth muscle cell cytoplasm occupied by synthetic organelles compared with cells of the media. Synthetic state cells in culture

are capable of proliferation, synthesis of extra-cellular matrix and are susceptible to lipid accumulation. Thus the altered phenotypic expression by smooth muscle cells in DIT's may explain the predilection of these sites for atherosclerosis.

## Does polyploidy occur in vascular smooth muscle in hypertension?

M.J. Black, J.H. Campbell, and I. Barr

Increase in ploidy (increase in the number of chromosomes from 2N to 4N) occurs in smooth muscle cells of large vessels during hypertension. However, it is the small resistance vessels and not the large vessels which play a major haemodynamic role in hypertension.

In the present study, using flow cytometric DNA analysis, it was found that the ploidy of enzyme-isolated smooth muscle cells of the caudal artery of the spontaneously hypertensive rat (SHR), did not change significantly with duration of hypertension.

In contrast there was a 19% increase in the number of cells of the SHR aorta expressing 4N chromosomes during the same period.

By growing smooth muscle cells from normal (non-hypertensive) animals in culture we were able to induce an increase in ploidy by selective nutrient deprivation such that the cells could not pass effectively through the cell cycle, and also by allowing the cells to undergo multiple divisions.

The results suggest that polyploidy is not involved in the etiology of hypertension and that it probably occurs in large arteries as a result of nutrient deprivation through the thick vessel wall and by cellular aging.

## Macrophage influences on smooth muscle phenotype and proliferation

R. Rennick, Z.L. Wang and J.H. Campbell

Circulating monocytes are derived from bone marrow precursors and differentiate into macrophages when moving from the circulation into a tissue. Greatly increased numbers of monocytes adhere to and penetrate the arterial endothelium in animals fed a high cholesterol diet. This occurs within two weeks after starting the diet, well in advance of the formation of the defined smooth muscle proliferative lesion. It has long been known that monocytes/macrophages, together with smooth muscle cells, are the source of lipid-filled "foam cells" of atherosclerotic plaques. But their early appearance in the lesion raises the possibility that they may play a wider role in the etiology of this disease.

We have shown that arterial smooth muscle can exist in a range of phenotypes related to functional demand, and that before the cells become capable of proliferating they must modulate from the contractile to synthetic phenotype. To determine whether the monocyte/macrophage can influence smooth muscle phenotype, the two cell types were grown together in culture. It was found that the change in smooth muscle phenotype to the synthetic state was stimulated by macrophages and that the macrophages also influenced cell growth. Both stimulators and inhibitors of cell growth were released by the macrophages. Which one was predominant depended on the concentration of macrophages and their state of activation.

Thus monocyte/macrophages may be in part responsible for the proliferative phase of atherosclerosis as well as contributing to the deposition of fat.

# Publications

## Hypertension and Circulatory Regulation Unit

**ANDERSON WP.**
Has anything been learned from 100 years of conflict about experiments on animals? Aust Soc Lab Animal Sci. Newsletter, Nov. 1984, pp. 10-12.

**ANDERSON WP, HARRISON DT.**
Blood pressure responses to prolonged infusions of adrenaline and noradrenaline in conscious dogs. Clin Exp Hypertens A6:1469-1484, 1984.

**ANDERSON WP, SELIG SE.**
The importance of renal prostaglandins in the control of renal blood flow and renin release. Proceedings of the Second Asian Pacific Congress of Nephrology, Melbourne, ed. GJ Becker, RC Atkins, PS Kincaid-Smith, 1983, pp. 24-30.

**ANGUS JA.**
Calcium antagonists-clinical pharmacology. Aust Prescriber 7: 46-47, 1984.

**ANGUS JA, COCKS TM.**
Endothelium-derived relaxing factor (EDRF) — progress with its isolation and properties. Proc Aust Physiol Pharmacol Soc. 15: 59-65, 1984.

**ANGUS JA, COCKS TM.**
Role of endothelium in vascular responses to norepinephrine, serotonin and acetylcholine. *In:* Mechanisms of Vasodilatation III; ed. PM Vanhoutte, S Vatner. Basle, Karger, 1984. pp. 43-52.

**BARON R, JÄNIG W, McLACHLAN EM.**
On the anatomical organisation of the lumbosacral sympathetic chain and the lumbar splanchnic nerves of the cat — Langley revisited. J Auton Nerv Syst. 12: 289-300, 1985.

**BOBIK A, BADOER E, ODDIE C, HEAD G, KORNER P.**
Contribution of central and peripheral mechanisms to the antihypertensive effects of α-methyldopa in the rabbit. J Cardiovasc Pharmacol 6: 1109-1114, 1984.

**BOBIK A, CAMPBELL JH, LITTLE PJ.**
Desensitization of the alpha$_1$ adrenoceptor system in vascular smooth muscle. Biochem Pharmacol 33: 1143-1145, 1984.

**BOBIK A, LITTLE PJ.**
Role of cyclic AMP in cardiac *B*-adrenoceptor desensitization. Studies using prenalterol and inhibitors of phosphodiesterase. J Cardiovasc Pharmacol 6: 795-801, 1984.

**BROUGHTON A.**
Left ventricular function in pressure overload hypertrophy: Basal and maximal inotropic state and pumping capacity in the anaesthetized open chest dog. PhD Thesis (Monash University), 1984.

**BROUGHTON A.**
Tachyarrhythmias during acute myocardial infarction and options for their electrical management. *In:* Acute Coronary Care. eds RM Califf, GS Wagner. Boston, Martinus Nijhoff, pp 243-255, 1985.

**BROUGHTON A, GALLAGHER JJ, GERMAN LD, GUARNIERI T, TRANTHAM JL.**
Differentiation of septal from free wall accessory pathway location: Observations during bundle branch block in reciprocating tachycardia in the presence of Type I antiarrhythmic drugs. Am J Cardiol 52: 751-754, 1983.

**BROUGHTON A, GRANT AO, STARMER CF, KLINGER JK, STAMBLER BS, STRAUSS HC.**
Lipid solubility modulates pH potentiation of local anesthetic block of Vmax reactivation in guinea pig myocardium. Circ Res 55: 513-523, 1984.

**CASSELL JF, CLARK AL, McLACHLAN EM.**
Differences in transient outward currents activated by depolarization in prevertebral and paravertebral sympathetic neurones of guinea pigs. J Physiol (Lond). 357:43P, 1984.

**COCKS TM, ANGUS JA.**
Bioassay of the release of endothelium-derived relaxing factor (EDRF) from isolated endothelial cells *in vitro. In:* Vascular neuroeffector mechanisms ed. J. Bevan et al. Amsterdam, Elsevier Science Publ (Biomedical Division) 1985, pp. 131-136.

**COCKS TM, ANGUS JA.**
Endothelium-dependent modulation of blood vessel reactivity. *In:* The Peripheral Circulation; Ed. S Hunyor, J Ludbrook, J Shaw, M McGrath, New York, Elsevier, 1984 pp. 9-21.

**COCKS TM, JENKINSON DH, KOLLER K.**
Interactions between receptors that increase cytosolic calcium and cyclic AMP in guinea-pig liver cells. Br J Pharmacol 83: 281-291, 1984.

**DUSTING GJ, ANGUS JA.**
Interaction of epoprostenol ($PGI_2$) with vasoconstrictors on diameter of large coronary arteries of the dog. J Cardiovasc Pharmacol. 6:20-27, 1984.

**ESLER M.**
How can we measure sympathetic nervous system "tone" in patients? Aust NZ J Med 14: 4-5, 1984.

**ESLER M. JENNINGS G, KORNER P, BLOMBERY P, SACHARIAS N, LEONARD P.**
Measurement of total and organ-specific norepinephrine kinetics in humans. Am J Physiol 247: E21-E28, 1984.

**ESLER M, WILLETT I, LEONARD P, HASKING G, JOHNS J, LITTLE P, JENNINGS G.**
Plasma noradrenaline kinetics in humans. J. Auton Nerv Syst 11: 125-144, 1984.

**ELSER M, JENNINGS G, LEONARD P, SACHARIAS N, BURKE F, JOHNS J, BLOMBERY P.**
Contribution of individual organs to total noradrenaline release in humans. Acta Physiol Scand Suppl 527: 11-16, 1984.

**GALLAGHER JJ, GERMAN LD, BROUGHTON A, GUARNIERI T, TRAN-THAM JL.**
Variants of the pre-excitation syndromes. *In:* Frontiers of Cardiac electrophysiology. Eds MB Rosenbaum, MV Elizari. Boston, Martinus Nijhoff, 1983, pp 724-772.

**GERMAN LD, GALLAGHER JJ, BROUGHTON A, GUARNIERI T, TRAN-THAM JL.**
Effect of exercise and isoproterenol during atrial fibrillation in patients with Wolff-Parkinson-White syndrome. Am J Cardiol 51: 1203-1206, 1983.

**HIRST GDS, MCLACHLAN EM.**
Effect of barium substitution on calcium currents in rat sympathetic ganglion cells. Proc Aust Physiol Pharmacol Soc. 15: 25P, 1984.

**HIRST GD, McLACHLAN EM.**
Post-natal development of ganglia in the lower lumbar sympathetic chain of the rat. J Physiol (Lond) 349: 119-134, 1984.

**JACKMAN G, ODDIE C, SKEWS H, BOBIK A.**
Plasma catecholamines-HPLC determination during alpha-methyldopa therapy. J Chromatogr 308: 301-305, 1984.

**JÄNIG W, McLACHLAN EM.**
On the fate of sympathetic and sensory neurons projecting into a neuroma of the superficial peroneal nerve in the cat. J Comp Neurol. 225:302-311, 1984.

**KORNER PI.**
Beta-blockers, autoregulation and experimental design. Hypertension 6:140-144, 1984.

**KORNER PI.**
Role of central monoaminergic systems and of beta-adrenoceptors in cardiovascular regulation. *In:* Alpha and Beta Adrenoceptors and the Cardiovascular System, Ed W

Kobinger and RP Ahlquist, Amsterdam, Excerpta Medica, 1984, pp 72-105.

**KORNER PI, HEAD GA, BADOER E.**
Effects of sino-aortic denervation on the acute circulatory responses to centrally administered 6-hydroxydopamine and of clonidine in conscious rabbits. J Cardiovasc Pharmacol. 6:909-913, 1984.

**KORNER PI, HEAD GA, BOBIK A, BADOER E, ABERDEEN J.**
Central and peripheral autonomic mechanisms involved in the circulatory actions of methyldopa. Hypertension, A6: (Suppl II) II-63 — II-70, 1984.

**KORNER PI (CHAIRMAN) MANAGEMENT COMMITTEE OF THE AUSTRALIAN NATIONAL BLOOD PRESSURE STUDY.**
Prognostic factors in the treatment of mild hypertension. Circulation 69:668-676, 1984.

**LITTLE PJ, CAMPBELL JH, SKEWS H, BOBIK A.**
Mechanism of isoprenaline induced desensitization of rabbit aortic smooth muscle *B*-adrenoceptor responses. Clin Exp Pharmacol Physiol 11: 503-512, 1984.

**McLACHLAN EM, HIRST GDS.**
Appearance of dendritic calcium currents in sympathetic ganglion cells during post-natal development in the rat. Proc Aust Physiol Pharmacol Soc 15: 24P, 1984.

**McLACHLAN EM, CLARK AL, CASSELL JF.**
Electrophysiological characteristics of two classes of sympathetic ganglion cells in the guinea pig. Proc Aust Physiol Pharmacol Soc 15: 140p, 1984.

**McLACHLAN EM, OLDFIELD BJ, SITTIRACHA T.**
Localisation of hindlimb vasomotor neurones in the lumbar spinal cord of the guinea pig. Neurosci Lett. 54: 269-275, 1985.

**ODDIE C, JACKMAN G, BOBIK A.**
Measurement of xamoterol in plasma and urine by high performance liquid chromatography. J Chromatogr 308: 370-375, 1984.

**OLDFIELD BJ, HOU-YU A, SILVERMAN AJ.**
A combined electron microscopic HRP and immunocytochemical study of the limbic projections to rat hypothalamic nuclei containing vasopressin and oxytocin neurons. J Comp Neurol 231: 221-231, 1985.

**SILVERMAN AJ, OLDFIELD BJ.**
Synaptic input to vasopressin neurones of the paraventricular nucleus (PVN). Peptides 5: (Suppl 1) 139-150, 1984.

**SILVERMAN AJ, OLDFIELD BJ, HOU-YU A, ZIMMERMAN EA.**
The noradrenergic innervation of vasopressin neurons in the paraventricular nucleus of the hypothalamus: an ultrastructural study using radioautography and immunocytochemistry. Brain Res 325: 215-229, 1985.

**SITTIRACHA T, McLACHLAN EM.**
The effect of dimethylsulphoxide on retrograde labelling of neurone cell bodies with horseradish peroxidase applied to damaged and undamaged axons of the rabbit sural nerve. Proc Aust Physiol Pharmacol Soc 15: 216P, 1984.

**STRAUSS HC, BROUGHTON A, STARMER CF, GRANT AO.**
pH potentiation of local anesthetic action in heart muscle. *In:* Cardiac Electrophysiology and Arrhythmias. Eds. DP Zipes, J Jalife. Orlando, Grune & Stratton, 1985, pp 217-224.

**TRANTHAM JL, GALLAGHER JJ, GERMAN LD, BROUGHTON A, GUARNIERI T, KASELL J.**
Effects of energy delivery via a His bundle catheter during closed chest ablation of the atrioventricular conduction system. J Clin Invest 72: 1563-1574, 1983.

**WESSELMANN U, McLACHLAN EM.**
The effect of previous transection of quantitative estimates of the preganglionic neurones projecting in the cervical sympathetic trunk of the guinea-pig and the cat made by retrograde labelling of damaged axons by horseradish peroxidase. Neuroscience 13: 1299-1309, 1985.

## In Press

**ANDERSON WP, RAMSEY DE.**
Blood pressure responses to ACTH and adrenaline infusions in dogs. Clin Exp Hypertens.

**ANDERSON WP, WOOD RL, KLINE RL, KORNER PI.**
Acute haemodynamic responses to unilateral renal artery stenosis in conscious dogs. Clin Exp Pharmacol Physiol.

**BARON R, JÄNIG W, McLACHLAN EM.**
The afferent and sympathetic components of the lumbar spinal outflow to the colon and pelvic organs in the cat: I. The hypogastric nerve. J Comp Neurol.

**BARON R, JÄNIG W, McLACHLAN EM.**
The afferent and sympathetic components of the lumbar spinal outflow to the colon and pelvic organs in the cat: II The lumbar splanchnic nerves. J Comp Neurol.

**BARON R, JÄNIG W, McLACHLAN EM.**
The afferent and sympathetic components of the lumbar spinal outflow to the colon and pelvic organs in the cat: III The colonic nerves, incorporating an analysis of all components of the lumbar prevertebral outflow. J Comp Neurol.

**BLAIR-WEST JR, GIBSON AP, WOODS RL, BROOK AH.**
Acute reduction of plasma vasopressin levels by rehydration in sheep. Am J Physiol.

**BOBIK A, JENNINGS G, JACKMAN G, ODDIE C, KORNER P.**
Pharmacological and biochemical evidence for a predominantly central hypotensive effect of alpha-methyldopa in man. Hypertension.

**COCKS TM, ANGUS JA, CAMPBELL JC, CAMPBELL GR.**
Release and properties of endothelium-derived relaxing factor (EDRF) from endothelial cells in culture. J Cell Physiol.

**KORNER PI, BROUGHTON A, JENNINGS GL, ESLER MD.**
Disparate hemodynamic amplifying capacities of the heart and arterial resistance vessels in hypertension and their consequences. In "The Heart and Hypertension" Ed FH Messerli, New Orleans, Ochsner Foundation.

**KORNER PI, JENNINGS GL, ESLER MD, BROUGHTON A.**
Role of cardiac and vascular amplifiers in the maintenance of hypertension and the effect of reversal of cardiovascular hypertrophy. Clin Exp Pharmacol Physiol.

**LEW MJ, ANGUS JA.**
Alpha$_1$ and alpha$_2$-adrenoceptor mediated pressor responses: are they differentiated by calcium antagonists or by functional antagonism. J Cardiovasc Pharmacol. 1985.

**OLDFIELD BJ, SILVERMAN AJ.**
A light microscopic HRP study of limbic projections to the paraventricular nucleus. Brain Res Bull.

**OLDFIELD BJ, SHEAPPARD A, NILAVER G.**
A study of the Substance P innervation of the intermediate zone of the thoracolumbar spinal cord. J Comp Neurol.

## Cardiovascular Metabolism & Nutrition Research Unit

**COHN JS, KIMPTON WG, NESTEL PJ.**
The effect of dietary casein and soy protein on cholesterol and very low density lipoprotein metabolism in the rat. Atherosclerosis 52: 219-231, 1984.

**DE JONG FA, HOWLETT G, ALDRED AR, FIDGE N, SCHREIBER G.**
Synthesis of rat apolipoprotein E by E coli infected with recombinant bacteriophage. Biochem Biophys Res Comm 119: 657-662, 1984.

**FIDGE N, LEONARD-KANEVSKY M, NESTEL P.**
The hormonal stimulation of high-density lipoprotein binding, internalization and degradation by cultured rat adrenal cortical cells. Biochem Biophys Acta 793: 180-186, 1984.

**FIDGE NH, NESTEL PJ.**
Identification of apolipoproteins involved in the interaction of human high density lipoprotein$_3$ with receptors on cultured cells. J Biol Chem 260: 3570-3575, 1985.

**KAGAMI, FIDGE N, SUZUKI N, NESTEL P.**
Characteristics of the binding of high-density lipoprotein$_3$ by intact cells and membrane preparations of rat intestinal mucosa. Biochim Biophys Acta 795: 179-190, 1984.

**NESTEL PJ.**
Time to treat cholesterol seriously. Aust NZ J Med 14: 198-199, 1985.

**NESTEL PJ, CONNOR WE, REARDON MF, CONNOR S, WONG S, BOSTON R.**
Suppression by diets rich in fish oil of very low density lipoprotein production in man. J Clin Invest 74: 82-89, 1984.

**NESTEL PJ, NOLAN C, BAZELMANS J, COOK R.**
Effects of a high-starch diet with low or high fibre content on postabsorptive glucose utilization and glucose production in normal subjects. Diabetes Care 7: 207-210, 1984.

**OHTA T, FIDGE NH, NESTEL PJ.**
Characterization of apolipoprotein A-IV complexes and A-IV isoforms in human lymph and plasma lipoproteins. J Biol Chem 259: 14888-14893, 1984.

**REARDON MF, NESTEL PJ, CRAIG IH, HARPER RW.**
Lipoprotein predictors of the severity of coronary artery disease in men and women. Circulation 71: 881-888, 1985.

**TURLEY SD, DIETSCHY JM.**
Modulation of the stimulatory effect of pregnenolone-16-carbonitrile on biliary cholesterol output in the rat by manipulation of the rate of hepatic cholesterol synthesis. Gastroenterology 87: 284-292, 1984.

## In Press

**BAZELMANS J, NESTEL PJ, O'DEA K, ESLER MD.**
Blunted norepinephrine responsiveness to changing energy states in obese subjects. Metabolism.

**COHN JS, FIDGE NH, NESTEL PJ.**
Initial plasma high density lipoprotein distribution in the rat. Effects of age, sex and fasting. Am J Physiol.

**COHN J, NESTEL PJ.**
Hepatic lipoprotein receptor activity in rats fed casein and soy protein. Atherosclerosis.

**FIDGE NH, KAGAMI A, O'CONNOR M.**
Identification of a high density lipoprotein binding protein from adrenocortical membranes. Biochem Biophys Res Comm.

**FIDGE N, NESTEL PJ.**
Metabolism of apolipoprotein C. Methods in Enzymology.

**KAGAMI A, FIDGE NH, NESTEL P.**
Specific binding of high density lipoprotein ($HDL_3$) is not related to sterol synthesis in rat intestinal mucosa. J Lipid Res.

**NESTEL PJ.**
Dietary factors affecting lipoprotein metabolism. *In:* Drugs Affecting Lipid Metabolism. Eds D Kritchevsky, R Paoletti. Amsterdam, Elsevier/North Holland.

