THE MEDICAL RESEARCH COUNCIL OF IRELAND

Annual Report
For the year ended December 31st, 1974

Obtainable from The Medical Research Council of Ireland,
9 Clyde Road, Dublin 4.
Price 25p
THE MEDICAL RESEARCH COUNCIL OF IRELAND

PROFESSOR D. K. O'DONOVAN, MD., PhD., FRCP., Chairman
Nominated by the Minister for Health.

PROFESSOR O. FITZGERALD, MD., MSc., FRCPI., FRCP. (Ed.), FRCP.,
Honorary Treasurer. Nominated by the Royal Academy of Medicine
in Ireland.

PROFESSOR P. B. B. GATENBY, MD., FRCPI., FRCP.
Nominated by the University of Dublin.

PROFESSOR J. W. HARMAN, MD., MSc., FCAP., Honorary Secretary.
Nominated by University College, Dublin.

PROFESSOR W. A. L. MACGOWAN, MD., FRCSI., FACS.
Nominated by the Royal College of Surgeons in Ireland.

PROFESSOR C. F. MccARTHY, MD., FRCPI., MRCP.
Nominated by University College, Galway.

PROFESSOR D. O'SULLIVAN, MD., FRCP., FRCPI.
Nominated by the National University of Ireland.

PROFESSOR R. A. Q. O'MEARA, MD., ScD., FRCPI., FTCD., Honorary
Treasurer. Nominated by the Royal College of Physicians of Ireland.

PROFESSOR J. D. SHEEHAN, MD., BSc., MRCP.
Nominated by University College, Cork.

Secretary:
LT. COMDR. E. J. FURNISS

Offices:
9 Clyde Road, Ballsbridge, Dublin 4.
SPECIAL COMMITTEES OF THE COUNCIL

CANCER
Professor R. A. Q. O'Meara, *Chairman*
Dr. G. J. Bourke
Dr. J. J. Fennelly
Mr. C. Galvin
Dr. J. Greally
Dr. D. O'B. Hourihane
Professor S. M. Lavelle
Mr. J. P. McMullin
Professor S. F. O'Beirn
Dr. M. J. O'Halloran
Dr. M. L. Conalty, *Secretary*

CARDIOVASCULAR DISEASES
Professor J. D. Sheehan, *Chairman*
Dr. S. Blake
Professor M. P. Brady
Professor W. G. Fegan
Professor P. FitzGerald
Mr. D. F. Kneafsey
Professor W. A. L. MacGowan
Dr. R. Mulcahy
Professor M. F. Murnaghan
Dr. G. Gearty, *Secretary*

GASTROENTEROLOGY
Professor O. FitzGerald, *Chairman*
Dr. B. Alton
Professor T. G. Brady
Professor P. G. Collins
Dr. E. Doyle
Dr. J. S. Doyle
Professor P. F. Fottrell
Dr. D. G. Weir
Dr. M. J. Whelton
Mr. K. F. McGeeey, *Secretary*

HAEMATOLOGY
Professor C. F. McCarthy, *Chairman*
Professor P. B. B. Gatenby
Professor S. M. Lavelle
Dr. H. C. Moore
Dr. L. G. O'Connell
Dr. J. P. O'Riordan
Dr. J. M. Scott
Professor I. J. Temperley
Dr. N. Clarke, *Secretary*
MENTAL HEALTH
Professor R. A. Q. O'Meara, Chairman
Professor P. G. S. Beckett
Professor I. W. Browne
Dr. F. Campbell
Dr. J. G. Cooney
Professor T. Lynch
Dr. S. D. McGrath
Dr. J. J. Stack
Dr. J. J. Cullen, Secretary

METABOLISM AND ENDOCRINOLOGY
Professor D. K. O'Donovan, Chairman
Dr. E. Bourke
Professor M. G. Harrington
Professor W. J. E. Jessop
Professor B. F. Leek
Professor R. E. Moore
Professor F. P. Muldowney
Dr. P. O'Carra
Professor D. O'Sullivan
Professor F. G. A. Winder
Professor R. P. Kernan, Secretary

MICROBIOLOGY, IMMUNOLOGY AND PATHOLOGY
Professor W. A. L. MacGowan, Chairman
Dr. M. L. Conalty
Dr. J. G. Devlin
Dr. C. T. Doyle
Dr. M. P. G. Little
Professor P. N. Meenan
Professor E. C. Moorhouse
Dr. J. B. O'Regan
Professor F. S. Stewart
Dr. J. A. Kirrane, Secretary

PREGNANCY AND CONGENITAL DEFORMITIES
Professor P. B. B. Gatenby, Chairman
Dr. S. F. Cahalane
Dr. V. Coffey
Dr. F. J. Geoghegan
Professor W. J. E. Jessop
Dr. F. Meehan
Dr. H. C. Moore
Dr. L. G. O'Connell
Mr. B. O'Donnell
Professor J. Masterson, Secretary
BIOMEDICAL GENETICS
   Professor J. W. Harman, Chairman
   Professor G. W. P. Dawson
   Professor L. K. Duncan
   Professor Ellen C. Moorhouse
   Professor F. S. Stewart
   Mr. D. White
   Professor J. Masterson, Secretary

WELLCOME TRUST MEDICAL SCHOLARSHIPS
   Professor J. W. Harman, Chairman
   Dr. M. J. Cullen, T.C.D.
   Professor C. F. McCarthy, U.C.G.
   Professor W. A. L. MacGowan, R.C.S.I.
   Professor M. F. Murnaghan, U.C.D.
   Professor D. J. O'Sullivan, U.C.C.
   Professor R. E. Moore, Secretary
ACKNOWLEDGMENTS

The Council acknowledges with thanks the generous financial support afforded by the following:

MESSRS. MAY & BAKER LTD.
MESSRS. ARTHUR GUINNESS SON & CO. LTD.
IRISH TOBACCO MANUFACTURERS ADVISORY COMMITTEE
IRISH CANCER SOCIETY
NATIONAL SCIENCE COUNCIL.
The Chairman and Members of the Medical Research Council have pleasure in presenting their report for the year 1974.

MEMBERSHIP OF THE COUNCIL

Professor P. B. B. Gatenby was nominated by the University of Dublin to fill the vacancy created by the retirement of Professor Jessop. Professor D. J. O'Sullivan was renominated by the National University of Ireland.

Professor J. W. Harman was appointed Honorary Secretary and Professor O. FitzGerald Honorary Treasurer.

The Council suffered a grievous loss in December with the death of Professor R. A. Q. O'Meara. Appointed to the Council in January 1956, Professor O'Meara was elected Honorary Treasurer in 1960, a position he held until his sudden death. During his long and distinguished career in medical research, he achieved international recognition for his work on cancer. His loss will be keenly felt on the Council, where his encyclopaedic knowledge in the medical field and his practical wisdom in financial matters will not easily be replaced.

FINANCES

The research funds made available to the Council during 1974 were as follows:

<table>
<thead>
<tr>
<th>THE MINISTER FOR HEALTH</th>
<th>£</th>
</tr>
</thead>
<tbody>
<tr>
<td>For general research</td>
<td>230,000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IRISH TOBACCO MANUFACTURERS ADVISORY COMMITTEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>For research on cancer under the direction of Professor R. A. Q. O'Meara</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GRANTS FROM VARIOUS SOURCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>For research on the chemotherapy of cancer and tuberculosis under the direction of Dr. V. C. Barry, M.R.C. Laboratories:</td>
</tr>
<tr>
<td>Irish Cancer Society</td>
</tr>
<tr>
<td>Arthur Guinness Son &amp; Co. Ltd.</td>
</tr>
<tr>
<td>National Science Council</td>
</tr>
<tr>
<td>Messrs. May &amp; Baker Ltd.</td>
</tr>
<tr>
<td>Dr. V. C. Barry</td>
</tr>
</tbody>
</table>

| TOTAL                        | £242,765 |

£242,765
The manner in which these funds were utilised during the year was as follows:

**FROM THE MINISTERS ANNUAL GRANT**

<table>
<thead>
<tr>
<th>General Research:</th>
<th>£</th>
<th>£</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fellowships – wholetime</td>
<td>32,478</td>
<td></td>
</tr>
<tr>
<td>Fellowships – part-time</td>
<td>9,782</td>
<td></td>
</tr>
<tr>
<td>Training Grants</td>
<td>2,407</td>
<td></td>
</tr>
<tr>
<td>Student Grants</td>
<td>2,500</td>
<td></td>
</tr>
<tr>
<td>Grants-in-aid</td>
<td>74,400</td>
<td></td>
</tr>
<tr>
<td>Cancer Research under the direction of Professor R. A. Q. O'Meara</td>
<td>3,000</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy of Cancer and Tuberculosis under the direction of Dr. V. C. Barry</td>
<td>76,500</td>
<td>201,067</td>
</tr>
</tbody>
</table>

**FROM SPECIAL GRANTS**

| Cancer Research under the direction of Professor R. A. Q. O'Meara | 6,692  |     |
| Chemotherapy of Cancer and Tuberculosis under the direction of Dr. V. C. Barry | 10,965 | 17,657 |

**ADMINISTRATION**

(6.6% of total) | 15,400 |

**£234,124**

A programme for national expansion of biomedical research formulated by the Medical Research Council was presented to the Minister in response to his request for its views on the grant policy. It adhered to the general pattern indicated in the previous annual report, which referred to a National Biomedical Research Institute, research units affiliated with medical school departments and hospital complexes and more comprehensive grant projects. Such a national policy is consistent with the Council's Articles of Association, which include as a specific objective "To establish an Institute for Research and to make all necessary provisions therefor" and as a primary objective "To organize and carry out research on any or all branches of medicine and in any or all sciences or subjects pertaining thereto". In context with these and other directives defining its terms of reference the broader program has been proposed together with a financial estimate requiring a minimal annual budget of £570,000, for consideration by the Minister. The Council now awaits his deliberations.

**GENERAL GRANT POLICY**

An analysis of the Council's traditional policy of research support illustrates a role which has been mainly passive in deployment of funds. To strengthen the research in the medical schools, and associated hospitals, its funds were allocated to those medical school departments demonstrably
The Council has practised a granting policy based on recognition of scientists and their creativity as a principal asset for expenditure. An analysis of the disposition of funds in grants awarded demonstrates the application of this approach to research support. In a recent grant period the financing of scientific personnel used 86% of the budget, compared with 11% and 3% spent respectively for materials and machinery. Over several decades this policy has fostered the growth of competent biomedical manpower in our Universities and hospitals. Continued development of this scientific resource is of promising value to the nation and a particular concern of the Council. It has posed two immediate problems; both the training of medical scientists and their subsequent employment in areas of medical research have become significant issues. While the absorption of scientific personnel into the structure of our national research endeavours is outside the purview of the Council’s current commitments, it is aware of the necessity for educational and hospital authorities to assume a responsibility in their arrangements for research as well as for service scientists. An expanded programme such as the Council advises, can partly implement this both with university research units and in a National Research Institute.

TRAINING OF MEDICAL SCIENTISTS

The considerable support of medical scientists has required the Council to take an interest in their training. In lieu of formal training programmes such as exist in other countries, a quasi-apprentice arrangement has prevailed. Many grants include provision for a graduate assistant who obtains a variable measure of training under supervision of the grant holder. Other young scientists may obtain fellowships at a stage of their career when training is incomplete; informal association with colleagues
in their institution replaces supervised training to some extent. Neither suffices as substitute for an essential training program necessary for the proper training of man-power required for effective biomedical research. Both suffer the defect of premature diversion of trainees into whole time research which tends to narrow activity and stultify future adaptability to changes in research. There is a real danger of the young scientist being locked into the machinery of a powerful and limiting research effort. The decision to continue support of scientists in training under research grants requires that the budget should include a training grant in order to assure a sustained level of training and also evidence that an adequate training program is available in the department. Otherwise the cost of the training is at the expense of the on-going research and results in no net saving of grant funds. The Council has been re-evaluating the merits of training grants in the light of such factors, and considering the proportion of assistant grants which properly belong in that category.

RESEARCH TRAINING FOR MEDICAL UNDERGRADUATES

The Council has expended annually a small sum in summer grants to medical students for a limited period of supervised training in about fifteen or more University departments and hospitals. Each student is assimilated into an on-going research project and briefly educated in research methodology. Familiarity is attained in instrumentation, data accumulation and precision techniques in a variety of disciplines, which include cytogenetics, pharmacology, molecular pathology and physiology. Subsequently students have become co-authors of several publications. The Council has also undertaken administration of a medical undergraduate scholarship scheme with funds granted by the Wellcome Trust which will support four medical students annually. This scheme will continue for 3 years and will be tenable in any of the schools in the Republic. Departments with adequate programmes are eligible to take such students and prepare them for an honours science degree in their discipline. The summer grants and Wellcome scholarships are intended to fill a gap in the practical bench experience of those medical students who have a leaning toward experimental science and encourage their recruitment into biomedical research.

APPRAISAL OF GRANT APPLICATIONS AND RENEWALS

A peer review system of applications, initiated several years ago has appeared to work reasonably well. Applications are processed by a relevant Committee which interviews the applicant and makes a recommendation to the Council concerning the feasibility and scientific quality of the project. Exploration of trends in biomedical research and proposals for initiation of studies in areas with national health problems is within their purview. In the proposed expanded program the Committees may find scope for wider responsibility and help determine the character of research units and the requirements of training programs.

In regard to the renewal of research support the Committees and Council reexamine the project and analyse the results achieved. The progress reports of the grant holder are acceptable as valid evidence of the development of a project; the presentation of them in our annual report is in
fact a form of priority publication. In the third year, when accomplishment of an objective may reasonably be expected, the decision of further renewal poses a more critical problem. More substantial and objective evidence of successful results is required for assessment. In general, scientific projects cannot be regarded as satisfactorily completed until the results of the research appear in a reputable journal. The Council utilizes this criterion, among others, in its evaluation of requests for continued support. While it is improper to defer final decision on the worthiness of research work to editors, the scrutiny of articles submitted to them for publication by expert editorial boards is tantamount to peer review. For this reason the Council places particular value on the standard of a grant holder's publications as a measure of accomplishment. Most publications are essentially progress reports and useful estimates of the originality of the research investment. With prevalent financial stringency curbing the disbursing of funds an assessment of research according to productivity and creativity is invaluable in making decisions. In the section on publications the range of reports issued by grant holders annually is presented, on which the Council draws for evaluation. Publications in transactions of societies are essentially priority claims. Articles in recognised journals represent the presentations of completed projects in whole or part.

**Biohazard of Genetic Recombination of DNA Molecules**

In response to the international concern with the potential hazard of genetic manipulation of micro-organisms and because of the interest of the Department of Health the Council established a special Committee of experts to advise it. It has met, examined the situation in this country and maintains communication with Committees in other countries. At the request of the European Science Foundation, several members of this Committee have been designated to take part in a European Working Party formed to consider the implications of the Ashby report and the magnitude of genetic manipulation as a health hazard. Currently no one in Ireland is performing the manipulations under review, but the techniques are sufficiently advanced for such manipulations to be undertaken in several institutions. It is too early in the development of this unique biological technique for a realisation of the impact it may have on human biology. While the universal alarm may have exaggerated the extent of the problem, the biomedical community has manifested a healthy reaction to the possible dangers of its own activity.

**European Medical Research Councils Group**

The Council maintains cooperation with medical research organizations of other European countries as a member of the European Medical Research Group. Meetings were held in Paris in February and Stockholm in October as specific areas of cooperation were examined. Discussions concerning problems of toxicology, clinical trials and the ethics of clinical research were continued from previous meetings. Between meetings Professor Zetterstrom of the Swedish Council visited Dublin to consult with a group of workers in order to assemble data concerning the international effort in the prenatal diagnosis of hereditary disorders. The
discussants represented several disciplines such as paediatrics, medical genetics, epidemiology, and clinical pathology. As it stands the Group has held biannual meetings mainly for exchange of valuable information and views concerning their various research policies and fundings.

**EUROPEAN SCIENCE FOUNDATION**

This year saw the Foundation become a reality. After a brisk meeting in Stockholm, at which it was voted to site the organization in Strasbourg, and the establishment of the statutes, it was formally ratified in November. The Medical Research Council is a member organisation from Ireland, together with the National Science Council and the Royal Irish Academy. Professor D. K. O'Donovan was our signatory. It is pertinent that the Foundation extended to the European Medical Research Council Group the opportunity of affiliation to it as a standing Committee with special conditions. The Foundation is financed mainly through its member organisations according to their country's GNP and population. It proposes to initiate organised research through formation of ad hoc Committees designed for the purpose and derived from the member organisations. Biomedical research is among its considerations.

**C.R.E.S.T.**

This body, formerly known as P.R.E.S.T. continued its activities in the field of medical research and public health through its sub-committee CRM. The Council's representative on CRM, Professor C. F. McCarthy, attended meetings in Brussels. Special working groups were formed by CRM on such subjects as deafness, traffic accidents, epidemiology, biology and biomedical engineering. The Council, at the request of the Department of Health, furnished a list of suitably qualified persons from which Irish representatives on these groups were selected. It is expected that visits by C.R.E.S.T. and CRM to member countries to familiarise themselves with methods of organisation and special problems will occur in the near future and it is hoped that the benefits of Ireland's membership of these bodies in the matter of financial support for research projects will soon be apparent.

**OTHER MATTERS**

*Post Doctoral Fellowships:* The Council was pleased to learn that Mr. Trevor J. I. McGill was awarded an International Research Fellowship for the year 1974–1975 and that Dr. T. F. Warner had been granted an extension of his existing fellowship for a further year.

**AWARDS**

The following awards were made during the year:

**GRAVES LECTURE**

(Awarded in collaboration with the Royal Academy of Medicine in Ireland).
PROFESSOR C. F. McCarthy, MD., FRCPI., MRCP. “Coeliac Disease –
The Irish Dimension”.

FELLOWSHIPS (WHOLETIME)

DR. ANNA F. Doyle, BSc., MA., PhD., Department of Biochemistry.
Trinity College, Dublin, under the direction of Professor F. G. A. Winder.

DR. C. FEIGHERY, MB., BCh., BAO., MRCPI., Sir Patrick Dun’s Hospital,
Dublin under the direction of Professor D. G. Weir.

DR. JUDITH S. MYLES, MB., BCh., BAO., BA., Sir Patrick Dun’s Hospital,
Dublin under the direction of Professor F. G. Fegan.

DR. J. F. O’LEARY, MB., BCh., BAO., Department of Surgery, University
College, Cork and St. Finbarr’s Hospital, Cork under the direction
of Mr. T. P. J. Hennessy.

DR. AIDEEN M. O’SULLIVAN, PhD., M.R.C. Laboratories, Trinity College,
Dublin under the direction of Dr. V. C. Barry.

FELLOWSHIPS (PART-TIME)

DR. MARIE GREALLY, MB., BCh., School of Pathology, Trinity College,
Dublin under the direction of Dr. J. Greally.

TRAINING GRANTS

MR. P. J. QUIGLEY, BSc. (H)., Department of Biochemistry, University
College, Dublin under the direction of Dr. P. Brennan.

RESEARCH ASSISTANT GRANTS

DR. J. COSTELLO, PhD., Meath Hospital, Dublin under the direction
of Professor E. Bourke.

MISS MARY P. DALY, BSc. (H)., St. James’s Hospital, Dublin under the
direction of Dr. V. Coffey.

MISS EMER DINEEN, BSc., St. Finbarr’s Hospital, Cork under the direction
of Dr. J. P. O’Regan.

MRS. MARY FEELEY, BSc. (H)., H.Dip.inEd., Department of Biochemistry,
University College, Galway under the direction of Dr. M. P. Coughlan/Dr. D. Johnson.

MR. H. GRAHAM, BSc. (H)., M.R.C. Laboratories, Trinity College,
Dublin under the direction of Dr. R. S. McElhinney.

MRS. ANNE HAYDEN, BSc., Department of Medicine and Therapeutics,
University College, Dublin under the direction of Professor O. FitzGerald.
Mr. E. L. Hickey, B.Agr.Sc. (Hort.), M.R.C. Laboratories, Trinity College, Dublin under the direction of Dr. V. C. Barry.

Mr. P. McKenna, BSc., Department of Pharmacology, University College, Dublin under the direction of Dr. M. P. Ryan.

Mr. J. J. Mitchell, MSc., Department of Physiology, University College, Dublin under the direction of Professor M. F. Murnaghan.

Mrs. Barbara Murray, BSc. (H)., MSc., Metabolic Unit, St. Vincent’s Hospital, Dublin under the direction of Professor F. P. Muldowney.

Miss Claire O’Leary, BSc., St. Finbarr’s Hospital, Cork under the direction of Dr. M. J. Whelton.

Mr. J. J. Rafter, BSc. (H)., Department of Biochemistry, University College, Dublin under the direction of Dr. M. E. Beary.

Miss Elizabeth Roche, BSc. (H)., Veterinary College, Dublin under the direction of Dr. B. J. Sheahan.

Grants-in-Aid

Dr. M. J. Carroll, Department of Biochemistry, Trinity College, Dublin.

Dr. F. R. Comerford, Department of Experimental Medicine and Practical Pharmacology, University College, Galway.

Dr. M. P. Coughlan/Dr. D. B. Johnson, Department of Biochemistry, University College, Galway.

Mr. T. V. Delaney, Department of Surgery, University College, Dublin.

