Intestinal absorption

Christie, DA; Tansey, EM

Wellcome Witnesses
to Twentieth Century Medicine

INTESTINAL ABSORPTION

A Witness Seminar held at the
Wellcome Institute for the History of Medicine,
London, on 9 February 1999

Witness Seminar Transcript edited
by D A Christie and E M Tansey

Introduction by Sir Christopher Booth

Volume 8 – September 2000

First published by the Wellcome Trust, 2000

The Wellcome Trust is a registered charity, no. 210183.

ISBN 978 184129 017 1

All volumes are freely available online at: *www.history.qmul.ac.uk/research/modbiomed/wellcome_witnesses/*

Please cite as: Christie D A, Tansey E M. (eds) (2000) *Intestinal Absorption*. Wellcome Witnesses to Twentieth Century Medicine, vol. 8. London: Wellcome Trust.

Key
Front cover photographs, L to R from the top:
Dr Derek Holdsworth
Dr Peter Hanson, Dr Richard Boyd
Dr Peter Williams, Professor Oliver Wrong
Professor Ramsey Bronk, Professor Michael Gardner
Lord Turnberg (chair), Professor Timothy Peters
Dr Richard Boyd, Professor Ramsey Bronk Dr Sheila Callender (1914–2004), Professor Hermon Dowling
Dr Roy Levin, Sir Christopher Booth

Back cover photographs, L to R from the top:
Professor Hermon Dowling, Sir Christopher Booth
Professor Michael Gardner, Dr Peter Hanson, Dr Richard Boyd, Professor Ramsey Bronk
Professor John Walker-Smith, Dr George Misiewicz
Dr Michael Hellier
Professor Roy Pounder
Dr George Misiewicz
Professor Oliver Wrong

CONTENTS

T

INTRODUCTION

The capacity to absorb substances from the external environment is one of the features that differentiates animate from inanimate matter. In mammalian species, this is predominantly the function of the small intestine. The absorbing surface of the small intestine is made up of innumerable villi, which greatly increase the surface area available for absorption. The villi are covered by a layer of absorbing cells which themselves have a surface which is greatly increased by the presence of microvilli which are the major feature of the absorbing surface of the small intestine. During the twentieth century, the remarkable functions of the cells of the small intestine have been the subject of intensive research. At first, there were many who considered that the absorption of substances from the intestinal tract was a process of passive diffusion, in which nutrients passed from the lumen of the intestine without the interposition of any active process on the part of the cells of the intestinal mucosa. The progress of research has shown, however, that absorption from the intestine is for the most part an active process, involving specific transport systems that carry sugars, amino acids, peptides, lipids and other substances into the body. Furthermore, the intestinal cells themselves are highly active metabolically, utilizing energy as do other cells for performing their functions. In addition, the process of digestion, originally thought to occur entirely within the intestinal lumen, has been shown to occur also within the microvilli of the intestinal cells. In addition, the cells themselves have an important function in transforming substances, for example lipids, during their passage across the intestinal mucosa.

The understanding of how the small intestine functions has been a major interest of physiologists throughout the past century. In addition, clinical research workers from different disciplines have also made major contributions. Renal physicians have shown how the reabsorption of substances in the renal tubule has mirrored absorption in the small intestine. Gastroenterologists have studied how absorption may be influenced by clinical procedures such as resection of the small intestine, as well as how dietary changes may affect absorptive processes. Haematologists have made major contributions to our understanding of the complex mechanisms involved in the absorption of iron or vitamin B_{12}. Others have studied disorders such as cholera in order to find out how best to rehydrate those unfortunate sufferers from fluid loss due to intense diarrhoea.

This Witness Seminar brings together scientists and clinicians who have been involved in recent years in unravelling the ways in which the process of absorption takes place in the small intestine. It has been a fascinating enterprise for all those involved, many of whom have taken part in this meeting. We are grateful for the time they gave us, not only in planning and holding this meeting, but also during the lengthy editorial process, which is described below.

Sir Christopher Booth
Wellcome Institute for the History of Medicine

WITNESS SEMINARS: MEETINGS AND PUBLICATIONS*

In 1990 the Wellcome Trust created the History of Twentieth Century Medicine Group to bring together clinicians, scientists, historians and others interested in contemporary medical history. Amongst a number of other initiatives, the format of Witness Seminars – used by the Institute of Contemporary British History to address issues of recent political history – was adopted, to promote interaction between these different groups, to emphasize the potentials of working jointly, and to encourage the creation and deposit of archival sources for present and future use.

The Witness Seminar is a particularly specialized form of oral history where several people associated with a particular set of circumstances or events are invited to meet together to discuss, debate, and agree or disagree about their memories. To date, the History of Twentieth Century Medicine Group has held 24 such meetings, most of which have been published, as listed in the Table below.

Subjects for such meetings are usually proposed by, or through, members of the Steering Committee of the Group, and once an appropriate topic has been agreed, suitable participants are identified and invited. These inevitably lead to further contacts, and more suggestions of people to invite. As the organization of the meeting progresses, a flexible outline plan for the meeting is devised, usually with assistance from the meeting's chairman, and some participants are invited to 'set the ball rolling' on particular themes, by speaking for a short period of time to initiate and stimulate further discussion.

Each meeting is fully recorded, the tapes are transcribed and the unedited transcript is immediately sent to every participant. Each is asked to check their own contributions and to provide brief biographical details. The editors turn the transcript into readable text, and participants' minor corrections and comments are incorporated into that text, while biographical and bibliographical details are added as footnotes, as are more substantial comments and additional material provided by participants. The final scripts are then sent to every contributor, accompanied by copyright assignment forms. All additional correspondence received during the editorial process is deposited along with the records of this meeting in the Contemporary Medical Archives Centre of the Wellcome Library.

As with all our meetings, we hope that even if the precise details of some of the technical sections are not clear to the non-specialist, the sense and significance of the events are understandable. Our aim is for the volumes that emerge from these meetings to inform those with a general interest in the history of modern medicine and medical science, to provide for historians new insights, fresh material for study, and prompt fresh themes for research, and to emphasize to the participants that events of the recent past, of their own working lives, are of proper and necessary concern to historians.

---

* The following text also appears in the 'Introduction' to recent volumes of *Wellcome Witnesses to Twentieth Century Medicine* published by The Wellcome Trust.

ACKNOWLEDGEMENTS

‘Intestinal Absorption’ was suggested as a suitable topic for a Witness Seminar by Sir Christopher Booth and Dr Tilli Tansey. We are particularly grateful to Sir Christopher Booth for assisting us in planning the meeting, helping to decide the topics to be discussed, and for writing such a useful Introduction to these published proceedings. We are equally grateful to Lord Turnberg for his assistance in organizing the format of the meeting and for his excellent chairing of the occasion. We also thank Professor Michael Gardner and Dr Gerald Wiseman for their permission to reproduce a number of illustrations, Dr Gordon Cook who read through an earlier draft of the transcript and offered us helpful comments and advice, and Dr Gordon Cook, Professor Hermon Dowling and Dr Roy Levin for their assistance in compiling the Glossary.

As with all our meetings, we depend a great deal on our colleagues at the Wellcome Trust to ensure their smooth running: the Audiovisual Department, the Medical Photographic Library, and the Publishing Department, especially Julie Wood who has supervised the design and production of this volume. Mrs Jaqui Carter is our transcriber, and Mrs Wendy Kutner and Mrs Lois Reynolds assist us in running the meetings. Finally we thank the Wellcome Trust for supporting this programme.

Tilli Tansey
Daphne Christie
Wellcome Institute for the History of Medicine

1993 Monoclonal antibodies[1]
Organizers: Dr E M Tansey and Dr Peter Catterall

1994 The early history of renal transplantation
Organizer: Dr Stephen Lock

Pneumoconiosis of coal workers[2]
Organizer: Dr E M Tansey

1995 Self and non-self: a history of autoimmunity[1]
Organizers: Sir Christopher Booth and Dr E M Tansey

Ashes to ashes: the history of smoking and health[3]
Organizers: Dr Stephen Lock and Dr E M Tansey

Oral contraceptives
Organizers: Dr Lara Marks and Dr E M Tansey

Endogenous opiates[1]
Organizer: Dr E M Tansey

1996 Committee on Safety of Drugs[1]
Organizers: Dr Stephen Lock and Dr E M Tansey

Making the body more transparent: the impact of nuclear magnetic resonance and magnetic resonance imaging[4]
Organizer: Sir Christopher Booth

1997 Research in General Practice[4]
Organizers: Dr Ian Tait and Dr E M Tansey

Drugs in psychiatric practice[4]
Organizers: Dr E M Tansey and Dr David Healy

The MRC Common Cold Unit[4]
Organizers: Dr David Tyrrell and Dr E M Tansey

---

[1] Tansey E M, Catterall P P, Christie D A, Willhoft S V, Reynolds L A. (eds) (1997) *Wellcome Witnesses to Twentieth Century Medicine*, vol. 1. London: The Wellcome Trust, 135pp.

[2] P D'Arcy Hart, edited and annotated by E M Tansey. (1998) Chronic pulmonary disease in South Wales coalmines: An eye-witness account of the MRC surveys (1937–1942). *Social History of Medicine* 11: 459–468.

[3] Lock S P, Reynolds L A, Tansey E M. (eds) (1998*) Ashes to Ashes: The history of smoking and health*. London: The Wellcome Trust, 228pp.

[4] Tansey E M, Christie D A, Reynolds L A. (eds) (1998) *Wellcome Witnesses to Twentieth Century Medicine*, vol. 2. London: The Wellcome Trust, 282pp.

The first heart transplant in the UK[5]
Organizer: Professor Tom Treasure

1998 **Haemophilia: recent history of clinical management**[6]
Organizers: Dr E M Tansey and Professor Christine Lee

Obstetric ultrasound: historical perspectives[7]
Organizers: Dr Malcolm Nicolson, Mr John Fleming and Dr E M Tansey

Post penicillin antibiotics[8]
Organizers: Dr Robert Bud and Dr E M Tansey

Clinical research in Britain, 1950–1980[9]
Organizers: Dr David Gordon and Dr E M Tansey

1999 **Intestinal absorption**[10]
Organizers: Sir Christopher Booth and Dr E M Tansey

The MRC Epidemiology Unit (South Wales)
Organizers: Dr Andy Ness and Dr E M Tansey

Neonatal intensive care
Organizers:Professor Osmund Reynolds, Dr David Gordon and Dr E M Tansey

British contributions to medicine in Africa after the Second World War
Organizers: Dr Mary Dobson, Dr Maureen Malowany,
Dr Gordon Cook and Dr E M Tansey

2000 **Childhood asthma, and beyond**
Organizers: Dr Chris O'Callaghan and Dr Daphne Christie

Peptic ulcer: rise and fall
Organizers: Sir Christopher Booth, Professor Roy Pounder and Dr E M Tansey

Maternal care
Organizers: Dr Irvine Loudon and Dr Daphne Christie

---

[5] Tansey E M, Reynolds L A. (eds) (1999) Early heart transplant surgery in the UK. *Wellcome Witnesses to Twentieth Century Medicine*, vol. 3. London: The Wellcome Trust, 72pp.

[6] Tansey E M, Christie D A. (eds) (1999) Haemophilia: Recent history of clinical management. *Wellcome Witnesses to Twentieth Century Medicine*, vol. 4. London: The Wellcome Trust, 90pp.

[7] Tansey E M, Christie D A. (eds) (2000) Looking at the unborn: Historical aspects of obstetric ultrasound. *Wellcome Witnesses to Twentieth Century Medicine,* vol. 5. London: The Wellcome Trust, 80pp.

[8] Tansey E M, Reynolds L A. (eds) (2000) Post penicillin antibiotics: From acceptance to resistance? *Wellcome Witnesses to Twentieth Century Medicine*, vol. 6. London: The Wellcome Trust, 71pp.

[9] Reynolds L A, Tansey E M. (eds) (2000) Clinical research in Britain, 1950–1980. *Wellcome Witnesses to Twentieth Century Medicine*, vol. 7. London: The Wellcome Trust, 74pp.

[10] Christie D A, Tansey E M. (eds) (2000) Intestinal absorption. *Wellcome Witnesses to Twentieth Century Medicine*, this volume. London: The Wellcome Trust, 81pp.

INTESTINAL ABSORPTION

The transcript of a Witness Seminar held at the Wellcome Institute for the History of Medicine, London, on 9 February 1999

Edited by D A Christie and E M Tansey

PARTICIPANTS

Dr Derek Bangham
Sir Christopher Booth
Dr Richard Boyd
Professor J Ramsey Bronk
Dr Sheila Callender
Professor Hermon Dowling
Professor Michael Gardner
Dr Peter Hanson
Dr Michael Hellier
Dr Derek Holdsworth
Dr Roy Levin
Professor Richard Naftalin
Professor Timothy Peters
Professor Roy Pounder
Dr Tilli Tansey
Professor Sir Leslie Turnberg* (Chair)
Professor John Walker-Smith
Dr Peter Williams
Professor Oliver Wrong

Others attending the meeting: Dr Gordon Cook, Dr Helen Cox, Dr George Misiewicz, Dr David Tyrrell, Dr Elise Vandervelde, Mrs Magda Whitrow, Dr Billie Williams

Apologies: Dr Roy Barry, Dr Brian Creamer, Dr John Cummings, Dr Ian Forgacs, Professor Ronald Girdwood, Dr David Gordon, Professor Cecil Kidd, Dr Lavinia Loughridge, Dr Dennis Parsons, Sir Robert Shields, Dr Gordon Sladen, Dr Jacob Sweiry, Professor Thomas Hastings Wilson, Dr Gerald Wiseman

* Now Lord Turnberg of Cheadle

Professor Sir Leslie Turnberg:[1] Thank you very much, Tilli [Tansey],[2] for inviting me to chair this meeting and for giving us the opportunity of getting together and meeting old friends. The idea behind this meeting is to try and fill in the background to some of the advances that were made in the field of intestinal absorption by people who were actually involved in the work at the time these advances were being made. I hope we will get a personal view from the people here today. We can get the cold facts from the published literature of course, but the excitement of the discoveries, the humour, the debates that went on, are very much in people's minds rather than on paper. It would be nice to try to capture the way the ideas emerged, how they came about. It's interesting that intestinal absorption is relatively new: it's within living memory that most advances in the field seem to have been made; within the living memory of at least some of us. So before we all forget, it would be nice to have a record of it.

The programme is divided roughly into two halves: the first half is rather more basic and animal based, whereas the second half focuses more on the 'human'; and we've asked a number of individuals to lead the discussion on various subjects.

Chris Booth has kindly agreed to give us a historical introduction, on which he is such an expert and authority, and it's a delight to welcome him and to ask him to do that.

Sir Christopher Booth:[3] Well, Sir Leslie, thank you for your very kind introduction and also for chairing this meeting. I am a stand-in for Dennis Parsons[4] and no substitute, as I am sure you will all recognize. If anybody really wants to know about the development of methods and techniques for the study of intestinal transport, I would refer them to Dennis Parsons's excellent article in the 1968 *Handbook of*

---

[1] Professor Sir Leslie Turnberg Kt FRCP FRCS (now Lord Turnberg of Cheadle) (b. 1934) was Professor of Medicine, University of Manchester, from 1973 to 1997, President of the Royal College of Physicians from 1992 to 1997, and Chairman, Public Health Laboratory Health Board, since 1997.

[2] Dr Tilli Tansey is Convenor of the History of Twentieth Century Medicine Group and Historian of Modern Medical Science, Academic Unit, The Wellcome Trust.

[3] Sir Christopher Booth Kt FRCP (b. 1924) trained as a gastroenterologist and was the first Convenor of the Wellcome Trust's History of Twentieth Century Medicine Group, from 1990 to 1996, and Harveian Librarian at the Royal College of Physicians from 1989 to 1997. He was Professor of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, from 1966 to 1977 and Director of the Medical Research Council's Clinical Research Centre, Northwick Park Hospital, Harrow, from 1978 to 1988. See Booth C C, Neale G. (eds) (1985) *Disorders of the Small Intestine*. Oxford: Blackwell Scientific Publications.

[4] Dr Dennis Parsons (b. 1917) worked in the Department of Biochemistry, University of Oxford, from 1946 to 1984 and was Fellow of Merton College, Oxford, from 1950 to 1984. He worked mainly on membrane and epithelial transport of fluid and solutes, on cell volume regulation, on distribution and functions of carbonic anhydrase isoenzymes and on models of epithelial transport. See, for example, Fisher R B, Parsons D S. (1950) The gradient of mucosal surface area in the small intestine of the rat. *Journal of Anatomy* 84: 272–282. op. cit. notes 5, 46, 47 and 129.

Physiology on intestinal absorption.[5] That article sets out the story of the development of methods for studying transport extraordinarily well. I think, though, that he was unduly modest in that article in the sense that he didn't deal as much with his own work with R B Fisher,[6] which was very important, as I would have expected him to do, but he showed a lot of the long past studies of intestinal absorption in the eighteenth and nineteenth centuries which were matters of very great concern to many people at the time.

One of the major things that people were interested in, for example, was the extent to which the lacteals were involved in absorption. People would link dyes to substances in the intestinal lumen in an experimental animal and then study the lacteals and find out what happened. There were also interesting experiments by people like Sanctorius Sanctorius who managed to weigh himself in a very sensitive balance and worked out what happened to his weight when he fed. And there were those who put food, for example, bread, into containers with holes in the side of them and swallowed them and then brought them back up again and found out how much digestion had occurred within the stomach or in the gut lumen. Many experiments of that sort took place from time to time.[7]

So far as our century is concerned, I think the people who were important were, first, the Professor of Physiology in the University of St Andrews in Dundee, Waymouth Reid, who was the first man to develop an *in vitro* system in which he separated the external mucosal from the internal serosal side of the intestine and was able to show that differences in pressure gradients would develop across them.[8] Waymouth Reid's work, which strongly suggested that absorption of fluids and other substances in the intestine was an active process, was forgotten completely for a very long time. By the 1930s, Verzár, working in Switzerland with his colleague McDougall, had published a book[9] in which after many decades of investigating intestinal absorption, mostly in experimental animals, and particularly the dog, they concluded that all absorption could be explained on the basis of osmosis and diffusion and that no active processes were involved. Now

---

[5] Parsons D S. (1968) Methods for investigation of intestinal absorption. In Code C F. (ed.) *Handbook of Physiology*, Alimentary Canal III, ch. 64. Washington: American Physiological Society, 1177–1216.

[6] Professor Michael Gardner wrote: 'Reginald Brettauer (David) Fisher CBE (1907–1986) gained his DPhil in 1933 under Sir Rudolph Peters in Oxford where he was University Demonstrator in biochemistry from 1933 to 1959. He held the Chair of Biochemistry in Edinburgh from 1959 to 1976, being the first non-medical man to be Dean of the Faculty of Medicine, and in retirement held an honorary position in the Department of Physiology, Oxford. He worked on numerous topics of physiological biochemistry, especially with whole-organ and quantitative approaches, including creatine metabolism, intestinal absorption, action of insulin; he influenced H W Davenport (when Davenport was a Rhodes scholar in Oxford in 1937) in elucidating the mechanism of gastric acid secretion.' Letter to Dr Daphne Christie, 28 April 2000.

[7] See, for example, Kleinzeller A, Kotyk A. (eds) (1961) *Membrane Transport and Metabolism*. Proceedings of a Symposium held in Prague, August 22–27, 1960. London: Academic Press. Wiseman G. (1964) *Absorption from the Intestine*. London: Academic Press. See also note 5.

[8] Edward Waymouth Reid FRS (1862–1948) was first Professor of Physiology at University College, Dundee, from 1889 to 1935. He carried out many experiments on intestinal absorption *in vitro*, including experiments with everted intestine. See, for example, Reid E W. (1900) On intestinal absorption, especially on the absorption of serum, peptone, and glucose. *Philosophical Transactions of the Royal Society* 102: 211–297. See also Cathcart E P, Garry R C. (1948) Edward Waymouth Reid. *Obituary Notices of Fellows of the Royal Society* 6: 213–218.

[9] Verzár F, McDougall E J. (1936) *Absorption from the Intestine*. London: Longmans, Green & Co.

that conclusion was interesting, because it certainly shaped the thinking for the next decade. What has happened in our lifetime, I think, is the transformation of what was regarded as a passive membrane into an organ, an organ that has metabolic functions, and intense activity in terms of its turnover and active processes for transport. Passive processes turn out to be of minor importance in terms of transfer of nutrients across the intestine, although they must be very much more important in the absorption of drugs.

There was one observation in Verzár's work that confused him. This was the report by von Tappeiner in 1878, that bile acids were selectively absorbed in the distal part of the intestine.[10] Now once somebody had suggested selective absorption in a different area of the intestine, that strongly indicated there must be some receptor, some active process involved in picking up that substance. Verzár was so confused by that, that in 1932 he invited a young research fellow of his, called Fröhlicher, to repeat the experiments that Tappeiner had done. Fröhlicher's experiments completely corroborated what Tappeiner had shown, namely that if you put conjugated bile acids into the distal intestine of a dog they are absorbed better than in the jejunum.[11] Verzár explained that anomaly, suggesting that something must have happened to the physico-chemical nature of the bile acid within the intestinal lumen. In the post-war period, the most important experiment was that carried out by Fisher and Parsons in 1949 and demonstrated at one Physiological Society Meeting.[12] They developed an *in vitro* system of studying absorption from the isolated intestine of the rat (Figure 1). They perfused the intestine with varying concentrations of glucose and galactose, and were able to show that the absorption was not linear which is what you would, of course, expect if it was passive transport, but that it was a saturable phenomenon of some sort. That experiment opened up a whole new era in which people were looking at kinetic responses in the intestine to determine whether or not there were active processes involved.

New techniques were then developed, such as the everted sac preparation, isolated brush-border techniques, the use of isolated segments of intestine, and Sheff and Smyth's *in vivo* observation of recirculation of the intestine that allowed people to look at saturation kinetics.[13] And all of those things seem to me to have developed following the Fisher and Parsons's experiment.

Turnberg: Thank you very much, Chris; that was a very nice general introduction. We are going to get down to specifics later on, but does anyone have any comments or questions on this broad-based introduction?

---

[10] Tappeiner H. (1878) Über die aufsaugung der gallensauren alkalien im dünndarme. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften* 77: 281–304. op. cit. note 9, 218–219.

[11] Fröhlicher E. (1936) Die resorption von gallensäuren aus verschiedenen dünndarmabachnitten. *Biochemische Zeitschrift* 283: 273–279.

[12] See Fisher R B, Parsons D S. (1949) A preparation of surviving rat small intestine for the study of absorption. *Journal of Physiology* 110: 36–46.

[13] These techniques are discussed later in the meeting, see, for example, pages 23 and 31. Sheff M F, Smyth D H. (1955) An apparatus for the study of *in vivo* intestinal absorption in the rat. *Journal of Physiology* 128: 67P. See also Wilson T H. (1962) (ed.) Methods, ch. 2. In *Intestinal Absorption.* London: W B Saunders Company, 20–39.

Dr Derek Holdsworth:[14] I agree with Sir Christopher that Verzár concluded that osmosis and diffusion accounted for almost all intestinal absorption, but in Verzár's book he shows his own data which demonstrated selective absorption for glucose and galactose, as compared with other monosaccharides, including fructose.[15]

Booth: Yes. I have never quite understood that. You are quite right that in the 1920s, I think, differential absorption rates had been shown between different sugars. This obviously indicated that something funny was going on, rather more than just diffusion, because equimolecular substances were absorbed at different rates and that had been shown long before. I think Cori had shown that, hadn't he, in respect of sugars?[16]

Dr Roy Levin:[17] I think the other feature is the huge concentration of sugars that the early people used; they weren't low levels, they were often one molar, half molar, or something like that. They were very, very large, so that it wasn't easy to sort out mechanisms. It was so much easier to look at it and say it was diffusion because of the large concentrations often put inside the intestine. So I think it wasn't until we began using much lower concentrations that one could start looking at active transport.[18]

Professor Timothy Peters:[19] I think you are absolutely right. One of the other observations that really nailed the diffusion hypothesis was the demonstration that

---

[14] Dr Derek Holdsworth FRCP (b. 1933) qualified in medicine at the University of Leeds in 1957. While Medical Registrar at the Royal Free Hospital, London, he wrote his MD thesis on monosaccharide absorption in man. Following a year (1964–1965) on an MRC Fellowship in the Physiology Department of Harvard University in the laboratory of Tom Hastings Wilson, he returned to London as Senior Lecturer in Medicine on the Medical Unit at St Bartholomew's Hospital in London. He moved to Sheffield in 1970, one attraction being the opportunity there to continue his interest in the physiology of absorption, whilst pursuing a clinical career.

[15] Dr Derek Holdsworth wrote: 'He [Verzár] concluded that an "active process" was involved in the absorption of these two sugars, attributing the selective absorption of glucose and galactose to their capacity for phosphorylation, which reduced the concentration of hexose within the cell, thereby keeping the diffusion gradient high. In coming to this conclusion, he quoted earlier experiments that showed inhibition of glucose absorption by metabolic inhibitors.' Note on draft transcript, 25 October 1999. See Verzár F, McDougall E J. (eds) (1936) op. cit. note 9, 113–149.

[16] Dr Derek Holdsworth wrote: '...The Coris [husband and wife (see note 67)] showed equal rates of absorption of these sugars, an artifact because they used intragastric introduction of the sugars – the rate was then dependent on gastric emptying as the rate-limiting factor.' Note on draft transcript, 25 October 1999. See Cori C F. (1925) The fate of sugar in the animal body. I. The rate of absorption of hexoses and pentoses from the intestinal tract. *Journal of Biological Chemistry* 66: 691–715. *idem* (1926) The rate of absorption of a mixture of glucose and galactose. *Proceedings of the Society for Experimental Biology* 23: 290–291. See also note 67.

[17] Dr Roy Levin (b. 1935) has been a Reader in Physiology at the University of Sheffield since 1977. He took his original degrees (BSc and MSc) in the Department of Physiology at the University of Liverpool studying with Professor Rod Gregory from 1953 to 1959. He moved to the Department of Physiology (now Department of Biomedical Science) at Sheffield University initially to work with Professor David Smyth on the effects of hormones and diet on intestinal absorption, and obtained his doctorate in 1964. His later studies involved investigating the effects of hormones and of changes in dietary intake on intestinal secretory function.

[18] Debnam E S, Levin R J. (1975) An experimental method of identifying and quantifying the active transfer electrogenic component during sugar absorption measured *in vivo*. *Journal of Physiology* 246: 181–196.

[19] Professor Timothy Peters DSc FRCP FRCPath (b. 1939) has been Professor and Head of Department of Clinical Biochemistry, King's College School of Medicine and Dentistry, London, since 1988 and was Clinical Director of Pathology, King's Healthcare, from 1992 to 1999. He was Head of the Division of Clinical Cell Biology, MRC Clinical Research Centre, Harrow, from 1979 to 1988 and Sub-Dean for Postgraduates and Higher Degrees, King's College School of Medicine, London, since 1988.