**NESTEL PJ.**
Nutrition, lipid metabolism and hyperlipidaemia in childhood. *In:* Clinical Nutrition of the Young Child. Ed Nestle Nutrition, Switzerland.

**NESTEL PJ.**
Treatment of hyperlipidemia. *In:* Cardiovascular Drug Therapy. Ed S Hunyor.

**NESTEL PJ.**
Management of Hyperlipidaemia. Australian Family Physician.

**NESTEL PJ, BAZELMANS J, REARDON MF, BOSTON RC.**
Lower triglyceride production during carbohydrate-rich diets through acarbose, a glucoside hydrolase inhibitor. Diabete & Metabolisme.

**NESTEL PJ, BILLINGTON T, BAZELMANS J.**
Metabolism of human plasma chylomicrons in rodent macrophages: capacity for interaction at *B*-VLDL receptor and for stimulation of cholesteryl ester formation. J Biol Chem.

**NESTEL PJ, WONG S, TOPPING D.**
Reduced triglyceride formation from long chain polyenoic fatty acids in perfused liver and hepatocytes of rats fed fish oils. Am J Clin Nutr.

**OHTA T, FIDGE NH, NESTEL PJ.**
Studies on the in vivo and in vitro distribution of apolipoprotein AIV in human plasma and lymph. J Clin Invest.

**SPADY DK, TURLEY SD, DIETSCHY JM.**
Low density lipoprotein uptake and cholesterol synthesis are regulated independently in the liver. J Lipid Res.

**WONG S, REARDON M, NESTEL P.**
Reduced triglyceride formation from long chain polyenoic fatty acids in rat hepatocytes. Metabolism.

## Cardiovascular Surgical Research Unit

**LUDBROOK J.**
Comparison of the reflex effects of arterial baroreceptors and cardiac receptors on the heart rate of conscious rabbits. Clin Exp Pharmacol Physiol II: 245-260, 1984.

**LUDBROOK J.**
Concern about gain: is this the best measure of performance of cardiovascular reflexes? Clin Exp Pharmacol Physiol II: 385-390, 1984.

**LUDBROOK J, GRAHAM WF.**
Circulatory responses to onset of exercise: role of arterial and cardiac baroreflexes. Am J Physiol. 248: H457-H467, 1985.

**LUDBROOK J, GRAHAM WF, POTOCNIK SJ.**
Acute deletion of arterial baroreceptor input in the conscious rabbit. Aust J Exp Biol Med Sci. 63: 231-240, 1985.

**LUDBROOK J, GRAHAM WF, QUAIL AF.**
Control of the circulation in exercise in rabbits. *In:* The Peripheral Circulation. Eds. S Hunyor, J Ludbrook, J Shaw, M McGrath. N.Y., Elsevier, 1984, pp. 259-265.

**ROSENFELDT FL, HARPER RW, WALL RE, UTHER JB, HILDER R, SHARDEY GC.**
A digital timing and display unit for intraoperative mapping of cardiac arrhythmias. Pace 7: 985-992, 1984.

### In Press

**FRAENKEL GJ, LUDBROOK J, DUDLEY HAF, HILL GL, MARSHALL VR.**
Guide for House Surgeons in the Surgical Unit. 8th ed. London, Heinemann.

**JOHNS JA, BLACK AJ, HARPER RW, ROSENFELDT FL, MIDDLEBROOK K, ANDERSON ST, FEDERMAN J, PITT A.**
Control of refractory supraventricular arrhythmias after unsuccessful closed-chest His bundle ablation. Pace.

**LUDBROOK J.**
Lower extremity varicosities. *In:* Current Surgical Therapy. Ed JL Cameron. Philadelphia, Marcel Decker.

## Cell Biology Laboratory

**CAMPBELL JH, CAMPBELL GR.**
Cellular interactions in the artery wall. *In:* The Peripheral Circulation, Ed. S Hunyor, J Ludbrook, J Shaw, M McGrath, New York, Elsevier 1984, pp 33-39.

**CAMPBELL JH, CAMPBELL GR.**
Cardiac muscle cells in culture: Microscopic Anatomy of the Mammalian Heart, Ed. MA Qayyam, Berlin, Veb Gustav Fischer Verlag, 1984.

**CAMPBELL GR, CAMPBELL JH.**
Smooth muscle phenotypic changes in arterial wall homeostasis: implications for the pathogenesis of atherosclerosis. Exp Mol Pathol. 42: 139-162, 1985.

**NICHOLS NR, McNALLY M, CAMPBELL JH, FUNDER·JW.**
Overlapping but not identical protein synthetic domains in cardiovascular cells in response to glucocorticoid hormones. J Hypertension 2: 663-669, 1984.

### In Press

**CAMPBELL JH, CAMPBELL GR.**
Chemical stimuli of the hypertrophic

response in smooth muscle. *In:* Hypertrophy of Smooth Muscle, Ed. C Seidel CRC Press Boca Raton.

**CAMPBELL JH, CAMPBELL GR.**
Endothelial cell influence on vascular smooth muscle phenotype. Ann Rev Physiol. 48:

**CAMPBELL GR, CAMPBELL JH.**
The smooth muscle cell. *In:* The Biology and Clinical Science of Atherosclerosis, ed. O Olsson, Churchill Livingston.

**CAMPBELL JH, CAMPBELL GR.**
(Authors of 3 chapters and editors of book). "Vascular Smooth Muscle in Culture", Boca Raton, CRC Press.

**CAMPBELL JH, REARDON MF, CAMPBELL GR, NESTEL PJ.**
Metabolism of atherogenic lipoproteins by smooth muscle cells of different phenotype in culture. Arteriosclerosis.

**CANALE ED, CAMPBELL GR, SMOLICH JJ, CAMPBELL JH.**
Cardiac Muscle: Handbuch der Mikroskopischen Anatomie des Menschen, Ed. O Oksche, L Vollrath, Berlin, Springer Verlag.

**CULLINAN V, CAMPBELL JH, MOSSE PRL, CAMPBELL GR.**
The morphology and cell culture of the striated musculature of the rat azygos vein. Cell tissue Res.

**MOSSE PRL, CAMPBELL GR, WANG ZL, CAMPBELL JH.**
Smooth muscle phenotypic expression in human carotid arteries. Comparison of cells from diffuse intimal thickenings adjacent to atheromatous plaques with those of the media. Lab Invest.

**SCHWARTZ S, CAMPBELL GR, CAMPBELL JH.**
Replication of smooth muscle cells in vascular disease. Circ Res.

**SOUTHWELL B, CHAMLEY-CAMPBELL JH, CAMPBELL GR.**
Tropic interactions between sympathetic nerves and vascular smooth muscle. J Auton Nerv Syst.

# Institute Services

## Biomedical Engineering

Most of the year's operations have been taken up by implementing a number of previously designed projects. In particular our interference-free computer trolleys required a substantial team effort.

We now have a compact mobile system of maximum flexibility with respect to both software and hardware applications.

With the gradual integration of more on-line computing equipment however, most laboratories experienced a need for new, specialised interfacing devices as few of them are commercially available. The future direction for our in-house resources appears to be mapped out.

The overall repair and maintenance load again increased over the year, as a consequence the engineering laboratory keeps a daily record on individual times spent on the various projects. This will facilitate future budgetting for technical man-power and skill requirements as well as help to decide on the cost-benefit or service contracts for new, even more complex instrumentation.

*Biomedical Engineering. Workshop Staff (l-r) F. Hannemann, K. Harvey, D. Bell, S. Carter and J. Baird.*

# Computers

Programmer: Mrs. J. Gipps

During the last year most programming time has been spent on developing programmes to be used for analysing data collected on dedicated computers in specific laboratories.

One example of this has been a programme developed for the Circulatory Control Laboratory. In this laboratory, data recording from Doppler flow meters of renal, mesenteric and hind limb blood flow together with blood pressure and heart rate is collected according to a set experimental protocol. From this data corresponding conductance values can be obtained. A simple mathematical model is then applied to estimate the blood volume of the animal during the time course of the experiment. The experimenter then has the ability to plot the data out in various ways and parameters from the experiment are subsequently deduced which are then stored in a summary data base for further analysis.

The development of such analysis programmes highlights the degree of interaction required between the experimenter and the programmer both in the validation of the results and the production of programmes which are easy to use by the experimenter.

Work has also been done on setting up data bases for both experimental and administrative purposes. On the time sharing computer this has been made possible mainly by the increased disk capacity of the computer.

# The Library

Librarian: M. Delafield

The library has had another very active year in supplying the bibliographic needs of the research staff. Where possible, subscriptions are taken to all journals whose contents are applicable to current Institute research. This reduces the necessity for photocopy requests to other libraries which may take 3-4 weeks to arrive. These outgoing requests have steadily reduced in number over the last four years — from 900 in 1981 to 600 in 1984.

A growing area of concern is the escalating cost of journal subscriptions which now consume 80% of the library budget. This rise is accentuated by the falling value of the Australian dollar. Subscription maintenance and selection in the future will thus be made more difficult. Monographs are also increasing in price. The average price of a book purchased for the library in 1984 was $77 compared to $68 in 1983.

Literature searches are conducted regularly using computer facilities to access the Medline data base in Canberra and to a lesser extent the more expensive, but also more extensive, Dialog Information Retrieval Service in California.

The library again acknowledges the great support and co-operation received during the year from the Alfred Hospital library and the University libraries of Melbourne, Monash and Latrobe.

# Staff Activities

## Hypertension and Circulatory Control Research Unit

Professor Korner attended the International Society of Hypertension Meeting, Interlaken, Switzerland in June 1984 and the Milano Medicine Meeting in Milan, October 1984 where he spoke on "Neural Mechanisms in Human Hypertension". He has again served as a member of the National Scientific Advisory Committee of the National Heart Foundation. He is a member of the Science and Industry Forum of the Australian Academy of Science; and a member of the Forward Planning Committee of the NH&MRC.

Dr. W.P. Anderson attended the International Congress of Nephrology Meeting in Los Angeles in June 1984. He gave lectures at Monash and Melbourne Universities and was visiting lecturer at the University of New England.

Dr. J. Angus attended the 9th International Congress of Pharmacology in London in August 1984 and spoke in the inaugural Debates on the Physiological role of autoinhibition in cardiac sympathetic transmission. He also spent one week at the Department of Analytical Pharmacology — Kings College Hospital, Denmark Hill, London followed by a week at the Biophysics Institute, University of Aarhus, Denmark. In October, he was invited to speak at the Second Ferdinand Nedee Conference in Corsendonc, Belgium. He served as a member of the Regional Grants Interviewing Committee (Sydney) of the NH&MRC.

Dr. Alex Bobik presented a paper on the regulation of cardiac beta adrenoceptors by cyclic AMP at the 9th International Congress of Pharmacology, London (1984).

Dr. T. Cocks presented papers at the International Congress of Pharmacology London August (1984); the satellite meeting on Neuroeffector mechanisms in Paris; the Department of Physiology and Pharmacology University of Rochester, Minnesota and to the Department of Pharmacology, University of West Virginia, USA.

Dr. P.K. Dorward attended the meeting of the International Society of Hypertension in Interlaken (June 1984) and visited the laboratory of Professor A.C. Barger, Harvard Medical School speaking at the Combined Hypertension Conference at the Brigham and Women's Hospital (January 1985).

Dr. M. Esler presented a paper at the International Society of Hypertension, Interlaken, Switzerland June 1984. He presented the Annual John Agar Memorial Lecture, Geelong Hospital November 1984. He co-ordinated the lectures on hypertension to final year medical students, Monash University.

Dr. G. Jennings attended the International Society of Hypertension Meeting in Interlaken, Switzerland June 1984 and the American Heart Association Meeting in Miami. He lectured to undergraduate and postgraduate medical nursing and pharmacy students.

Dr. E. McLachlan attended the Physiological Society Meeting, Cambridge July 1984; the International Union of Pharmacological Sciences Congress, London August 1984 and the satellite Symposium on Vascular Neuroeffector Mechanisms in Paris. She visited Physiology and Pharmacology Departments in the U.K. and she lectured to 2nd and 3rd year medical students at Monash University.

Dr. R. Woods attended the Meeting of the International Society of Hypertension in Switzerland, the International Congress of Nephrology in Quebec and a Symposium of Neuropeptides and Blood Pressure Control in Heidelberg.

## Cardiovascular Metabolism and Nutrition Research Unit

The tragic death of Michael Reardon in the second week of January 1985 has immensely saddened his colleagues in the Unit and left a void that will be very difficult to fill. A tribute to him appears on page 95.

Two of our three Ph.D. scholars, Jeff Bazelmans and Jeff Cohn have completed their courses and will be the Unit's first Ph.D. graduates.

The Annual Meeting of the Australian Atherosclerosis Society took place at the Institute in 1984 and all senior scientific staff members and Ph.D scholars presented papers. Guest speaker was Professor Richard Havel from San Francisco who was also an external adviser to the Institute. Members of the Unit are also closely involved in the Planning of the 7th International Atherosclerosis Symposium in Melbourne in October 1985.

Dr. P. Nestel was invited during 1984 to present review papers dealing with diet, hyperlipoproteinaemia and atherosclerosis as guest of the Japanese Atherosclerosis Society (Tokyo), the European Atherosclerosis Group (Perugia) and the Ninth Annual Hugh Lofland Conference (San Antonio). He also presented a paper at the American Heart Association Meeting in Miami.

Professor Nome Baker, Professor of Biochemistry at the University of California, Los Angeles, spent three months' sabbatical leave in the Unit collaborating on the lipoprotein kinetic work. His stimulating guidance in this difficult area was greatly appreciated.

Dr. S. Turley visited the National Institutes of Health in Bethesda and University of Texas Health Science Centre in Dallas, March 1984.

## Cardiovascular Surgical Research Unit

Professor J. Ludbrook attended the International Society of Hypertension, Interlaken, Switzerland, June 1984; visited Tianjin, China on behalf of the Australian Red Cross Society in November 1984. He was appointed chairman, Australia Red Cross National Blood Transfusion Committee, Member National AIDS Taskforce; Member Board of the Royal Children's Hospital Research Foundation; Member NH&MRC Regional Grant Interviewing Committee and Assigners Panel; Member NH&MRC Scholarships Committee.

Dr. R. Rosenfeldt was an invited speaker at the Auckland satellite Symposium on Cardiac Surgery held at Green Lane Hospital, April 1984. He taught Monash Medical students in cardiovascular surgery and cardiac physiology.

## Cell Biology

Dr. Julie Campbell presented a paper at the 3rd International Symposium on Cell Biology, Tokyo, August 1984; then at the invitation of Professor She Mingpeng, Deputy Director of the Institute of Basic Medical Sciences, Beijing and the Chinese Department of Health gave lectures and laboratory demonstrations in Beijing, Hangzhou and Shanghai, China, September 1984. She lectured to 3rd year Science students and ran practical sessions in the Department of Anatomy, University of Melbourne. Dr. Julie Campbell was President of the Australian Atherosclerosis Group and organised the Group's 11th Annual Conference which was held on 17 and 18 May at the Baker Institute and attracted over 70 participants. Dr. Richard Havel was the guest lecturer.

# Seminar Programme

| | | |
|---|---|---|
| 13 April | Workshop. New Perspectives on peripheral actions of catecholamines | |
| | Sources and Functions of peripheral catecholamines | Dr. C. Bell<br>University of Melbourne |
| | Actions of catecholamines of large arteries and resistance vessels | Dr. J. Angus<br>Baker Institute |
| | Mechanisms of action of catecholamines on arteriolar smooth muscle | Dr. G.D.S.Hirst<br>Australian<br>National University |
| 27 April | Responses of central respiratory neurones to excitation of peripheral chemoreceptors | Dr. J.Lipski<br>Australian<br>National<br>University |
| 1st June | Workshop. $Ca^{++}$ Antagonists | |
| | Pharmacology of calcium agonists and antagonists | Dr. W. Nayler<br>Austin Hospital |
| | Calcium antagonism in arterioles | Dr. G.D.S.Hirst<br>Australian National<br>University |
| | Clinical efficacy and pharmacokinetics of calcium antagonists | Dr. J.Horowitz<br>Austin Hospital |
| | Calcium antagonists — a sceptic's view | Dr. A. McLean<br>Alfred Hospital |
| 6th July | Workshop. Protein Structure and Function | |
| | Specificity of High Density Lipoprotein Cell Receptors | Dr. N. Fidge<br>Baker Institute |

| | | |
|---|---|---|
| | Variation in the three dimensional structure of influenza virus proteins and its role in influenza epidemics | Dr. P.Colman C.S.I.R.O. |
| | Amino acid composition of antigenic determinants: implications for a mechanism of neutralization of biological function | Dr. H.M.Geysen Commonwealth Serum Laboratories |
| | Unusual repeat structures in malaria antigens | Dr. R. Anders Walter & Eliza Hall Institute |
| | Current approaches to microsequencing of proteins using gas phase sequence technology | Dr. R. Simpson Ludwig Institute for Cancer Research |
| 22nd July | Workshop. Exercise | |
| | Adaption of skeletal muscle for physical exercise | Assoc. Prof. M. Gould Monash University |
| | Disturbances to the circulation and their control during exercise | Prof. J. Ludbrook Baker Institute |
| | CNS control of muscle performance and co-ordination | Dr. M. Horne Alfred Hospital |
| | How much exercise? The Baker Institute study | Dr. G. Jennings Baker Institute |
| | Hormones and Fuels during exercise | Dr. P. Nestel Baker Institute |
| | A controlled trial of exercise after myocardial infarction | Dr. A. Goble Austin Hospital |

| | | |
|---|---|---|
| 3rd August | Workshop. Studying the heart in health and disease. | |
| | Cardiac cells in culture | Dr. J. Campbell<br>Baker Institute |
| | The papillary muscle | Dr. C. Gibbs<br>Monash University |
| | The blood-perfused isolated working rat heart in vitro | Dr. D. Topping<br>C.S.I.R.O. |
| | The isolated supported dog heart operation | Dr. M. Rabinov<br>Baker Institute |
| | Models of the effect of ageing on the heart | Dr. D. Topping<br>C.S.I.R.O. |
| | Models of hypertension and cardiac hypertrophy | Prof. P.Korner<br>Baker Institute |
| | Pitfalls in the use of animal models to study the heart | Dr.F.Rosenfeldt<br>Baker<br>Institute |
| 14th September | Why do we have glucocorticoid hormones | Dr. J.Funder<br>Prince Henry's Hospital |
| 24th September | The development of synapses | Prof. M.Bennett<br>University of Sydney |
| 28th September | Homocystinuria — a non-lipid model of human vascular disease | Dr.D. Wilcken<br>Prince Henry's<br>Hospital, Sydney |
| 5th December | Omega — 3 fatty acids, their relation to epidemiology of acute myocardial infarction and their biological role in prevention cardiology | Dr.J. Dyerberg<br>Halborg<br>Hospital,<br>Denmark |

# Baker Institute Award

Mr. Darren Baillieu was presented with the Baker Award by Mr. John Habersberger at a small ceremony at the Baillieus' home on 15th May 1985. The ceremony was attended by Mr. Baillieu's family, Board Members and a number of staff.

The Baker Award is presented infrequently to individuals whose efforts on behalf of the Institute go beyond anything which might be expected of them through their formal association with the Institute. This was only the second occasion on which the Award has been made. Mr. Baillieu was a Trustee of the Institute for approximately twenty five years and was instrumental in raising funds for the building of the Institute's present premises.

*Mr. John Habersberger presents the Baker Award to Mr. Darren Baillieu (right). In the background are Mrs. Baillieu with Dr. T. Lowe, a former Director of the Institute.*

# In Memoriam

## Sir John Reid

On the 31st December 1984 the Baker Institute suffered a grievous loss by the death of its former board member Sir John Reid.

"Jock" became interested in the work of the Institute nearly twenty-five years ago and in 1977 became a member of a small advisory committee of businessmen appointed to assist the Trustees of the Institute in their current administration and long range planning. He brought his long experience of business affairs and sound judgment to bear on the problems facing the Institute particularly in financial planning.

Jock saw the need to change the structure of the Institute and played a leading role in the initiatives taken to constitute the Institute as a separate legal entity under its own Act of Parliament.

This was done in 1980 and Jock became one of the first directors of the reconstituted Baker Medical Research Institute. For the next four years Jock served as a director with dedication and enthusiasm. His advice, always given with courtesy and understanding, had a significant influence on the development of the Institute under its new structure.