Dr. C. Feighery, Sir Patrick Dun’s Hospital, Dublin.

Professor O. Fitzgerald, Department of Medicine & Therapeutics, University College, Dublin.

Mr. J. R. N. Flynn, Department of Surgery, Regional Hospital, Galway.

Dr. T. J. Foster, Department of Bacteriology, Trinity College, Dublin.

Professor J. Masterson, Department of Pathology, University College, Dublin.

Dr. P. A. O’Connor, Metabolic Unit, St. Vincent’s Hospital, Dublin.

Dr. J. P. O’Regan, St. Finbarr’s Hospital, Cork.

Dr. J. F. O’Leary, Department of Surgery, University College, Cork and St. Finbarr’s Hospital, Cork.
DR. M. P. RYAN, Department of Pharmacology, University College, Dublin.

DR. D. B. SHANLEY, Dublin Dental Hospital, Dublin.

DR. B. J. SHEEHAN, Veterinary College and Veterinary Research Laboratory, Dublin.

STUDENT GRANTS

MR. S. BOOLELL, under the direction of Dr. J. G. Devlin, St. Laurence's Hospital, Dublin.

MR. C. J. BUCKLEY, under the direction of Dr. M. T. Kane, Department of Physiology, University College, Cork.

MISS M. B. COLEMAN, under the direction of Professor M. G. Harrington, Department of Biochemistry, University College, Dublin.

MISS M. CORRY, under the direction of Professor J. Masterson, Department of Pathology, University College, Dublin.

MISS M. DUFFY, under the direction of Dr. H. Grimes, Department of Pathology, University College, Galway.

MISS R. FINN, under the direction of Dr. R. Mulcahy, St. Vincent's Hospital, Dublin.

MISS J. FOLAN, under the direction of Dr. M. Henry, Sir Patrick Dun's Hospital, Dublin.

MR. W. GRODIN, under the direction of Professor R. D. Thorne, St. Laurence's Hospital, Dublin.

MR. B. H. KHAMIS, under the direction of Dr. J. S. Doyle, St. Laurence's Hospital, Dublin.

MR. J. McCANN, under the direction of Mr. W. Hederman, Mater Misericordiae Hospital, Dublin.

MR. M. McGLINN, under the direction of Professor D. J. O'Donovan, Department of Physiology, University College, Galway.

MR. D. R. MULHALL, under the direction of Dr. M. J. Duffy, Department of Biochemistry, Trinity College, Dublin.

MR. B. MURRAY, under the direction of Professor D. G. Weir, Department of Biochemistry, Trinity College, Dublin.

MISS B. NOONE, under the direction of Professor J. K. Burns, Department of Physiology, University College, Galway.
Mr. M. O'Doherty, under the direction of Professor W. A. L. MacGowan, Department of Surgery, Royal College of Surgeons in Ireland, Dublin.

Mr. P. O'Neill, under the direction of Professor J. D. Sheehan, Department of Physiology, University College, Cork.

Miss M. Phelan, under the direction of Professor J. D. Kennedy, Department of Pathology, University College, Galway.

Mr. F. Shanahan, under the direction of Professor J. W. Harman, Department of Pathology, University College, Dublin.

Mr. J. D. Sweeney, under the direction of Dr. M. P. Coughlan, Department of Biochemistry, University College, Galway.

Mr. G. Tobin and Mr. P. Warde, under the direction of Professor C. W. M. Wilson, Department of Pharmacology, Trinity College, Dublin.

The following grants were renewed during the year:

FELLOWSHIPS (WHOLETIME)

Dr. G. Brow, Sir Patrick Dun's Hospital, Dublin.

Dr. G. O'Cuinn, Department of Biochemistry, University College, Galway.

Mrs. M. D. O'Donnell, Department of Medicine and Therapeutics, University College, Dublin.

Mrs. Vivien Reid, Cardiac Department, St. Vincent's Hospital, Dublin.

Dr. Honor Smyth, Department of Biochemistry, University College Dublin.

FELLOWSHIPS (PART-TIME)

Mr. D. Bouchier-Hayes, Department of Surgery, University College, Dublin and St. Vincent's Hospital, Dublin.

Dr. Victoria Coffey, Department of Social Medicine, T.C.D. Medical Unit, St. James Hospital, Dublin.

Dr. Bridget Egan-Mitchell, Department of Paediatrics, Regional Hospital, Galway.

Dr. I. Graham, Cardiac Department, St. Vincent's Hospital, Dublin.

Mrs. Nuala Mahon, Department of Medicine, University College, Dublin.
TRAINING GRANTS

Miss C. Spellman, Department of Obstetrics and Gynaecology, University College, Galway under the direction of Professor E. O'Dwyer.

RESEARCH ASSISTANT GRANTS

Miss C. Barrett, Metabolic Unit, St. Vincent's Hospital, Dublin.

Mr. P. Brown, Department of Clinical Medicine, Meath Hospital, Dublin.

Mr. D. E. Cannon, Department of Pathology, University College, Dublin.

Miss A. Corboy, Metabolic Unit, St. Vincent's Hospital, Dublin.

Mr. S. Cunningham, Department of Surgery, University College, Dublin.

Mrs. Eleanor Davis, Department of Clinical Medicine, Meath Hospital, Dublin.

Miss A. Dennehy, Department of Clinical Medicine, Meath Hospital, Dublin.

Miss M. Gannon, M.R.C. Laboratories, Trinity College, Dublin.

Miss H. Gorry, Department of Experimental Medicine, St. Luke's Hospital, Dublin.

Mrs. N. Hiney (nee Ring), Department of Biochemistry, University College, Dublin.

Miss R. Jacob, St. Finbarr's Hospital, Cork.

Miss Mary McDermott, Department of Physiology, University College, Dublin.

Miss M. McEllin, Department of Biochemistry, University College, Dublin.

Mr. J. J. Mitchell, Department of Physiology, University College, Dublin.

Mrs. P. A. Mooney, Department of Gastroenterology, Regional Hospital, Galway.

Mrs. C. O'Dwyer, Department of Biochemistry, Trinity College, Dublin.

Mr. V. Parameswaran, Endocrine Unit, St. Laurence's Hospital, Dublin.

Mrs. E. Rooney, Department of Biochemistry, University College, Galway.
MR. H. SANKARAN, Department of Biochemistry, University College, Dublin.

GRANTS-IN-AID

DR. M. E. BEARY, Department of Biochemistry, University College, Dublin.

MR. W. H. BEESLEY/DR. R. J. GAY, Department of Experimental Surgery, Trinity College, Dublin.

MR. D. BOUCHER-HAYES, Department of Surgery, University College, Dublin and St. Vincent’s Hospital, Dublin.

DR. W. A. BOGGUST, Cancer Research Unit, St. Luke’s Hospital, Dublin.

DR. G. BROW, Clinical Research Department, Sir Patrick Dun’s Hospital, Dublin.

PROFESSOR J. K. BURNS, Department of Physiology, University College, Galway.

DR. E. BOURKE, Department of Clinical Medicine, Meath Hospital, Dublin.

MR. J. T. CAHILL, Department of Surgery, University College, Dublin.

PROFESSOR P. J. CANNON, Department of Pharmacology, University College, Dublin.

DR. N. CLARKE, Department of Pathology, University College, Dublin.

DR. V. P. COFFEY, Department of Social Medicine, T.C.D. Medical Unit, St. James’s Hospital, Dublin.

DR. M. J. CULLEN, Department of Clinical Medicine, Meath Hospital, Dublin.

DR. J. G. DEVLIN, Endocrine/Metabolic Unit, St. Laurence’s Hospital, Dublin.

DR. P. F. DUGGAN, Department of Biochemistry, St. Finbarr’s Hospital, Cork.

PROFESSOR W. G. FEGAN, Sir Patrick Dun’s Hospital, Dublin.

PROFESSOR P. B. B. GATENBY, Department of Clinical Medicine, Meath Hospital, Dublin.

PROFESSOR M. G. HARRINGTON, Department of Biochemistry, University College, Dublin.
Dr. M. T. Kane, Department of Physiology, University College, Cork.

Mr. T. V. Keaveny, Department of Surgery, University College, Dublin.

Professor R. P. Kernan, Department of Physiology, University College, Dublin.

Dr. R. G. Luckwill, Department of Physiology, Trinity College, Dublin.

Professor C. F. McCarthy, Department of Gastroenterology, Regional Hospital, Galway.

Mrs. N. Mahon, Department of Medicine and Therapeutics, University College, Dublin.

Professor J. Masterson, Department of Pathology, University College, Dublin.

Dr. H. C. Moore, The Rotunda Hospital, Dublin.

Professor E. C. Moorhouse, Department of Clinical Microbiology, Royal College of Surgeons in Ireland, Dublin.

Dr. R. Mulcahy, Cardiac Department, St. Vincent's Hospital, Dublin.

Professor F. P. Muldowney, Metabolic Unit, St. Vincent's Hospital, Dublin.

Professor M. F. Murnaghan, Department of Physiology, University College, Dublin.

Dr. P. O'Carra, Department of Biochemistry, University College, Galway.

Professor D. J. O'Donovan, Department of Physiology, University College, Galway.

Dr. M. T. O'Hegarty, Department of Pathology, University College, Dublin.

Dr. J. Scott, Department of Biochemistry, Trinity College, Dublin.

Dr. H. Smyth, Department of Biochemistry, University College, Dublin.

Professor I. J. Temperley, School of Pathology, Trinity College, Dublin.
CHEMOTHERAPY

Medical Research Council of Ireland Laboratories, Trinity College, Dublin.

Director:

VINCENT C. BARRY, DSc., FICl., FRIC., MRIA.

CHEMISTRY:
Dermot Twomey, MSc., PhD., FICl., MRIA.
James G. Belton, MSc., PhD., FICl., MRIA.
Joan E. McCormick, MSc., PhD., ARIC.
Conor O'Callaghan, MSc., PhD.
R. S. McElhinney, BSc., PhD., FRIC., MRIA.
Marie Gannon, BSc.
Hugh Graham, BSc. (Appointed September 1, 1974).

BIOCHEMISTRY:
Aideen O'Sullivan, MSc., PhD. (Appointed October 1, 1974).

PHARMACOLOGY AND CELL CULTURE:
J. F. O'Sullivan, MSc., PhD., MICl.

MICROBIOLOGY, HISTOCHEMISTRY, ANTITUMOUR AND ANTIBACTERIAL TESTING:
M. L. Conalty, MD., FRCPath., DPH., MRIA.
Joan Byrne, BSc., PhD.
Edward Hickey, BSc. (Appointed from June 4, 1974).
ANTITUMOUR ACTIVITY

Of the several chemical types of compounds at present under study for antitumour activity the benzofuroxans and the tetrazoles, particularly the tetrazolopyridazines and the tetrazolophthalazines, are proving of particular interest. Activity against the P.388 lymphatic leukaemia of the same order, or better, as has been reported for such clinically interesting substances as 6-mercaptopurine, 6-thioguanine, 6-thiotepa, hydroxyurea, methyl GAG, streptozotocin, bleomycin and myleran has been observed. Very marked activity has also been observed against a variety of ascitic tumours, Ehrlich, Landschutz, and Sarcoma 180 in ascitic form. The tables in the following pages some of the more important results have been set out and the general findings are discussed. Many of our compounds are now being submitted for further evaluation under the scheme sponsored by the National Cancer Institute, Bethesda, through their centre at the Institut Jules Bordet in Brussels and such compounds, therefore, also bear a Bethesda (NSC) number. This numbering system has also been adopted by the “European Organisation for Research on Treatment of Cancer” (EORTC), to the Screening and Pharmacology Group of which Dr. Michael Conalty has recently been elected.

Benzofuroxans (See Table I)
The benzofuroxans were inactive against solid Sarcoma 180 but high activity was found with many of them against the Ehrlich ascites tumour (treatment once daily i.p. for 7 days) and against the P.388 leukaemia (treatment once daily i.p. on days 1, 5, 9 following implantation) (Table I). High activity was also noted against ascitic Sarcoma 180 and against the Landschutz ascitic tumour.

Table I. Activity of Some 4-Substituted 7-Nitro-Benzofuroxans Against Lymphatic Leukaemia P.388 in B6D2F1 mice.

<table>
<thead>
<tr>
<th>Compound and Number</th>
<th>Treatment Once Daily on Days 1, 5, 9 Post Day of Implant mg/kg i.p.</th>
<th>Survival Time. Test/Control Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. 2837</td>
<td>128</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>117</td>
</tr>
<tr>
<td>Compound</td>
<td>Structures</td>
<td>Toxicity</td>
</tr>
<tr>
<td>-----------</td>
<td>------------</td>
<td>----------</td>
</tr>
<tr>
<td>B. 2274</td>
<td><img src="image1" alt="Structure" /></td>
<td>128 64 32 16 8</td>
</tr>
<tr>
<td>B. 2368</td>
<td><img src="image2" alt="Structure" /></td>
<td>128 64 32 16 8 4</td>
</tr>
<tr>
<td>B. 2766</td>
<td><img src="image3" alt="Structure" /></td>
<td>64 32 16 8 4</td>
</tr>
<tr>
<td>B. 2767</td>
<td><img src="image4" alt="Structure" /></td>
<td>128 64 32 16 8 4</td>
</tr>
<tr>
<td>B. 2743</td>
<td><img src="image5" alt="Structure" /></td>
<td>128 64 32 16 8 4</td>
</tr>
<tr>
<td>B. 2748</td>
<td><img src="image6" alt="Structure" /></td>
<td>128 64 32 16 8 4</td>
</tr>
<tr>
<td>B. 2742</td>
<td><img src="image7" alt="Structure" /></td>
<td>256 128 64 32 16</td>
</tr>
</tbody>
</table>
The following structure-activity relationships have been observed against the Ehrlich and P.388 lines:

1. Where \( R = -\text{N} \):

   \[
   X \quad Y
   \]

   (a) \( X = \text{H} \) and \( Y = \text{aryl, substituted aryl or alkyl} \). Inactive against Ehrlich and P.388.
   (b) \( X = Y = \text{alkyl} \). Activity against Ehrlich but not against P.388 except where \( X = Y = \text{Me} \).
   (c) \( X = \text{alkyl}, Y = \text{aryl} \). Activity against Ehrlich but not against P.388.
   (d) \( X = Y = \text{hydroxyethyl} \). Marked activity against Ehrlich and moderate activity against P.388 (e.g. B.2837, NSC 228109).

2. Cycloalkylimino derivatives such as ethyleneimino, pyrrolidino, piperidino and homopiperidino etc. were, with the exception of the ethyleneimine compound (B.2274, NSC 228081), inactive.

3. Piperazinyl derivatives are highly active, particularly alkyl substituted compounds B.2368 (NSC 179940), B.2766 (NSC 228105), B.2767 (NSC 228106), the hydroxyalkyl compound B.2743 (NSC 228101), the aldehyde compound B.2748 (NSC 228102) and the carbethoxy compound B.2742 (NSC 228100), against both tumours. Table II setting out the activity of B.2368 against the Ehrlich ascites tumour illustrates the order of activity obtained with the piperazine derivatives.

4. On the other hand aryl and substituted aryl piperazinyl derivatives have little or no activity.

**Table II**

_Ehrlich Ascites Carcinoma_. Schofield albino mice, inoculum approximately \( 10^7 \) cells. Treatment once daily i.p. with B.2368 for 7 days commencing on day after implantation.

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>Inhibition of Ascitic Fluid on Day 7 (Method of Sugiura)*</th>
<th>Survival Time** T/C%</th>
<th>Survivors on Day Experiment Terminated (day 112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>Complete</td>
<td>168</td>
<td>0/10</td>
</tr>
<tr>
<td>18</td>
<td>Complete</td>
<td>277</td>
<td>1/10</td>
</tr>
<tr>
<td>12</td>
<td>Complete</td>
<td>324</td>
<td>6/10</td>
</tr>
<tr>
<td>8</td>
<td>Complete</td>
<td>372</td>
<td>5/10</td>
</tr>
<tr>
<td>5.4</td>
<td>Complete</td>
<td>384</td>
<td>8/10</td>
</tr>
<tr>
<td>Controls</td>
<td>–</td>
<td>Mean=21.1 days All dead by day 25</td>
<td></td>
</tr>
</tbody>
</table>


**Survivors killed on day 112 included in calculations as having died on day 113.
**Note:** On day 112 when the experiment was terminated all 20 survivors were tumour-free.

The extended dose range over which activity is observed with the more active agents is most encouraging. Also under study are 5-substituted 6-nitro-benzofuroxans which are providing interesting results. For example the analogue corresponding to B.2368 (Table I) was devoid of activity whereas compounds analogous to types 1c above were more active.

![Tetrazoles](image-url)

A wide variety of tetrazolo compounds is presently under investigation. The study arose from the discovery of growth inhibitory activity against HeLa cells *in vitro* (<1µg/ml) in the tetrazolo-pyridine compound 1, \( R = \text{NO}_2 \), although it had no antitumour activity *in vivo*. This led in turn to a study of pyridazine derivatives (2) the most active *in vivo* of which was that with \( R = \text{Cl} \), B.2557. (Table III—see page 26).

A further modification of the molecule led to benzo-derivatives of the pyridazine compounds – phthalazines (3). Again, variation of the \( R \) substituent greatly modified activity, the most active compound being the chloro derivative B.2834, NSC 179944. Triazolo-analogues appear to be inactive.

Modification of the heterocyclic ring resulted in compounds of type 4 (quinoxalines). As in the case of the phthalazine compounds activity was highest with \( R = \text{Cl} \), B.3023 (NSC 228175), but of a lower order than was observed with the phthalazines.

Type 5, 6 and 7 compounds are presently under study and are providing interesting results.
### Table III. Activity of Tetrazoles Against Lymphatic Leukaemic P.388 in BοDsF1 Mice.

<table>
<thead>
<tr>
<th>Compound and Number</th>
<th>Treatment Once Daily on Days 1, 5, 9 Post Day of Implant. mg/kg i.p.</th>
<th>Survival Time. Test/Control Per Cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. 2557</td>
<td>256 128 64 32 16</td>
<td>Toxic 135 129 126 117</td>
</tr>
<tr>
<td></td>
<td>![N-N] Cl</td>
<td></td>
</tr>
<tr>
<td>B. 2834</td>
<td>256 128 64 32 16</td>
<td>Toxic 115 (toxic) 160 153 117</td>
</tr>
<tr>
<td></td>
<td>![N-N] Cl</td>
<td></td>
</tr>
<tr>
<td>B. 3023</td>
<td>64 32 16 8</td>
<td>Toxic 123 139 121</td>
</tr>
<tr>
<td></td>
<td>![N-N] Cl</td>
<td></td>
</tr>
<tr>
<td>B. 3012</td>
<td>Inactive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>![N-N] Cl</td>
<td></td>
</tr>
</tbody>
</table>
**Iminochromens**

We have for some time been investigating the antitumour properties of derivatives of 3-carbamoyl-2-iminochroman \( C_6H_4O.C(:NH).C \) (CONHR)\( \cdot \)CH (8). It has been found that the formation of tricyclic condensation products from these compounds diminishes rather than enhances biological activity, and that antitumour properties are much more evident in the parent compounds (8). Derivatives of (8), where \( R \) represents alkyl, aryl and heterocyclic groups, have now been synthesised. These have shown significant activity when tested against the Ehrlich ascites tumour. In an attempt to further assess this activity, selected compounds have also been screened against the P.388 tumour. These tests have established that maximum activity is displayed by these compounds having alkyl substituents on the carbamoyl group; B.2960 NSC 227160 (8, \( R = \)Et) is the most active of these tested. The active dosage levels of these compounds are relatively high, but their lack of toxicity encourages us to experiment further with the object of enhancing the activity while preserving the basic iminochroman structure. Our studies in this field are continuing.

**Bis-thiosemicarbazide Derivatives**

Another investigation has been concerned with new bis(4-methylthiosemicarbazide) derivatives (cf. previous screening data reported by Barry et al. (Proc. Roy. Ir. Acad. 65B, 309, 1967). Some of these new compounds have shown significant antitumour activity against Sarcoma 180 in the mouse. Cyclisation of these bis-thiosemicarbazides to bis-(1, 2, 4-triazoline-3-thiones) appears to eliminate this activity.

In connection with this work, derivatives of 1-acyl-4-alkylthiosemicarbazide were also examined. An attempt has been made to vary the bis(4-methylthiosemicarbazide) structure, using the following reaction sequence:

\[
\begin{align*}
\text{EtOOC.CH}_2\text{CONNH}_2 & \quad \xrightarrow{(i)} \quad \text{EtOOC.CH}_2\text{CONHNHCSNHR} \\
\text{H}_2\text{HNOC.CH}_2\text{CO NHNCSNHR} & \quad \xrightarrow{(ii)} \quad \text{R}'\text{HNSCHNOC.CH}_2\text{CONHNHCSNHR} \\
\end{align*}
\]

This was only partially successful, since there was a tendency to cyclise during stage (ii), with formation of 5-carbazoylmethyl-1, 2, 4-triazoline-3-thiones. Hydrazones formed by some of these products have shown antibacterial activity in the primary screen; antitumour screening is not yet complete.
PUBLICATIONS AND COMMUNICATIONS


Steroid Catabolism in Choline-Deficient Rat Livers.