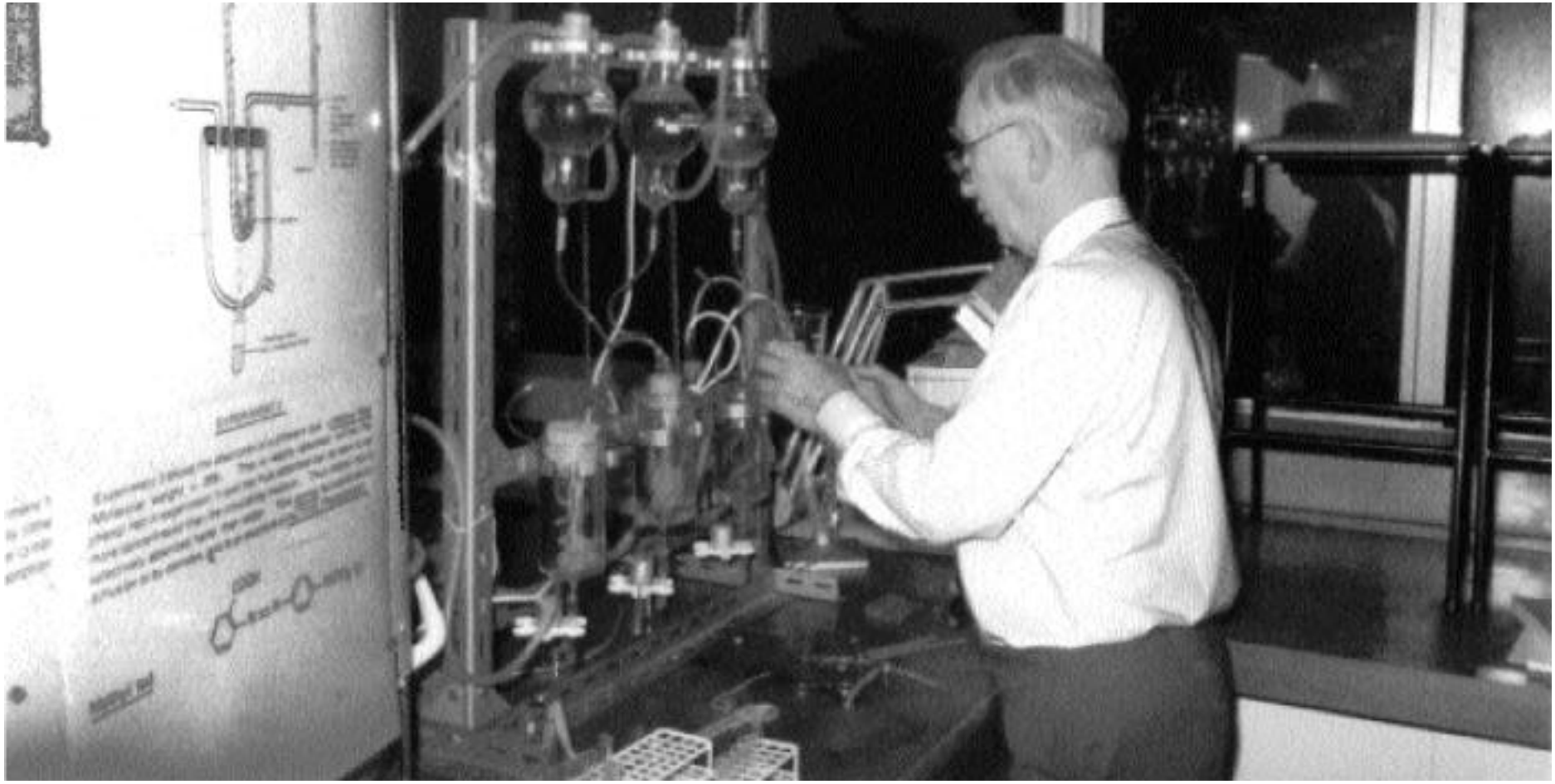


Figure 1. The late Professor R B Fisher setting up a demonstration of perfused small intestine at the Southampton meeting of the European Intestinal Transport Group in 1980. The apparatus here comprises three identical sets of Fisher and Parsons's apparatus modified by removal of the circulating serosal fluid. This demonstration showed the sustained absorption of fluid, but not phenol red, in the presence of luminal glucose by rat small intestine in vitro*; the failure of the intestine to absorb fluid when luminal glucose was absent; and the active transport against a concentration gradient of methyl red. A key feature of this preparation was that the organ was never deprived of adequate oxygen, even momentarily, during setting up from an anaesthetized animal and subsequently. © Professor Michael Gardner, 2000.*

absorption is energy requiring. Fisher, Parsons and other people, using metabolic inhibitors, showed that if they inhibited ATP production, then absorption was markedly inhibited.[20] I think that was a very seminal observation.

Booth: Could I just ask when was that done?

Peters: I would have to refer to Tom Hastings Wilson's book,[21] but it was way before the 1940s.[22]

Professor Hermon Dowling:[23] Two brief points if I may. First, Chris, I enjoyed your opening remarks which provided a broad and helpful perspective. You mentioned von Tappeiner's publication in 1878,[24] but, in fact, there was an earlier study eight years before that which implied that there was selective absorption of bile acids.[25] This was the prototypic substrate that was used to document segmental absorption of specific substances.

The second comment relates to the importance of passive diffusion, as opposed to active transport, in glucose absorption. I shared your views about this until the late 1960s when I worked with Franz Ingelfinger in Boston. He had done a lot of work using segmental perfusion techniques to study sugar absorption in humans[26] and with

---

[20] op. cit. notes 15 and 22. Professor Michael Gardner wrote: 'I think it was Smyth's group in Sheffield, rather than Fisher and Parsons, who explored effects of metabolic inhibitors, though Holdsworth draws attention in footnote 22 to even earlier work. Though Fisher and Parsons did not use metabolic inhibitors, their observations of Michaelis–Menten saturation kinetics (1953) and their findings of competition by phlorizin (1950), together with transport of glucose against a concentration gradient (1950) led them to conclude that a carrier-binding process was involved and also that active transport must be implicated (op. cit. notes 12 and 46). This predates Widdas's observations on carrier mediation in erythrocytes mentioned by Parsons (note 51), though it is after Shannon's work on kidney, mentioned on page 41, note 141.' Letter to Dr Daphne Christie, 28 April 2000. See Parsons B J, Smyth D H, Taylor C B. (1958) The action of phlorrhizin on the intestinal transfer of glucose and water *in vitro*. *Journal of Physiology* 144: 387–402.

[21] Wilson T H. (1962) op. cit. note 13.

[22] Dr Derek Holdsworth wrote: 'They were used much earlier, for example, by Wilbrandt and Laszt in 1932 (quoted by Verzár op. cit. note 9, 130–134. [Wilbrandt W, Laszt L. (1933) Untersuchungen über die ursachen der selektiven resorption der zucker aus dem darm. *Biochemische Zeitschrift* 259: 398–417]). Verzár quotes even earlier work on the effect of metabolic inhibitors on glucose absorption, for example, fluoride by Cohnheim in 1899, and Reid in 1900. Wilson and Wiseman used fluoride in their early everted sac in 1954 (op. cit. note 82).' Note on draft transcript, 25 October 1999.

[23] Professor Hermon Dowling MD FRCP (b. 1934) trained in medicine at the Queen's University, Belfast, graduating in 1959. In 1964 he moved to the Postgraduate School in London to work with Professor Sir Christopher Booth and spent ten years at Hammersmith (which included two years in Boston) before moving to Guy's Hospital and Medical School to become Professor of Gastroenterology in 1974. He is a Past-President of the European Society for Clinical Investigation and the British Society for Gastroenterology and was the Founding Secretary of the United European Gastroenterology Federation.

[24] op. cit. note 10.

[25] Schiff M. (1870) Gallenbildung, abhängig von der aufsaugung der gallenstoffe. *Archiv für die Gesamte Physiologie* 3: 598.

[26] See, for example, Gray G M, Ingelfinger F J. (1966) Intestinal absorption of sucrose in man: interrelation of hydrolysis and monosaccharide product absorption. *Journal of Clinical Investigation* 45: 388–398. Olsen W A, Ingelfinger F J. (1968) The role of sodium in intestinal glucose absorption in man. ibid. 47: 1133–1142.

Ward Olsen, clearly showed that the bulk of glucose absorption in the human jejunum took place by a passive process – rather than by active transport. As Roy Levin said, for many sugars and amino acids it's only when one gets down to low substrate concentrations in the intestinal lumen that the active transport processes begin. For the majority of ingested nutrients there is a huge concentration gradient from lumen to plasma (mucosa to serosa) after meals. This favours passive diffusion and, conceptually, active transport serves as an end-stage 'mop-up' function to transport very low residual concentrations of substrate and to stop back-diffusion. Now that may be a controversial idea which will be contested by many in this room but at least it was the concept that emerged at that time.

Turnberg: I think we will come to this controversy later on and I don't think we should open it now. Can I just comment on Dennis Parsons's role in all this? It's a great pity he's not here today because it does seem to me that he made a number of the seminal observations upon which others have built. Is that a correct perception or were there other leading figures then who had an equal or higher standing?

Booth: You would have to include David Fisher,[27] of course, with Parsons.

Professor Richard Naftalin:[28] I agree that Parsons did have a most important role; we will come to that later this afternoon, but I think one shouldn't forget Ussing's role. Although he wasn't directly involved in the intestine, his experiments were seminal in almost all the work of the 1950s, 1960s and 1970s in the intestine, and I think his work can't be underestimated.[29]

Turnberg: When did Jared Diamond and Peter Curran come in?[30] It was a bit later.

---

[27] See note 6.

[28] Professor Richard Naftalin (b. 1939) has been Professor of Epithelial Physiology at King's College, London, since 1991. He currently works on intestinal salt and water transport and glucose transport in red and white blood cells. His main interest is in regulation of salt and water transport in the colon by growth factors relating to dietary salt intake and to the effects of ionizing radiation on salt and water transport in the colon in relation to expression of adhesion molecules by epithelial cells and myofibroblasts.

[29] Hans Henrikson Ussing (b. 1911) graduated in physiology from the University of Copenhagen in 1934, was Research Professor and Head of the Isotope Division of the Department of Zoophysiology from 1951 to 1960, Head of the Institute of Biological Chemistry from 1960 to 1980, and then Emeritus Research Professor. His many experiments utilized a frog skin preparation mounted in a specially constructed apparatus, known as the 'Ussing chamber', where each surface was bathed in a separate fluid. Chemical manipulation and analysis of the medium on each side provided extensive information about transport processes across the skin. See, for example, Ussing H H, Zerahn K. (1951) Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiologica Scandinavica* 23: 110–127. Ussing H H. (1989) Epithelial transport: Frog skin as a model system. In Tosteson D C. (ed.) *Membrane Transport. People and Ideas*. Bethesda, MD: American Physiological Society, 337–362. For short-circuit current *in vitro* measurements made on segments of rat and rabbit terminal ileum, see, for example, Clarkson T W, Toole S R. (1964) Measurement of short-circuit and ion transport across the ileum. *American Journal of Physiology* 206: 658–668. op. cit. note 99 and page 29.

[30] See, for example, Curran P F, Solomon A K. (1957) Ion and water fluxes in the ileum of rats. *Journal of General Physiology* 41: 143–168. Diamond J M. (1966) A rapid method for determining voltage-concentration relations across membranes. *Journal of Physiology* 183: 83–100. *idem* (1971) Standing-gradient model of fluid transport in epithelia. *Federation Proceedings* 30: 6–13. Curran P F. (1965) Ion transport in intestine and its coupling to other transport processes. ibid. 24: 993–999. *idem* (1968) Coupling between transport processes in intestine. *Physiologist* 11: 3–23.

Booth: What was the date of the earliest work on the Ussing chamber?[31]

Naftalin: 1953.

Dr Michael Hellier:[32] The other people I feel we should pay credit to are Wilson and Wiseman with their everted sac in the 1950s.[33] I think they made a very major contribution providing a technique to enable us to demonstrate active transfer.

Booth: I think we shall be coming to that because that's a specific question we will address.

Turnberg: I was just thinking whether before Parsons there were some important advances on to which others latched? Ussing was certainly working on frog skin and looking at ion transport largely in that tissue and only later was the technique taken up by others to apply it to the intestine.[34]

Levin: In relation to Ussing, I think the importance of the technique was enormous, but also it was the first model of an epithelial cell that you could actually see polarization at the front and the back – the potassium and the sodium transport processes. I remember seeing it and it was burnt into my consciousness, and so that was the one that we all followed. Really, it was *the* model of the epithelial cell and it has survived for years.

Turnberg: I think we should move on because a lot of this will come out as we move to the next item on the agenda, which is sodium-linked glucose transport, amino acids and peptides, and Richard Boyd is going to lead off.

Dr Richard Boyd:[35] I have been in contact with Dennis Parsons[36] this week and he has given me something which he thought you might like to hear. He did make some interesting contributions with David Fisher.[37] This is dated 2 February 1999.

> 'Dear Richard, here, as promised, are my recollections of how the work on the intestine started in Oxford [Figure 2]. One of the things about memory is that with experiments, for example, when they go well, the

---

[31] op. cit. note 29. See also Ussing H H. (1954) Active transport of inorganic ions. *Symposium of the Society for Experimental Biology* 8: 407–422.

[32] Dr Michael Hellier MD MA FRCP (b. 1941) trained in medicine at Cambridge and St Thomas' Hospital, London. His MD thesis 'A study of amino acid and dipeptide absorption with observations on their interactions with water and electrolytes' was written whilst a lecturer at St Bartholomew's Hospital, London, in 1973. He continued his research on absorption in Tropical Enteropathy in South India with Professor Selwyn Baker. He is currently a gastroenterologist at Princess Margaret Hospital, Swindon.

[33] Wilson T H, Wiseman G. (1954) The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. *Journal of Physiology* 123: 116–125.

[34] op. cit. notes 29 and 31.

[35] Dr Richard Boyd (b. 1945) is currently Medical Tutor at Brasenose College, Oxford, and works in the Department of Human Anatomy and Genetics where he carries out research on membrane transport in epithelia. He trained in Dennis Parsons's laboratory from 1971 to 1975, having been taught by him when he was a medical student.

[36] See note 4.

[37] See, for example, notes 12 and 46.

Figure 2. Dr Dennis Parsons (left) talking to Dr Neil Ian McNeil and Professor Leslie Turnberg (back to camera) at the 1983 Falk Symposium on Intestinal Absorption and Secretion in Titisee, Black Forest. At the meeting, Parsons presented a valuable historical account of some of the key aspects of intestinal absorption, including an autobiographical note about his own involvement. [Parsons D S. (1984) Subsequence and consequence in studies on absorption in the intestine. In Skadhauge E, Heintze K. (eds) Intestinal Absorption and Secretion. *Lancaster: MTP Press, 1–17]. © Professor Michael Gardner, 2000.*

timescale is recalled as being short, but when they go badly the timescale seems extended. I have not looked at my notebooks, so I don't know exactly when we started to use rats for example, nor do I remember when we started to do kinetic studies of glucose absorption. I do remember reading A N Richards's micropuncture studies on glucose absorption in the renal proximal tubule, where he had found saturation kinetics,[38] and I found this to be very stimulating and these certainly preceded and probably catalysed some of our own work. I must mention Frank Caddick. Frank was the technician who worked with R B Fisher and me and was a marvellous assistant. You may remember that reducing sugars were measured by the reduction method of Hagedorn and Jensen.[39] This involved analysis of the total reducing sugar, the amount removed when you incubated the analysate with yeast and then the residual reducing component. So every stage had to be calibrated; there was certainly a serious analytical problem which Frank helped to solve. Who would go into all that now?

With an MRC grant I started to work with R B Fisher in the Biochemistry Department in Oxford. R A Peters was the Whitley Professor, Fisher was a university lecturer; he had spent most of the war on statistical problems, the bombing of strategic targets, and how to set fire to cities from the air. This work had taken place in Princes Risborough with J Bronowski[40] and others. After the war was over and having visited various bomb-targeted sites in Europe and Japan, Fisher returned to the Biochemistry Department and prepared to start work on intestinal absorption. His interest was stimulated by the work of E S London from Leningrad who had used the cannula, the 'London cannula', to study the composition of amino-nitrogen in the portal vein of dogs.[41] London had reported the presence of peptides in portal blood

---

[38] See, for example, Wearn J T, Richards A N. (1924) Observations on the composition of glomerular urine with particular reference to the problem of reabsorption in the renal tubules. *American Journal of Physiology* 71: 209–227. See also Walker A M, Bott P A, Oliver J, MacDowell M C. (1941) The collection and analysis of fluid from single nephrons of the mammalian kidney. ibid. 134: 580–595. Starr I. (1969) Alfred Newton Richards, scientist and man. *Annals of Internal Medicine* 71: 1–89.

[39] Hagedorn H C, Jensen B N. (1923) Zur mikrobestimmung des blutzuckers mittels firricyanid. *Biochemische Zeitschrift* 135: 46–58.

[40] Dr Jacob Bronowski (1908–1974) studied mathematics at Jesus College, Cambridge, obtaining his PhD in 1933 and becoming an Honorary Fellow in 1967. He was Senior Lecturer at University College, Hull, from 1934 to 1942, and carried out military research during the Second World War, remaining a government official until 1963. From 1964 he was a senior fellow and Director of the Council for Biology in Human Affairs at the Salk Institute for Biological Studies, San Diego, California. His many publications include *The Poet's Defence* (1939), *William Blake, A Man without a Mask* (1944) and *The Ascent of Man* (1973).

[41] Dr Efim Semenovich London (1869–1939) and his school in Leningrad developed a technique for portal vein sampling. The 'London cannula' was a metal cannula surgically fixed in close proximity to the portal vein or other large vessel in such a way that a sharp needle could be introduced through the cannula into the vessel for sampling. For a description, see London E S. (1929) Experimental fistulae of blood vessels. *Harvey Lectures* 23: 208–221.

during absorption of a meal. Fisher was attracted to this hypothesis, that it was peptides, rather than amino acids, that formed the major form of nitrogen traffic between the tissues in animals. London's experiments were done before the war and the methods of analysis of nitrogenous substances were not very good. It was thought that the more recent developments in chromatography, especially of paper chromatography which had been developed by Martin and Synge,[42] could be used in the projected studies. Fisher's views on peptide transport are expounded in his excellent monograph on protein metabolism.[43]

Before the war Fisher had worked on a variety of aspects of cardiac metabolism *in vitro*, using the rat heart in a Langendorff preparation. The heart, in a water-jacketed organ chamber, was perfused via gravity from a reservoir placed at a suitable height above it. The output from the coronary veins was returned to the reservoir by a water-jacketed return line at the bottom of which there was a glass nozzle through which gas (95 per cent O_2/5 per cent CO_2) was injected to form an ascending stream of bubbles in the returning fluid. This 'gas lift' was an excellent way of maintaining the fluid circulation through and around tissues *in vitro*. It seems to have been devised for heart perfusion experiments at University College London. At the end of the nineteenth century, gas lifts had been used by the London Metropolitan Water Board as pumps for mixing in water treatment systems. Gas lifts are used to circulate the fluids on both sides in the Ussing chamber.[44] In any case, Fisher proposed to use this system replacing the heart with a segment of small intestine. A key notion was that the fluid in the lumen was to be isotonic extracellular fluid, so that as in the case of the heart, the circulating fluid would be Krebs bicarbonate Ringer and the tissue would thus be supplied with oxygen from the mucosal surface. The length of the path of diffusion for oxygen across the serosal surface and muscularis was much too long to provide an adequate PO_2 at the epithelium, even with serosal fluid oxygenation with 100 per cent oxygen. All the glass vessels were water

[42] Paper chromatography, a technique for separating the components of complex mixtures, was developed as a research tool, for which Archer Martin shared the 1952 Nobel Prize for Chemistry with his coworker, Richard Synge. See Martin A J P, Synge R L M. (1941) A new form of chromatography employing two liquid phases: 1. A theory of chromatography, 2. Application to the micro-determination of the higher monoamino acids in proteins. *Biochemical Journal* 35: 1358–1368. See also James L K. (ed.) (1993) Archer John Porter Martin. In *Nobel Laureates in Chemistry 1901–1992*. Washington, DC: American Chemical Society and the Chemical Heritage Foundation, 352–355. *idem* Richard Laurence Millington Synge. ibid. 356–358. Martin A J P. (1964) The development of partition chromatography. In *Nobel Lectures: Chemistry, 1942–1962.* Amsterdam, the Netherlands: Elsevier, 359–371. Synge R L M. (1964) Applications of partition chromatography. ibid. 347–387. See also Gordon H. (1996) Richard Laurence Millington Synge FRS 1914–1994. *Biographical Memoirs of Fellows of the Royal Society* 42: 455–479.

[43] Fisher R B. (1954) *Protein Metabolism*. London: Methuen.

[44] op. cit. note 29.

jacketed and made in the glassblowers' workshop and were rather fragile and difficult to repair. In my view the design was unnecessarily complicated and I later made many modifications; for example, much to Fisher's disgust, I found that if you put a hypodermic needle through a thick-walled rubber tube, you got exactly the same effect as that produced by the glass nozzle.

By the time I arrived in the department, Fisher had assembled at least one set of circulation equipment but had not made serious use of it. He had decided to use guinea-pigs, but first they had to be anaesthetized because it was agreed that at no time was the mucosa to be anoxic, and that the mesenteric circulation was to be maintained until the luminal circulation was established. At the Nuffield Department of Anaesthetics at the Radcliffe Infirmary in Oxford, a physicist, Epstein, had produced a small portable anaesthetic machine for delivery of volatile anaesthetics to patients under field conditions. It was made of black plastic and was about 20 cm in diameter by 12 cm deep. Ether was in a compartment provided from a water-jacketed container. It was exactly the type of apparatus which appealed to Fisher. It was splendid looking, but proved to be quite useless and a lot of time was wasted in trying to get it to work. I actually found that it was much easier to use some ether on a piece of cotton wool gauze in a funnel. That worked well and we never looked back. But the guinea-pig experiments were failures. This was because of the presence of very significant peristalsis which interrupted the luminal flow circulation to such an extent that the epithelium became anoxic and the mucosa sloughed off. The peristalsis could not be reduced by altering perfusion pressure, so we tried the effect of adding pulses of hydrostatic pressure to the lumen.

At the end of the war there were vast amounts of surplus equipment available for virtually nothing and being quite a gadgeteer myself I constructed a pulsatile actuator which I called a pulsometer, electrically activated with a pneumatic valve, to pump air as a way of trying to overcome peristalsis. Although this equipment was most impressive, and indeed reduced peristalsis somewhat, it was not sufficient to provide a useful preparation. All very depressing. But guinea-pigs were herbivores, and so we started to use rats, omnivores, and right from the beginning the segments survived. Peristalsis only caused problems if the luminal hydrostatic pressure was low. In the Langendorff preparation the hearts were given glucose as a fuel, so it was provided for the rat intestine.

As regards the Krebs–Ringer, it was made up each day from stock solutions, the calcium always being added last, because it formed a precipitate, which tended to dissolve when the solution was warmed up and gassed, but it never went completely. Later on, A Baird Hastings, the great clinical chemist, was visiting the department and told us that Krebs had based the composition of

his Ringer solution on the ionic content of whole mammalian plasma, but because half the calcium ions of plasma are bound to proteins, the calcium content in Krebs–Ringer is too high by a factor of two. Henceforth, we cut the calcium content by half and precipitation was never a problem.

Just before the war an interesting book on intestinal absorption was published. This was, of course, written by Verzár and McDougall.[45] In that they reported that D-glucose, a hexose, was absorbed faster than the smaller pentose, D-xylose; thus the process of absorption was more complicated than merely simple diffusion. After the war there was great interest in 'active transport', transport 'uphill' against a concentration gradient, or, more strictly, an electrochemical gradient. Merely as a test of the viability of our intestinal segments, it was decided to see if they were capable of sustaining transport of glucose from the bulk phase of the lumen into the serosal fluid, the initial concentration in both sides being the same. We were soon able to show that such transport did occur, while at the same time the glucose concentration in the lumen fell below, and that in the serosal fluid rose above, the initial value. These concentration changes were observed in the presence of substantial fluid transport from the luminal to the serosal fluid. Galactose produced similar findings, except that the fluid transport was much less.[46] The stimulatory effect of glucose on fluid transport was examined later in experiments with Wingate.[47] A finding of importance in the glucose experiments was that the quantity that disappeared from the lumen greatly exceeded the amount that appeared in the serosal fluid. Measurement of the glucose retained in the intestinal wall at the end of the experiment did not account for this discrepancy. Later T H Wilson of Harvard, who had been working in Sheffield and had visited our lab in Oxford, showed that lactate was a significant metabolic product of glucose during its absorption from the intestine. This was of great importance in the disposal of absorbed carbohydrate, lactate being an excellent substrate for hepatic glycogenesis.[48] Nowadays, it is recognized that extrusion of two moles of lactate from one mole of absorbed glucose has consequences for enterocyte volume regulation. In experiments with galactose it was easy to show that the quantity absorbed from the lumen could be accounted for by the amounts appearing on the other side in the serosal fluid and by accumulation in the intestinal wall. It was not metabolized.

---

[45] op. cit. note 9.

[46] Fisher R B, Parsons D S. (1950) Glucose absorption from surviving rat small intestine. *Journal of Physiology* 110: 281–293. *idem* (1953) Glucose movements across the wall of the rat small intestine. ibid. 119: 210–223. *idem* (1953) Galactose absorption from the surviving small intestine of the rat. ibid. 119: 224–232.

[47] Parsons D S, Wingate D L. (1961) The effect of osmotic gradients on fluid transfer across rat intestine *in vitro*. *Biochimica et Biophysica Acta* 46: 170–183. *idem* Changes in the fluid content of rat intestine segments during fluid absorption *in vitro*. ibid. 184–186.

[48] See note 66.

A second test of the integrity of the circulated segment was to compare the cellular structure before and after absorption had occurred. This was satisfactory, the absorbing segments being characterized by the presence of dilated lymphatics in the villus cores, a sign indicative of raised tissue fluid pressure, as had been shown by Florey and colleagues in the Sir William Dunn School of Pathology in Oxford.[49] Another finding in both absorbing and control tissue was that at the tips of the villi degenerating cells could be seen, some in the process of desquamation. A search of the literature revealed that this was a natural phenomenon, the mucosal cell population continually being renewed by cells migrating up the villi surface and discarded at the tips. This was first described in the 1880s by Bizzozero and must be one of the earliest accounts of what is now called apoptosis.[50]

About this time there was a great interest in understanding the nature of the systems underlying sugar uptake in tissues. At the Department of Physiology at St Mary's Hospital in London, glucose uptake into erythrocytes was being measured by following the volume changes by nephelometry. W F Widdas was able to show that in this case the kinetics could be accounted for if the glucose molecules were transported by a small number of 'carrier' systems, each with a relatively high turnover number.[51] As with enzyme systems, the reaction had a maximum transport rate (V_{max}) and an affinity for the glucose measured as the concentration required to give half the V_{max}, which was called K_t. These experiments were going on at about the time that Fisher and I examined the kinetics of glucose and galactose transport and of the competition for transport between sugars. I cannot now remember the dates,[52] but we certainly became aware of Widdas's work.

The received wisdom of that time, at least as far as teaching was concerned, was that the products of digestion of starch were glucose molecules and of proteins, amino acids. But if we had read Starling and Cohnheim, we would have known otherwise.[53]

---

[49] Pullinger B D, Florey H W. (1935) Some observations on structure and functions of lymphatics: their behaviour in local oedema. *British Journal of Experimental Pathology* 16: 49–61.

[50] Bizzozero G. (1888) Über die regeneration der elemente der schlauchförmigen drüsen und des epithels des magendarmkanals. *Anatomischer Anzeiger* 3: 781–784. *idem* (1889) Über die schlauchförmigen drüsen des magendarmkanals und die beziehungen ihres epithels zudem oberflächenepithel der schleimhaut. Erste Mittheilung. *Archiv für Mikroskopische Anatomie* 33: 216–246. Information provided by Dr Richard Boyd, 3 May 2000.

[51] Widdas W F. (1954) Facilitated transfer of hexoses across the human erythrocyte membrane. *Journal of Physiology* 125: 163–180.

[52] Professor Michael Gardner wrote: 'Fisher and Parsons reported kinetics for glucose and galactose transport in 1953 (op. cit. note 46).' Note on draft transcript, 28 April 2000.

[53] See, for example, Starling E H. (1898) The mechanisms of the secretion of urine. In Schaefer E A. (ed.) *Textbook of Physiology*. Edinburgh: Pentland, 639–661. *idem* (1899) The glomerular functions of the kidney. *Journal of Physiology* 24: 317–330. op. cit. note 164.