Jock followed closely the research projects undertaken by the members of the Institute's staff and his personal interest did much to build morale.

Jock's contribution to the Institute cannot be over estimated and his death is a great loss.

J.C. Habersberger

## Mr John Milne

John Milne was Treasurer of the Baker Institute for two years from 1982 to 1984. He was also Managing Director of the Australia and New Zealand Banking Group Limited at the time of his death which culminated a dedicated career with ANZ that spanned 45 years. During the last four years of his life as ANZ Group's Chief Executive, he presided over the Group's significant growth and diversification into a major financial institution of world standing.

His contribution to the Baker Institute was also outstanding and as Treasurer he selflessly offered the Institute his business and professional acumen with dedication and commitment.

We will sadly miss but long remember John's friendship and contribution in making the Institute one of Australia's leading medical research establishments.

L. M. Muir

## Dr. Michael Reardon

Michael Reardon's death on January 8 at the age of 35 greatly saddened his many friends at the Institute. Since joining the staff in 1976, he had contributed greatly to the warm esprit de corps which prevails at the Baker. His enthusiastic involvement in scientific, social and administrative affairs and his humane and compassionate views on life are sorely missed.

He was one of the Canberra group and, like myself, transferred from the John Curtin School of Medical Research to continue our research work here at the Baker. He obtained his Ph.D on the subject of lipoprotein metabolism at the Australian National University. As a Post-Doctoral Fellow, he spent the following two years in Toronto continuing his lipoprotein studies and arrived at the Institute at the end of 1976. He quickly gained an international reputation in his field and made very many friends overseas.

Michael's tragic death has deprived us of a close friend and a fine scientist who was just reaching his peak.

Paul J. Nestel

# Community Health Services

## Heart Risk Evaluation

### Summary of Preliminary Analysis

During 1983, the Risk Clinic at the Institute participated in the Second National Heart Foundation's National Survey of cardiovascular risk factors. The results have only just been analysed and give a comprehensive picture of the "average" Australian adult. At the Institute we examined about 1000 people of the 7600 screened throughout Australia.

**Blood pressure**

According to the study's definition, about 1 in 6 men and 1 in 8 women were found to be hypertensive. 50% of men and 66% of women found to be hypertensive were currently taking tablets for raised blood pressure. 8% of men and 4% of women had diastolic blood pressures of 95 mmHg or more and were not on tablets.

A diastolic blood pressure of 95 mmHg or more was found in 11% of men and 6% of women whether they were on tablets for raised blood pressure or not. Of those who said they were on tablets for blood pressure, 2 in 5 men and 1 in 5 women had a diastolic blood pressure of 95 mmHg or more.

**Blood cholesterol and triglycerides**

19% of men and 21% of women (not taking the oral contraceptive pill) had plasma cholesterol levels of 6.5 mmol/l or more.

12% of men and 6% of women (not taking the oral contraceptive pill) had plasma triglycerides of 2 mmol/l or more.

**Smoking**

32% of men and 25% of women said they were current cigarette smokers.

45% of men and 39% of women who had smoked regularly said they were no longer smoking.

The average amount smoked daily was 21 cigarettes for male smokers and 16 for female smokers.

**Multiple major risk factors: High blood pressure, high blood cholesterol and cigarette smoking.**

Almost 50% of men and 42% of women had at least one of the three major risk factors.

11% of men and 7% of women had two or three major risk factors, the prevalence generally increasing with age.

**Overweight and obesity**

1 in 2 men aged 35 years and over and 1 in 2 women aged 50 years and over were overweight or obese.

In every age-group, men had a higher prevalence of overweight or obesity than women. This difference was more marked in the younger than in the older age-groups.

**Exercise**

1 in 10 men and 1 in 20 women exercise at a level commonly believed to confer a "training effect" on the heart and lungs.

About a half of men and women said they had walked for recreation in the preceding 2 weeks.

**Alcohol**

88% of men and 75% of women said they drank alcohol, the proportion of drinkers being lower in the older age-groups.

When younger adults drank they drank more heavily than the older age-groups, although they were likely to drink less frequently.

9% of all men and 6% of all women were classified as intermediate or high risk drinkers.

# Australian Nutrition Foundation

The office of the Victorian Division is situated at the Baker Institute.

An abundance of food and relative affluence in the Australian community does not guarantee good nutrition for everyone. In fact, many common diseases are nutrition-related. These diseases include obesity, heart disease, high blood pressure, stroke, gall bladder disease, non-insulin dependent diabetes, certain cancers, food intolerance and tooth decay.

To promote the health of Australians the Commonwealth Department of Health has adopted a Food and Nutrition Policy which includes the following dietary guidelines:

1) Choose a nutritious diet from a variety of foods
2) Control your weight
3) Avoid eating too much fat
4) Avoid eating too much sugar
5) Eat more breads, cereals (preferably wholegrain), vegetables and fruits
6) Limit alcohol consumption
7) Use less salt
8) Promote breast feeding

The Victorian Division of the ANF is taking an active part in a joint project together with the Health Commission of Victoria and the Department of Agriculture to support these guidelines and towards making healthy food choices easy choices.

Services include:

* The ANF Newsletter
* Nutrition Information Sheets
* Books and Booklets
* Posters
* Dial-a-Nutrition Talk (03) 520 2197 (24 hour service)
* Dial-a-Dietition for Nutrition advice Tuesdays 2pm — 5pm on (03) 596 5352
* Lectures, Forums and Seminars
* Articles for professional and consumer publications
* Expert advice for consumers and the media
* Co-operation with other organisations such as consumer groups and professional associations

The Australian Nutrition Foundation is a voluntary, independent community service organisation dedicated to providing a source of up-to-date, accurate information about food, nutrition and health.

Mrs. Lydia Nestel is the Executive Officer of the Victorian Division

# Financial Report

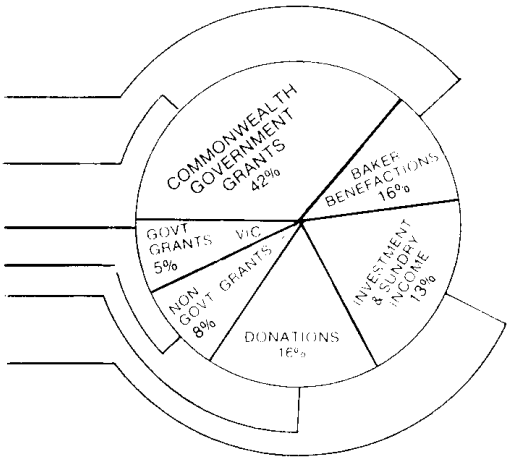
## BAKER MEDICAL RESEARCH INSTITUTE

### Year ended 31 December 1984

### Income and Expenditure at a glance

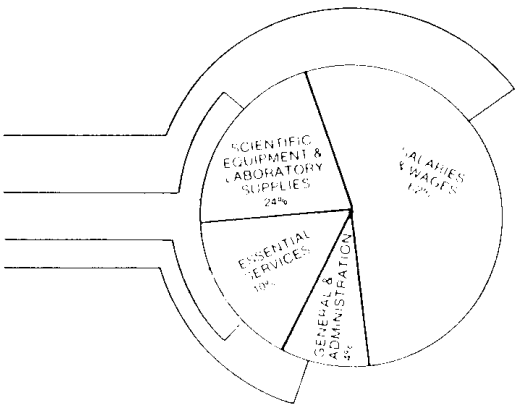
#### INCOME DERIVED FROM THE FOLLOWING SOURCES:

| 1983 000's | 1983 % | | 1984 000's | 1984 % |
|---|---|---|---|---|
| 442 | 15 | Baker Benefactions | 562 | 16 |
| 1230 | 41 | Government Grants — Commonwealth | 1439 | 42 |
| 201 | 7 | Government Grants — Victorian | 183 | 5 |
| 153 | 5 | Non-Government Grants | 253 | 8 |
| 498 | 16 | Donations | 546 | 16 |
| 515 | 16 | Interest from Investments and Sundry Income | 449 | 13 |
| 3039 | 100 | | 3432 | 100 |




#### EXPENDITURE DISTRIBUTED AS FOLLOWS:

| 1983 000's | 1983 % | | 1984 000's | 1984 % |
|---|---|---|---|---|
| 1865 | 62 | Salaries & Wages | 2166 | 62 |
| 716 | 24 | Scientific Equipment and Laboratory Supplies | 832 | 24 |
| 292 | 10 | Essential Services | 346 | 10 |
| 112 | 4 | General & Administration | 128 | 4 |
| 2985 | 100 | | 3472 | 100 |



# BAKER MEDICAL RESEARCH INSTITUTE

## Balance Sheet at 31 December 1984

| 1983 | ACCUMULATED FUNDS AND LIABILITIES | 1984 |
|---|---|---|
| | **OPERATING FUND** | |
| (89,026) | Accumulated (deficit) | **(343,543)** |
| 213,616 | Bank overdraft | **199,634** |
| 105,257 | Sundry creditors & Accrued Expenses | **61,401** |
| — | Provision for leave entitlement | **249,703** |
| $229,847 | | **$167,195** |
| | **ENDOWMENT FUND** | |
| 1,483,376 | Accumulated fund — Page 103 | **1,580,610** |
| $1,483,376 | | **$1,580,610** |
| | **RESEARCH SCHOLARSHIPS AND OTHER FUNDS** | |
| 220,246 | Restricted Fund — Page 103 | **116,461** |
| 88,713 | Edgar Rouse Memorial Fellowship Fund — Page 104 | **101,589** |
| 3,489 | Laura Nyulasy Scholarship Fund — Page 104 | **3,828** |
| 38,458 | William Buckland Research Fund — Page 104 | **38,456** |
| 4,852 | Lang Research Scholarship Fund | **4,852** |
| $355,758 | | **$265,186** |

These accounts should be read in conjunction with the notes on page

| 1983 | ASSETS | | | 1984 |
|---|---|---|---|---|
| | **OPERATING FUND ASSETS** | | | |
| 300 | Cash on hand | | | **300** |
| 38,733 | Sundry debtors & prepayments | | | **70,955** |
| 176,753 | Short term deposits held by the Institute | | | **95,940** |
| 14,061 | Cash held by ANZ Executors & Trustee Co. | | | — |
| $229,847 | | | | **$167,195** |
| | **ENDOWMENT FUND ASSETS** | | | |
| | Investments (at cost) | | | |
| | Held by the Institute | | | |
| 202,550 | Govt & semi-government stock | 202,550 | | |
| 194,612 | Shares & debentures in companies | 345,064 | | |
| 387,865 | Short term deposits | 259,114 | | |
| 138,500 | Mortgage loans | 118,200 | | |
| 923,527 | | | 924,928 | |
| | Held by ANZ Executors & Trustee Co. Ltd. | | | |
| — | Govt & semi-government stock | 25,581 | | |
| 81,678 | Shares & debentures in companies | 73,304 | | |
| 239,000 | Trust units | 340,824 | | |
| — | Short term deposits | 10,461 | | |
| 239,050 | Mortgage loans | 202,500 | | |
| 559,728 | | | 655,670 | |
| 121 | Cash at bank | | 12 | |
| $1,483,376 | | | | **$1,580,610** |
| | **RESEARCH SCHOLARSHIP AND OTHER FUND ASSETS** | | | |
| | Investments (at cost) | | | |
| | Held by the Institute | | | |
| 4,852 | Shares in companies | | 4,852 | |
| 308,926 | Short term deposits | | 219,868 | |
| 313,778 | | | | **224,720** |
| | Held by ANZ Executors & Trustee Co. Ltd. | | | |
| 8,253 | Shares in companies | 8,253 | | |
| 25,460 | Trust units | 32,928 | | |
| 8,234 | Short term deposits | 1,103 | | |
| 41,947 | | | 42,284 | |
| 33 | Cash at bank | | (1,818) | |
| $355,758 | | | | **$265,186** |

# BAKER MEDICAL RESEARCH INSTITUTE

## Year Ending 31 December 1984

| 1983 | STATEMENT OF MOVEMENT IN ACCUMULATED FUNDS | 1984 |
|---|---|---|
| | **OPERATING FUND — EXPENDITURE** | |
| 1,865,027 | Salaries & wages | **2,165,745** |
| 287,518 | Laboratory supplies & isotopes | **281,571** |
| 263,273 | Additional equipment & building costs | **421,942** |
| 42,197 | Library maintenance | **43,137** |
| 17,859 | Postage & telephone | **18,847** |
| 40,044 | Printing & stationery | **37,252** |
| 79,432 | Light & power | **84,973** |
| 32,400 | Insurance | **45,879** |
| 74,403 | Repairs & renewals | **62,351** |
| — | Animal house contribution | **10,032** |
| 50,000 | Collaborative grant — NH & MRC programme | **50,000** |
| 58,301 | Travelling expenses | **70,091** |
| 10,202 | Public relations | **11,257** |
| 2,717 | Stanhope Court | — |
| 99,294 | Fundraising expenses | **117,014** |
| 25,927 | Sundries | **32,563** |
| 30,395 | Clinical services | **16,250** |
| 6,212 | Data processing | **2,874** |
| 53,952 | Surplus for year | — |
| $3,039,153 | | **$3,471,778** |

| | | |
|---|---|---|
| 53,952 | Surplus/(Deficit) for year | **(40,049)** |
| (142,978) | Accumulated deficit as at 1 January 1984 | ***(303,494)** |
| (89,026) | Accumulated deficit as at 31 December 1984 | **(343,543)** |

* The accumulated deficit as at 1/1/84 includes annual leave and long service leave entitlements for all periods prior to 1984. In previous years, staff entitlements were not provided for, but were indicated in the notes to and forming part of the accounts.

| 1983 | | 1984 |
|---|---|---|
| | **DONATIONS FROM BAKER BENEFACTIONS** | |
| 11,569 | Statutory amount | 11,569 |
| 430,000 | Transfers from Endowment Fund | 550,000 |
| 441,659 | | **561,569** |
| | **GRANTS-IN-AID OF RESEARCH PROJECTS** | |
| 51,000 | Life Insurance Medical Research Fund of Australia and New Zealand | 17,000 |
| 1,230,079 | National Health & Medical Research Council | 1,439,359 |
| 94,070 | National Heart Foundation of Australia | 110,463 |
| 8,000 | Australian College of Surgeons | — |
| — | Alfred Hospital | 80,045 |
| 1,383,149 | | **1,646,867** |
| | **OTHER GRANTS** | |
| 9,500 | The James & Elsie Borrowman Research Trust | 9,500 |
| 2,800 | The William Buckland Research Fund | 4,000 |
| 201,000 | Victorian State Government | 183,500 |
| 125 | The Laura Nyulasy Research Scholarship Fund | 125 |
| 10,000 | Clive & Vera Ramaciotti Foundation | 30,621 |
| 32,457 | Merck Sharpe & Dohme | — |
| 255,882 | | **227,746** |
| | **INCOME FROM INVESTMENTS** | |
| 3,421 | Held by the Trustees of the Baker Institute Grant Trust | 5,097 |
| 246,553 | Other investment income | 274,546 |
| 249,974 | | **279,643** |
| | **OTHER INCOME** | |
| 269 | Rentals | — |
| 165,331 | Sundry sales, recoveries & refunds | 119,959 |
| 45,327 | Clinical services | 49,454 |
| 210,927 | | **169,413** |
| (443,700) | Deficit | **(586,540)** |
| | **PUBLIC & CORPORATE DONATIONS** | |
| 497,652 | (Net of Tranfers) | **546,491** |
| — | Deficit for year | **(40,049)** |
| $3,039,153 | | **$3,471,778** |

# BAKER MEDICAL RESEARCH INSTITUTE

## Year Ending 31 December 1984

## STATEMENT OF MOVEMENT IN ACCUMULATED FUNDS

| 1983 | | | 1984 |
|---|---|---|---|
| | **ENDOWMENT FUND** | | |
| 1,238,610 | Balance at 1 January 1984 | | **1,483,376** |
| 594,691 | Donations — Baker Benefactions | 593,605 | |
| — | T.E.&A. recovery | 2,269 | |
| 1,445 | Adjustment to investments | — | |
| 121 | Interest | 33 | |
| 526 | Profit on sale of shares & redemption of stocks | 101,274 | |
| — | Sale of motor vehicle | 5,500 | |
| 213,301 | Profit on sale of Stanhope Court units | — | |
| 570,084 | | | **702,681** |
| 2,048,694 | | | **2,186,057** |
| 92 | Bank charges | 30 | |
| 430,000 | Transfer to Operating Fund | 550,000 | |
| 4,173 | TE&A write off | — | |
| 131,053 | Building maintenance & renovations | 29,673 | |
| — | Cost of motor vehicles | 24,306 | |
| — | Brokerage fees | 1,438 | |
| 565,318 | | | **605,447** |
| 1,483,376 | Balance as at 31 December 1984 — Page 99 | | **1,580,610** |
| | **RESTRICTED FUND** | | |
| 154,955 | Balance at 1 January 1984 | | **220,246** |
| 11,569 | Baker Benefactions — Statutory amount | 11,569 | |
| 165,717 | Donations | 119,500 | |
| 15,311 | Investment income & bank interest | 11,112 | |
| 1,500 | Transfer from Operating Fund | 11,452 | |
| — | NH & MRC Grant — Turley 1985 | 22,333 | |
| 194,097 | | | **175,966** |
| 223,993 | | | **396,212** |
| 11,569 | Transfer to Operating Fund Baker Benefactions statutory amount Page 102 | 11,569 | |
| 106,561 | Donations, Grants & Other income | 129,842 | |
| 10,676 | Payments | 138,227 | |
| — | Bank charges & Federal Tax | 113 | |
| 128,806 | | | **279,751** |
| $220,246 | Balance at 31 December 1984 — Page 99 | | **$116,461** |

NOTE: Income received from Endowment Fund investments is credited direct to the Operating Fund.

# RESEARCH, SCHOLARSHIP & OTHER FUNDS

| 1983 | | | 1984 |
|---|---|---|---|
| | **EDGAR ROUSE MEMORIAL SCHOLARSHIP FUND** | | |
| 86,193 | Balance at 1 January 1984 | | **88,713** |
| 2,224 | Donations | 8,798 | |
| 10,280 | Investment income & bank interest | 11,113 | |
| — | TE&A recovery | 883 | |
| 12,504 | | | **20,794** |
| 98,697 | | | **109,507** |
| 8,361 | Payments | 7,913 | |
| 1,623 | Loss due to TE&A liquidation | — | |
| — | Federal tax | 5 | |
| | | | **7,918** |
| $88,713 | Balance at 31 December 1984 | | **$101,589** |
| | **LAURA NYULASY SCHOLARSHIP FUND** | | |
| 3,339 | Balance at 1 January 1984 | | **3,489** |
| 330 | Investment income | 499 | |
| — | TE&A recovery | 215 | |
| 330 | | | **714** |
| (180) | Loss due to TE&A liquidation | — | |
| — | Payments | 150 | |
| — | Adjustment | 225 | |
| (180) | | | **375** |
| $3,489 | Balance at 31 December 1984 | | **$3,828** |
| | **WILLIAM BUCKLAND RESEARCH FUND** | | |
| 32,661 | Balance at 1 January 1984 | | **38,458** |
| 5,797 | Investment income | | **4,208** |
| — | Payments | | **4,210** |
| $38,458 | Balance at 31 December 1984 | | **$38,456** |

# Baker Medical Research Institute

## NOTES TO AND FORMING PART OF THE ACCOUNTS FOR THE YEAR ENDED 31 DECEMBER 1984

### 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 the 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 convenants apply to that income.

Income from investments (except interest on short term deposits) is accounted for on a cash 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 1984 was approximately $7,500,000.

### 3. INVESTMENTS — ENDOWMENT FUND

The market value of shares in companies listed on the Australian Stock Exchange at 31 December 1984 was $608,567 (1983 $755,165).

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. STAFF ENTITLEMENTS

In prior years the Institute did not provide for long service leave or holiday pay in the accounts.

For the year ended 31 December 1984, liabilities have been raised in the accounts for holiday pay $213,418 (1983 $179,529 not provided for) and long service leave $36,285 (1983 $34,940 not provided for). Adjustments have been made to the Operating Fund for the prior year to reflect the liabilities at 1 January 1984.

