Histol Histopathol (1996) 11: 729-741

Histology and Histopathology

Invited Review

Adaptive remodelling of intestinal epithelium assessed using stereology: correlation of single cell and whole organ data with nutrient transport

T.M. Mayhew

Department of Human Morphology, Queen's Medical Centre, University of Nottingham, UK

Summary. Adaptation in the intestinal epithelium depends on cell number and the properties of individual cells but these responses operate within different time frames. Changes in number take days to accomplish but those in behaviour may occur within hours. This article reviews the value of stereology for characterising structural features of the average enterocyte and the entire organ (mammalian small intestine or avian lower intestine) during adaptation. Stereological data are correlated with the physiology and molecular biology of glucose and Na^+ transport. In small intestine, account is taken of vertical (crypt-villus) and longitudinal (craniocaudal) gradients and of adaptations to chemically-induced diabetes and diet. Results show that longer-term adaptation depends critically on epithelial renewal. In diabetic small intestine, changes in glucose transport are accompanied by changes in the number, but not morphology, of villous enterocytes. In avian lower intestine, increased Na^+ transport requires changes in cell number and the extent of their apical, but not basolateral, membrane surfaces. These changes allow opportunities to incorporate more (or more active) transport sites in apical and basolateral membrane domains of individual cells and of whole organs.

Key words: Intestinal epithelium, Enterocytes, Stereology, Whole organ, Single cell

Introduction

Intestinal epithelium is a continuously renewing epithelium. The progeny of stem cells in crypts (proliferative compartment) migrate onto villi (functional compartment) where they mature before being eventually extruded. In mammalian small intestine, cells take 2-6 days to migrate from crypt base to villous tip. In avian coprodaeum (lower intestine), the epithelium is renewed in 8-16 days.

Adaptation involves changes in villous surface area rather than number (in rat small intestine, the number of villi is constant most of the lifespan; Clarke, 1970, 1972; Forrester, 1972). The changes in surface area could be mediated by cell recruitment. However, intestinal epithelium may also adapt by altering the maturity and/or activity of its constituent cells. Alterations can occur in vertical (e.g. crypt-villus) and longitudinal (craniocaudal) directions and may have non-specific as well as specific effects on transepithelial transport (Karasov and Diamond, 1983). To some extent, alterations of cell structure and behaviour run in tandem. The earliest adaptive responses can occur in hours and without visible changes in gross or microscopical anatomy. They may involve, say, the synthesis or activation of specific transporters near or within cell membranes. Structural adaptations often take longer to detect. A change in cell number or membrane area may take days (epithelial turnover time) to accomplish.

Analysing relative contributions to adaptation made by changes in structure and behaviour requires the application of rigorous quantitative methods. Despite attention given over a decade ago to the paucity of information on villous and microvillous surface areas (Karasov and Diamond, 1983), there are still few reports on these functionally relevant quantities. The paucity does not reflect a lack of suitable quantitative techniques. Recently, there have been fundamental improvements in stereology and it is now the preferred choice for defining 3-dimensional biological structure from sections (Gundersen et al., 1988a,b; Cruz-Orive and Weibel, 1990; Mayhew, 1991, 1992; Mayhew and Gundersen, 1996). Provided that stereology is combined with randomised sampling of organs (Mayhew, 1988, 1990; Mayhew et al., 1990; Zoubi et al., 1994; Makanya et al., 1995), cell ultrastructure can be described in terms of size and the extent of microvillous and basolateral membranes. Values for the entire organ can also be estimated. The methods have proved valuable for quantifying epithelial structure during intestinal adaptation to a variety of circumstances (Ross and

Offprint requests to: Professor T.M. Mayhew, Department of Human Morphology, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH, UK

Mayhew, 1985; Mayhew and Middleton, 1985; Mayhew, 1987, 1990; Elbrønd et al., 1991; Warren, 1991; Mayhew et al., 1992a,b; Zoubi et al., 1994, 1995a,b).

Because functional morphology at a particular place and/or time depends on the number of cells as well the properties of average cells, both pieces of information are required before whole organ biology can be interpreted properly. This review illustrates the importance of monitoring cell number by deriving stereological data for average enterocyte and entire organ. Data from random tissue samples are correlated with the molecular biology and physiology of nutrient transport mechanisms. Transport of glucose (mammalian small intestine) and Na^+ (avian coprodaeum) are taken as examples.

Rat small intestine: basic morphology and glucose transporter localization

Glucose transport

Mammalian small intestine digests and absorbs luminal nutrients for which purpose its effective surface is amplified by various structural devices including villi and microvilli. Carbohydrates are hydrolysed by apical brush border enzymes and monosaccharides cross the epithelium by energy-dependent and energy-independent mechanisms. Active absorption of D-glucose is mediated by integral membrane proteins and initiated by a Na^+-dependent phlorizin-inhibitable glucose transporter (SGLT1) which is found in brush border microvillous membranes of enterocytes (Silverman, 1991; Wright, 1993; Yoshida et al., 1995). Entry is driven by the Na^+ gradient created by the Na^+/K^+ pump enzyme complex (Na^+/K^+-ATPase) which is located in basolateral membranes. Immunoelectron microscopy has shown in rats (Yoshida et al., 1995) that SGLT1 is found only in apical membranes of differentiated villous enterocytes. It is rare in, or absent from, crypt cells but present all along the villus and in increasingly higher amounts towards villous tips. In situ hybridization of SGLT1 mRNA shows a similar spatial distribution (Hwang et al., 1991; Smith et al., 1992). A downhill efflux of glucose occurs via one of the family of facilitative glucose transporters (GLUT2) which is also found in basolateral membranes (Thorens et al., 1990) all along the crypt-villus axis.

Cell morphology

The prominent brush border of columnar enterocytes is separated from basolateral membranes by tight junctions. These effectively seal the epithelium from luminal contents thereby restricting transport of most nutrients to the transcellular route. The average villous enterocyte (Mayhew, 1990; Zoubi et al., 1994, 1995a) is 26 µm long with a volume of 360 μm^3 and an apex bearing almost 500 microvilli of total area 200 μm^2 packed at a density 35-45 per μm^2 (Fig. 1). The average microvillus is 120 nm wide and 1.3 µm long. The basal membrane area is about 14 μm^2. If the basolateral and apical membrane areas are roughly equal (Buschmann and Manke, 1981a), lateral membrane area is probably 180 μm^2.

Crypt cells (Fig. 1) are 20 µm tall with a mean volume of 210 μm^3 and basal attachment of about 10 μm^2 to underlying basal lamina (Zoubi et al., 1994, 1995a). Shorter microvilli provide it with a smaller apical surface but precise values are not available.

Organ morphology

In adult rats (Mayhew and Middleton, 1985; Ross and Mayhew, 1985; Mayhew, 1987, 1990; Mayhew and Carson, 1989; Mayhew et al., 1989; Warren, 1991; Zoubi et al., 1994, 1995a), the effective area of the basic tube is amplified to 500-700 cm^2 per organ by villi and 1-1.2 m^2 by microvilli. Villous epithelial volume is about 1800 mm^3. Columnar enterocytes (total number $5x10^9$) reside transiently on the villous surface and are recruited from a crypt compartment which has a total

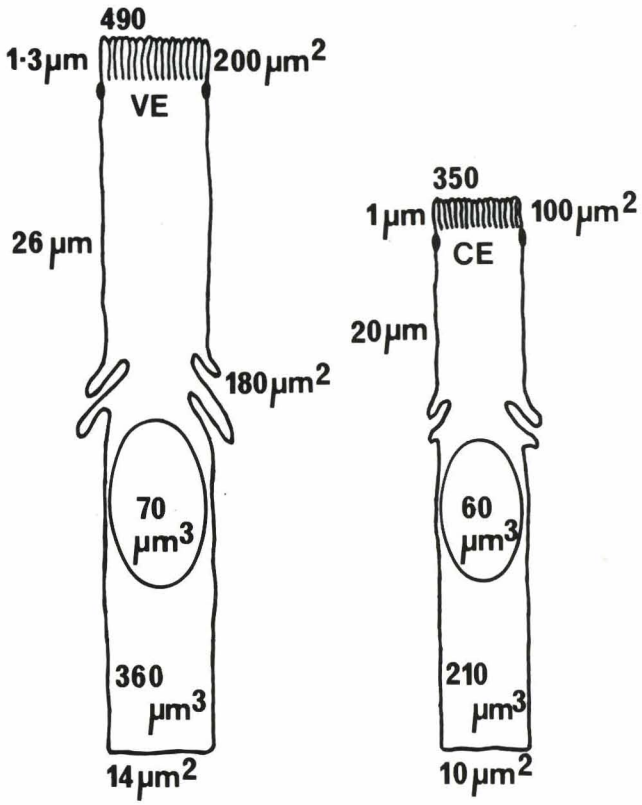

Fig. 1. Morphophenotypic features of the average villous enterocyte (VE) and crypt enterocyte (CE) in rat small intestine. For example, the average VE is 26 µm tall and bears 490 microvilli of mean height 1.3 µm and total area 200μm^2. The lateral membrane area is 180 μm^2 and the basal area is 14 μm^2. Cell volume is 360 μm^3 and nuclear volume is 70 μm^3. In addition, note that there are $5.1x10^9$ VEs and $3.3x10^9$ CEs per intestine. Heights and widths are to scale. Data based on Zoubi et al. (1994, 1995a) and values in the literature (see text). The lateral membrane area of CEs is not known.

volume of 700 mm^3 per organ and harbours more than $3x10^9$ epithelial cells.