> In spite of its limitations the recirculated intestinal segment was a very useful and stable preparation. Provided that glucose in the lumen was kept above 20 mmol/l, the preparation could survive and for demonstration purposes segments would continue to absorb for over three hours. However, special apparatus was required, it took time to learn the procedure, and most people took the easy way out by using, later on, everted sacs. For these to work, the incubation flask had to be shaken vigorously which can eventually cause damage if the experiment continues too long.
>
> Fisher had lots of ideas, some very good, but he wasn't a very good experimentalist, and he loved complicated apparatus. I liked to simplify. However, Fisher was a marvellous statistician. At the end of 1951, I left Fisher and moved to my own lab, and started to work using recirculated segments of rat intestine *in vivo*, looking at the effects of fluid transport on absorption and on acid–base changes during fluid absorption.[54] Later my continuing interest in fluid transport produced more *in vitro* experiments which, with David Wingate, showed that from a Ringer of particular osmotic pressure in the lumen, fluid could be transported across the intestinal wall into a fluid of lower osmotic pressure than that in the lumen. But the presence of glucose in the system assisted this transport greatly.'[55]

So that is Dennis Parsons's view of experiments carried out some 50 years ago. Like you, I think they were seminal experiments. It is interesting to see, running in parallel, the relevance of Richards's work in the kidney and of Widdas's work on the erythrocyte.[56]

To get the ball rolling I would like to move on and ask some questions. One obvious one is, why wasn't sodium-coupled glucose transport discovered in the UK and, more specifically, why wasn't it discovered either in Sheffield or in Oxford? Of course, the seminal work that led to our key modern view of how sodium-coupled glucose transport occurs was a model drawn by R K Crane and colleagues, published in an extraordinarily interesting symposium which was held in Prague in 1960.[57] It was put together by A Kleinzeller and was held at the height of the Cold War. At that symposium were a galaxy of young and later eminent scientists including two (Jens Christian Skou and Peter Mitchell) who later received Nobel Prizes. From the intestinal transport field Crane was the only participant, and he talked on the

---

[54] See note 61. op. cit. note 129.

[55] op. cit. note 47.

[56] op. cit. notes 38 and 51.

[57] Crane R K, Miller D, Bihler I. (1961) The restrictions on possible mechanisms of intestinal active transport of sugars. op. cit. note 7, 439–464. See also Crane R K. (1962) Hypothesis for mechanism of intestinal active transport of sugars. *Federation Proceedings* 21: 891–895.

structural features of hexoses required for transport. As an addendum, added after the meeting, he included the model, which for the first time shows the notion of flux coupling, sodium moving through a transport molecule in the same direction as glucose. There are a number of features of the model that nowadays are obviously problematic (for example, where he put the sodium pump). But without doubt this model, originally proposed at this obscure symposium in 1960, continues to dominate thinking right through to 1999. I would like to ask the question, why wasn't such a model put forward by able people such as Dennis Parsons and his colleagues in the 1950s or David Smyth and his colleagues in Sheffield at a similar time?[58]

Another question relates to the coming together of the field of 'bioenergetics'. This was developing with mitochondrial studies, led, of course, by Peter Mitchell. (Mitchell was also at the 1960 symposium in Prague, where he talked on transport in bacteria.) Why was it that at that time people working on mitochondrial ATP synthesis and chemiosmosis were so completely isolated from individuals working in the field of intestinal transport? Intellectually, that has subsequently emerged as one of the major syntheses in the last quarter of this century. I think it is interesting to examine the issue of how much interaction there was between these two fields at different times.

My third comment is really a very straightforward one. Biology has benefited hugely from interactions with chemists on the one side and physicists on the other. However, it seems to me that people who come from a biochemical background failed to take electricity sufficiently seriously and people who came from a biophysical (electrical) background failed to take biochemistry sufficiently seriously. When Richard [Naftalin] talks about Ussing's contribution in relation to this field, it becomes obvious that indeed he is a key figure.[59] I suspect, however, that the biochemical community were both ignorant of, and a bit frightened by, people who talked about electrical events; similarly I wonder if the biophysicists were not a bit baffled by the chemistry of hexoses!

Turnberg: Thank you very much, Richard. You have thrown up some big questions. Why wasn't glucose–sodium linkage discovered earlier?

Naftalin: I think what Richard said is largely true. One of the things I noticed was that he tended to telescope a certain amount of information [From the floor: Well he only had a quarter of an hour]. One of the key pieces of information which he didn't mention was the linkage between not glucose and sodium, but water and sodium. This was actually nailed by Curran in 1966. He showed, as I am sure we all know, that there is a linear relationship between net sodium movement and water movement.[60]

---

[58] Professor David Smyth FRS (1908–1979) was Professor of Physiology, Sheffield University, from 1946 to 1973, and Emeritus Professor from 1973 to 1979. See Barcroft H, Matthews D M. (1981) David Henry Smyth. *Biographical Memoirs of Fellows of the Royal Society* 27: 525–561.

[59] op. cit. notes 29 and 31.

[60] Curran P F. (1965) Ion transport in intestine and its coupling to other transport processes. *Federation Proceedings* 24: 993–999.

Now at that time I am almost sure that Parsons held the view that water transport was active and that sodium, in part at least, was coupled to water movement and not the other way round. And, in fact, if you look at Curran's experiment where he showed a linear relationship between water and sodium movement, as Parsons, I remember, said, 'Well, how do we know that it's not sodium-coupled water.' Of course, the answer was that you could inhibit the sodium transport with ouabain and that showed that water transport was directly related to sodium movement. From that comment one can see that Parsons either didn't want to recognize that connection or he actually was thinking the wrong way round. There's no great blame, because, in fact, water transport was considered active by Fisher and his contemporaries right up until 1966.[61] So I think that view should be considered and I would like to know what Richard [Boyd] thinks Parsons thought about that?[62]

Holdsworth: I remember visiting Parsons with Tony Dawson in 1962, because we had found that both glucose and galactose stimulated water absorption in human jejunum *in vivo* and we were anxious to have his comments. Parsons had shown that *in vitro* glucose stimulated water absorption but galactose didn't, and at that time he was very keen on the explanation that this was entirely due to the metabolic action of glucose.[63] In 1960 Curran had also demonstrated that active transport of sodium *in vitro* needed glucose, and he too attributed this to the role of glucose in providing metabolic

---

[61] Professor Michael Gardner wrote: 'Note that Fisher's early views on water transport in 1955 [Fisher R B. (1955) The absorption of water and of some small solute molecules from the isolated small intestine of the rat. *Journal of Physiology* 130: 655–664] included a solvent drag mechanism through a paracellular route which pre-empted the current ideas of Pappenheimer and colleagues [Pappenheimer J R, Reiss K Z. (1987) Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. *Journal of Membrane Biology* 100: 123–136. Pappenheimer J R. (1990) Paracellular intestinal absorption of glucose, creatinine, and mannitol in normal animals: relation to body size. *American Journal of Physiology* 259: G290–G299].' Letter to Dr Daphne Christie, 5 November 1999. Dr Dennis Parsons wrote: 'The late Paddy Fullerton (Mr leQuesne) and I showed (*c*. 1953) that at least *in vivo*, there was evidence that solvent drag was involved in the "absorption" of both glucose and xylose. If fluid absorption was zero, then glucose but not xylose was still absorbed. See Fullerton P M, Parsons D S. (1956) The absorption of sugars and water from rat intestine *in vivo*. *Quarterly Journal of Experimental Physiology* 41: 387–397.' Letter to Dr Daphne Christie, 5 April 2000.

[62] Professor Richard Naftalin wrote, in response to information requested by Dr Dennis Parsons and Dr Richard Boyd: 'The question of the manner of coupling of water to Na^+ and glucose flows is still very much an open one. Recently, Zeuthen and collaborators have advanced the interesting view that isotonic water flow in epithelia results from cotransport of water with Na^+ and glucose via the Na^+/glucose cotransporter [Loo D D F, Zeuthen T, Chandy G, Wright E M. (1996) Cotransport of water by the Na^+/glucose cotransporter. *Proceedings of the National Academy of Sciences of the United States of America* 93: 13367–13370]. ...As the cotransporters have a high *passive* water permeability, the process of sugar-driven water transport could be related to osmosis rather than cotransport. It would seem important to test this possibility by observing bi-directional water flows in the cotransport condition, as Meinild and colleagues (1998) have done with water flows to eliminate the possibility that sugar accumulation in "hot spots" within the oocyte cortex is not a cause of water flow. Spring has recently expressed some reservations about water cotransport [Spring K R. (1999) Epithelial fluid transport – a century of investigation. *News in Physiological Science* 14: 92–98]. He bases his criticism on firstly the absence of pinocytic vesicles in transporting epithelia – perhaps not surprising, as no claims were made for pinocytosis as the mechanism. Additionally, cotransported water would have only a small effect on fluid absorption in the proximal tubule (< 2 per cent of GFR) – this, of course, does not have much bearing on whether the process exists or not. Thirdly, he prefers the Curran three-compartment model referred to earlier.' Edited from a letter to Dr Daphne Christie, 14 April 2000.

[63] op. cit. notes 12 and 46.

substrate for the intestinal mucosa.[64] Conversely, in 1958, Ricklis and Quastel had shown, I think for the first time, that glucose transport *in vitro* was dependent on the presence of sodium.[65] Crane followed up this observation in the detailed study of the kinetics of sugar absorption at different concentrations of sodium which led to his hypothesis published in 1960.[66]

Peters: I am not sure that this is directly relevant, but I think we need to think of the environment in which Bob Crane was working in Missouri in the late 1940s. He was working for the two Coris who had just got the Nobel Prize.[67] In the lab at the same time was Earl Sutherland, who you may remember got a Nobel Prize for the discovery of cyclic adenosine monophosphate (cAMP), and Christian de Duve, also a Nobel Laureate, whom I worked with, was working on glucose 6-phosphatase. He discovered the lysosome because he was using acid phosphatase as a control for subcellular fractionation studies. So in that lab there was a lot of work going on, on the bioenergetics, on the cell biology, physiology and biochemistry of carbohydrate metabolism. I think that was probably part of the reason for Crane's discovery. The critical mass – not just one person working on his own, but several people looking at several aspects of carbohydrate metabolism from several different angles – provided a unique scientific environment.

Turnberg: Perhaps part of the reason was that he was working almost entirely *in vivo*, so that it was very difficult to get down to low sodium concentrations to study the effect of sodium on glucose transport. So the link may not have been very obvious.

Peters: It's actually difficult *in vivo* to demonstrate sodium dependence of glucose transport; you really have to have negligible levels of Na^+ in the control perfusates.

Levin: I came to Sheffield in 1959. I remember that particular conference.[68] The story was that the model of sodium linked with glucose carrier was prepared on the aeroplane going back, drawn on the back of an envelope, talking in the plane with somebody, and that's how it came about. Whether that's true or not I don't know, but

---

[64] Curran P F. (1960) Na, Cl, and water transport in rat ileum *in vitro*. *Journal of General Physiology* 43: 1137–1148.

[65] Ricklis E, Quastel J H. (1958) Effects of cations on sugar absorption by isolated surviving guinea pig intestine. *Canadian Journal of Biochemistry and Physiology* 36: 347–371.

[66] Crane R K. (1960) Intestinal absorption of sugars. *Physiological Reviews* 40: 789–825. *idem* (1960) Studies on the mechanism of the intestinal absorption of sugars. III. Mutual inhibition *in vitro*, between some actively transported sugars. *Biochimica et Biophysica Acta* 45: 477–482. *idem* (1962) op. cit. note 57. Dr Derek Holdsworth wrote: 'As far as the production of lactate during glucose absorption is concerned, this is really an artifact of the *in vitro* preparations. We found that lactate levels in human subjects rose minimally or not at all in mesenteric blood obtained at laparotomy when glucose was instilled into the jejunum, or when portal venous collaterals were sampled during glucose absorption [Holdsworth C D, Dawson A M. (1965) Fructose absorption in man. *Proceedings of the Society for Experimental Biology and Medicine* 118: 142–145].' Note on draft transcript, 25 October 1999.

[67] Professor Carl Cori (1896–1984) was Professor of Biochemistry, Washington University School of Medicine, St Louis, MO, from 1931 to 1967. He shared (with Gerty Cori) the 1947 Nobel Prize in Physiology or Medicine, for their discovery of the course of the catalytic conversion of glycogen. Professor Gerty Cori (1896–1957) was Professor of Biochemistry, Washington University Medical School, St Louis, MO, from 1947. See Houssay B A. (1956) Carl F and Gerty T Cori. *Biochimica et Biophysica Acta* 20: 11–16.

[68] The Symposium held in Prague in 1960 discussed by Dr Richard Boyd on page 17. op. cit. note 57.

it certainly wasn't at the conference.

Turnberg: Richard, do you want to comment?

Boyd: I really haven't got much to say except that I think Richard [Naftalin] has put his finger on one really important thing about cause and effect. There is an editorial in the *Lancet* in 1978 (which I suspect may have been written by somebody in this room), which states that the discovery that water absorption was linked to sodium and glucose absorption was one of the major medical advances of this century because it opened the door to oral rehydration therapies.[69] I think Richard has put his finger on a central problem (cause and effect) which is really not trivial. I don't think that this issue for the relationship between water and solute absorption was solved in the UK.

With respect to the important comment on the environment that Crane was working in at St Louis, I would disagree with the word 'bioenergetics'. I think what people knew about in the St Louis environment was excellent biochemistry; but I don't think they knew enough about membrane potential; and, with hindsight, I think that was the important weakness of the 'Crane hypothesis' as originally presented.

Professor Ramsey Bronk:[70] One point about the measurement of water movement and the transport of glucose and sodium is again that people in the 1950s and the early 1960s did not recall the things that A N Richards had said in earlier decades about water absorption in the kidney tubule.[71] If they had realized that water transport was driven by the osmolarity changes across the wall of the kidney tubule, they would have appreciated the fact that changes in osmolarity were also involved in water transport in the intestine.

Turnberg: The work on the red cell. When was that? [From the floor: 1953].[72]

Naftalin: Crane was able to look at the kinetics because he had a method to do it, fortunately. Looking at glucose transport in rats is incredibly laborious, as those of us who have done it know, whereas looking at it with rings, which is what Crane did, is relatively easy and you could, in fact, look at kinetic time dependence concentration of methods with ease. It would take many, many rats to do that sort of thing. So one has to look at the methodology, methodology is very important, and I think you are right.

Turnberg: I think we must move on, but no doubt this will come back to the everted sacs and the Ussing chambers, which we are going to hear a bit about now from Roy Levin.

---

[69] Anonymous. (1978) Water with sugar and salt. *Lancet* ii: 300–301.

[70] Professor J Ramsey Bronk (b. 1929), after graduating from Princeton University in 1952, went to Oriel College, Oxford University, on a Rhodes Scholarship as a research student in Dr R B Fisher's laboratory and completed his DPhil in biochemistry in June 1955. He worked as a research scientist at the National Institutes of Health in Bethesda, MD, until 1958 and was a member of the academic staff in the Department of Zoology at Columbia University from 1958 to 1966, spending the 1964–1965 academic year in Dr D S Parsons's laboratory in Oxford as a Guggenheim Fellow. In 1966 Bronk became the first Professor of Biochemistry at the University of York and continued to work on intestinal transport, becoming an Emeritus Professor in 1997.

[71] op. cit. note 38. Richards A N. (1938) Processes of urine formation. *Proceedings of the Royal Society of London* 126: 398–432.

[72] op. cit. note 51.

Levin: I came to Sheffield University, as I said earlier, in 1959, and Gerald Wiseman[73] had been in the department for over ten years. He couldn't manage to come down today but I went and talked to him for a couple of hours and also recorded his comments about how it all started. So I will say a little bit about the everted sac first of all.

Professor David Smyth[74] advertised for a lecturer in 1948 and Gerald Wiseman, who had done medicine in University College London, came up for an interview. Within three days he got the letter saying, 'Yes you are in, you are the Lecturer in Physiology'.

He started to work with Smyth, and at the time, of course, Krebs was there, in the Department of Biochemistry.[75] They had a very active Biochemistry Department at Sheffield. Physiology was about five or six strong, not particularly well known, but the Biochemistry Department was extremely well known and everybody knew Krebs. Everybody has forgotten now that Krebs worked and did the 'cycle' work in Sheffield, not in Oxford.[76] Wiseman worked with Smyth on trying to get information about the citric acid cycle in the dog's hind legs. It wasn't very successful.

As a medical student he had always thought that the idea that things were absorbed across the intestine by passive diffusion was absolute rubbish, but he couldn't prove it. At Sheffield he was surrounded by people who were doing estimations of amino acids with enzymes from bacteria, in the Department of Biochemistry which he said he was in most of the time, and hardly in Physiology at all. At the time (you must realize this was the late 1940s, or early 1950s), you couldn't estimate D- and L-amino acids very effectively. Moreover, you needed about a gram to do it! All of a sudden he came across people who said, 'Yes, I've got an enzyme, it will only take you six months to prepare it, and you can then estimate the L- and the D-forms'. So he left working with the dog's hind legs and started to do absorption of amino acids. He prepared the powders, which were enzymes of course, and estimated the amino acids by manometry. Gibson, who was present in the department, had told him how to do manometry and estimate the amino acids.[77]

---

[73] Dr Gerald Wiseman (b. 1923) was at University College London and University College Hospital, London, from 1942 to 1947. He joined the staff of the Physiology Department, Sheffield, in 1948 and retired as a Reader in Physiology in 1989. He is currently Honorary Lecturer in Biomedical Science, Sheffield. His main research was in intestinal absorption of amino acids, sugars and peptides and he gave his first description of active absorption of amino acids in the early 1950s. With Thomas Hastings Wilson, he introduced the sac of everted intestine technique. op. cit. notes 7, 33, 77 and 82. See also Figure 3. Copies of the tapes and transcript of an interview with Dr Gerald Wiseman, conducted by Dr Roy Levin on 5 February 1999, will be deposited with the records of this meeting in the Contemporary Medical Archives Centre of the Wellcome Library.

[74] See note 58.

[75] Sir Hans Adolf Krebs Kt FRS FRCP (1900–1981) was Lecturer in Pharmacology, University of Sheffield, from 1935 to 1938, Lecturer, Department of Biochemistry, University of Sheffield, from 1938 to 1945, and Professor from 1945 to 1954. He was Whitley Professor of Biochemistry, and Fellow of Trinity College, Oxford, from 1954 to 1967. He shared (jointly with Fritz Lipmann) the 1953 Nobel Prize for Physiology or Medicine for the discovery of the citric acid cycle (Krebs cycle). See Kornberg H, Williamson D H. (1984) Hans Adolf Krebs. *Biographical Memoirs of Fellows of the Royal Society* 30: 351–385.

[76] See note 75.

[77] Gibson Q H, Wiseman G. (1951) Selective absorption of stereo-isomers of amino-acids from loops of the small intestine of the rat. *Biochemical Journal* 48: 426–429.

At the time David Smyth wasn't involved in intestinal absorption and Gerald [Wiseman] went to that particular Physiological Society Meeting.[78] So he saw the Fisher and Parsons apparatus and thought that he could make a much better apparatus; that one was too complicated. So he made the Wiseman preparation which used three pieces of intestine, and he started to estimate amino acids. He said that at the time, because they were a small department, everybody talked with everybody else and met at the bar. He was walking past a laboratory, which was actually that of Bob Davies – a biochemist. He was looking at frogs' stomachs in manometers and incubating them and measuring acid secretion.[79] What attracted Gerald's attention was the fact that the manometers were being gassed with rubber tubes and as he was doing manometry, he thought, 'What on earth is he doing?' So he popped into the laboratory and said to him, 'Why are you gassing it?' and Davies said, 'Well, I have got stomachs inside'. Gerald said, 'Can you keep the stomachs alive?' and he said, 'Oh yes! They go on for a long time'. And then he thought, 'Well that's a good idea, maybe I should, in fact, take pieces of intestine and keep them alive outside of the body like Fisher and Parsons did.' The real problem was that he wanted to show that the amino acids could be pumped against concentration gradients, so he thought at the time (and it wasn't to do with oxygenation), 'If I turn the intestine inside out I will have a small serosal fluid volume where I could pick up quite a large degree of change in the concentration.' So he had a method for estimating amino acids. He had a very good glass blower, Mr Hadfield, in our department, who could make practically anything in glass that you wanted. Wiseman was surrounded by biochemists who helped him along. He had Krebs, who was a father figure to him. Krebs thought of him as a son and as the 'blue-eyed' boy in the Department of Physiology.

At the time in America, Tom Hastings Wilson[80] had just finished medicine. He didn't like medicine very much and decided, because his father, I think, was a Professor of Biochemistry, that he wanted to do purine absorption in the intestine and wanted to do a PhD with Krebs. He wrote to Krebs, and Krebs replied, 'Sure, come across'. So Tom Hastings Wilson came across to Sheffield to work with Krebs. He said he wanted to do purine absorption and Krebs said, 'I know nothing about purines, I know nothing about absorption, but there's a bright young man in physiology who's working on amino acids, go across and see him'. So Tom Hastings Wilson went to Gerald [Wiseman] and Gerald said, 'Well, I don't know anything about purines and absorption either, but I do know about amino acids and we are using this preparation'. That's how Tom Hastings Wilson and Gerald got together and developed the 'everted sac' (Figure 3).[81]

They worked with all sorts of animals. Gerald told me they did pigeons, rats, mice and hamsters, and in the end, because the sacs had to be shaken quite a lot to get good

[78] Dr Gerald Wiseman wrote: 'The meeting held in Oxford in 1949.' Note on draft transcript, 31 March 2000. See page 5, op. cit. note 12.

[79] Davies R E. (1948) Hydrochloric acid production by isolated gastric mucosa. *Biochemical Journal* 42: 609–621.

[80] See notes 14, 21 and 81.

[81] Some of the early work on intestinal absorption is given in Wilson T H. (1962) op. cit. note 13.

Figure 3. Dr Gerald Wiseman (left) and Dr Thomas Hastings Wilson (right) holding an enlarged photograph of a sac of everted small intestine of the hamster. This photograph was taken in 1973 in Wilson's laboratory at Harvard Medical School in Boston, Massachusetts, USA. © Dr Gerald Wiseman, 2000.

oxygenation, they had the problem of cell shedding, knocking off the mucosa. They found out that the best sacs and the ones that got damaged the least, were those from the golden hamster.[82] That was interesting in itself, because this hamster had only begun to come into Britain as an experimental animal, because they were found in Syria originally under a stone. I think there were about half a dozen hamsters, and they were brought across from what I shall call Israel, but what people will probably still say is Palestine. It was Krebs who actually imported the hamsters into England. These hamsters really were a relatively new experimental animal in those days and only just begun to be used, and he actually bred his own colony of hamsters. So again, it was the ability of choosing the right animal for a particular task.

It all started, I suppose, by the conglomeration of the right people at the right time. The amino acids were difficult to come by and were donated generously by a firm called Lights which doesn't exist now. It was a Sheffield-based firm and they gave Wiseman £250 worth of amino acids, which probably represents a gift of about £5000

[82] Dr Gerald Wiseman wrote: 'Golden hamster (*Mesocricetus auratus*).' Note on draft transcript, 31 March 2000. See, for example, Wilson T H, Wiseman G. (1954) op. cit. note 33. *idem* Metabolic activity of the small intestine of the rat and golden hamster (*Mesocricetus auratus*). *Journal of Physiology* 123: 126–130. Wilson T H, Vincent T N. (1955) Absorption of sugars *in vitro* by the intestine of the golden hamster. *Journal of Biological Chemistry* 216: 851–866. For comment on the introduction of hamsters into laboratory research see note 93.

to £10 000 nowadays. There were few grants in those days; nobody bothered with the Medical Research Council or the Science Research Council. Everybody funded themselves from the meagre funds that the departments had.

The key technique to begin with was the ability to estimate the D- and L-amino acids, and that was, I suppose, the beginning of the everted sac which then obviously had such a huge worldwide impact. The everted sac allowed you to show active transport for the first time, it was relatively easy to prepare, it allowed you to demonstrate fluid transfer and, of course, you could show transfer from the fluid outside to the fluid inside. It also allowed you to study the metabolism; you incubated the sac with the various compounds and then estimated how much was left, and it allowed you to control the ionic concentration.

To answer your [Richard Boyd] question.[83] I don't know why Sheffield didn't actually make that discovery of the link between Na^+ and hexose transfer, because Sheffield was the centre of intestinal absorption. For some reason people didn't take sodium out of the incubation fluid and link it together; I think they were too busy doing other things at the time. They weren't kinetic people at that particular time, although, to be fair, David Smyth later turned to kinetics.

After getting nowhere with the dog's hind legs, David Smyth decided to pack it in. Wiseman went to work in the States for a year, came back, and David Smyth said to him, 'Look, I've packed in the dog and I am going to work on the absorption of glucose, is that all right? You're doing amino acids and I'll do hexoses.' Gerald Wiseman said, 'Yes'. I think he couldn't very well say anything else could he, he was the Lecturer! So David Smyth started to work on the intestine and, of course, built up a large school.[84] I was one of the people that came in and worked with him on hormones and intestinal absorption. In the end, of course, he got the FRS and Gerald Wiseman didn't. I suppose these things happen in science and in universities.

I think the reason that the sac started to get supplanted by the sheet, was that it wasn't so easy to measure electrical activity across the sac. You could do it, but it wasn't easy to control, especially if you wanted to obtain the short-circuit current. You had to have a silver coil around the sac, have a central silver electrode and it was far from easy to set up. Silver seemed to poison the sac and it didn't do too well. I think that was the end of it.

But there was a man, Shabtay Dikstein, a pharmacologist, who came over to visit the [Sheffield] department from Israel. He was one of those incredible characters who had a mind that could literally do anything in about one-and-a-half days. He said, 'Let's

---

[83] See page 18.

[84] Dr Roy Levin wrote: 'Dr Brian Taylor worked on ions and fluid movement, Dr Roy Barry worked on fatty acid transfer, Dr Leslie Jervis on amino acid absorption and Dr Harry Newey on peptide and amino acid absorption. See, for example, Levin R J, Smyth D H. (1963) The effect of thyroid gland on intestinal absorption of hexoses. *Journal of Physiology* 169: 755–769. Levin R J, Newey A, Smyth D H. (1965) The effects of adrenalectomy and fasting on intestinal function in the rat. ibid. 177: 58–73.' Note on draft transcript, 26 May 2000.

measure the potential difference across the sac'. So he did just that! At the time there was just one abstract on the subject, it was in *Federation Proceedings*.[85] And this was in the early 1960s. I think they had shown that when you add glucose to the mucosal fluid bathing the sac you got an increase in the potential. What Dikstein did in about three days was to add everything to the sac and measure potentials.[86] I think that was one of the early illustrations of the link between sodium and hexoses. Some of the amino acids gave the potential as well. That was, I think, a really good demonstration that ions were being transported across. We had no idea of the sodium–hexose carrier model at the time because Crane hadn't yet taken his aeroplane and drawn on the back of his envelope![87] Gradually, of course, the glucose transfer potential or the hexose transfer potential (as it was later called) became incredibly useful, because you could do kinetic studies on the sacs and also on sheets very, very easily and they became the dominant way, even, with Ernie Wright when he cloned the carrier. They used electrical recording, now it is intracellular with microelectrodes, but they have been using that to show how the hexose goes across the cell to this day.[88]

I just would add one thing about a man called Csáky. He used the hexose, 3-O-methylglucose, which wasn't metabolized but was actively transported.[89] I think that part of the reason that Crane was able to do things was by using sugars which were actively transported but not metabolized. Glucose is very difficult to use, because it is metabolized, galactose messes up the intestine, but by using things like 3-O-methylglucose and D-xylose you could estimate them and do kinetics with them.[90] So I think Csáky shouldn't be forgotten, because I think he first utilized that 3-O-methylglucose which is still used today.[91]

Turnberg: OK. Comments. Memories. Corrections.

---

[85] Schachter D, Britten J S. (1961) Active transport of non-electrolytes and the potential gradients across intestinal segments *in vitro*. *Federation Proceedings* 20: 137.

[86] Barry R J C, Dikstein S, Matthews J, Smyth D H. (1961) Electrical potentials in the isolated intestine. *Journal of Physiology* 155: 17–18P. *idem* (1964) Electrical potentials associated with intestinal sugar transfer. ibid. 171: 316–338.

[87] See page 20.

[88] Professor Michael Gardner wrote: 'Wright's early Sheffield work was on electrophysiology of intestinal transport [see, for example, Wright E M. (1966) The origin of the glucose dependent increase in the potential difference across the tortoise small intestine. *Journal of Physiology* 185: 486–500], work that became relevant in his subsequent cloning studies of the sodium–glucose co-transporter when he was able to demonstrate that glucose induced a similar potential difference in oocytes with inserted cloned carriers as had been observed in the original whole intestinal tissue.' Letter to Dr Daphne Christie, 5 November 1999.

[89] See, for example, Csáky T Z, Glenn J E. (1957) Urinary recovery of 3-methylglucose administered to rats. *American Journal of Physiology* 188: 159–162. Csáky T Z. (1958) Active intestinal transport of 3-O-methylglucose. *Proceedings of the Fourth International Congress of Biochemistry*, 6–79.