### 5. CONTINGENCY 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.

# Donations 1984

| | |
|---|---|
| Victorian State Government | 183,500 |
| E.E. Stewart Trust | 39,389 |
| Anonymous | 30,000 |
| Utah Foundation | 19,415 |
| Ian Potter Foundation | 12,500 |
| Allan Williams Trust | 11,000 |
| Sterling Pharmaceuticals | 10,000 |
| Felton Bequest | 9,100 |
| Appel Family Bequest | 8,000 |
| Windemere Hospital Foundation | 8,000 |
| Kodak (Aust.) | 7,500 |
| Felton Bequest | 7,000 |
| Roche | 6,000 |
| ANZ Banking Group | 5,000 |
| Commonwealth Banking Corporation | 5,000 |
| William Angliss Charitable Fund | 5,000 |
| George Thomas & Lockyer Potter Trust | 5,000 |
| BHP Co. | 5,000 |
| Esso Australia Ltd. | 5,000 |
| Perpetual Trustees | 5,000 |
| Percy Baxter Charitable Trust | 4,000 |
| Collier Charitable Foundation | 3,000 |
| Executors & Trustee Co. | 3,000 |
| Mr. R.M. Sykes | 3,000 |
| American Home Assurance | 2,500 |
| Marian & E.H. Flack Trust | 2,500 |
| The Late Edward Wilson Foundations of the ANZ | 2,500 |
| ICI Australia Operations | 2,000 |
| E.B. Meyer Charity Fund | 2,000 |
| William Paxton Fund | 2,000 |
| Westpac Banking Corporation | 2,000 |
| Reckitt & Coleman | 2,000 |
| National Australia Bank | 2,000 |
| B.S. Robbins Nurseries Pty. Ltd. | 2,000 |
| Bell Charitable Fund | 1,500 |
| P.I. & J.M.N. Korner | 1,500 |
| Robert Bosch (Aust.) Ltd. | 1,200 |
| ACI Australia | 1,000 |
| APM Ltd. | 1,000 |
| Allied Mills Industries Pty. Ltd. | 1,000 |
| Blue Circle Southern Cement | 1,000 |
| Geoff Brady Motors | 1,000 |
| Brambles Industries Ltd. | 1,000 |
| David Bruce Cookes | 1,000 |
| CRA Services Ltd. | 1,000 |
| Caltex Oil (Aust.) Pty. Ltd. | 1,000 |
| Chamber of Manufacturers Ins. Ltd. | 1,000 |
| Ciba Geigy | 1,000 |
| G.J. Coles & Co. | 1,000 |
| Comalco | 1,000 |
| Containers Packaging | 1,000 |
| The Danks Trust | 1,000 |
| Mr. A. Douglas | 1,000 |
| Mr. L.J. Fitzgerald | 1,000 |
| George Weston Foods | 1,000 |
| Herald & Weekly Times Pty. Ltd. | 1,000 |
| Invicta Group Industries | 1,000 |
| Jacy Holdings (Aust.) Pty. Ltd. | 1,000 |
| James N. Kirby Foundation | 1,000 |
| Leighton Holdings | 1,000 |
| McCaughan Dyson & Co. | 1,000 |
| Nicholas Kiwi Pty. Ltd. | 1,000 |
| North Broken Hill Holdings Pty. Ltd. | 1,000 |
| Potter Partners | 1,000 |
| Red Tulip Chocolates Pty. Ltd. | 1,000 |
| Riteway Supermarkets | 1,000 |
| Royal Insurance Aust. Ltd. | 1,000 |
| Santos Ltd. | 1,000 |
| Sir Donald & Lady Trescowthick | 1,000 |
| Estate of Mrs. Anna White | 1,000 |
| H.P. Williams Estate | 1,000 |
| Idaps Australia Pty. Ltd. | 750 |
| Sir James McNeill | 750 |
| E.M. McLachlan | 650 |
| Mr. & Mrs. J.C. Habersberger | 540 |
| Mr. R.G. Ansett | 500 |
| Australian Guarantee Corporation | 500 |
| Australian Nutrition Foundation | 500 |
| Ballarat Courier | 500 |
| Blake & Riggall | 500 |
| Bunge Australia Pty. Ltd. | 500 |
| Carlton United Breweries | 500 |
| Commercial Union Assurance Co. | 500 |
| Commonwealth Dyers Association | 500 |
| Construction Engineering | 500 |
| Crawford Productions | 500 |
| Dalgety Holdings (Aust.) | 500 |
| Gordon & Gotch Pty. Ltd. | 500 |
| William Heath Trust | 500 |
| Hooker Corporation | 500 |
| Mrs. I. Hunter | 500 |
| Joubert & Joubert | 500 |
| Linfox Transport Group | 500 |
| Moore Paragon | 500 |
| Pierce Armstrong Foundation | 500 |
| H.J. Reece Pty. Ltd. | 500 |
| Mrs. Beth Smith | 500 |
| Mr. H.E. Towers | 500 |
| H.J. Reece Pty. Ltd. | 500 |
| Bunge Australia Pty. Ltd. | 500 |
| Gordon & Gotch Pty. Ltd. | 500 |
| Pacemaker Set | 475 |

| | |
|---|---|
| George F. Little Settlement | 470 |
| Dr. V.V. Bower | 450 |
| Mr. Fusspots Dry Cleaners | 400 |
| Mrs. M.C. Volum | 400 |
| Witchery Pty. Ltd | 400 |
| Mr. J. Moir | 350 |
| Budget Rent-A-Car | 320 |
| Mr. F.K. Alfredson | 300 |
| Colgate Palmolive Pty. Ltd. | 300 |
| Coopers & Lyband | 300 |
| Mrs. A. Dickason | 300 |
| Mr. V. Flynn | 300 |
| Falk Hanneman | 300 |
| Miss E.C. Hanson | 300 |
| Lily Industries | 300 |
| Mr. W.D. McPherson | 300 |
| Partnership Pacific Ltd. | 300 |
| Mr. E.A. Richards | 300 |
| Mr. A. Stickland | 300 |
| Air International | 250 |
| Alliance Holdings Ltd. | 250 |
| Australian Anglo American | 250 |
| E.L. & C. Baillieu | 250 |
| Mrs. A.L. Bottomley | 250 |
| Billy Guyatts | 250 |
| Cadbury Schweppes Pty. Ltd. | 250 |
| Capel Court Corportion | 250 |
| Containers Packaging | 250 |
| Mr. R. Croft | 250 |
| Custom Credit Corporation | 250 |
| Mr. K. Eisner | 250 |
| Mr. L. Janover | 250 |
| Johnson & Johnson | 250 |
| Lane Motors | 250 |
| Lantor of Australia | 250 |
| Malcolm Moore Pty. Ltd. | 250 |
| Meldrum, Burrows & Partners | 250 |
| Monier Ltd. | 250 |
| Monsanto | 250 |
| Sir Laurence & Lady Muir | 250 |
| Mr. F. Murphy | 250 |
| Repco Ltd. | 250 |
| Reserve Bank of Australia | 250 |
| Shell Co. of Australia | 250 |
| Tubemakers of Australia | 250 |
| Mr. H.E. Vivian | 250 |
| Wilkinson Sword Group | 250 |
| Wiretainers Pty. Ltd. | 250 |
| A.A. Wright Pty. Ltd. | 250 |
| Late George Adams Estate | 200 |
| Mrs. H. Allan | 200 |
| Australian Reinsurance Co. Ltd. | 200 |
| Barclays International Aust. | 200 |
| Chadstone Branch of C.W.A. | 200 |
| Mr. G.W. Chatfield | 200 |
| Mr. E.J. Collings | 200 |
| Corr & Corr Solicitors | 200 |
| Mr. M.A. Cuming | 200 |
| Mr. G. Dryen | 200 |
| Engelhard Industries Pty. Ltd. | 200 |
| Mr. R.G. Farrow | 200 |
| Glaxo Aust. Pty. Ltd. | 200 |
| Glo-Weave Investments Pty. Ltd. | 200 |
| W.R. Grace | 200 |
| Mrs. P. Greenshields | 200 |
| Mrs. B. Hewitt | 200 |
| Humes Ltd. | 200 |
| John Holland Management Services | 200 |
| Koppers Australia Pty. Ltd. | 200 |
| Mr. N.R. Korner | 200 |
| Mr. A.T. Marriott | 200 |
| Mildara Wines | 200 |
| Mr. A. Muir | 200 |
| Mr. E. Newman | 200 |
| Rev. W.A. O'Driscoll | 200 |
| Ord Minnett | 200 |
| Pressfast Industries Pty. Ltd. | 200 |
| Mrs. T. Santos | 200 |
| Mrs. M.A. Simpson | 200 |
| Mr. H.D. Stewart | 200 |
| Mrs. C. Sutherland | 200 |
| Mr. F.P. Thornton | 200 |
| Vic. Medical Postgraduate Foundation | 200 |
| World Services | 200 |
| Arthur Robinson | 180 |
| Miss E.M. Robinson | 180 |
| Prof. R.R. Andrew | 175 |
| Mr. & Mrs. W. Lewis | 175 |
| Mr. G.U. Pruscino | 175 |
| Electrolux Pty. Ltd. | 150 |
| Mr. L.S. Goulden | 150 |
| Mrs. T.M. Hoult | 150 |
| Mr. H. Janes | 150 |
| Rev. J.P. Leahy | 150 |
| Mr. I.T. Perkins | 150 |
| Mr. A. Pfeiffer | 150 |
| Mr. F.N. Secomb | 150 |
| Mr. R.B. Taylor | 150 |
| Mr. F.R. Townsend | 150 |
| Mr. & Mrs. N.S. Gibson | 130 |
| Mrs. N.L. Duncan | 125 |
| Mrs. R.S. Gadsden | 125 |
| Mr. D. Gibbs | 125 |
| Mr. B. Halpern | 125 |
| Mr. L.J. Hayes | 125 |
| Jabomi Developments | 125 |
| Mr. H. Schwartz | 125 |
| Mr. F.M. Spurway | 125 |
| Mr. L.M. Francome | 120 |
| Mrs. C.A. Linton-Smith | 120 |

| | |
|---|---|
| Mr. & Mrs. J.A. Cockburn | 110 |
| Mr. S.J. Cook | 110 |
| Mr. J.E. Graham | 110 |
| Dr. E. Messer | 110 |
| Mr. I.C. Aberdeen | 100 |
| Aberfoyle Industries Pty. Ltd. | 100 |
| Mrs. S.J. Anglam | 100 |
| Applied Chemicals | 100 |
| Mr. H.P. Atchison | 100 |
| Aussie Disposals | 100 |
| Aust. Agriculture Mach. Group | 100 |
| Aust. Shipping Cons. Pty. Ltd. | 100 |
| Miss P.F. Ayers | 100 |
| Baker Perkins | 100 |
| Mr. G.F. Bell | 100 |
| Mr. R.L. Bienvenu | 100 |
| Mr. D.W. Bingham | 100 |
| Mr. F.D. Bladin | 100 |
| Mr. & Mrs. D.S. Blair | 100 |
| Miss J.M. Braithwaite | 100 |
| Mrs. A.M. Buchanan | 100 |
| Mr. & Mrs. S. Bude | 100 |
| Miss E.F. Bunning | 100 |
| Mr. W. Burgoyne | 100 |
| Mr. M.A. Burne | 100 |
| Dr. F.P. Byrne | 100 |
| Mr. J.S. Campbell | 100 |
| Mr. L.W. Candy | 100 |
| Mr. G.E. Carrington | 100 |
| Dr. D. Chong | 100 |
| Mr. A.G. Coulthard | 100 |
| Miss H.J. Crawcour | 100 |
| Mrs. E. Creed | 100 |
| Mrs. S.J. Cruse | 100 |
| Du Pont (Aust.) Ltd. | 100 |
| Dr. R.G. Denardo | 100 |
| Mr. L.E. Dobson | 100 |
| Mrs. M.A. Dobson | 100 |
| Mr. A.F. Drayton | 100 |
| Mrs. A.C. Duffield | 100 |
| Mr. L.K. Farmer | 100 |
| P.D. Field | 100 |
| Fielder, Gillespie Davis Ltd. | 100 |
| Mrs. M. Fink, M.B.E. | 100 |
| Mrs. S. Fink | 100 |
| Mr. S. Fish | 100 |
| Forges Pty. Ltd. | 100 |
| Mr. J.E. Freeman | 100 |
| Mr. G. Gibson | 100 |
| T.J. Gillespie | 100 |
| Mr. J.S. Grey | 100 |
| Mr. John Grimwade, A.M. | 100 |
| Mr. & Mrs. G. Handbury | 100 |
| Guardian Property Management | 100 |
| Mr. W.J. Haysom | 100 |
| Mr. L.Q. Hogg | 100 |
| Mr. N.A. Jackson | 100 |
| James Hardy Paper & Packaging | 100 |
| Mr. & Mrs. A. Joske | 100 |
| Dr. H.B. Kay | 100 |
| Miss A.P. Kennedy | 100 |
| Mrs. E.E. Lamberd | 100 |
| Mrs. G.M. Love | 100 |
| Mr. & Mrs. E. Lyons | 100 |
| Miss J. Macdonald | 100 |
| Mallesons | 100 |
| Mrs. J. Mackay | 100 |
| Mrs. A. Marks | 100 |
| Mrs. G. Marks | 100 |
| Mr. A. Marshall | 100 |
| Mrs. D.M. Martin | 100 |
| Maunsell & Partners Pty. Ltd. | 100 |
| Mr. W. Maxwell | 100 |
| Mr. E.R. Meagher | 100 |
| Mr. S. Meyer | 100 |
| Mr. F.R. Monotti | 100 |
| Mr. I.R. Monotti | 100 |
| Mrs. M.E. Moodie | 100 |
| Miss D. Newton | 100 |
| Misses C. & M. Norman | 100 |
| Mr. A.A. Paige | 100 |
| Mr. J.G. Peppard | 100 |
| Phillips, Fox & Masel | 100 |
| Pilkington ACI | 100 |
| Plessey Australia Pty. Ltd. | 100 |
| Mr. D. Porter | 100 |
| Pratt Foundation | 100 |
| Miss N. Price | 100 |
| Mr. R.M. Reid | 100 |
| Mr. A.H. Richards | 100 |
| Mr. D.J. Roach | 100 |
| Mr. R.F. Row | 100 |
| Mr. & Mrs. C. Roxburgh | 100 |
| Mr. H. Sadler | 100 |
| Sally Browne Pty. Ltd. | 100 |
| Mr. G.J. Samuel | 100 |
| Mr. R.A. Samuel, M.B.E. | 100 |
| Mr. N. Scott | 100 |
| Mr. A. Sebire | 100 |
| Mrs. M. Shankly | 100 |
| Mr. K.A. Skinner | 100 |
| Mr. L. Smith | 100 |
| The Southsiders | 100 |
| Mr. K.H. Spencer | 100 |
| Mr. T.N.D. Stevens | 100 |
| Mrs. A.K. Stewart | 100 |
| Mr. Neil Stewart | 100 |
| Taylor & Roberts | 100 |
| Mr. W. Tope | 100 |
| Rogers Charitable Trust | 100 |

| | | | |
|---|---|---|---|
| Travencore Group Pty. Ltd. | 100 | Mr. G.N. Webster | 100 |
| Mr. & Mrs. A.E. Tully | 100 | Mr. L.W. Weickhardt | 100 |
| VACC Insurance | 100 | Mrs. P.E. Weilandt | 100 |
| Mrs. W.J. Wark | 100 | Mr. E. Yencken | 100 |

Hewlett Packard donated an 8 Channel FM Tape Recorder for the Cardiac Surgery Laboratory — estimated value $20,000

# Century Club Membership 1984

Allen Foundry Trust Fund
Mr. & Mrs. H. Arnhold
Associated Graphics Pty. Ltd.
Mr. L.J. Baldy
Mr. & Mrs. H. Beaconsfield
Mr. & Mrs. K. Belcher
Mr. R.W. Bell
Mr. N.J. Bertalli
Mr. J.M. Bland
Dr. I.R. Bock
Mr. R. Bolwell
Mr. M.D. Bridgeland
Mr. J. Brown
Mr. R.T. Brunskill
Mr. W.A. Burleigh
Mr. & Mrs. M. Butler
Mr. H.T. Cadwallader
Miss L. Cairns
Miss M.H. Calder
Miss M. Carlyle
Dr. L. Carp
Mr. R.J. Charleston
Sng. R/Adm. R.M. Coplans
Mr. G. Cormack
Mr. F. Crean
Dr. S.E. Csordas
Mr. & Mrs. K.M. Cunningham
Mr. W.E. Curham
Mrs. M.E. Cutler
Mr. F.D. Danglow
David Bull Laboratories Pty. Ltd.
Dame Joyce Daws
Prof. Derek Denton
Mrs. G.E. Downes
Mr. & Mrs. M.G. Downes
Mr. H. Dowling
Dr. W.C. Dwyer
Mrs. H. Eather
Mr. & Mrs. M. Eckhaus
Mr. H. Van Elkan
Ms. J.F. Eltham
Mr. R.T. Elvish
Mr. J. Engelbert
Mr. L. Fih
Florentino Restaurant
Forge's Pty. Ltd.
Mr. H.C. Foulkes
Dr. F.A. Fox
Furn-A-Home
Dr. J.M. Gardiner
Mr. V.G. Garner
Miss E. Gaylard
Mr. J.J. Gelb
Mr. B. Gelbart
Mr. E. Giorgione
Miss H.D. Glascodine
Dr. & Mrs. M. Gooey
Miss M. Greig
Mr. P. Grey
Mr. J. Guest
Mr. S.M. Guilfoyle
Mr. M.R. Ham
Mr. J.D. Harris
Mrs. J. Harris
Miss I.L. Hicks
Mr. J.M. Hilliard
Mr. T.G. Hinds
Sir John Holland
Mr. P.M. Hudson
Miss S.J. Hunt
Dr. S.J. Iceton
Mr. C.H. Ineke
Mr. & Mrs. F.T. Ireland
Jacqueline Eve Vic.
Mr. F.R. Jennings
Miss W. Johnson
Dr. F.C. Jones
Miss G. Jones
Mr. M. Joss
Mr. R. Jukes
Mr. M. Kaye
Mr. A.M. Kilfour
Mr. L.J. King
Mr. A. Kouymans
Mr. & Mrs. R. Lawton
Mr. J. Leicester
Dr. P. Leigh-Jones
Mr. J.B. Levingston
Mr. F. Liebmann
Mr. L. Long
Mr. R.J. Loughnan
Dr. D.N. Madill
Mr. B.A. Marquez
Martyniuk Family
Mr. & Mrs. J. Marsh
Ms. M. Matthews
Mayfair Fashions Pty. Ltd.
Mrs. Emily McGuinness
Mr. V.J. McLeod
Mr. J.D. Merralls
Miss M. Moore
Mr. R.G. Moore
Mr. & Mrs. A. Muraca
Rev. Father B.F. Murphy
Miss G.B. Murray
Mr. R.H. Needham
Mr. G. Niall
Mr. R.J. Noakes
Noble Park Newsagency
Miss S. O'Donnell
Dr. K. Oppenheimer
Mr. D. Palm
Mr. & Mrs. G.W. Parsons
Mr. J.N. Perry
Mr. H.I. Phillips
Mr. J.D. Phillips
Mr. I.F. Phipps
Mr. & Mrs. T.G. Pickford
Mr. G.A. Pinches
Mr. J.H. Pincott
Mr. R.K. Pitman
Miss E. Service
Mr. R.A. Waters
Mr. H.E. Watson
Mr. W.G. Wicks
Mr. K.O. Wilks
Mr. B.F. Williams
Mr. K.O. Woodward
Mr. A.J. Worner

# 36th Annual Report of The Alfred Hospital Clinical Research Unit

Clinical Research Unit
Clinical Pharmacology Unit
Vascular Laboratory

# Clinical Research Unit

## Staff List

**Director**
P.I. Korner, M.D., B.S., M.Sc., F.R.A.C.P., F.A.A.

**Deputy Director**
P.J. Nestel, M.D., B.S., F.R.A.C.P.

**Associate Director**
J. Ludbrook, M.D., Ch.M., D.Sc., B.Med.Sc., F.R.A.C.S., F.R.C.S.

**Medical Staff**
P.A. Blombery, M.B., B.S., B.Sc.(Med.), Ph.D., F.R.A.C.P. Vascular Physician
A. Broughton, M.B., B.S., Ph.D., F.R.A.C.P. Assistant Physician
M.D. Esler, M.B., B.S., B.Med.Sci., Ph.D., F.R.A.C.P. Assistant Physician
G. Hasking, M.B., B.S., F.R.A.C.P. Assistant Physician
G.L. Jennings, M.D., B.S., M.R.C.P.(U.K.), F.R.A.C.P. Staff Physician
A. Lim, M.B., B.S., F.R.A.C.P. Assistant Physician

**Biochemical Pharmacology Laboratory**
A. Bobik, Ph.D.(Syd.), M.Sc., B.Pharm. Officer-in-Charge
G. Jackman, Ph.D.(Lond.), B.Sc., A.R.A.C.I., A.R.C.S.
P. Little, Ph.D.(Syd.), M.Sc., B.Pharm.