Rat small intestine: vertical gradients

Along the crypt-villus axis, cells display gradual but marked changes in phenotype before the form and function of fully differentiated columnar enterocytes is expressed. Cells migrate from the crypt base into a proliferative and then a functional zone. Here, crypt cells start to mature more rapidly.

Glucose transport

The localization of SGLT1 and activities of brush border hydrolases vary vertically (Nordstrom et al., 1968; Galjaard et al., 1972; Both et al., 1974; Yoshida et al., 1995). In rabbits and rats, microvillous membranes of relatively less mature enterocytes (found towards the bases of villi) have a higher affinity and lower capacity form of Na^+-dependent glucose transporter than those at mid-villus and villous tip levels (Dudeja et al., 1990; Meddings et al., 1990).

Cell morphology

At the crypt-villus transition zone, there are increases in the size and content of cells and their organelles. Maturation involves acquisition of longer and more densely packed microvilli with associated hydrolases (Brown, 1962; McElligott et al., 1975; Dongen et al., 1976; Pothier and Hugon, 1980; Potten,

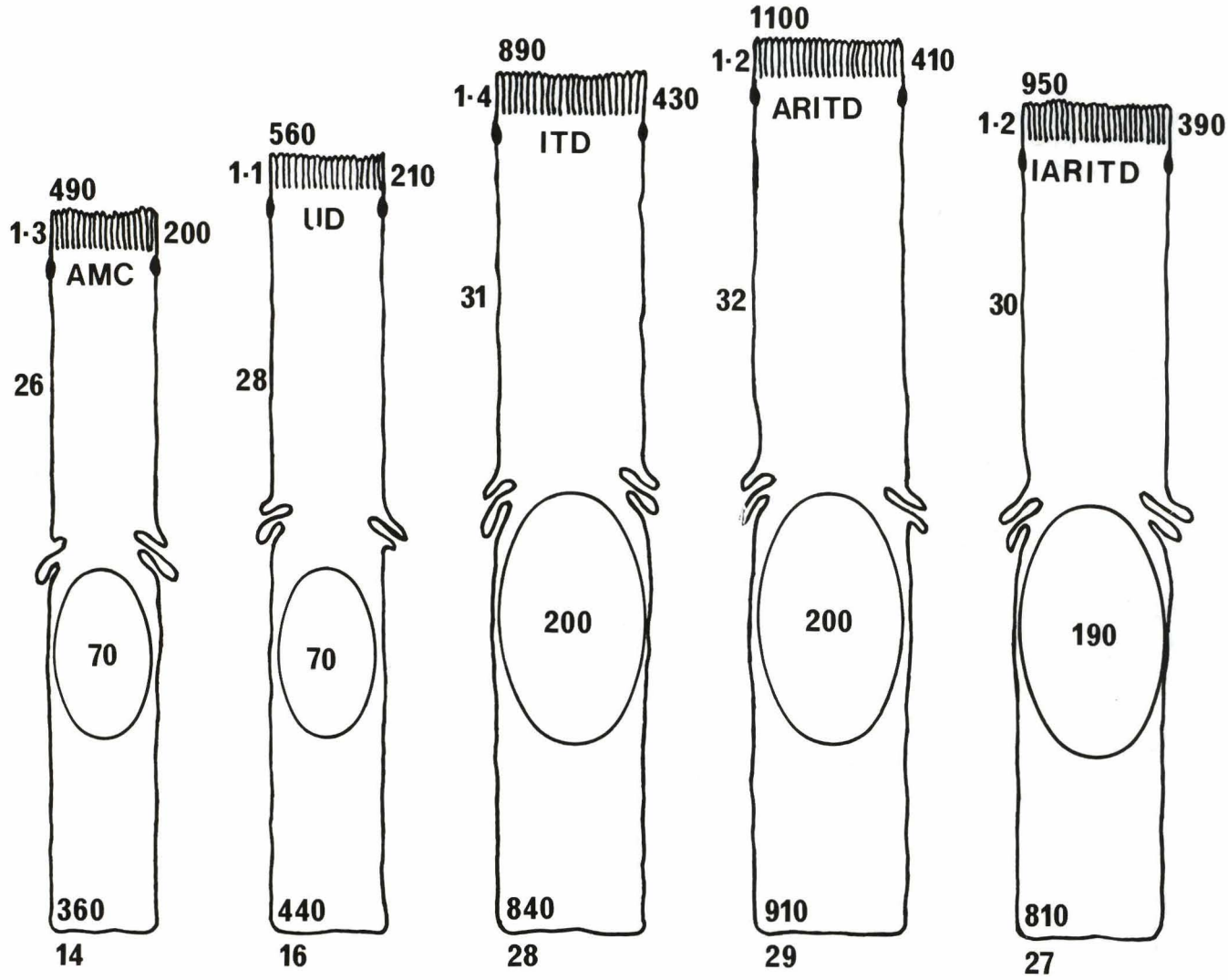

Fig. 2. Morphophenotypes of average villous enterocytes in small intestines of age-matched control (AMC, total cell number $5.1x10^9$) and streptozotocin-diabetic rats. The latter are untreated (UD, $9.1X10^9$ cells) or treated with insulin (ITD, $2.4X10^9$ cells), aldose reductase inhibitor (ARITD, $4.6x10^9$ cells) or insulin+inhibitor (IARITD, $2.3x10^9$ cells). For key to structural quantities, see Fig. 1. Data taken from Williams and Mayhew (1992) and Zoubi et al., (1995a,b). Comparative data for lateral membrane areas are not available.

1980; Leblond, 1981; Stenling and Helander, 1981; Abbas et al., 1989; Smith and Brown, 1989; Altmann, 1990; Potten et al., 1994; Zoubi et al., 1994, 1995a).

Investigations on age-matched control and streptozotocin-diabetic rats have revealed that cells become taller and more voluminous as they pass from crypts onto villi (Zoubi et al., 1994, 1995a). In control rats, height increased by 30% and volume by 70% (Fig. 1). The area of the cell base (or microvillous-free cell apex) increased (almost 40%) only in control rats. Control cells grew taller and wider whilst those in diabetic rats simply became taller (Figs. 2, 3). Indeed, in some regions along the intestine, diabetic cells became more slender as they crossed the crypt-villus transition (Zoubi et al., 1995a). Buschmann and Manke (1981b) argued that major alterations in cell width are unlikely since they would be disruptive to tissue architecture. This is consistent with lateral constraints imposed by the presence of tight junctions. Instead, increases in cell height may be mediated by partial unfolding of the interdigitations of lateral membranes.

Using different therapies (insulin and/or aldose reductase inhibition, ARI) to treat diabetes has confirmed that there are minor differences in cell width, but major changes in height, as cells pass from crypts to villi (Zoubi et al., 1995b). This suggests that cell width is determined before cells exit the crypt and that volume changes during migration are mediated by altering cell height (Figs. 2, 3). These considerations seem to apply particularly to differences within a treatment group. Between-group differences in villous cell volumes may be partly pre-set by intracryptal changes in cell width on which are superimposed changes in cell height.

Commentary

The evidence for increases in height, width and volume of enterocytes in passing from crypt to villus is clear although studies based on average cells in crypts and on villi may underestimate the magnitude of the differences involved. Morphometry of mouse villous cells at basal, intermediate and tip sites (Abbas et al., 1989) has revealed variations in cell width and microvillous length but not in mean cell height. Near villous tips, cells tended to be wider and have longer microvilli. Clearly, gradients of microvillous morphology accompany changes in transporters and hydrolases along the crypt-villus axis. Increases in surfaces of microvilli allow the possibility to incorporate