[90] Fordtran J S, Clodi P H, Soergel K H, Ingelfinger F J. (1962) Sugar absorption tests, with special reference to 3-O-methyl-d-glucose and d-xylose. *Annals of Internal Medicine* 57: 883–891. Csáky T Z, Ho P M. (1965) Intestinal transport of D-xylose. *Proceedings of the Society for Experimental Biology and Medicine* 120: 403–408.

[91] See, for example, Malo C. (1990) Separation of two distinct Na^+/D-glucose cotransport systems in the human fetal jejunum by means of their differential specificity for 3-O-methylglucose. *Biochimica et Biophysica Acta* 1022: 8–16.

Professor Oliver Wrong:[92] I don't want to impugn Hans Krebs in any way, but at my boarding school in Edinburgh in 1938 or 1939, our Scottish housemaster's daughter had a golden hamster.[93]

Booth: I can remember going back in the 1960s to visit Gerald Wiseman to learn about the everted sac. I hadn't realized at that time that David Smyth had taken it over and that Gerald was really running a rather private laboratory of his own in the department. What I recollect most about it was the water bath that was on a sort of dais in the middle of the laboratory and you had to go up two or three steps to get up to the water bath to put your material inside, almost like approaching an altar. He was tremendously helpful and encouraging.

Professor Michael Gardner:[94] Can I ask if anyone knows why David Smyth returned to the perfused preparation of Smyth and Taylor, because this was, in a sense, revisiting the Fisher and Parsons preparation?[95] It was the first removal, I think, of the serosal fluid which then led on to another generation of perfused preparations, but it seemed to be acknowledging some of the shortcomings of everted sacs then.

Levin: I don't know exactly why some of these things came around, but there was quite an active feeling of trying to get new techniques all the time. There was the

---

[92] Professor Oliver Wrong FRCP (b. 1925) graduated in 1947, trained in Oxford, Boston and Manchester, and worked in clinical problems of salt and water metabolism, mainly in relation to kidney disease but also in connection with the intestinal tract. In the 1960s, while on the staff at the Postgraduate Medical School, London, he introduced the method of *in vivo* faecal dialysis for analysis of stool water. He was Chairman of the National Kidney Research Fund from 1976 to 1980, and retired from the Chair of Medicine at University College London, in 1990, now Professor Emeritus. He is currently working on the genetics of renal tubular disease.

[93] Professor Oliver Wrong wrote: 'I have a vivid memory of this animal running over the carpet and tucking pieces of biscuit into its cheek pads, and it cannot have been later than 1939 as the girl then went away to help with the war effort. The point that I wanted to make is that hamsters had been introduced to the country many years before the studies referred to by Dr Levin (see page 24). It is indeed possible that Krebs was the first to import the animals into England (he came to England in 1933) but the dates don't fit and I suspect the story has been elaborated in the telling.' Letter to Dr Daphne Christie, 29 March 2000. Dr Roy Levin wrote: 'The hamsters of Krebs were golden hamsters.' Note on draft transcript, 26 May 2000. The Syrian hamster was introduced into laboratory research by Dr Saul Adler of the Hebrew University of Jerusalem, in 1931. See Adler S. (1948) Origin of the Golden Hamster, *Cricetus auratus*, as a laboratory animal. *Nature* 162: 256–257. For a review of this claim, and a discussion of the relationship between Syrian and Chinese hamsters, see Yerganiam G. (1972) History and cytogenetics of hamsters in pathology of the Syrian hamster. In Hornburger F. (ed.) *Progress in Experimental Tumor Research*, vol. 16. Basel: S Karger AG, 2–42.'

[94] Professor Michael Gardner DSc FIBiol (b. 1946) worked on intestinal absorption of sugars with R B Fisher in the Department of Biochemistry, University of Edinburgh, from 1969 to 1975, and subsequently on a variety of aspects of the physiological biochemistry of absorption, in Edinburgh and Bradford. These have included investigations on absorption of intact peptides and proteins arising from Fisher's early predictions, and development of the Fisher and Gardner technique for intestinal perfusion [Fisher R B, Gardner M L G. (1974) A kinetic approach to the study of absorption of solutes by isolated perfused small intestine. *Journal of Physiology* 241: 211–234]. Professor Gardner has been Professor of Physiological Biochemistry in the Department of Biomedical Sciences, University of Bradford, since 1996.

[95] A preparation in which no serosal fluid is used, so that the fluid transported by the intestine can be collected, and not diluted with serosal fluid. Smyth D H, Taylor C B. (1957) Transfer of water and solutes by an *in vitro* intestinal preparation. *Journal of Physiology* 139: 632–648. op. cit. notes 12 and 46.

Sheff–Smyth technique where you circulated the fluid through the lumen of the intestine by a gas lift;[96] there was the Smyth and Taylor preparation;[97] they were just going on all at the time, I think, where people were thinking, 'Let's try this, let's try that'. There was a very good glass-blower, Mr Hadfield, and so they used to say, 'See if you can do that'. It was just an active department at the time, but I don't really know why that was the particular one at that particular time. I never really talked to David Smyth about it; it's funny really, because I worked with him for very many years, but we never talked about the early history at all.

Turnberg: The use of the Ussing chamber for the intestine overtook these everted sacs a bit, because you could control the conditions across the intestine rather better, and I think that's what reduced the interest in everted sacs even though they were much easier and quicker to use. I think they were useful for the purpose to which they were put, but then the next technique came along and seemed to allow greater definition of what was going on.

Levin: I would agree that you can put drugs and inhibitors on the serosal surface, but what you can't do with the sheet is measure fluid transfer, especially absorption, very easily. So you have to say, 'Okay, I'm not interested in fluid transfer but only in the electrogenic ion transfer'. I don't understand to this day why you can't obtain really good fluid transfers in sheets as you can in sacs. Possibly the stretch on mounting the sheets activates the intestine's secretory mechanism?

Turnberg: I think Richard Naftalin tried, but it was with a very modified version of Ussing chambers.

Naftalin: Yes. We did. Tripathi and I spent quite a long time looking at water transport in Ussing chambers.[98] In a way I am a bit hesitant to talk about Ussing chambers in the presence of a major Ussing player. However, I was first introduced to Ussing chambers in 1972 when I went to work with Peter Curran and, I have to say, I was horrified. Most of you, I am sure, have worked with these things, but they are very delicate and rather clumsy instruments, frankly, and certainly liable to breakage and not particularly well designed for the rather simple thing that they had to do, which was basically measure the containers for flux experiments. So anyway, I was bemused by watching all these things going on and there was an even more horrific experiment: Curran's lab was looking at unidirectional fluxes. By this time they had understood that looking at bidirectional fluxes of ions and sugars across the sheets wasn't enough. You had to look at uptake across the mucosal surface as well and so by this time Curran's lab was looking at unidirectional flux plus bidirectional fluxes. And to look

[96] op. cit. note 13. See also Fullerton P M, Parsons D S. (1956) The absorption of sugars and water from rat intestine *in vivo*. *Quarterly Journal of Experimental Physiology* 41: 387–397.

[97] op. cit. note 95.

[98] See, for example, Naftalin R J, Tripathi S. (1985) Passive water flows driven across the isolated rabbit ileum by osmotic, hydrostatic and electrical gradients. *Journal of Physiology* 360: 27–50. *idem* (1986) The roles of paracellular and transcellular pathways and submucosal space in isotonic water absorption by rabbit ileum. ibid. 370: 409–432.

at unidirectional flux they had a modified Ussing chamber where they had one side of the tissue exposed to water vapour, and they had to do this in a fairly standard degree room which was highly saturated with water vapour. So it was quite a major effort to do this experiment and it was all done very rapidly over a period of two or three minutes. An enormous amount of preparation went into getting the tissue right and it's quite understandable that this technique didn't really catch on. But a number of people did do it. What I want to say is that the Ussing chamber was adapted to the intestine and that the primary exponent in the 1960s of this technique was certainly Stanley Schultz. His work, I think, more or less laid the foundations for our understanding of ion transport and nature of glucose–sodium coupling in the intestine. Schultz and Zalusky's work in 1964 was without doubt the best quantitative work that there was and probably has been.[99] That was really magnificent work. Frizzell and Schultz then used the Ussing chamber to look at paracellular fluxes using modified short-circuit current technique where the voltage is clamped at different voltages.[100] And they looked at the voltage-independent flux and this technique was full of assumptions and difficulties. What I want to say about the Ussing chamber is that it fulfilled a need in the 1960s. It was a marvellous system for getting quantitative data, net and unidirectional ion fluxes, and organic solute fluxes, but, as Roy has said, it didn't really allow movement of water to be measured accurately. In fact, it didn't measure sodium movement very accurately either, as Parsons and Wade showed in their paper – that there was a large accumulation of water and electrolyte in the extracellular matrix.[101] People had assumed that net flux across the mucosal surface is equal to net flux across the serosal surface and, of course, the assumptions of the short-circuit current are that that is exactly what happens. But Parsons had shown, and Tripathi and I had shown as well, that water flux across the mucosal surface is about five times greater than across the serosal surface and during the time that one is actually measuring net flux, most of the fluids and electrolytes are accumulating in the lamina propria, even in the strip preparation.[102] So by that time I realized that one needed a perfused preparation to bring one back to reality, I certainly didn't want to chase artifacts for the rest of my scientific days, which are numbered, but that didn't prevent a large number of people from going on. In my view, the Ussing chamber of technology became a club, it was a rather exclusive club and if you didn't actually apply the techniques as prescribed, then you were out. That was very much part of the reason why some of the British intestinal physiology was marginalized and why, in fact, America had such a hold over people like you [Turnberg]. So I think one has to view the segmentation of science, the regionalizing of science, very much in terms of who was doing it and how exclusive it was.

---

[99] Schultz S G, Zalusky R. (1964) Ion transport in isolated rabbit ileum. I. Short-circuit current and Na fluxes. *Journal of General Physiology* 47: 567–584. *idem* Ion transport in isolated rabbit ileum. II. The interaction between active sodium and active sugar transport. ibid. 1043–1059.

[100] See, for example, Frizzell R A, Schultz S G. (1972) Ionic conductances of extracellular shunt pathways in rabbit ileum. Influence of shunt on transmural sodium transport and electrical potential differences. *Journal of General Physiology* 59: 318–346. *idem* An overview of intestinal absorptive and secretory processes. *Gastroenterology* 63: 161–170.

[101] Parsons D S, Wade S A. (1982) Influence of vascular and lumen flow on sodium movements across anuran intestine *in vitro*. *Quarterly Journal of Experimental Physiology* 67: 323–324.

[102] Naftalin R J, Tripathi S. (1986) op. cit. note 98.

The other part, which I haven't mentioned, is the secretory story. That also raises lots and lots of problems. For example, one of the major findings in secretion was the inhibition of sodium chloride absorption.[103] Yet nobody has ever been able to see inhibition of sodium absorption in vesicles and nobody has ever been able to see inhibition of sodium chloride absorption in isolated cells. So it is an artifact of unidirectional flux measurement. That's just the fluxes recycled and it became obvious that this was the case when one looked at accumulation. There is a lot to be said against Ussing chambers.

Turnberg: And you have said a lot of it. I think it is a very accurate summary. However, Ussing chambers provided us with an enormous stimulus and a lot of interest. It was only as time went on that it became obvious that as we became more refined in our experimental ideas, it was recognized that we might be chasing artifacts and very interesting artifacts. The technique created many publications during the 1960s and 1970s.[104] And, of course, it was only when one tried to relate what was being found in Ussing chambers to *in vivo* behaviour, that there seemed to be quite a gap between what happened there and what seemed to happen in the Ussing chamber. But they had their uses and they were a considerable boost even though, like all these things *in vitro,* they introduced artifacts.

Dowling: I would like to take advantage of Roy Levin's presence here to ask him about two of the methods which the Sheffield team introduced to the world. First there was the Wilson and Wiseman everted sac technique which contributed so much to our understanding of gut physiology, but it did have problems when applied in certain situations. For example, when I was working with Sir Christopher Booth 35 years ago, we induced adaptive hyperplasia or hypoplasia in the small intestine, by a variety of methods. And when one is studying absorption by everted sacs made from either very thick (hyperplastic) or very thin (hypoplastic) segments of adapted intestine, you get artifacts with Wilson and Wiseman's *in vitro* system.[105] That was one of the reasons why we switched from their *in vitro* technique, to a second method pioneered in Sheffield – Sheff–Smyth's *in vivo* recirculation perfusion system, for our studies of intestinal absorption in the adapting intestine.[106]

Roy, you referred briefly to the Sheff–Smyth technique but I wonder if you could tell us a little more about it. It too was a wonderful system, but it could be fiendishly difficult to apply – particularly if one is doing kinetic studies. You will recall that the recirculation perfusion system includes a reservoir from which one takes samples at regular intervals (every ten minutes for approximately one hour) during the perfusion.

---

[103] Nellans H N, Frizzell R A, Schultz S G. (1973) Coupled sodium chloride influx brush-border of rabbit ileum. *American Journal of Physiology* 225: 467–475. *idem* (1974) Brush-border processes and transepithelial Na^+ and Cl^- transport. ibid. 226: 1131–1141.

[104] op. cit. notes 29 and 31.

[105] Early work includes, Dowling R H, Riecken E O, Laws J W, Booth C C. (1967) The intestinal response to high bulk feeding in the rat. *Clinical Science* 32: 1–9. Dowling R H, Booth C C. (1967) Structural and functional changes following small intestinal resection in the rat. ibid. 139–149.

[106] op. cit. note 13.

This means that the total substrate load presented to the intestine diminishes progressively with time as a result of sampling – as well as absorption. To make the necessary calculations which correct for this is extraordinarily difficult. The Sheff–Smyth technique was a wonderful method but it too had its limitations.

Levin: With the Sheff–Smyth technique, I think that is absolutely true. I mean the way we used to do it; we did it in chickens. I always had my favourite question, whenever people used to come to the department, Professor Smyth used to hand out little pieces of paper with a question, so that any foreign visitor that ever came and talked always had a barrage of questions at the end. He was always very good about that! And there used to be all these questions, and his one always, whatever it was, he always used to say (he stuttered a little bit), 'Ha...have you tried phlorizin?' And no matter what the system it was, phlorizin had to be tried in it. And my particular one was, 'That's very interesting, have you tried it in chickens?' because we were working with chickens. And when we were doing it in chickens the people in the group got their own back at me and said, 'That's very interesting, Roy, have you tried it in hamsters?'! The Sheff–Smyth technique was the technique we used for the kinetics, it took all day to do one kinetic experiment, one K_m, one V_{max} in one animal. That was the problem with it, I think. We used to circulate the solution with the amino acid or the sugar, and then estimate the whole lot after, but there was always the problem of a decreasing concentration. I am not quite sure that it made that much difference to the kinetics. The real weakness I thought of the Sheff–Smyth procedure was the high intraluminal hydrostatic pressure that it imposed on the intestine. On the one hand it made everything get in contact with the villi, but on the other hand it probably inhibited venous return. So what you had with the Sheff–Smyth technique, was, I think, an '*in vitro* preparation *in vivo*'. The blood flow to the intestine was probably reduced but it was kept alive, because the circulatory fluids were oxygenated in the Sheff–Smyth apparatus's gas lift. It was very useful to do lots of things with it, but there's a time with these things, and all of a sudden you know that you can't use the Sheff–Smyth procedure any more. (I have two of them in my drawer in the lab and my wife says they are not coming back into the house and I don't know what to do with these things. Perhaps give it to the Wellcome!) But I think you are absolutely right, I mean some people did use the geometric concentration, if you remember, and I did that and it didn't make any difference if you used the initial concentration. I think you can get too fussy. I always remember what someone once said of Bayliss's son, Leonard,[107] who was in University College and had a most brilliant mind, that he always had 15 reasons for not doing the experiment, brilliant ones, and so he never did any experiments. You can always, I think, look back at these preparations and say, 'Oh, it didn't do this, and it didn't do that'. But at the time, that's how science develops initially, by relatively crude methods. These are constantly refined.

---

[107] Leonard Bayliss (1900–1964) was a physiologist at University College London and well known for his work on the rheology of blood. He was the son of Sir William Bayliss Kt FRS (1860–1924) and nephew of Ernest Starling (1866–1927), both Professors of Physiology at University College London. See Winton F R. (1964) Dr L E Bayliss. *Nature* 204: 327–328.

Dr Peter Williams:[108] I just wondered if anybody knew what Ussing thought about his technique being used for the intestine.[109]

Turnberg: Ussing was using the frog skin and the frog skin is very much thinner than the intestine and doesn't have all the problems that Richard was referring to.

Williams: So it was not his technique?

Turnberg: It was his technique but others just adapted it for intestinal sheets. Then they thought they could improve it by stripping off the serosal layers and finish up with what might be presumed to act like a single layer of cells. Of course it doesn't. Richard Naftalin demonstrated that there are lots of things happening in the bit between one side and the other side. So it had been adapted for another purpose, for which Ussing hadn't envisaged it.

We must move on now to brush-borders. Dr Richard Boyd is going to tell us something about that.

Boyd: Oddly enough, this comes back to the point I was making about how mitochondrial bioenergetics met the field of intestinal absorption, because the key paper in this field, published in 1974, came from Isselbacher's lab where both Murer and Hopfer were working.[110] It was this synthesis, of somebody interested in the gastrointestinal tract and intestinal transport coming together with people who had seen how mitochondrial particles could be used to study the chemiosmotic hypothesis of Mitchell, that generated the very first unambiguous data on flux coupling. I think, therefore, that membrane vesicles, where you could control the composition of the media on either side of a membrane (not of a cell, but of a membrane), and where you could impose electrochemical gradients using ionophores (which were really important as experimental tools, again coming from the mitochondrial field) actually allowed proper, rigorous testing of this model and took it to a completely different level of analysis.[111] Having said that I know there are people here who are aware of some of the inevitable problems of membrane vesicle experiments. In my view, vesicles were nevertheless one of

---

[108] Dr Peter Williams CBE FRCP (b. 1925) was Director of the Wellcome Trust from 1965 to 1991 and Honorary Director, Wellcome Institute for the History of Medicine, from 1981 to 1983. He was President of the Royal Society for Tropical Medicine and Hygiene from 1991 to 1993.

[109] Professor Oliver Wrong wrote: 'Ussing applied his chamber with great success to frog skin, but it is unsuitable for a complex multilayered membrane such as the intestine. Alexander Leaf in Boston used it extensively in his fine studies of solute movement in toad bladder, working out the effects of vasopressin and aldosterone on transport of sodium, water and urea. Leaf particularly made the point that that this membrane, both functionally and morphologically, was only one cell thick. See Dibona D R, Civan M M, Leaf A. (1969) The anatomic site of the transepithelial permeability barrier in toad urinary bladder. *Journal of Cell Biology* 40: 1–7. Leaf A, Sharp G W G. (1971) The stimulation of sodium transport by aldosterone. *Philosophical Transactions of the Royal Society of London* 262: 323–332.' Letter to Dr Daphne Christie, 29 March 2000.

[110] Murer H, Hopfer U. (1974) Demonstration of electrogenic Na^+-dependent D-glucose transport in intestinal brush border membranes. *Proceedings of the National Academy of Sciences of the United States of America* 71: 484–488.

[111] See, for example, Murer H, Cassano G, Stieger B. (1983) Transport of sodium and chloride across the small intestinal brush border membrane: studies with vesicles. In Turnberg L A. (ed.) *Proceedings of the Third BSG.SK&F International Workshop*. Welwyn Garden City: Smith Kline & French Laboratories Limited, 8–12.

the major advances over the last 30 years, allowing one experimentally to get from the cell down to membranes and therefore directly on the path to molecules.

Bronk: I would like to make a different point, if I could. By the time we reached the 1970s it was quite clear that the brush-border apical membrane of the enterocytes was the site of sodium-dependent glucose transport. However, an important feature of intestinal absorption is to recognize that it is not just uptake across the apical membrane, but it is also the exit across the basolateral membrane into the vascular system that is very important. One of the innovations that Dennis Parsons and Peter Hanson were able to develop was the vascular perfusion preparation for rat intestine.[112] A colleague that I had in York, Peter Ingham, and I used the Hanson and Parsons preparation to show the sodium dependence of D-galactose uptake, and the fact that galactose was not accumulated within the intestinal mucosa unless galactose was perfused through the vascular side.[113] The exit of galactose across the basolateral membrane of the enterocytes was shown to be a passive process down a concentration gradient. With the vascular perfusion preparation we were able to show that although uptake across the apical membrane was sodium dependent, the exit across the basolateral membrane was not; also the uptake was phlorizin sensitive but the exit was not sensitive to phlorizin. In order to consider the absorption of a solute in the small intestine from the lumen to the vascular bed, it is necessary to investigate both uptake and exit of the solute from the enterocytes.

Dr Peter Hanson:[114] I would like to make some general comments about preparations. In the Parsons's laboratory, the vascularly perfused frog preparation came before vascularly perfused rat intestine. Vascularly perfused frog intestine is a much superior preparation in many respects. I think one lesson that perhaps wasn't used was that often lower organisms would have been a better bet than higher organisms to study basic transport mechanisms.

The other comment I have is that a number of us tried to use isolated intestinal epithelial cells to study transport mechanisms and to study metabolism and, with the exception of an American called Kimmich who used chick cells,[115] these were uniformly very unsatisfactory. I think we know now why they were unsatisfactory; it is that these cells were undergoing apoptosis, because detachment of epithelial cells from the basement membrane actually leads to apoptosis and this is quite a rapid process.

---

[112] Hanson P J, Parsons D S. (1976) The utilization of glucose and production of lactate by *in vitro* preparations of rat small intestine: effects of vascular perfusion. *Journal of Physiology* 255: 775–795. *idem* (1977) Metabolism and transport of glutamine and glucose in vascularly perfused small intestine rat. *Biochemical Journal* 166: 509–519.

[113] Bronk J R, Ingham P A. (1976) Evidence for carrier-mediated uptake and efflux of sugars at the serosal side of the rat intestinal mucosa *in vitro*. *Journal of Physiology* 255: 481–495. *idem* (1979) Sugar transfer from the lumen of the rat small intestine to the vascular bed. ibid. 289: 99–113.

[114] Dr Peter Hanson (b. 1949) graduated from Oxford University in Biochemistry in 1971, and was a PhD student and then MRC Research Assistant in Dr D S Parsons's laboratory (1992–1997) where he worked on metabolism in rat small intestine. From 1977 he has been at the Pharmaceutical Sciences Institute, Aston University, Birmingham, working on signal transduction in gastric mucosa.

[115] Kimmich G A. (1970) Preparation and properties of mucosal epithelial cells isolated from small intestine of the chicken. *Biochemistry* 9: 3659–3668.

Turnberg: Why did it work for the chicken?

Hanson: I really have no idea.

Bronk: With respect to apoptosis, I had a research student in my laboratory who did electron microscopy of isolated mucosal cells from the small intestine. One finding that shocked us was the fact that these cells were filled with apoptopic vesicles. I have no idea what triggered these changes.

Peters: Perhaps preceding these electrophysiological studies were the experiments when people were trying to isolate brush-border vesicles. I think the work of Miller and Crane isolating brush-borders in the early 1960s, and of Alec Eichholz trying to isolate brush-border subcomponents led to a greater understanding of their function.[116] At the same time George Hübscher in Birmingham was also developing methods for isolating brush-borders.[117] But I think the important step was when people moved from brush-borders to the isolation of membrane vesicles and again that was done in Crane's lab in Rutgers. The interesting observation was that if you made a microsomal preparation from enterocytes you got a mixture of brush-border vesicles, basolateral membrane vesicles, endoplasmic reticulum, etc., and the trick of adding Ca^{2+}, which precipitated all membranes, except brush-border vesicles, allowed people to isolate fragments of the brush-border that allowed the studies to be carried on that have been described. So I think in parallel with electrophysiological and the *in vivo* studies, was subcellular fractionation, and Robert Crane again made major contributions in that area.

Naftalin: I would like to ask two vesicle people whether they think that vesicle technology actually contributed anything very much to our knowledge of intestinal mechanisms?

Boyd: I think it has allowed us to test the Crane hypothesis and show that it was right. Until then, I don't think you could. Certainly there is that wonderful paper by Kimmich, using the preparation that Peter [Hanson] has alluded to, disproving the Crane hypothesis published in the *Journal of Membrane Biology* in 1973.[118] What these experimentalists had forgotten is that if you impose a reversed sodium gradient (making the cells full of sodium), you switch on the sodium pump, which is electrogenic; these cells probably had a membrane potential of –120mV so they were markedly hyperpolarized. It was a failure to realize that electrochemical gradients are not synonymous with chemical gradients that led to a conclusion that was spectacularly wrong. You couldn't do that experiment unambiguously with isolated cells but you could with membrane vesicles.

---

[116] Miller D, Crane R K. (1961) The digestive function of epithelium of the small intestine. 1. An intracellular locus of disaccharide and sugar phosphate ester hydrolysis. *Biochimica et Biophysica Acta* 52: 293–298. Eichholz A, Howell K E, Crane R K. (1969) Studies on the organization of the brush border in intestinal epithelial cells. VI. Glucose binding to isolated intestinal brush borders and their subfractions. ibid. 14: 179–192. Eichholz A, Crane R K. (1974) Isolation of plasma membranes from intestinal brush borders. *Methods in Enzymology* 31: 123–134.

[117] See, for example, Hübscher G, West G R, Brindley D N. (1965) Studies on the fractionation of mucosal homogenates from the small intestine. *Biochemical Journal* 97: 629–642.

[118] Kimmich G A, Randles J. (1973) Interaction between Na^+-dependent transport systems for sugars and amino acids. Evidence against a role for the sodium gradient. *Journal of Membrane Biology* 12: 47–68. op. cit. note 66.

I think they also had a role (and this is related to Ramsey Bronk's comment) in confirming directly that the input face and the output face, the apical membrane and the basal membrane of the epithelium, had quite different transport properties. This provided a direct test of the Ussing model of epithelial polarity in the small intestine.

Naftalin: These asymmetric properties had been recognized at least ten years before. Crane had done all that work in sheets. So the recognition that this transport system is different was quite extant in 1970.[119]

Turnberg: But not proven.

Naftalin: Well, what is proven?

Turnberg: You take the basolateral membrane and the apical membrane and show that they behave in the ways you've predicted.

Naftalin: Well, I agree that that's an advance, but not an advance in knowledge.

Gardner: Another area in which vesicles I think were useful, albeit a bit later on, was in proving proton-linked mechanisms for peptide transport.[120]

Turnberg: Any other comments? In which case we will move on. I have been asked to introduce electrolyte transport. Because this is going to be a personal view, it will, I'm afraid, be somewhat biased. I am going to concentrate on the human *in vivo* work, because I want to bring in John Fordtran and the people who worked with him as well as others who were working at about the same time, with the whole human gut. I think that he is a very important figure in the field, because he used what is an enormously crude technique of taking the whole human being and putting a tube down the gut, perfusing and making some observations which seemed to be of importance and, I think, that was an unusual thing to be able to do. He had been working with Ingelfinger in the 1950s and they had developed the 'triple lumen tube'. That tube allowed them to control the perfusate that was going down the intestine. Up to that time, if you used a double lumen tube, you had fluid coming in from the stomach and the pancreas and whatever else, to contaminate the fluid you infused, so you had really little idea of what the content was of the fluid that was going into your segment.[121]

---

[119] See, for example, Bihler I, Cybulsky R. (1973) Sugar transport at the basal and lateral aspect of the small intestinal cell. *Biochimica et Biophysica Acta* 298: 429–436. Naftalin R J, Curran P F. (1974) Galactose transport in rabbit ileum. *Journal of Membrane Biology* 16: 257–278.

[120] See, for example, Ganapathy V, Leibach F H. (1983) Role of pH gradient and membrane potential in dipeptide transport in intestinal and renal brush-border membrane vesicles from the rabbit. Studies with L-carnosine and glycyl-L-proline. *Journal of Biological Chemistry* 258: 14189–14192.