Work was continued on the interrelation between the enzymes of steroid catabolism and microsomal phospholipids. The cytoplasmic factor, which reactivates the microsomal steroid hydroxylases of choline deficient rat livers, was further characterised. It has the following characteristics:

(a) It is precipitated by 40% (NH₄)₂SO₄.

(b) Sephadex chromatography indicates a molecular weight of approximately 13,000. It may, under certain conditions, aggregate to form a larger molecular weight species.

(c) It is inactivated by trypsin digestion but unaffected by phospholipase C.

(d) Incubation of the partially purified protein with Ca⁺⁺ increases its activation.

This work indicates that phospholipid-protein interactions may be essential for some steroid hydroxylases and that lipids may play a role in determining the manner in which a steroid substrate gains access to the enzyme system as well as the chemical change it will undergo. If this is correct, enzymatic disruption of the microsomal phospholipids should produce changes similar to those of choline deficiency. To test this, microsomal preparations from rats on a normal laboratory diet were incubated with phospholipase C. Control and phospholipase C treated microsomes were then incubated with (4-¹⁴C)-androstenedione, as previously described. The steroid metabolites were extracted and separated. The changes produced by the phospholipase paralleled those of the choline deficiency. The overall metabolism decreased on treatment but the percentage conversion into 16α-OH-androstenedione increased significantly: control 9.4 ± 2.6; treated 39.6 ± 2.5. Reactivation of the steroid hydroxylases was also brought about by including the cytoplasmic fraction in the incubate.

16α-OH-androstenedione had already been tentatively identified by chromatographic procedures but positive identification was obtained by Mass Spectroscopy which was carried out by Dr. F. Martin of the Steroid Research Laboratory, University of Helsinki, Finland.
M. J. Carroll, BSc., MA., PhD.

Department of Biochemistry, Trinity College, Dublin.

*Mucopolysaccharide Synthesis in the Early Mouse Embryo.*

The trophoblast plays the essential role in preventing immunologic reactions against the foetus: Trophoblastic antigens are masked by some characteristic of the surface of the trophoblastic cells. The nature of the cell surface glycoprotein is being investigated using the technique of *in vitro* embryo culture.

Prepubertal hybrid (BALB/c x C57/BL6) female mice were superovulated by the injection of pregnant mare’s serum followed 48 hours later with human chorionic gonadotropin. After the second injection the females were caged with C3H/FeJ males and 2-cell embryos were flushed from the oviduct 36 hours after mating. These embryos were grown in culture to the expanded blastocyst stage (approximately 80 hours). The cleavage-stage embryo, growing *in vitro* was susceptible to damage by radiation but a level of 10 μCi 35S-sulphate/ml of medium was tolerated. The pre-blastulae embryo did not metabolise 35S-sulphate but the onset of blastocoeI formation was accompanied by an abrupt surge in the accumulation of 35S-sulphate. This embryonic radioactivity was mainly in the form of glycosaminoglycan and autoradiographic analysis suggested that the radio activity was concentrated on the surface of the trophoblast.

Michael P. Coughlan, MA., PhD., Desmond B. Johnson, BSc., PhD.

Biochemistry Department, University College, Galway.

*The novel use of enzymes in clinical analysis.*

The use of immobilised enzymes.

The enzymes chosen for initial study were hog liver urate oxidase (uricase), yeast alcohol dehydrogenase, milk xanthine oxidase and turkey liver xanthine dehydrogenase.

The immobilisation of uricase on porous glass and on Hornblende was studied. The silane method provided active stable preparations of the enzyme immobilised on glass and hornblende. Immobilisation did not significantly alter either the pH of optimal activity or the pH/activity profile. At concentrations up to 0.05 mM quantitative conversion of uric acid was achieved using columns of bound enzyme 2.5 cm in height and 1.3 cm in diameter and flow rates of 2 ml. min⁻¹ or less. Moreover, the immobilised enzyme may be used routinely in the estimation of Serum uric acid levels.
The immobilisation of alcohol dehydrogenase with a view to its use in the measurement of blood alcohol levels has been initiated. The yeast enzyme immobilised on porous glass was used at 20°C for a period of 1.5 hours per day and stored at 4°C when not in use. Under these conditions the preparation had a half life of ten days but could readily be used in the estimation of ethanol concentrations in test samples for fourteen days. The levels of ethanol measurable were as low as 0.5 mM which is just a small fraction of the blood levels permitted by law while driving. Work is continuing to provide a method for routine use in the determination of blood alcohol levels.

Milk xanthine oxidase and turkey liver xanthine dehydrogenase were successfully immobilised on hornblende by the silane method. However, immobilised preparations of the milk enzyme were markedly unstable (t½ @ 30°C~4 hr.). While immobilised preparations of the turkey enzyme were more stable than those of the milk enzyme, activity at 30°C did fall by about 10% over a 3 hour period. We are presently attempting to define the cause(s) for such instability and to find ways to overcome the problem since it is one which would be germane to any immobilised enzyme in clinical use.

Anna F. Doyle, BSc., MA., PhD.

Department of Biochemistry, Trinity College, Dublin.

Director: Prof. F. G. A. Winder, MA., ScD.

The Mode of Action of Isoniazid on Mycolic Acid.

It has been shown that Isoniazid exerts its exhibited action on mycobacteria by inhibiting the synthesis of mycolic acids. These are a group of long-chain α-branched, β-hydroxy acids which are structural components of the mycobacterial cell wall. The long-chain, or meromycolate, portion is some 40 to 60 carbon atoms long. The side chain has 24 carbons. The route of biosynthesis of mycolic acids is not known and will have to be elucidated, at least in part, if the mechanism of action of Isoniazid at the molecular level is to be determined. A cell-free system has been obtained from Mycobacterium smegmatis which, when incubated with suitable additives, will incorporate 14C from [14C] acetate into mycolic acids. It has been shown with reasonable certainty that this activity is not due to unbroken cells in the system. The different classes of mycolic acids (which differ in chain-length, unsaturation and substituent groups) are labelled to differing extents, and this pattern of differential labelling is different from that which occurs with whole cells. This cell-free system from M. smegmatis appears to be insensitive to isoniazid.

A cell-free system from Mycobacterium bovis BCG has now been obtained which also synthesises mycolic acid-like compounds. This system is particularly active in the synthesis of meromycolic acid-like
compounds, which presumably represent intermediates in the synthesis of the true mycolic acids. Isoniazid inhibits the synthesis of the meromycolic acids in this system, which suggests that the drug acts on the formation of these intermediates rather than on their conversion to true mycolic acids.

P. F. Duggan, PhD., DSc., FRIC., FICI., in collaboration with Rosemary Jacob, BSc.

St. Finbarr's Hospital and University College, Cork.

*Effects of Ions and Various Classes of Pharmacological Agents on the Calcium Pump System of Sarcoplasmic Reticulum.*

A more detailed look has been taken at the intermediate reactions involved in calcium transport by sarcoplasmic reticulum because of reports purporting to show that potassium ions, present intracellularly in high concentration, inhibit such calcium uptake. A series of papers have also shown that potassium decreases the concentration of phosphorylated intermediate formed during this ATP-dependent process. As such recovery of calcium is the basis of muscle relaxation and intracellular potassium seems an unlikely inhibitor the effects of potassium on calcium transport and phosphorylated intermediate were followed simultaneously under carefully controlled conditions. It was found, as previously reported, that potassium stimulated calcium transport while decreasing the equilibrium concentration of phosphorylated intermediate. This indicates a faster turnover of the transport system in the presence of potassium and confirms its stimulatory rather than inhibitory role in muscle relaxation.

Interestingly, guanidinium and formamidinium ions, polyatomic monovalent cations each of which has sp³ hybrid atomic orbitals and trigonal orientation, inhibit calcium transport whereas soluble enzymes with absolute requirements for monovalent cation are activated.

The use of sarcoplasmic reticulum vesicles as a model system for membrane anaesthesia studies was further developed. Substances from twelve classes of anaesthetic compounds were found to increase calcium binding. All inhibited uptake as their concentrations were increased. The biphasic action of membrane stabilisers, with membrane lysis occurring at elevated concentrations, is well established.

Whether the increased Ca²⁺ transport in muscle microsomal vesicles is caused by the same sequence of events that occurs during membrane anaesthesia is, of course, not known, but it can be hypothesised that if the membranes of excitable cells are made labile by removal of Ca²⁺ any substance which prevents or diminishes such a loss of Ca²⁺ would tend to render such cells less responsive to stimuli. The studies are being extended to nerve cells isolated from various parts of the brain.
Michael Gerard Harrington, MSc., PhD., FICI., in collaboration with Hariharaan Sankaran, MSc. (Madras).

Department of Biochemistry, University College, Dublin.

Effect of leucine on blood glucose, insulin and other components.

An increase in the concentration of certain amino acids in blood has been shown to increase the absorption of glucose. Casey and Harrington (M.R.C. Annual Report 1970) showed in perfusion experiments with rat intestine that increased levels of leucine enhanced the glucose uptake. Incubation studies with inverted sacs confirmed this effect.

Control rats injected with physiological saline did not show an increase in blood glucose level.

Eviscerated rat preparations injected with leucine (24.26 g.l⁻¹) in physiological saline showed an increase in blood glucose within 10 min. of administration. This increase was followed by a slower rate of decrease in blood glucose level over the next 30 min. period. Thin layer chromatography of deproteinised blood specimens, containing 14c-leucine as marker, revealed that 60% of the administered leucine was removed from the blood in 40 min. Injection of an equal volume of leucine in physiological saline to eviscerated rats did not appear to alter significantly the blood volume, as determined by the 125i-labelled serum albumin and in experiments using 14c-glucose. However, the haematocrit and red cell count values were significantly reduced.

Attempts to show that the increased glucose, assayed by glucose-oxidase method, arose from muscle were unsuccessful. No appreciable amount of glucose-6-phosphate was detected in the blood. In addition, the level of alkaline phosphatase decreased significantly after leucine administration. The reduction in the level of alkaline phosphatase was comparable to the decrease in the level of circulating immunoreactive insulin, as determined by a double-antibody precipitation method and suggesting thereby an increase in the blood volume. An investigation of the muscle glycogen level was undertaken, but practical difficulties in determining changes in muscle glycogen were not satisfactorily solved.

The incompatibility of results obtained for haematocrit and alkaline phosphatase levels on the one hand and the methods used for determining blood volume on the other hand has led to the conclusion that infusion of leucine induces a redistribution of compounds between the circulating fluid and the intracellular fluid compartments giving rise to the observed rise in circulating glucose levels. This hypothesis will be subjected to further investigation.

The effect of intravenous administration of the two amino acids, arginine and lysine, on blood glucose in eviscerated rats was studied.
Maximum concentration of arginine and lysine in physiological saline, which did not alter the viability of the preparation, failed to produce significant increase in the blood glucose level following the intravenous injection.

Padraig O'Carra, BSc., PhD., in collaboration with Standish Barry, BSc. and Jane Watt, BSc.

Department of Biochemistry, University College, Galway.

New approaches to the study of some metabolic oxidoreductase systems.

The purpose of this project is the development of new methods for the isolation and investigation of certain enzyme systems whose study has previously been greatly hampered by the limitations of 'conventional' enzymological methods. The new methods were to be based largely on the affinity chromatographic approach. However, our efforts to apply this approach in rigorous fashion revealed defects in concepts on which most applications of this approach have hitherto been based. We were therefore obliged to re-investigate and re-develop much of the basic groundwork. This developmental work has allowed us in the last year to apply the affinity approach, as originally intended, to the study of a number of oxidoreductase systems, aspects of whose metabolic roles remained obscure.

The following two examples typify such studies, which are being extended to a range of other enzymes.

*Biliverdin Reductase*

An affinity chromatographic system has been developed for this enzyme, based on biospecific adsorption of the enzyme on an immobilised NADP analogue followed by displacement with either soluble NADH or NADPH. The displacement behaviour with these ligands confirms that a single enzyme system is responsible for the NADH- and NADPH-dependent activities. The affinity system has been used to stabilise the enzyme and purify it to a specific activity of 260 (previous best was 8) and the kinetic and other properties of the enzyme have been more precisely established through studies using the highly purified preparations. However, the metabolic purpose of biliverdin reductase still remains puzzling, and the puzzle is further deepened by our discovery that human placenta is characterised by a very high level of this activity. The enzyme responsible for the placental activity seems to be functionally identical with the enzyme isolated from liver and spleen.
Isoenzymes of Lactate Dehydrogenase

A range of affinity systems based on immobilised analogues of pyruvate, NAD and AMP have been developed for the H and M type isoenzymes. Besides providing excellent purification methods, these systems have provided powerful new methods for investigating the functional distinctions between the isoenzymes. Our studies indicate that the key functional distinctions depend not on complex differences in substrate inhibition and abortive complex formation (an almost universally accepted view at the moment) but rather on simple differences in the Km values for lactate and possibly also on a differential sensitivity of the H type isoenzyme to inhibition by oxaloacetate. The X isoenzyme (which occurs specifically in spermatozoa, and whose differential function is unknown) is currently under investigation.

Gerard O’Cuinn, BSc., PhD.

Biochemistry Department, University College, Galway.

Director: Professor P. F. Fotrell, MSc., PhD.

Purification and characterisation of Intestinal peptide hydrolases.

Objectives:

(1) The cytosol of guinea-pig intestinal mucosa is known to contain at least seven peptide hydrolases which are probably responsible for the terminal stages of protein digestion. Particular interest was paid to the enzymes hydrolysing dipeptides. As two such enzymes (α and β) had previously been purified it was decided to purify and characterise a third dipeptide hydrolase, the γ peptide hydrolase or aminoacyl proline hydrolase. These purified enzymes might then be subjected to rigorous comparative studies for substrate specificity, subunit structures etc.

(2) Subcellular fractionation studies reveal that the brush-borders of guinea-pig intestinal mucosa contain a small proportion of the dipeptide hydrolase of these cells. An investigation was undertaken to determine the relationship between the peptide hydrolase activities of brush-border and cytoplasm.

(3) The peptide hydrolase activities (α, β and γ) from the intestinal mucosa of the guinea-pig have now been purified to homogeneity. It was proposed to investigate the extent to which these enzymes are immunologically related to (a) each other, (b) enzymes with similar substrate specificities and electrophoretic mobilities in other tissues of the guinea-pig and (c) similar enzymes in intestinal mucosa of different species.

Results:

(1) The purified γ-peptide hydrolase was found to preferentially hydrolyse peptides of composition X-Pro (where X=amino acid) but also hydrolysues peptides containing proline in the amino terminus and some...
peptides which do not contain proline at all. Kinetic studies with the three purified peptide hydrolase shows that while they have overlapping substrate specificities they are broadly complementary in their activities e.g. the β peptide hydrolase is highly reactive with peptides of composition Gly-X whereas the γ enzyme is poorly active with these substrates and the α enzyme is unreactive. The γ enzyme is the only enzyme capable of hydrolyzing peptides of composition X-Pro.

(2) Our studies reveal that peptide hydrolase activities released from brush-borders by a variety of solubilising agents are similar to cytosol peptide hydrolases in their electrophoretic mobilities and substrate specificities. No distinctive Leu-Leu hydrolase activity could be released from brush-borders by using either sonication, Triton X-100 or papain.

(3) The α, β and γ peptide hydrolases of guinea-pig intestinal mucosa have been found to be immunologically distinct. Antisera raised against each purified enzyme formed precipitin lines with or inhibited only the enzyme to which each was raised. The γ peptide hydrolase of guinea-pig intestine cross reacted with enzymes of similar substrate specificity in brain and kidney of the same species. However no cross reactivity could be detected between the β and γ peptide hydrolases of guinea-pig intestinal mucosa and enzymes of similar specificities in the intestines of rat, pig and cow.

Patrick J. Quigley, BSc.

Department of Biochemistry, University College, Dublin.

Director: Patrick J. Brennan, MA., MSc., PhD.

An examination of the effects of infection with enveloped animal viruses on some carbohydrate-containing components of host cells.

An acid-insoluble glycoprotein-rich fraction obtained from a variety of cell lines, which were grown in culture and labelled for short periods with 3H-galactose, was digested with Pronase and the soluble products fractionated by Sephadex G-50 gel filtration. Two major fractions were revealed; fraction 1 was excluded from the column and therefore has high molecular weight; fraction 2 was included and is of low molecular weight. When similar procedures were applied to herpes simplex virus (HSV)-infected cells, fractions 1 and 2 were greatly diminished and instead a virus-specified glycopeptide fragment of intermediate molecular weight appeared. Hydrolysis of the entire acid-insoluble fraction from normal cells showed that about 70% of the incorporated radioactivity resided in glucose and the remainder in galactose. In HSV-infected cells the capacity to incorporate radioactivity into glucose was almost completely lost and there was an enhanced ability to label the acid-insoluble galactose. Gas liquid chromatography of the acid-insoluble hexoses from normal cells showed a relationship of glucose to galactose of about 3:1 indicating
that the pattern of labelling was reflective of the overall distribution of the hexoses in the glycoprotein fraction.

In an effort to understand the molecular basis of the altered glycoprotein metabolism in HSV-infected cells a characterisation of fractions 1 and 2 from normal cells was undertaken. $^{35}$SO$_4$, $^3$H-glucosamine, $^3$H-galactose and $^3$H-glucose were incorporated into the high molecular weight fraction. Furthermore, ion-exchange chromatography showed that this fraction was largely anionic, suggesting that fraction 1 was proteoglycan in nature. Other evidence suggested that fraction 2 was composed of at least five small neutral and acidic glucose-containing glycopeptides.

The virtual absence of fractions 1 and 2 in HSV-infected cells seems to be due to the ability of the virus to first shut off host protein synthesis and the subsequent virus-induced proteins are not suitable receptors of certain sugars, principally glucose, for proteoglycan or glycoprotein synthesis.

These features do not seem to be generally common to all lytic virus-infected cells. Semliki Forest virus and polio virus infected cells exhibited the normal Sephadex G-50 profile.

Dr. John M. Scott, BSc., MA., PhD.

Biochemistry Department, Trinity College, Dublin.

The absorption and metabolism of folate in man and other mammals.

Folate within cells exists attached to long polyglutamyl conjugates. Work carried out in this laboratory has led to the development of new methodology which has shown that in adult mammalian cells the folate cofactors present are conjugated to chains of five and six glutamyl residues. One of the basic unanswered questions in the area of folate metabolism is why the cell makes polyglutamates since all folate entering the cell has only one glutamyl residue. It is clear from mutant studies that inability to carry out this process is incompatible with cell survival. We consider one of two possible explanations to be likely – either the formation of conjugates is necessary for retention and storage within the cell or conjugates of different chain length are required for different activities. Some work on the former concept has been done in this laboratory and it seems appropriate to investigate the latter concept simultaneously. The approach is to see if differences in the degree of polyglutamyl conjugation exists during the very different cellular metabolic states found in a developing animal. The experimental approach is to examine folate polyglutamate chain length in the liver of developing chick from as early as possible to maturity. It was first necessary to obtain a sufficient sample of liver containing enough radioactivity for analysis. This has been concluded and we have found it possible to use chicks as early as one week
old. The next objective was to decide upon the optimum dose of exogenous radioactive folate. The difficulty is that during development the size of the developing liver increases by a factor of 60. We have now determined a ratio of administered dose to expected liver size which while it gives good uptake does not oversaturate the ability of the cells to transport the administered folate. Work has now begun on determining the actual chain length of the liver folate from one week to full maturity at 14 weeks.

Honor Smyth, BSc., MSc., PhD., in collaboration with Antonia Corrigan (Dip. Appl. Biology), Declan Farrell, BSc., PhD. (Supported by Irish Cancer Society).

Department of Biochemistry, University College, Dublin.

Effects of enzymatic modification of the Tumour Cell surface.

Effects of Vibrio cholerae neuraminidase (VCN):

The objective is to determine why tumour immunogenicity is increased by exposure of ascites cells to VCN (which liberates the terminal sialic acid from surface glycoprotein) before inoculation into normally susceptible mice. Among various possible explanations are unmasking of antigens due to removal of sialic acid, or perhaps more efficient immunological processing due to a decreased cell surface negativity. Humoral and/or cellular immunity could be involved.

Four closely related rabbits were maintained for production of antisera against (A) intact, untreated ascites tumour cells and (B) VCN-treated (sialic acid depleted) tumour. The antisera were used for in vitro testing of agglutination and complement-mediated lysis of intact and VCN treated ascites cells.

VCN did not alter the agglutinability of ascites cells with antisera (A) or (B). Antiserum (A) against control cells gave a cytotoxic titre of 1/128 with both intact and VCN-treated cells. Antiserum (B) was much more potent as it gave a cytotoxic titre of 1/1024 with both intact and VCN-treated cells, i.e. an eight-fold increase in activity over the antiserum to cells whose surface sialic acid had not been removed. It was interesting to find that antiserum against VCN-treated cells was equally effective in lysing the untreated target cells. This is relevant to the earlier findings of other authors who showed that mice which had rejected VCN-treated cells could subsequently reject challenge with untreated tumour. The possibility that this approach might be useful in immunotherapy of human leukaemia is being followed up.

Our results suggest that the removal of surface sialic acid may facilitate detection of antigen by the antibody-forming cells. A series of repeat experiments are in progress, together with attempts to determine whether histocompatibility or tumour-specific antigens are involved, by allowing the antisera to react with various concentrations of normal mouse cells.
Carmel Spellman, BSc.