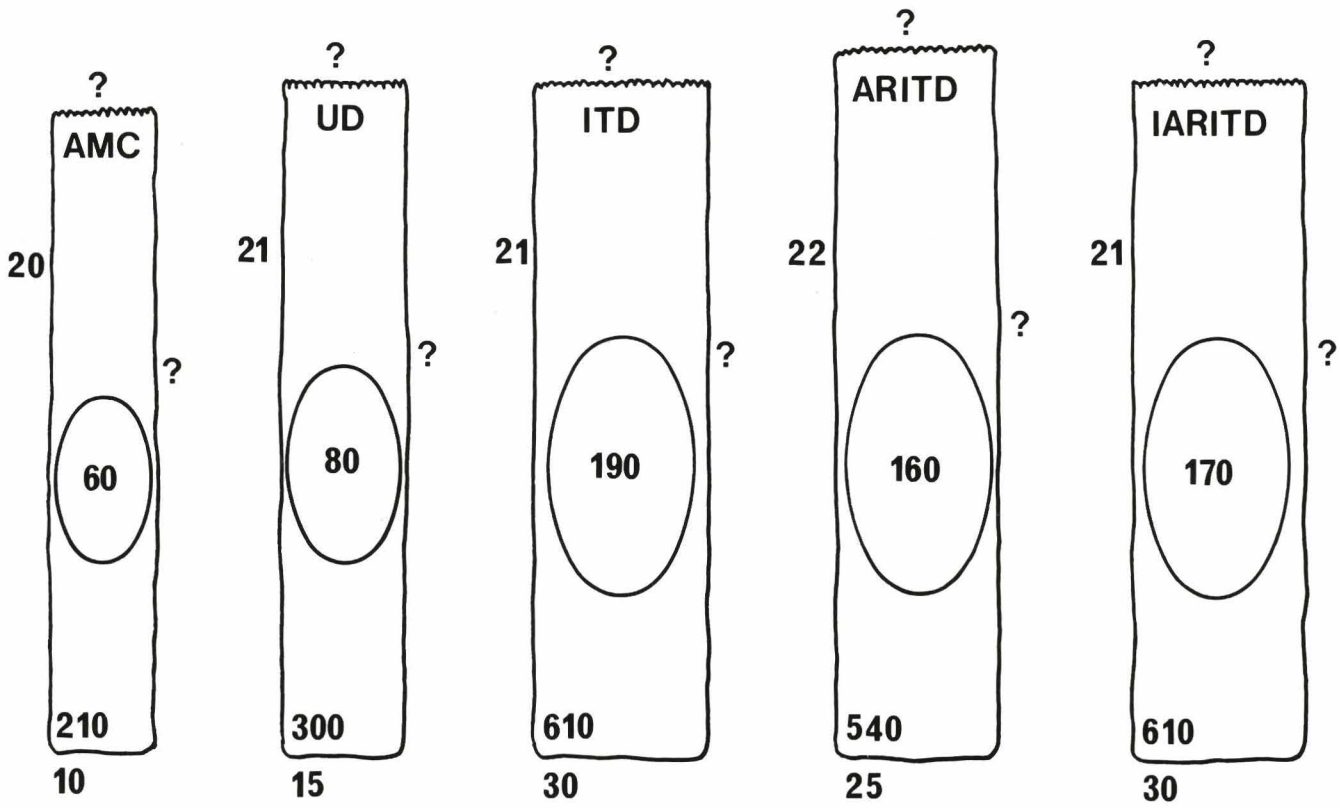

Fig. 3. Morphophenotypes of average crypt enterocytes in small intestines of control rats (AMC, total cell number 3.3×10^9), untreated streptozotocin-diabetics (UD, 5.9×10^9 cells) and diabetics treated with insulin (ITD, 2.1×10^9 cells), aldose reductase inhibitor (ARITD, 3.7×10^9 cells) or insulin+inhibitor (IARITD, 1.6×10^9 cells). For key to structural quantities, see Fig. 1. Data taken from Zoubi et al. (1995a,b). Comparative data for apical and lateral membrane areas, numbers and sizes of microvilli are not available.

greater numbers of transporters of the same or different properties. The presence of higher affinity and lower capacity transporters in less mature villous enterocytes may be a compensation for a lag in morphological development near the crypt-villus junction.

Mammalian small intestine: longitudinal gradients in rats and bats

Glucose transport

In rats, this is greatest in cranial regions but there may be species differences (Karasov and Diamond, 1983). It seems to vary with transport capacity and not with carrier affinity but further immunolocalisation studies are required to establish this. Gradients follow luminal nutrient concentrations suggesting that these induce local adaptive changes in packing densities or activities of transporters in membranes. SGLT1 immunohistochemistry has shown that this is localized in microvillous membranes all along the small intestine (Yoshida et al., 1995).

Cell morphology

In rats (Table 1), significant longitudinal variation exists for many variables, including cell volume, but not the numbers and surface areas of microvilli per villous enterocyte (Zoubi et al., 1994, 1995a). Greater cell volumes are found in cranial segments. However, whilst the packing densities of microvilli are constant between regions, the lengths and diameters vary locally and tend to be greater at cranial to middle regions (Mayhew and Middleton, 1985).

Table 1. Longitudinal variation in cell morphology and number in rat crypt and villus intestinal epithelium. Values are group means (SEM) for 6 rats (see Zoubi et al., 1995a).

VARIABLE	CRANIAL ·····			CAUDAL
Cell number ($x10^8$)				
Crypt	5.2 (1.4)	8.9 (0.7)	12.5 (3.1)	6.6 (1.8)
Villus	9.8 (1.0)	19.8 (3.0)	10.9 (1.0)	10.4 (3.0)
Cell height (μm)				
Crypt	20 (0.8)	20 (0.9)	21 (0.8)	20 (1.0)
Villus	31 (1.9)	29 (2.1)	27 (3.0)	23 (2.5)
Nuclear volume (μm^3)				
Crypt	81 (9.6)	75 (7.3)	44 (5.6)	58 (6.0)
Villus	103 (6.1)	66 (7.6)	67 (4.8)	62 (17.2)
Cell volume (μm^3)				
Crypt	390 (40)	230 (32)	170 (31)	220 (28)
Villus	530 (52)	340 (36)	340 (41)	330 (78)
Cell surface without microvilli (μm^2)				
Crypt	20 (2.7)	12 (1.8)	8 (1.2)	12 (1.8)
Villus	18 (1.5)	13 (1.9)	15 (1.7)	16 (2.3)
Surface with microvilli (μm^2)				
Villus	180 (27)	210 (34)	270 (35)	170 (26)
Number of microvilli per cell				
Villus	560 (46)	460 (67)	630 (72)	450 (68)

Organ morphology

Longitudinal gradients of epithelial structure exist and fluctuations in villous and microvillous surface areas are manifestations of this (Altmann and Enesco, 1967; Stenling et al., 1984; Mayhew and Middleton, 1985; Ross and Mayhew, 1985; Mayhew, 1987, 1990; Zoubi et al., 1994). More cranial segments tend to have more cells (Table 1), show greater absorptive surface areas and have higher proportions of villous cells (Altman and Enesco, 1967; Wright, 1980; Karasov and Diamond, 1983; Mayhew and Middleton, 1985; Mayhew, 1990; Zoubi et al., 1994).

Similar gradients are seen in other species. In a study on 5 intestinal regions in bats, there were gradients of morphology with greater surface areas and numbers of microvilli at cranial sites (see Table 2 and Makanya et al., 1995).

Commentary

Gradients of villous and microvillous surface in rats and bats are not linear but tend to reach a maximum in cranial to middle segments. The peak at mid-intestinal region in rats does not seem to match the cranial peak in glucose transport but this may be due, in part, to

Table 2. Longitudinal variation in numbers and sizes of microvilli, villous and microvillous surface areas in small intestines of three species of bat.

VARIABLE	BAT No.	CRANIAL ·····			CAUDAL
Diameter (nm)					
	1	100	80	80	90
	2	110	100	80	80	90
	3	130	110	110	110	130
Length (μm)					
	1	1.1	0.9	0.8	0.8	0.8
	2	2.7	2.5	2.1	2.1	2.7
	3	4.2	3.8	3.2	2.0	1.5
Number per μm^2 of cell apex					
	1	87	128	95	100	94
	2	51	53	67	75	45
	3	28	26	47	61	58
Number per region ($x10^{11}$)					
	1	1.2	1.6	0.9	0.4	0.4
	2	8.7	4.8	6.1	3.1	0.9
	3	2.6	1.6	1.8	1.9	0.9
Villous surface per region (cm^2)					
	1	14	13	10	4	4
	2	170	91	90	41	18
	3	90	61	39	31	16
Microvillous surface per region (cm^2)					
	1	420	330	170	85	88
	2	7930	3610	3160	1620	600
	3	4340	2040	1930	1240	550

Data based on Makanya et al. (1995). Note that microvillous areas and numbers are not corrected for section thickness effects. Key to bats: 1, *M. Inflatus* (insect-eater); 2, *E. wahlbergi* (fruit-eater); 3, *L. angolensis* (fruit-eater).

problems associated with choosing appropriate references for expressing physiological data (Karasov and Diamond, 1983). Findings suggest that microvilli vary in length, number and surface area to match the fluctuations in villous surface area. This implies that regional differences depend on not only cell number but also the maturational status of cells. It is not known if the incidence of transporter forms with different affinities and capacities alters along the intestine. Changes in the length of microvilli may provide a local means of partly compensating for reduced numbers of enterocytes. It is possible that these structural and functional adaptations are influenced by luminal factors including nutrient levels.

Rat small intestine: effects of experimental diabetes

Experimental diabetes may be induced using alloxan but most studies now use streptozotocin. Injection of the latter leads to loss of body weight, high blood glucose levels, polyuria, glucosuria, polydipsia, hyperphagia and premature death. Small intestines are heavier and enhanced water and nutrient transport are accompanied by changes in the sizes of crypts and villi (Jervis and Levin, 1966; Olsen and Rosenberg, 1970; Schedl and Wilson, 1971; Caspary, 1973; Lal and Schedl, 1974; Lorenz-Meyer et al., 1974; Nakabou et al., 1980; Karasov and Diamond, 1983; Stenling et al., 1984; Keelan et al., 1985).

Glucose transport

In diabetic rats, Na^+-dependent glucose uptake increases within 24 hours by induction of glucose transporters in microvillous and basolateral membranes (Karasov and Diamond, 1983; Ferraris and Diamond, 1986; Debnam et al., 1990; Debnam and Chowrimootoo, 1992). Later on, the 6-fold higher levels of nutrient transport do not match the 2-fold increases in villous surface area (Fedorak, 1990; Mayhew, 1990). This appears to reflect greater transport capacity rather than a change in carrier affinity but immunolocalisation studies have yet to confirm this. The signals responsible for elevated nutrient absorption are not understood but may include insulinopaenia, hyperglycaemia and high intracellular glucose (Karasov and Diamond, 1983).