[121] See, for example, Miller T G, Abbott W O. (1934) Intestinal intubation: a practical technique. *American Journal of the Medical Sciences* 187: 595–599. Blankenhorn D H, Hirsch J, Ahreus E H. (1955) Transintestinal intubation: technique for measurement of gut length and physiologic sampling at Kurcon loci. *Proceedings of the Society for Experimental Biology and Medicine* 88: 356–362. Fordtran J S, Rector F C, Locklear T W. (1967) Water and soluble movement in the small intestine of patients with sprue. *Journal of Clinical Investigation* 46: 287–298. op. cit. note 146. Intubation methods for humans are also described in Wilson T H. (1962) (ed.) Methods, ch. 2. op. cit. note 13, 27–28.

Using the triple lumen tube, it became possible to sample fluid at the entry point to the perfused segment of intestine. So that was the advance. Of course, that whole system has been criticized and I think it was Bob Shields, who's not here today, who showed a picture of the human intestine at laparotomy, all 'concertinaed' up. So, here we were thinking of a nice quiet 30-cm segment of intestine that was being perfused, and what Shields did when he opened the abdomen was to show that the whole thing was moving about and was really nothing like what everyone imagined.[122] Nevertheless, there were some interesting results that came out of this triple lumen tube.

There were others perfusing the intestine who were putting balloons in the intestine and blowing them up and perfusing a segment between the balloons in order to isolate it. But the problem with that system was it created some pain, it created peristalsis, and the whole thing tended to move down the intestine. You were never sure which region of the intestine you were perfusing.

So, the triple lumen tube seemed to help sort out some of the problems, but clearly left many others. The people who were working at about the same time perfusing human intestine were Syd Phillips at the Mayo Clinic, Konrad Soergel in Wisconsin, and in France, there was Bernier's group in Paris, with Rambaud and Modigliani. Also in the States John Banwell was perfusing the intestine. He was out for a time in Dacca with the people studying cholera, Greenough and Carpenter. They were using the same technique to look at the intestine in patients with cholera. And then in the UK, of course, there was Gordon Sladen, Tony Dawson and John Harries all perfusing the human intestine, and there are many others here who joined forces with them.

John Fordtran is still doing human gut perfusion experiments,[123] in Texas where he was born. He's a farmer's son and very much a man of the land. He was a Dallas graduate and very keen on Dallas, and in fact when he went to Boston to work with Ingelfinger that was a down point in his life, because it was the period between being in Dallas and getting back to Dallas. He was born and bred in Dallas and doesn't travel a great deal outside the United States and not all that often outside Dallas. He is a very affable, open man, as many of you will know.

He developed a very fruitful relationship with Floyd Rector, a renal physiologist, who was doing a lot of the work with renal tubules.[124] He was doing those shrinking drop

---

[122] Shields R. Personal communication. Information provided by Lord Turnberg, 23 May 2000. See also Cook G C, Carruthers R H. (1974) Reaction of human small intestine to an intraluminal tube and its importance in jejunal perfusion studies. *Gut* 15: 545–548.

[123] See, for example, Schiller L R, Santa Ann C A, Porter J, Fordtran J S. (1997) Glucose-stimulated sodium transport by the human intestine during experimental cholera. *Gastroenterology* 112: 1529–1535.

[124] See, for example, Kunau R T, Frick A, Rector F C, Seldin D W. (1968) Micropuncture study of the proximal tubular factors responsible for the maintenance of alkalosis during potassium deficiency in the rat. *Clinical Science* 34: 223–231. Andreucci V E, Herrera-Acosta J, Rector F C, Seldin D W. (1971) Measurement of single-nephron glomerular filtration rate by micropuncture: analysis of error. *American Journal of Physiology* 221: 1551–1559. Rector F C. (1983) Sodium, bicarbonate, and chloride absorption by the proximal tubule. ibid. 244: F461–F471.

experiments, putting two drops of oil in a renal tubule with micropuncture techniques and watching the shrinkage. Floyd Rector had a lot of basic information about transport in the kidney and John Fordtran used this ideology to interpret what was going on in the human intestine.

The other thing that he had going in Dallas was an amazing team of volunteers who were willing to swallow the tube. He would pay them, of course. We would put the tube down the night before and they would all turn up at the lab the next morning at about 6.00 a.m. to screen the tube and to make sure it was in the right place. Then we would start perfusing. We usually had two going at the same time so it was like a perfusion factory. There were, I think, a number of valuable advances that were being made by John Fordtran about the behaviour of the human intestine. The first thing that was of interest was how quickly the osmolality adjusted in the human jejunum to iso-osmolar. That was a very rapid process, and he proposed that the jejunum is very freely permeable to water. He showed that the volume of the meal goes up if you eat a carbohydrate meal, as fluid rushes in to equalize the osmotic pressure, and then goes down as the fluid leaves with the absorbed solute. He then did some very interesting things to try to calculate the relative permeability of the human jejunum compared with the human ileum and the colon. He used the Renkin reflection coefficient idea that he had got from Floyd Rector and put a number of variably absorbed solutes, like urea, xylose and sodium into the intestine, and measured the relative osmotic pressure gradients that they could create.[125] Because they are of different size and because they have a different ability to create an osmotic pressure he was able to calculate a reflection coefficient, and from those he made an assumption about the size of the pores through which water and solutes were moving. Now, all that from perfusing whole human intestine was quite a leap of imagination and, of course, he presumed for the purposes of these calculations, that the intestine was a single membrane with holes of a uniform size, which were the ones that he was determining the reflection coefficient for. He was saying that the intestine behaved in that way, and surprisingly enough he was able to produce figures which, I suspect, are not too far off. He showed that the jejunum is roughly twice as permeable as the ileum. That is, the presumed pores, through which water is flowing, are about twice the diameter of those in the ileum.

He then found himself face to face with Stanley Schultz, because Schultz, in the 1960s, was showing very clearly that glucose–sodium transport was an active process, and that was that.[126] What John Fordtran suggested from his experiments was that when you put glucose in the lumen it was absorbed rapidly and created an osmotic gradient down which water flowed across the mucosa. Furthermore, this water movement dragged sodium and small ions through the pores with it; that is ‘solvent drag’ sodium absorption. And he demonstrated this by influencing the movement of

---

[125] Fordtran J S, Rector F C Jr, Ewton M F, Soter N, Kinney J. (1965) Permeability characteristics of the human small intestine. *Journal of Clinical Investigation* 44: 1935–1944.

[126] op. cit. note 99.

water, by other sorts of osmotic pressure gradients (unlinked to glucose), and showed that sodium moved either way along with the flow of water. Furthermore, he showed that the predicted electrical potential was created which you would expect from moving ions with water.[127] Stanley Schultz was dead against this, since it contravened the glucose–sodium active transport hypothesis. Michael Field, who was in Boston, was also dead against it, and John Fordtran felt very beleaguered because he was basing all of this on whole human perfusion data, whilst Stanley Schultz was playing with very much more sophisticated techniques. At the end of the day I think there was a stand-off. I don't know whether Stanley Schultz ever agreed that there may be such a thing as solvent drag sodium absorption, but there was a lot of antagonism to the idea in those days.

When I got to Dallas and worked with John Fordtran he was interested in getting me to look at why sodium transport was stimulated by bicarbonate. If you put isotonic sodium chloride in the jejunum, it just sits there. But if you add sodium bicarbonate, sodium absorption is stimulated.[128] We eventually came up with the idea of a sodium–hydrogen exchanger. The reasons behind that were that you generate a high PCO_2 in the lumen during sodium bicarbonate absorption and that is due to hydrogen being secreted, which generates the high luminal PCO_2 and a 'disequilibrium pH'. The demonstration of a 'disequilibrium pH' was the proof, as far as Floyd Rector was concerned, and it was on this basis that the case was made. Of course the whole idea was presaged by Dennis Parsons, who had shown the same thing in 1956 in the rat intestine.[129] So we had come a long way round to showing that something similar happened *in vivo* in the human jejunum.

And then we perfused the ileum, which was rather more difficult to do. You had to wait longer for the tube to get down there, and therefore fewer people were perfusing the ileum in those days. But we had this marvellous team of volunteers!

On the basis of ileal perfusion experiments, we showed what we thought was a double-ion exchange; that is sodium chloride was absorbed against the concentration gradient compared with the jejunum. By analysing absorption rates at different concentrations of each ion we came up with a hypothesis that suggested that sodium was absorbed in exchange for hydrogen, and chloride was absorbed in exchange for bicarbonate. So there was a coupled anion and cation exchange and that was the 'double exchange'.[130]

---

[127] See, for example, Fordtran J S, Rector F C Jr, Carter N W. (1968) The mechanisms of sodium absorption in the human small intestine. *Journal of Clinical Investigation* 47: 884–900.

[128] Sladen G E, Parsons D S, Dupre J. (1968) Effects of bicarbonate on intestinal absorption. *Gut* 9: 731. Turnberg L A, Fordtran J S, Carter N W, Rector F C Jr. (1970) Mechanism of bicarbonate absorption and its relationship to sodium transport in the human jejunum. *Journal of Clinical Investigation* 49: 548–556.

[129] Parsons D S. (1956) The absorption of bicarbonate-saline solutions by the small intestine and colon of the white rat. *Quarterly Journal of Experimental Physiology* 41: 410–420.

[130] Turnberg L A, Bieberdorf F A, Morawski S G, Fordtran J S. (1970) Interrelationships of chloride, bicarbonate, disodium, and hydrogen transport in the human ileum. *Journal of Clinical Investigation* 49: 557–567.

That was a very exciting time to be in Dallas, when those ideas were emerging. When we presented these findings in Atlantic City at the American Society for Clinical Investigation there was considerable scepticism. Michael Field got up and said, 'It doesn't seem right' and, of course, it was all derived from a crude system of perfusing the human intestine. So proof of what we suggested took a long time in coming. There was no way we could determine at which cell membrane these presumed ion transport events were happening nor, even, whether it was simply a phenomenon due to perfusion, vascularity, or motility. All those compounding factors were in there and it was an enormous leap to interpret what we'd found in terms of ion exchangers. But, quite a few years later, a number of people showed that if you take apical membrane vesicles from ileal mucosa you can demonstrate anion and cation exchangers.[131] So it was remarkable that it was possible to develop those sorts of ideas from perfusing whole humans and I think John Fordtran deserves to be recognized for doing this type of work and delivering important insights into epithelial function.

Boyd: I am really interested to hear about the input of Rector, because I have always wondered about this play between the renal and the gastrointestinal. I can see how Fordtran got a lot out of Rector; what did Rector get out of Fordtran?

Turnberg: His name on the papers. Certainly on the early papers, they worked very closely together, on solvent drag, on reflection coefficients, and on our paper on the double ion exchange.[132]

Naftalin: Were you influenced by the work of the red cell chloride shift,[133] which is very similar in many ways to the work which you describe of the anion exchange and the movement of water?

Turnberg: Yes. You must remember that what we were doing was simply putting saline down the intestine, aspirating fluid and measuring the ions, and to get anything out of that which resembled anything that you could get in a single cell was, to me, remarkable. To relate what was happening to a single cell, like a red cell, to what was happening with this experimental technique was a leap which I found interesting. I still remain amazed that the whole intestine behaves as if it is a single layer of cells. So although we knew about ion exchangers in red cells it didn't mean that we made the immediate connection.[134]

Dowling: You referred to Floyd Rector, but I wonder if you would comment on the advances in renal transport which antedated those in gastrointestinal transport. It seems to me that in the world of renal physiology, sophisticated micropuncture

---

[131] Murer H, Sugrist-Nelson K, Hopfer U. (1975) On the mechanism of sugar and amino acid interaction in intestinal transport. *Journal of Biological Chemistry* 250: 7392–7396. Hopfer U. (1977) Isolated membrane vesicles as tools for analysis of epithelial transport. *American Journal of Physiology* 233: E445–E449.

[132] op. cit. notes 125, 127, 128 and 130.

[133] op. cit. note 51.

[134] See, for example, Hoffman J F. (1966) The red cell membrane and the transport of sodium and potassium. *American Journal of Medicine* 41: 666–680.

techniques had been developed by Berliner and others[135] at a time when gastrointestinal physiologists were using rather simple, crude techniques. Is that right?

Turnberg: I suppose what Floyd Rector always said was that the intestine is one big tubule. He thought of it in terms of a tubule and was happy that it was easier to perfuse than the micropuncture techniques you need for the renal tubules.

Booth: Can we just ask what date that was?

Turnberg: That would be about the late 1950s, early 1960s.

Booth: If I can just comment on [Malcolm] Milne's[136] work, I think that might be worth doing. The recognition that there was a renal transport defect in Hartnup disease which was reflected in the intestine was made by the clinician Malcolm Davenport Milne, first at the Hammersmith and later at the Westminster, after he had been appointed Professor there.[137] But the work on Hartnup disease was started at Hammersmith in about 1959. Most people have been talking so far of physiologists who come to the intestine through physiology. Malcolm Milne came to the intestine through non-ionic diffusion. He was interested in the influence of pH on the excretion of weak acids and bases in the kidney, and he'd worked particularly on the drug mecamylamine.[138] He then realized from Dent's work[139] that indoleacetic acid was excreted in the urine of patients with Hartnup disease, a beautiful example of a weak acid. So he invited Dent to send a patient of his with Hartnup disease to Hammersmith to study the non-ionic diffusion of indoleacetic acid. He also knew that indoleacetic acid was a bacterial breakdown product of the amino acid tryptophan. He knew, too, that the defect in the renal tubule involved the monoamino monocarboxylic amino acids, including tryptophan. He was sitting in his bath in East Sheen one day and he suddenly leapt up and said, 'My God, the reason there's indoleacetic acid in the urine is that there is malabsorption in the intestine'. That's how he came to the intestine and performed all that beautiful work. I remember his first patient being shown on a grand round at Hammersmith. He had

---

[135] See, for example, Sakai F, Jamison R L, Berliner R W. (1965) A method for exposing the rat renal medulla *in vivo*: micropuncture of the collecting duct. *American Journal of Physiology* 209: 663–668. See also Windhager E E. (1987) Micropuncture and microperfusion, ch. IV. In Gottschalk C W, Berliner R W, Giebisch G H. (eds) *Renal Physiology. People and Ideas.* Bethesda, MD: American Physiological Society, 101–129. op. cit. notes 38 and 124.

[136] Professor Malcolm Davenport Milne FRCP FRS (1915–1991) was Professor of Medicine, University of London, at Westminster Medical School, from 1961 to 1980, then Emeritus. See Peart S. (1995) Malcolm Milne. *Biographical Memoirs of Fellows of the Royal Society* 41: 299–307.

[137] Milne M D. (1967) Hereditary abnormalities of intestinal absorption. *British Medical Bulletin* 23: 279–284. *idem* (1969) Hartnup disease. *Biochemical Journal* 111: 3P–4P. See also Wilson T H. (ed.) (1962) Amino acids. Comparison of amino acid transport in kidney and intestine. op. cit. note 13, 128–130.

[138] Stone C A, Torchiana M L, Navarro A, Beyer K H. (1956) Ganglionic blocking properties of 3-methylamino-isocamphane hydrochloride (mecamylamine): a secondary amine. *Journal of Pharmacology and Experimental Therapeutics* 117: 169–183. Milne M D. (1965) Influence of acid-base balance on efficacy and toxicity of drugs. *Proceedings of the Royal Society of Medicine* 58: 961–963.

[139] For example, Dent and Schilling's studies on amino acid absorption. Dent C E, Schilling J A. (1949) Studies on the absorption of protein: the amino-acid pattern in the portal blood. *Biochemical Journal* 44: 318–333.

done this work with a biochemist called Dalgliesh who had done the paper chromatography of all the bacterial breakdown products in the urine. He showed the patient, with pieces of paper chromatography all over the place, sat down at the end of the grand round presentations and got thunderous applause from about 300 people. The Professor of Medicine at that time was a very distinguished cardiologist, Sir John McMichael,[140] who wasn't to be outdone. He stood up and looked at Milne, took out his stethoscope and said, 'Milne, I believe your patient has a systolic murmur'.

Holdsworth: If I can continue with this theme of the relationship between renal and intestinal transport, I am reminded that Wilbrandt said in 1959 that the membrane carrier hypothesis for glucose transfer was introduced by Shannon in 1939,[141] who showed saturation kinetics for glucose absorption in the renal tubule, and suggested 'reversible combination with some cellular element which is present in constant but limited amounts'. But the concept was not transferred to intestinal absorption until much later.[142]

Booth: Can I just ask one question? The two other people who always seem to be forgotten in the field of human intubation studies are Harold Schedl and Jim Clifton who was very active in Iowa City.[143] Now what did they do?

Levin: I can tell you because I was in Iowa City on sabbatical in 1964–5. They actually used prisoners in the University Hospital. They could come out of the prison and stay on the wards and they would swallow the tubes and be perfused, and they got off some of their sentence. It was actually the absorption of substances like methionine that they were doing at that particular time, but I remember seeing them. They were in pyjamas at the time so that they couldn't escape!

I want to go down a slightly different track, as we are off electrolyte transport and on to glucose again, I want to bring it back. Just to say that you have got to have a bit of luck in life because even if you do the right thing at the wrong time (*viz* too early) you are often never recognized. There's a very interesting early paper about intestinal secretion. You talked about absorption, but this is secretion really, especially as you are

---

[140] Sir John McMichael Kt FRCP FRS (1904–1993) was Professor and Director of the Department of Medicine at the Postgraduate Medical School at Hammersmith between 1946 and 1966, Director of the British Postgraduate Medical Federation from 1966 to 1971, and a Trustee of the Wellcome Trust from 1960 to 1977. His research interests were predominantly in the field of cardiology and he was the first in Britain to apply the technique of cardiac catheterization. See Dollery C. (1995) Sir John McMichael. *Biographical Memoirs of Fellows of the Royal Society* 41: 283–296.

[141] Shannon J A. (1939) Renal tubular excretion. *Pysiological Reviews* 19: 63–93. Wilbrandt W. (1959) In Geigy Colloquium I, *Kohlenhydratstoffwechsel im Kinderalter*. Basel: S Karger. See also Kleinzeller A, Kotyk A. (eds) (1961) Topic 3. Transport of sugars across cellular and biological membranes. In *Membrane Transport and Metabolism*. London: Academic Press, 341–464. Professor Michael Gardner wrote: 'According to D M Matthews (1991) (see note 173), Dr Gerald Wiseman (Sheffield) said that his work on amino acid absorption in the 1950s had been inspired by a paper by Shannon and Fisher (not R B Fisher) on renal reabsorption of glucose in the 1930s.' Letter to Dr Daphne Christie, 28 April 2000.

[142] See note 20.

[143] op. cit. notes 145 and 146.

coming on to oral rehydration, I will just reactivate it. Charlie Tidball in Washington University, way back in the early 1960s, had shown that it was chloride secretion that was the ion that powered intestinal secretion (in dogs). He published that in the *American Journal of Physiology*[144] and also had an abstract in *Federation Proceedings* and nobody picked it up for many years. Everybody said, 'No, it doesn't matter, it's not interesting'. It was the right thing to be done but at the wrong time, and nobody has ever commented about Charlie Tidball at all, but I think he was one of the first, if not the first, to show that it was chloride transport that was going on in the intestine. That was the ion that powered diarrhoea. It's funny if you are with the right thing, but at the wrong time.

Dowling: You were asking about Schedl and Clifton. They were interested in diabetes mellitus and its effects on intestinal transport. They also studied segmental intestinal absorption in patients with proximally confined coeliac disease and showed that as one goes down the gut, the profile of absorptive efficiency/unit length intestine was, as expected, relatively low in the jejunum but unexpectedly high in the ileum.[145] And although they didn't interpret their results as such, this was actually one of the first demonstrations in humans that when there is damage to the proximal intestine, there is compensatory or adaptive segmental hyperfunction in the ileum.

Holdsworth: Schedl and Clifton also published in *Nature* in 1963,[146] a paper that demonstrated net stimulation of sodium transport by glucose in human jejunum *in vivo*, and I think they should be given credit for that.

Turnberg: Now we have a series of topics which we are going to try to pick up. The first is oral rehydration which was, from the therapeutic point of view, a remarkable advance. It was thought that this was based directly on the results of physiological perfusion experiments. Whether it was or not is not so clear, but John Walker-Smith will enlighten us.

Professor John Walker-Smith:[147] Thank you very much, Mr Chairman. I am speaking as a clinician who has benefited from the development of oral rehydration therapy as a powerful tool for treating children with acute diarrhoeal disease rather than as one who has in any way contributed to its development. Through the centuries acute diarrhoeal disease (cholera in particular), especially in infants and children, has been

---

[144] Tidball C S. (1960) Active chloride transport during intestinal secretion. *Federation Proceedings* 19: 127. *idem* (1961) Active chloride transport during intestinal secretion. *American Journal of Physiology* 200: 309–312.

[145] See, for example, Schedl H P, Clifton J A. (1961) Kinetics of intestinal absorption in man: Normal subjects and patients with sprue. *Journal of Clinical Investigation* 40: 1079–1080.

[146] Schedl H P, Clifton J A. (1963) Solute and water absorption by the human small intestine. *Nature* 199: 1265–1267.

[147] Professor John Walker-Smith FRCP FRACP (b. 1936) graduated from the University of Sydney and began his career in adult gastroenterology at the Hammersmith Hospital, London, and Royal Prince Alfred Hospital, Sydney, before changing to paediatric gastroenterology. He held academic appointments at the Medical College of St Bartholomew's Hospital and Queen Elizabeth Hospital for Children from 1973 to 1995, and then transferred to the Royal Free Hospital, London, as Professor of Paediatric Gastroenterology.

a major cause of death – from dehydration in most cases. From the early part of the century investigators have sought possible aetiological agents with varying success. However, regardless of causation, it is, of course, dehydration which kills babies, children and adults. From the 1960s the advent of the technique of oral rehydration therapy has given a huge boost to the effective and practical management of dehydration due to acute diarrhoea. Yet it is interesting that paediatricians, with one notable exception, Dilip Mahalanabis,[148] actually played very little part in this development. There was, in fact, quite strong opposition against this treatment by the paediatric establishment when it was first introduced.[149]

The development of oral rehydration therapy came from three directions. First, there was the basic *in vitro* physiological work which has already been discussed. The work of Schultz and Zalusky in their classic paper of 1964, that glucose stimulated sodium transport across a piece of rabbit ileum, was of particular importance.[150] Second, there were the physiological studies in man such as those of John Fordtran in the classical paper of Malawer and colleagues[151] as well as Gordon Sladen and Tony Dawson in their 1969 study amongst others.[152] Third, in the field, practical work such as that of Captain Phillips, who was the first to successfully use oral rehydration therapy in clinical practice in two individuals and then in an uncontrolled trial.[153] Unfortunately, there were some deaths during this trial. Subsequently, there was general acceptance of oral rehydration therapy based on controlled clinical trials in the field, particularly in South-East Asia and particularly Dacca and Calcutta. The work of Nalin was particularly important for oral rehydration therapy in children. Nalin was the first to show in a paediatric cholera epidemic that oral rehydration therapy was effective.[154] This was followed by the important work of Hirschhorn in gastroenteritis.[155] However, when we look at the historical perspective, the development of oral rehydration therapy is like a patchwork quilt, with the *in vitro* observations, the perfusion studies

---

[148] See page 44, op. cit. note 157.

[149] Professor John Walker-Smith wrote: 'This largely stemmed from the fear of hypernatraemia related to the relatively high sodium content of the solution recommended by WHO and UNICEF.' Letter to Dr Daphne Christie, 4 October 1999.

[150] op. cit. note 99.

[151] Malawer S J, Ewton M, Fordtran J S, Ingelfinger F J. (1965) Interrelation between jejunal absorption of sodium, glucose and water in man. *Journal of Clinical Investigation* 44: 1072.

[152] See, for example, Sladen G E, Dawson A M. (1969) Interrelationships between the absorptions of glucose, sodium and water by the normal human jejunum. *Clinical Science* 36: 119–132.

[153] Phillips R A. (1964) Water and electrolyte losses in cholera. *Federation Proceedings* 23: 705–712. See also *idem* (1967) Twenty years of cholera research. *Journal of the American Medical Association* 202: 610–614. Ruxin J N. (1994) Magic bullet: the history of oral rehydration therapy. *Medical History* 38: 363–397.

[154] Nalin D R, Cash R A, Islam R, Molla M, Phillips R A. (1968) Oral maintenance therapy for cholera in adults. *Lancet* ii: 370–373. Nalin D R, Cash R A. (1971) Oral or nasogastric maintenance therapy in pediatric cholera patients. *Journal of Pediatrics* 78: 355–358.

[155] Hirschhorn N, McCarthy B J, Ranney B, Hirschhorn M A, Woodward S T, Lacapa A, Cash R A, Woodward W E. (1973) *Ad libitum* oral glucose-electrolyte therapy for acute diarrhoea in Apache children. *Journal of Pediatrics* 83: 562–571.

and the clinical studies occurring independently and then being linked together some time after the event. Indeed, those working with patients were not always well informed concerning, or did not accept, the work of the physiologists. Ruxin in 1994 published a very interesting paper in *Medical History* which reviewed these issues.[156] In fact, although scientists and research clinicians were convinced that oral rehydration therapy worked, based on elegant trials, it was not until the Bangladesh war of independence, when Dilip Mahalanabis and his colleagues actually used oral rehydration therapy very effectively in refugee camps in an emergency situation, that oral rehydration therapy became widely used in practice.[157] Mahalanabis applied it in a disaster situation for the first time, whereas others had applied it under more controlled research situations. It was this practical application which persuaded UNICEF and the World Health Organization to appreciate the clinical value of oral rehydration therapy.

Here in Britain we had a very different situation from the developing world. By the time oral rehydration therapy was developed children were no longer dying on a large scale from acute diarrhoeal disease. Infant diarrhoea mortality had fallen very greatly. At the Queen Elizabeth Hospital in the 1970s we were horrified that a few children were still dying in our wards from gastroenteritis with hypernatraemic dehydration; indeed, we had deaths in hospital up until 1979 in our gastroenteritis unit. So, because of this continuing low mortality associated with hypernatraemia we were at first cautious about the high sodium content of oral rehydration solution. Possibly we were wrong, because when this solution is given properly it is safe, but as observations from Egypt made clear, uncontrolled use of oral rehydration solution based on a powdered solution given incorrectly as an over-concentrated solution could lead to hypernatraemic dehydration. There was indeed reasonable cause for concern at that time, but retrospectively we were a bit unwise, I think, in slowing introduction of oral rehydration therapy to the UK. Now oral rehydration therapy has been shown to be an extremely effective therapy in many studies. Indeed, we paediatricians are greatly in debt to the variety of investigators that led to the development of oral rehydration therapy. One can now weave together a comprehensive logical story, from *in vitro* observations, to field work, to clinical use, but the actual events were not quite so logical as it might seem when we view events retrospectively.

I am a little bit too young to have been personally involved in the early work on oral rehydration therapy but I would be interested to hear from other people here how they feel that the physiological work, for example, contributed directly to the development of oral rehydration therapy.

Turnberg: Thank you very much. There's this nice logical progression of ideas; that glucose stimulates sodium and water absorption, deduced from the physiological

---

[156] Ruxin J N. (1994), op. cit. note 153.

[157] Mahalanabis D, Choudhuri A B, Bagchi N G, Bhattacharya A K, Simpson T W. (1973) Oral fluid therapy of cholera among Bangladesh refugees. *Johns Hopkins Medical Journal* 132: 197–205. Rahaman M M, Aziz K M, Patwari Y, Munshi M H. (1979) Diarrhoeal mortality in two Bangladeshi villages with and without community-based oral rehydration therapy. *Lancet* ii: 809–812.

experiments we have been talking about. Although the intestine is secreting away at a great rate in the presence of cholera, it is still capable of absorbing salt and water, if you give the right substrates into the lumen; hence why not try this in humans with cholera, and give them glucose and other solutes to stimulate the sodium and water absorption. It all sounds very logical, except I am not sure that it did happen quite like that. It would be very nice if it had, but it does seem that people were already feeding infants rice water and, I think, Carpenter and others in Dacca and Bangladesh were putting in fluids with different solutes before the physiological basis for what was being done was recognized. I think the idea of giving rice water as a stimulus to try and help to rehydrate people was already going on to a significant extent before the physiological knowledge came along. Is that correct?