Department of Biochemistry, University College, Galway.

Director: Professor E. O'Dwyer, Department of Obstetrics and Gynaecology, University College, Galway and Regional Hospital Galway.

Enzymes Involved in Carbohydrate Metabolism In Human Endometrium.

The purpose of the present study was to compare the level of Lactate Dehydrogenase (LDH) in normal endometrium with the level in hyperplastic endometrium and carcinoma of the endometrium. Previous studies from this laboratory involving 208 women have established normal values for a number of enzymes of carbohydrate metabolism in endometrium. These studies have also shown that interesting alterations occur in the isoenzymic composition of human endometrium following the change from proliferative to secretory phase. For instance, in normal endometrium during the proliferative phase the % of LDH-M sub-units was 11%. This increased to 32% during the secretory phase. In some human cancer tissues there is usually an increase in total LDH levels compared with the tissue of origin and a concomitant increase in the % of LDH-M sub-units.

The levels of LDH were very high in endometrium from 43 cases of endometrial hyperplasia, the mean level was over 4 fold greater than values previously cited for control biopsies in the proliferative phase. The levels of LDH were higher still in the six cases of carcinoma of the endometrium examined to date. The values of LDH-M sub-units in the hyperplastic endometrium were 5 to 6 fold higher than normal; likewise in the cases of carcinoma of the endometrium very high levels of LDH-M sub-units were encountered. Relatively high LDH values and the predominance of LDH-M sub-units are characteristic of many tumours. These studies are continuing. Because of the possible diagnostic implications of this study, efforts are now being made to measure total LDH and the percentage of M type sub-units in plasma from patients with hyperplastic or carcinoma of endometrium.

CLINICAL MEDICINE

Edmund Bourke, MD., MRCPI., FRCP. (Edin.).

T.C.D. Department of Clinical Medicine, Meath Hospital, Dublin.

Studies in Metabolic Acidosis.

In rats, on a constant nitrogen intake HCl acidosis reduced urea excretion with an equimolar increase in NH₃ excretion and no change in their sum. By contrast, in the guinea pig, where ammonium excretion is small, HCl administration did not effect urea excretion. Ammonium
administered as \( \text{NH}_4 \text{ HCO}_3 \) was excreted mainly in the form of urinary urea, whereas that administered as \( \text{NH}_4 \text{Cl} \) was excreted mainly in the form of \( \text{NH}_4^+ \). Methionine sulfoximine did not impair the increased ammonium excretion induced by acidosis, but did lead to a marked decrease in plasma glutamine levels and an overall increase in total urinary urea and ammonium nitrogen excretion. The data are compatible with a new interpretation of the adaptations in nitrogen metabolism which result from metabolic acidosis. Thus when amino nitrogen is converted to urea, two bicarbonate ions are lost, but two new bicarbonate ions are also formed from the resultant carbon skeleton. Acidosis reduces this hepatic mechanism of disposal of waste nitrogen. Instead the renal extraction and metabolism of glutamine increases. The amino nitrogen is eliminated as \( \text{NH}_4^+ \) by a mechanism which does not utilise bicarbonate ions. Nonetheless the carbon skeleton remains and ultimately generates bicarbonate. The net effect of the altered mechanism of waste nitrogen excretion is a gain in bicarbonate to counteract the acidosis.

Michael J Cullen, MB., BCh.

Department of Clinical Medicine, Trinity College and Meath Hospital, Dublin.

Studies of the Metabolism of Thyroid Hormones.

The main work of the year concentrated on further documentation of effects of hypothyroidism and thyrotoxicosis on the relative impact and sources of the two thyroid hormones thyroxine (\( T_4 \)) and triiodothyronine (\( T_3 \)). The latter is metabolically more potent and known to be secreted by the thyroid but in addition is formed outside the thyroid during the peripheral metabolism of thyroid hormones.

The study is designed to measure the \textit{in vivo} metabolism of \( T_4 \) and \( T_3 \) using a double isotope technique for the kinetics of peripheral metabolism of the hormones and the conversion of \( T_4 \) to \( T_3 \). An important additional feature is the direct measurement of \textit{in vitro} conversion of \( T_4 \) to \( T_3 \) using a third isotope \( ^{14}\text{C}-\text{labelled } T_4 \) in each sample studies.

In \textit{vitro} conversion of \( ^{14}\text{C}-T_4 \) to \( ^{14}\text{C}-T_3 \) was measured in 42 experiments and can be expressed as the ratio at equilibrium of \( ^{14}\text{C}-T_3 / ^{14}\text{C}-T_4 : = 0.56 \pm 0.08 \) (mean \( \pm \) S.E.). Appropriate corrections were made in the \textit{in vivo} conversion estimations.

The studies in \textit{vivo} have been completed in 20 experiments involving 14 patients (2 normal volunteers, 6 thyrotoxic patients and 6 hypothyroid patients). Studies in 4 patients have been completed in the euthyroid state following correction of their hormone imbalance. Thyrotoxic subjects converted 50.9% of \( T_4 \) degraded per day to \( T_3 \). This represents 73% of daily \( T_3 \) production from extrathyroidal sources (i.e. \( T_4 \)). The equilibrium serum ratio of \( ^{125}\text{I}-T_3 / ^{125}\text{I}-T_4 \) was 1.78%. These values are in
excess of normal controls and in one patient have been shown to reduce to 42% $T_4$ to $T_3$ conversion per day and 1.2% $^{125}$I-$T_3$/$^{125}$I-$T_4$ respectively. Hypothyroid patients though producing much less $T_4$ daily nevertheless converted 61.3% to $T_3$ daily. This was seen in 3 patients to reduce to 43% with therapy of $L$-thyroxine 150 ug per day. The equilibrium ratio in hypothyroid patients was 3.56% and returned to 1.4% on the above therapy.

In our laboratory some 3,500 estimates of $T_3$ by radioimmunoassay have been performed. The normal range is 98–195 nanograms per 100 ml (mean 153). Thyrotoxic subjects had $T_3$ levels of $609 \pm 114$ ng/per 100 ml. Hypothyroid patients levels were $40 \pm 24$ ng per 100 ml.

**Conclusions:** The combination of isotopic and stable hormone measurements cited supra show that substantial proportions of $T_3$ produced daily in thyrotoxicosis and hypothyroidism derive from $T_4$ by peripheral conversion. This phenomenon contributes to the pathophysiology of these conditions in an important way in view of the higher metabolic impact of $T_3$.

James G. Devlin, MD., MSc., FRCPI., in collaboration with V. Parameswaran, MSc. and Mary Ryan.

Endocrine/Metabolic Laboratory, St. Laurence's Hospital, Dublin.

1. **Insulin Immunology.** 2. **Insulin Insensitivity.**

**Insulin Immunology:**

A further twelve months has been added to the study of the immune response to highly purified Mono Component Porcine Insulin in the treatment of diabetes mellitus. Confirmation has been obtained that this preparation is poorly immunogenic in man in this country. Less than 20% of approximately 50 patients who have been studied have developed a detectable immune response and in none of these patients was the immune response sufficient to influence insulin resistance. There was no evidence of local allergy or hypersensitivity. A study of the kinetics of the antibody in those patients in whom it developed is under-way and attempts are being made to ascertain whether a cellular immune response can be demonstrated.

All patients were tissue typed with a panel of antisera using the Terasaki micro cytotoxicity technique. There was no obvious difference in HL-A frequency distribution in the patients studied and in a control population of over 100 patients nor was there any difference in HL-A frequency distribution between those patients who developed an immune response and in those in whom such a response was not obtained.
Insulin Insensitivity:

The above study has demonstrated the existence of two populations of patients receiving Mono Component Insulin in whom no antibody developed which is determined by the amount of insulin they require. A study therefore has been commenced to ascertain whether there is a demonstrable resistance factor in those patients requiring higher than a physiological quantity of exogenous insulin in the absence of an immune response. A preliminary study of methods of assessing insulin sensitivity using the procedure of insulin injection using Actrapid. 1 unit per kilogram of body weight has demonstrated a relationship between the delta fall in glucose as related to the fasting blood sugar and insulin levels. The procedure is still being investigated to obtain a technique which will not result in insulin levels above a maximum physiological levels i.e. in the region of 120 – 150 µU/ml. In this preliminary study approximately 20 subjects have been investigated. In addition results have been obtained in patients with gross obesity and in patients with pituitary insufficiency following partial hypophysectomy.

Conleth Feighery, MB., MRCPI.

Sir Patrick Dun’s Hospital and Trinity College, Dublin.

Directors: Prof. D. G. Weir, MD., FRCPI. and J. F. Greally, MB., MScPath.

A study of humoral and cell mediated immunity in the pathogenesis of auto-immune liver disease.

The primary objective of isolating a liver specific protein (LSP) has now been achieved. This was carried out in the same manner as other workers but modifications of their methods have been introduced. Obtaining fresh post-mortem liver proved initially difficult. When suitable liver was obtained this was homogenised and then ultracentrifuged. The original author used a two step gel filtration technique, with Sephadex G 100 and Sephadex G 200. It has now been found possible to forego the G 100 column step. Instead of Sephadex G 200 which can prove difficult to use, Sepharose 6B has been substituted.

The liver specific protein is contained in the first protein peak eluted from the 6B column. Antisera to this protein antigen was kindly donated by Dr. Roger Williams, King’s College Hospital, London. Using the Ouchterlony technique a single precipitin line formed between this antisera and the liver protein. This confirmed the antigenicity of this protein to be the same as that isolated by the other workers. A more sensitive countercurrent immuno-electrophoresis technique for the detection of this reaction has now also been developed. This can now be used as a sensitive method for detecting antibodies to liver specific protein in patients with auto-immune liver disease.
Antisera to LSP has now been successfully raised in rabbits. This antisera gives precipitin lines identical to the donated antisera. One rabbit died during the immunisation programme. Histological examination showed extensive liver damage.

Lymphocytes, donated by patients with auto-immune liver disease, will be set up in culture in the presence of LSP. The perfection of this technique is at an advanced stage. The antigen has proved relatively unstable when not suspended in the eluting tris buffer. The current difficulty lies in the fact that this buffer is incompatible with the lymphocyte culture medium.

John R. N. Flynn and Sean F. O'Beirn

Department of Surgery, University College, Galway.

The Potato as a Source of Dietary Fibre.

The present study investigated the use of the potato as a source of dietary fibre in normal subjects. Dietary fibre, stool weights, bowel transit times, colorectal pressures, body weight and blood lipids were studied on subjects before commencing on a potato intake of up to 1 Kg. daily and later after a minimum of eight weeks on this diet these studies were repeated. Subjects were divided into two groups on the basis of their pre-diet fibre intake. Group I of whom there were five subjects were consuming over 5.0 Gm. daily whilst Group II in which there were eighteen consumed less than 5.0 Gm. of fibre.

Results:

Stool Weights: Twenty-four hour stool weights showed significant changes in Group I and II. Group I 149 Gm. to 249 Gm. (P < 0.025) Group II 125 Gm. to 249 Gm. (P < 0.005).

Bowel Transit Times: measured by radiopaque pellets. Group I showed a shortened transit time 46 to 33 hours (P < 0.25). Group II showed significant shortening of transit 62 to 35 hours (P < 0.01).

Colorectal Pressures: Measured using open tipped catheters attached to Statham transducers.

Group I showed no significant changes in any of the pressure parameters. Group II showed a significant drop in mean peak heights, 35 cm H2O to 21 cm H2O (P < 0.0025). Percentage of events over 50 cm H2O, 23 down to 6 (P < 0.0005) and motility Index, 3133 down to 1686 (P < 0.005). There were no significant changes in the number of complete waves or the percentage activity.

Total Body Weights: There were no significant changes, though 6 showed no change, four gained weight and thirteen lost weight.
Blood Lipids: Cholesterol, Lipoproteins and Triglycerides were studied. There were no significant changes.

Conclusions: Potato is an acceptable source of dietary fibre. Increased potato intake in subjects with a low fibre intake will cause:
1. Increased stool weights.
2. Decreased bowel transit time.
3. Lower Colorectal pressures.

Professor P. B. B. Gatenby, MD., FRCPI., FRCP. (Lond.), in collaboration with Professor E. Bourke, MD., MRCPI., FRCP. (Edin.), and Peter Brown, BSc.

Department of Clinical Medicine, Meath Hospital, Dublin.

Measurement of Erythropoietin Excretion in Haematological Disease.

The bioassay for erythropoietin recently established in our laboratory has been further developed and improved. A standard preparation of erythropoietin, prepared in our laboratory and calibrated against the International Reference Preparation (I.R.P.) of erythropoietin, is now included in each assay at three dose levels with a log interval of four. A log-dose response regression is obtained which is linear in the range 0.05 to 0.80 units, and the slope of this regression has remained constant from assay to assay (11.75 ± 2.34). The normal range of erythropoietin excretion is significantly greater in males (1.38 – 5.00 units/day) than in females (0.76 – 1.54 units/day) (p < 0.01). These findings are in agreement with the recent literature. Erythropoietin excretion was invariably decreased to undetectable or barely detectable levels in patients of both sexes with polycythaemia vera, whereas markedly elevated levels were found in haemolytic anaemia, sideroblastic anaemia and the anaemia of leukaemia, as well as in aplastic anaemia where erythropoietin excretion ranged from 45 to 163 units/day. The erythropoietin levels found in secondary polycythaemia, although elevated, were less than expected (2 to 7 units/day). A possible explanation for this is the stabilisation of erythropoiesis at a higher level under hypoxic conditions. We have noted one such situation where a patient with elevated erythropoietin early in the course of secondary polycythaemia was found to be within the normal range one year later. Inappropriately low erythropoietin excretion has also been found in lymphosarcoma with anaemia. Further studies in both these conditions are in progress. In one patient with haemolytic anaemia, 24 hour erythropoietin excretion fell from 13.55 units to zero after the patient had undergone splenectomy. Further research into post-splenectomy erythropoietin levels is in progress.

44
The isolation of the glycocalyx of human small intestinal cells and the investigation of its composition and properties.

The objective of this project was to study the adenosine triphosphatase (ATPase) enzyme system in human small intestinal mucosa, with particular reference to (a) subcellular localisation (b) separation and purification and (c) characterisation. Most of the work on ATPase has been done on animal or bacterial tissue. The study of this enzyme in human intestinal epithelial cells is particularly interesting, as it could also function in these cells in the transport of organic compounds, such as sugars and amino acids.

Human intestinal specimens were obtained from patients undergoing surgical resection of the small bowel.

Brush-border fractions were prepared, and identified by phase contrast microscopy and marker enzymes. The fraction of the cell which did not contain brush-borders was subfractionated into a soluble fraction (containing mitochondrial protein) and a particulate fraction which contained lateral membranes and microsomes. ATPase activity was assayed in all fractions.

The results of this study showed that the human small intestinal mucosa contained multiple ATPase systems, i.e. Mg$^{2+}$ activated ATPase, Na$^+$/K$^+$ stimulated ATPase, and Ca$^{2+}$ stimulated ATPase. The distribution and subcellular localisation of these enzymes are shown in Table 1.

The ATPase enzyme systems were successfully purified and separated, using Sephadex G-100. The purified enzymes were subsequently characterised, and molecular weights, Km values, substrate specificities, pH optima, and reactions to inhibitors were examined. A summary of the enzymatic properties is shown in Table 2. Results showed that two distinct types of Na$^+$/K$^+$ ATPase exist in the human enterocyte. The stability of these two enzymes was studied at 0-4°C. The addition of 250mM Sucrose to the mitochondrial Na$^+$/K$^+$ ATPase prevented ageing of the enzyme.

This study has demonstrated that the Na$^+$/K$^+$ ATPases in the human intestinal mucosa differ in several ways from similar ATPase system in animal intestinal epithelial cells, e.g. localisation within the cell, occurrence of two forms of this enzyme in the enterocyte, and reaction to inhibitors.

We have also demonstrated the occurrence of a Ca$^{2+}$ stimulated ATPase in the lateral membranes, an enzyme which has not previously been reported in intestinal epithelium.
### Table I

Distribution of ATPase activity.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Mg²⁺ ATPase</th>
<th>Na⁺⁺K⁺ ATPase</th>
<th>Ca²⁺ ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total homogenate</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Brush borders</td>
<td>80</td>
<td>45</td>
<td>0.0</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>10</td>
<td>55</td>
<td>0.0</td>
</tr>
<tr>
<td>Lateral membranes</td>
<td>10</td>
<td>0.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### Table II

Properties of purified ATPases

<table>
<thead>
<tr>
<th>Properties</th>
<th>Mg²⁺ ATPase</th>
<th>Na⁺⁺K⁺ (B/B)</th>
<th>Na⁺⁺K⁺ (M)</th>
<th>Ca²⁺ ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>ph optimum</td>
<td>8.0 - 8.5</td>
<td>8.0</td>
<td>6.5</td>
<td>7.4</td>
</tr>
<tr>
<td>% Inhibition by Oligomycin</td>
<td>0.0</td>
<td>10</td>
<td>20</td>
<td>0.0</td>
</tr>
<tr>
<td>Diethylmaleimide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substrate specificity</td>
<td>ATP, GTP, CTP</td>
<td>ATP</td>
<td>ATP, ATP, GTP, CTP</td>
<td></td>
</tr>
</tbody>
</table>

M – Mitochondrial B/B – Brush borders, Conc. of inhibitors: 1mM.

Risteard Mulcahy, MD., FRCP., FRCPI., in collaboration with Dr. Ian Graham, MB., MRCP., Miss Chriss O'Doherty, SRN., Mrs. Vivien Reid, Dip.Diet.

Cardiac Department, St. Vincent's Hospital, Dublin.

(1) *The secondary prevention of coronary heart disease*; (2) *Dietary control of the hyperlipidaemias*.

An enquiry into determinants of prognosis in patients with established coronary heart disease continued during 1974. Five hundred and forty-two male and one hundred and thirty-seven female patients under 60 years with first myocardial infarction have been followed for periods of up to 13 years. Two hundred and fifty-two males have been followed for 4 years or more and an analysis of their survival experience has been reported (British Cardiac Society, 1974). Prognosis was related to initial and follow-up characteristics. Of eleven initial characteristics studied only the severity of the initial attack bore any significant relationship to long-term prognosis. Complicated cases, such as those with left ventricular failure, have a significantly worse survival experience.
As regards follow-up characteristics, patients who continued to smoke heavily had a significantly worse prognosis compared to those who stopped or who reduced substantially or who had not smoked at the time of the attack. Blood cholesterol levels were lower amongst survivors although the difference did not reach the level of significance. The blood pressure figures of non-survivors was significantly lower than those of survivors at the last follow-up examination. Survivors and non-survivors could not be distinguished by any other follow-up characteristics.

This work continues and it is hoped that, with more sophisticated and newer statistical techniques, the entire cohort can be studied over variable periods of follow-up. We continue the study of the statistical techniques of survivorship in association with Gilbert McKenzie of The Department of Social and Preventive Medicine, The Queen’s University, Belfast.

Our study on the effect of long-term dietary measures on the various hyperlipidaemias continues under the direction of Mrs. Vivien Reid. A preliminary report on this work is in preparation for publication.

Professor F. P. Muldowney, MD., FRCP. (Ed.).

Metabolic Unit, St. Vincent’s Hospital, Department of Medicine, University College, Dublin.

Parathyroid Hormone Excess.

The objectives of our work were to define the relative roles of parathyroid hormone (PTH) and/or 25, OH; cholecalciferol (Vit. D) in the abnormal phosphaturia and bicarbonaturia of primary and secondary hyperparathyroidism.

The original design required modification because financial restrictions did not permit the immediate acquisition of personnel and equipment necessary for Vit. D assay. Accordingly, our efforts were directed towards defining urinary and serum ionised calcium and P.T.H. levels in kidney stone subjects with ‘idiopathic hypercalciuria’ (I.H.).

Twenty-five I.H. subjects were studied and compared with twenty-seven primary hyperparathyroid subjects. It was found that both groups were essentially similar, suggesting that I.H. is commonly a mild or early ‘normocalcaemic’ variant of primary hyperparathyroidism. Measurements of serum ionised Ca and PTH were crucial pointers, and provided a diagnostic combination not previously defined in the world literature. Ten of eleven subjects submitted for neck exploration to date have been shown to have a parathyroid adenoma.

Conclusions: Recurrent renal stone formation with high urine Ca is commonly due to masked primary hyperparathyroidism. Combined ionised Ca and PTH measurements are important diagnostic markers of this condition.
That Parathyroid Hormone (P.T.H.) influences acid-base balance by altering renal tubular bicarbonate reabsorption.

Objective: In the previous grant period we had studied the effect of P.T.H. suppression on renal bicarbonate excretion and had found that in primary hyperparathyroidism and in secondary hyperparathyroidism of intestinal malabsorption, urinary bicarbonate excretion decreased during calcium infusions. During this period the effect of P.T.H. suppression in uraemic subjects was studied.

Methods: 4 studies were performed in 3 subjects. Each subject had a G.F.R. of 10 mls/min. and was clinically stable. In order that changes in urinary bicarbonate might be better seen each subject ingested a constant daily amount of NaHCO₃ sufficient to maintain a normal plasma bicarbonate.

