INTESTINAL ABSORPTION

Edited by D. H. Smyth

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Preface

It might be asked if there is a need for yet another large review on Intestinal Absorption, and the answer is that this is still a rapidly expanding field of interest both from the medical and scientific points of view. There is ample evidence for this in the number of papers which continue to be published, and the bulletin on Intestinal Absorption issued by the Biomedical Information Project of the University of Sheffield lists about 150 titles per month, and there is still no sign of any diminution in this rate. There are in fact so many papers that those interested in intestinal absorption have to be specialists in one particular field, but must at the same time be aware of the general developments in the subject as a whole. The last major review was the excellent volume in the American Handbook published in 1968, already six years ago, and indeed a number of the contributors to that volume have taken part in the present work.

Some observations made in the introduction to a volume of the British Medical Bulletin on Intestinal Absorption some years ago are still pertinent. Progress in the experimental sciences is not continuous, but proceeds in phases of rapid expansion alternating with periods of slower growth. This is partly because of a fundamental law governing the progress in experimental science which states that if you think of anything easy to do which has not been done before, further investigation shows either that it is not easy, or that it has been done before. One way of escaping from the grip of this law is to avoid finding out or to ignore what has been done before. This book is not intended for those seeking this solution. But this rigorous law is periodically relaxed, and this happens when a new technique is discovered. There is then a sudden surge of publications to exploit the new technique. It is easy to date the present tide of advance in intestinal absorption to the introduction of an effective *in vitro* technique by R. B. Fisher and D. S. Parsons in 1949, followed

by the development of the everted sac by T. H. Wilson and G. Wiseman in 1954. While these workers popularized *in vitro* techniques, they did not in fact introduce them, and this was done more than fifty years ago by Weymouth Reid, whose remarkable work seems to have escaped serious notice by the physiologists of the day.

But perhaps the real credit for *in vitro* intestinal studies and indeed *in vitro* studies in everything should go to Sidney Ringer, who first introduced the idea of replacing the life-giving blood with a salt solution and hence led the way for the highly unphysiological *in vitro* experiments. It was indeed the introduction of salt solutions for keeping isolated tissues alive that made modern physiology and biochemistry possible, and it is well that Ringer should be remembered chiefly by Ringer solution rather than by the experiments he did with it, important though they were. Ringer's most famous lineal scientific descendant is Hans Krebs, whose name, although associated with at least two major discoveries in biochemistry, is still probably most widely used in referring to Krebs' solution, and indeed it is Krebs' bicarbonate saline which has mainly been used for the *in vitro* intestine. In the early days of the *in vitro* intestine a great many things were said about the unphysiological nature of the preparation and particularly when it was exposed to the insult of being turned inside out in the everted sac technique. But unphysiological approaches are paradoxically the way to advances in physiological knowledge, and most major advances in our knowledge of how living tissues work have come from using living tissues in conditions very different from their normal ones. Ringer was the great apostle of unphysiological experiments, and his disciples do not need to make apologies for continuing his tradition.

Early studies of the intestine emphasize the important role of the cells lining the gut, and Hiedenheim spoke of the 'Triebkraft' or driving force of these cells. Hiedenheim was involved in the old controversy on vitalism, and his unfashionable vitalistic term perhaps prevented full recognition of the importance of his ideas on the intestinal cell. A later generation was explaining the movement of fluid in terms of classical osmosis, and did not require the Triebkraft of the epithelial cell. Modern work has fully substantiated Heidenheim's idea and we now know that movement of water depends on forces generated by the activity

of the living cell. The undesirable connotation of vital forces of Latin derivation (vita = life) has been neatly avoided by substituting biophysical forces of Greek derivation (βίος = life) to everyone's complete satisfaction.