Walker-Smith: There was quite a body of experience at the Queen Elizabeth Hospital for Children from 1952, using an oral glucose electrolyte solution as recommended by the Medical Research Council. Winifred Young used such a glucose electrolyte solution for treatment of mild cases of gastroenteritis. The glucose content was thought to be an important calorie source and there was no appreciation whatever of the notion of oral rehydration therapy. A glucose electrolyte solution was used for inpatients and a sucrose electrolyte solution for outpatients with gastroenteritis. I inherited that approach. It was a breakthrough when the work of the physiologists gave a rational basis for a correctly formulated oral rehydration solution. In fact, the solution introduced by Winifred Young had a glucose content which was too high. Indeed, when this solution has been used in animal models it has a powerful osmotic effect. So when that solution was used in severe cases, particularly rotavirus gastroenteritis, osmotic diarrhoea would have resulted.

One of the issues for paediatricians was, it is all very well for oral rehydration therapy to work in cholera but what about rotavirus gastroenteritis, where it was known from the work of Barnes at the Royal Children's Hospital in Melbourne that there could be an almost flat small intestinal mucosa.[158] In actual fact, oral rehydration therapy is effective in most such cases. It may be that the extent of the small gut damage is most important.

Hellier: I think you are right, there was a change of attitude regarding treatment of people dying of diarrhoea. From our experience in South India, where 30 000 people died of dehydration from what we termed 'tropical sprue' in the early 1960s, the change of attitude was that up until that time if you had diarrhoea you didn't drink, because that seemed the best way to stop the diarrhoea. The last thing you wanted to do was to pour fluid in if you had diarrhoea. It was when the change of attitude came about that people actually should drink, irrespective of what they drank, that mortality started to fall.

Turnberg: And what was the attitude change due to?

---

[158] See, for example, Walker-Smith J. (1978) Rotavirus gastroenteritis. *Archives of Disease in Childhood* 53: 355–362. Davidson G P, Barnes G L. (1979) Structural and functional abnormalities of the small intestine in infants and young children with rotavirus enteritis. *Acta Paediatrica Scandinavica* 68: 181–186.

Hellier: I think it may well have stemmed from the realization that water and electrolyte absorption could be stimulated by nonelectrolytes. It came about in the mid-to-late 1960s.

Dowling: To continue the theme that the renal physicians and physiologists had something to teach gastrointestinal physiologists: in my early postgraduate days, I trained in Belfast with Graham Bull.[159] He visited the cholera research unit in Dacca, and there he met, and became friendly with, an American called Bob Phillips who was studying fluid and electrolyte balance in cholera patients. Bull always claimed that Phillips was one of the first to show the importance of adding glucose to rehydration solutions, to enhance sodium and water absorption in cholera.[160] Now is that correct or not?

Booth: My knowledge of this field is derived from the excellent book by Seal and van Heyningen on the history of cholera and particularly the history of the Dacca unit.[161] I think the story of the American involvement with cholera is an example of an advantage which derived from a military alliance, the South-East Asia Treaty Organization. It was they who funded Bob Phillips and his work. Phillips was working in the Philippines and then went to Taiwan and from Taiwan went to head the unit in Dacca, which was where he built up the cholera research unit. The story there goes that Phillips tried a couple of cases of oral rehydration, I think in Taiwan, before he went to Dacca, but I may be wrong about that. Certainly the people in Dacca were told by Bob Phillips under no circumstances were they to indulge in oral rehydration therapy because it had proved to be wrong. Now Phillips was a very commanding character with certain personal problems of his own; that made him a somewhat difficult director of the Institute in Dacca. They really didn't get ahead with it until he had gone. I think the people in Dacca following Phillips were the ones who deserve credit for having introduced the oral treatment, remembering, of course, that it wasn't they who said it was the oral treatment of diarrhoea, it was the oral treatment of *cholera* they were concerned with. Others said, 'Right let's try this for infantile diarrhoea throughout the world'.

Professor Roy Pounder:[162] Adding to that, speaking as an adult gastroenterologist, these solutions not only liberated travellers around the world, but, importantly, made

---

[159] Sir Graham Bull Kt FRCP (1918–1987) was Lecturer in Medicine at the Postgraduate Medical School in Hammersmith, London, from 1947 to 1952, Professor of Medicine, The Queen's University, Belfast, from 1952 to 1966, and Director of the MRC Clinical Research Centre, from 1966 to 1978. He was Chairman of the Ciba Foundation Executive Committee from 1977 to 1983 and second Vice-President of the Royal College of Physicians, from 1978 to 1979.

[160] See, for example, Taylor J, Hirschhorn N, Phillips R A. (1967) Enhancement by intestinal glucose lavage of net sodium and water absorption in acute cholera patients. *Federation Proceedings* 26: 384.

[161] Seal J R, van Heyningen W E. (1983) The SEATO cholera research program and the Pakistan-SEATO cholera research laboratory in Dacca. In *Cholera. The American Scientific Experience 1947–1980*. Boulder, CO: Westview Press, Inc, 95–118. See also Phillips R A. (1967) Twenty years of cholera research. *Journal of the American Medical Association* 202: 610–614.

[162] Professor Roy Pounder MA, MD, DSc(Med), FRCP (b. 1944) qualified in medicine at Cambridge and Guy's in 1969. He trained under Sir Christopher Booth, Sir Francis Avery Jones, George Misiewicz and Brian Creamer. Appointed to the Royal Free by Dame Sheila Sherlock in 1980, he is now Professor of Medicine and Director of the Centre of Gastroenterology at the Royal Free and University College Medical School, London.

possible the management of the short bowel in people with Crohn's disease. Work at St Mark's Hospital defined how you can get the last bit of life out of the small intestine in people with a very short bowel, by forcing salt in, rather than losing it out of the stoma.

Walker-Smith: A further comment on Captain Phillips.[163] He was actually working on the wrong premise. He thought that in cholera there was a 'poisoning' of the intestinal sodium pump and that this was the functional disturbance in cholera. He was trying to 'unpoison' the pump. So pathophysiologically, he was completely wrong. However, the worst thing that happened to him was that owing to his success with his first two patients he planned another *ad hoc* study. He then went on holiday leaving others to do the study. When he came back after holidaying, five of the patients in the new study had died. He was very shocked by that outcome and thereafter rather lost heart. He certainly was not an heir of the physiologists, he was working independently, and he seemed to be indifferent to the precise amount of glucose that was present in the solutions that were given.

Turnberg: I think it is quite interesting that Michael Field, doyen of the Ussing chamber and *in vitro* techniques, and John Fordtran, the whole-human gut perfusion man, jointly were given the King Faisal Prize for developing the scientific basis for treatment of diarrhoeal disease.

Now, peptide absorption is the next topic. Mike Hellier might lead the discussion.

Hellier: Up until the end of the last century it was very much assumed that protein was absorbed in the form of peptides. But Cohnheim in 1901 discovered 'erepsin' and showed it could digest peptones (peptides) down to free amino acids.[164] Really, from this time onwards right through to the end of the late 1960s it was taken for granted that protein was absorbed in its free amino acid form, and any suggestion that peptide absorption might be of significance in protein absorption was rather overlooked.

But I think the credit for raising the profile of peptides again goes to the Westminster group where Craft, Milne, Matthews, Asatoor and Navab started to look at peptides in the late 1960s.[165] They looked at absorption in animal studies and showed that amino acids could be absorbed faster when presented as a dipeptide solution than as free amino acids which favoured intact peptide absorption. They took advantage of a patient with Hartnup disease, and did oral absorption studies which showed that the patient seemed to absorb neutral amino acids when given in the form of peptides.

---

[163] op. cit. notes 153 and 161.

[164] Otto Cohnheim (1873–1913) looking for the synthesis of protein in the intestinal mucosa during absorption in the dog found that the mucosa could break down 'peptones' (peptides) to free amino acids. He called the chemical responsible for the hydrolysis 'erepsin'. See Cohnheim O. (1901) Die umwandlung des eiweiss durch die darmwand. *Zeitschrift für Physikalische Chemie* 33: 451–455. *idem* (1902) Trypsin und Erepsin. ibid. 36: 13–19.

[165] See, for example, Craft I L, Geddes D, Hyde C W, Wise I J, Matthews D M. (1968) Absorption and malabsorption of glycine and glycine peptides in man. *Gut* 9: 425–437. Asatoor A M, Bandoh J K, Lant A F, Milne M D, Navab F. (1969) Absorption of amino acids and the dipeptide, carnosine, from the gut in normal subjects and a case of Hartnup disease. *Clinical Science* 37: 568.

Well, it was about this stage that I came on the scene. In 1969 I had succeeded in passing my membership examination [for the Royal College of Physicians] and thought that from then onwards it would be downhill and easy, but my supervisor quickly corrected me of this idea and said, 'Now the difficult times are just starting, you must do some research and get an MD'. So I thumbed through the back of the *British Medical Journal* and saw a job advertised at St Bartholomew's Hospital. The job was to look at cystinuric patients, because there was a unique collection of cystinuric patients at St Bartholomew's, thanks to Dr Dickie Watts. He had more cystinuric patients than anyone else in the country and I was to devise an oral-load test to try and look for subgroups of cystinuric patients and perhaps use it for looking for partial defects. There was also a chap called Derek Holdsworth at Bart's at the time and I was told he was a rising star in British gastroenterology and that I could do worse than to go and work with him. So I ended up working for Derek on Richard Watts's cystinuric patients at St Bartholomew's. However, three months later I was no further on. It was quite clear that an oral absorption test in cystinuria was going to be of no use, unlike in Hartnup disease. My fluorometric assay for arginine wasn't working and wouldn't work and was never likely to work. So I went through the phase that I think a lot of researchers go through, of utter gloom, and decided that I might cut my losses, get out, and get back into clinical medicine. And then, fortuitously, as so often happens, a bright idea came along, I am not quite sure whose bright idea it was, probably Derek's, aware of Milne's and Matthews's work.[166] Here we had a unique collection of cystinuric patients, we knew they couldn't absorb the dibasic amino acids and therefore if we were to use dibasic amino acids in peptide form this really should provide the chance to demonstrate fairly clearly whether peptides could be absorbed intact. This involved initially assessing the kinetics of dibasic amino acid absorption which had not been done in man before, and we had no idea what the K_t and V_{max} figures might be. Initially we thought they might be comparable to those of the neutral amino acids.

At Bart's, we had refined a perfusion technique which meant that we could look in a rather more detailed way at absorption. The first thing to do was to look at arginine. Derek volunteered to swallow this terrible tube and I seem to remember we met about four o'clock in the morning on the medical unit at Bart's. It took about six hours to get the tube into position and then we started to perfuse concentrations of arginine from 10 to 300 millimolar which we thought was the range needed to be covered. Derek started to vomit and develop abdominal pain and had terrible diarrhoea. I then followed this a week later and had exactly the same experience and finally we realized that, in fact, the absorption kinetics of the dibasic amino acids were quite different from those of the neutral amino acids and the V_{max} figures were probably a tenth of those for the dibasic amino acids. Well, at last we managed to establish the absorption characteristics of the dibasic amino acids. We managed to

[166] See, for example, Milne M D, Asatoor A M, Edwards K D G, Loughridge L W. (1961) The intestinal absorption defect in cystinuria. *Gut* 2: 323–337. Matthews D M, Lis M T, Cheng B, Crampton R F. (1969) Observations on the intestinal absorption of some oligopeptides of methionine and glycine in the rat. *Clinical Science* 37: 751–764.

find a cystinuric patient prepared to undergo a perfusion study and sure enough the patient could not absorb the free dibasic amino acids, but to our delight, absorbed arginine and lysine quite normally in the form of dipeptides. This, we felt, was convincing evidence in favour of intact peptide absorption. We presented this work at the Medical Research Society in 1970.[167]

We went on to look at other peptides to see if we could discover more about their mechanism of transport. We were looking at glycyl-lysine and wondered whether in fact the dipeptide was being absorbed using the glycine transport mechanism. So we then looked at lysyl-lysine, because that obviously wouldn't be able to use a neutral amino acid transport mechanism. Lysyl-lysine was absorbed normally as well, so that really suggested that the absorption mechanism was different. We looked at the rates of the absorption of amino acid and peptide solutions and demonstrated what Matthews and Milne showed, that the amino acids actually got in faster when given as peptides than as free amino acids.[168] Ultimately we went on to look at electrolyte and water absorption in association with amino acid and peptide absorption, and from then on the work moved into the area of nutrition and the relevance of peptide absorption in nutritional solutions, but that is another story.

Turnberg: Thank you very much indeed. The use of a couple of basic defects in transport proved very valuable in illuminating the physiological basis.

Gardner: It's perhaps worth looking a bit earlier than this at the ideas of David Fisher, because by 1954 when he published his monograph on protein metabolism,[169] he had become convinced, more on theoretical grounds[170] than anything else, that peptide absorption must have been a reality. I suspect that when he started to work with Dennis Parsons[171] the intention always was to investigate peptide absorption rather than glucose absorption but, of course, because of their obsession with getting the preparation to work *in vitro*, that was why they stuck to glucose for so long. So certainly Fisher had set the scene and I think that subsequently some of the interest perhaps fell away because of the Sheffield group's very effective concentration on amino acid transport, which then, of course, set the scene for stereospecific active transport of amino acids, and so peptide absorption and metabolism was lost sight of.

---

[167] Hellier M D, Perrett D, Holdsworth C D. (1970) Dipeptide absorption in cystinurea. *British Medical Journal* iv: 782–783.

[168] Hellier M D, Holdsworth C D, Perrett D, Thirumalai C. (1972) Intestinal dipeptide transport in normal and cystinuric subjects. *Clinical Science* 43: 659–668.

[169] op. cit. note 43.

[170] Professor Michael Gardner wrote: 'Fisher repeatedly drew attention to the fact that the known activities of gastric and pancreatic proteases and peptidases were seriously inadequate to account for the speed of protein digestion if absorption were to be wholly in the form of free amino acids.' Letter to Dr Daphne Christie, 5 November 1999.

[171] Professor Michael Gardner wrote: 'This was confirmed by Parsons in a historical lecture at a Falk Symposium (see Parsons D S. (1984) Subsequence and consequence in studies on absorption in the intestine. In Skadhauge E, Heintze K. (eds) *Intestinal Absorption and Secretion*. Lancaster: MTP Press, 1–17).' Letter to Dr Daphne Christie, 5 November 1999. See also Figure 2.

I think also that Fisher's original view was of a considerable resynthesis in the gut during protein simulation and I am not sure, but I suspect, he was concerned with the product precursor relationships of protein digestion, and I also suspect that he was then realising the role of amino acids in lipoprotein synthesis in whole body assimilation of a meal. I think, though, there is no doubt that it was the inborn errors of amino acid absorption which then made it possible to make the biggest advances in peptide absorption. It is worth, I think, perhaps cross-referring to the work of David Matthews who had been in Sheffield before and so he had cut his teeth in David Smyth's lab,[172] and, of course, Matthews was a key figure in promoting work on peptide transport in a variety of organs, but I think there are several cross-working networks there which were very influential.[173]

Holdsworth: I well remember David Matthews's initial involvement in the field of peptide absorption. In 1962 we both worked at the Royal Free Hospital, London, David in Chemical Pathology, myself in the Department of Medicine. He approached me one day with an idea for a possible absorption test for the diagnosis of idiopathic steatorrhoea, as we then called adult coeliac disease, which was to administer oral glycyl-glycine, and measure glycine in venous blood. David was not the sort of person to normally show excitement, but even he was obviously excited when the blood levels he measured in normal volunteers that I carried out for him in pilot studies were higher after diglycine than after equimolar glycine. However, he did not publish this until 1968.[174]

Hellier: Newey and Smyth had actually demonstrated glycyl-glycine intact in the

---

[172] Matthews D M, Smyth D H. (1954) The intestinal absorption of amino acid enantiomorphs. *Journal of Physiology* 126: 96–100.

[173] Professor Michael Gardner wrote: 'The history of ideas on protein and peptide absorption has been comprehensively reviewed in the monograph by the late David Matthews (Matthews D M. (1991) *Protein Absorption: Development and present state of the subject*. New York: Wiley-Liss) and in his earlier works, for example, Matthews D M. (1975) Intestinal absorption of peptides. *Physiological Reviews* 55: 537–608. Matthews D M, Payne J W. (eds) (1975) *Peptide Transport in Protein Nutrition*. Amsterdam: North Holland. Matthews D M. (1977) Memorial lecture: protein absorption – then and now. *Gastroenterology* 73: 1267–1279.' Letter to Dr Daphne Christie, 5 November 1999. See also Smyth D H. (1961) Studies on the transport of amino acids and glucose by the intestine. op. cit. note 7, 488–510.

[174] See, for example, Matthews D M, Crampton R F, Lis M T. (1968) Intestinal absorption of peptides. *Lancet* ii: 639–640. Matthews D M, Craft I L, Geddes D M, Wise I J, Hyde C W. (1968) Absorption of glycine and glycine peptides from the small intestine of the rat. *Clinical Science* 35: 415–424. See also note 173. Dr Derek Holdsworth wrote: 'By this time Matthews was working in the Chemical Pathology Department at the Westminster Hospital, where, by coincidence, Milne was Professor of Medicine, and was carrying out studies on Hartnup disease that led to him publishing in 1970 his conclusion that intact peptide absorption could occur in man and be of nutritional significance. Matthews was, of course, alert to the possibility of intact peptide absorption from his contact with the work on dipeptide absorption in the Department of Physiology at Sheffield when he worked there (see Asatoor A M, Cheng B, Edwards K D, Lant A F, Matthews D M, Milne M D, Navab F, Richards A J. (1970) Intestinal absorption of two dipeptides in Hartnup disease. *Gut* 11: 380–387). These were the "cross-working networks" that Gardner mentions, as I remember them. The other being that after leaving the Royal Free to work for a year with Tom Wilson in the USA, I moved to St Bartholomew's Hospital where there was a unique opportunity to study patients with cystinuria, which Michael Hellier did so effectively. All those whose names I have mentioned used to meet in a London pub from time to time in an informal "Transport Club", which I am sure both Sir Christopher Booth and Professor Timothy Peters will remember (see page 65).' Note on draft transcript, 25 October 1999.

blood, but this was criticized because it was felt at that time that glycyl-glycine was an atypical peptide and not reflective of peptides as a whole.[175]

Turnberg: Where does the work of Adibi in the States fit in with all that; was he ahead or behind?

Gardner: Almost simultaneous, in 1968.[176]

Hanson: Who was the first user of non-hydrolysable dipeptides at that time? That, I think, would have been quite an important development.

Gardner: I think the answer to that is that Matthews, in anticipating the wide occurrence of peptide transport mechanisms, struck up relationships with the microbial transport people, especially John Payne, then in Durham. I think they then hit on the idea of using glycyl-sarcosine as the non-hydrolysable peptide, because it was recognized that peptides were a valuable nutrient and must be taken up by bacteria. That was the *raison d'être* for the first of the two Ciba Foundation Symposia, 'Peptide Transport in Bacteria and Mammalian Gut',[177] and then I would say that Matthews and Payne were co-partners in plugging the similarity of intestinal and microbial transport mechanisms.[178]

Hellier: You referred to Adibi. We were aware of Adibi's work. He had the advantage that has been alluded to earlier, that he had local prisoners 'volunteering' to be perfused. We had to try and persuade recalcitrant medical students and medical colleagues. I was having to get the dipeptides made by Fox Chemicals in Los Angeles when they were hit by an earthquake, and back home we had a postal strike in the United Kingdom, so my paper was put back by four months and I had envisaged Adibi getting all the answers first, but we just beat him to it.

Peters: The first absorption studies with a non-hydrolysable peptide utilized carnosine by Malcolm Milne. I was working on peptidases at that time and one of the important observations that Malcolm made was to ask, 'Why don't patients with Hartnup disease or cystinuria show protein malnutrition? If they couldn't absorb essential amino acids like tryptophan and lysine, why were they not grossly malnourished?' I think the question circulated in his mind, and he then said, 'Well, there must be a way of bypassing it.'

I would like to think that perhaps the work we were doing on dipeptidases was relevant. At that time people were thinking that proteins were absorbed like

---

[175] Newey H, Smyth D H. (1959) The intestinal absorption of some dipeptides. *Journal of Physiology* 145: 48–56. *idem* (1967) Absorption of nutrients from the intestine. Assessment of absorptive capacity. *Proceedings of the Nutrition Society* 26: 5–12.

[176] Adibi S, Phillips E. (1968) Evidence for greater absorption of amino acids from peptide than from free form in human intestine. *Clinical Research* 16: 446.

[177] Elliott K, O'Connor M. (eds) (1972) *Peptide Transport in Bacteria and Mammalian Gut*. Ciba Foundation Symposium. Amsterdam: Elsevier.

[178] Professor Michael Gardner wrote: 'The second Ciba Foundation Symposium was on peptide transport and hydrolysis. See Elliott K, O'Connor M. (eds) (1977) *Peptide Transport and Hydrolysis.* Ciba Foundation Symposium. Amsterdam: Elsevier.' Letter to Dr Daphne Christie, 5 November 1999.

carbohydrates, and there would be a brush-border dipeptidase. Histochemically people had demonstrated a leucine amino-peptidase. At that time in 1967 I had just started my PhD at the Hammersmith, Adrian Douglas was working on peptidases in coeliac disease,[179] and I was asked to look at the physiology of peptide absorption. We looked at where in the cell dipeptidases were localized and to our surprise they weren't in the brush-border, they were all inside the cell.[180] Certainly in my mind, that observation, linked with Malcolm Milne's findings, brought it all together. It was the inherited defects that really nailed the conclusions.

Booth: Is it worth just asking what is the biggest size of peptide that can get across the mucosal membrane? Four or five, six or seven? Anybody know? Is it guess work?[181]

Dr Derek Bangham:[182] I refer to the interesting property of gut mucosal cells of some species to transfer certain intact whole proteins selectively across a cell barrier. In the mid-1950s at the National Institute for Medical Research an American showed with histological methods that homologous gamma globulin was transported through gut columnar cells in suckling mice and rats.[183] R J Jerry and I showed the selective transmission and survival of orally fed I^{131} gamma globulin, but not albumin, into rats younger than three weeks.[184] Newborn calves, however, absorbed I^{131} serum proteins unselectively.[185] Later we showed in Rhesus monkeys there was almost no absorption from the amniotic fluid swallowed by the fetus, but there was selective transmission of gamma globulins into the fetal circulation across the placenta; very little albumin and no transferrin crossed the placenta (in the monkey the placental structure is closely similar to that of humans).[186]

---

[179] Douglas A P, Booth C C. (1970) Digestion of gluten peptides by normal human jejunal mucosa and by mucosa from patients with adult coeliac disease. *Clinical Science* 38: 11–25.

[180] Booth C C, Peters T J, Doe W F. (1977) Immunopathology of coeliac disease. *Ciba Foundation Symposium* 46: 329–346.

[181] Professor Michael Gardner wrote: 'Absorption of synthetic octapeptides via the paracellular pathway has been studied by Pappenheimer [see, for example, Pappenheimer J R, Dahl C E, Karnovsky M L, Maggio J E. (1994) Intestinal absorption and excretion of octapeptides composed of D amino acids. *Proceedings of the National Academy of Sciences of the United States of America* 91: 1942–1945]. It is now also clear that biologically significant amounts of intact protein can be absorbed, especially via the M-cell route [see, for example, Gardner M L G. (1988) Gastointestinal absorption of intact proteins. *Annual Review of Nutrition* 8: 329–350. *idem* (1994) Absorption of intact proteins and peptides. In Johnson L R. (ed.) *The Physiology of the Gastrointestinal Tract*. New York: Raven Press, 1795–1820].' Letter to Dr Daphne Christie, 5 November 1999.

[182] Dr Derek Bangham FRCP (b. 1924) was Head of the Division of Biological Standards at the National Institute for Medical Research (NIMR) from 1961 to 1972. He was later Head of the Hormones Division of the National Institute for Biological Standards and Control (NIBSC), from 1972 to 1987.

[183] Clark S L. (1959) The ingestion of proteins and colloidal materials by columnar absorptive cells of the small intestine in suckling rats and mice. *Journal of Biochemical and Biophysical Cytology* 5: 41–50.

[184] Bangham D R, Terry R J. (1957) Absorption of ^{131}I-labelled homologous and heterologous serum proteins fed orally to young rats. *Biochemical Journal* 66: 579–583, 584–587.

[185] Bangham D R, Ingram P L, Roy J H, Shillam K W G, Terry R J. (1958) Absorption of ^{131}I-labelled serum and colostral proteins from the gut of the young calf. *Proceedings of the Royal Society* 148: 184–191.

[186] Bangham D R. (1960) Transmission of homologous serum proteins to the foetus and to the amniotic fluid in the Rhesus monkey. *Journal of Physiology* 153: 265–289.

Turnberg: Thank you very much. We must move on. Now, Chris Booth will tell us about the exciting work on B_{12} absorption localization.

Booth: I think we mentioned earlier that entrance to gut work came in different ways. Physicists, physiologists and others can take an interest in the gut, and renal physicians like Malcolm Milne can come round to the gut. In the case of Sheila Callender and myself, we were both introduced to gut transport through the field of haematology. I should perhaps stress that I started my research career as a registrar in haematology in Sir John Dacie's department with David Mollin and it was there that I started work on vitamin B_{12}. At that stage, the B_{12} which was available was labelled as cobalt^{60} and it had a very low specific activity, but we had a friend called J E Bradley who had a colleague in the Birmingham cyclotron who was able to produce some cobalt^{56}. The cobalt^{56} was of very much higher specific activity than cobalt^{60}, which was being used in the American studies at that time. We gave this radioactive cobalt to Lester Smith at Glaxo. I think Lester Smith is one of the great unsung heroes of the modern world. He was a wonderful scientist, a most generous man, and I owe him much, because he produced this very highly radioactive B_{12}. My mentor, David Mollin, wanted to inject this into the blood to study the clearance of vitamin B_{12} from the blood. In vitamin deficiencies, if you inject a vitamin which is lacking, it disappears from the blood very quickly as it is taken up by the tissues. However, if you inject a tracer dose of B_{12} into a patient with severe deficiency of B_{12}, then the clearance from the blood is very slow. The reason for this is that there is a binding protein in the blood, which binds the B_{12} and traps it there. I wasn't terribly interested in that, but it seemed to me that we might for the first time be able to measure vitamin B_{12} in the blood after an oral dose, the object of the exercise being to distinguish normal people from those with Addisonian pernicious anaemia who had intrinsic factor deficiency and therefore could not absorb B_{12}. We fed our radioactive B_{12} to some normal people to see what happened. Normally if you do a glucose tolerance test, you take blood at half-hourly intervals for two hours after giving oral glucose and that covers the absorption phase of glucose. So I did that and to my surprise absolutely nothing appeared in the blood at all and we were puzzled by this. So we decided we'd extend the experiment to six hours and then found that at three hours we began to get an increase of radioactivity in the blood and there was a significant amount there at six hours. We went on to feed radioactive B_{12}, measure the radioactivity in the blood up to 24 hours, and found this fascinating phenomenon that nothing appeared in the blood for three hours after you fed the radiolabelled B_{12}, then it went up and came down as the B_{12} was cleared from the body into the lumen.[187] The question, of course, was why the delay happened, and clearly there were two options. One was that the B_{12} went into the intestinal mucosa, was held up there, and only later released, or secondly it could be that the B_{12} was absorbed lower down in the intestine than we had thought.

---

[187] Booth C C, Mollin D L. (1956) Plasma, tissue and urinary radioactivity after oral administration of ^{56}Co-labelled vitamin B_{12}. *British Journal of Haematology* 2: 223–236. Booth C C, Chanarin I, Anderson B B, Mollin D L. (1957) The site of absorption and tissue distribution of orally administered ^{56}Co-labelled vitamin B_{12} in the rat. ibid. 3: 253–261.

At that time there was considerable doubt about the site of absorption of B_{12}. W B Castle, who had discovered the intrinsic factor in Boston in the 1920s, had published an article claiming that absorption must occur high up in the intestine.[188] His reason for stating that was from studies of infection in Finland of people with the fish tapeworm, *Diphyllobotrium latum.* He pointed out that intubation studies had been done in Finland which had shown that anaemia only occurred when the parasite was in the upper intestine, not when it was in the lower. Of course, the reason for that was that until the patient was sick the tapeworm didn't really get up the intestine.