Each study consisted of 3 consecutive 24 hour periods, of which the first was the control, the second was the infusion period and observations continued for the following 24 hours. Calcium was infused at doses from 15 mg/Kg to 25 mg/Kg over periods of between 4 and 7 hours.

Results: In one of the studies the serum calcium did not rise high enough to provide adequate suppression of P.T.H. and this study is discarded. Two of the remaining studies showed a similar response during which serum P.T.H. levels fell significantly 6 – 12 hours after serum calcium reached its maximum levels, remained suppressed for another 6 – 12 hours and returned to control levels midway during the 3rd day. Urine bicarbonate excretion was diminished over the same period and regained preinfusion levels also on the 3rd day. In the remaining study no change in urine bicarbonate excretion was observed despite adequate serum calcium levels. Serum P.T.H. values for this study are awaited.

Conclusions: These studies provide evidence that in uraemia renal tubular reabsorption of bicarbonate is depressed by high circulating levels of P.T.H. and combined with our previous studies provide an explanation for the metabolic acidosis of hyperparathyroidism.
Regulation of Polysaccharide Digestion.

A study was made of methods of labelling α-amylase. The usual method of iodine labelling – the chloramine-T method – was shown to destroy enzyme activity. One mg of chloramine-T per mg of enzyme destroyed 60% of the catalytic activity within 10 seconds. Further studies were made on the solid-state lactoperoxidase method. It was found that the catalytic activity of α-amylase labelled by this method was dependent on the iodide/amylase (I/A) molar ratio e.g. ratios of 0.8 and 3.3 reduced the enzyme activity to 75% and 32.5% respectively. Full catalytic activity was preserved when I/A ratios less than 0.6 were used. This 1261-labelled pancreatic amylase behaved in a similar manner to unlabelled enzyme with respect to (a) its ability to combine with substrate, and (b) its rate of disappearance from the plasma when injected intravenously to dogs.

This iodinated amylase was used to study the theory of pancreatic amylase adsorption to intestinal mucosa. These studies were performed in vitro using canine and porcine jejunal strips. Very little (<5%) adsorption was shown to occur and the results do not support the adsorption theory of Ugolev.

The primary bile acid chenodeoxycholic acid (CDCA) was shown to inhibit α-amylase hydrolysis of both soluble and insoluble starch. Line-weaver-Burk plots demonstrated that competitive inhibition was predominant with both substrates. Ki values were 0.15 mmol/l (insoluble starch) and 0.26 mmol/l (soluble starch). These low values suggest that CDCA could be a depressor of α-amylase action in vivo since CDCA concentration in intestinal contents in man is in the region of 2 mmol/l. Naturally occurring inhibitors of salivary and pancreatic amylase are also under investigation in order to elucidate their role in the regulation of carbohydrate digestion.

A long-term feeding study is in progress of the effects of a CDCA diet on the enzyme composition of mouse pancreas and intestine.

Dr. M. J. Whelton, BSc., MD., FRCPI, MRCP. (London) in collaboration with Claire O'Leary, BSc.

St. Finbarr's Hospital, Cork.

Endogenous Faecal Calcium.

Excessive endogenous faecal calcium (E.F.C.) loss may be an important route for loss of body calcium. Such loss, if excessive, may be clinically
significant in patients with liver disease or malabsorption states. In the present study, the patient is given 20/UCi. $^{47}$Ca. (½ life 4.6 days) intravenously in normal saline. 24 hour urine and faecal collections are commenced at the time of the injection and these continue for seven and eight days respectively. The daily urinary calcium is measured by titration with E.D.T.A. The daily urinary $^{47}$Ca. and faecal $^{47}$Ca. are counted and the percentage of the dose calculated.

Endogenous faecal calcium is then calculated as follows:

$$E.F.C. = \frac{\text{Faecal }^{47}\text{Ca}}{\text{Urinary }^{47}\text{Ca} + \text{urinary calcium}}$$

The results of nine studies are summarised in the table. Five results are outside the upper limits of normal (1 – 3 mg/kg/day) as defined by Nordin and Smith (1965). Three of these had liver disease and in one the level of E.F.C. was very strikingly elevated. All three had evidence of cholestasis at the time of the study. The two other abnormal results were in a patient with coeliac syndrome and one with Sarcoidosis.

These findings confirm our previous suggestion that E.F.C. may be elevated in some patients with liver disease. In such patients it may well be a significant route of calcium loss leading ultimately to bone thinning and fracture. (See table on opposite page)

**CLINICAL SURGERY**

W. G. Fegan, FRCSI., in collaboration with Mary Henry, R. D. Quill, Judy Myles, Gary Brow, P. J. Byrne.

Clinical Research Department, Sir Patrick Dun’s Hospital, Dublin.

*Aspects of venous insufficiency and the aetiology of varicose veins.*

More progress has been made in the investigation of deep vein thrombosis and superficial phlebitis during pregnancy and in the puerperium. Figures extracted from the Maternal Mortality Reports clearly show the relationship between increased age, high parity and death due to pulmonary embolism.

The project to screen patients in the Rotunda Hospital using strain gauge plethysmography in cases of suspected deep vein thrombosis is under way. Data on these patients is being collected to see if these are in fact mainly high risk cases. The Doppler is also being used to screen the patients and the results of the Doppler and strain gauge will be correlated. Arrangements have been made to do ascending phlebography when necessary. From this study we intend to investigate the value of prophylactic anti coagulation in high risk cases at delivery or during prolonged bed rest.
## Endogenous Faecal Calcium

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Initial</th>
<th>N.O.S.</th>
<th>Age</th>
<th>Weight</th>
<th>Diagnosis</th>
<th>Stool Mean Daily Ca (mg/kg/day)</th>
<th>Urine Mean Daily Ca (mg/kg/day)</th>
<th>Mean 24 hr. urine specific activity Ca (mg/kg)</th>
<th>Mean Faecal Calcium Mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>J.P.</td>
<td>61</td>
<td>61</td>
<td>46.6 kg.</td>
<td>Active Cirrhosis</td>
<td>2.6129</td>
<td>1.3175</td>
<td>147.1113</td>
<td>590.1985</td>
</tr>
<tr>
<td>2</td>
<td>N.O.S.</td>
<td>58</td>
<td>58</td>
<td>26.4 kg.</td>
<td>Large duct biliary obstruction</td>
<td>1.0462</td>
<td>1.0950</td>
<td>147.1113</td>
<td>590.1985</td>
</tr>
<tr>
<td>3</td>
<td>J.K.</td>
<td>57</td>
<td>57</td>
<td>82.8 kg.</td>
<td>Non functioning Gall-Bladder</td>
<td>2.1066</td>
<td>2.6733</td>
<td>147.1113</td>
<td>590.1985</td>
</tr>
<tr>
<td>4</td>
<td>D.C.</td>
<td>70</td>
<td>70</td>
<td>70.0 kg.</td>
<td>Infective Hepatitis</td>
<td>1.7562</td>
<td>2.0666</td>
<td>147.1113</td>
<td>590.1985</td>
</tr>
<tr>
<td>5</td>
<td>D.O.L.</td>
<td>48</td>
<td>48</td>
<td>101.3 kg.</td>
<td>Large duct biliary obstruction</td>
<td>2.6801</td>
<td>1.3598</td>
<td>147.1113</td>
<td>590.1985</td>
</tr>
<tr>
<td>6</td>
<td>T.W.</td>
<td>58</td>
<td>58</td>
<td>53.2 kg.</td>
<td>Large duct biliary obstruction</td>
<td>2.6335</td>
<td>2.1971</td>
<td>147.1113</td>
<td>590.1985</td>
</tr>
<tr>
<td>7</td>
<td>P.H.</td>
<td>57</td>
<td>57</td>
<td>70.0 kg.</td>
<td>Sarcoidosis</td>
<td>1.7606</td>
<td>0.8403</td>
<td>147.1113</td>
<td>590.1985</td>
</tr>
<tr>
<td>8</td>
<td>P.McD.</td>
<td>70</td>
<td>70</td>
<td>58.6 kg.</td>
<td>Coeliac Syndrome</td>
<td>0.5685</td>
<td>0.4258</td>
<td>147.1113</td>
<td>590.1985</td>
</tr>
<tr>
<td>9</td>
<td>T.W.</td>
<td>54</td>
<td>54</td>
<td>46.8 kg.</td>
<td>Herpes Zoster</td>
<td>1.3679</td>
<td>1.3567</td>
<td>147.1113</td>
<td>590.1985</td>
</tr>
</tbody>
</table>
Skin Blood Flow in Crural Areas: This work has been continued and 30 limbs with evidence of venous insufficiency have now been studied. There was considerable variation in the skin blood flow and no significant difference between the whole group of abnormal limbs and normal limbs. However, in patients with venous insufficiency or lymphoedema complicated by cellulitis skin blood flows were markedly increased.

Venous Calf Pump Function: Further studies have been made using strain gauge venous occlusion plethysmography to measure the volume of blood expelled from the calf during plantar flexion. A device has been developed to control accurately both the range of and resistance to plantar flexion. In normal subjects, when resistance is constant there is a significant correlation between the volume of blood expelled from the calf and the permitted range of plantar flexion. Further studies on patients with venous insufficiency are in progress.

The range of ankle movement (flexion extension) has been measured in 112 limbs with venous insufficiency and 50 normal controls. There is a progressive reduction in the range of movement with the increasing age in both normals and abnormals. There was a significant reduction in range of movement compared with normals matched for age in limbs with varicose ulcers. In limbs which had been ulcerated in the past, but not at the time of examination the range of movement was reduced compared with normals, but not significantly so.

Venous Auto Transplants in Dogs: 7 auto transplants from jugular or opposite femoral to femoral vein have been performed. Immediate post operative venography reveals stenosis in two cases and thrombosis was present in one of these. The remainder were patent post-operatively. All grafts subsequently became thrombosed within 3 – 12 days. Subcutaneous heparin was administered to four animals and did not prevent thrombosis. Further studies to evaluate other methods of avoiding thrombosis following vein auto transplants are in progress.

Judith Sally Myles, MB., BAO., BA., in collaboration with Dr. Mary Henry, Mr. Robert Quill and Mr. P. J. Byrne.

Clinical Research Department, Sir Patrick Dun’s Hospital, Dublin.

Director: W. G. Fegan, MCh., FRCSI.

Distensibility changes, hormonal levels and flow patterns during the menstrual cycle and on oral contraceptive therapy.

The venous volume at pressures of 60, 40 and 20 mm. Hg. were studied on 3 occasions during the menstrual cycle in 12 normal females and used as a reflection of venous distensibility. All subjects were asymptomatic and had no palpable varicosities. The calf distensibility was studied using mercury-in-rubber strain gauge plethysmography. Using this technique
venous filling, venous emptying and calf volumes at 60, 40 and 20 mm. Hg. (with proximal thigh cuff inflation) were recorded. The leg volumes were measured using water displacement, by a simple application of Archimedes Principle, in an effort to pin-point ovulation. Each patient was screened with diagnostic ultrasound, using the Parks 806 Directional Doppler, for the presence or absence of reverse flow at the medial condyle. If reverse flow was present the Doppler Audio output was recorded for sonogram analysis to detect turbulence. 10 cc. of blood was taken, immediately spun down and the serum frozen for oestradiol and progesterone assay in the future.

The same protocol has been expanded to patients on oral contraceptive therapy. It was found, whilst conducting this study, that normal people appear to have transient reverse flow and therefore, a large number of normal asymptomatic patients are being screened for reverse flow. This is being done at a random point in their menstrual cycle again using diagnostic ultrasound. So far 38 females have been tested and the total number is to be 200 in all.

Findings (Table I)

No significant difference in calf distensibility, (at proximal thigh cuff pressures of 60, 40 and 20 mm. Hg.) at menstruation, ovulation, or the random readings was found.

This difference from previous publications on this subject may be attributable to the small number already studied and therefore, a much larger number of females are to be studied in this series.

Table II

Reverse flow: It is concluded from these preliminary results that the distribution of incidence of the reverse flow follows a pattern during the menstrual cycle. It is expected to find a different distribution in those patients on oral contraceptives and if so the changes may well be attributable to hormonal differences at various points in the cycle.

It has also been noted that many of the patients coming to the varicose vein clinic complain primarily of premenstrual pain in the veins. It is therefore proposed to screen these patients for reverse flow to find out if this symptomatology is attributable to transient or permanent incompetence.
Table I

<table>
<thead>
<tr>
<th></th>
<th>Menstruation</th>
<th>Ovulation</th>
<th>Random</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Venous Outflow</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>45.6</td>
<td>55.0</td>
<td>40.0</td>
</tr>
<tr>
<td>S.D.</td>
<td>±16.6</td>
<td>±15.6</td>
<td>±12.46</td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td><strong>Arterial Inflow</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.38</td>
<td>3.52</td>
<td>2.24</td>
</tr>
<tr>
<td>S.D.</td>
<td>±1.4</td>
<td>±3.2</td>
<td>±1.14</td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

Venous Volume (Mls)

<table>
<thead>
<tr>
<th></th>
<th>Menstruation</th>
<th>Ovulation</th>
<th>Random</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximal Thigh Tourniquet Pressure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 mmHg.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.8</td>
<td>2.9</td>
<td>2.8</td>
</tr>
<tr>
<td>S.D.</td>
<td>±0.6</td>
<td>±0.38</td>
<td>±0.47</td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>40 mmHg.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.59</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>S.D.</td>
<td>±0.5</td>
<td>±0.27</td>
<td>±0.49</td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>20 mmHg.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.25</td>
<td>1.3</td>
<td>1.23</td>
</tr>
<tr>
<td>S.D.</td>
<td>±0.45</td>
<td>±0.39</td>
<td>±0.45</td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>11</td>
<td>10</td>
</tr>
</tbody>
</table>

Legend
N = Number of patients studied in each group.
S.D. = Standard Deviation.

There was no significant difference between the 3 stages of the menstrual cycle for any of the parameters studied (using the mercury-in-rubber strain gauge technique).

DENTAL MEDICINE

Diarmaid B. Shanley, BDS., MScD., FDS., RCSEd.

Dublin Dental Hospital and University of Liverpool.

The effect of four implant materials on the induction of bone and cementum in the periodontium.

Periodontal disease causes more tooth loss than all other factors by destroying the supporting tissues of the teeth. The treatment of the condition consists of controlling the aetiological factors, primarily bacterial plaque, and correcting, by surgical methods, the tissue defects produced by the disease process. Increasing emphasis has recently been placed on the use of grafts to help restore the lost alveolar bone. The purpose
Table II

% of Normal Females with Reverse Flow during the Menstrual Cycle
of this study is to investigate the effects of four materials, commonly used as implants in osseous surgery, to determine their effect on bone and cementum induction and also their effect on the wound healing process in the periodontal tissues.

In each maxillary quadrant (2) of six Rhesus Monkeys (12 quadrants), five surgical defects were created (60 sites). One site was used as a control and the following materials were implanted:

(a) Fresh autologous bone (from ileum).
(b) Frozen allogenic bone (from ileum).
(c) Frozen autologous bone (from ileum).
(d) Fresh cortical bone chips.

Monkeys were sacrificed so that the quadrants which were operated yielded twelve periods of wound healing between one day and 240 days. Blocks were decalcified, double embedded in paraffin, sectioned and stained with the haematoxylin and eosin for histological detail. Azure - A was also used to highlight new bone formation.

Results so far indicate that the osteogenic inductive effects of the implant materials were more effective in the connective tissue than in the implantation sites. It also appeared that the cellular elements implanted with the graft did not differentiate into osteoblasts or cementoblasts.

The variations in the inductive potentials of these materials were not as apparent here, as in those studies on the abdominal walls of small laboratory animals. The reported effects on inhibition of cementogenesis were not as consistently found as other reports have suggested. The study is progressing.

EXPERIMENTAL MEDICINE

W. A. Boggust, MA., PhD., MSc. (Dunelm), in collaboration with the late Professor R. A. Q. O'Meara, MD., ScD., FRCPI., SFTCD., L. J. Peters, MD. and Hilda Gorry, BSc.

Department of Experimental Medicine, Trinity College and Saint Luke's Hospital, Dublin.

Factors related to tumour invasiveness and phospholipid metabolism in human cancers.

A. The blocking of collagen-degrading activity in extracts of human cancer tissues by various drugs has been shown to involve more than one kind of inhibitory process.
The chelators EDTA and o-phenanthroline are inhibitory and remove Ca$^{2+}$ ions and Fe$^{3+}$ or Mn$^{2+}$ ions respectively, required for activation of protein hydrolases. They differ also in that unlike EDTA, phenanthroline is dependent for its inhibitory effect upon the presence of a dialysable component without which it is inactive.

The anti-metastatic drug ICRF 159, also found to be an inhibitor, is chemically labile under biological conditions and may participate in various forms including the unchanged bis-diketopiperazine and after hydrolysis, the EDTA-type product or its precursor diamide, possibly having competitive inhibitory properties.

Combinations of phenanthroline and ICRF 159 at low concentrations, each separately inducing only minimal inhibition, are synergistic and cause a substantial reduction in enzyme activity in vitro. Experimental studies of metastasis formation in mice inoculated with primary tumour which was later excised, showed that although phenanthroline induced some improvement in the survival rate of animals treated with ICRF 159, phenanthroline alone was ineffective, presumably due to a deficiency of the dialysable tissue factor, whereas ICRF 159 alone was effectively antimetastatic.

Cathepsins from lysosomes and other enzymes degrading tissue matrix components in cancers are being evaluated and some susceptibility to ICRF 159 inhibition has been demonstrated. This further supports the view that a number of enzymic tissue processes may be involved in the release of malignant cells from a tumour into the circulation.

B. HeLa cells are being cultured and preliminary assays have been carried out to establish data for the differential uptake of $^{32}$P orthophosphate into newly synthesised phospholipids for comparison with data obtained from human cancers. These results together with information about peptidases and other hydrolytic enzymes from cells cultured under standardised normal conditions will be compared with those obtained when controlled modifications are introduced into the conditions of growth.

Francis R. Comerford, MD.

Department of Experimental Medicine, University College, Galway.

Director: Professor Sean Lavelle, MD.

An ultrastructural evaluation of the Reactivity of Synovial Membrane.

The objective of this study is to define, at an electron microscopic level, the varieties of morphologic response occuring in synovial membrane following injury. It is anticipated that the results will be relevant to human chronic articular disease. Initial experiments have included the following:
1. **Response of synovial membrane to magnesium Trasilicate.** Rabbit knee joints were injected with 0.5 to 50.0 mgs of sterile magnesium trisilicate. Clinical Arthritis was not noted. Animals were sacrificed at three, ten and fourteen days and two observations were made at 100 days. Changes ranged from focal and marked infiltration to minimal thickening of the membrane and vacuolation of the cells. Complex inclusion resembling myelin bodies were noted within macrophages.

2. **Arthus Reaction in Synovial Membrane.** Rabbits were immunised with horse spleen ferritin in complete Freund's adjuvant. After skin testing at 21 days, intra-articular injections of 2.5. mgs. of sterile ferritin were administered. Animals were sacrificed at three, ten and twenty-one days. Synovial membrane was processed for electron microscopy. Serum was tested for antiferritin antibodies using a semi-quantitative immunodiffusion technique. Early observations showed an intense focal inflammation involving synovial membrane and subsynovial areas. There was a polymorpho-nuclear (PMN) leukocytic infiltration. Aggregates of ferritin were observed within macrophages and in PMN’s. These were often associated with floccular material in membrane-limited inclusions. Necrosis of superficial cells and fibrin deposition were occasionally seen. Later observations showed some thickening of the membrane but little evidence of acute inflammation. Ferritin was occasionally seen within subsynovial macrophages.

These preliminary observations indicate the range of abnormality seen in synovial membrane. Certain insults are well tolerated. A partial resolution of the inflammatory response secondary to the Arthus reaction is seen. The antigen (ferritin) decreases in amount as the process progresses and, at the times of observation, is located primarily within phagocytic cells.

### EXPERIMENTAL SURGERY

W. H. Beesley, MD., MCh., FRCSI. in collaboration with R. Gay, FRCSI.

Department of Experimental Surgery, Trinity College, Dublin.

*An investigation into an operation for the treatment of gastric dumping.*

Using adult mongrel dogs, three animals were given a Poly type partial gastrectomy, with a standard stroma of 2.5 cm, together with an anterior and posterior truncal vagotomy. After a four week recovery period barium meal studies were performed using 100 gm of dog food plus 100 mls 'micropaque' to establish the gastric emptying pattern of these control preparations. Following three satisfactory barium studies, each dog underwent a jejunal circumferential myotomy 2.5 to 5 cm distal to the gastroenteric stroma, as described in our previous communication. After a further four weeks each of these animals then underwent three more barium studies to determine the pattern of gastric emptying. On completion of these studies a second jejunal myectomy was performed distal to the
first one, and further barium studies were carried out. The results suggest that the myectomy again delays gastric emptying, and at the present time these results are being subjected to statistical analysis.

A further two dogs had a partial gastrectomy, anterior and posterior truncal vagotomy, and a jejunal myectomy carried out all at the same operation. These animals had considerable post operative problems with gastric emptying, including much vomiting. It was concluded that in dogs such a procedure is not satisfactory, and it could not be advised in humans.

However, it is likely that a later jejunal myectomy is a safe and satisfactory procedure for delaying gastric emptying in dogs who have had a partial gastrectomy and vagotomy. If this is so, it may have applicability to humans.