The study of intestinal absorption offers opportunities to people of very widely different skills, varying from those who try to formulate the problems in terms of irreversible thermodynamics to those who think in terms of the clinical problems of the person unable to absorb enough of the nutrient substances he requires. Between these are the large number who think of one aspect of the absorptive process, and try to formulate the problems in such terms as is possible by their limited knowledge of fundamental science and their awareness of the dangers in making too many approximations and assumptions to make biological observations fit mathematical expressions. These volumes contain therefore many different approaches to the problems of the intestine. It purposely does not include detailed discussion of clinical problems, as these have been the subject of many symposia and many discussions in recent years. If it encourages its readers to broaden their interests and make an effort to come to grips with new and unfamiliar expertise, it will have served its purpose.

D. H. Smyth

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CHAPTER 1

Intestinal Structure in Relation to Absorption

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1.1 GENERAL STRUCTURAL FEATURES

1.1.1 Introduction

This section attempts to assemble those elements of the structure of the small intestine that are most pertinent to absorption. At the moment of absorption almost every substance is in a form smaller than the resolution of the electron-microscope, most cell 'pores' are also invisible and their sizes hypothetical and most substances are absorbed without any change being seen at light or ultrastructural level. Perhaps the most meaningful factor that structure could contribute to an understanding of absorption is a measurement of surface area, but as Wilson [1] rightly says, 'Despite wide interest in the small intestine brought about by modern biopsy techniques and absorption studies, quantitative knowledge of the basic structure of the small intestine remains slight' [1]. The relation of epithelial cell to capillary and lymphatic are probably crucial though little is known about blood flow and its control in this area. The ultrastructure of the epithelial cell is documented in great detail though this does not necessarily help in understanding absorptive processes. Of more value is the localization of enzymes, and this well shown by histochemical techniques (see chapter 2). Recent work using histochemical and immune labelling methods with electron microscopy has helped to place certain enzymes with greater accuracy, so that the gap between the membrane structure of the brush border as conceived by the physiologist and visualized by the microscopist is narrowing.

1.1.2 Surface area

This highly important factor has received scant attention, particularly in human disease. Surface area is increased by the mucosal folds of Kerkring (valvulae conniventes), by villi and by the microvilli of the brush border. Mucosal folds are prominent in man but absent in many smaller laboratory animals. Villi are strikingly longer in the jejunum than the ileum. It has been estimated that all these structures increase the surface area of the small intestine by a factor of 600 [2]. By careful measurements from histological sections of the length of the surface outline and the mucosal-serosal ratios enough data has been obtained to enable a calculation of surface area to be made. The results in the dog, cat and rat show a diminution of surface area

from the jejunum to the ileum; the fall in surface area is linear with distance along the intestine [3, 4, 5]. Wilson has made a detailed study of surface area in man from post-mortem specimens [1]. There is a fourfold difference between jejunum and ileum but this is not linear; the large surface area of the upper jejunum falls away sharply, while in the ileum the decline is much less marked.

The microvilli of the brush border make a big increment in surface area; estimates have been made of 14 to 39 times [6, 7]. Whether all the surface area so calculated is available for absorption is unknown. Jejunal villi are closely packed and the lower parts may not be exposed to luminal contents. Furthermore the glycocalyx of the brush border creates a microenvironment and similarly it cannot be assumed that all the surface area of this part of the cell is available. More work is needed to elucidate this problem.

1.1.3 The mucus layer

Mucoprotein secreted by goblet cells coats the epithelium and envelops the luminal contents. The function of this is poorly understood. Freeze dried secretions of the whole gut with contents intact shows the relation of mucus to epithelium and lumen. In the duodenum and jejunum mucus is scanty and wispy and the contents are fluid and well dispersed. By contrast, in the ileum there is a thick layer of mucus between the villi and the luminal content, which is usually more solid. This layer is situated above the villi so that little content is ever seen between the villi. Bacteria are frequently seen in the mucus layer and it has been suggested that this is the true habitat of the flora rather than the lumen [8].