Fedorak (1990) has suggested that diabetics have more glucose carriers compared with age-matched controls and that glucose binding extends from the villous tips to enterocytes at lower villous levels, even into intervillous areas. Membrane vesicles isolated from brush border preparations show higher levels of glucose uptake than in controls. Moreover, the differences are confined to preparations from the middle and lower regions of villi (Dudeja et al., 1990). Similar increases in total hexose transport are associated with early expression of transporters by less mature enterocytes (Burant et al., 1994). SGLT1 and GLUT2 contents increased up to 6-fold in enterocytes from diabetic rats.

Responses to diabetes may also vary with intestinal location. Recruitment of glucose transporters into lower villous areas occurs in the ileum but not the jejunum (Fedorak, 1990). This offers a molecular explanation for the greater impact of diabetes at caudal sites (Mayhew, 1990). Similar patterns of sucrase expression have been observed (Hoffman et al., 1992).

The earliest adaptations to experimental hyperglycaemia may be mediated by basolateral rather than apical membranes (Karasov and Debnam, 1987; Cheeseman, 1992). Mid-villus sites are important with changes occurring at apical and basolateral sites and the transport capacity of basolateral membranes being retained during migration along the crypt-villus axis (Debnam et al., 1990). Increased glucose efflux seems to result from an increased density of carriers in basolateral membranes but a rapid response to hyperglycaemia does not require large changes in the amounts of transporter protein (Cheeseman, 1992). In addition, the catalytic subunit of Na^+, K^+-ATPase exists in two forms which vary in their sensitivity to ouabain. Both forms exist in the basolateral membranes of enterocytes and experimental diabetes leads to a dominance of the ouabain-insensitive form. This can be mimicked in vitro by exposing cells to glucagon (Fedorak, 1990). Diabetic rats display elevated levels of this hormone.

Cell morphology

The main features are summarised in Figs 2 and 3. Diabetes does not alter the mean length, diameter or packing density of microvilli (Fedorak, 1990; Mayhew, 1990; Williams and Mayhew, 1992). Consequently, changes in the absolute numbers and surface area of microvilli are commensurate with those in villous surface area. This led to the suggestion that the key adaptive response must be cell proliferation (Mayhew, 1990). Supporting evidence for a preferential effect in caudal regions was found (Mayhew and Carson, 1989; Mayhew, 1990). Longitudinal differences in crypt cell volume, area and height were not detected in diabetic rats and nor was there variation in nuclear volume, cell volume, area and height of villous cells (Zoubi et al., 1995a).

Later studies confirmed the primary importance of cell proliferation (Zoubi et al., 1994, 1995a,b). Adaptation was examined in groups of 23 week old streptozotocin-diabetic rats (12 weeks duration) and age-matched controls. In each animal, 4 intestinal segments were examined. Although diabetics contained 80% more villous enterocytes than controls, their morphophenotype (volume, height, apex area, microvillous area, microvillous number and nuclear volume) was unaltered (Fig. 2). In contrast, crypt cell proliferation was accompanied by changes in cell morphology (Fig. 3). Cells and their nuclei were 40-50% bigger than in controls. Increases in cell number and volume accounted for greater crypt volume and diameter. Since crypt cell height did not alter, cells grew by becoming wider.

Longitudinal differences were found for the numbers and sizes of crypt cells and their nuclei.

It appears that there are constraints on the growth in height of crypt and villous cells in diabetes since this dimension does not alter. This might be related to physical factors (e.g. packing of cells in crypts and villi), or functional disadvantages consequent upon them (e.g. the impact on nutrient transport of unstirred layer effects (Karasov and Diamond, 1983).

As yet, there are no design-based studies on basolateral membranes to match those on apical membranes. Buschmann and Manke (1981a,b) relied on samples of hamster villous cells cut longitudinally. These «local vertical windows» should have been analysed using sine-weighted lines (Baddeley et al., 1986) but were not. In addition, cell volumes were based on models. For these reasons, their area estimates are biased to unknown degrees. On the assumption that basal membrane area approximates apex area (minus microvilli) and that lateral membranes account for 46% of total plasmalemma, our figures suggest a total basolateral membrane surface per normal cell of 200 μm^2 of which about 180 μm^2 is lateral membrane. If area per cell does not alter (as for apical and microvillous area), these figures would also apply to enterocytes from diabetic rats.

Organ morphology

A constant number of villi per intestine (Clarke, 1970, 1972; Forrester, 1972; Warren, 1991) implies that cell proliferation and/or hypertrophy contribute to altered transport. In fact, both occur. Intestinal water and DNA contents and protein/DNA ratio are greater in experimental diabetes (Schedl and Wilson, 1971; Nakabou et al., 1974). There is increased crypt activity and a shorter villous turnover time (Miller et al., 1977). Jejunal enterocytes near villous tips are taller and tissue sections display more cell profiles (Stenling et al., 1984; Keelan et al., 1985). Some of these changes preferentially affect caudal sites (Olsen et al., 1974; Miller et al., 1977; Stenling et al., 1984; Mayhew and Carson, 1989; Hoffman et al., 1992).

Stereological investigations on streptozotocin-diabetic rats and their age-matched controls confirmed some of the structural changes. Absorptive surface areas and volumes of villi were elevated and there were changes in villous height and shape (Mayhew and Carson, 1989). Crypt mitotic figures and crypt volume increased, the latter by changes in diameter but not length (Mayhew et al., 1989). Longitudinal differences in crypt cell number disappeared in diabetics (Zoubi et al., 1995a).

Diabetic animals contained 80% more enterocytes than controls and the proportions of villous and crypt cells were maintained. Given 140,000 villi and 18 crypts per villus, results suggest complements of 36,000 cells per villus and 1,300 cells per crypt in normal rats (Zoubi et al., 1994, 1995a). Predicted numbers for diabetic rats are 65,000 and 2,300 respectively. The near-doubling of cell numbers paralleled increases in villous surface area and crypt volume (Mayhew and Carson, 1989; Mayhew, 1990; Williams and Mayhew, 1992) demonstrating that changes are exclusively, or predominantly, the result of cell division.

After 12 weeks of diabetes, the complement of villous enterocytes increased by $4x10^9$ (Zoubi et al., 1995a). This is a net recruitment of 2 millions enterocytes per hour for the entire small intestine. This is an underestimate of total cell production because some cells are lost by extrusion from villous tips. However, with 140,000 villi, the net recruitment is just 14 cells per hour onto each villus. This is about 10% of the normal net cell influx of 130-180 cells/villus/hour (Appleton et al., 1980; Leblond, 1981).

Various factors influence intestinal epithelial renewal, including transforming growth factor-α epidermal growth factor, trefoil peptides, enteroglucagon (Alison and Sarraf, 1994) and a protein (or peptide) recently extracted from irradiated small intestine (Potten et al., 1994). Studies on isolated enterocytes suggest that hyperplasia in diabetes is associated with increased activity of ornithine decarboxylase and contents of polyamines (Younoszai et al., 1993). Administration of the ornithine decarboxylase inhibitor, difluoro-α-methylornithine, reduced or prevented hyperplasia in diabetics and retarded epithelial growth in control rats.

Commentary

Changes at cell and organ levels help to account for the long-term (beyond 3-5 days of induced diabetes) increases in absorption of glucose and other nutrients (Karasov and Diamond, 1983). This implies that a full physiological and morphological adaptation depends on total replacement of the epithelium and this is supported by the failure to observe any change in villous enterocyte morphophenotype in diabetics. Since the structure of microvilli on villous enterocytes is unaltered, the early expression of SGLT1 may be due to early incorporation into membrane or to modifications rendering it more active.

Rat small intestine: effects of alternative therapeutic interventions for treating diabetes

Glucose transport

Increased glucose absorption and elevated hydrolase activities in experimental diabetes are prevented or reduced by insulin therapy (Olsen and Rosenberg, 1970; Caspary et al., 1972; Olsen et al., 1974).

Cell morphology

Stereological studies examined the effects of alternative therapies (insulin, ARI) on streptozotocin-diabetic small intestines. Treated diabetic rats were

divided into 3 groups: those given daily subcutaneous injections of Ultralente insulin, ARI by gavage or insulin and ARI (Williams and Mayhew, 1992; Zoubi et al., 1995b). In crypts, insulin increases cell size beyond that in untreated diabetes. ARI also tends to increase cell sizes (Fig. 3). On villi, insulin affects all variables except cell height and number of microvilli per cell (Fig. 2). Cell volumes and widths are greater than in untreated diabetics or controls. The surface area and number of microvilli per cell are also greater. ARI has significant effects but cell and microvillous dimensions are not normalised.

Insulin does not restitute normal longitudinal differences in crypts but does normalise some of those on villi. Similar results are found with ARI which also restores normal longitudinal gradients of crypt cell volumes. The combined use of insulin and ARI returns fewer longitudinal differences to normal patterns (Zoubi et al., 1995b).