**Scientific and Technical Staff**
A. Bruce, SRN
D. Burton, B.Sc.(Hons) (to February, 1985)
E. Dewar, B.Sc.
G. Mill, B.Sc.(Hons)
A. McKenzie
L. Nelson, B.Sc.
C. Oddie, M.Sc.
L. Rowlands, B.Appl.Sc.
P. Scott, B.Appl.Sc.
H. Skews, B.Sc.(Hons)
J. Snell, M.Pharm.

**Dietitian**
R. Chuang

**Registrars**
S. Purser, M.B., B.S.
D. Mutimer, M.B., B.S
J. Sasadeusz, M.B., B.S.
J. Waterston, M.B., B.S.

**Residents**
N. Ferris, M.B., B.S.
E. Redmond, M.B., B.S.
N. Levick, M.B., B.S.
P. Braun, M.B., B.S.
M. Siddins, M.B., B.S.

**Laboratory and Ward Sister**
S. Scealy, SRN

**Hypertension Clinic Sister**
M. Henderson, SRN

**Charge Nurse Ward 2A**
A. Story, SRN

**Secretary**
C. Harwood

**Projects**
Redevelopment of essential hypertension after cessation of long term therapy.

Circulatory fitness in man.

Pathogenesis and therapy of congestive heart failure.

Echocardiographic analysis of cardiac dimensions and contractility.

Assessment of sympathetic function in man.

## Summary

Work is centred upon the mechanisms, prevention and treatment of the common cardiovascular diseases in man. Much of our recent effort has been in the field of hypertension and how this can be influenced by regular exercise.

We used a new approach to investigate the cardiovascular "benefits" of exercise. There is a strong feeling that exercise helps prevent heart disease, but there is little hard data on the longterm effects of exercise, the mechanism involved, or of the amount of ex-

ercise required to achieve benefit. Normal volunteers were studied at the end of one month periods at 4 different randomised levels of activity chosen to represent: (i) minimal activity (including a period in hospital), (ii) sedentary activities, (iii) "social" exercise (40 minute periods on an exercise bicycle 3 times weekly), (iv) "competitive" level (daily 40 minute exercise periods). From our measurements of changes in major cardiovascular risk factors, haemodynamics, metabolic and hormonal factors at each level of activity we hoped to form conclusions on the long-term effects of exercise on the circulation and metabolism and about the amount required to improve cardiovascular health. We found that the benefits of exercise included a fall in blood pressure and cholesterol, reduction in the activity of the sympathetic nervous system and improved glucose utilization. Most of these changes were evident after three times weekly exercise with only small additional benefit when exercise was performed seven times each week.

In view of the unequivocal and substantial effect on blood pressure we have now entered a group of patients with essential hypertension into a study of similar design. Preliminary results suggest that in hypertension there is a similar fall of 10-20 mmHg as in normal subjects. The fall is associated with a reduction in vascular resistance and in plasma noradrenaline, which is a measure of activity of sympathetic nervous system. It is clear that the value of using exercise to help in the therapy of hypertension has been underestimated. We do not yet know the mechanism for the fall in blood pressure although we have evidence that changes in sympathetic activity is involved. The results of these studies are now being applied and a large number of patients with mild and borderline hypertension attend our laboratory for an exercise program supervised by Lisa Nelson. Their results will help to clarify the value of exercise in management.

A new Cardiac Ultrasound and Doppler machine was purchased through the Ethel Mary Baillieu Fund and has had a major impact on our work. We can visualize the heart and the thickness of the muscle of its various chambers. After drug treatment the degree of reversal of left ventricular thickening is important in determining the rate of redevelopment of hypertension when the drugs are stopped in patients who have been treated for several years. With good reversal we may in future be able to select patients whose blood pressure can be maintained without drugs at normal levels. We are now searching for drug combinations which will not only result in good control of blood pressure, but which will reverse the structural changes in the heart and blood vessels as rapidly as possible. The new machine has been invaluable in our studies of cardiac failure and exercise. It also allows visualization of atherosclerosis of the carotid arteries, an important cause of stroke. Dr. Eljas Laufer has assisted greatly in this work.

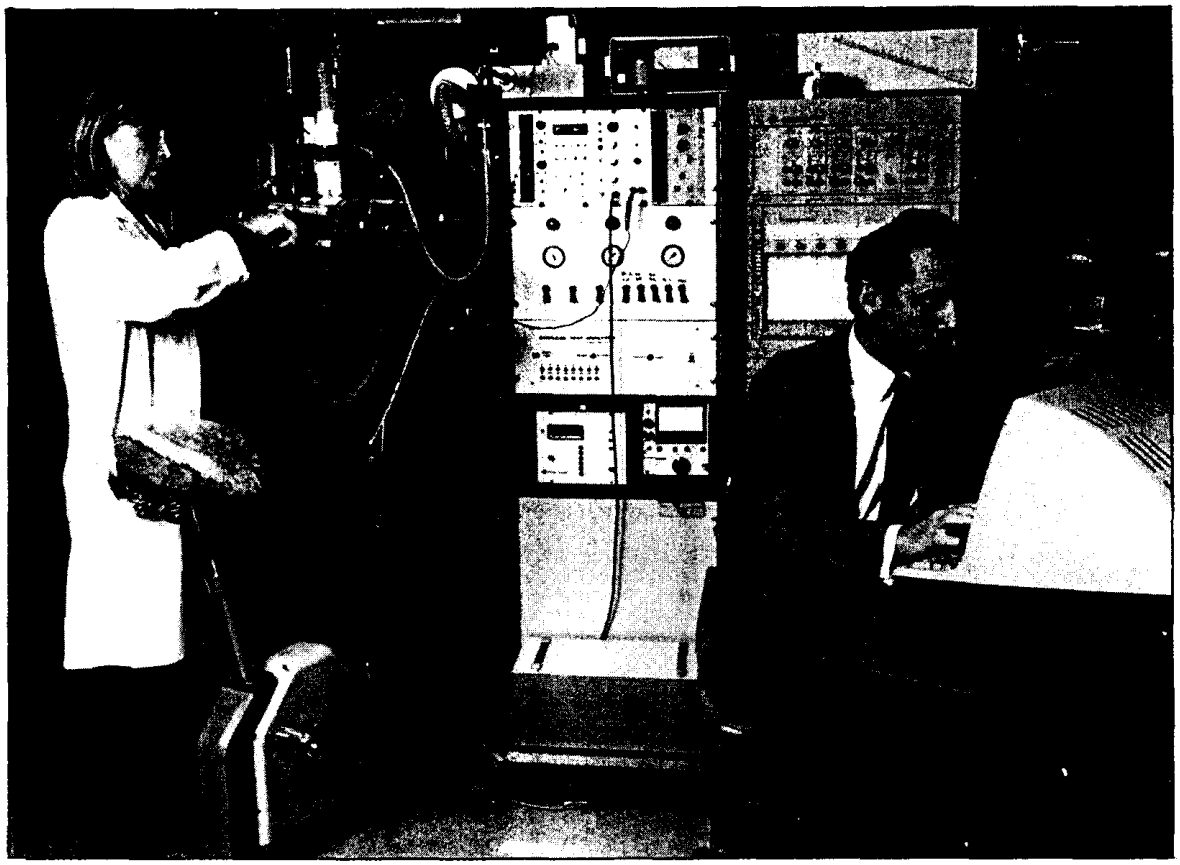
### Effects of Exercise on Cardiovascular Risk

L. Nelson, G. Jennings, M. Esler, D. Burton, P. Nestel, J. Bazelmans, P. Korner

We have studied four levels of activity in randomised order in 12 sedentary normal subjects. Activity levels, each of one month"s duration, were (i) a period of minimal activity, (ii) normal sedentary activities, (iii) 40 minutes at 60-70% of maximum work capacity (Wmax) on a bicycle ergometer thrice weekly and (iv) similar exercise daily. At the end of each month we measured heart rate, blood pressure and systemic haemodynamics and assessed sympathetic nervous activity and insulin sensitivity, lipids and various hormones. The measurements were performed 48 hours from the end after each activity.

Minimal activity, which included two weeks in hospital, had only minor effects on heart rate, blood pressure, haemodynamics and noradrenaline "spillover" rate, but impaired glucose utilisation by significantly reducing insulin sensitivity.

Exercise three times/week lowered resting blood pressure by 10/7 mmHg ($P < 0.05$) due to reduction in total peripheral resistance ($P < 0.01$). Similar haemodynamic changes were observed with the daily exercise schedule. After exercise at the rate of 3 times/week there was a significant increase in insulin sensitivity (29%, $P < 0.05$), but the latter declined significantly on the 7 times/week



*Lisa Nelson and Dr. Garry Jennings calibrating the exercise equipment.*

schedule. Total plasma cholesterol was reduced after daily exercise, but no changes were observed in triglycerides or HDL cholesterol.

Increased physical activity thus induced several favourable reductions in cardiovascular risk. The major benefit on blood pressure was similar with the 3 times/week and 7 times/week exercise schedules. But the metabolic benefit declined on the 7 times/week schedule, suggesting that maximum benefit may be associated with moderate exercise at the rate of 3-4 times/week

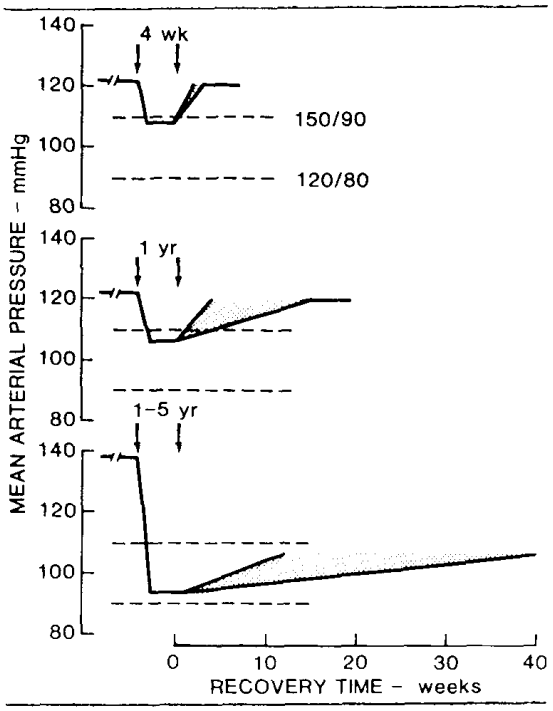
### Does Treatment of Hypertension Normalise Left Ventricular Mass?

G. Jennings, E. Laufer, G. Hasking

Antihypertensive therapy prevents the development of left ventricular hypertrophy (LVH) in animals with various types of experimental hypertension. Similarly, clinical studies have shown reduction in LV mass determined echocardiographically, but there is little information on the extent to which treatment can restore LV mass to normal.

We have measured LV mass in 30 normal subjects (age 17-55 years) and compared the results with those in 11 subjects with essential hypertension (age 29-64 years) who had received antihypertensive therapy (beta blocker + diuretic) which had lowered their blood pressure to $< 160/90$ mmHg on each clinic visit for at least 2 years. The drugs were then stopped and we monitored the redevelopment of hypertension and the changes in cardiac wall dimensions over the subsequent 3 months. LV mass was measured by two dimensional echocardiography, using the average measurements obtained by three "blinded" observers.

We found that LV mass of the normal subjects was significantly related to age and body surface area (BSA) by the relationship: LV mass = 184.5 BSA — 0.77 age — 88.17, $r = 0.73$, $P < 0.001$. But no correlation could be demonstrated with resting blood pressure, family history of hypertension or habitual level of activity.




*The recovery of BP after stopping therapy for hypertension after 4 weeks (upper), 1 year (middle) and a longer period (lower). With increasing duration of effective BP control, hypertension returns more slowly, reflecting better reversal of the secondary effects of hypertension on the circulation.*

In 5/11 patients with hypertension, LV mass was within 10% of the value predicted by the formula obtained in normal subjects. This subgroup of patients had received effective antihypertensive therapy for 5 years or longer, but the 6 patients in whom LV mass was > 10% above the predicted value had been treated for only 1-4 years. We found that LV mass was significantly related to total peripheral resistance after autonomic blockade ($r = 0.68$, $P < 0.05$), which is a measure of smooth muscle tone and is elevated in hypertrophy of the peripheral vasculature associated with hypertension. There was also a significant correlation between LV mass at the end of therapy and the systolic blood pressure observed 3 months after stopping therapy ($r = 0.72$, $P < 0.01$).

Over the 3 months off therapy, average supine blood pressure rose by 14/7 mmHg (both $P < 0.05$). There was a greater rise in standing blood pressure which averaged 21/16 mmHg (both $P < 0.01$). However, LV mass did not increase over this period.

Thus, patients with essential hypertension, which has been well treated for > 5 years, LV mass is closely similar to that of normotensive subjects of the same age and BSA. But in patients treated for shorter periods LV hypertrophy is usually still present. The amount of residual cardiac hypertrophy is a useful clinical measurement and is more direct than measures of hypertrophy of the peripheral vasculature. It appears to be an important determinant of the rate of redevelopment of hypertension on stopping therapy. It may be of value in relation to predicting outcome of non-drug treatment of hypertension, such as regular exercise, at the end of a period of drug therapy.

## How Hypertension Redevelops After Cessation of Long Term Therapy

G. Jennings, P. Korner, E. Laufer, M. Esler, D. Burton, A. Bruce, M. Henderson

The circulatory abnormalities in essential hypertension are generally regarded as due to a fundamental cause(s) of the hypertension and secondary mechanisms which occur as part of the body's homeostatic response to the elevated blood pressure (BP). Of particular importance is hypertrophy (i.e. enlargement) of the muscles of the heart and vessels which amplify cardiovascular stimuli. We have previously examined the effects on the non-autonomic component of total peripheral resistance (TPR) of one year's treatment of patients with essential hypertension. Non-automatic TPR is a measure of vascular structural changes and is the main contribution to elevated TPR in untreated hypertension. We found that non-automatic TPR was almost completely restored to the normal range after one year's normotension, suggesting substantial diminution in the amplifying capacity of the vessels. An interesting finding in these patients was that cardiac index one week after stopping therapy was significantly higher than in non-hypertensive subjects, so that the year of therapy had altered the haemodynamic pattern from one typical of established hypertension to one characteristic of mild or borderline hypertension. One explanation for these changes in haemodynamic pattern is that treatment for this period resulted in more complete reversal of vascular than cardiac hypertrophy. In this group the blood pressure has returned to pretreatment values in 10/13

patients after 5 weeks without therapy. In 3 patients the return was more gradual — 3, 4 and 8 months.

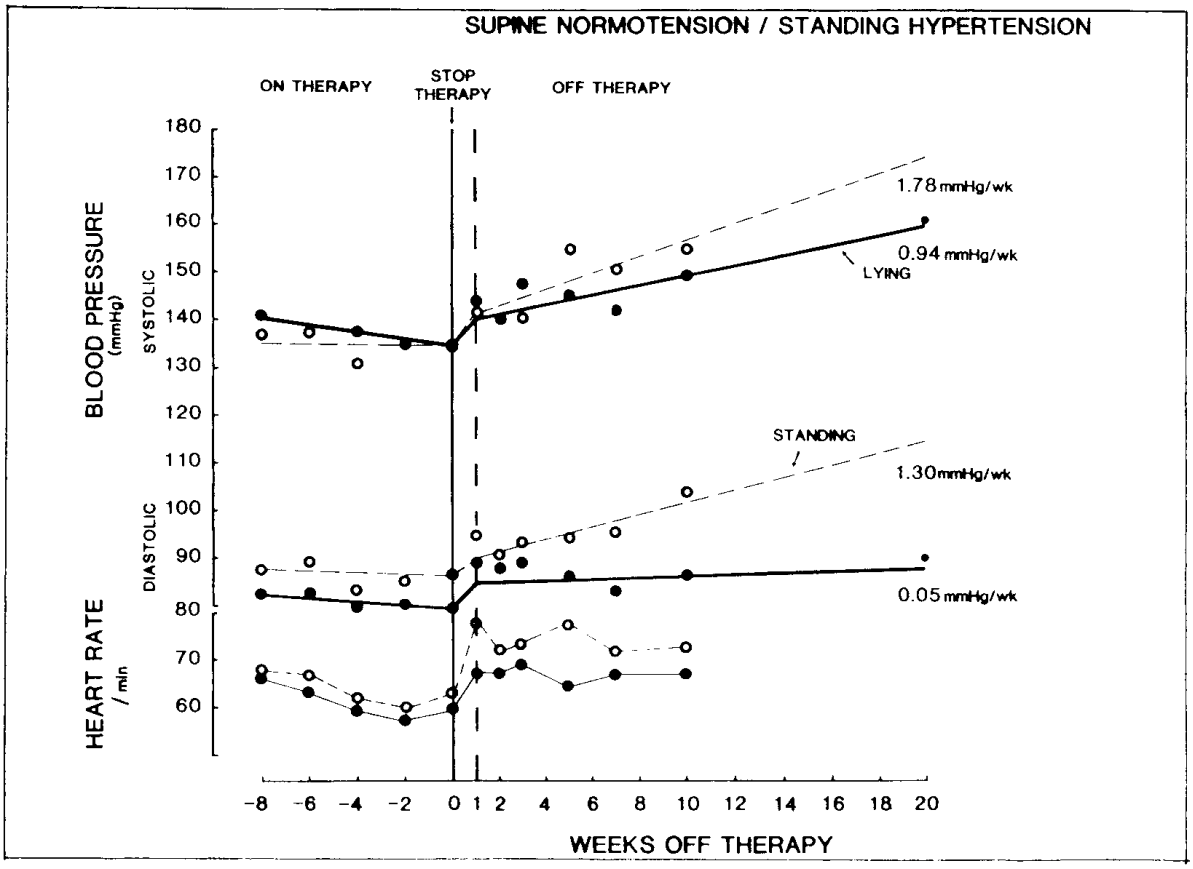
In the present study, we studied patients treated for longer than one year. Our hypothesis was that the longer period might result in further regression of cardiac hypertrophy and that as a result the redevelopment of hypertension when treatment was stopped might be more gradual.

After an average of 7.5 (1.5 — 27) years of successful treatment, supine blood pressure returned towards pretreatment levels at a slower rate than in patients treated for only one year. In both groups there was regression of muscle enlargement of the vessels during therapy, as justified by TPRI values after autonomic blockade. The two groups had hypertension of similar severity before treatment. In the group treated for more than one year the blood pressure was slightly lower during therapy (mean MAP 94 mmHg) than in the group treated for only one year (mean MAP 105 mmHg) although drug regimen was similar.

Longer effective therapy may have allowed more complete regression of cardiac hypertrophy. Only patients with "normalised" blood pressures for more than 3 years had normal LV mass. On stopping therapy, cardiac index measured was lower in the present group than in the earlier series studied after one year's therapy. Although at entry all patients had supine systolic BP on therapy < 160 mmHg, this variable on the last day of therapy was directly related to LVMI. A similar relationship was apparent at measurements made 10 weeks later. Our findings suggest that the lower the residual left ventricular hypertrophy, the slower the return of BP.

