Research

BAKER INSTITUTE

ALFRED HOSPITAL

1966

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

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

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

FORTIETH ANNUAL REPORT

of

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

(Including Alfred Hospital Clinical Research Unit)

TENTH ANNUAL RESEARCH REPORT

of

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

REPORTS

of

ALFRED HOSPITAL RESEARCH FELLOWS

1966

ALFRED HOSPITAL, PRAHRAN, VICTORIA, AUSTRALIA

BAKER MEDICAL RESEARCH INSTITUTE

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	N. E. JONES
	K. N. MORRIS, M.B., M.S., F.R.C.S., F.R.A.C.S.
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	J. C. STEWART, M.B., B.S., F.R.C.S., F.R.A.C.S.
	DIRECTOR OF CLINICAL RESEARCH UNIT (ex officio)

ALFRED HOSPITAL RESEARCH FELLOWS, 1966

"Edward Wilson Memorial":	T. G. JONES, M.B., B.S. (Lond.), M.C.Path.
"Victor Y. and Margaret Kimpton":	J. A. OWEN, B.Sc., M.D., Ch.B., Ph.D. (Edin.), M.C.P.A.
"Frederick and Esther Michaelis": *"Connibere Bequest"*:	N. KATHLEEN TAYLOR, M.B., B.S.
"Collier": *"George Merriman"*:	JEAN TOLHURST, D.Sc.
"A. A. Swallow": *"H. M. Black"*:	R. J. SAWERS, M.B., B.S., F.R.A.C.P., M.C.Path.
"Amelia Haigh Estate" (Heart Disease): *"Edward Wilson Memorial"*:	A. D. McCUTCHEON, M.D., M.R.A.C.P.

APPOINTED TO RESEARCH FELLOWSHIPS FOR 1967

"Edward Wilson Memorial":	F. W. GURR, M.B., B.S., M.R.A.C.P.
"James Richardson": *"Sartori"*:	PINCUS TAFT, M.D., F.R.A.C.P.
"Connibere Bequest": *"Sol Green"*:	G. C. ENNIS, M.B., B.S., M.R.A.C.P.
"S. W. Jones": *"E. H. Flack"*:	J. B. St. JOHN, M.B., B.S., M.R.A.C.P. A. PITT, M.D., M.R.A.C.P.
"Dr. Henry Laurie": *"E. H. Flack"*: *"H. M. Black"*:	J. B. SWANN, M.B., B.S.
"J. F. Mackeddie":	J. A. MIRAMS, M.B., B.S., M.R.A.C.P.
"Victor Y. and Margaret Kimpton": *"Frederick and Esther Michaelis"*:	J. A. OWENS, B.Sc., M.D., Ch.B., Ph.D. (Edin.), M.C.P.A.
"Connibere Bequest": *"R. B. McComas"*:	J. T. SYKES, F.R.C.A.
"Amelia Haigh Estate": *(Rheumatoid Arthritis)* *"George Merriman"*: *"A. A. Swallow"*:	G. BUCKLE, B.Sc.

TRAVEL GRANT

"J. H. Patterson Travelling Scholarship":	G. R. STIRLING, M.B., B.S., F.R.A.C.S.

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Investigations by Research Fellows in Other Departments of Alfred Hospital

INTRODUCTION

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

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

The appointment of Dr. T. E. Lowe as Director of the Clinical Research Unit in 1948 was followed by his appointment as Director of the Baker Medical Research Institute in 1949, and since that time the Committee has become concerned with an increasing interest and responsibility not only for clinical research conducted within the Clinical Research Unit, but also with Research Fellows who work in various departments of the Hospital, supported from specific research funds bequeathed in trust to Alfred Hospital.

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

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

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

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

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

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

BAKER MEDICAL RESEARCH INSTITUTE

STAFF

Director: T. E. LOWE, D.Sc., M.D., F.R.C.P., F.R.A.C.P.

Associate Directors: P. FANTL, D.Sc., F.R.A.C.I. (to 30/5/66)
A. J. BARNETT, M.D., F.R.A.C.P., M.R.C.P.
WINIFRED G. NAYLER, D.Sc. (from 1/6/66)

Administrative Assistant: R. BLAKEMORE, LL.B.

Graduates:
Mrs. V. CARSON, M.Sc.
D. A. COVENTRY, M.D., M.R.A.C.P.
C. C. CURTAIN, D.Sc., Ph.D., F.R.A.C.I.
Mrs. N. DOREVITCH, B.Sc.
Mrs. J. GUTHRIE, B.Sc.
Miss N. LOBB, B.Sc.
I. McINNES, F.R.C.S., F.R.A.C.S.
Miss J. PRICE, B.Sc.
D. RACE, M.B., B.S.
M. ROSENBAUM, M.D., M.R.A.C.P.
Miss N. RULEVICH, B.Sc.
J. B. SWANN, M.B., B.S.

Technical:
S. HART (Laboratory Supervisor)
J. L. BREMNER
Mrs. E. MORTENSEN
Mrs. R. SABO
Mrs. B. SKYM

Clerical:
Mrs. I. R. ROBINSON
Mrs. B. ASHTON (to 20/5/66)
Mrs. R. BRETT
Miss M. GAYTON
Mrs. H. WINSTANLEY (from 23/5/66)
Mrs. H. P. MORRIS (part-time)

Laboratory Assistants:
D. BREEN (to 10/6/66)
R. CAMP
Miss K. CLARKSON (from 12/4/66)
Miss D. DAVIS
Miss J. FINDLAY
K. HARVEY
Miss F. HIRSCH
Miss D. HUGHES (to 3/3/66)
Miss R. KNOWLES
C. LEWIS (from 11/7/66)
Miss V. MACK
Miss M. SHIERS
Miss R. TURNBULL

WARD STAFF

Registrar: S. A. CANTOR, M.B., B.S.

Resident Medical Officers:
C. T. K. STUART, M.B., B.S.
K. W. WALDRON, M.B., B.S.
B. J. DOWTY, M.B., B.S.
G. J. G. ANDERSON, M.B., B.S.

Sister: J. W. SIMON

Staff Nurses:
E. M. BELL (1/1/66 to 6/2/66)
T. A. CANNINGTON (12/9/66 to 31/12/66)
S. CARTER (1/1/66 to 15/2/66)
P. ERSKINE (14/12/65 to 8/5/66)
J. HOMEWOOD (7/2/66 to 18/9/66)
M. R. PEARCE (9/5/66 to 16/10/66)
D. PITCHER (17/10/66 to 31/12/66)

ALFRED HOSPITAL RESEARCH FELLOWS

"Amelia Haigh Estate" (Heart Disease):
"Edward Wilson Memorial": A. D. McCUTCHEON, M.D., M.R.A.C.P.

"Edward Wilson Memorial": T. G. JONES, M.B., B.S. (Lond.), M.C.Path.

ANTI-CANCER COUNCIL RESEARCH FELLOW

"A. A. Thomas": C. KIDSON, Ph.D. (Lond.), M.B., B.S., B.Sc. (Med.)

NYULASY SCHOLARSHIP

J. HASKER, B.Sc. (1965)

ANNUAL REPORT OF THE DIRECTOR OF THE BAKER INSTITUTE

A major event of 1966 was the commencement of the re-building of the Institute which, when completed, will greatly facilitate future research activities.

As part of its re-building programme, the Hospital has made provision for the removal of Ward 8 (the Clinical Research Ward) from its site alongside the Institute laboratories into the main hospital complex, and the Board of Management of the Hospital agreed to make the site of Ward 8 available for extensions to the Institute. Commencement of re-building of the Institute has necessitated the removal of Ward 8 to a temporary site, and until the new main ward block is completed the clinical activities of the Institute group will be physically divorced from the laboratory facilities, but with as little disturbance as possible to the functional integration of clinical and laboratory projects. Ultimately it is planned that the new ward and the Institute will be linked by an overhead connecting bridge, and the cherished physical unity restored.

Stage **I** of the new Institute building commenced in mid-1966 on the Ward 8 site and completion is anticipated early in the second half of 1967. Subject to the availability of funds, it is planned to proceed with the erection of Stage **II** immediately after Stage **I** is occupied.

The new building is being erected in reinforced concrete, with brick panels in conformity with the general design of the new hospital buildings. It will be completely air-conditioned, so that the carefully controlled and dust-free environment so necessary to modern biological research can be provided.

The three floors of approximately 180 x 75 feet each will provide adequate space to house the presently grossly overcrowded research staff and equipment and to provide for future expansion of activities and the installation of additional equipment. Adequate space for library, seminar and staff facilities will now be possible.

Thomas E. Lowe

During the planning of this building much thought was given to the patterns of medical research likely to evolve in the future. Although in recent years the advancing front of medicine has moved from clinical studies to laboratory orientated projects and this trend is likely to continue, our experience with studies on Kinekard have emphasized, if indeed such is necessary, the need for close laboratory and clinical co-operation. We have, therefore, endeavoured to design laboratories which are standardized in lay-out, which can be used for a variety of disciplines and which will provide suitable facilities for clinically based research projects.

Detailed accounts of research work in progress during the year are given in the scientific section of this report, and the following brief account of a few of the projects is intended to give an indication of the interests of our workers. These cover, amongst others, various facets of the physiology and diseases of the heart and blood vessels, studies concerning the production of protein molecules and nucleic acids by cells and studies at the clinical level of a variety of diseases.

ENERGY PRODUCTION IN THE MYOCARDIUM

In the body blood is contained in a closed system of blood vessels and is circulated through them by a complex pump (the heart). The heart is composed of millions of muscle cells arranged to form a series of pumping chambers through which the flow of blood is controlled by valves. The pumping power of the heart is provided by the rhythmic shortening and lengthening of these muscle cells which derive their fuel from substances in the blood itself. The way in which cardiac muscle cells extract these substances from the blood and derive from them the energy for their rhythmic contraction and relaxion has formed the basis of many of our investigations for many years.

One of the critical factors in this contractile process is the concentration of calcium within the cells and this year studies have been made concerning the influence of certain cardiac drugs on the distribution of calcium within these cells.

Because the coronary arteries supply the heart muscle with its nutrients, the study directed to the action of drugs on these vessels has implications for the treatment of cardiaç disease.

KINEKARD

Various investigations into the working of the heart and blood vessels showed that a fraction, which contained a substance with a powerful action on the heart and blood vessels, could be isolated from blood plasma. This fraction has been called Kinekard and is apparently found in all species. Studies during the past year have shown that kinekard is a previously unknown substance and that in some diseases of man its concentration in the blood is altered. In some, but not all, cases of high blood pressure this level is raised, in some patients with myocardial infarction the level is low, and in some with pituitary disease it is high. Other studies have demonstrated that kinekard has an action on other muscles of the body such as those in the alimentary canal.

Although the chemical structure of kinekard is still unknown and it has only been isolated in minute quantities, it appears to be a substance of great importance to the body.

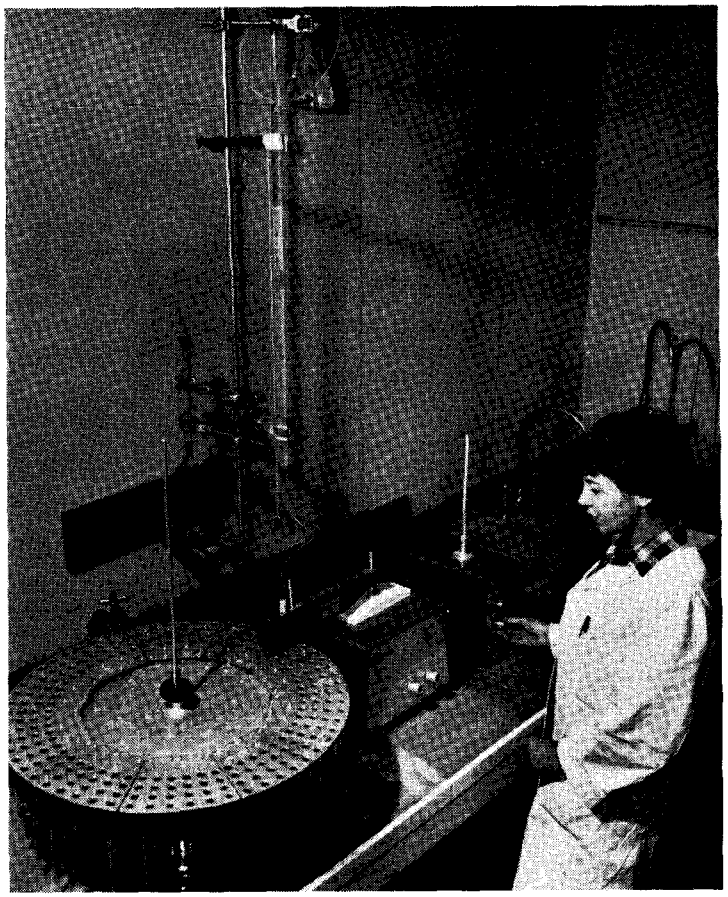
HYPERTENSION

Over the past two decades the outlook for patients with high blood pressure has been dramatically changed by the introduction of drugs capable of controlling blood pressure levels for long periods. During this time drug companies have introduced many new drugs, and throughout the world a great deal of clinical investigation has been necessary to determine the value and indications for use of these products. Sixteen years ago we established a small clinic for the treatment of patients with high blood pressure and so have been able to study many of the new drugs as they become available. This has helped to accelerate the routine use of suitable drugs in the treatment of hypertension. As the perfect drug for the control of blood pressure is not yet available this project must continue.

ARTERIAL OBSTRUCTION

One of the distressing complications of disease of arteries is obstruction to the blood flow in a limb which may lead to death of part of the limb and the consequent need for amputation. For many years surgeons have been devising methods to save these threatened limbs by procedures that either remove the obstruction and so restore the continuity of the vessel or else bypass the obstruction with a tube which may be made either of synthetic material or a vein grafted from elsewhere.

For many years physicians in the Institute have been co-operating with surgeons of the Hospital in the management of these patients and the data recorded in the detailed report show the steady improvement in results that has been obtained.



Fractionating Kinekard.

STAFF

Dr. Winifred Nayler assumed the duties of Associate Director in June, in succession to Dr. P. Fantl, who then commenced an extended period of leave but plans to return to work on a part-time basis during 1967.

Dr. C. C. Curtain has been appointed a Principal Research Scientist at the C.S.I.R.O. in Melbourne, and ended his long association with the Institute at the end of the year.

Dr. Chev Kidson, who held the A. A. Thomas Fellowship of the Anti-Cancer Council of Victoria for several years, worked in the Institute on a project in molecular genetics. He has been appointed Director of the Department of Molecular Genetics at Syntex Research Center, Palo Alto, U.S.A., and left us at the end of the year. Arrangements have been made for Dr. P. Hughes to hold this Fellowship and continue work on a related project from the beginning of 1968.

Dr. D. Race, who has been working in the Department of Surgery at the State University of New York (Buffalo), returned during the year, and is continuing his studies in the field of haemodynamics.

Dr. T. G. Jones, from Westminster Hospital, London, has held the Edward Wilson Memorial Fellowship during the year and has co-operated with Dr. P. Fantl in various investigations into the blood coagulation mechanism.

OVERSEAS VISITS

Dr. Winifred Nayler visited U.S.A., England and Europe to discuss problems connected with the Kinekard project.

Dr. C. C. Curtain visited Papua-New Guinea to continue field studies in the project on Autoantibodies in Tropical Populations.

Dr. C. Kidson made separate visits to California and Japan in connection with his study of molecular genetics. In Japan he attended the **IXth** International Cancer Congress to present some aspects of his work.

Dr. M. Rosenbaum left early in the year to take up the Overseas Fellowship granted to him by the National Heart Foundation of Australia. He is pursuing a course of study at the Bockus Research Institute in Philadelphia, U.S.A.

Dr. R. G. Wyllie spent the first half of the year on a tour of duty in South Vietnam with the Alfred Hospital Medical Team. At the end of the year he left to continue his studies in histochemistry at Johns Hopkins Medical School, Baltimore, U.S.A.

RESEARCH ASSISTANCE

Many of the investigations recorded in this Report have been supported by funds provided by the Life Insurance Medical Research Fund of Australia and New Zealand, the Anti-Cancer Council of Victoria, the National Heart Foundation of Australia, the Asthma Foundation of Victoria and Alfred Hospital Research Funds, and this assistance is gratefully acknowledged.

It is a pleasure to record thanks for generous donations from those whose names are listed in the various financial reports.

Many organisations have made gifts to the Institute library, and our thanks are expressed to them, to various libraries that have loaned us journals, and particularly to the librarians whose assistance is greatly valued.

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

It is a pleasure for me to thank the Trustees of the Institute and the Board of Management of the Hospital for their continued generous support and to thank members of the staff and research fellows for their co-operation during the year.

T. E. LOWE.

31st December, 1966.

Addendum

Prior to printing this report, the Government of the State of Victoria has announced a gift of $200,000 towards the cost of Stage **II** of the re-building programme. This generous donation will enable Stage **II** to be proceeded with as soon as Stage **I** is occupied. The assistance given is gratefully acknowledged.