But there were other views. Frank Gardner, a physician in Boston, had seen patients who had abnormalities of the intestine which involved the distal intestine and who became B_{12} deficient.

We went on to do studies in the rat and we showed that when you fed radiolabelled B_{12} to a rat in a physiological dose, you got no radioactivity in the blood for one hour and during that one hour, all the B_{12} was locked up in intestine. To discover its site of absorption we had to chop the intestine up into lengths to find out where the B_{12} was. With a very small oral dose the radioactive B_{12} was in the mid-intestine. As you increased the dose, it went down into the distal intestine too, which was in keeping with the idea of increasing saturation of a receptor in the intestine. We went on to do a similar experiment in man in which we fed radiolabelled B_{12} to patients who were going to have part of their stomachs taken out by a surgeon for peptic ulcer. We deliberately measured the blood and showed the expected feature of the blood levels going up at three hours and we were able to show that at three hours if you took a Geiger counter and ran it round the intestine, all the B_{12} was in the distal intestine.

The point about Frank Gardner's studies on patients with gut abnormalities who didn't absorb B_{12} was that many of them had bacterial overgrowth in the intestine, and it was this that inhibited B_{12} absorption. So the crucial question was not just that they didn't absorb B_{12}, but whether, if you gave them an antibiotic and then measured B_{12} absorption, you could then show normal absorption. This had been shown by Sheila Callender's group in Oxford in patients with jejunal diverticulosis.[189] When they fed antibiotics the B_{12} absorption went up. But we found in some dozen patients that the only ones in whom the absorption got better after antibiotics, were those who had an ileum. We went on to study patients with gut resection, and showed what we expected, that there was malabsorption of B_{12} if the ileum had been removed. I was then Malcolm Milne's registrar, and we had a patient with Crohn's disease of the distal ileum. She was quite unable to absorb B_{12} and I remember Malcolm Milne looking at this patient and he said, 'Just like your rats, Christopher, just like your rats' and I said, 'Yes'. We published that paper in the

[188] Castle W B. (1953) Development of knowledge concerning the gastric intrinsic factor and its relation to pernicious anaemia. *New England Journal of Medicine* 249: 603–614. Wiseman G. (1964) op. cit. note 7, 299–324.

[189] See, for example, Badenoch J, Bedford P D, Evans J R. (1955) Massive diverticulosis of the small intestine with steatorrhoea and megaloblastic anaemia. *Quarterly Journal of Medicine* 24: 321–333. See also Anonymous. (1967) Jejunal diverticulosis and B_{12} deficiency. *The London Clinical Medical Journal* 8: 10–11.

Lancet in 1959.[190] In the following year the *Year Book of Medicine* came out. The editor of the gastrointestinal section was the great American physiologist, Franz J Ingelfinger, and he chose to launch an attack upon this paper which lasted for two whole pages of that fine print that they used in the *Year Book of Medicine*, saying that he didn't believe a word of it and that it couldn't have been proved and it wasn't true.[191] I wrote to him to object. But it soon became quite clear from other studies that B_{12} is normally absorbed in the ileum. I have a suspicion that I must have been in some way influenced at that time by Milne's concept of the gut as a renal tubule, because that was the way that he was thinking.

The work was followed up by Bob Donaldson in the United States, using isolated brush-borders from experimental animals and showing that the intrinsic factor bound radiolabelled B_{12} would only be taken up by the brush-borders of the ileum and not by the jejunum.[192] Subsequently a considerable amount of work has been done isolating the receptors in the ileum that take up B_{12}.

Now one other point about B_{12}. The requirement of B_{12} in man is something between 1 and 4 micrograms per day, and if you don't get that you get pernicious anaemia, which is what happens to people who have no intrinsic factor. In fact, the B_{12} absorption is the only one which demonstrates the concept of mucosal block, that is the idea that you can increase the dose of what you are feeding, and then you reach a stage where no more will go in. It is not like glucose, where when you give increasing doses of glucose the receptor becomes saturated as you increase it and you get a curvilinear relationship. With B_{12} and intrinsic factor you don't. You get a linear relationship up to a certain point and in most people the limit is about 2 micrograms, something like that. So that is your maximum capacity to absorb B_{12} and it's restricted to your ileum.

Turnberg: Thank you very much. Any comments on B_{12}?

Boyd: Two questions. One, I would like to hear about the origin of the Schilling test. Two, were the structural studies on B_{12} and the work of Dorothy Hodgkin completely unrelated to your interest in epithelial transport of this vitamin?

Booth: Well, Dorothy Hodgkin[193] first. She was working entirely on crystallography looking at the molecular structure, but she was interested in all that was going on. We

---

[190] Booth C C, Mollin D L. (1959) The site of absorption of vitamin B_{12} in man. *Lancet* i: 18–21.

[191] Ingelfinger F J. (1960) The digestive system. Site of absorption of vitamin B_{12} in man. In Beeson P B, Muschenheim C, Castle W B, Harrison T R, Ingelfinger F J, Bondy P K. (eds) *Year Book of Medicine.* Chicago, IL: The Year Book Publishers, 506–507.

[192] See, for example, Donaldson R M, Jr Mackenzie I L, Trier J S. (1967) Intrinsic factor-mediated attachment of vitamin B_{12} to brush borders and microvillous membranes of hamster intestine. *Journal of Clinical Investigation* 46: 1215–1228. Mackenzie I L, Donaldson R M. Jr (1972) Effect of divalent cations and pH on intrinsic factor-mediated attachment of vitamin B_{12} to intestinal microvillous membranes. ibid. 51: 2465–2471.

[193] Dorothy Mary Hodgkin FRS (1910–1994) was Research Fellow at Somerville College, Oxford, from 1936 to 1977 and Wolfson Research Professor at the Royal Society from 1960 to 1977. A crystallographer of distinction, she was awarded the Nobel Prize for Chemistry in 1964 for discoveries, by use of X-ray techniques, of the structure of molecules, including penicillin, vitamin B_{12} and insulin. She was made a member of the Order of Merit in 1965 and was awarded the Lenin Peace Prize in 1987. See Ferry G. (1998) *Dorothy Hodgkin. A Life*. London: Granta Books.

had a meeting in 1956 in Hamburg. Dorothy Hodgkin came to that meeting with Lester Smith and we were all there together, clinicians and various people from Europe and America. It was at that meeting that Dorothy Hodgkin got agreement to the nomenclature for the new molecule and the different bits of it. She was a bright, breezy, lovely young lady in those days. I knew her in later years when she had become very crippled by rheumatoid arthritis, but she was tremendously excited by everything that was happening and was always very interested in everything.

The first question was about the Schilling test, named after Robert F Schilling, who was a research worker in Boston.[194] The point about B_{12} is that if you feed it by mouth and then look at the urine, you don't get any radiolabelled B_{12} coming out in the urine at all. It all gets locked up in the body in some way or another. So that if you want to do a test of absorption using oral doses of B_{12}, you can't do it just by feeding B_{12}. On the other hand, if you inject B_{12} as a large, subcutaneous intramuscular injection at the same time, then much of it will flood out into the urine immediately. Schilling took advantage of this and said that if you fed a physiological dose of B_{12}, say 1–2 micrograms, and then gave an injection of, say, 1000 micrograms, then it would wash around 30 per cent of radiolabelled B_{12} out into the urine.[195]

Dr Sheila Callender:[196] We abandoned the Schilling test quite quickly because we then had the whole-body counter, which made life very much easier.[197]

Booth: Well, I suppose you could say that the Schilling test was the poor man's absorption test, if you didn't have a whole-body counter. But it was very useful clinically.

Pounder: I'd not heard about the B_{12} absorption levelling off. It makes you wonder whether there would be an overload disease – yet we see plenty of patients who get

---

[194] See page 72.

[195] Silberstein E B. (1969) The Schilling test. *Journal of the American Medical Association* 208: 2325–2326. Anonymous. (1969) Schilling test of vitamin B_{12} absorption. *British Medical Journal* i: 300–301. Rinsler M G, Booth C C. (1985) Intestinal function tests. In Booth C C, Neale G. (eds) *Disorders of the Small Intestine.* Oxford: Blackwell Scientific Publications, 28.

[196] Dr Sheila Callender MD FRCP (b. 1914) qualified in medicine in 1938 at St Andrews. She spent three years in pathology in Dundee and developed a special interest in haematology. In 1942 she joined Professor L J Witts in the Nuffield Department of Medicine in Oxford, and in 1946 joined Dr Carl Moore and his department in St Louis, Missouri, USA, who were studying iron metabolism using radioactive iron as a tracer. On returning to Oxford she introduced the technique especially for studies of iron absorption from foods.

[197] The whole-body counter offered a convenient and reliable method of measuring retention or loss of radioactivity *in vivo*. Dr Sheila Callender wrote: 'The Schilling test was certainly useful but it was a relatively crude measurement. It required the cooperation of the subject to ensure an accurate collection of urine as indeed did the collection of faeces for the measurement of unabsorbed radioactive B_{12} for the estimation of absorption [Callender S, Turnbull A, Wakisaka G. (1954) Estimation of intrinsic factor of Castle by use of radioactive vitamin B_{12}. *British Medical Journal* i: 10–13]. We abandoned both these methods when, in 1966, Warner and Oliver in Oxford designed a simple whole-body counter, using the shadow shield technique, which enabled direct measurement of absorption [Warner G T, Oliver R. (1966) A whole-body counter for clinical measurements using the 'shadow shield' technique. *Physics in Medicine and Biology* 16: 83–94. Callender S, Witts L J, Warner G T, Oliver R. (1966) The use of a simple body counter for haematological investigations. *British Journal of Haematology* 12: 276–282].' Note on draft transcript, 25 April 2000.

addicted to little red injections, who don't seem to come to any harm from being awash with vitamin B_{12}.

Booth: No, I think the advantage of B_{12} is that it is entirely water soluble, so it just gets excreted in the urine. That's why water-soluble vitamins have never caused an overload problem. The only vitamins that cause overload problems are the fat-soluble vitamins D and A.

Wrong: It's a very minor point, but, of course, B_{12} clearance was used for a time for measurement of glomerular filtration.[198] It was entirely in accord with what Chris has just said.

Turnberg: Let's discuss bile acids now. Who else but Hermon Dowling to talk about these?

Dowling: Thank you. Well it seems appropriate I should be talking about bile acid absorption immediately after Sir Christopher's contribution, because my story forms a logical sequence to his. And although he might imply that vitamin B_{12} absorption is complicated, it's nothing compared to bile acids! We aren't talking about the absorption of just one substrate, but about the absorption of perhaps ten or more different bile acids.

As you know, the liver synthesizes two primary bile acids – cholic acid and chenodeoxycholic acid – from cholesterol. After conjugation (or amidation) to glycine or taurine, the four resultant bile acids are then secreted into bile and pass into the intestine where colonic bacteria biotransform them into the two secondary bile acids – deoxycholic acid and lithocholic acid. Some of these secondary bile acids are then absorbed and return to the liver where they, in turn, are conjugated with glycine or taurine before they join their 'parents' in the enterohepatic circulation. Intestinal bacteria can also oxidize and/or reduce the primary and secondary bile acids to form keto bile acids and β-epimers. So altogether, there are at least ten to 15 different bile acids, all of which can be absorbed from the stomach, duodenum, jejunum, ileum and the colon – albeit with very different efficiencies. So it takes somebody either very naive or very ignorant to start studying bile acid absorption – and I was both of these.

I too would like just to give a rather personal account. When Tilli Tansey approached me, if I could paraphrase what she said, it was something like, 'Tell us how it was for you' and that's exactly what I would like to do.

Forty years ago Chris Booth and colleagues published a paper in the *British Journal of Surgery*[199] showing that if one resects the jejunum, the residual ileum became quite hypertrophic, whereas if one resects the ileum, the remaining jejunum does not show

---

[198] Nelp W B, Wagner H N, Reba R. (1964) Renal excretion of vitamin B_{12} and its use in measurement of glomerular filtration in man. *Journal of Laboratory and Clinical Measurement* 63: 480–491. Breckenridge A, Metcalfe-Gibson A. (1965) Methods of measuring glomerular filtration rate. A comparison of inulin, vitamin B_{12} and creatinine clearances. *Lancet* ii: 265–267.

[199] Booth C C, Evans K T, Menzies T, Street D F. (1958–9) Intestinal hypertrophy following partial resection of the small bowel in the rat. *British Journal of Surgery* 46: 403–410.

the same degree of adaptive growth. Chris, maybe you can confirm this, but I think that that particular study was one of a series in which you looked at the sites of absorption of different substrates – including intrinsic factor-bound vitamin B_{12}. And to prove that B_{12} was absorbed in the terminal ileum, you showed that if one removes the ileum, one gets B_{12} malabsorption whereas if one performs a jejunectomy (as a control), one does not [**Booth**: That's correct].[200]

Well, having performed these surgical procedures and measured B_{12} absorption, when the rats were subsequently opened up, they showed these very striking adaptive changes in the gut. At that point I joined Chris as a very junior research fellow and my task was to study these adaptive phenomena.

At that time, the registrars and research fellows working with Sir Christopher set up a journal club and once a month we would organize a working supper in each other's homes (with people like Soad Tabaqchali, Graham Neale and several others). At one of these evening journal clubs, I had to review a paper by a young Australian called Marc Playoust. He had gone to work, I think, with Kurt Isselbacher at the Massachusetts General Hospital (although the publication in question was with Leon Lack and Weiner).[201] His study was based on Lack and Weiner's observations, which extended the very old studies by Schiff and Tappeiner, that the ileum was the active transport site for conjugated bile acids.[202]

Chris had done much to popularize the idea that the Schilling test of vitamin B_{12} absorption could be used as a test of ileal function. Given Playoust's findings it was a logical extension of Chris's ideas to suggest that it should be possible to devise a clinical test of ileal function using bile acids, rather than vitamin B_{12}. Indeed, this was the basis of proposed studies in Boston with Franz Ingelfinger and Donald Small (one of Ingelfinger's protégés).

At that time, Donald Small's interest was not so much in the physiology of bile acid absorption, but rather in the pathogenesis of gallstones. But today we are not talking about bile acids in the liver: nor are we talking about the absorption of a nutrient – but rather about the absorption of endogenous detergents which facilitate the digestion and absorption of dietary fats, sterols and fat-soluble vitamins. Moreover as clinicians know, abnormal bile acid metabolism can cause many problems.[203] For

---

[200] Booth C C. (1961) The metabolic effects of small intestinal resection in man. *Postgraduate Medical Journal* 37: 725–739.

[201] Playoust M R, Lack L, Weiner I M. (1965) Effect of intestinal resection on bile salt absorption in dogs. *American Journal of Physiology* 208: 363–369. op. cit. note 212.

[202] Professor Dowling wrote: 'Playoust showed that in the dog, ileal resection markedly impaired the enterohepatic circulation of bile acids while jejunal resection did not.' Note on draft transcript, 12 June 2000. See Baker R D, Searle G W. (1960) Bile salt absorption at various levels of rat small intestine. *Proceedings of the Society for Experimental Biology* 105: 521–523. op. cit. notes 10, 25 and 212.

[203] See, for example, Dowling R H. (1972) The enterohepatic circulation. *Gastroenterology* 62: 122–140. Small D M, Dowling R H, Redinger R N. (1972) The enterohepatic circulation of bile salts. *Archives of Internal Medicine* 130: 552–573. Hofmann A. (1978) The enterohepatic circulation of bile acids. In Sleisenger M, Fordtran J T. (eds) *Gastrointestinal Disease*. London: W B Saunders, 418–429. *idem* (1989) Enterohepatic circulation of bile acids. In Schultz S G, Forte J G, Rauner B B. (eds) *Handbook of Physiology*. New York: Oxford University Press, 567–596.

example, jejunal bile acid deficiency can impair the absorption of dietary lipids. And when excess bile acids spill into the colon they can cause diarrhoea, inhibit water and electrolyte transport, influence motility, regulate peptide hormone release, promote oxalate absorption, and so on. They may even play a role in the development of colorectal cancer. So for all these reasons, it seemed important to study further the effects of ileal resection on bile acid metabolism.

At that time, several investigators had shown that patients with ileal resection often had severe diarrhoea, steatorrhoea and malabsorption of cholesterol and fat-soluble vitamins whereas those with jejunal resection did not. On this basis, they reached the logical, but erroneous, conclusion that the ileum must be the site of absorption of dietary fat, sterols and fat-soluble vitamins. We now know, of course, that these phenomena are largely a consequence of bile acid malabsorption.

To study the effects of ileal resection on the enterohepatic circulation of bile acids, we had to adopt indirect approaches – rather than measuring bile acid absorption directly. One such approach is to study bile fistula models but, as you know, once you cannulate the bile duct and drain the bile acid pool to the exterior, there are all sorts of artifacts and you are no longer studying normal physiology. To overcome this problem, Donald Small proposed that we should study Rhesus monkeys and apply a technique first developed in humans by Isaksson and Thureborn.[204] This involved a chronic biliary fistula and the use of an extra-corporeal stream-splitting device, coupled to a drop counter. Drops of fistula bile passed through the drop counter and the stream-splitter was programmed to divert every twentieth drop of bile into a sampling system and to return the other 19 drops to the upper intestine (through a return cannula in the distal common bile duct).[205] In other words, the 5 per cent bile diversion provided a representative sample of bile for analysis, but ensured that the enterohepatic circulation remained virtually intact. It enabled us to sample bile without significantly distorting the normal physiology.[206]

One related question which we addressed was as follows: if you haven't got an ileum (as a result of resection or bypass), does the rest of the gut take over bile acid

---

[204] See Thureborn E. (1962) Human hepatic bile: composition changes due to altered enterohepatic circulation. *Acta Chirurgica Scandinavica* 303: 1–63. Isaksson B, Thureborn E. (1963) A method for continuous collection of hepatic bile at 'intact' entero-hepatic circulation in man. *Second World Congress of Gastroenterology, Munich, 1962.* 3: 3.

[205] See, for example, Dowling R H, Mack E, Picott J, Berger J, Small D M. (1968) Experimental model for the study of the enterohepatic circulation of bile in rhesus monkeys. *Journal of Laboratory and Clinical Medicine* 72: 169–176. Dowling R H, Mack E, Small D M. (1970) Effects of controlled interruption of the enterohepatic circulation of bile salts by biliary diversion and by ileal resection on bile salt secretion, synthesis and pool size in the rhesus monkey. *Journal of Clinical Investigation* 49: 232–242. *idem* (1971) Biliary lipid secretion and bile composition after acute and chronic interruption of the enterohepatic circulation in the Rhesus monkey. IV. Primate biliary physiology. ibid. 50: 1917–1926.

[206] Professor Hermon Dowling wrote: 'We then used this model in Rhesus monkeys to study graded interruption of the enterohepatic circulation either with the stream splitter (10, 20, 33, 66 or 100 per cent bile diversion) or as a result of resecting the distal one-third or two-thirds of the small intestine.' Note on draft transcript, 7 June 2000. op. cit. note 205.

transport?[207] This, of course, was the theme of the work that Chris Booth had started me on when I was working on adaptation. And we used just this technique – not just perfusing the gut and measuring luminal disappearance with all the artifacts that this involves – such as non-specific binding to surface mucus, bidirectional transport, etc. Instead we used bile fistula ileectomized rats, and perfused the residual jejunum or colon with isotopically labelled bile acids. Once absorbed, bile acids go to the liver and pass out in the bile, so the appearance of labelled bile acids in bile is proof positive that they have been absorbed.[208]

One other comment about bile acids. As many of you know, for several years we were trying to dissolve cholesterol gall stones with oral bile acid treatment[209] and at that time, it became important to understand something about the pharmacokinetics and bioavailability of the ingested bile acids. To do this, we had to modify an approach frequently used by clinical pharmacologists – that is, looking at the area under the serum bile acid concentration–time curves after the oral ingestion of different bile acid doses. With this approach one normally needs a 'gold standard' as a reference point. That gold standard is usually obtained by giving the same dose of the drug intravenously, and looking at the serum concentration–time curve. However, one cannot use this approach to study bile acid absorption in humans, because intravenous bile acids are toxic.

Earlier, Les [Turnberg] was talking about John Fordtran. On one occasion, he visited us at Guy's Hospital when we discussed the problem. He proposed that we should intubate the gut and infuse different bolus doses of bile acids (plus markers) down the proximal lumen of a double-lumen tube. Provided we confirmed that no bile acids remained in the luminal fluid aspirated from the district port of the double lumen tube, we could use resultant concentration–time curves as *bona fide* reference standards to study the pharmacokinetics and bioavailability of both chenodeoxycholic acid and ursodeoxycholic acids.[210]

The final thing that I would say is that although my hope was to follow in Chris's footsteps by developing a bile acid absorption test as a measure of ileal function (analogous to the Schilling test), I never accomplished that aim. Instead, this goal was achieved simultaneously by two separate groups – Alan Hofmann and Hans Fromm,

---

[207] Dowling R H, White J, Perry P M. (1973) Conservation of intestinal bile acid concentration after ileal resection. *Helvetica Medica Acta* 37: 103–111. Mok H Y, Perry P M, Dowling R H. (1974) The control of bile acid pool size: effect of jejunal resection and phenobarbitone on bile acid metabolism in the rat. *Gut* 15: 247–253.

[208] Professor Hermon Dowling wrote: 'Mike Perry, a surgical fellow at the that time, showed that after ileal resection, there was an adaptive increase in jejunal, but not in colonic, bile acid absorption.' Note on draft transcript, 12 June 2000.

[209] Bell G D, Whitney B, Dowling R H. (1972) Gallstone dissolution in man using chenodeoxycholic acid. *Lancet* ii: 1213–1216. Iser J H, Dowling R H, Mok H Y I, Bell G D. (1975) Chenodeoxycholic acid treatment of gallstones: a follow-up report and analysis of factors influencing response to therapy. *New England Journal of Medicine* 293: 378–383. Maton P N, Murphy G M, Dowling R H. (1977) Ursodeoxycholic acid treatment of gallstones: dose–response study and possible mechanisms of action. *Lancet* ii: 1297–1301.

[210] See Ponz de Leon M, Loria P, Carulli N, Murphy G M, Dowling R H. (1980) Intestinal solubilization, absorption, pharmacokinetics and bioavailability of chenodeoxycholic acid. *European Journal of Clinical Investigation* 10: 262–271.

(his then young German research fellow) at the Mayo Clinic, and Sherr and colleagues at the Johns Hopkins Hospital in Baltimore. This was, of course, the ^{14}C-glycine-labelled glycocholate bile acid breath test which is still used in some centres today.[211]

Chris may remember that when Alan Hofmann synthesized this isotopic bile acid he generously sent some of it for us to try out in one of our patients with ileal resection. This extremely valuable sample had been flown across the Atlantic. But when we fed it to our poor patient, she promptly vomited it all over the floor. Chris tried to persuade me that we should follow Malcolm Milne's example because he had scooped up some vomit containing ingested isotopes and reused it, but we didn't follow that example!

So with these few reminiscences, I would welcome your comments and questions.

Turnberg: Thank you very much, very nice to hear it. Any comments on bile acids?

Booth: Can you show any form of saturation kinetics of bile acid transport either in *in vitro* systems or in man?

Dowling: As noted earlier at this meeting, the kinetics of bile acid transport in different segments of the gut were worked out in the early 1960s, by Lack and Weiner.[212] However, the topic was revisited subsequently by others, including John Dietschy, who worked with Jared Diamond on the importance of unstirred layers in bile acid transport.[213] They redefined this in considerable detail, as did Henrik Westergaard, and Eugene Schiff, both of whom worked with John Dietschy in this field[214] More recently the kinetics of bile acid absorption from the intestine was re-examined by Hofmann and colleagues, in San Diego.

The concept that the active transport site in the terminal ileum is responsible for *all* bile acid absorption, is potentially misleading. Of course, the ileum is the site for active bile acid transport and it is very important for the trihydroxy bile acid, cholic acid. But passive diffusion is also important[215] and in the jejunum and colon, this is

---

[211] A test that is used both as a measure of ileal function and as a screen for bacterial overgrowth in the small intestine. See Fromm H, Hofmann A F. (1971) Breath test for altered bile acid metabolism. *Lancet* ii: 621–625. Sherr H P, Sasaki Y, Newman A, Banwell J G, Wagner H N Jr, Hendrix T R. (1971) Detection of bacterial deconjugation of bile salts by a convenient breath-analysis technic. *New England Journal of Medicine* 285: 656–661. See also page 70.

[212] Lack L, Weiner I M. (1961) *In vitro* absorption of bile salts by small intestine of rats and guinea pigs. *American Journal of Physiology* 200: 313–317. *idem* (1966) Intestinal bile salt transport: structure-activity relationships and other properties. ibid. 210: 1142–1152. *idem* (1973) Bile salt transport systems. In Nair P P, Kritchevsky D. (eds) *The Bile Acids, Physiology and Metabolism.* New York: Plenum Press, 33–54.

[213] See, for example, Dietschy J M. (1968) Mechanisms for the intestinal absorption of bile acids. *Journal of Lipid Research* 9: 297–309. op. cit. note 214.

[214] See, for example, Schiff E R, Dietschy M. (1969) Current concepts of bile acid absorption. *American Journal of Clinical Nutrition* 22: 272–278. Dietschy J M. (1973) Mechanism of bile acid and fatty acid absorption across the unstirred water layer and brush border of the intestine. *Helvetica Medica Acta* 37: 89–102. Westergaard H, Dietschy J M. (1974) Delineation of the dimensions and permeability characteristics of the two major diffusion barriers to passive mucosal uptake in the rabbit intestine. *Journal of Clinical Investigation* 54: 718–732.

[215] op. cit. notes 205 and 207.

the principal transport mechanism for glycine conjugated, and unconjugated bile acids. In the absence of an ileum, hepatic supplies of taurine for bile acid conjugation rapidly become exhausted as a result of which, most of the bile acids become conjugated with glycine.[216] Fortuitously, this acts as conservation mechanism whereby, even in the absence of an active bile acid transport system in the ileum, at least some of the glycine conjugated bile acids can still be absorbed from extra-ileal sites, by diffusion.[217]

I might add that molecular medicine arrived fairly late in the bile acid field, but about five years ago, Paul Dawson from North Carolina identified and cloned the ileal bile acid transporter gene, and this sodium-linked bile acid transporter gene has now been defined in some detail.[218]

Turnberg: We will move on now to iron absorption. This is an interesting area for me, because I started out my research by measuring iron absorption in patients in a rather crude way and I always remember thinking that the doyenne of iron absorption was Sheila Callender. I used to quote her work all the time, and I'm delighted that here she is going to talk about iron absorption.

Callender: It seems almost incredible that when I was a student in the 1930s, iron deficiency when not clearly related to blood loss was referred to as 'idiopathic iron deficiency'. However, Stanley Davidson and colleagues had shown the very high frequency of iron deficiency in the poorer parts of places like Aberdeen and Edinburgh, and had suggested that this was nutritional in origin.[219] Also, Witts had shown that a high proportion of patients with iron deficiency had achlorhydria.[220] He thought this might be an aetiological factor and suggested the name 'simple achlorhydric anaemia'. A significant breakthrough came with McCance and Widdowson's meticulous iron balance studies of 1937, which showed that there was negligible excretion of iron from the gut.[221] This posed the problem of how the absorption of iron was controlled to prevent the development of iron overload. Little progress was made until the development of radioisotopes as tracers in the early 1940s. Then there was an explosion of investigations, notably in the USA, in St Louis in Carl Moore's laboratory, where I went to work in 1946. This group had shown that ferrous iron was much better absorbed than the ferric form, and we soon also showed

---

[216] Chadwick V S, Modha K, Dowling R H. (1973) Mechanism for hyperoxaluria in patients with ileal dysfunction. *New England Journal of Medicine* 289: 172–176.

[217] Professor Hermon Dowling wrote: 'Indeed, we found that after distal small bowel resection in the Rhesus monkey, approximately 50 per cent of the circulating bile acids could still be reabsorbed from the non-ileal sites (op. cit. note 205).' Note on draft transcript, 12 June 2000.

[218] Wong M H, Oelkers P, Craddock A L, Dawson P A. (1994) Expression cloning and characterization of the hamster ileal sodium-dependent bile acid transporter. *Journal of Biological Chemistry* 269: 1340–1347. Dawson P A, Oelkers P. (1995) Bile acid transporters. *Current Opinion in Lipidology* 6: 109–114.