The rate of return of pretreatment values of the BP on standing was almost double the corresponding value whilst lying down. Standing heart rate was also higher. This suggests some enhancement of postural reflexes, and may represent sympathetic

*Systolic and diastolic blood pressure and heart rate before and after stopping therapy measured supine (unbroken lines, closed dots) and standing (broken lines, open dots). The rate of increase of blood pressure was determined from the slopes of linear regression equations relating BP with time. All subjects are included up to 10 weeks of therapy. The 20 week point is the mean of 4 subjects.*



overactivity. Haemodynamic measurements and assessment of sympathetic activity were made only in the supine posture. The former showed a slight increase in TPRI during the period off therapy and the latter remained normal throughout. Exaggerated sympathetic reflex effects on TPRI or cardiac output may have played some role in the recovery of blood pressure, despite the absence of abnormalities in the supine posture. Sodium intake, BP or renin levels did not appear to contribute to the haemodynamic changes after therapy was stopped.

The haemodynamic pattern that develops after stopping drugs resembles that of mild hypertension, in that there is more rapid increase in standing and systolic blood pressures than lying or diastolic. We conclude than the residual amount of left ventricular hypertrophy appears an important determinant of the rate of return of hypertension after long-term therapy.

### New Drugs for Congestive Heart Failure

G. Hasking, L. Nelson, C. Oddie, A. Bobik, G. Jennings

We have begun studies on two drugs, Ro 13-6438 and milrinone, which have positive inotropic and vasodilator effects. We found that Ro 13-6438 increased resting cardiac output without important effects on heart rate and blood pressure. However, our pharmacokinetic data demonstrated that the clearance of the drug was reduced in patients with heart failure. Plasma levels and half-time were considerably increased. These properties make the drug less useful for such patients than was expected from studies in animals and normal subjects.

# References

(see also Baker Institute Report)

**ESLER M, JENNINGS G, LEONARD P, SACHARIAS N, BURKE F, JOHNS J, BLOMBERY P.**
Contribution of individual organs to total noradrenaline release in humans. Acta Physiol Scand, Suppl. 527:11-16, 1984.

**ESLER M, WILLETT I, LEONARD P, HASKING G, JOHNS J, LITTLE P, JENNINGS G.**
Plasma noradrenaline kinetics in humans. J Auton Nerv Syst 11:125-144, 1984.

**JENNINGS G, KIAT H, NELSON L, KELLY MJ, KALFF V, JOHNS J.**
Enalapril for severe congestive heart failure: A double-blind study. Med J Aust 141:723-726, 1984.

**O'HEHIR R, ESLER M, JENNINGS G, LEONARD P, LITTLE P, JOHNS J, PANETTA F.**
Two patients with abnormalities of regional sympathetic nervous tone. Aust NZ J Med 14:855-859, 1984.

## In Press

**JENNINGS GL, KORNER PI, LAUFER E, ESLER MD, BURTON D, BRUCE A.**
How hypertension redevelops after cessation of long-term therapy. J Hypertens.

**JENNINGS GL, NELSON L, ESLER MD, LEONARD P, KORNER PI.**
Effects of changes in physical activity on blood pressure and sympathetic tone. J Hypertens.

**KORNER PI, JENNINGS GL, ESLER MD, BROUGHTON A.**
Role of cardiac and vascular amplifiers in the maintenance of hypertension and the effect of reversal of cardiovascular hypertrophy. Clin Exp Pharmacol Physiol.

# Clinical Pharmacology Unit

**Director:**
Dr. A.J. McLean, B.Sc. (Med), M.B.,B.S., Ph.D. (Mon), F.R.A.C.P.

**Scientific Staff:**
Dr. H.R. Corbett, B.V.Sc. (Melb).
Dr. P.M. Harrison, B.Tech.(Hons), PhD.
Mrs. L. Demos, B.Pharm.

**Technical Staff:**
Mrs. C. Cahill, B.Appl.Sci.
Mrs. A. Tonkin, B.Appl.Sci.

**Clinical Fellow:**
Dr. P. McCarthy, M.B.B.S., F.R.A.C.P.

**Students:**
Mrs. A. Byrne, B.Sc.
Mr. G. Farrugia, B.Sc.
Mr. D. McMillan, B.Sc.(Hons)

## Projects

Audit and rationalization of peptic ulcer therapy.

Audit and rationalization of oncological therapy.

Clinical pharmacology of anticonvulsant drugs.

Metronidazole usage in surgical patients.

Computer-based study of outpatient polypharmacy.

Hepatic blood flow and extraction of high clearance drugs.

The Clinical Pharmacology Department has now become an autonomous department within the Alfred Hospital. However, clinical and scientific links with the Clinical Research Unit and Baker Institute are actively maintained. Our involvement in clinical therapeutics has broadened:— the unit is now represented on the Ministerial Working Party on "Drug Use in Victorian Hospitals" and also on the "Antibiotic Usage Project" of the Victorian Medical Postgraduate Foundation Advisory Committee.

## Summary

**Cancer:** Our most recent initiative has been in the therapy of haematological and solid-tissue malignancies. We have carried out detailed audits within the Alfred and had access through the Ministerial Working Party to the data through the entire State of Victoria. Additionally we have extended our analysis to include other modes of cancer therapy (surgery, radiology) and care. The outcome of this analysis has been that oncological therapy is not optimal in Victoria due to a lack of radiotherapy resources, with consequent excessive reliance on chemotherapy. Assuming known disease incidence, it has been possible to demonstrate that the Alfred Hospital should be developed as a major therapy centre for oncological disease with integrated surgical radiotherapeutic and chemotherapeutic services serving the Monash affiliated hospitals and the population east and south of the line of the Yarra River.

**Peptic ulcer:** Our research into ulcer therapeutics had yielded two avenues of progress. Based on our demonstration that healing rates and relapse rates vary independently among accepted therapeutic agents, we have examined quantitatively in a group of patients under therapy, the potential influence of healing rate versus relapse rate on population outcomes. In summary, relapse rates are much more important than healing rate in determining longterm outcomes. This is seen in Fig. 1, which shows that a drug with poor healing properties (which would normally result in exclusion by regulatory authorities) and favourable relapse properties (i.e. a low relapse rate) can yield a markedly superior longterm result when compared to a drug associated with rapid healing rates and unfavourable relapse properties (i.e. a high relapse rate). These findings indicate that our current approaches to the selection of anti-ulcer medications (i.e. developmental, regulatory and clinical choices) are based

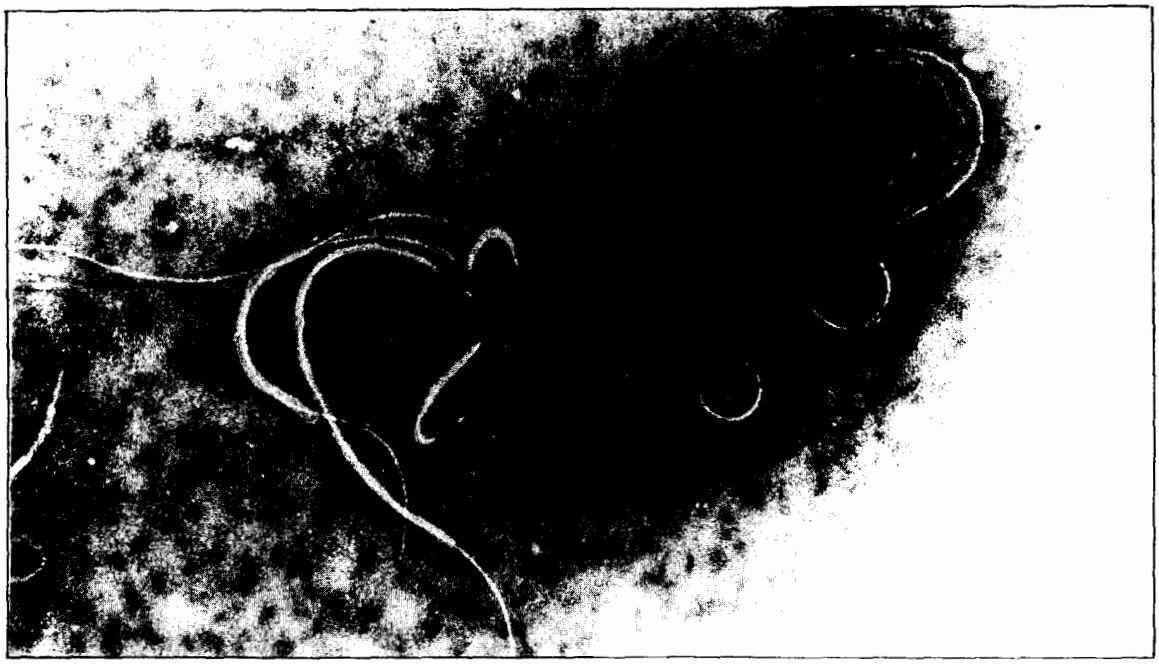
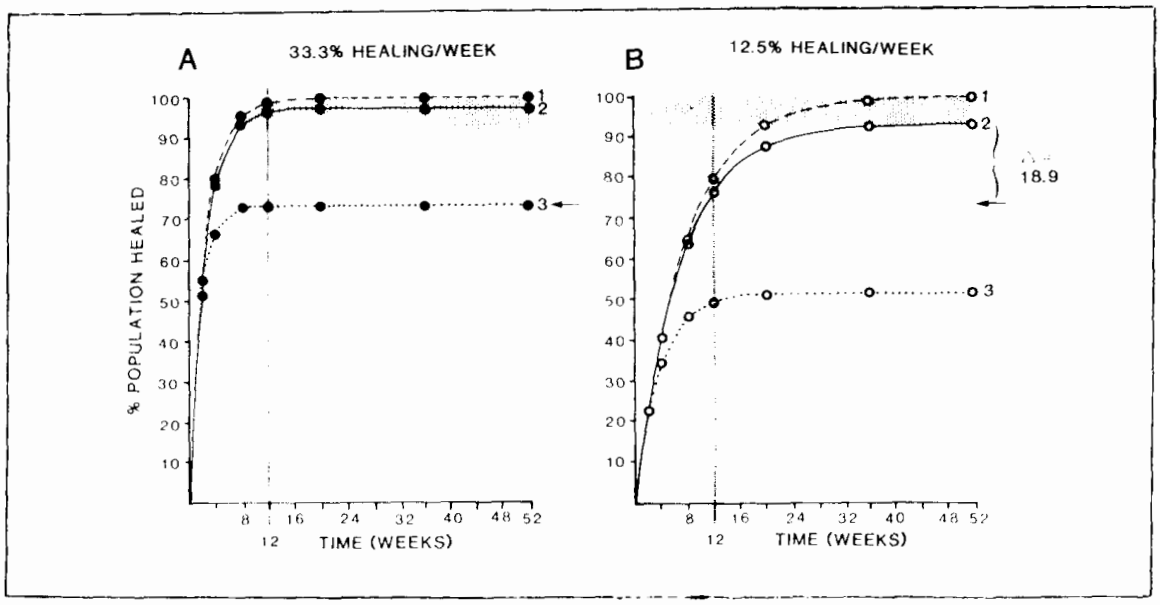


*Figure 2. Electron micrograph of Campylobactor-like organism associated with chronic gastritis and peptic ulcer. It is a gram negative, rod shaped, flagellated bacterium.*

on a potentially misleading measure of drug action (i.e. healing rate).

The evidence that relapse rates can be influenced by prior drug therapy over the following 12 month period raised important issues about mechanisms. It was difficult to postulate local changes in the physiology of the gastrointestinal tract which would "carry over" for such long periods of time. Accordingly, pathological phenomena had to be invoked. The description of a new species of gastrointestinal micro-organism (Campylobacter pyloridis — Figure 2) in association with both chronic gastritis and peptic ulceration provided one possible mech-

*Figure 1. Illustration of the influence of ulcer relapse rates at two healing rates; 33.3% healed/week – Panel A, 12.5% healed/week – Panel B. The numbers (1, 2, 3) indicate relapse rates of 0.4%/month, 4%/month and 40%/month respectively.*



anism. We are investigating whether bacterial action causes peptic ulcer relapse, attempting to test Koch's postulates in our study populations. To study possible associations between the organism and the state of the ulcer syndrome in a large number of patients, we have been performing biopsies and looking at serological markers of bacterial colonization of the gastrointestinal tract. This area of research may have implications for the therapy of peptic ulcer. Our work on ulcer therapeutics reinforces the point made by Professor Korner in the 1983/84 Baker Institute Annual Report:— "With real understanding (of the causes of disease) comes more specific, more effective, and usually *cheap* remedies".

### Splanchnic Physiology & Hepatic Drug Clearance

In our studies of splanchnic vascular physiology and hepatic function, we have established that presystemic drug interactions involve mechanisms other than altered splanchnic blood flow. We are systematically exploring other mechanisms, including alterations in functional state of metabolic enzymes and in the plasma protein binding of hepatic substrate. Our re-evaluation of the potential role of protein binding change as a limiting constraint on high clearance substrates is now widely accepted. Progress in our studies has involved the development of new analytical methods to allow comprehensive description of substrate (propranolol) metabolism and the development of a variety of "in vitro" techniques.

### Drug Prescribing

Another major initiative has been in the area of drug use, using computer-based retrieval techniques in the area of prescribing. Using an extreme definition of polypharmacy ( $\geq 10$ different medications during any one period of attendance) we have established that polypharmacy is frequent (3.5% of the overall population), related to age (incidence of polypharmacy versus age by decade, $r^2 = 0.992$) with cardiovascular agents heavily represented in the older age group. Duplication of therapies for angina and cardiac failure were particularly common in the elderly as was the use of psychotropic agents and non-prescription drugs (represented in 46%, 64% and 97% of polypharmacy scripts respectively).

We wish to acknowledge the generous provision of space within the Baker Institute, the unstinting help of the Alfred Hospital Biological Research Unit staff and the assistance of numerous collaborators.

# References

**BYRNE AJ, McNEIL JJ, HARRISON PM, LOUIS W, TONKIN AM, McLEAN AJ**
Stable oral availability of sustained release propranolol when co-administered with hydralazine or food; evidence implicating substrate delivery rate as a determinant of presystemic drug interactions. Brit J Clin Pharmacol 17 (Supp 1): 45s-50s, 1984.

**BYRNE AJ, MORGAN DJ, HARRISON PM, McLEAN AJ**
Variation in hepatic extraction ratio with unbound drug fraction: Discrimination between models of hepatic drug elimination. J Pharmacol Sci 74: 205-207, 1985.

**COLLIER G, McLEAN A, O'DEA K**
Effect of co-ingestion of fat on the metabolic responses to slowly and rapidly absorbed carbohydrates. Diabetologia 26: 50-54, 1984.

**EFTHIMIOU H, MORGAN DJ, IOANNIDES-DEMOS L, RAYMOND K, McLEAN AJ**
Influence of chronic dosing on theophylline clearance. Br J Clin Pharmacol 17: 525-530, 1984.

**HARRISON PM, TONKIN AM, McLEAN AJ**
Determination of 4-amino-3-(p-chlorophenyl) butyric acid (baclofen) in plasma by high performance liquid chromatography. J Chromatogr 339: 424-428, 1985.

**HARRISON PM, TONKIN AM, McLEAN AJ**
Simple and rapid analysis of atenolol and metoprolol in plasma using solid-phase extraction and high-performance liquid chromatography. J Chromatogr. 339: 429-433, 1985.

**HEINZOW B, CORBETT H, CONSTANTINIDES S, BOURNE R, McLEAN AJ**
Interaction between oral hydralazine and propranolol. 1. Changes in absorption, presystemic clearance and splanchnic blood flow. J Pharmacol Exp Ther 229: 509-514, 1984.

**MELANDER A, McLEAN A**
Effect of food on the absorption and metabolism of drugs. Internal Medicine for the Specialist 5: 51-60, 1984.

**McLEAN AJ**
Rational choice of drugs for ulcer therapy, (Letter) Med J Aust. 141: 726-727, 1984.

**McLEAN AJ**
Sampling time for therapeutic drug monitoring. Prescriber. 7: 16-17, 1984.

**McLEAN AJ**
Drug usage in patients with liver disease. Saudi Med J 6: 1-2, 1985.

**McLEAN AJ, BARNED J, IOANNIDES-DEMOS L, NEL D, TONG N, MERCER W, WOOD TJ**
Computer based prescription audit as a research, education and management tool. Aust Health Rev 7: 260-268, 1984.

**McLEAN AJ, HARRISON PM, IOANNIDES-DEMOS L, BYRNE AJ, McCARTHY P, DUDLEY FJ**
Microbes, peptic ulcer and relapse rates with different drugs. (Letter) Lancet 2: 525-526, 1984.

**McLEAN AJ, IOANNIDES-DEMOS LL, SPICER WJ**
Current clinical applications and dose regimens of metronidazole and related nitroimidazoles. Med J Aust. 141: 163-166, 1984.

**McLEAN AJ, IOANNIDES-DEMOS LL, SPICER WJ**
Cost of nitroimidazoles (Letter) Med J Aust 141: 601- 602, 1984.

**McLEAN AJ, McCARTHY P, DUDLEY FJ**
Cytoprotective agents and ulcer relapse. Med J Aust 142 (Spec Suppt Feb 4): S25-S28, 1985.

**McLEAN AJ, TONKIN A, McCARTHY P, HARRISON P**
Dose-dependence of atenolol-ampicillin interaction. Brit J Clin Pharmacol 18: 969-971, 1984.

**SOMOGYI AA, KONG CB, GURR FW, SABTO J, SPICER WJ, McLEAN AJ**
Metronidazole pharmacokinetics in patients with acute renal failure. J. Antimicrob Chemother 13: 183-189, 1984.

## In Press

**HARRISON PM, TONKIN CM, CAHILL AJ, McLEAN AJ**
The rapid and simultaneous extraction of propranolol, its basic metabolites and conjugates from plasma and assay by high performance liquid chromatography. J Chromatogr.

**IOANNIDES-DEMOS LL, McLEAN AJ, WODAM J, TONG N, HEINZOW U, HORNE M, HARRISON PM, GILLIGAN BS**
Availability of drug assay results and dosage advice improves antiepileptic care in a specialist neurology outpatient clinic. Clin Exp Neurol 21, 1985.

**McLEAN AJ, HARRISON PM, IOANNIDES-DEMOS L, BYRNE AJ, McCARTHY P, DUDLEY FJ**
The choice of ulcer healing agent influences duodoneal ulcer relapse rate and longterm clinical outcome. Aust NZ J Med 15, 1985.

**McLEAN AJ, HARRISON PM, BYRNE AJ, McCARTHY P, DUDLEY FJ**
Relapse rates are more important than healing rates in determining the longterm outcome of duodenal ulcer therapy. Gastroenterology.

**McLEAN AJ, KNIGHT R, HARRISON PM, HARPER RW**
Clearance-based oral drug interaction between verapamil and metoprolol and comparison with atenolol. Am J Cardiol.

# Vascular Laboratory

**Head**
P.A. Blombery, B.Sc.(Med.), M.B., B.S., Ph.D., F.R.A.C.P.

**Technical Staff**
M. Persich, B.Sc.
J. Hilliard, B.Appl.Sc.

**Projects**

Hand blood flow in Raynaud's phenomenon

Cardiovascular effects of bucindolol

Effect of beta-blockers in intermittent claudication

Dosage of aspirin and platelet function

Computer follow-up of vascular surgery

## Summary

The major interests of the Vascular Laboratory has been the mechanisms involved in Raynaud's phenomenon and the assessment of new forms of therapy for this disorder, and the role of risk factors and place of medical treatment in atherosclerosis affecting the large peripheral arteries.

Raynaud's phenomenon is characterised by an abnormal constrictor response to the cold of the small vessels of the hands and feet. We have defined the role of irreversible structural changes in the small arteries of the extremities by measurement of blood flow in the hands at different temperatures. By raising the central body temperature of such patients with the use of electric blankets and hot water, we induced maximal vasodilatation in these small vessels, so that any reduction in blood flow observed under these circumstances provided an index of the extent of structural narrowing of the arterial supply of the hand. Using this technique, we have observed structural changes to be present in the hands of patients with scleroderma and the CRST syndrome — a variant of scleroderma — with a maximum blood flow only one-third that of normal. This explains the relative lack of effect of potent vasodilating drugs in such patients, which depend on the presence of reversible spasm rather than irreversible change for their maximal benefit. By measuring hand blood flow in a large number of patients with Raynaud's phenomenon, we have defined a new subset of patients who have evidence of minor structural changes in the vessels on blood flow testing and who also have an antibody in their blood to cell nuclei, normally seen in patients with the disorder called systemic lupus erythematosis. Our patients, however, show none of the clinical manifestations of lupus or other connective tissue diseases and it remains to be seen whether this is a very early stage of lupus or whether it will remain a separate disorder in its long-term progression.