LIST OF ORGANISATIONS WHICH HAVE MADE GIFTS TO THE LIBRARY DURING THE YEAR

Australian Medical Association.
Adelaide Children's Hospital.
Anti-Cancer Council of Victoria.
A.N.Z.A.A.S.
Austin Hospital.
College of Physicians and Surgeons, New York.
Commonwealth Department of Health.
Commonwealth X-Ray and Radium Laboratory.
Department of Health, New Zealand.
Department of Territories, Canberra.
Halstrom Institute of Cardiology, Sydney.
Instituto de Biologia y Medicina Experimental, Buenos Aires.
Institut Pasteur, Algiers.
Institute of Dental Science.
Institute of Medicine and Veterinary Science, Adelaide.
Kanematsu Memorial Institute, Sydney.
Medical Research Council, London.
Middlesex Hospital Medical School.
National Heart Foundation, Australia.
National Institute of Nutrition, Japan.
New York State Department of Health.
New York University College of Medicine.
New Zealand Medical Research Council.
Ophthalmic Research Institute of Australia.
Queensland Institute of Medical Research.
Rockefeller Institute, New York.
Royal Children's Hospital, Melbourne.
Royal Melbourne Hospital.
Royal Prince Alfred Hospital, Sydney.
Royal Women's Hospital, Melbourne.
St. Vincent's Hospital, Melbourne.
St. Vincent's School of Medical Research, Melbourne.
South African Institute of Medical Research.
Strangeways Research Laboratories, Cambridge.
Staten Seruminstitut, Copenhagen.
University of Melbourne.
University of Otago, New Zealand.
University of Queensland.
University of Sydney.
Universitatis Mariae Curie Sklodowska, Poland.
Walter & Eliza Hall Institute, Melbourne.
Wellington Medical Research Foundation.
World Health Organisation.

ALFRED HOSPITAL RESEARCH FELLOWS IN THE INSTITUTE

1949 - 1966

Anderson, R. McD., 1953-55
Andrew, R. R., 1949-55
Barnett, A. J., 1949-50
Baumgarten, A., 1962-64
Beavis, E. L. G., 1955-56
Boake, W. C., 1958
Breidahl, H. D., 1952-53
Burnside, K. B., 1951
Cooper, E., 1962
Duffy, D. G., 1952-55
Ferguson, I. A. L., 1957-58
Fowler, R., 1953-54
Francis, J. K., 1956-57
Fraser, J. R. E., 1957
Gardiner, J. M., 1952
Goble, A. J., 1951
Hudson, B., 1952
Jamieson, K., 1954
Kay, H. B., 1959-60
Kincaid-Smith, P., 1959-60
McCutcheon, A. D., 1959, 65-66
McDonald, W., 1960-61
McNeur, J. C., 1955
McRae, C. J., 1955
Murfitt, L., 1955
Newman, H. C., 1954
Parsons, P. J., 1951
Quinn-Young, M., 1956
Race, D., 1959-63
Sawers, R. J., 1953-60
St. Clair, W. A., 1955
Silberberg, F. G., 1953
Stern, W., 1954-55
Stirling, G. R., 1955
Wagner, G., 1958

OVERSEAS FELLOWS

Dawson, J. B., 1961-63 (Oxford)
Emslie-Smith, D., 1955-56 (Dundee)
Hamilton, M., 1954 (London)
Jones, T. G., 1966 (London)
Lumb, F. H., 1960-61 (London)
Marshall, R. J., 1957 (Belfast)
Robertson, P. G. C., 1963-64 (Dundee)
Simpson, F. O., 1958-59 (Edinburgh)
Stevenson, M. M., 1957 (Belfast)
Thomson, J. W. W., 1959 (Edinburgh)

REPORT OF SCIENTIFIC INVESTIGATIONS

ENERGY PRODUCTION IN THE MYOCARDIUM†‡

W. G. Nayler, N. Dorevitch, J. Guthrie, J. M. Price, and T. E. Lowe

IONIC STUDIES‡

The Effect of Cardioactive Drugs on the Uptake and Release of Calcium from the Sarcoplasmic Reticulum.

Excitation-contraction coupling in cardiac muscle depends upon the availability of ionized calcium. Relaxation occurs when the intracellular concentration of ionized calcium falls below a critical level. This reduction in intracellular calcium is probably achieved through the sarcoplasmic reticulum, a fine membranous network which is dispersed throughout the sarcoplasm and which can accumulate calcium ions against a large concentration gradient. During the past year experiments have been carried out to determine whether or not cardioactive drugs interfere with the calcium-accumulating activity of the sarcoplasmic reticulum, and thereby either increase or decrease the amount of ionized calcium which is available for participation in excitation-contraction coupling. The drugs studied include tetraethylammonium chloride, acetylcholine and caffeine.

Sarcoplasmic reticulum was isolated from other cellular components by differential centrifugation and, after incubation with Ca^{45}-labelled Tyrode solution, was separated from the suspending media by millipore filtration. ATP, creatine phosphate and oxalate all increased the rate at which the reticulum accumulated ionized calcium.

Both the uptake and release of ionized calcium from the reticulum was enhanced if tetraethylammonium chloride was present, but unless a precipitating agent such as oxalate was added, uptake and release balanced. Acetylcholine, when added after incubation had commenced, caused an increase in the rate at which ionized calcium was released from the reticulum.

Calcium Exchange and Drug Antagonism

Isolated guinea-pig atria actively accumulate ionized calcium, detected as Ca^{45}. Experiments have shown that caffeine which has a marked positive inotropic effect on cardiac muscle, increases the rate at which calcium ions are accumulated by isolated atria. Adenosine, which depresses cardiac contractions, decreased the rate at which atria accumulate ionized calcium. The negative inotropic effect of adenosine on cardiac muscle is antagonized by caffeine and this antagonism was found to be reflected in the altered calcium exchangeability detected when both drugs were present. It is concluded that the antagonistic action of these two drugs on cardiac muscle can be accounted for in terms of their action on calcium exchange.

PHARMACOLOGICAL STUDIES†

The Effect of Drugs on the Lipid Facilitated Transport of Ionized Calcium

There is now abundant evidence to show that excitation-contraction coupling in cardiac muscle fails in the absence of ionized calcium and that during excitation there is an inward movement of ionized calcium from the extracellular phase through or across the cell membrane into the intra-cellular phase. In cardiac muscle relaxation occurs if the intracellular concentration of ionized calcium falls below a critical level. Such a reduction in the intracellular concentration of ionized calcium results from the calcium-accumulating activity of the sarcoplasmic reticulum within each muscle cell and from the displacement of ionized calcium from the intracellular to the extracellular phase. Previous investigations have shown that lipids extracted from cardiac microsomal fractions may provide the basis for such a system of calcium transport.

Investigations carried out during the current year have shown that drugs which precipitate

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

cardiac arrhythmias, including isoprenaline, noradrenaline and adrenaline, stimulate the lipid facilitated transport of ionized calcium (detected as Ca^{45}). Antiarrhythmic drugs, including a variety of β-adrenergic antagonists in addition to propranolol, MJ 1999 and Kö -592, resemble propranolol in that they inhibit or impair the ability of these lipids to promote the transfer of ionized calcium across a lipid-aqueous interface.

The effect of β-adrenergic antagonists on the lipid-facilitated transport of ionized calcium may possibly be related to the negative inotropic actions of such drugs.

Effect of Hypothermia on the Action of Certain Cardioactive Drugs

Previous investigations in these laboratories have shown that the direct effect of certain drugs on myocardial contractility is modified during hypothermia. The positive inotropic effect of adrenaline and noradrenaline on isolated Langendorff-perfused mammalian hearts was found to be abolished and in some cases reversed during perfusion at 15°C. Experiments during this past year have shown that the inotropic action of other cardioactive drugs is modified during hypothermia. Thus the action of the xanthine derivatives, caffeine and aminophylline, on myocardial contractions was found to be enhanced during hypothermia so that concentrations of these drugs which produce only a positive inotropic effect at 35°C. produce sustained contracture at 15°C.

Isoprenaline stimulated the β-adrenergic receptors and therefore differs from either adrenaline or noradrenaline which stimulate both β and α receptors. Since the positive inotropic effect of adrenaline reflects stimulation of the β receptors experiments were performed to determine whether or not the effect of isoprenaline was modified during hypothermic conditions. The results indicated that the effect of isoprenaline on cardiac contractions and on the phosphorylase enzyme is abolished if the myocardium is maintained at or below 15°C. Similarly, the dilator action of isoprenaline on the peripheral vasculature of intact dogs was found to be completely abolished when preparations were maintained at 15°C.

The Direct Action of Coronary Vasodilator Drugs

In accordance with Ahlquist's dual receptor hypothesis the coronory circulation is believed to contain both α adrenergic constrictor and β adrenergic dilator receptors. Experiments therefore were planned to determine whether or not the action of certain coronary vasodilotor drugs was mediated via these adrenergic receptors. The drugs studied included adenosine, dipyridamole (Persantin), and diphenylhydantoin (Dilantin).

Adenosine caused an immediate and marked increase in coronary flow in isolated, and therefore non-innervated, guinea-pig and rat hearts which were perfused under conditions of constant perfusion pressure. Prior perfusion with α adrenergic antagonist phenoxybenzamine blocked the coronary dilator action of adenosine but failed to inhibit the adenosine induced bradycardia. This finding suggests that adenosine interacts with other receptors in the myocardium in addition to those of the α adrenergic system. Dipyridamole resembled adenosine in that its direct dilator effect on the coronary circulation was blocked by the prior addition of phenoxybenzamine. These results may mean that inhibition of the α adrenergic receptors is involved in the coronary dilator action of both adenosine and dipyridamole.

Dilantin resembles adenosine and dipyridamole in that its addition to isolated hearts perfused under conditions of constant perfusion pressure results in an increase in coronary flow which is abolished by prior α adrenergic blockade. However, in contrast to either adenosine or dipyridamole, dilantin caused a small increase in the size of cardiac contractions despite the accompanying bradycardia. This dilantin-induced bradycardia and the positive inotropic response was abolished or markedly decreased if the β adrenergic receptor blocking drug, propranolol, was present.

CARDIOACTIVE PLASMA SUBSTANCES†

T. E. Lowe, J. Swann, N. Dorevitch, W. G. Nayler and V. Carson

Previous investigations have shown that blood plasma contains, in addition to the already well recognised cardioactive substances, a highly potent fraction with a relatively low molecular weight and a marked positive inotropic action. This fraction, **kinekard,** has a marked pressor effect on the peripheral circulation which appears to be mediated through the α-adrenergic system. During this past year experiments have been directed towards providing clear-cut differences between the mode of action of kinekard and that of the catecholamines.

Dose response curves relating to the action of adrenaline and kinekard on two smooth muscle preparations — aortic strip and ileum, have been constructed. These show that, although the dose-response curve for the action of adrenaline on these preparations resembles that of kinekard, the curves are not parallel nor do they tend towards common maxima.

A second point of differentiation between kinekard and the catecholamines is found in the fact that the action of kinekard does not depend upon the availability of tissue stores of catecholamines nor is its action potentiated by prior reserpinization. Thirdly, the action of kinekard on a variety of isolated smooth muscle preparations is not potentiated by cocaine nor does it stimulate free fatty acid metabolism.

Plasma Levels of Kinekard and Cardiovascular Disease

In 1965 we reported on the plasma kinekard concentrations in health and disease, and noted that, with an assay based on inotropic action, some patients had plasma concentrations that were either below or above the normal range. During this current year the number of patients examined has been considerably increased and it now appears that some correlation between plasma kinekard concentration and certain cardiovascular syndromes probably does exist. Especially likely is a relationship between the severity of hypertensive disease (essential hypertension) and plasma kinekard concentration.

The previously reported finding that plasma kinekard concentration is independent of age or sex was confirmed.

The severity of hypertensive disease (essential hypertension) in a patient was assessed by allotting points to each of four facets of the disease; retinal changes, renal function, ease of control of blood pressure by therapy and a clinical assessment of the patient's well being.

The results of this survey indicated that in the normal individual the plasma concentration of kinekard falls within narrow limits; in the hypertensive group a correlation was found between the severity of the hypertensive disease and the plasma kinekard concentration. Patients suffering from myocardial infarcts, however, appeared to have a greater than normal chance of having a low concentration of kinekard.

Patients with pituitary-hypothalamic disorders appeared to have a greater than normal chance of a high plasma concentration of kinekard.

PERIPHERAL CIRCULATION AND LEFT VENTRICULAR WORK STUDIES‡

W. G. Nayler, I. McInnes, D. Race, J. Swann, V. Carson and T. E. Lowe

Effect of Some Hypotensive and Anti-arrhythmic Drugs on the Circulation

The dog heart-lung bypass preparation developed to study the factors involved in controlling the distribution of blood throughout the various vascular fields has been used to study the effect of several recently introduced antihypertensive drugs on the peripheral circulation. One such drug, diazoxide (7-chloro-3-methyl-1, 2, 4-benzothiadiazine, 1, 1-dioxide), is a non-diuretic thiazide derivative which, when given intravenously, causes a marked and sustained fall in systemic blood

pressure. This hypotensive action was found to be associated with changes in the regional distribution of blood in the peripheral circulation such that blood flow through the coronary vessels and through the superior and inferior venae cavae was increased whereas flow through the renal, splanchnic and vena azygos beds was diminished. Further investigations suggested that this hypotensive drug exerted a direct vasodilator effect on the vasculature of the coronary circulation, and on the inferior and superior venae cavae. This vasodilator effect was not mediated through the β-adrenergic system nor was it abolished by ganglionic blockade. Although diazoxide decreased the resistance to blood flow in the coronary circulation the capacity of the left ventricle to perform mechanical work was diminished by this drug.

The therapeutic use of β adrenergic antagonists for treatment of angina pectoris and in the treatment of certain cardiac arrhythmias, including those due to cardiac glycoside intoxication, precipitated a series of experiments designed to investigate the effect of the available β-antagonists on the cardiovascular system. These studies indicated that all the available antagonists, including propranolol, MJ1999 (dl.4-(2-isopropyl amino-1-hydroxyethyl)methanesulphonanilide and Kö-592 (1-(3-methylphenoxy)-3-isopropylamino propranolol HCl) caused a decline in coronary blood flow. The direct effect of these antagonists on the contractions of isolated papillary muscle preparations varied, propranolol causing the most marked decline in contractile activity. This negative inotropic action could not be accounted for in terms of depressed oxidative metabolism. This conclusion was substantiated in other studies in which the ability of the left ventricle to perform mechanical work was measured before and after the addition of the various β-adrenergic antagonists.

Diphenylhydantoin (Dilantin) was introduced for the treatment of epilepsy, but recently has been shown to possess antiarrhythmic properties. When administered to dogs on heart-lung bypass dilantin caused a reduction in the resistance to blood flow in the coronary vasculature. Left ventricular work studies indicated that, in contrast to propranolol, it does not markedly alter the work capacity of the left ventricle. The coronary vasodilator effect of dilantin is not blocked by β-adrenergic nor by ganglionic blockade.

BIOCHEMICAL BASIS OF DRUG ACTION

V. Carson

Action of Drugs on Metabolic Pathways

As a logical extension of the investigations designed to determine the effect of cardioactive drugs on left ventricular work capacity and on the rate at which the myocardium utilizes oxygen experimental procedures have been developed to study the metabolic pathways involved. Standard Warburg manometric techniques were used to study the effect of three recently developed β adrenergic antagonists and of three sympathomimetic amines on the respiratory enzymes in homogenized rat heart preparations. The results indicated that neither the antagonists nor the agonists in this series changed the rate at which homogenized heart muscle utilized oxygen.

The techniques used to determine creatine phosphate, ATP, ADP and AMP in heart muscle have been investigated and established so that these can be applied to and correlated with the left ventricular work studies described in another section of this report.

Assay of Pepsinogen in Serum

Preliminary work has been done to develop a satisfactory method for the assay of serum pepsinogen.

A. J. Barnett and S. Cantor

heart rate and cardiac return have been made, so that stroke volume, total peripheral resistance and left ventricular work could be calculated.

Catapresan (ST155)

The haemodynamic changes produced in normal persons by catapresan, a new drug introduced for the treatment of arterial hypertension, were described last year. This year we have studied the effect of intravenous injection of this drug in 7 hypertensive subjects.

Haemodynamic measurements were made before administration of the drug, with the patient resting horizontally, tilted to 60° from the horizontal for 2 minutes, exercising on a bicycle-ergometer, and during the Valsalva manoeuvre. These measurements were repeated 30 minutes and 60 minutes after the intravenous injection of 150 μg catapresan. In two cases, blood pressure tracings were recorded during the injection and at intervals of 5 minutes thereafter.

During the injection of catapresan there was a transient mild rise in blood pressure. After 5 minutes a definite hypotensive effect was present and this continued for approximately 60 minutes. It occurred in all cases.