[219] Davidson L S P, Fullerton H W, Campbell R M. (1935) Nutritional iron-deficiency anaemia, with special reference to prevalence and age and sex incidence. *British Medical Journal* ii: 195–198.

[220] Witts L J. (1930) Simple achlorhydric anaemia. *Guy's Hospital Reports* 80: 253–296. *idem* Achlorhydria and anaemia. *Practitioner* 124: 348–357.

[221] McCance R A, Widdowson E M. (1937) Absorption and excretion of iron. *Lancet* ii: 680–684.

that iron was much better absorbed in iron-deficient patients than in normal subjects.[222] This was using inorganic ferrous iron salts, clearly not completely applicable to absorption of iron from foods. Now, a number of people began to devise ways of labelling foods with radioactive iron, for example by growing vegetables and cereals in hydroponic tanks. When I came back to Oxford we continued this work and confirmed that the iron in cereals labelled with radioactive iron was very poorly absorbed in contrast to that from inorganic iron salts. We also labelled eggs by injecting birds with ^{59}Fe and showed a very poor absorption from this source.[223] It appeared that absorption of inorganic iron in food was greatly influenced by the composition of the diet. Acid in the stomach increased the absorption of inorganic iron, and, notably, ascorbic acid – for example in orange juice – which kept the iron in the ferrous form and also formed small molecular weight complexes, enhanced absorption. We went on to show that there were other factors which would depress absorption, such as phytates and phosphates in cereals and the phosphoprotein in eggs.

We then turned our attention to haem iron absorption. For a long time iron in haem had been thought not to be available for absorption. In fact, Douglas Black and Joan Powell,[224] when I arrived in Oxford, were carrying out a study on iron-deficient patients and I was rather surprised to see these patients in the ward with blood dripping into their stomachs from bottles of blood. Black and Powell were able, by careful iron-balance studies, to show that a proportion of the iron was absorbed from the blood, and, in fact, three out of four patients showed a slight reticulocytosis and a small response to the treatment with the haem iron.[225] Much later we went on to study haem iron absorption using rabbit blood labelled biologically with ^{59}Fe. We injected rabbits with radioactive iron and took samples of their blood. When it had reached a peak of activity, we gave haemolysates of the blood to various volunteers, both iron deficient and normal, using the amount of blood containing 5-milligram iron, i.e. the amount we had been using with the inorganic iron studies. We were able to show that the haem iron absorption was unaffected by substances like phytates, phosphates, and ascorbic acid, quite unlike absorption from inorganic iron. Also, the peak of plasma iron absorption of the radioactive iron following the dose was delayed. Instead of appearing quite early, it was delayed up to 100–200 minutes after the dose, so we assumed that the iron was being absorbed further down the intestine.

I went on with John Badenoch to look at patients with steatorrhoea and coeliac disease. These conditions are characterized by sensitivity to gluten in wheat. This results in

---

[222] Moore C V, Dubach R, Minnich V, Roberts H K. (1944) Absorption of ferrous and ferric radioactive iron by human subjects and by dogs. *Journal of Clinical Investigation* 23: 755–767. Badenoch J, Callender S. (1954) Use of radioactive iron in investigation of anaemia. *British Journal of Radiology* 27: 381–386.

[223] Callender S T, Marney S R, Warner G T. (1970) Eggs and iron absorption. *British Journal of Haematology* 19: 657–665.

[224] Black D A K, Powell J F. (1942) Absorption of haemoglobin iron. *Biochemical Journal* 36: 110–112.

[225] Callender S T, Mallett B J, Smith M D. (1957) Absorption of haemoglobin iron. *British Journal of Haematology* 3: 186–192. Anand B S, Callender S T, Warner G T. (1977) Absorption of inorganic and haemoglobin iron in coeliac disease. *British Journal of Haematology* 37: 409–414.

damage to the villi in the upper part of the small intestine leaving a flat mucosal surface. We found that inorganic iron was very poorly absorbed; in fact this absorption could be used as a pointer to a diagnosis of malabsorption syndrome.[226] In contrast, the haem iron absorption was normal in these patients. This again suggested that the haem iron was absorbed much further down the intestine where there was no mucosal damage.[227]

It no longer seems surprising to look back on the very high frequency of iron deficiency in women in the 1930s during the depression, because I know from my own experience of 'district deliveries' in Dundee, many women had multiple pregnancies increasing iron requirement and also they were existing mainly on diets of bread and tea. Tea has been shown by Disler and colleagues to be another factor which exerts an inhibitory effect on iron absorption.[228] So gradually, the whole picture has emerged. There isn't anything 'idiopathic' about iron deficiency, but there are multiple factors in the absorption of iron, which determine the cause of anaemia.

Booth: Can I just chip in an additional point. Sheila Callender referred to the ladies in Dundee living on tea and bread. When I was in Dundee we used to call it 'whippet disease'. Certainly when the miners in Fife kept whippets in food-rationing time, the meat was given to the whippet and nobody else got it, so we used to call it 'whippet disease'.

Callender: In my time before the war they were existing on the dole of 30 shillings a week, and if ever there was a scrap of meat, it was given to the men in the family, because they had to keep their strength up!

Turnberg: What about the mechanisms for haem iron absorption? Has there been any further progress on how it gets in?

Callender: For inorganic iron the first idea was Granick's – who suggested that apoferritin was formed in response to iron in the mucosal cell and this was converted to ferritin and from that there was passage on to the serosal surface and into the blood.[229] Where the apoferritin was saturated it was referred to as a mucosal block; it's not as simple as that, certainly. We now think that the ferritin is really a mechanism for bypassing the absorption and that most of the ferritin iron is shed from the gut.

---

[226] Badenoch J, Callender S T. (1960) Effect of corticosteroids and gluten-free diet on absorption of iron in idiopathic steatorrhoea and coeliac disease. *British Journal of Haematology* 23: 135–146.

[227] Dr Sheila Callender wrote: 'All these studies indicate that absorption of iron occurs largely in the upper part of the small intestine, and depends on the iron being present in a soluble form. Any other substance which converts it into an insoluble state will inhibit absorption. Conversely, anything which increases solubility will enhance absorption. Potentially iron can be absorbed from any level of the intestine but the more alkaline medium lower down prevents this, except in the case of haem iron with is not dependent on such factors.' Note on draft transcript, 25 April 2000.

[228] Disler P B, Lynch S R, Charlton R W, Torrance J D, Bothwell T H, Walker R B, Mayet F. (1975) The effect of tea on iron absorption. *Gut* 16: 193–200.

[229] See, for example, Granick S. (1946) Increase of the protein apoferritin in the gastrointestinal mucosa as a direct response to iron feeding. The function of ferritin in the regulation of iron absorption. *Journal of Biological Chemistry* 164: 737–746. See also Drysdale J W, Shafritz D A. (1975) *In vitro* stimulation of apoferritin synthesis by iron. *Biochimica et Biophysica Acta* 383: 97–105. Zahringer J, Konijn A M, Baliga B S, Munro H N. (1975) Mechanism of iron induction of ferritin synthesis. *Biochemical and Biophysical Research Communications* 65: 583–590.

The rate of erythropoiesis, and the state of the iron stores, are both important factors in regulating absorption through the cell.[230]

Turnberg: And haem. Is haem split before the iron gets into the cell? In some way digested?

Callender: Probably most of the haem is split in the cell and is absorbed after it has been liberated there. Hence the lack of effect on absorption of other factors in the diet.

Booth: We know a lot in terms of *in vitro* systems for glucose, galactose, dibasic and amino acids and so on. Do we have comparable data for iron in respect to kinetics of absorption and so on?

Peters: Iron absorption is enormously complicated. Tim Cox and I worked very much in this area. Tim did his MD thesis with me and determined the kinetic constants for iron absorption in humans.[231] There is evidence that haem–iron can also be broken down within the lumen of the gut in the presence of ascorbate. A chap with the splendid Irish name of Poroc O'Cora from Galway showed that chemically iron could be released from haem within the lumen of the gut. The remaining haem can be taken into the cell and the iron released by haem oxygenase.

Tansey: It strikes me that during the course of this meeting, the scientists and clinicians have been singing from different hymn sheets. Was there much interaction between the two groups?

Dowling: I think Tilli is right to draw attention to this. On the other hand, I think the apparent split is not quite as great as it might seem. For many years we had a 'Transport Club' where basic scientists and clinicians worked together on all aspects of transport. Moreover, when I started working with Chris Booth, one of the first things he did was to send me off to David Smyth's lab in Sheffield (where I met Roy Levin for the first time) to learn some basic methods of studying intestinal transport. Later, when I arrived in Boston, I spent three months working exclusively on the physical chemistry of bile acids – titration curves in a test tube from which we derived the pH of precipitation, and the pK_as of the bile acids, and the ratio of ionized versus non-ionized bile acid species (which we have just been talking about).[232] I think that such approaches are vital.

Turnberg: But you had to go to America to do it!

Dowling: I did and that's 35 years ago. Today our work is on bile acid absorption from the colon as a function of transit-induced changes in intracolonic pH (probably as a

---

[230] Dr Sheila Callender wrote: 'It is possible that some of the iron is released within the lumen of the gut but findings of the *in vivo* studies strongly suggest that the great part of the iron is liberated within the mucosal cell and is therefore unaffected by intraluminary factors.' Letter to Dr Daphne Christie, 25 October 1999.

[231] See, for example, Cox T M, Peters T J. (1979) The kinetics of iron uptake *in vitro* by human duodenal mucosa: studies in normal subjects. *Journal of Physiology* 289: 469–478.

[232] Dowling R H, Small D M. (1968) The effect of pH on the solubility of varying mixtures of free and conjugated bile salts in solution. *Gastroenterology* 54: 1291. Roda A, Hofmann A F, Mysels K J. (1983) The influence of bile salt structure on self-association in aqueous solutions. *Journal of Biological Chemistry* 258: 6362–6370.

result of changes in short-chain fatty acid absorption).[233] It's a question we have been addressing because bile acids (particularly deoxycholic acid) have been implicated in the pathogenesis of gallstone disease and colorectal cancer. Slow transit constipation influences colonic luminal pH, and the solubility and bio-availability of the nascent deoxycholic acid. In this situation, the physical chemistry of the secondary bile acids becomes vitally important – lessons first learnt 35 years ago.

Booth: I wonder whether I might just put in a plug for the Medical Research Council. When I was working at Hammersmith, I think I was made a Professor for the first time in 1966 and I remember just then applying to the MRC for a research grant. In those days there was a thing called Group Structure. You could apply for an MRC Group and this would support perhaps a lecturer, a couple of technicians, some equipment, and the rest of it, and that lasted for five years. At the end of the five years, in those halcyon days, the university had to take over your funding. That was a rule and had been negotiated by that great supporter of medical research in this country, Sir Harold Himsworth.[234] I remember applying for one of his groups in 1967. We got our group. But the point about it was the agreement that the other group that they supported was an intestinal absorption group. That was in Sheffield and under David Smyth. We were expected to get together and, as Hermon has said, we did.[235]

Walker-Smith: In relation to Dennis Parsons, it is interesting how one can have a long-term influence. A young medical student was inspired by him whilst doing his BSc to take up gastroenterology, in fact paediatric gastroenterology. He was Ian Sanderson, who has recently been appointed Professor of Paediatric Gastroenterology at Bart's. His inspiration was through Dennis Parsons, which led him to work with Dr Tony Dawson, and to be where he is now.

Turnberg: I think the whole issue of how clinicians and basic scientists work together is an enormously tricky one. You ought to have a meeting on that, because it clearly is one of the difficulties of UK medical research and it's not one that can be overcome immediately. There is undoubtedly a problem but there is a lot of effort going on to try and get over it, and I think it is being successfully overcome in some but not all places around the UK. In the past we were riddled with antagonisms between basic

---

[233] See, for example, Veysey M J, Thomas L A, Mallet A I, Jenkins P J, Besser G M, Wass J A, Murphy G M, Dowling R H. (1999) Prolonged bowel transit increases serum deoxycholic acid: a risk factor for octreotide induced gallstones. *Gut* 44: 675–681. Dowling R H, Veysey M J, Pereira S P, Hussaini S H, Thomas L A, Wass J A, Murphy G M. (1977) Role of intestinal transit in the pathogenesis of gallbladder stones. *Canadian Journal of Gastroenterology* 11: 57–64.

[234] Sir Harold Himsworth KCB FRCP FRS (1905–1993), a distinguished clinical scientist, was Secretary of the Medical Research Council from 1949 to 1968. He was appointed Professor of Medicine and Director of the Medical Unit at University College Hospital, London, in 1939. His major project, the Clinical Research Centre, was described as a dream inspired by Sir Thomas Lewis. See Gray J, Booth C. (1994) Sir Harold Himsworth. *Munk's Roll* 9: 238–241. See also Reynolds L A, Tansey E M. (eds) (2000) Clinical Research in Britain, 1950–1980. *Wellcome Witnesses to Twentieth Century Medicine*, vol. 7. London: The Wellcome Trust, 74pp.

[235] Professor Timothy Peters wrote: 'I think we should also record the contributions of the Wellcome Trust in supporting training and research in intestinal transport through Prize Studentships, Fellowships and Senior Fellowships over the past three decades.' Note on draft transcript, 8 October 1999.

and clinical scientists. I think there were some exceptions. The Hammersmith group, Chris Booth's group, a group here and there, but overall in gastroenterology there was first of all a suspicion that the basic scientists were working in fields which were not relevant – relevance was the key – and on the basic science side a sort of snootiness that the clinicians really were very amateurish and not to be trusted. Those were not the attitudes which encouraged collaboration and it's taken a long time to get through that. My own impression is that things are improving.

Booth: Can I just make a point about America and Britain, because there is a very real distinction that can be drawn here. The Association of American Physicians was founded in 1886 and when it was founded it was a group that was to bring in all American physicians, a term which includes scientists as well as physicians, so that men like Van Slyke (a biochemist) and Oswald Avery (a microbiologist) and William Welch, who was a pathologist, were members. William Welch was president of that group in 1901 when he made a classical speech on the importance of the full-time system in medicine. The British Association wasn't founded until 20 years later by William Osler in 1907 and it was restricted predominantly to people doing internal medicine in university departments in Britain. It's only been the last three or four years that we have had any basic scientists in that group. A fundamental distinction.

Bangham: Could I just add that during the 1950s at the National Institute for Medical Research, for about ten years or more, half the staff were medically qualified and in one of the Divisions, Virology, that of Sir Christopher Andrewes, 17 of the scientists were medically qualified. The proportion has gradually diminished since, but in those days it was very firmly a good mixture of medical and basic scientists.

Dowling: I am reminded of one occasion when the British Society of Gastroenterology organized annual satellite meetings in conjunction with a pharmaceutical company. Our chairman (Les Turnberg) organized one of these sessions on Intestinal Absorption and Secretion. At that meeting, one of the most fascinating talks was given by Mike Berridge about the insect salivary gland and the IP-3 second messenger system. There was also a talk, by Don Powell of Chapel Hill, North Carolina, about mesenchymal–epithelial interactions in transport – a talk given by a clinical investigator.[236] So that's a good example of how you can have basic scientific and clinical interaction. I do think that's the sort of model we ought to be working on.

Turnberg: So there's hope for the future even if the history isn't as good as we'd like.

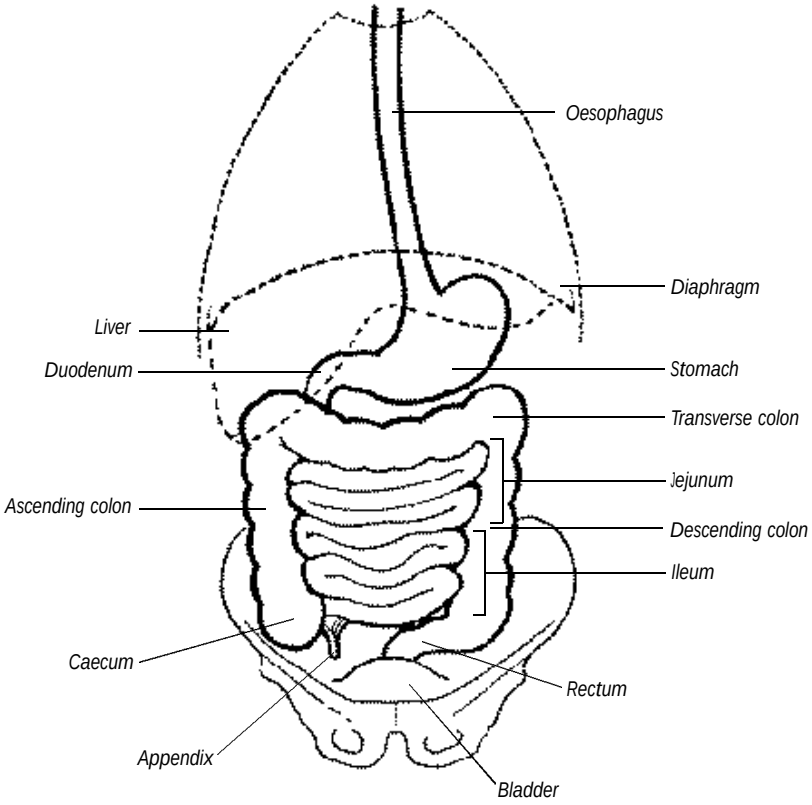
I think we have had quite a long session and we have covered an enormous amount of ground. I am intrigued that we did cover the whole of this agenda, because I suspect we could have done just half of it, and filled the time, but we have kept going, and I have found it absolutely fascinating. I am very grateful to everyone for giving their all and telling us about their own historical recollections, and I am very grateful to Tilli

[236] Berridge M J. (1982) Phosphatidylinositol hydrolysis: a general transducing mechanism for calcium-mobilising receptors. op. cit. note 111, 28–34. Powell D W. (1982) Neurohumoral control of intestinal secretion. ibid. 42–45.

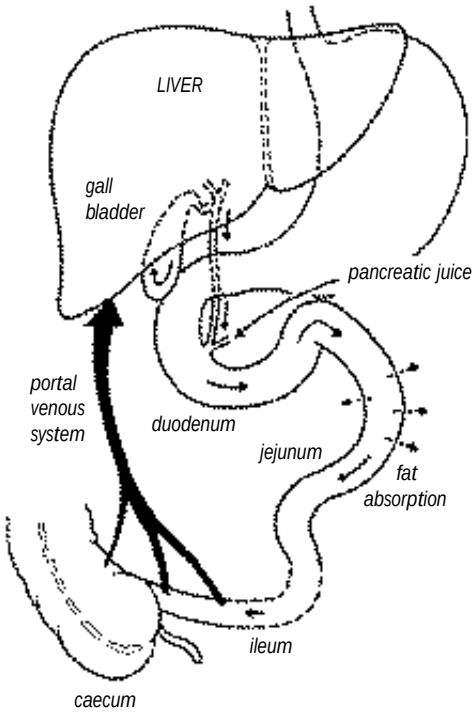
Tansey and Chris Booth and the Wellcome Trust for putting up with us, and putting it on. Thank you.

Tansey: Can I also add the thanks of the Wellcome Trust to all of you for coming to this day and also particularly to Sir Leslie Turnberg for chairing the meeting. It's really been a splendid occasion and there have been many issues to the interest of historians that have emerged today. When Chris Booth and I first proposed this topic some of our colleagues were very dismissive, they saw this as a very esoteric part of contemporary medicine and thought that we would only get people to talk for ten minutes. The fact that we have kept going for four hours and could keep going for much longer, shows that we were right to pursue this. Thank you all very much for coming.

THE DIGESTIVE TRACT [1]



THE ENTEROHEPATIC CIRCULATION OF BILE ACIDS [2]



[1] Adapted from Green J H. (ed.) (1976) Digestion. In *An Introduction to Human Physiology*, ch. 9. Oxford: Oxford University Press, 104.

[2] Adapted from Haselwood G A D. (1978) The biological importance of bile solutions. In Neuberger A, Tatum E L. (eds) *Frontiers of Biology*, vol. 47. Amsterdam, New York, Oxford: North-Holland Publishing Company, 4.

GLOSSARY

Achlorhydria
The absence of hydrochloric acid from gastric secretion.

Addisonian anaemia
See Pernicious anaemia .

Affinity constant [K_t (or K_m)]
A measure obtained in kinetic studies of the affinity of a substance for a carrier or enzyme site (K_m is half V_{max}).

Apoptosis
Programmed cell death which in the intestinal mucosa leads to shedding of the cells.

Bile
A golden yellow fluid that is secreted by the liver. It is stored and concentrated in the gallbladder which contracts in response to food and expels the concentrated bile into the duodenum, via the common bile duct. Bile is a complex aqueous medium, greater than 90 per cent water, which contains lipids (bile acids, phospholipids and cholesterol), bile pigments (mainly conjugated bilirubin), electrolytes and small amounts of proteins.

Bile acids
Bile acids are amphilic ('loving' both water and fats/lipids), 24-carbon, steroid-ring, detergent molecules, which are synthesized in liver from cholesterol. Their principal functions in the liver are to stimulate blood flow and solubilize biliary cholesterol, and in the intestine to promote the digestion and absorption of dietary triglycerides, sterols and fat-soluble vitamins. In biological fluids, most bile acids are present as their sodium salts, and occasionally as their calcium or potassium salts.

Bile acid breath test
A test utilizing a ^{14}C-labelled conjugated bile acid which is given by mouth; the amount of $^{14}CO_2$ appearing in the breath provides a measure of bacterial deconjugation and metabolism of the labelled bile acid (op. cit. note 211).

Bile duct
Any of the ducts that convey bile from the liver.

Bile salts
The salts (usually sodium) of bile acids such as cholic and chenodeoxycholic acids (for example, sodium glycocholate and sodium taurocholate); necessary for the emulsification of fats.

Biliary fistula
An abnormal communication between the biliary tree and either the surface of the body or an internal organ. Biliary fistulae occur spontaneously in disease but can also be created surgically – usually by inserting a tube or cannula into the common bile duct.

Brush-border
Microvilli on the free surfaces of certain epithelial cells, particularly the absorptive surface of the small intestine and the proximal convoluted tubules of the kidney.

Citric acid cycle
See Krebs cycle.

Cobalt^{56}
A radioisotope of cobalt having a half-life of only 72 days, used in diagnostic tests for the evaluation of vitamin B_{12} absorption (see page 53).

Cobalt^{60}
A radioisotope of cobalt having a half-life of 5.25 years, used in diagnostic tests for the evaluation of vitamin B_{12} absorption (see page 53).

Coeliac disease (idiopathic steatorrhoea)
A cause of malabsorption, genetically determined but associated with dietary gluten intake; there are significant changes in the small-intestinal mucosa.

Crohn's disease
One of the two types of inflammatory bowel disease. It may affect the small and large intestines, the pathological changes being marked as severe.

Cystinuria
An abnormal presence of the amino acid cystine in the urine. The disorder is caused by an autosomal recessive trait that impairs cystine reabsorption by the kidney tubules. In high concentration cystine tends to cause kidney or bladder stones.

Enterohepatic circulation
Substances which are secreted by the liver into bile, pass down the intestine, are reabsorbed, return to the liver (usually in the portal vein) and are then resecreted into bile, are said to undergo an enterohepatic circulation. The best known example is the enterohepatic of the naturally occurring bile acids (op. cit. notes 203 and 205 and page 69).

Enteropathy
A disease or other disorder of the small intestine.

Erepsin
A mixture of protein-digesting enzymes secreted by the intestinal glands.

Erythrocyte (red blood cell)
In mammals erythrocytes lack a nucleus; haemoglobin is a major constituent.

Erythropoiesis
The process of red blood cell (erythrocyte) production, which normally occurs in the blood-forming tissue of the bone marrow.

Hartnup disease
A genetically determined (autosomal recessive) defect in renal and intestinal transport of certain neutral amino acids – including methionine and tryptophan.

Hypernatraemic dehydration
Dehydration associated with an excessive concentration of sodium in circulating blood. This condition is virtually confined to infants who are usually sodium- and water-depleted ('dehydrated') from diarrhoea, but have lost more water than salt (because of insensible water losses from their large surface area in relation to body mass) so their serum sodium concentration actually rises.

Hypertonic solution
A solution in which the concentration of solutes exceeds that of an isotonic solution.

Intrinsic factor
A glycoprotein secreted by the parietal cells of the gastric mucosa as a component of gastric juice and essential for the absorption of vitamin B_{12}.
Its absence leads to pernicious anaemia .

Ionophore
An antibiotic that carries specific ions across a membrane, such as the plasma membrane of bacterial or animal cells or the mitochondrial membrane, e.g. valinomycin.

Jejunal diverticulosis
A clinical condition in which diverticuli of varying sizes develop along the mesenteric border of the small intestine. It is often associated with stagnation of luminal contents, bacterial overgrowth and malabsorption of fat and vitamin B_{12}.

Krebs bicarbonate Ringer
See Krebs–Ringer solution .

Krebs cycle (citric acid cycle)
A sequence of enzymatic reactions involving the metabolism of carbon chains of monosaccharides, fatty acids and amino acids to yield carbon dioxide, water and high energy phosphate bonds (see note 75).

Krebs–Ringer solution
Ringer's solution modified with sodium bicarbonate, magnesium sulphate and phosphate buffer.

K_t (or K_m)
See Affinity constant .

Lacteals
Lymph vessels containing chyle draining the small intestine; these are termed lacteals because of their milky white appearance conferred on them by the presence of chylomicrons. Digested fats are absorbed into the lacteals.

Langendorff preparation
A preparation of an isolated mammalian heart in which the aorta is cannulated and perfused, thus supplying blood flow to the coronary circulation.

Mecamylamine
A non-depolarizing ganglionic blocking agent causing a decrease in the volume and acidity of gastric secretion, and a reduction in the tone and motility of the gastrointestinal tract in humans.

3-O-methylglucose
A non-metabolizable monosaccharide (hexose).

Pepsin
An enzyme secreted by the stomach that breaks peptide bonds and thus initiates the process of breaking the proteins in food into their constituent amino acids.

Peptone
A large protein fragment produced by the action of enzymes on proteins in the first stages of protein digestion.

Pernicious anaemia (Addisonian anaemia)
The defective production of red blood cells caused by a lack of vitamin B_{12}.

Phlorizin
A plant glycoside which inhibits intestinal (and renal tubular) absorption of glucose and galactose. [See Newey H, Parsons B J, Smyth D H. (1959) The site of action of phlorrhizin in inhibiting intestinal absorption of glucose. *Journal of Physiology* 148: 83–92. op. cit. note 20.]

PO_2
Partial pressure (P) of oxygen (O_2) measured in mmHg.

Reticulocyte
An immature red blood cell (erythrocyte); they normally comprise about 1 per cent of the total red cells and are increased (reticulocytosis) whenever the rate of red cell production increases.

Reticulocytosis
An increase in the proportion of immature red blood cells (reticulocytes) in the bloodstream.

Ringer's solution
An artificial physiological salt solution containing sodium chloride, potassium chloride, calcium chloride and distilled water. It is used for maintaining organs or tissues alive outside the animal or human body for limited periods. This was introduced by Sydney Ringer (1835–1910) from University College London.

Schilling test
Developed by the American haematologist Robert F Schilling (b. 1919) as a test for measuring vitamin B_{12} absorption. Vitamin B_{12} tagged with radioactive cobalt is administered orally, and a large intramuscular injection of nonradioactive B_{12} is given to 'flush' the orally administered B_{12} into the urine. Gastrointestinal absorption is measured by determining the radioactivity of urine samples collected over a 24-hour period (op. cit. notes 195 and 197).

Sheet
An epithelium removed from animals or humans and mounted as a membrane between two chambers. In the case of the intestine (large, small or rectum) it can be mounted with or without their smooth muscle coats.

Short circuit current
A measure of net ion electrogenic transport.

Solvent drag
The passive movement of solute secondary to movement of fluid across a membrane.

Steatorrhoea
Excess faecal fat excretion – usually due to malabsorption and/or maldigestion of dietary fat. It is a feature of coeliac disease and tropical sprue.

Tropical sprue
A clinical syndrome usually occurring in tropical countries, typified by chronic diarrhoea, weight loss and malabsorption; there may be an association with vitamin deficiencies.

V_{max}
The maximum rate at which the enzyme or carrier transfers or breaks down a substance (see K_m).

INDEX: SUBJECT

INDEX: NAMES