1.1.4 Villous shape

The text-book picture of a finger-shaped villus is not invariably found in animals or man. The new-born of almost all mammals do indeed have these villi, but at the time of weaning most small animals suffer a change in villous shape to triangular or leaf-like villi [9] (Fig. 1.1). In the rat these may become joined so that ridges with triangular projections run across the long axis of the bowel. This change is more marked in the jejunum than the ileum. It is due to environmental factors, as can be demonstrated by a reversion to finger villi in closed segments of

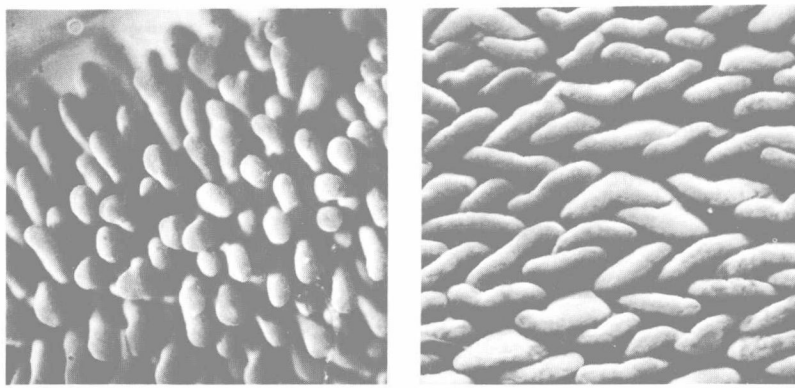

Fig. 1.1. Dissecting microscope photographs of rat jejunal mucosa. A, at ten days old and B, in the adult. X 41.

intestine that have been surgically separated from the continuity of the bowel [10].

In adult man, in countries with a Western culture, the small intestinal structure is little altered from the foetal structure (Fig. 1.2). Leaf-like villi are frequently encountered in the duodenum and upper jejunum mixed with finger forms. Occasionally convolutions are found in the normal population and there may be some small local variation even within the British Isles [11]. In other parts of the world the 'normal' mucosal structure may be a convoluted or a mixture of leaf and convoluted mucosal shapes. This has been reported in Thailand [12], East [13] and West Pakistan [14], India [15], Uganda [16] and parts of the Carribean [18]. Thus the majority of the human population probably has a small intestinal structure with a comparatively diminished surface area. Again there is good reason to believe that this is environmental in origin. Diminished absorption of xylose and other evidence of marginal malabsorption has been demonstrated in as much as a third of the apparently healthy population in some areas [13, 14].

In some animals that might be used for absorption studies striking changes of villous shape have been reported due to infection and infestation. Pout documents extreme and extensive mucosal change in lambs producing at times a flat mucosa in association with coccidia and helminths [18].