The changes in the circulation produced by tilting after the administration of the drug were in general similar to those produced by tilting before the drug was administered. In 2 cases only was there an exaggerated fall in blood pressure produced by tilting following the drug. A normal increase in blood pressure during exercise occurred following the drug. The catapresan-induced change in heart rate was not marked and was variable in direction. Changes in heart rate with tilting and exercise were not modified. In all cases cardiac output in the recumbent position fell following catapresan the peripheral resistance showed a normal rise on tilting and fall on exercise.

Catapresan did not modify the rise in blood pressure following the Valsalva manoeuvre; central venous pressure showed only a slight fall.

In summary, the haemodynamic studies indicated that the fall in blood pressure following the intravenous administration of catapresan was brought about by a fall in cardiac output with little change in the systemic peripheral resistance. The mechanism of the fall in cardiac output has not been elucidated. Responses to tilting, exercise and the Valsalva manoeuvre were usually normal after catapresan.

Haemodynamic Response to Exercise in Control Subjects and Following Myocardial Infarction

Other workers have shown that following a myocardial infarction cardiac output falls, and that in patients studied months or years after a myocardial infarction those making a satisfactory recovery and returning to previous work differ from those unable to return to work in that the former show a normal increase in the stroke volume on exercise, whereas the latter do not. This inability to produce a normal increase in cardiac output would therefore seem to be an indication of cardiac damage, inability to produce a normal increase in cardiac output in response to exercise appears to be an indication of impaired cardiac function. Evidence is lacking on the rate of return of cardiac function after myocardial infarction and we decided to study this problem.

The selection of a suitable exercise test, severe enough to produce definite haemodynamic changes, but mild enough to be used safely in myocardial infarction patients and

which could be performed without undue psychological stress, presented problems. The following test was eventually evolved. After introducing catheters into the superior vena cava (for measurement of central venous pressure and injection of cardiogreen dye) the brachial artery was cannulated (for intra-arterial pressure measurements and dye concentration curves), the subject was seated on a bicycle ergometer. Measurements of cardiac output, intra-arterial and central venous blood pressures were taken at rest, whilst bicycling at successive levels of 100, 200 and 400 kiloponds per minute, each for 5 minutes, observations being repeated during the fifth minute at each level of exercise. These procedures were repeated 5 and 10 minutes following cessation of exercise. E.C.G. recordings were taken before, during and after exercise. Cardiac patients were not allowed to continue past the 200 k.p.m. exercise level at their first test.

Patients aged 30-60 years without history of either cardiac disease or severe debilitating condition were chosen as controls. When tested, however, a high proportion had an abnormal E.C.G. or other features suggesting that their cardiovascular system was not "normal" and only 7 "normals" were studied. The effect of work at 100 k.p.m. was slight and the most apparent changes were present under a work load of 200 and 400 k.p.m. Marked variations occurred between individual subjects.

In the control patients a moderate step-wise increase in blood pressure was noted in 43 subjects and a little change in the other four. There was a step-wise increase in heart rate, of varying in degree between subjects.

Cardiac output similarly showed a step-wise increase with work, the biggest jump usually being with exercise from 0 to 200 k.p.m. Stroke volume showed the greatest rise with exercise and in 4 of the 7 cases it was maximal at 200 k.p.m. Changes in the left ventricular work per minute and left ventricular stroke work in general followed those of the cardiac volume and cardiac stroke volume respectively, except that there was always a further increment in work between 200 and 400 k.p.m. related to the rise in blood pressure.

Five myocardial patients were studied one month after infarction. With one exception, a man with a severe infarction who was not exercised beyond the 100 k.p.m. level, the responses were within the normal range.

Three patients have been studied up to 6 months after infarction. In 2 of these an increased cardiac output at the most severe level of exercise (400 k.p.m.) was found from 3-6 months after the infarction. In the third case, in which an attack of chest pain associated with E.C.G. evidence of further cardiac damage was experienced between the 3 and 6 months study, there was no such increase.

The observations suggest that, unless further damage occurs, cardiac function after myocardial infarction may continue to improve up to 6 months. The study is, as yet, only in an early phase.

MOLECULAR BIOLOGY*** *

C. C. Curtain, C. Kidson, N. Lobb, and N. Rulevich

Our work in molecular biology is centred mainly on a study of the regulation of gene expression. This is central in the understanding of such complex biological phenomena as cell differentiation, neoplasia, immunological specificity and the general organization of biological information. The fundamental question is: How is one gene "turned on" while another gene is "turned off" and how can this be expressed in molecular terms? This problem can be further resolved into two steps: The regulation of RNA synthesis–transcription–and the regulation of protein synthesis–translation. Our approaches to the problem, through nucleic acid and protein chemistry, concern both transcription and translation. Genetic

and immunological analyses are being applied where possible.

These studies have involved the development of a number of new techniques and the use of a number of different experimental systems. The latter have been chosen for their suitability for the study of each aspect of the problem and range from micro-organisms to mammals, including man. Of the specific projects in this programme, several concern protein synthesis or the characterization of genetically determined protein variants; these projects are concerned particularly with the immunoglobulins and hence with the genetic basis of immunological specificity. One concerns hormone action in the control of gene expression in mammals, a question which is relevant to differentiation, neoplasia and a number of other biological problems. Another on chemical carcinogenesis is proving to be a fascinating source of data on the role of miscoding of genetic information in neoplastic transformation, whilst that on the regulation of transfer RNA synthesis concerns the general problem of decoding in the translation process, providing at the same time some insight into the genetic relationships between virus and host cell.

Finally, our continuing interest in population genetics in Melanesia represents an attempt to interpret more completely at the molecular level many genetic and paragenetic phenomena in man. These populations continue to be a source of valuable biological material not available elsewhere.

NUCLEIC ACID

DNA Secondary Structure and Gene Function

Previous studies demonstrated that DNA is partly denatured in the region of the replication point and that it undergoes renaturation rather slowly subsequent to the formation of new DNA strands. In these studies it was observed that rather more DNA exhibited partial strand separation than would be expected to be associated with replicating regions.

Early studies which examined DNA secondary structure by countercurrent distribution during greatly divergent conditions of bacterial growth suggested that partial strand separation might also occur into messenger RNA. This observation has been confirmed in two ways: (1) DNA isolated from bacterial cells grown under conditions of amino acid starvation and transferred to enriched media showed a marked reduction in the extent of denaturation concomitant with repression of a substantial portion of the genome; (2) DNA isolated from cells grown under conditions of amino acid starvation in the presence of actinomycin D, a specific inhibitor of RNA synthesis, showed similar structural changes concomitant with repression of the genome.

The implications of these data in the control of gene expression are considerable. Since the two strands of DNA must be separated for at least a number of nucleotide pairs to permit the transcription of one of these strands into RNA, the differential expression of cistrons comprising the genome requires a means of selective regional strand separation. The information for this selection must reside in part at least in the primary nucleotide base sequence of the DNA; possibly it may also reside in part in associated proteins.

DNA Secondary Structure and Genetic Recombination

Similar methods have been employed to study the way in which donor DNA is incorporated into the recipient genome in *Bacillus subtilis* during transformation. It is clear that any double-stranded DNA is able to penetrate successfully into the recipient cell and that this donor is incorporated gradually, mainly in regions of recipient DNA molecules which are partially denatured. Attempts to localize quiescent replication points in the recipient chromosomes have shown that these are in fact widely scattered throughout countercurrent distribution profiles of the DNA. Since donor DNA, labelled with ^{3}H-thymidine, only assumes random distribution with respect to recipient DNA after recipient cells have been induced to recommence logarithmic growth, it is evident that integration of donor at pre-existing replication points in recipient DNA is unlikely to account for the observed integration kinetics.

Phenographic techniques have been used recently to show that donor DNA is indeed incorporated at positions in the recipient genome which are random with respect to pre-existing replication points. We have therefore suggested that recombination may involve integration of donor DNA at active transcription sites in the recipient genome rather than (or in addition to) replication regions.

Hormone Regulation of RNA and Protein Biosynthesis

Previously we demonstrated that a number of steroid hormones exert a selective effect on the synthesis of messenger RNA in rat and mouse tissues *in vivo*. Subsequently it was found that cortisol could exert similar selective effects on messenger RNA synthesis on rabbit lymph node cells *in vitro*, although the net result was a decrease in the rate of RNA synthesis. Detailed kinetic studies using this *in vitro* system have now revealed the course of events to be' (1) a very rapid decrease in RNA synthesis, initiated in seconds, maximal in 5 minutes corresponding to the time required for maximal cortisol uptake; (2) a short lag period of about 10 minutes; (3) a "burst" of increased protein synthesis maximal at 15 or 20 minutes: (4) a final decrease in protein synthesis.

The RNA effect is largely reversed by pre-incubation of the cells with puromycin, an inhibitor of protein synthesis, suggesting that the cortisol effect involves peptide(s) with a relatively short half-life (10 to 20 minutes). The kinetics of RNA synthesis indicate that this peptide(s) must be present in the cells before the steroid is added.

At the present time it seems clear that some steroid hormones can rapidly regulate the synthesis of selected RNA species and that this control system may involve steroid-protein interaction. Cell fractionation studies and experiments examining the binding of isotopically labelled steroids to cellular proteins indicate, however, that most of the steroid is in free solution in the cell. Thus, other important regulating mechanisms, not involving protein-binding, are possibly involved; some, such as the alteration of specific transfer RNA secondary structure and hence coding properties, are being investigated.

Another series of experiments has examined DNA secondary structure during cortisol action in rat liver cells and rabbit lymph node cells in suspension culture. DNA was isolated from cell cultures at various intervals after the addition of the steroid, purified, and secondary structure analyzed by thermal denaturation by reaction with ^{14}C-formaldehyde and by counter-current distribution in an alkoxyethanol-trilithium citrate solvent system. Lymphoid cell DNA showed a reduction with time in the number of partially denatured regions, while liver cell DNA showed an increase with time of the number of partially denatured regions. In both cases the secondary structure of the DNA reverted to control patterns 60-90 minutes after cortisol addition. These alterations showed a correlation with the contrary effects of the steroid on messenger RNA synthesis in the two cell types in culture: lymphoid cell messenger RNA synthesis was decreased and liver cell messenger RNA synthesis was increased, but both reverted to control values after 60-90 minutes cortisol. The cortisol effect on messenger RNA synthesis was selective in both cell types, as shown by counter-current distribution. These observations provide further evidence to support the above-mentioned bacterial model of strand separation during DNA-primed RNA synthesis. This means that the steroid hormone most probably exerts its genetic effects via protein associated with or interacting with DNA rather than by direct interaction with DNA, since it can cause opposite net effects in the two cell types. The observation that in both cases messenger RNA synthesis is selectively altered suggests, however, that the basic mechanism is the same in both cell types.

Hormone Control of Enzyme Synthesis and Activity

In vivo kinetic studies on the effect of steroids on the enzyme glutamic pyruvic transaminase in rat liver have confirmed other reports that this enzyme is specifically induced by cortisol. The maximum induction occurred

only after several days of chronic steroid administration to intact animals but in adrenalectomised rats a peak induction period of 3-4 hours was observed, followed by a fall in enzyme level, although the half-life of the enzyme protein as measured by actinomycin-chase was > 24 hours. It seems probable that this secondary fall is due to inhibition of the enzyme activity by a cortisol metabolite.

Kinetic studies of steroid interaction with partially purified enzyme protein *in vitro* suggest that cortisol may activate the enzyme allosterically. This raises the possibility that induction of synthesis of the enzyme might be due to interaction of cortisol with a partially complete monome peptide chain resulting in an increased rate of chain completion and reinitiation. Taken together with the inhibition of formed enzyme by a cortisol metabolite (which might also act as a co-repressor of enzyme synthesis) it appears that total synthesis of glutamic pyruvic transaminase would depend on the relative amounts of cortisol and metabolite present at the site of polysomal synthesis at a given time. Other steroids such as testosterone also probably control the activity and synthesis of this enzyme.

MOLECULAR GENETICS*** *

C. C. Curtain, A. Baumgarten[1], E. Giles[2], N. Lobb, T. Golab, C. Kidson, P. Hughes[3], J. Blunck[3], and J. Baldwin.[4]

MOLECULAR BASIS OF CHEMICAL CARCINOGENESIS

The general pattern of action of carcinogenic and non-carcinogenic aminoazo dyes on gene transcription is now reasonably clear. Carcinogenic dyes slowly induce selective changes in the pattern of messenger RNA synthesis which are reversible for a time but later become irreversible. Non-carcinogenic dyes produce slower rates of change and these remain reversible for longer periods. The fact that it takes days or weeks to detect such changes suggests that, in contrast to hormones, these carcinogens do not show analogy with bacterial inducers and that the changes observed are probably secondary to earlier events.

Methylation of transfer RNA and ribosomal RNA as well as of DNA is important in inducing correct conformation and hence function in these molecular species. Abnormal methylation has been observed in a number of formed tumours, and has given rise to the theory that anomalous methylation might be a factor in some forms of carcinogenesis. Studies which have examined transmethylation of transfer RNA from ^{14}C (methyl) methionine in rat liver following administration of the carcinogenic and non-carcinogenic aminoazo dyes have shown that within four hours grossly anomalous transmethylation does occur. From a control ratio of ^{14}C-methyl-pyrimidine nucleotides/^{14}C-methyl-purine bases of 20 : 1, the ratio in the presence of a carcinogenic dye was found to fall to values in the range 8 : 1 to 2 : 1. While final identification of all methylated bases has not yet been completed, the dramatic change in pyrimidine/purine methylation may play a critical role in the early stages of aminoazo dye carcinogenesis. Since methylated nucleotide bases in RNAs are important determinants of secondary structure and of their specific code-recognition functions, this observation suggests that some steps in aminoazo dye carcinogenesis may involve non-mutational alterations of genetic code translation in the early stages.

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MOLECULAR TAXONOMY

Two approaches have been used to study genetic relatedness among Australian marsupials at the molecular level. The first has consisted of comparing countercurrent distribution patterns of single-stranded DNA preparations made from either liver or kidney of the koala, wombat and ringtail possum. This method fractionates single-stranded DNA by nucleotide base composition and base sequence and provides reproducible profiles of DNA from a single source. The patterns of the three marsupial species were found to be markedly different.

The second approach is a more traditional one; the finger-printing of haemoglobins from these animals by two-dimensional paper chromatography of tryptic digests. Again, the fingerprints of the three species are markedly different.

Whilst these data must be interpreted cautiously, it is evident that the differences in genome structure in the koala, the wombat and the ringtail possum are considerable, whether one measures either the total DNA sequence or the amino acid sequence of a small number of cistrons. If these methods do indeed measure genetic relatedness, the inference is that these Australian marsupials are widely separated in terms of molecular evolution, perhaps more widely than might be expected on morphological grounds. However, these methods, and others, clearly illustrate the limitations of any system of taxonomic classification.

COMPUTER SIMULATION IN POPULATION AND MOLECULAR GENETICS

This project was begun with the aim of exploring the potentialities of the fast general purpose digital computer for constructing models of the hormonal programming of mammalian RNA and protein biosynthesis as a help in the prediction of likely components of the system, and to study the analogy between mammalian hormones and the chemical inducers and corepressors of bacterial enzyme synthesis. We were also interested in the molecular bases of hormone selectivity and the role played by competitive and allosteric phenomena. Further aims were to analyse the published data for the kinetics of known synthetic systems (such as the isoleucine operon of Changeux and the histidine operon of Ames and Hartman) in an attempt to study such factors as template stability and reutilization modulation, coding and the probability of template translation.

Like all chemical reactions the steps involved in the processes of protein biosynthesis may be assumed to be governed by the law of Guildberg and Waage, i.e., the rate of a reaction is proportional to the concentration of the reactants. The application of this law to a given reaction system leads to a set of simultaneous, ordinary non-linear differential equations. The difficulties associated with the solution of these are well known and are emphasized by the nature of the processes involved in the transcription and translation of the genetic code. Here, small changes in the input lead to a large change in the output, i.e., the equations are "ill conditioned" or inherently unstable, making the conventional Runge-Kutta methods of solution unsuitable. The most closely analgous problems in physics are those involving the determination of the flow of heat in a body and the determination of electric and magnetic fields in regions with irregular boundaries. These equations may be solved by what are called relaxation methods.

Using this approach it was possible to produce a simplified model of the Jacob-Monod hypothesis and then test it against published data for the kinetics of induced enzyme synthesis in various bacteria. At this stage two things became evident. First, the time resolution of the published data was inadequate for a critical test of the model, and, secondly, anything more complex than the simplest model involving a single cistron and its product would severely tax the capacity of the medium-sized general purpose computer available to us. In an attempt to improve the time resolution of the kinetic data protein biosynthesis studies a metabolite metering device was designed and built. It consists of a digital clock controlling a network of solenoid valves which can meter

compressed air impulses to a series of automatic pipettes which deliver the desired metabolites, inducers and repressors into the culture vessel. The device has been tested electronically and mechanically but has not yet been applied to actual studies in protein biosynthesis. The problem of computer speed can only be solved either by the availability of time on a much faster digital computer or the availability of hybrid type computers in which the steps involving differentiation are carried out by electronic analog devices although the machine still has the logic and memory of a digital computer. Such machines have been built in centres overseas for the studies in problems involving the programming of large numbers of differential equations and it is possible that this type of computer may become more widely available in the future.