Organ morphology

In crypts, insulin has significant effects on all variables except epithelial height. It, and ARI, tend to normalise cell numbers. On villi, insulin has significant effects on all variables except epithelial height. Cell numbers are reduced to below those found not only in untreated diabetic rats but also in controls. ARI has significant effects on all variables except epithelial height. Again, cell numbers return towards normal values. The numerical ratio of villus: crypt cells is unaltered by insulin or ARI (since these do not change during diabetes).

Commentary

Insulin, but not ARI, normalises the volumes of villi and crypts and villous surface area (Mayhew et al., 1989) in parallel with glucose transport and hydrolase activities. ARI actually leads to crypt hypertrophy beyond that found in untreated diabetics. In this sense at least, the effects of ARI on the crypts are less beneficial than those of insulin. Insulin and ARI tend to reduce cell numbers (though not necessarily to normal values) but fail to conserve or restore normal cell morphophenotype. This is surprising given that insulin therapy normalises intestinal absorption, blood glucose levels, enzyme activities (Caspary, 1973; Olsen et al., 1974; Young et al., 1987; Hoffman et al., 1992) and villous and crypt sizes (Mayhew and Carson, 1989; Mayhew et al., 1989). It seems that the morphological change which restores volumes and absorptive surfaces of villi and volumes of crypts is the presence of fewer but larger cells.

Studies on villous enterocytes in diabetic rats have demonstrated that insulin affects the extent to which microvilli amplify the apical cell surface via changes in their length, diameter and packing density (Williams and Mayhew, 1992). ARI affected amplification mainly by altering packing densities. The studies by Zoubi et al. (1995b) indicate that these events are accompanied by increases in the number and surface of microvilli per average cell in insulin- and ARI-treated rats and that these, in turn, are determined by the increase in cell apex area. This may explain why insulin therapy normalises villous and microvillous surface areas in the whole organ (Mayhew and Carson, 1989; Mayhew et al., 1989; Williams and Mayhew, 1992).

The insulin effects on morphology are intriguing. Its cellular effects are not markedly different from those of ARI save for a more exaggerated depletion of enterocyte number. The depletion of endogenous insulin triggered by administering streptozotocin elicits cellular changes which vary between crypts and villi and are not restored by injecting exogenous insulin. Insulinopaenia increases cell numbers in crypts and villi and leads to crypt cell hypertrophy without consequent changes in the volumes of villous cells. Injecting exogenous insulin does not normalise cell size. On the contrary, it leads to further cell hypertrophy. Finally, it tends to restore the numbers of crypt cells but produces lower than normal complements of villous enterocytes.

Insulin affects the activities of brush border hydrolases and there are binding sites for insulin and insulin-like growth factor (IGF) on enterocytes (Forgue-Lafitte et al., 1980; Gallo-Payet and Hugon, 1984; Laburthe et al., 1988; Park et al., 1990; Heinz-Erian et al., 1991). The expression of IGF receptors on crypt cells may indicate a role in the control of cell growth but, unfortunately, little is known about their incidence, activity or regulation in diabetes. It has also been suggested that insulin affects DNA synthesis but recent evidence (Goodland et al., 1993) indicates that it does not play a cardinal role in regulating cell proliferation. The hyperplasia of diabetes may be a consequence of hyperphagia.

Insulin influences intestinal absorption of polyamines (important in maturation and cell proliferation during weaning) and stimulates ornithine decarboxylase, the rate-limiting enzyme of polyamine synthesis, in mature cells (Buts et al., 1994). Inhibitors of this enzyme prevent hyperplasia in diabetic rats (Younoszai et al., 1993).

Bat small intestine: effects of fruit- and insecting-eating diets

Carnivores and herbivores of the same vertebrate class tend to differ in intestinal length and transport rates. Herbivores tend to have longer intestines and higher glucose transport rates (Karasov and Diamond, 1983). Bats are unique amongst mammals in their capacity for active flight. This is energy-expensive and it might be expected that respiratory, cardiovascular and gastrointestinal adaptations might accompany the higher metabolic demands. In a recent pilot study, we examined the features of 3 bats in order to establish a reproducible protocol for comparing intestinal morphology in bats with different lifestyles (Makanya et al., 1995). The

specimens and diets were *Miniopterus inflatus* (body weight 9 g, insect-eater), *Epomophorus wahlbergi* (69 g, fruit-eater) and *Lisonycteris angolensis* (76 g, fruit-eater).

Glucose transport

Whilst glucose transport in bats is probably essentially similar to that in other mammals, physiological studies indicate the absence of certain active transport mechanisms (Keegan, 1980). Despite this, sugars are absorbed at faster rates than in rat intestine (Keegan, 1977)

Cell morphology

There are no data for the attributes of average cells but there are for microvillous dimensions (Tables 2 and Makanya et al., 1995). Mean microvillous diameters per intestine varied from 88 to 115 nm, heights from 1 to 3 µm and mean areas from 0.24 to 1.11 μm^2. After correcting for section thickness effects, the data suggest that microvilli were packed on the cell apex at densities of 70 (*M. inflatus*), 40 (*E. wahlbergi*) and 30 (*L. angolensis*) per μm^2.

Organ morphology

There are few quantitative data for the villous and microvillous surfaces of bat intestines. For this reason, the relationship between gut morphology and diet has, hitherto, not been clear. In one fruit bat, the greater transport capacity has been attributed to the presence of a large mucosal surface area an enterocytes well-endowed with microvilli (Keegan and Modinger, 1979).

In the study by Makanya et al. (1995), estimated surface areas of villi were 44 (*M. inflatus*), 410 (*E. wahlbergi)*) and 237 cm^2 (*L. angolensis*). The corresponding microvillous surface areas and numbers, corrected for section thickness, were 0.1, 1.2 ad 0.8 m^2 and 3, 16 and $7x10^{11}$ per intestine. When normalised for body weights, corrected microvillous surfaces were 80, 170 and 100 cm^2/g respectively.

Commentary

The functional surfaces of fruit bats appears to be more extensive than those of insect-eaters. Differences between bats are achieved by adaptations at several levels of structural organisation. These include increases in intestinal length, circumference and villous and microvillous amplification. The latter is accomplished mainly by altering the lengths of microvilli, although diameters may also alter with consequent changes in packing densities.

In rat small intestine (Mayhew, 1990), microvilli are shorter, thicker and less densely packed. Despite these differences, absolute microvillous surface area in bats (0.1-1.2 m^2) is not much lower than that in rats (0.9-1.2 m^2) once allowance is made for section thickness effects (Mayhew and Middleton, 1985; Mayhew, 1990; Makanya et al., 1995). However, when referred to body weights, the relative surface areas of microvilli are much greater in the bat (80-170 cm^2/g) than in the rat (14-40 cm^2/g). It is not known if these differences in morphology are accompanied by species differences in transporter affinity and/or capacity.

Avian coprodaeum and transepithelial Na^+ transport

The avian coprodaeum, part of the cloaca, has several functions which include ion transport, osmoregulation and storage of urine and faeces. Like mammalian small intestine, this is a tight epithelium although the tall columnar cells lack the highly organised brush border typical of small intestinal enterocytes.

Sodium transport

The organ affords an excellent system for studying Na^+ transport and its structural correlates (Eldrup et al., 1980; Elbrønd et al., 1991; Mayhew et al., 1992a,b). No other vertebrate system can match the variation in amiloride-blockable Na^+ transport which shows good correlation with levels of dietary NaCl and plasma aldosterone (Choshniak et al., 1977; Bindslev, 1979; Thomas and Skadhauge, 1982; Skadhauge et al., 1983; Clauss et al., 1987; Skadhauge, 1989).

Transporters are localised in the apical and baso-lateral membrane domains of coprodaeal enterocytes. Passive influx of Na^+ into cells is rate-limiting and occurs via apical Na^+ channels. It can be promoted by aldosterone and inhibited by the diuretic amiloride. The apical Na^+ channels have been identified by immuno-chemistry and are related to those found in bovine renal epithelial cells (Smith et al., 1993). The numbers of Na^+ channels vary with dietary NaCl load but the underlying mechanism is not fully understood. The total number of channels may be constant and the ratio of open-to-closed channels vary (Tousson et al., 1989) or the number of channels within the apical membranes may alter (Asher et al., 1992). At present, the latter is the more likely given that transfer of coprodaeal cell mRNA from birds on a low NaCl diet induces elevated Na^+ transport in *Xenopus oocytes* (Garty and Asher, 1991).

Efflux occurs via active basolateral ATPase-dependent, oxygen-consuming and ouabain-inhibitable Na^+/K^+ pumps. Enzyme histochemistry has shown hat the latter are located on the cytoplasmic aspect of basolateral membrane domains (Dantzer et al., 1988; Mayhew et al., 1992b). Adaptation to a low NaCl diet leads to a 100-fold greater net transport and 9-fold higher level of plasma aldosterone. However, the increase in activity of enzymes associated with the basolateral Na^+/K^+ pumps, notably potassium-dependent paranitrophenyl phosphatase (Dantzer et al., 1988), is considerably less and this suggests that adaptation of

Na^+ transport is regulated within apical membrane domains. Morphological studies support this notion.