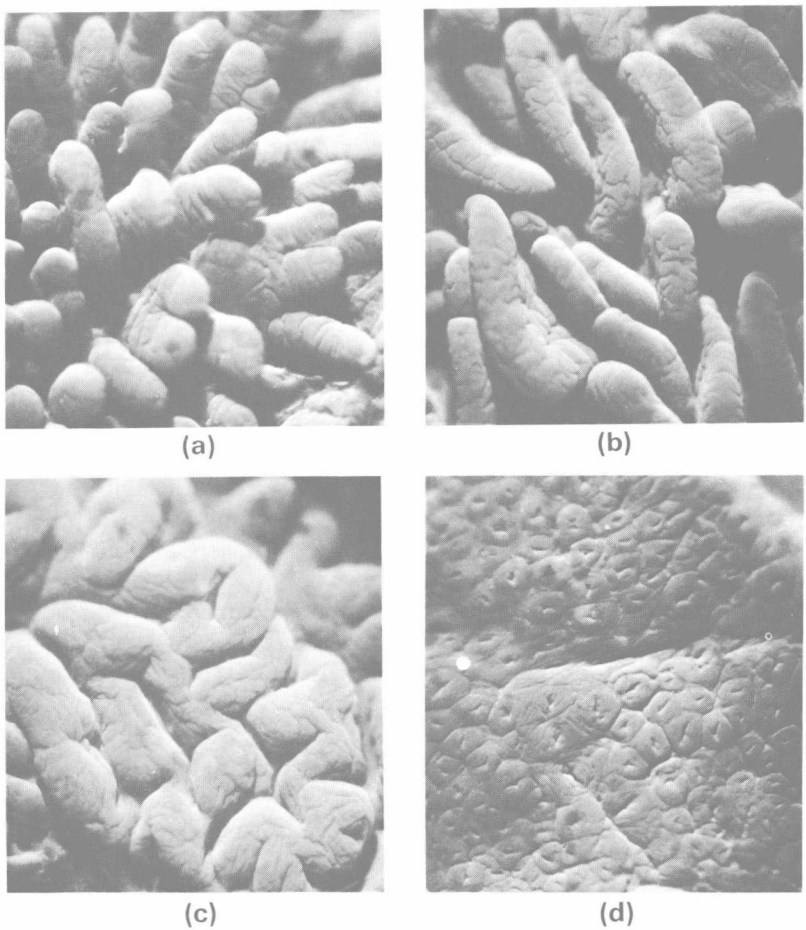

Fig. 1.2. Dissecting microscope photographs of human jejunal mucosa. A, finger-shaped villi; B, leaf-shaped villi; C, convolutions and D, a flat mucosa. A and B are within normal limits for Western man. X 33.

In human disease states mucosal changes can be found. The most important is the coeliac syndrome (adult coeliac disease, idiopathic steatorrhoea) where the upper small intestinal mucosa becomes diffusely flattened (Fig. 1.2). The lesion is usually marked and definitive, total villous atrophy, though occasionally milder changes, partial villous atrophy, are encountered. The lesion gradually regresses in the distal bowel

so that in the terminal ileum normal appearances are found. The extent of the lesion usually correlates with the severity of the malabsorption so that it is the integrity of the ileum that determines much of the disease pattern.

1.1.5 Cell turnover

The small intestinal epithelium, in common with the rest of the gut, is highly dynamic being in a continuous state of division, migration and loss [16, 20, 21]. It is, however, much more active in this respect than the stomach or colon. The patterns of cell turnover in the small intestine are well documented but there is almost complete ignorance about controlling mechanisms.

Cell division occurs only in the crypts which are often looked upon as areas totally devoted to replication, though there is considerable evidence of secretion in this zone. Mitotic activity is readily visible and may be counted to arrive at a mitotic index. If the total population of cells is known and the duration of mitosis is measured or assumed then the turnover time can be calculated. Blocking of mitosis at the metaphase with colchicine is frequently used for increasing the efficiency of mitotic counts and estimating mitotic duration.

By the use of a combination of mitotic counting and autoradiography after pulse labelling with tritiated thymidine the individual events in the mitotic cycle can be timed [22]. The sequence is composed of a synthetic (S) phase in which DNA is doubled, a post-synthetic gap (G2), the act of mitosis and then a post-mitotic gap (G1). In man the following times have been documented: S phase is about 14 hours, G2 2 to 7 hours, mitosis 1 hour, while G1 is very variable as some cells will cease dividing while others may enter a prolonged interphase (G0) [23]. The production of new cells can be expressed as a birthrate; in the human ileum 0.8 cells/100 crypt cell/hour and in the jejunum 1 cell/100 crypt cells/hour [23, 24, 25].

The cells undergo several divisions in the crypts as they migrate up the gland and at the crypt-villous junction undergo a rapid maturation to become an adult epithelial cell. This is marked by a more columnar appearance of the cell and a prominent brush border. Goblet cells appear to arise from the same stem cell as epithelial cells but mature earlier and are seen

fully distended with mucus in the crypts. They migrate in the same way as epithelial cells.