The relationships involved in population genetics, particularly those found in the study of gene flow, bear many resemblances to the equations of motion programmed for our molecular model. Using the same sub-programmes we have explored the possibility of constructing models of the relationships between the insular or pseudoinsular populations which may be found in the highlands of New Guinea. These models have had some success in enabling us to study the effects of different kinds of marriage proscriptions, random fluctuations, migration and selective forces on the gene pools of given communities.

THE DISTRIBUTION OF VARIOUS GENETIC MARKERS IN NORTH-EAST NEW GUINEA

The genetic diversity found in New Guinea may have arisen through the mechanisms of natural selection, migration, mutation pressure or, divided, as the island is into many relatively isolated populations, the random effects on the gene pool of various disasters, warfare, and the sampling error inherent in a small population, acting singly or in combination.

As a region for the investigation of these phenomena the Markham Valley of North-east New Guinea provides a remarkable range of environment and culture. In the area studied, a rectangle about 60 by 80 miles, extending north-west from Lae at the mouth of the Markham River, the villages ranged in altitude from sea level to 5000 feet and both New Guinea's principal linguistic stocks, Melanesian and non-Austronesian, are present in roughly equal numbers. The markers studied were the Gc (group specific), haptoglobin and transferrin polymorphisms, the erythrocyte glucose-6-phosphate dehydrogenase deficiency (G6PD) and the presence or absence of the gene for β thalassaemia. In the areas studied the haptoglobin gene frequencies varied from 90.0% to 61.4% and the genes present were $Tf^{D}1$ and in low frequency Tf^{B}Lae. Overall, no apparent correlations were found between the frequencies of these genes and altitudes, languages or distances up the valley of the villages studied. It was felt that the arguments we have put forward earlier emphasizing the role which genetic drift plays in determining gene distributions in New Guinea could also be applied to explain distribution of haptoglobin and transferrin genes in the Markham River Valley. The highest G6PD deficiency frequency was found in the sea level village of Labubutu (18.2%). Low frequencies of 1-2% were found in the villages of the Markham Valley flatlands and at a much greater altitude on the eastern edge of the Eastern Highlands. Other mountain villages at roughly the same elevation, however, demonstrate quite different G6PD deficiency frequencies ranging from 2.0% in Binumarien at 4500 feet to 8.5% in Mamaban at 4000 feet. This genetic heterogeneity of the G6PD marker is difficult to interpret in terms of simple selective pressure.

GENETIC CONTROL OF IMMUNOGLOBULIN SYNTHESIS

Separate Localization of the Immunoglobulin Factors Gm(a), Gm(b) and Gm(x) in Lymphoid Tissue obtained from Individual of $Gm^{ax/ab}$ Genotype.

Using fluorescent antibodies we have already shown that in Caucasians of phenotype Gm(ab) the Gm(a) and Gm(b) immunoglobulin markers are localized in separate plasma cells of the medullary cords of the lymph nodes and in the red pulp of the spleen. In Caucasian populations Gm(a) and Gm(b) behave as alternate

alleles. However, practically all non-Caucasian peoples are 100% Gm(a+) and the Gm(b) marker, when it occurs, is always associated with Gm(a). The Gm(ab) character has been postulated by Steinberg to be a product of the Gm(ab) gene. This gene occurs with 100% frequency in Negroes and in varying frequencies in Asians and Micronesian populations. It also occurs in the Melanesians of New Britain and the mainland of New Guinea in frequencies ranging from 25-100%.

The immunoglobulin markers Gm(a) and Gm(b) were localized by a fluorescent antibody in separate populations of plasma cells in the medullary cords of lymph nodes obtained from Melanesians of $Gm^{ax/ab}$ genotype. These results suggest that Gm(a) and Gm(b) were coded by separate although closely associated cistrons with separate regulator genes. In individuals possessing Gm(ab) the cistrons are on the same chromosome, in Caucasian populations which possess Gm(a) and Gm(b) they are not.

REGULATORY GENES CONTROLLING THE SYNTHESIS OF H-CHAINS OF HUMAN IMMUNOGLOBULIN-G

In this project we have used two IgG H-chain genetic markers, Gm(a) and Gm(b) to analyse more critically the synthesis of immunoglobulins in phytohaemagglutinin stimulated cultures of human peripheral blood lymphocytes. With donors of serum phenotype Gm(a) many of the cultured cells showed a positive reaction with fluorescent antibody to the Gm(a) marker. Unexpectedly, however, a small but significant number of cells were also positive with fluorescent anti-Gm(b). Cultures from Gm(b) donors also gave rise to two populations of cells, one reacting with anti-Gm(b), the other with anti-Gm(a). These results suggest that the serum phenotype of an individual need not reflect the genotype of the cells producing IgG. Possibly, regardless of serum phenotype, the structural cistrons for both Gm(a) and Gm(b) are normally present in the genome, but the phenotype depends upon the inheritance of regulator genes which control the expression of these structural cistrons. An individual whose serum phenotype is Gm(a+b−) may possess both Gm(a) and Gm(b), together with a regulator gene R(b) which causes the repression of Gm(b). When his cells are cultured in the presence of PHA the regulator gene or its products are inactivated, allowing the expression of Gm(b).

These remarks are of general application to the detailed genetic analysis of the control of immunoglobulin synthesis. With a panel of antisera to the other Gm factors the system could be extended considerably to give a much more complete picture of the genome concerned with immunoglobulin synthesis.

AUTOANTIBODIES IN TROPICAL POPULATIONS

A. Baumgarten[1], C. C. Curtain, T. Golab, J. Gorman[2], C. Kidson and C. Rutgers[3].

Previously we have reported cold haemagglutinins of relatively high titre (64-600), high incidence (10-16%) and unusual specificity in the Melanesian populations of the Gazelle Peninsula of New Britain. The widespread occurrence of these cold-reacting erythrocyte autoantibodies poses a number of intriguing questions which are relevant to the general problem of autoimmunity. Briefly, are they antibodies to parasitic, bacterial or viral antigens which cross react with erythrocyte antigens, perhaps so weakly that they require a low temperature to stabilize the union; are they formed in response to the production of modified erythrocyte antigens following haemolysis of viral or malarial origin, or are they by-products of the high rate of immunoglobulin synthesis found in tropical peoples? In an attempt to answer some of these questions, we studied two separate populations, one group living at sea level and in the lowlands of the Gazelle Penin-

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sula of New Britain, and the other living at 7000 feet at Laiagan in the Western Highlands of New Guinea. Strong cold haemagglutination, ranging in incidence from 50 to 90% was observed in both populations. It was judged by the reactions with cells from a panel of donors that many of the antibodies were directed against an antigen or antigens of unknown specificity occurring in varying strengths on different adult erythrocytes.

In contrast to the observation made by Harboe that only K-light chains are found in cold haemagglutinins obtained from patients suffering from the cold haemagglutination syndrome, we found that very few of the cold haemagglutinins in our New Guinea collections were of K-chain type; the majority appeared to have K and λ chains. This conflicts with Harboe's hypothesis that K-chains may be needed for cold haemagglutination. The heavy chains from the cold haemagglutinins were separated and fingerprinted. It was found that the cold haemagglutinins from the New Guinea sera had very different peptide patterns from those isolated from sera of patients suffering from chronic cold haemagglutination syndrome or lymphoma. In contrast to the relative uniformity of those occurring in these conditions, the New Guinea cold haemagglutinins appear in their complexity to be closer to whole 19S macroglobulin.

The genesis of the cold haemogglutinins is still obscure at Laiagam, an area virtually free of mosquito-borne diseases, in particular malaria and haemorrhagic virus infections, it seems unlikely that the cold haemagglutinins present in the population could have arisen from antigenic stimulation by the by-products of red cell breakdown.

If intense reactivity of the reticuloendothelial system alone will cause the appearance of cold haemagglutinins then we would again expect their incidence and titre to be lower in the population of Laiagam where the mean gammaglobulin levels were significantly lower than that of any of the groups studied on the Gazelle Peninsula of New Britain. However, our results show that, although there may be differences in specificity, the incidence and titre of cold haemagglutinins at Laiagam are not lower, but even higher than those found in many of the New Britain populations.

It is well known that certain infections give rise to cold haemagglutinins of varying specificity. It is possible that cold haemagglutinins observed in New Guinea may arise as the result of stimulation by microbial antigens which are closely related to erythrocyte antigens. The reactions of cold haemagglutinins with the erythrocyte autoantigens takes place only below the normal body temperature, and hence, suppression of the production of these antibodies by the mechanisms of immunological tolerance need not occur and they may therefore reach high titres. This escape from the mechanisms of immunological tolerance may also apply to antibodies which may be fortuitously produced by a hyperactive reticuloendothelial system.

We have also investigated the incidence of serum antibodies which are capable of fixing complement with tissue extracts and which are also capable of localizing in sections of kidney, spleen, and brain as detected by the fluorescent antiglobulin test. This year it has been possible to collect fresh postmortem serum and tissues at Port Moresby, and it has been found that these antibodies appear to be iso- rather than auto- antibodies. That is, they give much lower titres with autologous tissues than with isologous tissues. The genesis of these antibodies may also be explained by supposing that they are the result of infection by microorganisms which possess antigens which cross-react with those of human tissues. Since the autoantibodies formed are not of cold reacting variety they are suppressed by the normal mechanisms of immune tolerance, whilst the isoantibodies remain.

P. Fantl, R. J. Sawers[1] and T. Gwyn Jones

Osmotic Stability of Blood Platelets

Platelet-containing plasma obtained from citrated or EDTA treated blood of man, monkeys (*Macacus irus*), dogs, sheep, rabbits, cats, calves, pigs, horses and brush-tailed possums (*Trichosurus vulpecula*), were diluted with solutions of differing osmolarities.

Additions of isotonic sodium chloride produced little change in absorbency at 610 mμ at 30°C during 15 minutes. By lowering the osmolarity a transient drop in absorbency occurred which was followed by a gradual rise.

Mixture of 0.37 mM stearate and human platelet-containing plasma resulting in 200 and 300 m-osmole respectively produced similar absorbency changes as did mixtures with inorganic salts of similar osmolarities. Mixtures containing 0.82 mM stearate and human platelet-containing plasma showed, after lowering the osmolarity from 300 to 200 mM, the same drop of absorbency as was found in previous experiments but this stearate concentration damaged the platelets. On the other hand, 0.9 mM oleate did not have a greater effect on absorbency of platelet-containing plasma than did inorganic salts of equal osmolarities.

Platelets are distended but not aggregated by stearates. Hypertonic solutions produce shrinkage of platelets associated with increase in absorbency. Hypertonic sodium chloride and saccharose produce permanent increases in absorbency. In human platelet-containing plasma the increased absorbency caused by urea is transient and declines.

The Varying Influence of Urea on Blood Platelets

In order to obtain some information about the mechanism by which urea produces osmotic changes in platelets, experiments with "fresh" and "stored" citrated human platelet-containing plasma were carried out. The results show that the reversal of absorbency which follows hypotonic swelling of fresh platelets is reduced to 6% of that obtained with fresh platelet-containing plasma after 7 hours' storage at 4°C. However, the reversal of absorbency due to urea in "fresh" and "stored" platelet-containing plasma was not significantly different.

That the osmotic changes of platelets are independent of adenosine-5'-diphosphate (ADP) is seen first by the fact that, at 1000 m-osmole, urea inhibited platelet aggregation following addition of ADP. However, at a urea concentration of 820 mg.% (440 m-osmole) in plasma, which is in the pathological range, ADP aggregation is not impaired.

The reversal of absorbency following hypotonic swelling and hypertonic shrinking have $Q_{10} > 1$, indicating chemical-enzymic reactions.

The presented experiments indicate further that the platelet components associated with the reversal of absorbency are, in the presence of urea, more stable than those associated with reversal of absorbency following hypotonic platelet swelling.

It appears that human and pig platelets are not permeable to hypertonic sodium chloride, but readily permeable to sucrose and slowly permeable to urea. The platelets of other mammals and of a marsupial are not permeable to hypertonic sodium chloride, but are readily permeable to saccharose and urea. The marked species differences in reactivity of platelets to hypertonic urea indicate that the envelope of the human and pig platelet is different from that of other mammals and a marsupial.

The Effect of Fluoride on Blood Platelets

The addition of NaF to platelet-containing plasma of mammals and marsupials produced, after a delay period, irreversible reduction of light absorbence which is dependent on fluoride concentration.

The reduction of absorbency was found to be greater in citrate than in EDTA platelet-containing plasma. Fluoride affected both

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platelet-containing plasma and isolated platelets.

N-ethylmaleimide prevented absorbency changes induced by NaF. ATP had no influence on the fluoride reaction. NaF-induced swelling of platelets was observed during storage of platelet-containing plasma in the cold for several hours; the fluoride reaction had a $Q_{10} > 1$. Fluoride treated platelet-containing plasma gave a shortened plasma recalcification time and reduced clot retraction.

It is concluded that the NaF effect is independent of ADP, that fluoride reacts slowly with a -SH platelet component and that divalent cations may be involved.

The Influence of Pyrazole Derivatives on Blood Platelets

In contrast to hypertonic urea, which causes an immediate but transient increase in absorbency of human platelet-containing plasma, hypertonic antipyrine causes an immediate reduction in absorbency. The urea effect is characteristic for human and pig platelets; antipyrine affects both human and dog platelets. Antipyrine in isotonic concentration causes little change in absorbency of platelet-containing plasma, but platelet aggregation by ADP is inhibited at pH values between 7.1 to 7.9.

Butazolidine, Tanderil and Anturan are more potent as inhibitors of platelet ADP aggregation than antipyrine.

Disorders of Haemostasis

The study of a wide range of disturbances causing either thrombosis or abnormal bleeding was made in both old and new patients; brief comments on some of these follow:—

The stimulating effect of plasma infusions on Factor **VIII** in patients with von Willebrand's disease was further examined for diagnostic and therapeutic purposes. Serial infusions in one patient undergoing surgery resulted in long sustained elevation of Factor **VIII;** the pre-infusion plasma activity of Factor **VIII** was 5% of normal, but during treatment the activity did not drop below 30% when infusions were given at three-day intervals. However, limitations of the value of plasma as a diagnostic agent is apparent when Factor **VIII** levels are high, i.e., 30% - 40% of normal. In an attempt to differentiate between the diagnosis of the carrier state of haemophilia A with prolonged skin bleeding time, and von Willebrand's disease in a female patient, although a plasma infusion resulted in complete correction of the Factor **VIII** deficiency it did not cause a sustained rise. The usefulness of serum as a diagnostic agent is being investigated again as it contains the stimulating factor, while lacking in Factor **VIII** activity.

The variable response to transfused Factor **VIII** in patients with haemophilia A undergoing surgery has been further examined and confirmed. While most patients' requirements are similar, a few appear to require appreciably less Factor **VIII** concentrates to maintain elevation of the plasma concentration of the factor. The phenomenon does not appear to be due to inhibitors of the immune type as the "half life" of the transfused Factor **VIII** is the same in all patients, and requirements do not increase during convalescence.

One patient with haemophilia A did develop an inhibitor within a few days of an operation. This inhibitor showed the unusual feature of different activities against the Factor **VIII** of plasma from different species. When the patient's plasma was tested against human Factor **VIII** the activity of the inhibitor was 7 units per ml; the inhibitor activity against both pig and beef Factor **VIII** was only 0.7 units per ml.

Two patients with acquired haemorrhagic disorders showed unusual features. One presented with unexplained abdominal pain and later abnormal bleeding. She was shown to have the chronic defibrination syndrome with associated red cell fragmentation. These disturbances were caused by haemangiosarcoma of the liver. The other patient was seen while convalescing from an operation which had been complicated by severe post-operative bleeding. Despite extensive investigation, the only abnormality found was instability of her whole blood and whole plasma clot. Clot prepared from saline-diluted plasma was stable. As lysis was not prevented by ε-amino-caproic

acid, it was thought she may have had an abnormal fibrinogen. However, as the abnormality disappeared spontaneously after one week, it was attributed to the presence of undetectable breakdown products resulting from acute fibrinolysis occurring after operation.

Several members of a family who suffered from recurrent venous thrombosis were shown to be deficient in progressive anti-thrombin; this defect was not demonstrated in several other patients with isolated attacks of thrombosis.

Thrombosis Associated with Deficiency of Heparin Co-factor

The antithrombins are responsible for limiting the extent of thrombin action in the human body. Originally separated into four fractions found in normal physiological conditions and two found only in certain disease, it is now suggested that there is one thrombin inhibitor in the human system under physiological conditions. Reduction in the level of the antithrombins is believed to be responsible for certain hypercoagulable states. A family which has a high incidence of thrombotic episodes has been studied from this viewpoint. This study has accompanied laboratory investigation of the characteristics of heparin co-factor (antithrombin **II**) and antithrombin **III.** The difference between the level of plasma and serum antithrombin has been confirmed and experiments which attempt to assess the rate of re-appearance of these factors after they have been reduced by intravenous thrombin in animals are in progress. It also appears that the level of these antithrombins can be correlated with the state of hepatic function being markedly decreased in liver failure.