T. V. Keaveny, BSc., MCh., FRCSI. & Ed., in collaboration with S. M. K. Cunningham, BSc.

Department of Surgery, University College, Dublin.

Director: Professor P. FitzGerald, MSc., MD., MCh., FRCSI., FACS.

Effect of Allopurinol and other therapies in experimental haemorrhagic shock.

Experimental haemorrhagic shock is associated with a rise in plasma uric acid levels and a breakdown of tissue ATP and other purine nucleotides. To investigate the mechanism of action of allopurinol and the role of the purine loss in irreversible haemorrhagic shock, the effect of shock on ATP, ADP and AMP concentrations in liver, spleen, duodenum and pancreas was studied in mongrel dogs and in dogs pretreated with sodium allopurinol (50 mgs/Kg.).

Haemorrhagic shock was induced in anesthetised dogs by arterial bleeding to 300 mm Hg. The blood was reinfused when 20 per cent of the shed blood had returned spontaneously. Biopsies for determination of adenine nucleotides were removed 3 hours after reinfusion.

Plasma uric acid concentration in the shocked animals rose from 0.21 mgs/100 mls. to 3.95 mgs/100 mls. at the time of reinfusion and decreased thereafter. Allopurinol pretreatment completely eliminated the rise in plasma uric acid.
Liver ATP was reduced in all shocked animals and allopurinol pretreatment did not improve this. Pancreatic ATP and the total of ATP + ADP + AMP were greatly reduced in the shocked animals and were not significantly different from controls in allopurinol pretreated animals. The concentration of ATP and of ATP + ADP + AMP was significantly higher in allopurinol pretreated than in shocked animals. (P < 0.0005 and P < 0.005 respectively).

Plasma B Glucuronidase and acid phosphatase increased throughout the experimental period and there were significant (P < 0.05) differences between shocked and allopurinol pretreated animals 3 hours after reinfusion. At this time the plasma B Glucuronidase in control and allopurinol pretreated animals was 89.3 ± 20.6 and 55.7 ± 9.8 U/ML respectively and the plasma acid phosphatase was 74.3 ± 4.1 and 30.2 ± 10.7 mU/ml respectively.

**Conclusions**: Of the organs tested for their response to pretreatment by allopurinol in haemorrhagic shock the pancreas was shown to be the most responsive. Allopurinol also reduced the release of the potentially harmful lysosomal enzymes.

**James F. O’Leary, MB., BCh., BAO. (N.U.I.).**

University Department of Surgery, St. Finbarr’s Hospital, Cork.

**Director**: Thomas P. J. Hennessy, MCh., FRCS.

*The influence of surgical procedures on the canine gastric pacesetter potential.*

Gastrointestinal motility is controlled by the underlying pacesetter potential or basal electrical rhythm (BER). Since motility disturbances are associated with certain forms of gastric surgery we investigated the effect of three of these procedures on the BER, namely segmental resection (SR), pylorus preserving gastrectomy (PPG) and Billroth I gastrectomy.
Five animals in each group were studied using monopolar stainless steel electrodes implanted on the stomach and duodenum. Recordings were obtained before and after operation and at varying intervals from 5 to 18 weeks.

All animals showed immediate slowing of the gastric and duodenal BER below the anastomosis in both the SR and PPG groups (Table I).

Table I. Mean frequency of antral and duodenal BER before and after resection (cycles/min.).

<table>
<thead>
<tr>
<th></th>
<th>Gastric Frequency</th>
<th></th>
<th>Duodenal Frequency</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-op.</td>
<td>Immed.</td>
<td>5 - 8 weeks</td>
<td>Pre-op.</td>
</tr>
<tr>
<td>S.R.</td>
<td>4.4</td>
<td>2.4</td>
<td>4.4</td>
<td>16.7</td>
</tr>
<tr>
<td>PPG</td>
<td>4.9</td>
<td>2.8</td>
<td>4.5</td>
<td>16.2</td>
</tr>
</tbody>
</table>

Retrograde conduction of the gastric BER was observed in all the SR animals and in one PPG preparation. Two SR animals demonstrated retrograde duodenal conduction into the antrum.

The BER frequencies returned to normal in four animals in each group within two months, with re-establishment of conduction across the anastomosis. Four SR animals demonstrated retrograde gastric activity up to four months after operation.

Identical results were obtained in a further group who had a truncal vagotomy prior to gastric resection.

Following Billroth I gastrectomy, four animals developed temporary slowing of the duodenal frequency (15.7 to 14.5 cycles/min.). The gastric frequency remained unchanged. Enhanced duodenal fast spike activity was also observed and appeared to be permanent.

These results indicate the temporary nature of the changes in BER frequency following gastric resection with associated re-establishment of normal conduction. Intermittent retrograde conduction persists following resections which retain more than 2 cms. of distal stomach and may be the cause of continuing motility disturbances. The enhanced duodenal fast activity following Billroth I resection reflects the disruption of coordination with the distal antrum and suggests hypermotility of the proximal duodenum which may account for the rapid emptying after this operation.

This work is continuing with emphasis on the effect of feeding and of pyloroplasty on the duodenal BER.
Heterozygote Detection in Cystic Fibrosis.

A. As a supplement to the initial studies already reported, further investigation of erythrocyte sedimentation in the presence of ouabain were carried out on patients with cystic fibrosis, compulsory heterozygotes and a series of control subjects. The test was modified to dispense with the need for preliminary overnight refrigeration of blood samples and a comparison of the original with the modified technique confirmed the validity and efficiency of the latter. The results obtained in the control series indicate the existence of a polymorphism in relation to the sedimentation behaviour of different individuals red cells in the presence of ouabain. A pattern practically invariably seen in CF affected and carriers is also observed in approximately 15% of control individuals. This figure appears much too high to represent only the proportion of the general population who are carriers of the CF gene but it might be postulated that it is representative of a population subtype in which the effect of the CF gene may be most strongly manifested.

B. The correlation between the results obtained with the technique referred to above and those of a recently described test for CF involving immobilisation and agglutination of Proteus vulgaris in suspension is also being investigated. Preliminary results indicate good but not universal agreement. Both tests are also being applied to a series of hospitalised patients with chronic obstructive airway disease to investigate the possibility of an increased prevalence of hetero- or even homozygosity for the CF gene among patients in this clinical category.

Investigation into the mechanism of R factor mediated tetracycline resistance.

R factor determined tetracycline resistance in enteric bacteria is thought to involve a plasmid-coded protein being incorporated into the bacterial membrane which prevents the cell accumulating the drug. The initial objective of this research was to analyse genetically tetracycline resistance
determined by one R factor (R100-1) to identify the number of genes involved, their function and the control of their expression.

First however, Tetr determinants of several different R factors were characterised phenotypically into several classes on the basis of the level of resistance conferred to tetracycline and minocycline. All determinants were shown to be inducible, requiring exposure to subinhibitory drug concentrations for maximal expression of resistance.

Tetracycline sensitive (Tets) mutants of R100-1 have been isolated. One type of mutant was both Tets and transfer-defective (Tra-). Complementation tests between these Tets Tra- deletion mutants and mutants of a related plasmid (Flac) have allowed the tet genes to be mapped on the plasmid between traA and traJ. In addition, the order of some other R factor markers was verified, and two new promoters in the R factor transfer operon were postulated.

Among single-site Tets mutants of R100-1, both nonsense (amber-suppressible) and missense lesions have been characterised. Complementation tests so far indicate that the product of the Tetr structural gene region is a single polypeptide.

In addition to the plasmid mutants described above, a mutant was isolated which increased the level of plasmid determined resistance to both chloramphenicol and tetracycline but which was located on the host Escherichia coli K12 chromosome (in the cmlB gene). R factor tetracycline resistance was expressed constitutively in this strain. Experiments are in progress to test if cmlB strains of E. coli have a decreased ability to accumulate these drugs. This might explain the higher resistance levels when R factors are carried by this strain being due to synergistic action between the plasmid and chromosomal resistance determinants.

Professor E. C. Moorhouse, DPH., MRC.Path.

Department of Clinical Microbiology, Royal College of Surgeons, Dublin.

Studies on the epidemiology of R factors.

Overseas Student Survey

(a) Acquisition of drug resistant Escherichia coli carrying R factors (R+).
(b) Acquisition of new E. coli serotypes.

A total of 60 students from foreign countries were admitted to this survey. Results (published in M.R.C. report, 1973) showed that 40% of these students were excreting drug resistant E. coli carrying R factors (R+) on arrival and all excreted R+ E. coli during the first three months of their stay in this country. About 60% of the strains isolated during the
survey have been serotyped with "O" antisera. The untypable strains will be tested in the near future against a wide range of "H" antisera. It is hoped that it will be possible to classify the R factors carried by the resistant strains using R factor compatibility groups.

PAEDIATRICS

Bridget Egan-Mitchell, MB., DCh.
Department of Paediatrics, Regional Hospital, Galway.

Director: Professor B. McNicholl, MD., FRCP., FRCPI., DCh.

Intestinal Mucosal Enzymes in Childhood.

Objectives: The main initial objective was clinical cooperation in studies on intestinal peptidases in general and in coeliac disease. Studies have shown absence of any specific peptidase deficiency, normal enterokinase levels and have also succeeded in isolating and purifying intestinal peptidases for the first time.

Additional clinical research in recent years has concentrated on (a) Definition of incidence clinical picture and role of enzymology. (b) Permanence of gluten intolerance in coeliac disease. (c) The degree of mucosal recovery in treated coeliac disease.

Findings: (a) The first valid incidence study related to population and births showed an incidence of less than 1/400 births. The incidence of constipation and of absence of diarrhoea have been described (10% and 4% respectively). New aspects of early diagnosis have been described (b) In the largest trial of gluten tolerance in coeliac disease reported to date it has been shown that it may take three or more years before the recovered mucosa reverts to a flat state; gluten intolerance is probably permanent. (c) In the largest group of coeliacs in remission hitherto studied, 36 children were shown to have normal morphology of mucosa, normal enterocyte height, normal intra epithelial lymphocyte counts, sucrase and alkaline phosphatase. Mucosal lactase however remained significantly low and is probably the most sensitive indicator or persisting gluten intolerance.

Conclusions: Coeliac disease is of high prevalence in the West of Ireland, and its diagnosis can be established in some cases within a few weeks of onset. Some atypical features have been described and quantitated for the first time. New and disturbing information as to the variability of gluten intolerance has been gained. All clinical and mucosal morphological and enzymological abnormalities return to normal with successful treatment except mucosa lactase. Return of mucosal lymphocyte population to normal with adequate treatment may have favourable implications concerning risks of reticulo-endothelial malignancy.
Noel Clarke, MA., BSc., MD., MRCPL, MRC.Path., in collaboration with V. McCabe, BE., MI.Mech.E., MIEI.

Department of Pathology, University College, Dublin.

The Detection of Platelet Aggregation in Whole Blood.

Rationale: It is generally accepted that platelet aggregation is an important early event in the intravascular process of thrombosis during which a blood vessel may become blocked and deprive a vital organ of its blood supply. The precise mechanism of arterial or venous thrombosis is not known, although many biological reagents which may be involved have been identified. Indeed we do not know whether the involvement of platelets in thrombosis is an active or a passive process. An active process would mean that the platelets were themselves intrinsically over-reactive. A passive process would mean the platelets were normal but were caused to aggregate or adhere by a factor or factors in their environment such as endothelial damage, turbulent flow, abnormal plasma constituents, etc.

It might be reasoned that if one could titrate the sensitivity of platelets to react against a number of known thrombotic agents one might derive an indicator of 'thrombotic tendency'.

When the circular rotor was developed we were not satisfied with the precision and accuracy of results which could be derived from existing knowledge. Therefore many aspects of rotor optics were investigated in relation to this system. We have determined the ideal wavelength, importance of collimating the incident beam, light path depth, cuvette shape, cuvette photodetector distance. Light emitting diodes have been used.

Aggregometer: The design and construction of an automatically controlled d.c. rotor system with bi-directional rotation under step and sinusoidal signals was completed.

Multiple Cuvette Rotor: A major problem was the impracticality of performing the desired number of tests on the amount of blood which circular rotors required. This difficulty was solved by segmenting the rotor head into multiple cuvettes which each held 0.4 ml. blood. In this way it is possible to carry out large numbers of tests on a single blood but also to run the appropriate standards in parallel.
A survey of normal dermatoglyphic patterns in an Irish population.

A sample of 30 individuals (14 female and 16 males) was analysed as a pilot study to determine the correct number required for a survey of the Irish population. The right and left digit and palm prints were analysed for 8 different parameters. Three of these, being independent variables, were analysed statistically. The method used was to obtain the mean (X) and the variance (\(\delta^2\)) of the variable. The final standard error was then chosen and also the confidence interval. The standard error will be \(\pm 4\) with a 95\% confidence interval. These figures were then inserted into the formula that gives \(n\) or the number required for the population survey i.e.

\[
\text{S.E.} = 1.96 \times \frac{\delta}{\sqrt{n}}
\]

\[
n = \frac{(1.96)^2 \times 2748.3}{(4)^2}
\]

\(n = 687\)

**Note:**
S.E. = 4.  
Confidence interval of 95\% = 1.96.  
\(\delta^2\) (for total finger ridge count) = 2748.3.

This number (\(n = 687\)) being the largest \(n\) of the 3 variables will be taken as the number required for the survey. Statistical analysis has been carried out by me to date, but I am now receiving advice from the Statistics Department, T.C.D. on more detailed sampling procedures which take into account such factors as geographic location, social status, age and sex of the population to be sampled.

H. C. Moore, MD. (Lond.), FRC. (Path.).
The Rotunda Hospital Laboratory.

*The mechanism of the effect of pregnancy and allied states on experimental hypertension in the rat; relation to human pregnancy hypertension.*

The method for the measurement of blood pressure in rats now used is the photoelectric method of the Huntingdon Research Centre. Towards
the end of the year this method was used in conjunction with the older Friedman and Freed technique.

Following the report of 1973 it was decided to include non-pregnant animals in the experiments. A surprising finding was that steroid hypertension could not be maintained in the absence of the kidneys; the animals were maintained alive by intermittent peritoneal dialysis. Further experiments were planned and are still in operation using the two methods of blood pressure measurement as given above.

A difficulty was that peritoneal dialysis of itself reduced the blood pressure in intact hypertensive animals and the difference between these animals and nephrectomised animals will have to be further elucidated.

It had been suggested to us that DOCA given subcutaneously twice a week for 3 to 4 weeks would produce metacorticoi hypertensive animals. I gave DOCA intramuscularly for this period in a number of animals and the blood pressure failed to rise. This experimental method has been abandoned.

Catherine Mullins, MSc.

The Children's Hospital, Temple Street, Department of Biochemistry, University College, Dublin.

Director: Seamus F. Cahalane, MB., PhD., FRCPath.

A Survey of Metabolic Abnormalities in Mentally Abnormal Patients.

The object of this study was to develop a screening method which could be applied in the detection of aminoacidemia in large populations. The present popular and effective form of collection of blood samples, from large populations, is by finger or heel puncture and impregnation of the whole blood on filter paper cards. The method was developed to utilize blood specimens collected in this form.

The screening method designed and the experimental work leading to its optimisation were described earlier. This method involves preliminary elution of the amino acids from the blood impregnated paper, followed by chromatography, staining, densitometric scanning of the amino acid patterns and numerical analysis of the traces obtained. Ninety per cent of the amino nitrogen present on blood impregnated paper is recovered. The amino acids are separated by unidimensional double development into ten peak systems.

The population studied was one for which blood samples had already been collected and consisted of a cross section of the inmates of mental hospitals. This population was separated into three groups according to clinical diagnosis: mentally defectives, schizophrenics and neurotics. In
addition, a control group of blood samples from 200 normal children was included.

Visual observation of 2,000 chromatograms and examination of the densitometric scans of 738 chromatograms showed no gross abnormalities. For twenty samples from each clinical group and the control group the densitometric scans were transformed into a number series which was amenable to numerical analysis. By means of numerical analysis the groups were compared to each other. Significant differences in some amino acids were found between the control group of children, aged four to fourteen years and the adult groups in accordance with results reported in the literature. Analysis of blood samples from a group of neurotics were significantly different from the other adult groups in one peak system, that of asparagine, lysine, citrulline, serine and hydroxyproline.

Mary T. O’Hegarty, BSc., PhD.
Pathology Department, University College, Dublin.

In vitro effects of chemical carcinogens on cell surface.

A series of experiments has been done to investigate the effects of 20-methylcholanthrene (MCA) on Chinese Hamster Ovary (CHO) cells. Since other workers have shown that the toxicity of carcinogenic polycyclic hydrocarbons to cells in culture was related to their carcinogenic potential, measurement of cloning efficiency of test and control cultures was done in addition to morphological study. Cells were seeded at low density (100 cells/flask) to obtain colonies. The flasks were divided into three batches, one to serve as controls, one as solvent control and one as test cultures. Test cultures were treated with MCA (initially dissolved in dimethylsulphoxide, DMSO) at varying concentrations (10 μg/ml, 7.5 μg/ml, 5 μg/ml, 2.5 μg/ml) for a period of 7 days, after which time the carcinogen was removed and fresh medium added. Solvent control cultures were treated with DMSO for the same period. Control cultures were incubated with normal culture medium only. Two days later, colony counts were done on sample flasks from each batch to determine cloning efficiency. The results of these counts indicated that neither the carcinogen nor the solvent in which the carcinogen had been dissolved exerted any significant toxic effect on the cells, thus suggesting (1) that MCA, at the concentration used, did not cause transformation of the cells or (2) the cells were already transformed before addition of MCA. Light microscopic examination of the stained colonies revealed no evidence of transformation in any of the cultures at this stage. The remaining flasks were maintained for a further 25 days in culture medium. Some flasks received changes of medium only; in the case of others, cells were harvested, counted and subcultured twice during this period. Cell counts showed no significant difference in growth rate between test and control cultures.
Representative cultures from each batch were treated with ruthenium red (to reveal cell coat), fixed, dehydrated and embedded in-situ. Light microscopical examination of these preparations showed no significant differences in degree of organisation of growth pattern between control and test cultures. In both cases there were areas of increased cell density separated by zones of lower density where cells appeared to have grown in monolayers.

For electron microscopy, sections from both a 'low' and a 'high' density area of each culture were cut. Electron microscopical examination of the sections showed: (1) in 'low' density areas growth was between 1 – 3 cells deep, while in 'high' density areas, growth was generally between 4 – 7 cells deep, i.e. 'piling' had occurred, and there was a variable but usually considerable amount of cell 'debris' between the intact cells. (2) No consistent difference in amount or distribution of ruthenium-red-positive cell coat between test and control cultures was observed. The majority of cells had a cell coat which completely invested the cell membrane but in areas where intact cells were surrounded by cell debris, cell coat was frequently reduced or entirely absent.

I. J. Temperley, MD., FRCPI., MRC.Path., in collaboration with J. Scott.
Moyne Institute, Trinity College, Dublin.

Food folates.

There are eight different active forms of the vitamin folic acid within cells.

Investigations by us and others have however shown that cells whether bacterial, plant or mammalian contain predominant pools of only two forms, 10-formyltetrahydrofolate and 5-methyltetrahydrofolate. Thus, in food in the first instance the vast majority of the folate present is going to be divided equally between these two forms. A good deal of information is available on the chemical stability of these two major food folates which would indicate that 10-formyltetrahydrofolate is extremely unstable and 5-methyltetrahydrofolate is quite stable. Thus the view held to date by nutritionalists would be that almost all of the ingested folate of value would come from the latter form. Our earlier studies which concentrated on a comparison of all of the folates began to show that, contrary to what was expected, the 5-methyltetrahydrofolate was nutritionally quite unstable and 10-formyltetrahydrofolate was much more stable. This finding has been pursued and confirmed. We have now shown that 10-formyltetrahydrofolate is rapidly converted to the chemically and nutritionally very stable 5-formyltetrahydrofolate and this explains its unexpected nutritional stability. We are now convinced that 5-methyltetrahydrofolate forms a hitherto unrecognised degradation product which is nutritionally inactive. A comprehensive paper on this study is in the course of preparation.
PHARMACOLOGY

P. J. Cannon, MB., MSc.

Department of Pharmacology, University College, Dublin.

ECG amplifiers.

Work has continued on the construction of three ECG amplifiers compatible with existing equipment, as each amplifier forms only a part of a recording system for spatial vectorcardiography. A general purpose power supply unit has already been built, and the amplifiers are being designed to fit into this power supply. Each amplifier will have a gain of about 2,000, to give a useful output voltage and enable them to be used with a variety of general purpose oscilloscopes. It is intended to study slow ECG changes, and so the time constant has been set at 5.6 seconds.

Some difficulties in obtaining suitable coupling capacitors have been overcome. The output stage is rather unsatisfactory at the moment, its performance not matching that of the prototype; but we hope to solve this problem soon. It will then only be necessary to duplicate to complete the three amplifiers. A switching unit and a Frank Lead Selector box will then be constructed. The amplifier can also be used for taking scalar ECG’s.

The work is designed to produce a cost-effective instrument for the particular purpose of spatial analyses of slow potentials already described in the literature (by Cannon and others) and previously alluded to in reports to the MRCI.

Michael P. Ryan, BSc., PhD., in collaboration with Patrick McKenna, BSc.

Department of Pharmacology, University College, Dublin.