Cell morphology

Studies at this level emphasise that adaptation is greater apically (Fig. 4). Changes in the surfaces of microvilli per cell are not accompanied by those in the surfaces of basolateral membranes (Dantzer et al., 1988; Elbrønd et al., 1991; Mayhew et al., 1992a,b). Of the total organ adaptation (see below), roughly 20% of the increase in number of microvilli might be due to cells bearing more microvilli (Mayhew et al., 1992a). In hens on a high NaCl diet, the membrane signature of the average cell was 32 μm^2 (microvilli) and 950 μm^2 (basolateral membranes, of which 17 μm^2 is basal). Cells from hens on a low NaCl diet possessed, on average, 55% more microvillous membrane area but the same area of basolateral membranes (Mayhew et al., 1992b). The expanded apical membrane area of the average cell was attributable to longer (34%) and more densely packed (38%) microvilli.

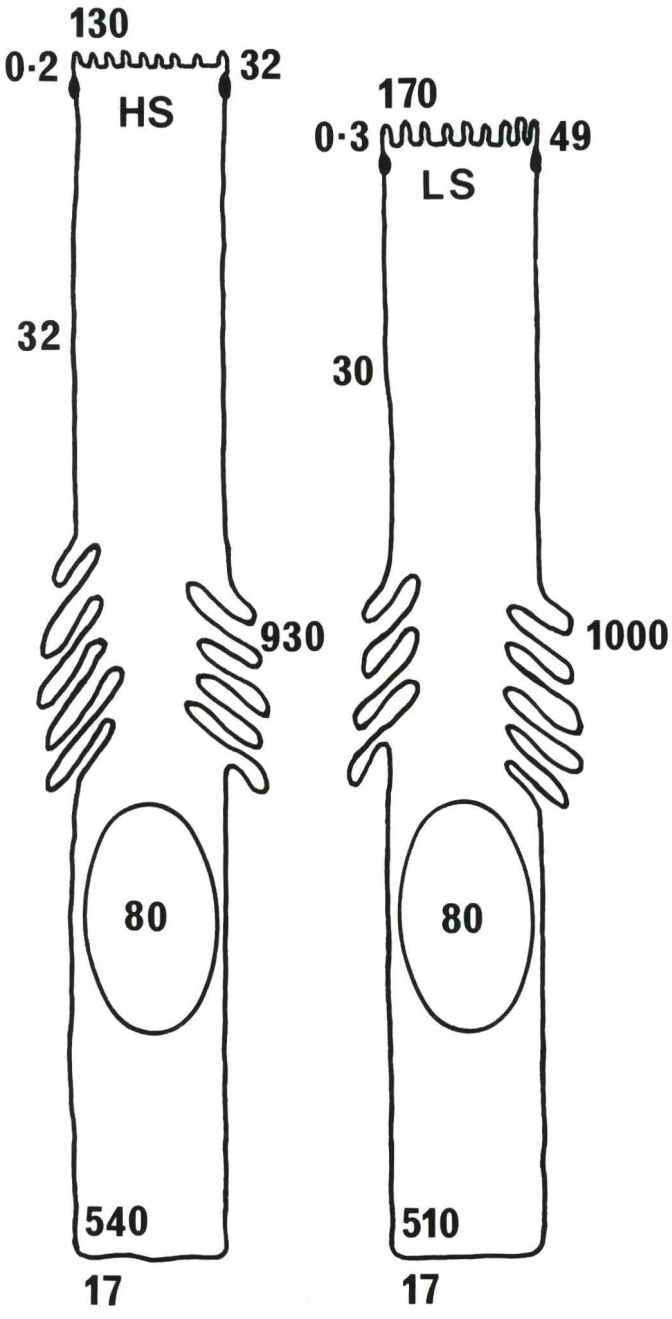

Fig. 4. Morphophenotypes of average villous enterocytes in coprodaea of domestic hens on high-salt (HS, total cell number $2.7x10^8$) and low-salt (LS, $4.2x10^8$ cells) diets. For key to structural quantities, see Fig. 1. Data taken from Elbrønd et al. (1991) and Mayhew et al. (1992a,b).

Organ morphology

The coprodaeum responds to changes in Na^+ absorption by altering microvillus and basolateral membrane surface areas. Both are achieved partly by altering the total number of cells, from about $2.7x10^8$ to $4.2x10^8$ per organ (Dantzer et al., 1988; Elbrønd et al., 1991; Mayhew et al., 1992a,b). In the entire coprodaeum, microvillus area increases from 90 to 200 cm^2. Of the total organ adaptation, roughly 80% of the total increase in number of microvilli can be explained by cell proliferation (Mayhew et al., 1992a). Lateral membranes increased in area from 2500 to 4000 cm^2 and basal membranes from 45 to 68 cm^2 per organ (Mayhew et al., 1992b). Though the basolateral membrane signature of the average cell did not alter after adaptation to a low NaCl diet (see above), the larger complement of cells explained the overall increases in membrane area per organ.

Commentary

These studies have confirmed that the differentiated columnar enterocyte (specifically, the «light» cell, Mayhew et al., 1992b) is the mediator of these changes rather than some subset of so-called «supercells» (Eldrup et al., 1980). They also suggest that there is an increase in the number or activity of Na^+/K^+ pump sites per unit surface of basolateral membrane and that there may be an alteration in the control ratio of about 1500 Na^+/K^+ pump sites per Na^+ channel (Mayhew et al., 1992b). Since pump activity is driven by ATP, the observed increase in activity of succinic dehydrogenase in mitochondria within enterocytes is consistent with enhanced basolateral transport. Expansion of microvillous membrane area on the average cell (Elbrønd et al., 1991; Mayhew et al., 1992a,b) to accommodate a greater number of apical Na^+ channels (Asher et al., 1992) is, to some degree, consistent with freeze-fracture analyses. These have suggested that increased numbers of rod-shaped particles (putative Na^+ channels) are inserted into apical membranes (Eldrup et al., 1980). However, these were a subset of mitochondria-rich «dark» cells, the numbers and morphophenotype of

which could not account for the whole organ changes observed (Mayhew et al., 1992b).

In conclusion, morphological adaptations to altered Na^+ transport in coprodaeum involve increases in the total extent of apical and basolateral membranes. Adjustments at the cellular level are focussed on the apical membranes which contain more (or more active) transport channels. Basolateral area per cell does not alter but it is likely that these membranes contain increased numbers of pump sites. As with mammalian small intestine and its responses to experimental diabetes, the longer term adaptation of avian coprodaeum to reduced dietary NaCl depends on a renewal of the entire epithelium. However, the morphophenotype of the coprodaeal enterocyte is quantitatively different from its small intestinal counterpart. This is particularly conspicuous when the areas of apical and lateral membrane domains are compared (Figs. 1, 4).

Acknowledgements. I am grateful to all the colleagues in Aberdeen, Benghazi, Copenhagen, Nairobi and Nottingham who have contributed to these stereological studies on vertebrate intestines.

References

Abbas B., Hayes T.L., Wilson D.J. and Carr K.E. (1989). Internal structure of the intestinal villus: morphological and morphometric observations at different levels of the mouse villus. J. Anat. 162, 263-273.

Alison M.R. and Sarraf C.E. (1994). The role of growth factors in gastrointestinal cell proliferation. Cell Biol. Int. 18, 1-10.

Altmann G.G. (1990). Renewal of the intestinal epithelium: new aspects as indicated by recent ultrastructural observations. J. Electr. Microsc. Tech. 16, 2-14.

Altmann G.G. and Enesco M. (1967). Cell number as a measure of distribution and renewal of epithelial cells in the intestine of growing and adult rats. Am. J. Anat. 121, 319-336.

Appleton D.R., Sunter J.P. and Watson A.J. (1980). Cell proliferation in the gastrointestinal tract. Pitman Medical. Tunbridge Wells.

Asher C., Singer D., Eren R., Yeger O., Dascal N. and Garty H. (1992). NaCl dependent expression of an amiloride-blockable Na^+ channel in *Xenopus oocytes*. Am. J. Physiol. 262, G244-G248.

Baddeley A.J., Gundersen H.J.G. and Cruz-Orive L.M. (1986). Estimation of surface area from vertical sections. J. Microsc. 142, 259-276.

Bindslev N. (1979). Sodium transport in the hen lower intestine. Induction of sodium sites in the brush border by a low sodium diet. J. Physiol. 288, 449-466.

Both N.J. de, Dongen J.M. van, Hofwegen B. van, Keulemans J., Visser W.J. and Galjaard H. (1974). The influence of various cell kinetic conditions on functional differentiation in the small intestine of the rat. A study of enzymes bound to subcellular organelles. Dev. Biol. 38, 119-137.

Brown A.L. (1962). Microvilli of the human jejunal epithelial cell. J. Cell Biol. 12, 623-627.

Burant C.F., Flink S., DePaoli A.M., Chen J., Lee W-S., Hediger M.A., Buse J.B. and Chang E.B. (1994). Small intestine hexose transport in experimental diabetes. Increased transporter mRNA and protein expression in enterocytes. J. Clin. Invest. 93, 578-585.

Buschmann R.J. and Manke D.J. (1981a). Morphometic analysis of the membranes and organelles of small intestinal enterocytes. I. Fasted hamster. J. Ultrastr. Res. 76, 1-14.

Buschmann R.J. and Manke D.J. (1981b). Morphometric analysis of the membranes and organelles of small intestinal enterocytes. II. Lipid-fed hamster. J. Ultrastr. Res. 76, 15-26.