Heparin Rebound Phenomenon

The re-appearance of heparin after adequate initial neutralization by protamine was described by Fantl in 1960. Recent investigators state that this was encountered only if the sulphate of protamine was used and did not occur with the chloride salt. This has been partly confirmed, and it has also been found that a considerable individual variation in the degree of heparin release occurs. Attention is now being directed towards the possibility that the difference in the behaviour of the two protamine salts does not lie in the type of salt but in the presence of an impurity affecting the stability of the heparin-protamine complex.

Thrombolytic Therapy

Considerable interest in the possibility of removing intravascular blood clots has been shown recently. The indications for this type of therapy are becoming more clearly defined and at the moment its use is confined to central retinal artery occlusion.

HYPER AND HYPOTENSIVE STATES

A. J. Barnett and S. Cantor

HYPERTENSION

The hypertension clinic, commenced 16 years ago, is concerned with the follow-up of patients with severe hypertension who have been admitted to hospital for the control of their hypertension; most have had Grade III or IV eye signs. Such cases are now rare, so that the intake into the clinic is low. To date, 194 patients have been admitted to the clinics; 71 have died, 105 are currently attending, and 11 patients were admitted during 1966.

The most commonly used treatment is a combination of guanethidine, methyldopa and a thiazide.

Methyldopa and Positive Coomb's Test

Following reports from Great Britain that about 20% of patients being treated with methyldopa develop a positive Coomb's test and a very small percentage of patients treated with this drug develop haemolytic anaemia, it was decided to examine the patients in our

clinic for positive Coomb's test and evidence of haemolysis. Eighty patients were under treatment with methyldopa. Coomb's tests were performed in 55 of these patients with positive tests in 8. No positive Coomb's tests were found in 25 hypertensive patients not taking methyldopa. A raised reticulocyte count (3%) was found in 3 of the 8 patients with positive Coomb's test, but also in 3 of the 6 patients without positive Coomb's test.

Our results confirm the claim that positive Coomb's test may occur in patients being treated with methyldopa although the incidence in our series is less than that reported from Great Britain. We have not experienced any cases of haemolytic anaemia in patients being treated with methyldopa.

New Hypotensive Drugs

(a) **Debrisoquin ("Declinax")*** is a new hypotensive drug which is reputed to act on post-ganglionic sympathetic pathways. In this respect its action is similar to guanethidine, but it differs from guanethidine in that the onset and decay of its action is more rapid.

In 10 patients with severe hypertension being treated with guanethidine and other drugs, the effect of withdrawal of guanethidine and substitution of debrisoquin was noted.

In 6 patients equivalent blood pressure control was achieved with guanethidine and debrisoquin. However, in three cases, treatment with debrisoquin had to be terminated, in 2 because of side effects and in 1 because of other (probably unrelated) symptoms. In 3 patients control was better with guanethidine. In 1 patient control with both drugs was ineffective. The daily dose of debrisoquin was 2 to 4 times that of guanethidine.

Side effects included diarrhoea, abdominal pain, faintness, blurred vision. It was concluded that debrisoquin is an active hypotensive drug. However, in this small series of severe hypertensives, it compared unfavourably with guanethidine in respect to control of the hypertension and side effects.

(b) **Catapresan:** The haemodynamic effects of Catapresan (ST155) in man have already been described in this report.

The hypotensive effect of an oral dose of 150μg was tested in seven patients. A moderate hypotensive effect was observed in six subjects. In only one of these cases was there a significant postural effect. The hypotensive action was established after 1-2 hours and lasted from 4-8 hours. The only side effect detected was drowsiness.

Continued treatment with Catapresan was attempted in three patients; in two patients the treatment was terminated because of possible side effects—soreness of the chest and paraesthesia in one case, Raynaud's phenomenon and paraesthesia in the other case. One patient is still under treatment.

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* Supplies of Debrisoquin ("Declinax") were kindly supplied by Roche Products Pty. Ltd.

A. J. Barnett, K. N. Morris[1], I. A. Ferguson[2], K. Stuchbery[1] and G. Stafford[1]

Reconstructive Arterial Surgery

During 1966 eighty operations for relief of occlusive arterial diseases have been performed, this being less than for the previous year. The new techniques commenced in 1965 have been continued and vein grafting is now established as the method of choice for long-standing occlusion of the superficial femoral artery and balloon thrombectomy for acute occlusions.

The following table shows the procedure adopted in operations in 1966:

Vein graft	36
Synthetic graft	12
Endarterectomy or removal of old thrombus	14
Embolectomy or removal of recent thrombus	6
Miscellaneous and complicated procedures	12
	80

Embolectomy or removal of recent thrombus was by means of the Fogarty balloon catheters. These were also employed in 5 of the complicated procedures.

Review of Arterial Grafting Results

Arterial grafting for the relief of symptoms of arterial occlusion, particularly intermittent claudication and ischaemic rest pain, was introduced in the Alfred Hospital in 1955. To date, 309 arterial grafting operations have been performed. The results of 299 of these are available for review and comprise 45 arterial homografts, 44 teflon grafts, 118 dacron grafts, and 92 autogenous vein grafts. It was considered that now is an appropriate time to assess the results of reconstructive arterial surgery in general and the various types of arterial graft in particular.

The data show that there are 4 consecutive periods and that the favoured grafting material was arterial homograft (1953-58), teflon (1959-61), dacron (1961-66) and autogenous vein (1963-66). There is an overlap of the dacron and vein periods as a few early trial vein grafts were inserted in 1963 before this became the method of choice and dacron grafts are still being used for grafts to large arteries (from aorta to common femoral) and occasionally to bypass a block of the superficial femoral artery. The age of the patients in the homograft and teflon periods was similar, about 50% of the patients being over 60 years of age; in the dacron and vein periods, there is a higher proportion of older patients with approximately 60% over 60 years of age. Distal ischaemic symptoms either acute or chronic indicate more severe ischaemia than does claudication alone. In the homograft and teflon periods, approximately 50% of cases had these symptoms of severe ischaemia; in the dacron and vein periods, as many as 65%. The most common anatomical site of the graft was femoro-popliteal, which accounted for practically all the grafts in the homograft, teflon and vein periods. In the dacron period there is a larger proportion of grafts involving larger vessels with 26 of the 118 (22%) being aorto-iliac or aorto-femoral.

The patency rates of the various grafts is shown in the table, where the percentage patency is expressed in two ways: (a) the patent grafts as a percentage of the total grafts (assuming that in those dead or lost the graft would have blocked), and (b) the patent grafts as a percentage of those in known survivors, assuming that those dead or lost would have a blockage rate in proportion to those studied. Both these methods give only an approximation of the true state of affairs, which probably lies somewhere in between. The tables indicate that, although there is a high immediate success rate (85% or over) by all methods, when judged by the late blockage rate (1 year and 4 years), the synthetics are apparently inferior to homografts. Vein grafts have not been used long enough for 4 year patency figures, however, the patency at 1 year (75%) is much higher than with any of the other methods. This is not due to better subject material because the severity of ischaemia was on the average more severe and the patients older than in the arterial homograft and teflon series.

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TABLE

Patency rates of the various type of graft.

(a) **Expressed as percentage of total grafts:**

	Percent patent at		
	Discharge	1 year	4 years
Arterial homograft	93 n = 46	59 n = 46	39 n = 46
Teflon	86 n = 43	40 n = 43	9 n = 43
Dacron	97 n = 118	43 n = 105	11 n = 44
Autogenour vein	91 n = 92	75 n = 83	

(b) **Expressed as a percentage of grafts in known survivors:**

	Percent patent at		
	Discharge	1 year	4 years
Arterial homograft	93 n = 46	60 n = 45	39 n = 42
Teflon	86 n = 43	17 n = 41	12 n = 37
Dacron	85 n = 118	48 n = 89	30 n = 37
Autogenour vein	92 n = 91	77 n = 79	

CLINICAL TRIALS OF ANTI-ANGINAL DRUGS

S. Cantor and A. J. Barnett

Comparison Between Vialibran and Iproveratril

An attempt was made to evaluate the clinical usefulness of a relatively new and poorly studied coronary vasodilator, Vialibran (N-piperonyl-N-benzhydryl piperazine dichlorhydrate), by comparing its effect with that of a known clinically useful coronary vasodilator, Iproveratril (α isopropyl α (N-methyl-N - homoveratryl) - γ - aminopropyl)3,4 - dimethoxyphenylacetonitrile hydrochloride; 'Isoptin'. Eight patients received three courses of treatment, one of vialibran, one of iproveratril and one of placebo. The patients, all males aged between 40 and 60 years, had suffered from angina pectoris for from three to twelve years. All cases showed E.C.G. evidence of ischaemia but myocardial infarction had not occurred within the preceding three months.

After an initial two week control period, treatment with the appropriate drug or placebo was started. Criteria regarded as indicative

of improvement were a decrease in the number of anginal attacks, a decrease in the number of glyceryl trinitrate tablets taken, increased resistance to known precipitating factors, and exacerbation of symptoms after cessation of drug therapy.

These tests showed that both vialibran and iproveratril can relieve angina pectoris and that both drugs do so with the same frequency of success. Since considerable individual variation in the response to these drugs was evident a larger clinical trial is needed to confirm the effectiveness of vialibran as an anti-anginal agent and to explore possible toxic side effects.

ULCERATIVE COLITIS

A. D. McCutcheon

Ulcerative colitis is a condition of unknown aetiology in which the pathological features have often been described using ordinary histochemical stains. A fresh study of the condition was undertaken, using a variety of enzyme stains. Blocks of tissue were taken from fresh operative specimens, from 4 sites—terminal ileum, ascending colon, transverse colon and descending colon or rectum. Sections from each region were stained for succinic dehydrogenase, lactic dehydrogenase, glucose-6-phosphate dehydrogenase, non-specific esterase, adenosine triphosphatase, alkaline phosphatase and with periodic acid schiff, alcian blue, acid fuchsin and chlorantine fast red; (in collaboration with Dr. R. Wyllie). The material included 6 normals, 6 patients with ulcerative colitis, and 2 with carcinoma of the large bowel.

These stains were used to study selectively the various tissues involved in the inflammatory process; ganglia by esterase, LDH and ATPase; mast cells by alcian blue and acid fuchsin; elastic tissue by acid fuchsin; arterioles and capillaries by alkaline phosphatase and the larger vessels by ATPase. In ulcerative colitis it is possible to find relatively normal areas near areas of gross pathological change. By studying the more normal tissue in which the earliest pathological changes are present, it was hoped to gain some insight into the pathogenesis of the condition.

The main findings were: vacuolation of superficial epithelium with loss of basement membrane in the earliest mucosal lesions and marked dilation of small blood vessels immediately beneath the mucosal epithelium. No definite pattern could be attributed to changes in number or staining of mast cells. A curious feature of the SDH stain is its patchy localization in isolated glands in both normal and abnormal tissue. This seems to represent patchy distribution of the enzyme rather than an artefact.

OESOPHAGEAL MOTILITY

D. A. Coventry

In the past year 22 recordings of oesophageal motility have been made in 20 patients. The apparatus used consisted of four water-filled open-tipped polythene tubes attached together so that the openings were 5 cm. apart. The pressures were recorded via four strain gauges linked to a multichannel recorder, recording on ultraviolet light sensitive paper. This apparatus provided linear pressure tracings which were found to be accurate by two independent checking manoeuvres. Variable paper speeds were used to study the pressure response to swallowing in the region of the oesophageal sphincters.

The patients studied included 12 patients with scleroderma and 8 patients with either dysphagia or other evidence suggestive of diffuse oesophageal spasm. In the patients with scleroderma the main finding was reduction of

oesophageal peristalsis. Other findings included poor delineation of the gastro-oesophageal sphincter, increased resting pressure in the body of the oesophagus and normal sphincteric responses in the region of the pharyngo-oesophageal sphincter. The manometric assessment of oesophageal motility was found to correlate well with the radiological assessment of motility in these patients.

Amongst the patients with other disorders, two with mild dysphagia were found to have normal oesophageal motility patterns. Three patients with suspected oesophageal spasm and one elderly patient with dysphagia revealed only minor abnormalities. In two patients definite evidence of oesophageal spasm was found.

It is felt that oesophageal manometry provides useful information in some patients with oesophageal disorders and that it is best used as a complementary diagnostic test. When taken in conjunction with clinical, radiological and endoscopic findings, oesophageal manometry has been of use in planning treatment for the individual patient. Further clinical information may be obtained by the combination of motility recordings and cine-radiography in the future and simultaneous recording of pressure, potential differences and pH should increase the value of the procedure as a research tool.

SCLERODERMA

A. J. Barnett and D. A. Coventry

Survey

The survey, commenced in 1965, of patients with scleroderma seen in this hospital has been continued. This has comprised a follow-up of 27 patients described in 1959 plus an additional 34 cases, giving a total of 61 cases.

The present follow-up states of these 61 patients is shown below.

Follow-up States	Number of Patients 1959 Series	Additional Cases	Total
Patients died	11	3	14
Patients alive and condition known	13	26	39
Patients lost from follow-up	3	5	8
	27	34	61

Thirty-one of the 39 known surviving patients have been fully surveyed by means of detailed history, clinical examination and tests.

From a study of all the data, we have made observations on the clinical types of the disease, age and sex incidence, involvement of individual systems and course of the disease. We have looked for possible aetiological factors and assessed the effects of the treatments used.

Scleroderma can conveniently be divided into three types, distinguished by the extent and rapidity of development of the skin involvement. In Type I the skin involvement is limited to the digits, in Type II it spreads slowly to involve other parts, particularly the face and neck; in Type III it spreads rapidly to involve most of the body.

The disease affects females more frequently than males (ratio 2.5 : 1), with a large pre-

dominance of females in Type II. The disease may commence at any age, but the highest incidence of onset is from 40 to 60 years. In addition to cutaneous and vascular disturbances which occur in all patients, gastrointestinal, cardiac, pulmonary and locomotor-system involvement is common in all three types.

In general the severity of the involvement tended to progress with time. Fourteen of the 61 patients were known to have died (4 Type I, 4 Type II and 6 Type III). In none of the Type I cases did it appear that death was related to scleroderma, but it was definitely or probably related in all of the Type II and Type III cases. In all the Type II cases death occurred more than 10 years after the onset, but in 5 of the 6 Type III cases within 5 years of the onset.

No definite causative factor was discovered. In particular we could find no evidence for an auto-immune basis for the disease.

No specific therapy is available. In some cases improvement in the skin follows use of adrenal cortico-steroids, and in some cases the circulation is improved by sympathectomy. However, much help can be given to these patients by appropriate symptomatic treatment.

Alimentary Tract Involvement

Abnormalities thought to be due to associated involvement of the alimentary tract are found in many patients suffering from scleroderma of the systemic sclerosis type. In a series of 61 patients with this disease alimentary abnormalities were found to be the most common form of visceral involvement. Thirty-one patients from this group were studied carefully by means of a full clinical examination and a series of special investigations.

The most common abnormal findings were associated with reduction of oesophageal and intestinal motility. Using both radiological techniques and oesophageal manometry, reduced oesophageal peristalsis and its attendant problems of oesophageal dilatation, hiatus hernia or oesophageal stricture, was found in 80% of patients. In 58% this was associated with dyspepsia and in 50% dysphagia was present. Gastric abnormalities were not encountered and none of the 31 patients had any evidence of duodenal ulceration.

Small intestinal function was disturbed in many patients. Steatorrhoea was detected in 28% of the patients studied. Generally this was mild, but in one patient it was more severe and associated with other clinical and laboratory evidence of intestinal malabsorption. This patient had marked small bowel dilatation and reduced intestinal motility. He has shown a good response to treatment with oral broad spectrum antibiotics. It was considered that malabsorption in this instance was due to overgrowth of micro-organisms in the small bowel secondary to the reduced motility and dilatation. This mechanism may also explain the frequency of mild steatorrhoea seen in the other patients. Mucosal biopsies of the small intestine in 4 patients did not show any gross morphological change although some degree of cellular infiltration could be seen in the mucosal layer.

Minor changes in liver function tests were seen in 61% of patients. The most common abnormal finding was elevation of the serum γ-globulin level. It was considered that this probably was indicative of the nature of the disease rather than true hepatic involvement. None of the 31 patients had any clinical evidence to suggest the presence of liver disease.

Analysis of the whole series of 61 patients showed that alimentary involvement occurred progressively from the time of onset of the disease and that adrenal cortico-steroid therapy was not helpful in the treatment of the alimentary lesions. Only one of fourteen deceased patients died from an alimentary cause.