Complete maturation of cells is not accomplished until they are between a third and half-way up the villous as judged by histochemical findings. The migration of cells is readily visualized by autoradiography with tritiated thymidine which enables accurate migration times to be measured. The cells appear to flow continuously up the villi and in most small animals the turnover time is of the order of 2 days. In man a turnover time of 4 to 5 days has been recorded but there have been few measurements [23, 24, 25]. The rate of movement of cells up the villus can be expressed as a flow rate. In man this is of the order of 1 cell position per hour in the jejunum and 0.8 cell positions per hour in the ileum [23, 25]. There is now good evidence that not only do the epithelial cells divide and migrate but the fibroblasts which cover the basement membrane of the crypts divide at the same rate [26, 27]. These labelled cells migrate synchronously with the epithelial cells. It is not known whether the basement membrane also migrates but the simultaneous movement of epithelial cell and fibrocyte would give support to this idea. If so, then the whole absorbing unit, epithelial cells, basement membrane and fibrocyte, would maintain a constant relationship.

At the villous tips the epithelial cells are shed from distinct sites called extrusion zones. These can usually be seen on light microscopy as either a cleft or a small streamer of cells entering the lumen. The cells appear somewhat shrivelled or pyknotic in this area but are otherwise shed intact into the lumen.

Another approach to measuring cell turnover is to quantitate the cell loss into the lumen. If all the cells can be recovered and if the nuclei are intact then a chemical measurement of DNA should act as a cell count [28]. This approach has been perfected by Croft and gives a fairly reproducible method that can be used in animal or man [29]. The main difficulty is to be sure that all the cells have been washed from the segment of the lumen being studied, as it is easy to imagine that cells may be caught in mucus. Using this technique in man, the cell loss from a 5 cm segment of jejunum is about half a million cells per minute and therefore for the whole small intestine may be calculated to be between 20 and 50 million per minute [30]. A different approach to measuring cell loss has been developed by

Clarke [31]. In the rat he has been able to trap the cells shed into the mucus overlying villi during a timed period. With suitable staining the cells can be directly counted over each villus and this gives a highly sensitive method of calculating turnover.

The mass of cells shed is impressive. In man, Croft's data gives a figure of 250 gms per day which is in close agreement with Leblond's estimate of half a pound [21]. When faecal excretion of a substance is being used in balance experiments to measure absorption this endogenous component may be a significant factor.

Cell turnover is a highly sensitive process and may be altered by a large number of physical, nutritional and chemical changes. Irradiation, antimitotic and antimetabolic drugs will inhibit mitosis so that division will be stopped or slowed [32, 33]. Deficiencies of vitamin B_{12} [34] and folic acid also reduce mitosis, but iron deficiency apparently has no effect [35]. A dimunition of mitosis is not accompanied by an immediate dimunition of cell loss and villi shrink, sometimes to a striking degree. Physical or chemical trauma to villi increases cell loss and cell turnover undergoes a compensatory increase [36]. However, division may be unable to match loss and the adult cell population will fall, again producing smaller villi though these are now of a different shape as will be outlined below. The flora of the small intestine influences cell turnover; in the germ-free animal cell division is halved but gell life is doubled and the turnover time is therefore twice as long [37, 38]. Abrupt alterations of flora, as at weaning, may also change cell turnover [39]. Nutritional factors are primarily seen in pregnancy and lactation where hyperphagia is the determining factor; villi become larger and cell turnover is increased [40]. By contrast, in protein malnutrition and starvation cell division decreases [41, 42]. Even brief periods of starvation may slow cell turnover and there is a demonstrable increase after normal feeding [43].

1.1.6 Cell turnover and villous shape

In states of diminished cell turnover villi become smaller but retain their shape; by contrast, with increased cell turnover villi metamorphose into the range of shapes already described. These changes are usually brought about by physical or chemical changes in the lumen or by infestation which cause an increased