Buts J.P., Keyser N., Romain N., Dandrifosse G., Sokal E. and Nsengiyumva T. (1994). Response of rat immature enterocytes to insulin: regulation of receptor binding and endoluminal polyamine uptake. Gastroenterology 106, 49-59.

Caspary W.F. (1973). Effect of insulin and experimental diabetes mellitus on the digestive absorptive function of the small intestine. Digestion 9, 248-263.

Caspary W.E., Rhein A.M. and Creutzfeldt W. (1972). Increase of intestinal brush-border hydrolases in mucosa of streptozotocin diabetic rats. Diabetologia 8, 412-414.

Cheeseman C. (1992). Role of intestinal basolateral membrane in absorption of nutrients. Am. J. Physiol. 263, R482-R488.

Choshniak I., Munck B.G. and Sakdhauge E. (1977). Sodium chloride transport across the chicken coprodeum. Basic characteristics and dependence on sodium chloride intake. J. Physiol. 271, 489-504.

Clarke R.M. (1970). Mucosal architecture and epithelial cell production rate in the small intestine of the albino rat. J. Anat. 107, 519-529.

Clarke R.M. (1972). The effect of growth and fasting on the number of villi and crypts in the small intestine of the albino rat. J. Anat. 112, 27-33.

Clauss W., Durr J.E., Guth D. and Skadhauge E. (1987). Effects of adrenal steroids on Na transport in the lower intestine (coprodeum) of the hen. J. Membrane Biol. 96, 141-152.

Cruz-Orive LM. and Weibel E.R. (1990). Recent stereological methods for cell biology: a brief survey. Am. J. Physiol. 258, L148-L156.

Dantzer V., Møller O. and Skadhauge E. (1988). Morphological and enzymic adaptation to aldosterone of the epithelium of the caudal hindgut of the hen. J. Physiol. 396, 30P.

Debnam E.S. and Chowrimootoo G. (1992). Streptozotocin diabetes and sugar transport by rat ileal enterocytes: evidence for adaptation caused by an increased luminal nutrient load. Biochim. Biophys. Acta 1107, 86-92.

Debnam E.S., Ebrahim H.Y. and Swaine D.J. (1990). Diabetes mellitus and sugar transport across the brush-border and basolateral membranes of rat jejunal enterocytes. J. Physiol. 424, 13-25.

Dongen J.M. van, Visser W.J., Daems W.Th. and Galjaard H. (1976). The relation between cell proliferation, differentiation and ultrastructural development in rat intestinal epithelium. Cell Tissue Res. 174, 183-199.

Dudeja P.K., Wall R.K., Klitzke A. and Brasitus T.A. (1990). Intestinal D-glucose transport and membrane fluidity along the crypt-villus axis of streptozotocin-induced diabetic rats. Am. J. Physiol. 259, G571-G577.

Elbrønd V.S., Dantzer V., Mayhew T.M. and Skadhauge E. (1991). Avian lower intestine adapts to dietary salt (NaCl) depletion by increasing transepithelial sodium transport and microvillous membrane surface area. Exp. Physiol. 76, 733-744.

Eldrup E., Møllgaard K. and Bindslev N. (1980). Possible epithelial sodium channels visualized by freeze-fracture. Biochim. Biophys. Acta 596, 152-157.

Fedorak R.N. (1990). Adaptation of small intestinal membrane transport

processes during diabetes mellitus in rats. Can. J. Physiol. Pharmacol. 68, 630-635.

Ferraris R.P. and Diamond J.M. (1986). Use of phlorizin binding to demonstrate induction of intestinal glucose transporters. J. Membrane Biol. 94, 77-82.

Forgue-Lafitte M.E., Marescot M.R., Chamblier M.C. and Rosselin G. (1980). Evidence for the presence of insulin binding sites in isolated rat intestinal epithelial cells. Diabetologia 19, 373-378.

Forrester J.M. (1972). The number of villi in rat's jejunum and ileum: effect of normal growth, partial enterectomy and tube feeding. J. Anat. 111, 283-291.

Galjaard H., Meer-Flieggen W. van der and Both N.J. de (1972). Cell differentiation in gut epithelium. In: Cell differentiation. Viza D. and Harris H. (eds). Munksgaard. Copenhagen. pp 322-328.

Gallo-Payet N. and Hugon J.S. (1984). Insulin receptors in isolated adult mouse intestinal cells: studies in vivo and in organ culture. Endocrinology 114, 1885-1892.

Garty H. and Asher C. (1991). Does aldosterone induce de novo synthesis of Na^+ channels? In: Aldosterone: fundamental aspects. Bonvalet J.P., Farman N., Lombes M. and Rafestin-Oblin M.E. (eds). John Libbey Eurotext Ltd. INSERM, pp 273-283.

Goodland R.A., Lee C.Y., Gilbey S.G., Ghatei M.A. and Bloom S.R. (1993). Insulin and intestinal epithelial cell proliferation. Exp. Physiol. 78, 697-705.

Gundersen H.J.G., Bagger P., Bendtsen T.F., Evans S.M., Korbo L., Marcussen N., Møller A., Nielsen K., Nyengaard J.R., Pakkenberg B., Sørensen F.B., Vesterby A. and West M.J. (1988a). The new stereological tools: disector, fractionator and point-sampled intercepts and their use in pathological research. APMIS 96, 857-881.

Gundersen H.J.G., Bendtsen T.F., Korbo L., Marcussen N., Møller A., Nielsen K., Nyengaard J.R., Pakkenberg B., Sørensen F.B., Vesterby A. and West M.J. (1988b). Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 96, 397-394.

Heinz-Erian P., Kessler U., Funk B., Gais P. and Kiess W. (1991). Identification and in situ localisation of the insulin-like growth factor II/mannose-6-phosphate (IGF-II/M6P) receptor in the rat gastrointestinal tract: comparison with the IGF-I receptor. Endocrinology 129, 1769-1778.

Hoffman L.A., Yen S. and Chang E.B. (1992). Regional alterations in intestinal sucrase expression in STZ-treated chronically diabetic rats. Digest. Dis. Sci. 37, 1078-1083.

Hwang E.S., Hirayama B.A. and Wright E.M. (1991). Distribution of the SGLT1 Na^+/glucose contransporter and mRNA along the crypt-villus axis of rabbit small intestine. Biochem. Biophys. Res. Commun. 181, 1208-1217.

Jervis E.L. and Levin R.J. (1966). Anatomic adaptation of the alimentary tract of the rat to the hyperphagia of chronic alloxan-diabetes. Nature 210, 391-393.

Karasov W.H. and Debnam E.S. (1987). Rapid adaptation of intestinal glucose transport: a brush-border or basolateral phenomenon? Am. J. Physiol. 253, G54-G61.

Karasov W.H. and Diamond J.M. (1983). Adaptive regulation of sugar and amino acid transport by vertebrate intestine. Am. J. Physiol. 245, G443-G462.

Keegan D.J. (1977). Aspects of assimilation of sugars by *Rousettus aegypticus*. Comp. Biochem. Physiol. 58A, 349-352.

Keegan D.J. (1980). Lack of active glucose transport system in the bat, *Rousettus aegypticus*, intestine. S. Afr. J. Sci. 76, 570-571.

Keegan D.J. and Modinger R. (1979). Microvilli of the intestinal mucosal cells of the bat, *Rousettus aegypticus*. S. Afr. J. Zool. 14, 220-223.

Keelan M., Walker K. and Thompson A.B.R. (1985). Intestinal brush border membrane marker enzymes, lipid composition and villus morphology: effect of fasting and diabetes mellitus in rats. Comp. Biochem. Physiol. 82A, 83-89.

Laburthe M., Rouyer F. and Gammeltoft S. (1988). Receptors for insulin-like growth factors I and II in rat gastrointestinal epithelium. Am. J. Physiol. 245, G457-G462.

Lal D. and Schedl H.P. (1974). Intestinal adaptation in diabetes: amino acid absorption. Am. J. Physiol. 227, 827-831.

Leblond C.P. (1981). The life history of cells in renewing systems. Am. J. Anat. 160, 114-158.

Lorenz-Meyer H., Gottesburen H., Menge H., Bloch R. and Riecken E.O. (1974). Intestinal structure and function in relation to blood sugar levels and food intake in experimental diabetes. In: Intestinal adaptation. Dowling R.H. and Riecken E.O. (eds). Schattauer Verlag. Stuttgart. pp 189-191.

Makanya A.N., Mayhew T.M. and Maina J.N. (1995). Stereological methods for estimating the functional surfaces of the chiropteran small intestine. J. Anat. 187, 361-368.

Mayhew T.M. (1987). Quantitative ultrastructural study on the responses of microvilli along the small bowel to fasting. J. Anat. 154, 237-243.

Mayhew T.M. (1988). Geometric model for estimating villous surface areas in the rat small bowel is justified by unbiased estimates obtained using vertical sections. J. Anat. 161, 187-193.

Mayhew T.M. (1990). Striated brush border of intestinal absorptive epithelial cells: stereological studies on microvillous morphology in different adaptive states. J. Electron Microsc. Tech. 16, 45-55.