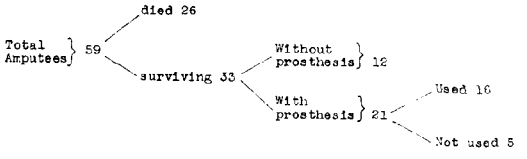
LOWER LIMB AMPUTEES

A. J. Barnett, D. J. Guthrie[1], J. McLean[2], and J. E. Crawshaw[2]

A prospective study has been carried out of all patients who had a lower limb amputation in 1964.

A follow-up of these patients was commenced in July, 1965, and a preliminary report was made in December, 1965. In the present study, all surviving patients (with the exception of 3 who had moved to country centres) were interviewed by a social worker. These patients (with the exception of one who died while the study was in progress) were examined by a physician and rehabilitation officer.

The following is a summary of break-down according to survival of patients, supply and use of prosthesis.



Why the prostheses were not supplied to 12 patients and why 5 patients to whom they were supplied failed to use them was studied. In review, there were adequate causes (poor general health, repeat amputation, ischaemia of the other limb) for not supplying the prosthesis in the 12 cases. In the 5 patients not using their prosthesis this was due predominantly to poor general health and psychological factors. In all cases the prosthesis had been supplied more than 6 months after amputation. In 6 of 15 patients examined with their prosthesis, the gait was regarded as faulty.

Home arrangements were usually not disturbed by amputation, although activity rating showed a down-grading. Purchase of a prosthesis placed a financial burden on the patients: 11 patients (including 5 pensioners) paid the full cost and another 5 paid part cost (usually one-third). In many cases amputation was associated with severe psychological stress and subsequent depression.

As a result of our study, we recommended that:

(a) a register of amputees be continued;

(b) there should be more careful psychological preparation of patients for amputation and postoperative support through the period of depression;

(c) care be exercised in the selection of patients for prosthesis;

(d) a follow-up system to correct faulty gait and give continuing guidance be instituted; and

(e) means of giving financial help to amputees should be explored.

[1] Rehabilitation Officer, Alfred Hospital.
[2] Medical Social Work Dept., Alfred Hospital.

PUBLICATIONS IN 1966

ENERGY PRODUCTION IN THE MYOCARDIUM†‡

Ionic Studies‡

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LECTURES DELIVERED DURING 1966

"Medical Aspects of Management of Occlusive Arterial Disease" — *Royal Australasian College of Surgeons, Melbourne.*	A. J. BARNETT
"The Value of Rectal Biopsy" — *Royal Australasian College of Physicians, Melbourne.*	D. A. COVENTRY
"The Genetic Control of Human Immunoglobulin Synthesis" — *Walter & Eliza Hall Institute, Melbourne.*	C. C. CURTAIN
"Cold Haemagglutination in Human Tropical Populations" — *CSIRO Division of Animal Health, Melbourne.*	C. C. CURTAIN
"Endemic Cold Haemagglutination" — *XIth Congress of the International Society of Blood Transfusion, Sydney.*	C. C. CURTAIN
"Immunocytochemical Localization of the Products of the Gm^{ab} and Gm^{ax} Alleles in Human Lymphoid Tissue" — *XIth Congress of the International Society of Blood Transfusion, Sydney.*	C. C. CURTAIN
"The Action of Kinekard on Smooth Muscle" — *Australian Physiological Society, Melbourne.*	N. DOREVITCH
"Hypercoagulability" — *Alfred Hospital Clinical Society, Melbourne.*	T. G. JONES
"Current Molecular Biology in U.S.A." — *Molecular Biology Seminar, University of Melbourne.*	C. KIDSON
"Regulation of DNA Transcription in Bacterial and Animal Cells" — *Institute of Nuclear Medicine, Hiroshima, Japan.*	C. KIDSON
"Transmethylation of Nucleic Acids in Amino-azo Dye Carcinogenesis."	C. KIDSON

"Molecular Biology of Carcinogenesis" — *Department of Pathology, Monash University, Melbourne.*	C. KIDSON
"Chemical Approaches to the Study of Gene Regulation" — *Department of Biochemistry, University of Melbourne.*	C. KIDSON
"Hormones: Nature and Actions" — *Department of Biochemistry, University of Adelaide.*	C. KIDSON
"Regulation of RNA and Protein Biosynthesis in Higher Organisms" — *Department of Biochemistry, University of Adelaide.*	C. KIDSON
"Hormone Control of RNA and Protein Biosynthesis" — *Department of Biochemistry, University of Adelaide.*	C. KIDSON
"Integration and Transfer of Biological Information" — *Department of Biochemistry, University of Adelaide.*	C. KIDSON
"DNA Secondary Structure and Regulation of Transcription" — *Syntex Institute, Stanford University, California, U.S.A.*	C. KIDSON
"Hormone Regulation of RNA and Protein Biosynthesis" — *Syntex Institute, Stanford University, California, U.S.A.*	C. KIDSON
"Hormonal Programming of Gene Expression" — *Syntex Institute, Stanford University, California, U.S.A.*	C. KIDSON
"Selection and Drift in Human Populations Studies in Melanesia" — *Department of Genetics, University of Hawaii, Honolulu.*	C. KIDSON
"Hormone Regulation of Nucleic Acid Biosynthesis" — *Department of Biological Sciences, Flinders University, South Australia.*	C. KIDSON
"Kinekard" — *Cardiac Society of Australia and New Zealand, Adelaide.*	T. E. LOWE
"Antiarrhythmic Drugs and Ca Ions" — *Department of Pharmacology, Downstate University, New York, U.S.A.*	W. G. NAYLER
"Studies on the Effect of Some Hypotensive Drugs on the Peripheral Circulation" — *Cardiac Society of Australia and New Zealand, Adelaide.*	W. G. NAYLER
"Kinekard" — *Tufts Hospital, Boston, U.S.A.*	W. G. NAYLER
"Ca Ions and Antiarrhythmic Drugs" — *Department of Physiology, University of London.*	W. G. NAYLER
"Kinekard — A Cardioactive Plasma Component" — *University of Ann Arbor, Michigan, U.S.A.*	W. G. NAYLER
"Effect of Antiarrhythmic Drugs on Calcium Transport" — *Australian Physiological Society, Melbourne.*	W. G. NAYLER
"The Mode of Action of ST155, a New Hypotensive Drug" — *Research Laboratories, Boehringer Ingelheim, Germany.*	W. G. NAYLER
"Kinekard" — *National Institutes of Health, Bethesda, U.S.A.*	W. G. NAYLER
"Kinekard" — *Geigy Research Laboratories, New York, U.S.A.*	W. G. NAYLER
"Kinekard" — *Research Laboratories, Merck Sharpe & Dohme, Philadelphia, U.S.A.*	W. G. NAYLER
"Hypothermia and the Action of Certain Drugs" — *Australian Physiological Society, Melbourne.*	J. M. PRICE

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

Revenue Account for the Year Ended 31st December, 1966

EXPENDITURE		INCOME		
Salaries and Wages	94,537	Donations —		
Laboratory Supplies	14,488	Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions		84,000
Library Maintenance	6,664	Other		100
Isotopes	1,375	Grants in Aid of Research —		
Postage and Telephone	1,611	National Heart Foundation of Australia	11,522	
Printing and Stationery	2,731	Anti-Cancer Council of Victoria (A. A. Thomas Fellowship)	15,690	
Light and Power	800	Life Insurance Medical Research Fund of Australia and New Zealand	14,360	
Insurance	2,509	Asthma Foundation of Victoria	4,298	
Repairs and Renewals	1,018	World Health Organization	268	
Animal House Contribution	4,000			46,138
Sundries	7,502	Interest from Investments —		
Travelling Expenses	3,785	Held by Trustees of the Baker Institute Grant Trust	1,700	
Data Processing	1,444	Endowment Fund	711	
Surplus for Year	2,395	Interest from Bank Accounts	695	
				3,106
		Sundry Sales and Recoveries		11,515
	$144,859			$144,859

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

Balance Sheet at 31st December, 1966

FUNDS AND LIABILITIES			ASSETS		
Funds —			Fixed Assets —		
Accumulated Revenue Brought Forward	4,401		Furniture and Fixtures [Note (i)]		—
Add Surplus for Year	2,395		Investments, at cost —		
	6,796		Commonwealth Inscribed Stock (Quoted market value, $581,921)	587,760	
Less Furniture and Fixtures Written Off	6,991		Treasury Bonds	8,020	
Accumulated Loss	(195)		Shares in Listed Company (Quoted market value, $27,306)	25,154	
Restricted Funds	11,041		Unlisted Investments	100	
Endowment Funds	56,280		Held by Trustee Company	2,868	
Development Fund	668,117		Short Term Deposits	95,432	
Laura Nyulasy Research Scholarship Fund	2,868				719,334
		738,111	Current Assets —		
Current Liabilities —			Cash at Banks on Current and Deposit Accounts	14,322	
Sundry Creditors	1,810		Sundry Debtors and Prepayments	14,167	
Accrued Expenses	7,902				28,489
		9,712			
		$747,823			$747,823

AUDITORS' REPORT TO THE TRUSTEES OF THE THOMAS BAKER, ALICE BAKER AND ELEANOR SHAW MEDICAL RESEARCH INSTITUTE

In our opinion the above Balance Sheet, together with the notes thereto, is properly drawn up to show a true and fair view of the state of the Institute's affairs at 31st December, 1966.

Melbourne,
15th May, 1967.

FLACK & FLACK, *Chartered Accountants.*
Honorary Auditors.

NOTES TO THE BALANCE SHEET

(i) Expenditure included in present or past periods on the acquisition of laboratory equipment and motor vehicles and on buildings, improvements, furniture and fittings have been charged against appropriate funds, grants or revenue accounts.

(ii) At 31st December, 1966, the Institute had a capital commitment, not shown above, in respect of a contract for a new building to stage 1, of $554,000. This expenditure is to be charged to the Development fund in future periods.

(iii) Retention monies paid to 31st December, 1966, in respect of the contract for a new building, amount to $16,500, and have been deposited in a bank account. The release of these funds to the builders is dependent on the satisfactory completion of the contract.

(iv) In addition to receiving income from investments shown above, the Institute receives interest on $34,000, 5% Commonwealth Inscribed Stock, which is held by the Trustees of the Baker Institute Grant Trust for the benefit of the Institute.

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

Year Ended 31st December, 1966

DEVELOPMENT FUND

Balance at 31st December, 1965		590,551
Add —		
Baker Benefactions	224,961	
Donation, Mrs. W. Crane	100	
Interest Received	33,426	
		258,487
		849,038
Deduct —		
Building Costs		180,921
Balance at 31st December, 1966		$668,117

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

Donations Received During Year to 31st December, 1966 for The Endowment Fund

Marian and E. H. Flack Trust	700
The William Angliss (Victoria) Charitable Trust Fund	500
The George F. Little Trust	474
Mr. and Mrs. Edgar Rouse	110
Essex Laboratories Pty. Ltd.	100
Mr. Seigfried Meyer	100
Mr. Robert Hinds	50
Donations in Memory of — Sir John Allison, Elstyn M. Baker, Marjorie Baker, Arthur Bradbury, Gladys Clarke, William S. Cox, Dr. Cyril Dickson, Beatrice Edwards, Fred Faram, E. W. E. Fraser, Sir Norman Gregg, Harry Hill, H. F. Hoey, Ivar Hultman, Marjorie E. Lucey, Isabel J. McKernan, R. W. Marriott, Ernest Mason, Alfred Victor Martin, Ralph Metcalfe, Herbert Pacini, W. J. Reynolds, L. S. Robb, Mrs. Henry Charles Slocombe, Sir William Spooner, Albert H. Thomas, Dr. Jean Tolhurst, Brigadier R. W. Tovell, Max Tweedi, Ernest Julian Van de Velde, E. Ward, Sir Arthur Warner, were received from — Mr. W. A. J. Baker, Mr. R. Blakemore, Miss N. E. Cameron, Mrs. W. Crane, Eagle Star Insurance Co. Ltd., Mr. J. C. Habersberger, Misses Dorothy and Jean Jeffrey, Mr. G. Knox, Mr. H. C. Morton, Mr. Edgar Rouse, Miss A. Tovell, Mr. G. A. Warner	444
	$2,478

ALFRED HOSPITAL DIABETIC AND METABOLIC UNIT

1967

STAFF

Honorary Consulting Physicians:	EWEN DOWNIE, M.D., F.R.C.P., F.R.A.C.P. BRYAN HUDSON, M.D., Ph.D., M.R.C.P., F.R.A.C.P.
Honorary Consulting Biochemist:	JOSEPH BORNSTEIN, D.Sc., M.D., F.R.A.C.P.
Physician-in-Charge:	PINCUS TAFT, M.D., F.R.A.C.P.
Honorary Physician:	HARALD BREIDAHL, M.D., M.R.C.P., F.R.A.C.P.
Registrar:	P. ZIMMET, M.B., B.S.
Biochemists:	DORA WINIKOFF, M.Sc. JUNE SHEATH, M.Sc. DORIS PAGE, B.Sc.
Technical Staff:	Mr. W. HUDSON Miss I. EKKEL Miss R. WITCHELL Miss J. KINDLER Mrs. F. RABOLD (part-time)
Secretary:	Miss J. SHARP

DIABETIC CLINIC

Clinical Assistants:	MARGARET SANDERS, M.B., B.S. GORDON ENNIS, M.B., B.S., M.R.A.C.P.
Chiropodist:	MAIDA O'CONNOR, F.Ch.A.V., M.Ch.I.A.

ENDOCRINE CLINIC

Clinical Assistants:	D. P. CAMERON, M.B., B.S., M.R.A.C.P. GORDON ENNIS, M.B., B.S., M.R.A.C.P. J. R. STOCKIGT, M.D., M.R.A.C.P.

RESEARCH FELLOWS

E. H. Flack Medical Research Scholarship:	J. R. STOCKIGT, M.D., M.R.A.C.P.
Burroughs Wellcome Research Fellow:	GORDON ENNIS, M.B., B.S., M.R.A.C.P.

HONORARY RESEARCH FELLOWS

MARGARET SANDERS, M.B., B.S.
E. L. G. BEAVIS, M.B., B.S., D.G.O., M.R.C.O.G., F.R.C.S.

ANNUAL REPORT

During this year, in the course of their investigation and treatment, 235 patients with various endocrine disorders have passed through our acute ward and 393 through the convalescent ward. There have been approximately 800 attendances at the Endocrine Outpatient Clinic and some 1300 patients have paid in excess of 700 visits to Diabetes Clinics. Clinical problems often present themselves — problems of diagnosis and of treatment, which are of direct concern to the patient as they affect his present and future health.

From the biochemical, biological and ecological patterns of disease as we see them in patients presenting, certain problems become posed and hypotheses constructed to explain them. Experiments can then be designed to test these hypotheses.

Systematic review of the large numbers of diagnostic tests, old established and newly developed, undertaken in the laboratory of the Unit, when considered in relation to the clinical history of patients undergoing these studies, define not only the scope and usefulness of such investigations and help clarify their diagnostic role, but also may define unusual and unexpected patterns which add to knowledge regarding the genesis and evolution of disease.

The application of new techniques of investigation to old problems is often helpful in advancing knowledge although sometimes, unfortunately, the new information obtained compounds pre-existing confusion.

In a variety of situations, increasing demand for facilities calls for modification of procedures in order that service requirements can be met. The diagnostic tests undertaken, if modified for this reason, must be so monitored as to be certain that reliability of results can be assured.

The reporting of findings occupies an integral role in research, for in this way the experience of all is shared and the constantly growing reservoir of knowledge can be tapped freely by all.

These various approaches to clinical investigation have particular application to the Diabetic and Metabolic Unit and are described in the report of research activities. In the main, the investigative work undertaken relates closely to the service responsibilities of the department. It is closely integrated, too, with a teaching responsibility in this aspect of Medicine. During the past year Dr. Cheah Jin Seng has spent some five months with us studying methods of radioimmunoassay as part of a postgraduate study programme prior to his return to Singapore to resume duties in the Department of Medicine. We have been able to offer terms of training in clinical medicine to medical students taking elective terms in the Unit. This year Mr. D. Ireland spent six weeks with us during which time, among other duties, he made ophthalmometric measurements on patients with normal thyroid function to serve as a control group for comparison with similar measurements made in patients suffering from various thyroid disorders. Dr. Gordon Ennis, who has worked as Burroughs Wellcome Research Fellow for the past two years studying glycerol metabolism, has been awarded the Cleveland Fellowship and has taken up duties in the Department of Medicine in Western Reserve University, Cleveland, U.S.A.

No report of the research activities of the Diabetic and Metabolic Unit would be considered complete without acknowledgement of the support—material and moral, which is given us by organisations and individuals. It is with a deep sense of the significance of their gifts that this acknowledgement is made. To the members also of the Honorary Medical Staff of the Alfred Hospital and of the Head and Staffs of the departments of Medicine, Surgery and Biochemistry of Monash University, grateful acknowledgement is made of their continued collaboration and assistance.

31st December, 1967. PINCUS TAFT.