The relationship of intracellular magnesium and potassium.

Magnesium (Mg) is primarily located within cells and is second only to potassium (K) as the major intracellular cation. Muscle K deficiency is a known concomitant of Mg depletion. The present studies were directed towards the intracellular events accompanying Mg deficiency, since there is a paucity of such data in both experimental and clinical Mg deficiency, and on their relationships to K depletion.

Male Wistar rats, averaging 150 grams, were dietarily deprived of Mg for 42 – 48 days. Controls were pair-fed on electrolyte-free diet with daily gavage of electrolytes including Mg. At the termination of the experiments, 24 hour urine samples were collected from each rat. Animals were then sacrificed, hearts were removed in toto, and cardiac mitochondrial
fractions were prepared by centrifugation. Mitochondrial function studies were carried out at 30° using glutamate as substrate. Oxygen consumption was measured polarographically with a Clark oxygen electrode.

Mg-deficient animals, despite pair-feeding, showed a significantly smaller weight gain compared to control animals. Both plasma Mg and urinary Mg output were greatly decreased during Mg deficiency.

The indices of mitochondrial function studied were as follows: $Q_0 = \text{n moles } O_2 \text{ consumed/mg mitochondrial protein/min. during state 4 respiration (before addition of ADP) and during state 3 respiration (rapid } O_2 \text{ consumption during the phosphorylation of ADP)}; \text{respiratory control index (R.C.I.) } = \frac{O_2 \text{ consumed in the presence of added ADP to that in the absence of added ADP}.}$ The R.C.I. is a quantitative measure of the degree of "coupling" of oxidation to phosphorylation. The data are shown in the table. Though there was a tendency towards decreased $O_2$ consumption during state 3 and decreased R.C.I. in mitochondrial fractions from Mg-deficient animals, the mean values for these parameters were not significantly different, statistically, from the control values. The finding that mitochondrial Mg was not decreased in cardiac tissue during Mg deficiency extends earlier reports on the ability of muscle to retain Mg during dietary-induced Mg-deficiency.

Means of enhancing muscle Mg loss and the effects of K deficiency and of Mg plus K deficiency on mitochondrial function are being investigated.

<table>
<thead>
<tr>
<th>Mitochondrial Function Indices (means ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (7)</td>
</tr>
<tr>
<td>$O_2$ consumption</td>
</tr>
<tr>
<td>$n$ moles $O_2$/mg protein/min.</td>
</tr>
<tr>
<td>State 4</td>
</tr>
<tr>
<td>12.6±8.3</td>
</tr>
<tr>
<td>State 3</td>
</tr>
<tr>
<td>49.6±10.3</td>
</tr>
<tr>
<td>R.C.I.</td>
</tr>
<tr>
<td>4.74±1.5</td>
</tr>
<tr>
<td>Mg content</td>
</tr>
<tr>
<td>$\mu$ moles/g. protein</td>
</tr>
<tr>
<td>41.6±11.1</td>
</tr>
</tbody>
</table>

| Mg Deficient (9)                              |
| $O_2$ consumption                             |
| $n$ moles $O_2$/mg protein/min.                |
| State 4                                       |
| 11.1±2.7                                      |
| State 3                                       |
| 37.6±12.2                                     |
| R.C.I.                                        |
| 3.68±1.7                                      |
| Mg content                                    |
| $\mu$ moles/g. protein                        |
| 43.4±16.8                                     |

Physiology

C. S. Breathnach, MD., PhD., in collaboration with J. Moynihan, PhD.

Physiology Department, University College, Dublin.

Respiratory Function of the Placenta.

The study proposes to examine the metabolic and respiratory function of the placenta throughout gestation by making parallel determinations of foetal and maternal blood gas and plasma substrate values at the same time as determining blood flows in the vessels serving the placenta.
Blood flow through the two uterine horns in pregnant sheep have been estimated simultaneously (Table).

<table>
<thead>
<tr>
<th></th>
<th>day of gestation</th>
<th>horn a</th>
<th>horn b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singlet pregnancy</td>
<td>121</td>
<td>461</td>
<td>363</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>252</td>
<td>178</td>
</tr>
<tr>
<td>Twin pregnancy</td>
<td>115</td>
<td>215</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td>123</td>
<td>152</td>
<td>248</td>
</tr>
<tr>
<td></td>
<td>129</td>
<td>187</td>
<td>152</td>
</tr>
</tbody>
</table>

The differences between the values found in the two horns is only slightly wider in the singlet pregnancy because the placental cotyledons are diffusely distributed through both uterine horns and are not confined to the so-called pregnant horn.

J. K. Burns, BSc., MB., ChB. (Galway), PhD. (Glas.).

Department of Physiology, University College, Galway.

Relation between blood levels of ACTH and duration of human labour.

It has previously been suggested that prolongation of human labour (in comparison with non-human primates) is due to the unique and profound increase in oestriol output during the latter half of human pregnancy. Oestriol has been found to cause inhibition of uterine contractility in laboratory animals. If it has a similar effect in women it may be expected to prolong labour. We however have found lack of correlation between urinary oestriol output and duration of human labour.

A neuroendocrine cycle (Burns, 1971) has previously been put forward to explain physiological prolongation of the duration of human labour. The degree of psychological stress is difficult to measure and present techniques do not seem to give accurate results. It is desirable that a physiological parameter be used for measurement of the degree of stress. In the present investigation blood levels of ACTH were measured and the relationship was examined between these and the duration of human labour.

Blood levels of ACTH were measured, using an immunoassay method (Croughs, Tops & de Jong, 1973) in 40 patients during the last 2 weeks of pregnancy. Subjects were chosen who were free of serious medical disease and who had no apparent obstetrical disorder. The durations of the stages of labour were measured and their relation examined to blood levels of ACTH. These levels were markedly elevated in comparison with non-pregnant women (P < 0.01) and a correlation was found for linear regres-
sion, higher ACTH levels being found in women with longer durations of labour. Results support a relation between the degree of stress, as indicated by blood levels of ACTH, and the durations of human labour (first stage).

Michael T. Kane, MAgrSc., PhD.

Department of Physiology, University College, Cork.

Investigation of Metabolic Patterns in Preimplantation Embryos.

The aim of this project has been to determine the presence and importance in the preimplantation rabbit embryo of the different energy-supplying metabolic pathways. The use of metabolic inhibitors in this study has proved a particularly fruitful approach. Ova were cultured from the one-cell stage in different inhibitors and examined microscopically over a five day period.

Three inhibitors of oxidative phosphorylation viz. cyanide, dinitrophenol and oligomycin were used. All of these inhibitors stopped growth at the one-cell stage when used at normal inhibitory levels. Signs of ovum damage usually became apparent as early as 15 minutes after the start of incubation. This provides strong evidence of the existence of an oxidative phosphorylation system at the one-cell stage and is the first such evidence for any mammalian ovum.

Two inhibitors of Krebs cycle were studied, fluoracetate and malonate. Fluoracetate stopped growth at the one-cell stage but malonate had no effect. It is probable that the failure of malonate to have any effect was due to a failure of permeability as malonate has been shown to have no effect on stages of the mouse embryo which are known to have Krebs cycle activity. The fluoracetate and oxidative phosphorylation results suggest that a fully active Krebs cycle is present and essential in the one-cell ovum.

One glycolytic inhibitor, 2-deoxy-glucose was used. This inhibitor allowed development of one-cell ova to the morula stage but prevented blastocyst formation. This effect was shown in a glucose-free medium suggesting that endogenous stores of glycogen were being metabolised. The specificity of the effect was shown by the fact that it could be overcome by the addition of glucose to the medium.

The existence of a ouabain sensitive sodium pump in the ovum was suggested by the fact that development of one-cell ova to blastocysts was completely prevented by 10⁻³M ouabain.

These results indicate (1) that oxidative phosphorylation and Krebs cycle activity are present in the one-cell ovum, (2) that glycolysis is not essential until the transition from morula to blastocyst and (3) that a sodium pump is present in the cleavage stages of the ovum.
Professor R. P. Kernan, DSc., PhD., in collaboration with Mary MacDermott, MSc.

Department of Physiology, University College, Dublin.

Measurement of chloride and potassium activity within cells of muscle and other tissues by means of ion selective microelectrodes.

Chloride-selective microelectrodes were prepared by inserting electrolytically etched and chlorided silver wires into standard microcapillaries of 0.5 μm tip diameter. The chloride sensitive tip of about 5 μm diameter was recessed about 20μm from the tip opening and was sealed to the glass at about 100 μm from there by silanes. Chloride activity was measured from the p.d. developed between this electrode and the standard KCl-filled microelectrode after calibration in solutions containing concentrations of KCl ranging from 1 to 100 mM. The electrodes used registered a potential change of 57 - 58 mV for a decade change in chloride activity. These electrodes were then used to measure the chloride activity within fibres of frog sartorius muscles, or more precisely to measure the chloride equilibrium potential across the fibre membrane.

Intracellular chloride activity was varied by soaking the isolated frog sartorii in modified Ringer-Conway fluid to which solid KCI had been added to give concentrations of 10, 25, 50 and 100 mM. In these solutions the product of extracellular concentrations [K]₀ x [Cl]₀ was increased above the product of intracellular concentrations so that the ions entered the fibres towards a Donnan equilibrium and to equilibrium with the membrane potential.

Membrane potential of the muscle fibres, Eₘ, was first measured in these solutions with the KCl-filled electrode, the Cl-electrode serving as an external electrode. The latter was then inserted into the fibres so that the p.d. between the electrodes was a measure of intracellular chloride activity. The e.m.f. produced by moving both microelectrodes from bath fluid into the fibres corresponded to chloride equilibrium potential across the membrane and this was plotted against Eₘ. The results for normal and high-K ringer are shown in Figure 1. There appears to be good agreement between Eₘ and Eₐ₁ except in normal ringer where Eₐ₁ was significantly less than Eₘ. Mean values found in normal ringer were Eₘ-90.1 ± 0.4mV, Eₐ₁-80.3 ± 0.7mV. Assuming the same activity coefficient 2C₁ inside and outside the fibres, [Cl]ᵢ based on Eₐ₁ would be 3.1mEq/l, that based on Eₘ would be 2.1mEq/l. Although this suggested that these ions were not passively distributed across the membrane it seems unnecessary to suggest the presence of a Cl-pump in the membrane as the sodium leak known to take place into the fibres under these conditions could provide the energy for Cl-uptake. We have also tested Cl-specific microelectrodes containing liquid ion exchanger Corning 477315 which have a fast response but these had a high impedance and low specificity for chloride.
Figure 1. Membrane potentials plotted against chloride equilibrium potentials. Broken line joins mean values (open circles) for 100, 50, 25, 10 and 2.5 mM-K in external fluid. Theoretical (solid) line is also inscribed.

R. G. Luckwill, BSc., PhD., MA.

Department of Physiology, Trinity College, Dublin.

Thermoregulation in newborn rabbits.

The firing rate of certain cells within the central nervous system of mammals reflect the thermal status of the animal and lead to the operation of the heat production or heat loss mechanisms. Many workers have studied the sites of such cells in adults, but since the heat production mechanisms differ in newborn rabbits, it seems possible that the central mechanisms may differ also.

Previous results from this laboratory have suggested that thermal responsive cells are not concentrated into the classical thermosensitive areas of the anterior/preoptic hypothalamus in the newborn rabbit but are more widely distributed throughout the thalamus and hypothalamus.
In recent experiments a systematic search of the anterior and posterior thalamus and hypothalamus has been carried out, using standard micro-electrode techniques, for cells responsive to a generalised peripheral thermal stimulus (heating or cooling the whole body in a metal trough in which the animal lay). As the microelectrode was lowered through the brain any cells encountered were tested for thermal responsiveness and their position plotted on previously prepared maps.

Experiments have been carried out on 30 New Zealand White rabbits ranging in age from 5 to 14 days. A total of 130 cells were tested for thermal responsiveness and of these 9 were considered to respond. All of these cells were in the posterior hypothalamus area or the dorsal medial thalamic nucleus.

The technique of micro-iontophoresis is also being developed using 5-barrelled microelectrodes, and it is hoped in the future to be able to test any thermoresponsive cells found for sensitivity to specific transmitter substances. This technique has so far been tried on 12 animals. No thermal responsive cells have been tested but preliminary results have shown that the activity of a number of cells is depressed by Gamma-aminobutyric acid and excited by acetylcholine.

Professor M. F. Murnaghan, MD., MSc.

Department of Physiology, University College, Dublin.

(a) Effect of different physiological salt solutions on ventricular contractility and the inotropic response to autonomic nerve fibre stimulation.
(b) A new circumferential electrode for use with circular pieces of cardiac muscle.

A strip of the right ventricle of a rat was immersed in a physiological salt ("Ringer") solution and driven via punctate electrodes at a rate of 3 Hz; the autonomic nerve fibres were stimulated via field electrodes during the refractory period of the muscle by the method described previously (Irish J. Med. Sci. 143, 183, 1974).

12 different solutions were compared with Tyrode to determine which was the most suitable; 2-5 experiments were carried out with each. Tyrode (T), Krebs-Henseleit (KH), Modified Krebs (MK), McEwen (ME), Chenoweth (C) and Locke 3 (L3) were gassed with 5% CO₂ in oxygen; Locke-Intestine (LI), Locke-Burn (LB), Locke-lower calcium (LC), Dale (D), Phosphate-Locke (PL), and Douglas and Rubin (DR) were gassed with oxygen and Modified Locke (ML) with 1% CO₂ in oxygen. LI, LC, D and ML contained 6 mM NaHCO₃, LB 1.8 mM and L3 24 mM. Their relative effectiveness on control contractility were C, MK > L3 > D > KH, ME > LI, LC > T, ML > LB, PL, DR and o on the positive and negative inotropic responses to autonomic nerve fibre stimulation were MK, LI > KH > LC > L3, D, C, LB > ME > T, ML > DR > PL and KH > T, L3 > MK, C respectively.
The results with rat ventricular strips confirm that obtained previously with rat atria that some ‘Ringer’ solutions including Tyrode are not satisfactory when recording contractility. In addition the choice of the ‘ideal’ solution varies with the type of muscle and according to the property being investigated.

(b) A circular piece of rat right ventricular wall was stimulated between a circumferential wire and a hook which attached the centre to a force transducer so that the muscle formed a cone. The adrenergic nerve fibres in the muscle were field stimulated between the circumferential wire and a circular wire above the muscle. The force of contraction and positive inotropic response of the circular muscle was always greater than that of the strip. In a comparison of Tyrode (T), McEwan (ME) and Locke 3 (L3) solutions, L3 was > ME > T for contractility and ME was > T > L3 for positive inotropism.

Daniel J. O’Donovan, PhD.

Physiology Department, University College, Galway.

Relationship between acid-base disturbance and ammonia metabolism.

The cerebral enzyme activity in control and ammonia-toxic rats was examined. Two procedures were employed to produce ammonia toxicity. Acute toxicity was induced by the intraperitoneal injection of 10 m. moles of ammonium acetate per Kg. body weight. Rats were made chronically toxic with ammonia by 4 intraperitoneal injections of 5 m. moles of ammonium acetate per Kg. body weight at intervals of 2 hours. Ammonia analysis was performed on the brain which was immersed in liquid nitrogen immediately after decapitation. Brain enzyme assays were performed immediately after sacrificing the animals or the samples were stored at −20°C.

Both procedures for inducing ammonia toxicity caused a substantial increase in brain ammonia concentration. The ammonia concentration in the cortex was greater than in the base of the brain for the control and ammonia-toxic groups. Convulsions and coma developed within 20 minutes after the induction of acute ammonia toxicity. Chronic ammonia toxicity resulted in drowsiness, but not convulsions or coma. Brain ammonia levels were higher 20 minutes after the induction of acute ammonia toxicity than at 8 hours after the induction of chronic ammonia toxicity. In acute experiments brain ammonia concentrations reached maximum values between 5 and 15 minutes after intraperitoneal injection of ammonium acetate.

Acute and chronic ammonia toxicity did not significantly influence the activities of glutamic dehydrogenase, glutamine synthetase, Na+ –K+ ATPase or pyruvate kinase in brain tissue.
RONAN G. O'REGAN, MB., BSc., PHD.

Department of Physiology, University College, Dublin.

Sinus nerve efferents.

The carotid body is supplied with sympathetic fibres which course in both the gangliogglomerular and sinus nerves. An investigation has been carried out in order to determine if the excitatory effects of this innervation upon chemoreceptor discharge can adequately account for the hyperventilatory changes which occur during electrical stimulation of the pre-ganglionic cervical sympathetic trunks.

Twenty-two cats were used, thirteen of which underwent mid-collicular decerebration while the remainder were anaesthetized with pentobarbitone sodium. In decerebrate cats, breathing either room air \((P_aO_2 = 85-110\) torr) or oxygen \((P_aO_2 = 410-650\) torr), electrical stimulation of the pre-ganglionic cervical sympathetic caused substantial increases of ventilation, effects which were usually abolished by cutting the sinus nerves. Ventilatory changes were much less marked in anaesthetized animals. The discharges of 36 of 48 chemoreceptor afferent preparations obtained from sinus nerves of both normoxic decerebrate and normoxic anaesthetized cats were not excited during sympathetic stimulation and those preparations that did show accentuations of discharge did so to a small extent. Indeed, only 4 preparations demonstrated elevations of discharge exceeding 25 per cent of their control values. In hyperoxia no effects of the sympathetic upon chemoreceptor activity was noted. The trivial chemoreceptor responses to sympathetic activation seem inadequate to explain the hyperventilatory effects. The possibility of an involvement in the ventilatory effects of those sympathetic components which, arising from the superior cervical ganglion course centrally in the sinus nerve, was examined. Section of these neural elements before they joined the sinus nerve caused a variable reduction of the hyperventilatory effects of preganglionic sympathetic stimulation. Electrical stimulation of these sympathetic fibres usually increased ventilation. In 25 percent of cats fibres of the internal carotid sympathetic nerve contributed to the hyperventilation.

This investigation suggests that a portion of the hyperventilation during sympathetic activation may be mediated by fibres not destined to supply the carotid body.

SOCIAL MEDICINE

VICTORIA P. COFFEY, LRCP., PHD., MFCM.

Department of Paediatrics, Trinity College, Dublin.

Congenital abnormalities.

The National Register of Congenital Abnormalities continues to be maintained. In 1972 we had records for 11 Counties; in 1973 we had reports from 15 Counties and in 1974, 17 Counties sent in details, while three other Counties sent in some incomplete details.
The number of deliveries investigated in the 17 Counties during 1974 was 57,741 (almost the same number as was recorded for 15 Counties in 1973). The number of abnormal babies recorded was 601 for both years so the over-all incidence of congenital abnormalities remains the same. What was found unusual was that there was a slight rise in the incidence of Anencephaly in 1974 (3.4 as against 3.3 per 1,000 deliveries) whereas in 1972 the rate was 1.9/1000. This shows that the increase reported last year in this congenital anomaly continues to rise slightly.

Of major interest is the fact that in Offaly where 901 births were investigated in the County Hospital, Tullamore, the incidence of Congenital Defects was 24.4/1000 deliveries, Anencephaly incidence being 6.0/1000. On the three previous occasions when I investigated the deliveries in Offaly I always recorded a very high incidence of congenital anomalies. I will report this finding to the County M.O.H.

Apart from Offaly, Dublin still continues to have the highest incidence of Anencephaly in the 17 Counties. A detailed incidence rate of the various anomalies in each county will be forwarded to the Council shortly.

In the study of Inborn Errors of Metabolism, the work continues to increase. In 1974, 2,723 specimens were received from 58 Hospitals or Institutions; 616 of these were abnormal biochemically, some severely so. Some interesting results are developing in the treatment of Gargoylism but the investigations are still being pursued. A case of argino Succinic acidurea was successfully treated during the past year.

VETERINARY MEDICINE

B. J. Sheahan, MVB., MS., PhD., MRCVS., in collaboration with Miss L. Roche, BSc.

Veterinary College, Ballsbridge and Veterinary Research Laboratory, Abbotstown, Co. Dublin.

Director: W. J. C. Donnelly, BVSc., MS., MRCVS.

Cell Culture Techniques in the Elucidation of an Animal Model for GM1 Gangliosidosis.

The recognition of GM1 gangliosidosis in calves of the Friesian breed in Ireland [Donnelly, Sheahan and Rogers (1973), J. Path. 111, 173-179], presented a unique opportunity for laboratory studies on gangliosidosis. The bovine disease resembles human GM1 gangliosidosis in that there is autosomal recessive inheritance and a reduction in the activity of tissue B-galactosidase [Donnelly, Sheahan and Kelly (1973), Res. vet. Sci. 15, 139-141]. Ultrastructural changes in the brains of affected calves also closely parallel those in man [Sheahan & Donnelly (1974), Acta neuropath. 30, 73-84].
The primary aim of the first year of this project was to devise suitable methods and conditions for (1) establishing fibroblast cell lines from affected and control animals, (2) sub-zero storage and recovery of these cell lines and (3) preparing cultured fibroblasts for examination by electron microscopy. Concurrently studies were continued on the nature of the enzymatic defect and the ultrastructural changes in the brains and selected organs of affected calves.