Mayhew T.M. (1991). The new stereological methods for interpreting functional morphology from slices of cells and organs. Exp. Physiol. 76, 639-665.

Mayhew T.M. (1992). A review of recent advances in stereology for quantifying neural structure. J. Neurocytol. 21, 313-328.

Mayhew T.M. and Carson F.L. (1989). Mechanisms of adaptation in rat small intestine: regional differences in quantitative morphology during normal growth and experimental hypertrophy. J. Anat. 164, 189-200.

Mayhew T.M. and Gundersen H.J.G. (1996). «If you assume, you can make an ass out of u and me»: a decade of the disector for stereological counting of particles in 3D space. J. Anat. 188, 1-15.

Mayhew T.M. and Middleton C. (1985). Crypts, villi and microvilli in the small intestine of the rat. A stereological study of their variability within and between animals. J. Anat. 141, 1-17.

Mayhew T.M., Carson F.L. and Sharma A.K. (1989). Small intestinal morphology in experimental diabetic rats: a stereological study on the effects of an aldose reductase inhibitor (ponalrestat) given with or without conventional insulin therapy. Diabetologia 32, 649-654.

Mayhew T.M., Dantzer V., Elbrønd V.S. and Skadhauge E. (1990). A sampling scheme intended for tandem measurements of sodium transport and microvillous surface area in the coprodaeal epithelium of hens on high- and low-salt diets. J. Anat. 173, 19-31.

Mayhew T.M., Elbrønd V.S., Dantzer V. and Skadhauge E. (1992a). Quantitative analysis of factors contributing to expansion of microvillous surface area in the coprodaeum of hens transferred to a low NaCl diet. J. Anat. 181, 73-77.

Mayhew T.M., Elbrønd V.S., Dantzer V., Skadhauge E. and Møller O. (1992b). Structural and enzymatic studies on the plasma membrane

domains and sodium pump enzymes of absorptive epithelial cells in the avian lower intestine. Cell Tissue Res. 270, 577-585.

McElligott T.F., Beck I.T., Dinda P.K. and Thompson S. (1975). Correlation of structural changes at different levels of the jejunal villus with positive and negative net water transport in vivo and in vitro. Can. J. Physiol. Pharmacol. 53, 439-450.

Meddings J.B., DeSouza D., Goel M. and Thiessen S. (1990). Glucose transport and microvillus membrane physical properties along the crypt-villus axis of the rabbit. J. Clin. Invest. 85, 1099-1107.

Miller D.L., Hanson W., Schedl H.P. and Osborne J.W. (1977). Proliferation rate and transit time of mucosal cells in the small intestine of the diabetic rat. Gastroenterology 73, 1326-1332.

Nakabou Y., Okita C., Takano Y. and Hagiira H. (1974). Hyperplastic and hypertrophic changes of the small intestine. J. Nutr. Sci. Vitaminol 20, 227-234.

Nakabou Y., Ishikawa Y., Misaki A. and Hagihira H. (1980). Effect of food intake on intestinal absorption and hydrolases in alloxan-diabetic rats. Metabolism 29, 181-185.

Nordstrom C., Dahlqvist A. and Josefsson L. (1968). Quantitative determination of enzymes in different parts of the villi and crypts of rat small intestine. J. Histochem. Cytochem. 15, 713-721.

Olsen W.A. and Rosenberg I.H. (1970). Intestinal transport of sugars and amino acids in diabetic rats. J. Clin. Invest. 49, 96-105.

Olsen W., Agresti H.L. and Lorentzsonn V.W. (1974). Intestinal disaccharidases in diabetic rats. In: Intestinal adaptation. Dowling R.H. and Riecken E.O. (eds). Schattauer Verlag. Stuttgart. pp 179-187.

Park J.H., Vanderhoof J.A., Blackwood D. and MacDonald R.G. (1990). Characterisation of type I and type II insulin-like growth factor receptors in an intestinal epithelial cell line. Endocrinology 126, 2998-3005.

Pothier P. and Hugon J.S. (1980). Characterization of isolated villus and crypt cells from the small intestine of the adult mouse. Cell Tissue Res. 211, 405-418.

Potten C.S. (1980). Stem cells in small-intestinal crypts. In: Cell proliferation in the gastrointestinal tract. Appleton D.R., Sunter J.P. and Watson A.J. (eds). Pitman Medical. London. pp 141-154.

Potten C.S., Booth C., Chadwick C.A. and Evans G.S. (1994). A potent stimulator of small intestinal cell proliferation extracted by simple diffusion from intact irradiated intestine: in vitro studies. Growth Factors 10, 53-61.

Ross G.A. and Mayhew T.M. (1985). Effects of fasting on mucosal dimensions in the duodenum, jejunum and ileum of the rat. J. Anat. 142, 191-200.

Schedl H.P. and Wilson H.D. (1971). Effects of diabetes on intestinal growth and hexose transport in the rat. Am. J. Physiol. 220, 1739-1745.

Silverman M. (1991). Structure and function of hexose transporters. Annu. Rev. Biochem. 60, 757-794.

Skadhauge E. (1989). Hormonal regulation of sodium absorption and chloride secretion across the lower intestine of birds. Zool. Sci. 6, 437-444.

Skadhauge E., Thomas D.H., Chadwick A. and Jallageas M. (1983). Time course of adaptation to low and high NaCl diets in the domestic fowl: effects on electrolyte excretion and on plasma hormone levels (aldosterone, corticosterone and prolactin). Pflugers Archiv. 396, 301-307.

Smith M.W. and Brown D. (1989). Dual control over microvillus elongation during enterocyte development. Comp. Biochem. Physiol. 93A, 623-628.

Smith M.W., Turvey A. and Freeman T.C. (1992). Appearance of phloridzin-sensitive glucose transport is not controlled at mRNA level in rabbit jejunal enterocyte. Exp. Physiol. 77, 525-528.

Smith P.R., Saccomani G., Bradford A.L., Dantzer V., Benos D.J. and Skadhauge E. (1993). Immunochemical identification of amiloride-sensitive sodium channels from an intestinal epithelium. Cell Tisuue Res. 272, 129-136.

Stenling R., Hagg E. and Falkmer S. (1984). Stereological studies on the rat small intestinal epithelium. III. Effects of short-term alloxan diabetes. Virchows Arch. (B) 47, 263-270.

Stenling R. and Helander H.F. (1981). Stereologic studies on the small intestinal epithelium of the rat. 1. The absorptive cells of the normal duodenum and jejunum. Cell Tissue Res. 217, 11-21.

Thomas D.H. and Skadhauge E. (1982). Time course of adaptation to low and high NaCl diets in the domestic fowl. Effects of electrical behaviour of isolated epithelia from the lower intestine. Pflugers Archiv. 395, 165-170.

Thorens B., Cheng Z-Q., Brown D. and Lodish H.F. (1990). Liver glucose transporter: a basolateral protein in hepatocytes and intestine and kidney cells. Am. J. Physiol. 259, C279-C285.

Tousson A., Alley C.D., Sorcher E.J., Brinkley B.R. and Benos D.J. (1989). Immunochemical localization of amiloride-sensitive sodium channels in sodium-transporting epithelia. J. Cell Sci. 93, 349-362.

Warren M.A. (1991). Adaptations of the rat small intestine to a single and a double period of undernutrition. J. Anat. 176, 89-97.

Williams M. and Mayhew T.M. (1992). Responses of enterocyte microvilli in experimental diabetes to insulin and an aldose reductase inhibitor (ponalrestat). Virchows Arch. (B) 62, 385-389.

Wright E.M. (1993). The intestinal Na^+/glucose cotransporter. Annu. Rev. Physiol. 55, 575-589.

Wright N. (1980). Cell proliferation in the normal gastrointestinal tract. Implications for proliferative responses. In: Cell proliferation in the gastrointestinal tract. Appleton D.R., Sunter J.P. and Watson A.J. (eds). Pitman Medical. London. pp 3-25.

Yoshida A., Takata K., Kasahara T., Aoyagi T., Saito S. and Hirano H. (1995). Immunohistochemical localization of Na^+-dependent glucose transporter in the rat digestive tract. Histochem. J. 27, 420-426.

Younoszai M.K., Parekh V.V. and Hoffman J.L. (1993). Polyamines and intestinal epithelial hyperplasia in streptozotocin-diabetic rats. Proc. Soc. Exp. Biol. Med. 202, 206-211.

Young G.P., Morton C.L., Rose I.S., Taranto T.M. and Bhathal P.S. (1987). Effects of intestinal adaptation on insulin binding to villus cell membranes. Gut 28, 57-62.

Zoubi S.A., Mayhew T.M. and Sparrow R.A. (1994). Crypt and villous epithelial cells in adult rat small intestine: numerical and volumetric variation along longitudinal and vertical axes. Epith. Cell Biol. 3, 112-118.

Zoubi S.A., Mayhew T.M. and Sparrow R.A. (1995a). The small intestine in experimental diabetes: cellular adaptation in crypts and villi at different longitudinal sites. Virchows Arch. 426, 501-507.

Zoubi S.A., Williams M.D., Mayhew T.M. and Sparrow R.A. (1995b). Number and ultrastructure of epithelial cells in crypts and villi along the streptozotocin-diabetic small intestine: a quantitative study on the effects of insulin and aldose reductase inhibition. Virchows Arch. 427, 187-193.