In patients with atherosclerosis affecting the arteries to the lower limbs, we have been interested in two areas. There is evidence that the blood platelets in such patients become activated, releasing vasoconstrictive substances which may aggravate the disturbance in peripheral circulation as well as possibly causing further progression of their atherosclerosis. Because of these abnormalities, it has been suggested that drugs which affect platelet function, such as aspirin, may be useful in the management of peripheral vascular disease. Previously, large doses of aspirin, commonly 1.2 gm per day, have been administered to obtain these effects, but on the basis of theoretical evidence, it is possible that a very much lower dose of aspirin may be equally if not more effective. We have assessed the effects of very low doses of aspirin from 25 to 100 mg per day on platelet function in such patients, performed in collaboration with Prof. Firkin in the Department of Medicine. Early results indicate that a dose as low as 50 mg is effective in inhibiting the excessive stickiness and activation of the blood platelets in patients with peripheral vascular disease. Such a low dose of aspirin has a much lower incidence of side effects than the doses normally used in therapeutics and may be a useful adjunct in the management of such patients. A con-

trolled trial is now planned to determine the efficacy of low dose aspirin in preventing thrombosis of bypass grafts in the lower limbs.

Beta-blocking drugs, used in the treatment of hypertension and angina, have been reported to cause deterioration in the symptoms of patients with peripheral vascular disease. It is not know what the frequency of this side effect is in such patients, or whether there is any relationship between particular features of the individual beta-blockers and the severity of this side effect. We are performing a double-blind trial comparing the effect of pindolol — a beta-blocker with intrinsic sympathomimetic activity — and metoprolol — a cardioselective beta-blocker — with placebo in patients having both intermittent claudication due to narrowing of the arteries to the lower limbs and hypertension. Although the code for this trial has not yet been broken, there has been no evidence of an increased incidence of claudication in these patients during any phase of the study. Although we have noticed differences during the various phase in the hand blood flow responses to temperature, these results would suggest that worsening of symptoms of intermittent claudication during beta-blocker therapy is an uncommon side effect and that avoidance of this group of antihypertensive drugs in patients with peripheral vascular disease is unnecessary as a routine.

Our computerised vascular register now contains over 3,000 operations performed during the last ten years and is proving of great benefit in determining the long-term success rate of various forms of vascular reconstructive procedure. Using a programme developed in the Vascular Laboratory, we have compared the long-term patency rate of bypass grafts using arm veins for conduits rather than leg veins and found no evidence of a significant difference between the two. In most surgical centres, the leg vein is considered the most appropriate conduit for vascular surgery and in their absence, various forms of prosthetic material are usually used which have very much inferior results. The results of our comparison indicate that arm vein is a more suitable alternative.

## Projects

### Hand Blood Flow in Raynaud's Phenomenon

P.A. Blombery and M. Persich

There have been conflicting reports about the relative roles of structural changes in small arteries, the local vascular response to cooling and the effects of sympathetic activation in the pathophysiology of Raynaud's phenomenon. The aim of this study was to determine the effect of a range of hand temperatures on hand blood flow, both under resting conditions, during sympathetic activation by the cold pressor test and after maximal vasodilatation produced by body heating in three groups of patients with Raynaud's phenomenon — 9 with primary Raynaud's disease, 8 with scleroderma or CRST syndrome, 8 with undifferentiated connective tissue disease, and 8 normals. Hand blood flow at 20°C and 30°C was similar in normals and patients with scleroderma, but was significantly lower in patients with primary Raynaud's disease. In contrast, hand blood flow at 40°C and during body heating was 31 ml/100 ml/min in normals, 20.4 ml/100 ml/min in Raynaud's disease, 14.2 ml/100 ml/min in undifferentiated connective tissue disease, and 10.9 ml/100 ml/min in scleroderma. The flows in scleroderma and undifferentiated connective tissue disease were significantly lower than normal. The cold pressor test resulted in similar vasoconstrictions in all groups of patients and normals.

We conclude that (i) there is evidence of structural change in the hand blood vessels in scleroderma and in undifferentiated connective tissue disease in contrast to primary Raynaud's disease, (ii) there is no abnormal sympathetic responsiveness in patients with Raynaud's phenomenon, and (iii) patients with primary Raynaud's disease show increased local response to cooling, suggesting that the disease is caused by a smooth muscle abnormality.

### The Cardiovascular Effects of Bucindolol

P.A. Blombery and G.L. Jennings

Bucindolol is a new orally active, antihypertensive drug which lowers blood pressure by several mechanisms including

vasodilatation, beta-blockade and alpha-blockade. The haemodynamic effects of administration of 0.3 mg/kg intravenously and oral doses of 50 and 200 mg were assessed in ten normal subjects.

Intravenous administration had no effect on resting blood pressure, cardiac output or peripheral vascular resistance. In contrast, oral administration of 200 mg resulted in a modest reduction in arterial pressure with an increase in cardiac output and a fall in total peripheral resistance. There was an associated fall in forearm vascular resistance at the highest oral dose. The vascular response in the hand was variable and with intravenous administration of bucindolol, there was a reduction in hand blood flow, also seen after oral administration of 50 mg. After administration of 200 mg of bucindolol orally, hand blood flow increased to pre-dose levels. The relative roles of vasodilatation and alpha-blockade were assessed by determining the haemodynamic responses in both the systemic circulation and forearm circulation to tilt, and by measuring the reduction in hand blood flow which occurred after performing the cold pressor test. Bucindolol had no effect on the increase in total peripheral resistance induced by tilting, but at the same time there was a significant impairment in the forearm vascular response to tilt. The response to the cold pressor test was attenuated by 30 percent after both intravenous and oral administration of bucindolol. Resting heart rate was reduced by 8 beats/min after both intravenous and oral administration.

These results suggest that bucindolol is an effective hypotensive agent when administered orally at a high dose and that its blood pressure lowering effects are mediated mainly by direct vasodilatation. The beta-blocking effect of the drug prevents the increase in heart rate, seen with other vasodilating agents. There is evidence of mild alpha-blockade demonstrable in the forearm and hand circulations, but this effect does not make a significant contribution to the overall reduction in peripheral vascular resistance. The drug may be useful in the long-term treatment of hypertension.

# References

**ESLER M, JENNINGS G, KORNER P, BLOMBERY P, SACHARIAS N, LEONARD P.**
Measurement of total and organ-specific norepinephrine kinetics in humans. Am J Physiol 247:E21-E28, 1984.

**ESLER M, JENNINGS G, LEONARD P, SACHARIAS N, BURKE F, JOHNS J, BLOMBERY P.**
Contribution of individual organs to total noradrenaline release in humans. Acta Physiol Scand Suppl 527:11-16, 1984.

**KOPIN IJ, BLOMBERY P, EBERT MH, GORDON EK, JIMERSON DC, MARKEY SP, POLINSKY RJ.**
Disposition and metabolism of MHPG-$CD_3$ in humans: Plasma MHPG as a principal pathway of norepinephrine metabolism and as an important determinant of CSF levels of MHPG. *In:* Frontiers in Biochemical and Pharmacological Research in Depression (ed. E Usdin, M Asberg, L Bertilsson, F Sjoqvist) New York, Raven Press, 1984.

# 28th Annual Report of Ewen Downie Metabolic Unit

# Ewen Downie Metabolic Unit

# Annual Report 1984

## Staff List

**Director**
J.R. STOCKIGT, M.D., F.R.A.C.P.

**Deputy Director**
D.J. TOPLISS, M.B., B.S.(Hons), F.R.A.C.P.

**Active Consultant**
PINCUS TAFT, M.D., F.R.A.C.P.

**Visiting Physician**
H.D. BREIDAHL, M.D., F.R.C.P., F.R.A.C.P.

**Assistant Physicians**
D.W. LORDING, M.B., B.S., B.Med.Sci., F.R.A.C.P.
A.N. HUNTER, M.B., B.S., F.R.A.C.P.

**Honorary Consulting Physician**
BRYAN HUDSON, M.D., Ph.D., F.R.A.C.P.

**Honorary Consulting Biochemist**
JOSEPH BORNSTEIN, D.Sc., M.D., F.R.A.C.P.

**Clinical Assistants**
F.E.H. RUDOLPH, M.D.
R.W. SIMPSON, M.A., B.Med.Sci., B.M., B.Ch., M.R.C.P., F.R.A.C.P.
P.J. FULLER, M.B., B.S., B.Med.Sci.(Hons)

**Research Fellow**
K.N. WYNNE, M.Sc., Ph.D.

**Senior Scientist**
J.W. BARLOW, M,.Sc., Ph.D.

**Research Officers**
C-F. LIM, M.Agr.Sc.
C. BINGHAM, M.Sc. (to Sept 1984)

**Biochemists**
VIRGINIA MOHR (nee Stevens), B.Sc.
STEPHANIE DYER, B.Sc.
ANNE GIBBS, (nee Ioannou), B.App.Sci. (to Jan 1984)
BRENDA LE GRAND, B.Sc. (from Jan 1984)

**Registrar**
P.S. HAMBLIN, M.B., B.S.

**Resident Medical Officers**
HILARY J. DICKSON, B.Med.Sci, M.B., B.S.
I.T. MEREDITH, B.Sc.(Hons), M.B., B.S.
P.S. TALMAN, B.Sc.(Hons), M.B., B.S.
G.C. BEACOM, M.B., B.S.

**Nurse Technologist**
FIONA LONG, S.R.N.

**Ward Sister**
MARY ESPARON, S.R.N.

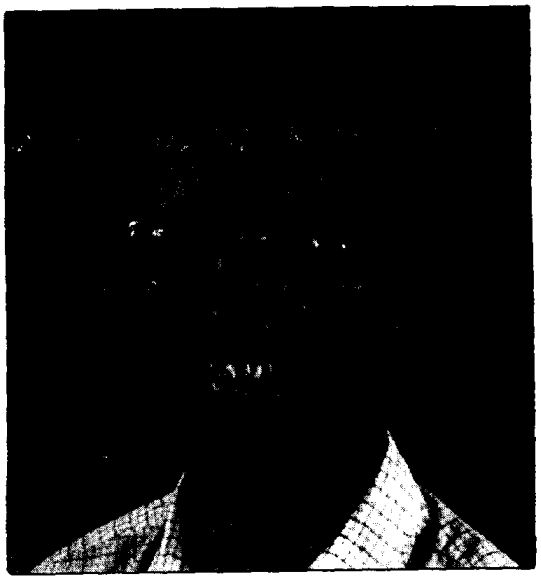
**Technical Staff**
IDA EKKEL, A.A.I.M.T.
FREDERICKE RABOLD

**Secretary**
HILARY HAMMOND

*Dr. Harald Breidahl retired from the senior visiting staff of the hospital at the end of 1984. A graduate of Melbourne University in 1948, he was appointed to the Unit in 1963. Dr. Breidahl served as Chairman of Medical Staff 1979-80 and was Vice President of the International Diabetes Federation 1976-1982. Dr. Breidahl will continue as an active consultant in 1985.*

*Dr. Douglas Lording, a graduate of Monash University in 1971 was appointed visiting endocrinologist in November 1984, to succeed Dr. Breidahl. After training in endocrinology at Prince Henry's Hospital and the Alfred Hospital he was appointed assistant endocrinologist in 1978. His special interests are in reproductive endocrinology and diabetes.*

*Virginia Mohr (nee Stevens) graduated B.Sc. from Monash University in 1977 and completed a M.Sc. qualifying year in 1984. She has been awarded the Alfred Hospital Whole-time Medical Specialists' Travelling Fellowship for 1985 and will visit laboratories in Minneapolis, Los Angeles, and Nashville as well as presenting work on abnormal thyroid hormone binding in Australian aborigines at the American Endocrine Society meeting in Baltimore in June 1985.*



*Dr. John Barlow graduated B.Sc. from Monash University in 1974 and was awarded M.Sc. in 1977. He joined the Unit in 1977 and has been senior scientist since 1982. His Ph.D. on familial dysalbuminaemic hyperthyroxinaemia was completed in 1984. He has been awarded a Neil Hamilton Fairley Applied Health Sciences Fellowship by the National Health and Medical Research Council and will be engaged in postgraduate work at the Institute of Cellular and Molecular Pathology of the University of Louvain in Brussels during 1985 and 1986.*

## Introduction

During 1984 the Unit continued its dual role as a research and clinical service department of the hospital. Thyroid disease continues to be the main aspect of research, but the clinical workload continues to be broadly based in all aspects of endocrinology and diabetes. We are increasingly called on to function as a reference centre for the assessment of diagnostically-difficult thyroid problems, especially in relation to newly recognized binding abnormalities.

We continue to enjoy helpful and valued collaboration with the Medical Research Centre Prince Henry's Hospital, Monash University Department of Medicine, the Baker Institute and Clinical Research Unit, and the Howard Florey Institute University of Melbourne.

Financial support, either direct, or in the form of equipment or travel funding from the following sources is gratefully acknowledged:

Estate of the late Vincenza Acton
Estate of the late H. Vistaline
G.M. Rollason Trust
Vivian Hill Trust
Alfred Hospital Whole-time Medical Specialists' Private Practice Fund
Research support from the National Health and Medical Research Council and the Alfred Hospitals Medical Research Committee is also acknowledged.

## List of Projects

Thyroid hormone pathophysiology

a. Thyroid hormone changes in critical illness
b. Effect of low affinity competitors on thyroid hormone plasma binding
c. Effect of frusemide on circulating thyroid hormone levels
d. Abnormal thyroid hormone binding in Australian aborigines
e. Expression of thyroid hormone action in hepatocytes

Evolution of mineralocorticoid hypertension.

Structure-activity relationships of steroid hormones.

Biologically-active principles in coffee preparations.

## Thyroid Hormone Research

Our major interest in the past year has been in the factors which alter thyroid hormone delivery to target tissues with particular reference to: (i) unusual binding abnormalities that may confound the biochemical diagnosis of thyroid disease; (ii) the effect of competitors, either exogenous or endogenous, that alter hormone binding in the circulation; (iii) factors that modify the diagnostic features of thyroid disease in the presence of associated disease.

In the circulation, thyroid hormones are carried in reversible association with various plasma proteins that differ widely in their affinity and capacity. This reversible association is in fact a rapidly-exchanging equilibrium, giving the protein-bound fraction a "reservoir" function which replenishes the free level as hormone is taken up by tissue receptors or degraded. For thyroxine, the free fraction is minute, being only one part in 3000 of the total. Direct measurements of the free concentration are not normally possible with any degree of precision, but numerous ingenious tricks have been developed to allow an estimate of the free concentration to be made. One standard method is to measure the extent of binding after dilution of serum and to infer from this, a free fraction for undiluted serum. While giving a valid comparison of binding for one hormone alone in different sera, this approach is not valid when low affinity competitors are studied.

It has long been known that a number of commonly used drugs (e.g. salicylate, diphenylhydantoin) can compete for $T_4$ and $T_3$ binding sites in serum, leading to acute displacement of hormone. It is now suggested that endogenous inhibitors may have a similar effect, especially in renal failure or critical illness. Generally, the drug inhibitors are bound to plasma proteins with much lower affinity than $T_4$. As serum is diluted, the effect of lower affinity competitors is gradually lost in any assay system for the following reason. At dilutions where hormone is still highly bound, binding of a lower affinity competitor has markedly diminished, leading to a bound/free relationship that differs greatly from undiluted serum in vivo. In effect, the reservoir or store of bound drug, available to replete the free concentration with progressive dilution, will become depleted more rapidly for lower affinity ligands. Hence, in order to make valid studies of the effect of low affinity competitors, it has been necessary to develop improved methods of studying binding precisely in undiluted serum. Major difficulties include the following: (i) The minute free fraction of $T_4$ means that a change in binding from 99.97% to 99.94% doubles the active hormone fraction; (ii) Impurities in labelled hormone. Even a minute contamination with free radioactive iodine, or an iodothyronine which does not bind, may greatly distort the apparent free fraction; (iii) The effect of transient competitors of short half-life, which may alter both hormone clearance and delivery to tissue, yet be undetectable in a subsequent serum sample; (iv) The presence of multiple serum binding sites for thyroid hormones, making it difficult to study interaction with a single serum protein.

The first two problems have been ad-

dressed by a technique of equilibrium dialysis with high volume of dialysate. Using 0.5 ml undiluted serum, less than 0.3% of the added [$^{125}$]I-$T_4$ enters the dialysate, so that even for samples showing the highest and lowest possible binding, variations in the dialysand (serum) count rate can be ignored in calculating the free fraction, making it necessary to sample only the dialysate. However, with very high binding, the majority of counts in the dialysate represents contaminants, rather than free [$^{125}$]I-$T_4$. Precipitation with magnesium chloride in the presence of added excess unlabelled thyroxine, results in a pellet of authentic hormone, the count rate allowing direct calculation of free fraction in undiluted serum. This technique, which measures the free fraction in undiluted serum with < 5% coefficient of variation, has been used to compare the relative importance of various competitors, including frusemide and free fatty acids.

The question of transient circulating competitors for hormone binding to plasma proteins has so far received little attention, but may be of great importance with the drug frusemide, shown to be the most potent drug inhibitor of thyroid hormone binding. Apart from an effect of high dose frusemide to lower circulating thyroid hormone levels, by allowing more rapid clearance of the higher free fraction, it is likely that this drug makes more hormone available to the tissue receptors which initiate thyroid hormone action. Hence, intermittent doses of a potent binding competitor of short half-life could lead to pulses of excess free hormone which might sustain tissue hyperthyroidism.

The presence of multiple classes of thyroid hormone binding sites in serum makes it difficult to study the interaction of hormone with a particular protein without laborious isolation procedures. However, by adjustment of hormone concentrations and serum dilution, it is possible to achieve substantial specificity. We have previously shown that artificially high hormone concentrations (50-1000 fold added excess of $T_4$) can be used to isolate the abnormal albumin-$T_4$ binding site of familial dysalbuminaemic hyperthyroxinaemia. The reverse approach, extreme serum dilution (1:10,000) lowers the total thyroxine concentration to about $10^{-11}$ molar. At such hormone concentrations only proteins with this order of affinity will participate in binding. Binding is still easily measurable (about 50%) and is specific for thyroxine binding globulin, as confirmed by the total absence of detectable binding in sera from subjects with hereditary deficiency of this protein.

These techniques form the basis of our current studies of factors that may influence thyroid hormone binding so as to alter clearance and delivery to tissues.

## Projects

### Changes in Thyroid Stimulating Hormone During Recovery from Hypothyroxinaemia of Critical Illness

P.S. Hamblin, D.J. Topliss, V.S. Mohr, S. Dyer, J.R. Stockigt.

The profound hypothyroxinaemia which can occur in apparently euthyroid subjects during critical systemic illness is usually regarded as predictive of high mortality. The development of the 'low $T_4$ syndrome' is associated with markedly enhanced $T_4$ clearance from blood and it has been suggested that circulating inhibitors of thyroid hormone plasma binding may be liberated from damaged tissues. Both basal TSH and TRH responsiveness are depressed in severe illness despite low free $T_4$ levels. When recovery from the low $T_4$ state occurs, the mechanisms of recovery are unclear, although transient elevations of TSH have been observed in some studies. In previous studies conclusions have usually been based on single samples.

In order to clarify the relationship between $T_4$ and TSH during recovery from critical non-thyroidal illness with hypothyroxinaemia, we studied multiple serial samples (5-20/subject) during six episodes of $T_4$ recovery in five clinically euthyroid patients who were hypothyroxinaemic during critical systemic illness (major burns, acute renal failure, severe metabolic acidosis, sepsis). These subjects had $T_4$ levels less than 30 nM for at least 48 h (range 2-9 days) followed by a $T_4$ rise of more than 25 nM in less than 96 h. The TSH assay had a detection limit of < 0.5mU/L with c.v. < 6% at 1-10 mU/L, allowing us to detect TSH changes within the normal range. The samples used for these

studies were taken for routine diagnostic indications and were made available by the Biochemistry Department and Renal Unit, Alfred Hospital.