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

Alfred Hospital Research Funds.
Boots Pure Drug Co. (Aust.) Pty. Ltd.
Burroughs Wellcome & Co. (Australia) Ltd.
Mrs. M. Clark.
Difrex Laboratories Pty. Ltd.
Dunklings Pty. Ltd.
Eli Lilly & Co., Indianapolis, U.S.A.
Hoechst Pharmaceuticals.
Pfizer Corporation.
Sandoz Ltd.
Syntex Pharmaceuticals Ltd., Berkshire, U.K.
The Upjohn Company, Kalamazoo, U.S.A.
William R. Warner & Co. Pty. Ltd.

THE METABOLISM OF GLYCEROL

Gordon Ennis and Doris Page

Studies in glycerol metabolism have continued during this year.

Three obese female patients were studied. Their metabolic clearance rates of glycerol were determined by constant infusion of H_3 glycerol. 50 μCi glycerol-2-H_3 were infused over one hour, and blood taken at 30, 40, 50 and 60 minutes. During the later stages of the infusion it was shown that a constant plasma level of glycerol-2-H_3 had been achieved. Thus it could be assumed that at this stage a glycerol space had been uniformly labelled with the tracer substance and that the rate of removal of labelled and stable glycerol from the pool was equal to the rate of its introduction and production.

Under these circumstances Metabolic Clearance Rate (MCR) is derived from the formula

$$MCR = \frac{\text{cpm/day infused}}{\text{cpm/litre plasma}}$$

In the three patients the MCR of glycerol were 5800, 5500 and 5600 litres per day under the standard conditions of an overnight fast.

After a week of complete caloric starvation the procedure was repeated in two of the patients and the MCR was found to be unaltered. This was to be expected since the clearance rate is a function of those organs which remove and metabolise glycerol, and not of the state of the adipose tissue.

However, it is known that the liver, the kidneys, and the intestinal wall are the only tissues which contain α-glycerokinase, the enzyme required to activate glycerol to α-glycerophosphate, a process that is necessary for the further metabolism of glycerol, so that it would be expected that the MCR would not exceed the combined blood flow through these organs. We assume, therefore, that the results we have obtained are erroneously high, as it is unlikely that this figure would be more than about 2500 to 3000 litres per day, even in the grossly obese.

We have as yet studied no normal subjects by this method. However, the results we have obtained by continuous infusion of unlabelled glycerol (a method which for other substances gives higher values for MCR than when calculated from radioactive tracer infusions) were in the range of 1500 to 3000 litres per day, which is within the expected range of values.

The possible sources of error in our results using tracer techniques could lie either in falsely high calculation of the rate of infusion of the radioactivity, or a falsely low estimation of the plasma level of H_3 glycerol achieved during the constant infusion. All investigations of these possible sources of error have so far failed to reveal the reason, and they are being continued.

SEPARATION OF PLASMA GLYCEROL BY THIN LAYER CHROMATOGRAPHY

Gordon Ennis

Until recently, the method for measurement of tritiated glycerol in this laboratory has been that of Havel, which involves oxidation of glycerol, and collection and counting of the released formic acid containing the tritium. Because, in certain experiments, we were obtaining values for plasma H_3-glycerol which were possibly lower than expected, it was decided to compare results obtained by chromatographic separation of glycerol-2-H_3 from other plasma substances which would be expected to contain tritium from the metabolism of H_3-glycerol — namely, glucose, the organic acids and water.

METHOD

Plasma samples are deproteinized with trichloracetic acid and the excess trichloracetic acid extracted by washing with ether. Plasma

lipids are removed by shaking the deproteinized extract with chloroform, and the glucose is removed with copper sulphate and calcium hydroxide. The resultant extract is then treated in the manner described by Nikkala and Ofala (Life Sci. 3, 1964, 234).

The extract is dried by lyphilization and redissolved in methanol. The salt free filtrate is then evaporated and applied quantitatively to a silica gel-G thin layer chromatography plate with methanol-water 1:1. The chromatography solvent is ethyl acetate, 58 : isopropylalcohol, 25 : water 17. The glycerol spots are identified by spraying the plate with ammoniacal silver nitrate. The spots are eluted with methanol-water and after evaporation Bray's scintillator is added and the samples counted in a liquid scintillation counter.

RESULTS

This method has been found to give satisfactory separation of glycerol from the organic acids. Recovery of added radioactivity is in the range of 50 - 60% and the precision of the method is ± 5% in individual samples.

This method will now be applied to routine separation of H_3 glycerol during studies of glycerol metabolism.

THYROID FUNCTION TESTS

Dora Winikoff[1]

A considerable time in 1967 has been devoted to reviewing and streamlining our routinely used thyroid function tests for the diagnosis of thyroid disease. The ever increasing demand on our services has forced us to seek more efficient methods and introduce automation.

PROTEIN BOUND IODINE (PBI).

The use of small tubes in the manual ashing technique has increased output fourfold. In addition, one standard curve (based on a blank and three quality control sera in duplicate) is constructed for two batches of patients' samples. The ashing step is carried out at the one time for all samples in the run. At the colorimetry stage (which has to be completed within two hours) the standards are split and one aliquot of each duplicate is used for each batch of samples (60 tests).

TRIIODOTHYRONINE RESIN UPTAKE (RU)

The procedure described last year has been modified to include an automatic sample changer[2] for the count on the supernatant solution. The results are expressed in terms of resin uptake.

This technique has been compared with the commercially available "TBI" (Mallincrodt) and "Triosorb" (Abbotts) kits for T_3 resin uptake.

It was found that although these simpler techniques were suitable for routine use, the results were not as consistent and the normal range much narrower than for our own method. Consequently the overlap, particularly for the hypothyroid patients, was much greater.

The same difficulties were encountered when bulk T_3 I^{125} was substituted for the bulk T_3 I^{131} which we are currently using.

ELECTROPHORETIC INDEX (EI)

This parameter of thyroid function (the method being described in 1965) has proved of inestimable value. With the advent and use of many new hormonal preparations (oestrogens, androgens, corticosteroids and oral contraceptives) the results of PBI and RU tests are often contradictory, deviating in opposite directions from the normal range, but the use of EI will, by defining abnormal thyroxine binding, indicate the fact that hormones are being administered to the patient.

[1] With technical assistance of R. Witchell and J. Kindler.

[2] This instrument was kindly donated by "Syntex Pharmaceuticals Ltd.".

Two individual tests (or one in duplicate) can therefore be run on each strip. Again the automatic and more sensitive counter made the use of narrow strips possible without the need to increase the amount of radioactive thyroxine since this has been shown to alter the thyroxine bound globulin-albumin ratio.

The long lasting isotope T_4 I^{125} (Amersham) was tried and proved of equal value although the normal range is slightly displaced.

THE USE OF "ISOPOR" RESIN FOR THE PBI ASSAY

In recent years the value of PBI estimation has greatly diminished. Among other explanations the use of a large number of iodine containing drugs adversely affect this parameter of thyroid function by adding exogenous iodine to the endogenous thyroxine source. We investigated the "Isopor" anion exchange resin "Permutit De-Acidite FF-IP" which removes iodine from contaminated samples. The difference in PBI level before and after a passage through the resin gives a guide to the level of true hormonal iodine in the patient's blood. The procedure is simple. 2.5 ml. of serum are shaken mechanically for 15-20 min. with 0.5 gm. of resin. The serum is separated and PBI estimation commenced immediately.

Following the intake of iodides the use of "Isopor" was shown to achieve a fall in PBI to a fraction of the original level. Organic iodine compounds are harder to remove, some contaminated sera being unaffected. In normal samples, PBI before and after treatment with resin does not vary more than 10%. We have taken a difference of 20% or more to indicate the presence of contamination by exogenous iodine.

In 100 patients with PBI higher than expected the above outlined procedure contributed to correct diagnosis in over 60% of cases. Moreover, in many instances it avoided the necessity to undertake more complicated and time consuming additional diagnostic tests.

THE THYROID AND THE "PILL"

Dora Winikoff

The work on the effects of oral contraceptives on thyroid function tests which commenced in 1964 is continuing. We have established that

(1) The ovulation inhibitors invariably alter the thyroid parameters from pretreatment values;

(2) The magnitude of deviation can be correlated with the amount and length of administration of the oestrogen component; and

(3) The progestogens, apart from norethynodrel, have no effect and do not synergise the action of oestrogen.

During the current year we have studied the effects on thyroid function tests of long-term administration of cycle-to-cycle variations and of patient-to-patient variations.

It was found that interpatient variations are far greater than cycle-to-cycle fluctuations, which are also considerable. With this experience we feel that we are able to diagnose patients with thyroid disease while on the "pill".

This investigation has also extended to the use of Chlormadinone at 0.5 mg. levels for contraceptive purposes. The results are being correlated with other endocrine effects of this steroid.

Work is also in progress on the establishment of a "free thyroxine" assay in order to study the influence of oral contraceptives on this metabolically important component of the plasma pool of thyroxine.

PLASMA INSULIN LEVELS IN PATIENTS WITH SUSPECTED INSULINOMA

Doris Page

During 1966-67 the fasting plasma insulin concentration and the insulin levels during standard intravenous tolbutamide tolerance tests have been measured in 19 patients in whom the diagnosis of insulinoma was suspected. At subsequent operation, six of these patients were found to have an insulinoma.

The mean fasting insulin level of the 13 patients in whom a negative diagnosis was made was 13 ± 3 μU/ml. with a range of 6-17 μU/ml. This is directly comparable with that found in our series of 50 normal volunteers, where the mean fasting insulin level was 16 ± 7 μU/ml., and the range 0-30 μU/ml. However, 5 of the 6 patients who were subsequently shown to have an insulinoma had fasting insulin levels far in excess of the upper limit of the normal range. The levels were 50, 117, 55, 84 and 250 μU/ml. The fasting insulin concentration in the remaining patient was measured on three occasions, the highest level recorded being only 29 μU/ml.

Plasma insulin levels were determined at intervals during one hour after intravenous tolbutamide. The maximum insulin response occurred within 5 minutes in 18 of the 19 patients. The mean rise in plasma insulin five minutes after tolbutamide in patients with no insulinoma was 45 ± 26 μU/ml. (range 14-107 μU/ml.) and in patients with an insulinoma was 121 ± 57 μU/ml. (range 56-> 250 μU/ml.). The plasma insulin level in two of the patients with insulinoma rose to beyond the upper limit of the assay. The large variation in the insulin response to tolbutamide seen in the normal group makes this a less reliable indication of an insulinoma than the fasting insulin level.

A fasting insulin value in excess of 30 μU/ml. (mean normal + 2 S.D.) and a rise in the plasma insulin level of more than 71 μU/ml. (mean normal + 1 S.D.) five minutes after a standard tolbutamide load appears to be diagnostic of an insulinoma.

However, one patient who had an insulinoma and had fasting insulin levels below 30 μU/ml. had an abnormally elevated response to tolbutamide by the above criteria in only one of the three tolbutamide tests performed. Some low fasting blood sugar levels were measured but no abnormal depression of the blood sugar was noted during any of the tolbutamide tests. Apparently in this particular patient, the tumour produced an insulin or insulin-like substance which differed immunologically to native insulin and did not respond to tolbutamide.

Two patients with an insulinoma in whom grossly elevated fasting plasma insulin levels were noted had normal fasting insulin levels on another occasion. This observation is in agreement with the clinical finding of episodic hypoglycaemia in patients with insulinoma, and it indicates that the estimation of more than one fasting plasma insulin may be necessary to detect an abnormally elevated insulin level in the presence of an insulinoma.

FOETAL INSULIN LEVELS AND THE TRANSPORT OF INSULIN ACROSS THE HUMAN PLACENTA

Doris Page and P. Taft

(in conjunction with P. S. Paterson and E. C. Wood, Monash University Department of Obstetrics and Gynaecology, Queen Victoria Hospital)

Although much larger molecules than insulin, such as some gamma - globulins, are known to cross the human placenta, the results of studies on transplacental insulin transport are conflicting. The use of Saling's ingenious technique for obtaining blood samples from the foetal scalp during labour, and the employment of a sensitive immunoassay for measuring insulin levels has enabled us to study the movement of unlabelled insulin

across the placenta during labour, rather than as in earlier studies, observing the movement of radioactively labelled insulin and making all observations at the time of birth, when acute changes may be taking place.

Response of mother and foetus to maternal insulin injection

During the first stage of labour, 7 normal patients received 0.1 U/kg. insulin intravenously and blood samples were taken from a maternal vein and from the foetal scalp before and after the injection. Both the maternal and foetal glucose levels decreased, but although the mean maternal insulin concentration rose to more than four times the pre-injection level, the rise in foetal insulin was insignificant. Thus exogenous insulin does not cross the placenta during labour.

Response of mother and foetus to maternal glucose load

A further 11 normal patients received 25 gm. glucose intravenously in an experiment similar to that outlined above. A large and similar rise in maternal and foetal glucose levels was noted, demonstrating the free passage of glucose across the placenta. The maternal hyperglycaemia evoked a large insulin response in the mother but no significant rise in the mean foetal insulin level was noted.

Thus neither did the endogenous insulin produced by the mother cross the placenta to the foetus, nor did the foetus show any acute insulinogenic response to foetal hyperglycaemia.

Experiment	Mean Change in Glucose Levels (mg. %)		Mean Change in Insulin Levels (μU/ml.)	
	15 min.	60 min.	15 min.	60 min.
Maternal	+125	+16	+45	+43
25 gm. Glucose I.V.				
Foetal	+120	+20	+3	+3
Maternal	—35		+64	
0.1 Insulin/kg. I.V.				
Foetal	—17		+3	

Concentration of insulin in cord plasma in relation to maternal insulin levels — basal values

We have found no significant difference between the mean cord and maternal insulin levels in both normal patients and diabetic patients on dietary control. However, in diabetic patients receiving insulin therapy, the cord insulin level was much higher than the maternal concentration. The plasma from insulin treated patients often contains insulin antibodies which interact with the immunoassay system, leading to the finding of erroneously high insulin concentrations.

However, this does not invalidate the observation of a large difference between maternal and cord insulin levels. No explanation can be offered at this time for the higher foetal insulin level.

Patients	No.	Mean Maternal Insulin Concentration μU/ml. $\pm$ S.D.	Mean Cord Insulin Concentration μU/ml. $\pm$ S.D.
Normal Controls	7	17 ± 7	14 ± 6
Diabetic Patients on Diet Alone	7	30 ± 8	31 ± 24
Diabetic Patients on Insulin Therapy	9	79 ± 63	170 ± 95

We found no statistical correlation between individual maternal and cord insulin levels in any of the groups, thus supporting the notion that the placenta is impermeable to insulin.

PLASMA IONIZED CALCIUM IN HYPOMAGNESAEMIC STATES

Paul Zimmet, Harald Breidahl and Winifred Nayler[1]

For some years, it has been known that in clinical situations where hypocalcaemia and hypomagnesaemia coexist, that administration of magnesium (orally or parenterally) will correct both of these abnormalities. This is most commonly seen in patients with malabsorption syndromes.

We have studied two patients with hypocalcaemia and hypomagnesaemia — the first patient suffered from intestinal lymphangiectasia, the second from adult coeliac disease. Both patients exhibited tetany, and were shown to have very low plasma ionized calcium levels.

The patients were given intravenous infusions of magnesium lasting two hours, and plasma levels of magnesium, calcium (both total and ionized) and phosphate, were measured pre-infusion, hourly for four hours, and at 6, 8 and 22 hours. Ionized calcium levels were measured by a biological technique using the isolated frog's heart.

During infusion, total calcium levels rose significantly ($>$ 1.5 mEq/L.) — the rise being accounted for wholely by an increase in the ionized calcium fraction.

It would appear that the administered magnesium causes mobilization of ionized calcium from either the exchangeable bone pool, or muscle cells, in hypomagnesaemic individuals.

The true significance of this can only be assessed after similar infusions have been performed in hypocalcaemic, normomagnesaemic individuals, and ionized calcium levels measured. These studies are in progress at present. However, overseas workers have reported no change in total plasma calcium levels during magnesium infusion, so it is unlikely that any change in ionized levels does occur.

POTASSIUM DEPLETION AND CARBOHYDRATE MEABOLISM

J. R. Stockigt

The aim of this programme has been to investigate the effect of potassium depletion on insulin release and carbohydrate metabolism. A mild diabetic state has frequently been observed in association with potassium depletion. It has been proposed that potassium depletion impairs the release of insulin from the pancreas.

An isotope dilution method of measuring exchangeable potassium with K^{42} was established, and this gave a precise index of the total body potassium status. This measure-

[1] Baker Medical Research Institute.

ment was made several times in each subject, and the result correlated with standard tests of insulin release and carbohydrate tolerance, i.e., oral glucose tolerance tests (G.T.T.), intravenous G.T.T., and intravenous tolbutamide test.

After basal studies in normal subjects potassium depletion was induced with oral ion exchange resin, and the subjects retested. Two patients with Conn's syndrome were studied preoperatively while potassium depleted and after correction of potassium depletion by removal of the aldosterone - producing adenoma.