Fibroblast cultures have been established from 2 gangliosidosis and 2 control calves. The average time to obtain satisfactory monolayers in Leighton tubes on primary culture was 14–21 days. Subculture intervals in Leighton tubes and Falcon flasks were about 5 days. These intervals were not appreciably altered by variations in the concentration of foetal calf serum in culture media supplemented with vitamin concentrate and non-essential amino acids. Fibroblasts from an affected and a control calf have been prepared for ultrastructural studies. Different generations of the cell lines treated with DMSO have been stored in liquid nitrogen and successfully recovered at intervals up to 12 days after freezing.

These studies have shown that fibroblast cell lines can be established from calves with GM1 gangliosidosis. Storage and recovery of the cells at various generations is feasible thus providing a continuing source of material. Studies are proceeding on the lysosomal enzyme pattern in cultured fibroblasts from control and affected calves. It is also intended to compare the pattern of B-galactosidase activity in cultured fibroblasts with that in liver homogenates.

STUDENT GRANT REPORTS

S. Boolell

St. Laurence’s Hospital, Dublin.

Director: Dr. J. G. Devlin.

Investigation of insulin insensitivity in normal subjects and in a group of diabetics composed of possible diabetics, insulin dependent diabetics and diabetics on oral hypoglycaemic drugs.

This study was part of a project designed to relate the sensitivity of body tissues to insulin and that sensitivity to percentage adiposity in a group of seven normal healthy subjects, eight possible pituitary diabetics, eight insulin dependent diabetics and one diabetic stabilised on oral agents. Also investigated were three subject who were admitted in the acute phase of ketoacidosis. Tentative conclusions were reached regarding the relationship between fasting insulin levels, the fall of blood glucose as a percentage of fasting blood glucose level, and insulin sensitivity.
C. J. Buckley

Department of Physiology, University College, Cork.

*Director:* Dr. M. T. Kane.

*Sodium transport in rabbit ova.*

Sodium transport was studied in rabbit ova. An effort to measure the uptake and entrusion of radioactive sodium by ova was unsuccessful due to the small tissue mass of the ova. Culture of ova to blastocysts was prevented by low levels of ouabain suggesting the presence of a ouabain sensitive sodium pump.

Catherine M. B. Coleman

Department of Biochemistry, University College, Dublin.

*Director:* Professor M. G. Harrington.

*Polyunsaturated fatty acid composition of muscle fat of cattle fed special diets.*

GLC analysis was used to determine the rate of incorporation of polyunsaturated fatty acids (PUFA) into adipose tissue and the PUFA content of cooked *M. longissimus dorsi* of veal calves fed milk replacer diets containing elevated levels of PUFA. PUFA-fed animals showed a 3-4 fold increase over control animals, and had elevated serum cholesterol levels. Cooking of meat caused little change in PUFA content and organoleptic evaluation failed to distinguish between treated and control animals.

Martina Corry

Department of Pathology, University College, Dublin.

*Director:* Professor J. Masterson.

*New banding techniques for human chromosome identification.*

Banding techniques have proved to be invaluable in the identification of the individual chromosomes involved in aneuploidies, translocations and structural anomalies. Various banding techniques were investigated and evaluated, the agents used were:

(a) quinacrine
(b) proteolytic enzymes
(c) salt solutions
(d) buffers
(e) alkalis
(f) urea
(g) heat
(h) detergents

The results indicated that the most satisfactory technique for routine diagnostic cytogenetic studies, was one which involved pretreatment of flame-dried chromosome preparations with both trypsin and saline sodium citrate. Chromosomal G-bands obtained by this method were sharp and well-defined, and fully adequate for individual chromosome identifications.

Martina Duffy
Department of Pathology, University College, Galway.

Director: Dr. Helen Grimes.

Aminolevulinic acid dehydrogenase activity in a hospital population.

One hundred and nineteen patients at Galway Regional Hospital had blood aminolevulinic acid dehydrogenase activity (ALAD) and lead concentration measured. ALAD activity correlated inversely with blood lead level and was not significantly affected by the disease states investigated. Geriatric patients tended to have lower enzyme activity and higher lead levels than the other age groups.

Rosemary Finn
St. Vincent’s Hospital, Dublin.

Director: Dr. R. Mulcahy.

Coronary heart disease study.

Work included a review of the charts of 589 patients admitted to the coronary care unit in 1973 with definite or possible coronary heart disease. Precise details of diagnosis, complications and outcome were noted. The immediate and long term prognostic indicators of patients with myocardial infarction including a review of all x-rays to record cardio-thoracic ratio were also examined. Assistance was also given with a long term dietary study and in lipid clinical activities.
Jean C. Folan, BSc.

Sir Patrick Dun's Hospital, Dublin.

Director: Dr. Mary Henry.

The neurohistology of normal and varicose veins.

A study of the neurohistology of normal and varicose veins using a silver technique has revealed an innervation pattern, no difference having as yet been noted between the two types of vein. An initial trial using a fluorescence technique to demonstrate the adrenergic innervation was also attempted.

W. Grodin

St. Laurence's Hospital, Dublin.

Director: Professor R. D. Thornes.

Dinitrochlorobenzene skin sensitivity in cancer.

This was a study of the usefulness of dinitrochlorobenzene (DNCB) skin sensitivity as a screening device to pick out patients with depressed delayed hypersensitivity, particularly those resulting from cancer. Following Professor Thornes's investigation into the results of the traditional dosage for the skin testing it was decided to use the lower dosages of 20, 10 and 5 micrograms, and to compare the delayed hypersensitivity of cancer patients with a population of other chronically ill patients, matched for sex and age. We sensitised 23 pairs of patients and tested them for their sensitivity to DNCB.

Contrary to what we had expected these dosages applied to the skin are insufficient to stimulate a delayed hypersensitivity reaction. This fact, while disappointing, is useful to Professor Thornes's ongoing research into depressed delayed hypersensitivity in cancer patients. It also points the way for a second effort in this area using different dosages, and perhaps utilising the technique of Bone et al in their series of bowel cancers in the U.K.

B. H. Khamis

St. Laurence's Hospital, Dublin.

Director: Dr. J. Stephen Doyle.

Evaluation of transmucosal potential difference as a potential tool for diagnosis of gastritis.

Following some initial technical problems, a mongrel dog with a chronic Heidenhain Gastric pouch preparation was prepared with a modified Gregory Cannula inserted in the mouth of the pouch.
Using standard method of measuring potential difference the gastric transmucosal potential difference was measured with the mucosal lead passed through the Gregory Cannula and the reference electrode placed subcutaneously.

Three solutions were then prepared, one of standard acid solution, second special bowel solution and the third control solution.

The Heidenhain pouch was filled with the control solution for a set period and hydrogen ion flux and transmucosal potential difference was measured. Similar measurements were done during periods when the simple acid solution was used and also when the specially prepared bile solution was induced into the pouch.

A significant fall in transmucosal potential difference and a marked increase in hydrogen ion flux was obtained following use of the bile solution. These procedures were associated with direct gastric mucosal biopsies. A total of three experiments of this nature were done on the final preparation.

It was concluded that the measurement of transmucosal difference in the stomach of dogs could be used as a worthwhile clinical method of estimating a breakdown of the "mucosal barrier". It was concluded that this method could, with further elaboration, be a potential tool for the diagnosis of gastritis in humans.

J. J. McCann

Mater Misericordiae Hospital, Dublin.

Director: W. P. Hederman, FRCS.

Chemical Sympathectomy. A Retrospective Study.

309 patients presenting with peripheral vascular disease or hyperhidrosis had 485 chemical sympathectomies (phenol) within a two and a half year period. 68% needed no further treatment. 22% had surgery combined with the procedure and 10% needed surgery at a later date.
M. McGlone

Department of Physiology, University College, Galway.

Director: Professor D. J. O’Donovan.

Effects of calcium chloride, ammonium chloride and sodium bicarbonate on the acid-base status of male albino Wistar rats.

The effects of NH₄Cl, CaCl₂ and NaHCO₃ on the acid-base status of male albino Wistar rats were investigated. Both NH₄Cl and CaCl₂ increased urinary titratable acidity, but decreased blood PH, urine PH and urine flow rate. These changes were greater for CaCl₂ than for NH₄Cl. Addition of CaCl₂ to the drinking water decreased fluid intake, which resulted in a dramatic decrease in body weight.

D. Mulhail

Department of Biochemistry, Trinity College, Dublin.

Director: Dr. M. J. Duffy.

Subcellular distribution of substance P in bovine hypothalamus and substantia nigra.

Subcellular distribution of substance P as measured by radioimmunoassay was studied in fractions and subfractions from bovine hypothalamus and substantia nigra. Most of the substance P was found in the crude mitochondrial fraction. Subfractionation of the crude mitochondrial fraction by density gradient centrifugation showed most of substance P was present in nerve ending particles. Subcellular particle integrity was assessed using enzyme markers.

B. Murray

Department of Biochemistry, Trinity College, Dublin.

Director: Professor D. G. Weir.

Studies on the existence and nature of a pancreatic intrinsic factor (P.I.F.) and its possible role in B₁₂ malabsorption and/or free intrinsic factor (I.F.) degradation.

It had previously been reported (Weir, Temperley and Collery, Quar. J. Med., 36, 605, 1967; Temperley, Weir, Collery and Scott, Gastroenterology, 57, 273, 1969) that normal duodenal juice contains a factor which inhibits the binding of Vitamin B₁₂ by human intrinsic factor. The object of the present study was to confirm and extend these original observations.
That normal human duodenal juice does in fact inactivate human intrinsic factor was confirmed. It was found in addition that this inhibition appears to be due to two separate entities. (I) a high molecular weight compound, heat labile not removed by treatment with albumin-coated charcoal. (II) a low molecular weight heat stable compound removable by treatment with albumin-coated charcoal.

Brid Noone

Department of Physiology, University College, Galway.

Director: Professor J. K. Burns.

Effect of three combined oestrogens on uterine contractility.

Six dose levels of combined oestrogens (oestriol + oestrone + oestradiol) were used, and their effects on uterine contractility were recorded.

Mature, female virgin rats were injected with the combined oestrogen solution (dissolved in alcohol) intramuscularly for three consecutive days. On the fourth day, they were sacrificed, the uterus was removed and contractions recorded on single channel recorders. The small dose levels employed caused an increase in uterine contractility. Moderate dose levels decreased the number of uterine contractions and high dose levels resulted in no significant change. Inhibition of contractility was not found at low and high dose levels of oestrogens. Partial inhibition was marked at low dose levels and moderate following injection of larger amounts of oestrogens.

With oestrogens, contractions were regular and larger. High doses of oestrogens produced physical changes in the uterine horns, which were oedematous and distended. Ovaries showed similar changes and were haemorrhagic. The highest dose levels produced hypertrophy of the wall in the uterine horns, and an increase in vascularity.

M. A. O'Doherty

Department of Surgery, St. Laurence’s Hospital, Dublin.

Director: Professor W. A. L. MacGowan.

Review of the treatment of varicose veins.

A review of the results of operative treatment of varicose veins during the period 1964–1974 at St. Laurence’s Hospital was carried out. One hundred and fifty-seven case records were examined and 120 patient enquiries made by a questionnaire about the post operative result.
There was an 82.5% satisfactory result ranging over a follow-up period of from 6 months to 10 years. This result compares favourably with those reported either by operation or sclerotherapy. There were no deaths or limb loss but 7.6% had post-operative complications of a minor nature.

P. O'Neill, BSc.

Department of Physiology, University College, Cork.

Director: Professor J. D. Sheehan.

Effect of prostaglandin E\textsubscript{1} and acetylsalicylic acid on cholinergic transmission in the guinea-pig.

Prostaglandin E\textsubscript{1} potentiates the response to cholinergic nerve stimulation in guinea-pig ileum. Acetylsalicylic acid diminishes both twitch response and acetylcholine output. These effects can be reversed by applied E\textsubscript{1}. Similar results were obtained in the trachea. E\textsubscript{1} diminished the cardiac chronotropic effect of vagal stimulation. In the phrenic nerve-diaphragm preparation neurotransmission was unaffected.

Margaret Phelan

Department of Pathology, University College, Galway.

Director: Professor J. D. Kennedy.

\textit{Alpha\textsubscript{1} antitrypsin deficiency in the West of Ireland.}

During a period of four months, 36 patients at Galway Regional Hospital were suspected of alpha\textsubscript{1} antitrypsin deficiency. Ten of these had levels corresponding to the homozygous state, eight to the heterozygous state. A 10% incidence of the heterozygous state was found in 106 healthy school children.

F. Shanahan

Department of Pathology, University College, Dublin.

Director: Professor J. W. Harman.

\textit{Systems of anion transport in mitochondria.}

The aim of the project was to elucidate some of the biochemical features of anion transport through muscle mitochondrial membranes. The procedure used involved isolation of mitochondria from pigeon skeletal muscle by centrifugation and subsequent measurement of mitochondrial metabolism, in the presence or absence of various substrates and under precisely defined conditions, by the Warburg manometric technique.
J. D. Sweeney

Department of Biochemistry, University College, Galway.

Director: Dr. M. P. Coughlan.

Effects of ascorbate, sulphonamides and tuberculostatic agents on milk xanthine oxidase.

The effects of a number of chemotherapeutic agents on the activity of xanthine oxidase were determined. Streptomycin did not inhibit activity as had been claimed by other workers and while ascorbate did inhibit activity at high concentrations it did not form a non-dissociable complex with the enzyme. Nicotinic acid and isonicotinic acids were competitive inhibitors of the enzyme regardless of the electron acceptor used. However, p-amino salicylate, p-amino benzoate and isoniazid apparently effected a differential inhibition of activity. In the case of p-amino salicylate it was shown that the observed inhibition of activity was due to interaction between the drug and uric acid, the product of the reaction, rather than to inhibition of the enzyme itself.

G. Tobin and P. Warde

Department of Pharmacology, Trinity College, Dublin.

Director: Professor C. W. M. Wilson.

Investigation of the effects of fenfluramine on ascorbic acid metabolism in normal subjects.

The clinical results have confirmed the laboratory findings in guinea-pigs carried out in this department that the anti-obesity action of fenfluramine depends on its action on ascorbic acid metabolism. It has been shown that this anti-obesity action can be inhibited in humans by concomitant administration of supplementary Vitamin C in the diet.
PUBLICATIONS AND ABSTRACTS


O'CARRA, P. with DELANEY, M. Lactate dehydrogenase: Affinity chromatographic studies of the relationship between abortive complex


COMMUNICATIONS


JOHNSON, D. B. Studies on the immobilisation of uricase. Biological Sciences Section of the Royal Academy of Medicine in Ireland, July 1974.


KERNAN, R. P. and McDERMOTT, MARY. Effects of nerve block and of
denervation on membrane potential and on electrolyte and water
fluxes in rat skeletal muscles in vivo. Physiol. Instut. Homburg,

MCCARTHY, C. F. The incidence, familial occurrence and aetiology of
celiac disease in Ireland. 5th World Congress of Gastroenterology,
Mexico City. 1974.

MCCARTHY, C. F. Celiac disease its Irish dimension. The Graves Lecture.
1974.

1974.

MCCARTHY, C. F. Carcinoma of the small bowel. European Association

MCCARTHY, C. F. Aspects of celiac disease. Department of Medicine,
University of Bristol. 1974.

MOONEY, P. A. ATPases in the human small intestine. Biological Section

MOORHOUSE, E. C. Antibacterial drug prescribing by doctors in the Irish

MOORHOUSE, E. C. Antibiotics – uses and abuses in hospital and general

MOORHOUSE, E. C. Incidence of R factor bacteria in Ireland. Symposium

MULCAHY, R. et al. The primary and secondary prevention of coronary
heart disease. Midland Postgraduate Symposium, Coventry. April
1974.

MULCAHY, R. et al. Determinants of prognosis in patients with coronary

MULCAHY, R. et al. Experience of cardiopulmonary resuscitation in a


MULCAHY, R. et al. The primary and secondary prevention of coronary
heart disease. Organon Laboratories, OSS, Holland.


MULDOWNEY, F. P. Diagnosis of normocalcaemic hyperparathyroidism. Renal Seminar, Barnes Hospital, St. Louis, April 1974.


O'CARRA, P. Complicating factors in affinity chromatography. Round Table meeting of experts concerned with fractionation and purification of biological materials (sponsored by the Council of Europe sub-group EFRAC) Bruges, May 1974.


O'CARRA, P. Why is biliverdin converted to bilirubin? Bilirubin meeting, Hemsedal, Norway, September 1974.


O'CARRA, P. Heme catabolism. Invited paper delivered at Rockefeller University, New York, November 1974.


PARAMESWARAN, V. with DEVLIN, JAMES G. Immune response to mono component insulin. Royal Academy of Medicine in Ireland, January 1974.


SHANLEY, D. B. Use of bone grafts in periodontology. Annual meeting of the Dental Faculty in Royal College of Surgeons in Ireland, November 22, 1974.

SMYTH, H. and FARRELL, D. Recent findings related to the altered properties of neoplastic cell surfaces. Annual meeting of the Irish Association for Cancer Research, 1974.

SMYTH, H. with FARRELL, D. Effect of neuraminidase on the antigenicity of Landschutz ascites tumour cells. Annual meeting of the Biological Sciences Section, Academy of Medicine, 1974.
Index to Personal Names

Alton, B., 4
Barrett, Catherine, 18
Barry, S., 34, 92
Barry, V. C., 8, 9, 14, 15, 21
Beary, Maura E., 15, 19, 29
Beckett, P. G. S., 5
Beasley, W. H., 19, 58
Belton, J. G., 21, 28
Blake, S., 4
Boggust, W. A., 19, 56, 89
Boolell, S., 16, 80
Boucher-Hayes, D. J., 17, 19
Bourke, E., 5, 14, 19, 39, 44, 89
Bourke, G. J., 4, 91, 92
Brady, M. P., 4
Brady, T. G., 4
Breathnach, C. S., 71
Brennan, P., 34
Brown, P., 18, 44
Brown, P. J., 50, 52, 90
Cahalane, S. F., 5, 67
Cahill, J. T., 19
Campbell, F., 5
Cannon, D. E., 18
Cannon, P. J., 19, 70
Carroll, M. J., 15, 30
Clarke, N., 4, 19, 65, 89
Coffey, Victoria, 5, 14, 17, 19, 78
Coleman, Catherine M. B., 16, 81
Collins, P. G., 4
Corcoran, Victoria, 5, 14, 17, 19, 78
Conalty, M. L., 4, 5, 21, 28
Cooney, Catherine M. B., 16, 81
Collins, P. G., 4
Corner, F. R., 15, 57, 89, 95
Conley, J. G., 5
Corbett, J., 14
Costello, J., 14
Coughlan, M. P., 14, 15, 17, 30, 88, 89, 90
Cullen, J. J., 5
Cullen, M. J., 6, 19, 40, 89
Cunningham, S., 18, 59
Daly, Mary P., 14
Davies, Eleanor, 18
Dawson, G. W. P., 6
Delanev, Ann, 18
Delaney, T. V., 15
Devlin, J. G., 5, 16, 19, 41, 80, 90, 95, 98
Dineen, Emer, 14
Donnelly, W. J. C., 79, 93, 94
Doyle, Anna F., 14, 31
Doyle, C. T., 5
Doyle, E., 4
Doyle, J. S., 4, 16, 83
Duffy, Martina, 16, 82
Duffy, M. J., 16, 85
Duggan, P. F., 19, 32, 90
Dunne, L. K., 6
Egan-Mitchell, Bridget, 17, 64, 90, 91
Farrell, D., 38, 94, 98
Fenley, Mary, 14
Fegan, W. G., 4, 14, 19, 50, 52, 90, 95
Feighery, C., 14, 15, 42
Fennelly, J. J., 4
Finn, Rosemary, 16, 82
FitzGerald, O., 3, 4, 8, 14, 15
FitzGerald, P., 4, 59
Flynn, J. R. N., 15, 43
Folan, Jean C., 16, 83
Foster, T. J., 15, 62, 95
Fottrell, P. F., 4, 35, 90, 91, 93, 94, 97
Galvin, C., 4
Gannon, Marie, 18, 21, 28
Gratenby, P. B. B., 3, 4, 5, 8, 19, 44
Gay, R. J., 19, 58
Geeley, G. F., 4, 91, 92
Geoghegan, F. J., 5
Graham, H., 14, 21
Graham, I., 17, 46
Green, J., 4, 14, 42, 66
Greely, Marie, 14, 66
Grimes, Helen, 16, 82
Gorry, Hilda, 18, 56
Grodin, W., 16, 83
Harman, J. W., 3, 6, 8, 17, 87
Harrington, M. G., 5, 16, 19, 33, 81
Hayden, Anne, 14
Hederman, W. P., 16, 84
Hennessy, T. P. J., 14, 60
Henry, Mary, 16, 50, 52, 83
Hickey, E. L., 15, 21
Hiney, Norma, 18
Hourihane, D. O’B., 4
Jacob, Rosemary, 18, 32
Jessop, W. J. E., 5, 8
Johnson, D. B., 14, 15, 30, 89, 90, 95
Kane, M. T., 16, 20, 81, 91, 95
Keaveney, T. V., 20, 59
Kennedy, J. D., 17, 87
Kernan, R. P., 5, 20, 74, 91, 95, 96
Khams, B. H., 16, 83
Kiriins, J. A., 5
Kneafsey, D. F., 4
Lavelle, S. M., 4, 57
Leek, B. F., 5
Little, M. P. G., 5

99