Every $T_4$ rise was associated with a concomitant TSH increase of at least 2.5 mU/L. Each TSH increase of > 2mU/L was associated with a $T_4$ response. In the two studies with samples < 6 h apart, the TSH increase preceded the $T_4$ rise. During recovery, maximum TSH values were clearly above normal in two episodes (20, 15 mU/L), marginally elevated in two (6, 8 mU/L) and remained within the normal range in two (2.9, 3.3 mU/L). All patients were biochemically euthyroid at follow-up 4-20 weeks later.

This study demonstrates a close association between changes in TSH and $T_4$ during recovery suggesting that the $T_4$ response is TSH-mediated and that the thyroid is responsive to TSH during this phase. The TSH increase during recovery may represent a return of appropriate thyrotropin responsiveness to hypothyroxinaemia, or may be due to intrinsic pituitary recovery independent of circulating thyroid hormone levels. If $T_4$ recovery requires TSH, as this close correlation suggests, then circumstances that impair TSH secretion during prolonged critical illness (e.g. starvation, dopamine, glucocorticoids) may prolong the deficiency of circulating thyroid hormones and might result in eventual tissue hypothyroidism. The role of sustained hypothyroxinaemia in contributing to the outcome of prolonged critical illness remains uncertain.

**Frusemide as a Competitor for Serum Binding of Thyroid Hormones: In Vivo and In Vitro Studies and Comparison with Other Known Competitors**

C.F. Lim, V.S. Mohr, S.A. Dyer, F. Long, P.S. Hamblin, D.J. Topliss, J.W. Barlow, K.N. Wynne, J. Sabto, J.R. Stockigt.

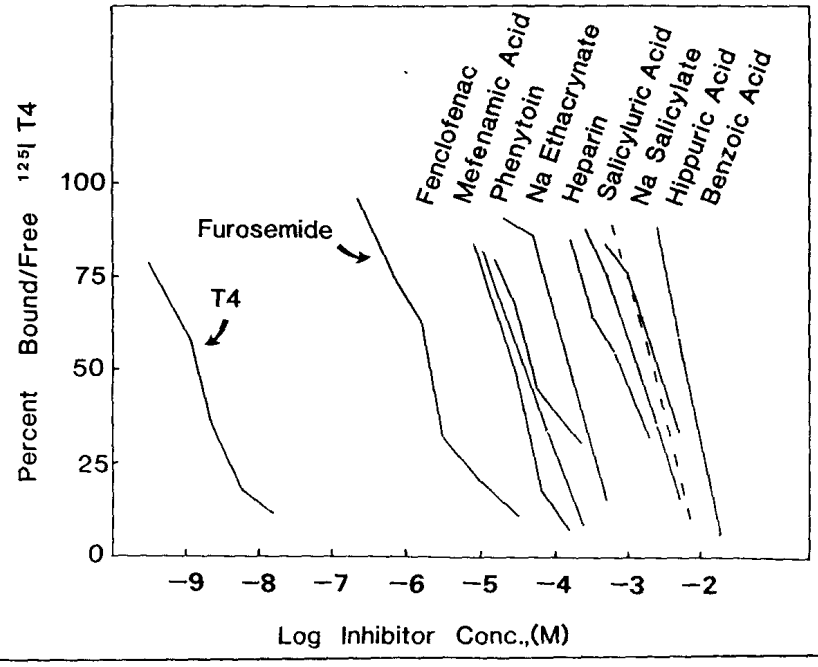
We have previously shown that frusemide is a low affinity competitor for plasma binding of $T_4$. As with other low affinity competitors, its effect diminishes with serum dilution in vitro, making it difficult to estimate the minimum in vivo inhibitory concentration from studies of diluted serum. We therefore studied the effect of frusemide on the percent free fraction of $[^{125}I]$-$T_3$ and $[^{125}I]$-$T_4$ in undiluted serum by equilibrium dialysis (37 C, Tris buffer, $MgCl_2$ precipitation). Frusemide in dialysand was measured by high performance liquid chromatography. In pooled normal serum, an increase of 20% and 50% in $T_4$ free fraction resulted from serum frusemide of 7 ug/ml and 30-40 ug/ml respectively. The threshold effect on $T_3$ free fraction was similar. In order to establish whether inhibition was accentuated with subnormal concentrations on plasma proteins, a serum pool from subjects with liver disease and hypoalbuminaemia (albumin 18 g/L, thyroxine binding globulin 9 mg/L (N 15-25), bilirubin 39 uM) was studied. A 20% increase in $T_4$ free fraction occurred at serum frusemide of < 2 ug/ml and 50% increase at 4-5 ug/ml.

Five chronic renal failure patients on peritoneal dialysis were studied after a single oral dose of 500 mg frusemide. After 4-5 h, a 26% mean increase in $T_4$ free fraction coincided with mean serum frusemide of 10 ug/ml. Resin $T_3$ uptake increased and total $T_3$ and $T_4$ decreased. Thyroxine binding globulin and reverse $T_3$ remained unchanged. As in the previous study, the effect of frusemide on $T_4$ free fraction was reproduced by frusemide added to serum in vitro.

Inhibition of $[^{125}I]$-$T_4$ binding by frusemide was compared with other known inhibitors (1:10 serum, 4 C, Tris buffer, dextran-charcoal separation). We also studied the effect of synthetic 2-hydroxybenzoylglycine which has recently been identified as an endogenous inhibitor of drug binding in patients with chronic renal failure. Comparative molar concentrations for 50% inhibition were in the ratio $T_4$ 1; frusemide $1 \times 10^3$; fenclofenac $1 \times 10^4$; 8-anilino-1-naphthalene sulphonic acid $3 \times 10^4$; merthiolate $4 \times 10^4$; sodium ethacrynate $1 \times 10^5$; heparin $7 \times 10^5$; 2-hydroxybenzoylglycine $1 \times 10^6$; salicylate $2 \times 10^6$; N-benzoyl glycine $3 \times 10^6$; and benzoic acid $4 \times 10^6$ (Fig 1).

These studies demonstrate that frusemide, either ingested or added to serum in vitro, inhibits $T_3$ and $T_4$ serum binding with threshold (20% increase in free fraction) at about 7 ug/ml. Threshold is lower if albumin and/or thyroxine binding globulin are subnormal, indicating that nephrotic or cirrhotic subjects may be especially sensitive to its inhibi-

*Comparison of frusemide with other inhibitors of thyroxine binding to serum proteins. Frusemide is more potent on a molar basis than any other inhibitor tested. (Serum dilution 1:10, dextran-charcoal separation.)*




tory effect. Frusemide in a large single dose, lowers $T_3$ and $T_4$ in serum and increases $T_3$ resin uptake and frusemide is more potent on a molar basis than previously-known inhibitors of $T_4$ binding. While normal diuretic doses of frusemide are unlikely to achieve inhibitory concentrations, the high doses given to patients with oliguric renal failure, who show delayed clearance of the drug, will achieve inhibitory concentrations. Frusemide must be considered in seeking a cause for low $T_4$ levels in critical illness.

## Diagnostic Significance of Sensitive Measurement of Thyroid Stimulating Hormone; Correlation with the Response to Releasing Hormone

V.S. Mohr, D.J. Topliss, S.A. Dyer, J.R. Stockigt

Routine assays of TSH cannot reliably distinguish between euthyroid and hyperthyroid states but modified assays have been developed in an attempt to make this distinction. Basal TSH levels correlate with TSH increment to TRH (TSH) in normal subjects and the use of basal TSH levels as an alternative to the TRH test in suspected hyperthyroidism has been suggested.

We evaluate a sensitive TSH assay (detection limit 0.3 mU/L) in order to assess how well the assay can discriminate hyperthyroid from euthyroid subjects, and to examine whether basal TSH levels are a good predictor of the TSH increment to TRH in various thyroid disorders.

TSH levels in 41 untreated hyperthyroid subjects ($FT_4I$ > 160, normal < 140; $FT_3I$ 3.0, normal < 2.7) were undetectable in 38 and detectable in 3 (range < 0.30-0.71 mU/L). In 38 euthyroid subjects with no history of thyroid disease from the hospital population TSH was <1 mU/L in 17, but only two were undetectable, (mean 1.44, range < 0.3-5.2 mU/L).

Serum samples from 100 consecutive TRH tests (200 *u*g i.v.) were evaluated. Undetectable basal TSH levels ( <0.3 mU/L) were found in both hyperthyroid and some euthyroid patients suspected of hyperthyroidism. We examined whether TSH could separate these groups in 8 hyperthyroid ($FT_4I$

> 165, $FT_3$ > 2.6) and 10 euthyroid ($FT_4I$ < 40, $FT_3I$ < 2.4) subjects. It was noteworthy that 7 of the euthyroid group had multinodular goitres. TSH remained undetectable after TRH in all with hyperthyroidism and in 3 euthyroid subjects with multinodular goitres. In the other 7 euthyroid subjects TSH ranged from 0.25-4.9 mU/L.

This study shows that with a sensitive assay, basal TSH levels in hyperthyroidism differ from those in the euthyroid state. A basal TSH level below the assay detection limit ( < 0.3 mU/L) had a diagnostic sensitivity for hyperthyroidism of 93% and specificity of 95%. The predictive value of an undetectable TSH was 95% for hyperthyroidism. A detectable TSH level had a 92% predictive value for euthyroidism. The TSH response to TRH was highly correlated with the basal TSH, but the width of confidence limits prevented accurate prediction of individual responses. Especially with multinodular goitre, TSH may discriminate between hyperthyroidism and the euthyroid state, when basal TSH fails to do so.

## Meetings and Conferences

Dr. Barlow and Dr. Stockigt attended the 7th International Congress of Endocrinology in Quebec, July 1984. Both presented papers, and Dr. Stockigt was an invited speaker on the subject of euthyroid hyperthyroxinaemia. Subsequently, Dr. Stockigt presented a paper on congenital adrenal hyperplasia in adults at a satellite meeting in New York. Dr. Barlow visited laboratories in Los Angeles and at the National Institute of Health in Bethesda. Dr. Topliss attended the European Thyroid Association meeting in Rotterdam and the American Thyroid Association meeting in New York in September, where he presented our work on frusemide and thyroid hormone binding.

Papers were presented at the annual meeting of the Endocrine Society of Australia in Melbourne by Stephanie Dyer, Virginia Mohr, Chen-Fee Lim, Shane Hamblin and John Barlow. Dr. Topliss was a member of the program-organizing committee for that meeting. Dr. Stockigt was elected to the Council of the Endocrine Society of Australia for 1985-87.

Dr. Breidahl attended the annual meeting of the European Association for Study of Diabetes, in London in September. He was elected one of the five members of the Nominating Committee of the International Diabetes Federation at its first meeting in London held to coincide with the EASD meeting.

During 1984 members of the Unit gave lectures and presentations in numerous metropolitan and country centres as part of continuing education and family medicine programmes, under the auspices of the Victorian Medical Postgraduate Foundation.

## Teaching and Seminars

Drs. Breidahl, Taft, Lording, Topliss and Stockigt gave seminars and lectures in the 6th year clinical teaching programmes and participated in the teaching of general medicine, diabetes and endocrinology for 4th, 5th and 6th year Monash University medical students.

Postgraduate teaching sessions were held weekly during the first half of 1984 for candidates for Part 1 FRACP. In addition, clinical lunchtime seminars were held twice monthly throughout the year. The following topics were presented in 1984:

- Osteoporosis — new diagnostic techniques
- Osteoporosis — questions of management
- Iopanoic acid in therapy of severe hyperthyroidism
- Acromegaly — problems in diagnosis
- Spontaneous intermittent hypothyroidism
- Frusemide therapy and thyroid function
- Amiodarone and thyroid function
- Mortality in diabetic ketoacidosis; 1974-1983 at Alfred Hospital
- Idiopathic hypoglycaemia in adulthood
- Nelson's syndrome — therapeutic possibilities
- Cushing's syndrome due to adrenal adenoma
- Diagnosis of diabetes insipidus
- Lymphoma of the thyroid
- Insulinoma
- Haematologic changes in thyroid disease

# Ewen Downie Metabolic Unit

## Published

**BARLOW JW.**
Familial euthyroid thyroxine excess: A phenomenon of enhanced thyroxine-binding to albumin. Ph.D. thesis. Monash University 1984.

**BARLOW JW, LIM C-F, STEVENS V, TOPLISS DJ, WYNNE KN, STOCKIGT JR.**
Furosemide is a competitive inhibitor of plasma binding of $T_4$ and $T_3$. Proceedings of the 7th International Congress of Endocrinology, Abstract 271, Quebec 1984.

**BARLOW JW, STOCKIGT JR.**
Familial dysalbuminaemic hyperthyroxinaemia: Implications for delivery of circulating thyroxine. Proceedings of the 27th Annual Meeting of the Endocrine Society of Australia, Abstract 24, Melbourne, August 1984.

**BECK-PECCOZ P, ROMELLI PB, CATTANEO MG, FAGLIA G, WHITE EL, BARLOW JW, STOCKIGT JR.**
Evaluation of free $T_4$ methods in the presence of iodothyronine autoantibodies. J Clin Endocrinol Metab 58:736-739, 1984.

**BREIDAHL H.**
Foot problems of the diabetic. Patient Manag 8:73-88, 1984.

**BREIDAHL HD.**
Treatment of non-insulin-dependent diabetes. In: Diabetes Mellitus: A Guide to Treatment, Ed. P. Taft, Balgowlah (NSW), Adis Health Science Press, 1984, pp 38-48.
Diabetes Mellitus: A Guide to Treatment, Ed. P. Taft, Balgowlah (NSW), Adis Health Science Press, 1984.

**DYER SA, MOHR VS, WHITE EL, BARLOW JW, STOCKIGT JR.**
Identification of plasma binding abnormalities as the cause of euthyroid hyperthyroxinaemia. Proceedings of the 27th Annual Meeting of the Endocrine Society of Australia, Abstract 23, Melbourne, August 1984.

**FRAILLON D, WYNNE KN, FUNDER JW.**
Further studies on neomycin and experimental hypertension. Clin Exp Pharmacol Physiol 11:339-342, 1984.

**FULLER PJ, LIM ATW, BARLOW JW, WHITE EL, KHALID BAK, COPOLOV DJ, LOLAIRS, FUNDER JW, STOCKIGT JR.**
A pituitary tumor producing high molecular weight ACTH-related peptides: Clinical and cell culture studies. J Clin Endocrinol Metab 58:134-142, 1984.

**HAMBLIN PS, MOHR VS, STOCKIGT JR, TOPLISS DJ.**
Iopanoic acid is of minimal therapeutic benefit in the treatment of severe hyperthyroidism: conclusions from a case study. Clin Endocrinol (Oxf) 22:503-510, 1985.

**HAMBLIN PS, TOPLISS DJ, MOHR VS, DYER S, STOCKIGT JR.**
Changes in TSH during recovery from hypothyroxinaemia of critical illness. Proceedings of the 27th Annual Meeting of the Endocrine Society of Australia, Abstract 55, Melbourne, August 1984.

**HEALY DL, LORDING DW.**
Amenorrhoea, galactorrhoea and impotence. In: Tutorials in Postgraduate Medicine. Clinical Endocrinology, Eds. M.W. Keynes, Fowler P.B.S. Kingswood Tadworth (Surrey), William Heinemann Medical Books, 1984, pp. 525-538.

**HUNTER AN, MATTHEW J.**
Complications of diabetes. In: Diabetes Mellitus: A Guide to Treatment, Ed. P. Taft, Balgowlah (NSW), Adis Health Science Press, 1984, pp 104-12.

**LIM C-F, MOHR VS, DYER SA, LONG F, HAMBLIN PS, TOPLISS DJ, BARLOW JW, WYNNE KN, SABTO J, STOCKIGT JR.**
Frusemide as a competitor for serum binding of thyroid hormones: in vivo and in vitro studies and comparison with other known competitors. Proceedings of the 27th Annual Meeting of the Endocrine Society of Australia. Abstract 52, Melbourne, August 1984.

**LORDING DW.**
Pathogenesis, classification, presentation, diagnosis and natural history. In: Diabetes Mellitus: A Guide to Treatment, Ed. P. Taft, Balgowlah (NSW), Adis Health Science Press, 1984, pp. 1-10.

**LORDING DW.**
Reproductive endocrinology. In: A Practical Approach to Endocrine Disorders, Ed. R. Larkins. Oxford, Blackwell Scientific Publications, 1985.

**MARKS R, SAWYER M, BARLOW JW.**
Glucocorticoid-induced vasoconstriction in canine skin. Arch Dermatol Res 277:318-322, 1985.

**MOHR VS, TOPLISS DJ, DYER SA, STOCKIGT JR.**
Diagnostic significance of sensitive TSH measurement: correlation with TSH response to TRH. Proceedings of the 27th Annual Meeting of the Endocrine Society of Australia, Abstract 54, Melbourne, August 1984.

**REISNER GS, LORDING DW.**
Experience with the Jonas penile prosthesis in the treatment of impotence. Andrologia 16:256-258, 1984

**ROBERTS C, TAFT P, WALQVIST M.**
Diet in diabetes. In: Diabetes Mellitus: A Guide to Treatment, Ed. P. Taft, Balgowlah (NSW), Adis Health Science Press, 1984, pp 54-75.

**STOCKIGT JR.**
Euthyroid hyperthyroxinemia. In: Endocrinology, Eds. F. Labrie, L. Proulx. Proceedings of the 7th International Congress of Endocrinology, Quebec City July 1984. Amsterdam, Elsevier, 1984, pp. 1057-1060.

**STOCKIGT JR, BARLOW JW.**
The diagnostic challenge of euthyroid hyperthyroxinemia. Aust NZ J Med 15:277-284, 1985.

**STOCKIGT JR, HAMBLIN PS, LONG F, TAFT P.**
Pituitary-adrenal suppression after long-term treatment of virilizing congenital adrenal hyperplasia. Congenital Adrenal Hyperplasia Meeting, July 1984, New York City, Abstract.

**STOCKIGT JR, LIM CF, BARLOW JW, STEVENS V, TOPLISS DJ, WYNNE KN.**
High concentrations of furosemide inhibit plasma binding of thyroxine. J Clin Endocrinol Metab 59:62-66, 1984.

**STOCKIGT JR, LIM CF, BARLOW JW, WYNNE KN, MOHR VS, TOPLISS DJ, HAMBLIN PS, SABTO J.**
Interaction of furosemide with serum thyroxine binding sites: in vivo and in vitro studies and comparison with other inhibitors. J Clin Endocrinol Metab 60:1025-1031, 1985.

**STOCKIGT JR, LIM C-F, MOHR VS, DYER SA, LONG F, HAMBLIN PS, TOPLISS DJ, BARLOW JW, WYNNE KN, SABTO J.**
Inhibition of $T_4$ binding by furosemide (Fr): in vivo and in vitro studies. Proceedings of the 60th meeting of the American Thyroid Association, Abstract 37, New York City, Sept 1984.

**STOCKIGT JR, STEVENS V, DYER S, WHITE EL, BARLOW JW.**
Diagnosis of plasma binding abnormalities in euthyroid hyperthyroxinemia. Proceedings of the 7th International Congress of Endocrinology, Abstract 2462, Quebec 1984.

## In Press

**STOCKIGT JR.**
The renin angiotensin system in aldosterone deficiency states. In: The Renin Angiotensin System in Diagnosis, Ed. C.I. Johnston, London, Gower Medical Publishers.

# How to Support Medical Research at The Baker Medical Research Institute

The Institute depends to a considerable extent upon private support in the form of grants, donations and legacies. All support of this kind is devoted entirely to medical research, is appropriately acknowledged and gratefully received.

The Institute's programme is entirely in the field of cardiovascular research. Disorders of the heart and cardiovascular system result in more deaths than any other single cause, are responsible for about 25% of all premature deaths and much serious illness. The Institute is trying to find the causes in order to arrive at improved methods of prevention and treatment.

**Donations to the Institute are tax deductible.**

Savings can be achieved if funds intended as a bequest are given as a donation, deductible from income. A bequest to the Institute may have only a marginal impact on the residue of an estate. Advice from an accountant or solicitor may be advisable.

Persons contemplating donations or bequests are welcome to visit the Institute by appointment.

For further information contact:
The Director
Baker Medical Research Institute
Commercial Road, Prahran 3181
(Telephone: 522 4313)

**The following is a suggested form of 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 $ .....free of all duties, for which the written acknowledgement of the Senior Executive Officer shall be sufficient discharge.**