It has been demonstrated that potassium deficits of 300-500 milliequivalents are associated with delayed and diminished plasma insulin response to oral glucose, and with an abnormal glucose tolerance test resembling that found in maturity onset diabetes. This effect is less marked following intravenous glucose. Preliminary data suggest that potassium depletion diminishes the effect of intravenous tolbutamide.

DIABETIC ACIDOSIS

P. Taft, P. Zimmet, J. Sheath, G. Ennis and J. Owen[1]

The main purpose of this work is:—

(1) To define the acid radicles contributing to the acidosis.

(2) To assess degree of osmolar changes before and during treatment, and to define major contributing factors (i.e., sugar, urea) to the increase in osmolality.

(3) To measure base deficit in an attempt to rationalise the basis of alkali replacement in acidotic individuals.

(4) To study the distribution of keto acids before and during treatment.

In thirteen patients presenting in diabetic acidosis, the following levels were measured at 0, 4, 8 and 24 hours.

(1) Blood pH and gases.

(2) Serum electrolytes and urea.

(3) Blood sugar.

(4) Osmolality.

(5) Ketone bodies (acetone, aceto-acetic acid and β-hydroxy butyric acid).

(6) Free fatty acids.

(7) Glycerol.

(8) Lactate and pyruvate.

Several interesting points arise from the work.

(a) The high levels of serum osmolality seen in untreated diabetic acidosis do not appear to bear any direct relationship to blood glucose, urea or serum electrolyte levels but probably are a reflection of the dehydration seen in these patients.

(b) Judging from the base deficit shown in these patients, the amount of alkali replacement necessary (either sodium lactate, or bicarbonate) is probably less than is usually given in clinical practice.

(c) Before treatment, the β-hydroxy butyric acid:aceto acetic acid plus acetone ratio is 1.7, but this ratio is reversed following treatment, being 0.60 at 24 hours.

(d) The estimated acid load (lactate, pyruvate, ketones, etc.) that we have measured, does not seem to account for the total base deficit in these patients. There may be some other acid product (that we are not measuring) that might account for this. A method for measuring citric acid levels has been established and interest will centre on levels of this acid in future patients studied.

[1] Biochemistry Department.

URINARY GONADOTROPHIN EXCRETION IN SECONDARY AMENORRHOEA

Ida Ekkel and Pincus Taft

In the majority of patients suffering secondary amenorrhoea in whom no organic cause can be found, the estimation of urinary "general" gonadotrophins (using the immature mouse uterine assay) indicates a normal excretion of these hormones. A study of 13 such patients with normal urinary gonadotrophins has been made in which the urinary follicle stimulating hormone (FSH) and luteinising hormone (LH) levels have been individually measured along with urinary oestrogen. The patients appeared to fall into two groups in that ovarian activity as judged by oestrogen levels was normal in half and inadequate in the remainder. However, the ratio of FSH to LH was essentially similar in the two groups, being approximately 1 to 1.3. This observation appears to exclude the possibility that an abnormal ratio of pituitary gonadotrophin secretion of the individual hormones is responsible for the existence of amenorrhoea in face of normal "general" gonadotrophin urinary levels.

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"Medical Uses of Radioisotopes" — *Med. Women's Soc. of Victoria.*	H. D. BREIDAHL
"Diabetes — Cradle to Grave" — *Queen Victoria Hospital Clinical Society.*	H. D. BREIDAHL
"Obesity" — *Queen Victoria Hospital Clinical Society.*	H. D. BREIDAHL
"Modern Therapy of Diabetes" — *College of General Practitioners Victorian Faculty.*	H. D. BREIDAHL
"The Diabetic and Exercise" — *6th Congress of International Diabetes Federation.*	H. D. BREIDAHL
"The Endocrine Profile in Secondary Amenorrhoea" — *Royal College of Obstetricians* and *Gynaecologists' Australian Regional Council Meeting.*	PINCUS TAFT
"The Influence of Human Pituitary and Chorionic Gonadotrophin on the Testis in Oligospermia" — *Endocrine Society of Australia.*	PINCUS TAFT
"The Site and Mode of Action of Insulin in Intermediary Metabolism" — *Royal Australasian College of Surgeons.*	PINCUS TAFT
"The Clinical Uses of Human Gonadotrophins" — *Royal Australasian College of Physicians.*	PINCUS TAFT
"Pitfalls in Estimation of Protein Bound Iodine" — *Association of Hospital Scientists.*	D. WINIKOFF
"Diagnosis of the Thyroid Function in the Laboratory" — *Annual Meeting of the Australian Association of Clinical Biochemists, Victorian Branch.*	D. WINIKOFF
"Oral Contraceptives and Thyroid Function Tests. The Role of Progestogens" — *Annual Meeting of the Endocrine Society of Australia.*	D. WINIKOFF
"Counting Techniques and Laboratory Practice. Electrophoresis — Application to Thyroid Disease" — *Royal Melbourne Institute of Technology.*	D. WINIKOFF
"Intestinal Lymphangiectasia with Hypomagnesaemic Tetany" — *Royal Australasian College of Physicians.*	P. ZIMMET and H. D. BREIDAHL

REPORT OF INVESTIGATIONS BY RESEARCH FELLOWS OF ALFRED HOSPITAL IN OTHER DEPARTMENTS

OPTIC ATROPHY

G. W. Crock[1]

During 1967, the Melbourne University Department of Ophthalmology was greatly assisted by a Grant from the Ringland Anderson Fund. The Grant was used to continue our work on ocular angiography.

We have made the first detailed study of vascular changes in the optic nerve of patients suffering from optic atrophy. The results were reported in the Lancet, July 1967.

J. M. Parel, of the Melbourne University Department of Ophthalmology, designed and built an automatic retinal fundus camera with the assistance of Australian Optical Manufacturers. This apparatus is based on the Zeiss fundus camera of Dr. Hans Littman, the Chief Designer for Carl Zeiss, Oberkochen, who has personally undertaken the construction of certain lenses in Parel's design.

Prior to fluorescein angiography, only subjective data on optic atrophy could be recorded. With the automatic fundus camera, objective measurements of the vascular changes at the disc are possible. A much greater understanding of the pattern of vessel changes involved in optic atrophy can now be obtained from the improved data recording facilities.

In 1968 the programme will continue with the development of automatic intravascular injection equipment, and electronic monitoring of the fluorescein passage through the retina and optic nerve.

It is intended to apply this new equipment to a clinical study of vascular changes at the optic nerve in several blinding diseases, particularly in chronic simple glaucoma.

THE RESULTS OF PLASTIC REPAIR OF THE MITRAL VALVE

Aubrey Pitt, Rena Zimmet and S. T. Anderson[2]

This study aims to evaluate results of repair of the mitral valve in patients suffering from mitral incompetence.

Preliminary results indicate that marked improvement may result haemodynamically although only a partial relief of mitral incompetence is achieved.

STUDIES IN LEFT VENTRICULAR FUNCTION

Aubrey Pitt and S. T. Anderson[2]

Left ventricular function is altered by graded doses of Angiotensin which increase left ventricular afterload. Curves are constructed relating left ventricular stroke work to left ventricular end - diastolic pressure.

Ten patients have been studied and preliminary results indicate that β-sympathetic blockade using Inderal or Trasicor depresses the abnormal left ventricle. In patients with normal left ventricular function there is little change following β-sympathetic blockade.

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[1] Department of Ophthalmology, Melbourne University.

[2] Cardiovascular Diagnostic Service.

THE RELATIONSHIP OF POTASSIUM IN THE GENESIS OF VENTRICULAR ARRHYTHMIAS FOLLOWING MAJOR CARDIAC SURGERY

S. T. Anderson and Anne Shanahan[1]

One hundred patients are being studied to assess the effects of potassium added to the pump prime and intravenous fluids during cardio-pulmonary bypass surgery.

Before potassium was added 42% of fifty patients had episodes of ventricular ectopic beats, 4% had ventricular tachycardia and 16% had ventricular fibrillation.

After the potassium supplements were used only 16% of fifty patients had evidence of ventricular ectopic beats and there was a 2% incidence of ventricular tachycardia.

THE USE OF XYLOCAINE IN THE TREATMENT OF VENTRICULAR ARRHYTHMIAS

S. T. Anderson and Aubrey Pitt[1]

To date twenty-five patients have received intravenous Xylocaine for the treatment of ventricular arrhythmias, including ventricular extra-systoles and ventricular tachycardia. Xylocaine has proved successful in 80% of these patients. No cardiac effects have been noted. With large doses of Xylocaine, drowsiness may result and in two patients muscular twitching was noted. These side effects are readily reversible if the dosage of the agent is reduced.

A survey is currently being undertaken to assess the usefulness of Xylocaine in preventing arrhythmias in patients admitted to the Coronary Care Unit with acute myocardial infarction.

TREATMENT OF ANGINA PECTORIS WITH TRASICOR

Aubrey Pitt[1]

Trasicor is a new β-sympathetic blocking agent. Its usefulness in the treatment of angina pectoris in a group of Out-patients is being assessed using a double blind control study.

STUDIES IN CLINICAL CHEMISTRY

J. A. Owen[2]

BIOMETRICAL STUDIES

The aim of this project, which is continuing, is to obtain information on the frequency distribution of biochemical changes in disease, which will aid the interpretation of biochemical findings in individual patients. Frequency distribution of the results of various tests in healthy persons and in patients have been prepared using computer techniques and the fit of the data as normal, log-normal and three parameter log-normal distribution examined. Data so far obtained have already led to a modification of the "normal range" of some tests.

A study (carried out in conjunction with Mr. R. Bywater) involving the results of 1000 oral glucose tolerance tests, has provided data which could lead to a re-evaluation of the significance of a particular result. We have failed to obtain evidence of a bimodal distribution of glucose tolerance which has been claimed to exist in the population at large.

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[1] Cardiovascular Diagnostic Service.
[2] Biochemistry Department.

In these studies, and in the studies on laboratory error, we are greatly indebted to Dr. C. Bellamy, of the Computer Centre, Monash University, and to his staff for help on numerous occasions.

STUDIES ON LABORATORY ERROR

This is a continuation of early studies and is being carried out in conjunction with Dr. D. G. Campbell, Royal Women's Hospital, and various staff members of the Biochemistry Department of the Alfred Hospital and the Royal Women's Hospital.

The aim of the project is an improvement in the accuracy of biochemical tests. Studies have comprised defining and measuring the components of laboratory error. Random and systematic error has been examined by preparing large pools of plasma and analyzing portions daily, or more frequently. Most of the work to date has concerned measurement of plasma electrolytes. In these tests systematic error between batches accounts for about 50% of the error.

Other studies are concerned with errors arising out of the use of Auto-Analysers and with the incidence and causation of gross blunders. An investigation into the efficiency of quality control based on patients' results has shown that this is not as effective as conventional quality control achieved by including known material in each analytical batch.

BIOCHEMICAL STUDIES ON THE FUNCTION OF ISOLATED PIG LIVER

These studies are being carried out jointly with members of the Monash University Department of Surgery (M. C. Douglas, P. Jablonski, J. McK. Watts), and are a continuation of previous studies. Since the last report the role of conjugation of bromsulphthalein and its excretion by the liver, and to a lesser extent, the kidney, has been extensively studied. Use of ^{75}Se-methionine has shown that there is fairly rapid incorporation of the amino-acid into plasma protein. This ability of the liver could form the basis of a liver function test for use in everyday clinical work.

DERMAL NECROSIS TEST FOR DETECTION OF BACTERIAL ENDOTOXINS

F. W. Gurr[1] and R. C. Atkins[2]

Local clinical experience in the diagnosis and management of patients suffering from severe bacterial infection has emphasized the need for a reliable and sensitive laboratory test capable of detecting the presence of circulating bacterial toxins. Although many methods for assay of bacterial toxins have been reported all depend on a biological response and are thus beset by inherent problems of time lag, methodology and standardization.

However, Thomas in 1956 described a dermal necrosis response to bacterial endotoxins which has been shown to possess advantages over other tests in terms of sensitivity and reproducibility. This test is performed in rabbits by simultaneous intravenous injection of endotoxin and intradermal injection of adrenalin. A positive response is marked by the development of an area of haemorrhagic dermal necrosis at the site of intradermal injection. The response appears within 4 hours and is fully developed by 24 hours. Characteristics of this test which favour its clinical application include ease of performance, all or none positive response and a relatively short latent interval.

A review of reports describing the rabbit dermal necrosis response to bacterial endotoxin administration suggested that laboratory characterization was incomplete and revealed that results of attempted clinical evaluation were inconclusive. In particular, although negative results have been recorded in patients with proven bacteraemia, no attempts to increase the sensitivity of the test have been described.

[1] Director, Dialysis Unit.
[2] Research Assistant.

A research study was therefore begun to evaluate the dermal necrosis test in the laboratory, study of its clinical application and increase its sensitivity. All investigations were matched by appropriate control studies and measures were adopted to avoid contamination of samples or apparatus by bacteria, bacterial toxins and detergents. Initial studies on selected rabbits using commercially prepared endotoxin and adrenalin tartrate were performed with particular care in relation to details of technique and definition of criteria determining recognition of a positive response.

Positive Dermal Necrosis Response

The characteristic skin lesion was observed following intravenous injection of bacterial endotoxin and was reproducible at certain dose levels. Descriptive criteria for recognition of the lesion were developed in detail.

False Positive Responses

Skin lesions developing at the site of the intra-dermal adrenalin injection in control animals were studied as a source of error in interpretation of results. It was found that in certain rabbits an atypical reaction to intradermal adrenalin injection alone resulted in skin necrosis, but that the appearance of such lesions permitted differentiation from the dermal necrosis response to endotoxin administration.

Local Variation in Dermal Reactivity

Simultaneous intradermal adrenalin injection at varying sites in rabbits receiving intravenous endotoxin showed a complete lack of dermal reactivity in relation to this test when the ear was chosen as the site of intradermal adrenalin injection. This local absence of dermal reactivity occurred despite the simultaneous production of well developed dermal necrosis on abdominal wall sites of adrenalin injection.

Sensitivity of Standard Dermal Necrosis Test

Performance of the test using endotoxin from four species of gram negative organisms demonstrated that, while dermal necrosis occasionally followed administration of 1mcgm of endotoxin, consistently positive responses did not occur until endotoxin dosage was elevated to 100 mcgm.

Variation in adrenalin dosage did not appear to influence sensitivity of the test.

Histological Studies

Examination of histological sections from areas of dermal necrosis following endotoxin injection showed predominant eosinophil infiltration. This finding, which was confirmed by specific staining, stands in contrast to previous reports describing predominant polymorphonuclear leucocyte infiltration. Preliminary immunofluorescent antibody studies failed to demonstrate endotoxin localized at the site of dermal necrosis.

Thorotrast Augmented Dermal Necrosis Test

Failure to produce dermal necrosis in rabbits following injection of plasma from patients with symptomatic bacteraemia has been recorded in several studies. The possibility is thus suggested that the standard dermal necrosis test as described by Thomas cannot detect levels of circulating endotoxin at lower concentrations in the clinically significant range. Although preliminary inactivation of the reticuloendothelial system by thorotrast injection has been shown to greatly enhance many biological properties of endotoxins, application of this effect has not been recorded in relation to the dermal necrosis response.

Experiments demonstrated that the preliminary administration of thorotrast increased the sensitivity of the dermal necrosis test by a factor of 100 to yield consistently positive responses to endotoxin at dosages of only

1 mcgm. Histological examination of the lesions produced showed an appearance identical to that of dermal necrosis following the unmodified test. Further experiments were performed to determine optimal dose and time factors for Thorotrast administration.

CLINICAL EVALUATION

Simultaneous evaluation of the standard dermal necrosis test and the thorotrast augmented dermal necrosis test was performed using plasma from septic patients and healthy controls. Results support the belief that this investigation will prove of value in the definitive and early diagnosis of systemic toxaemia following bacterial infection. A higher incidence of positive responses in relation to the thorotrast augmented test suggests that the increased sensitivity demonstrated in the laboratory is maintained under clinical circumstances.

SUMMARY

The necessity for a reliable test to detect circulating bacterial toxins prompted the laboratory and clinical evaluation of the rabbit dermal necrosis test described by Thomas. Details of technique and characteristics of the lesions have been studied to permit standardization of the procedure and reduce errors in interpretation of results.

Augmentation of the test by a factor of 100 has been achieved by preliminary thorotrast administration and clinical application of the dermal necrosis test has yielded results supporting its value in the early and definitive diagnosis of bacterial toxaemia.

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"Clinical Studies on the Effect of a β-Blocking Agent on Left Ventricular Function" — *Cardiac Society of Australia and New Zealand.* — Aubrey PITT

"Coronary Arteriographic Findings in Young Adults" — *Cardiac Society of Australia and New Zealand.* — Aubrey PITT

"Comparison of Left and Right Myocardial Blood Flow Determined by Xenon^{133}" — *Australian Society for Medical Research.* — Aubrey PITT

"Patho-physiology of Heart Failure" — *Melbourne Medical Postgraduate Committee.* — Aubrey PITT

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