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Changes in intestinal cell proliferation, absorptive capacity and structure in young, adult and old rats

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INTRODUCTION

The influence of development and age on the structure and function of the gastrointestinal tract is of considerable interest and importance, especially in light of the 'greying' of the western population (Holt, 1986; Schneider, 1988). Age-related changes in absorption could have considerable importance for the uptake of drugs and nutrients (Annesley, 1989; Armbrecht, Doubek & Porter, 1988) and age-related changes in proliferation could help explain why cancer risk increases with the fourth power of age (Franks & Teich, 1986).

Intestinal function is determined by both enterocyte maturity and the number of functional cells (villus cell population) which, in turn, is the balance between villus cell influx and cell loss. Cell influx into the villus is the product of crypt cell production and the number of crypts feeding cells into the villus (Goodlad & Wright, 1982; Goodlad, 1989). All of these parameters may alter with both development and age but, as is often the case, conflicting data have been published in the literature.

Crypt cell production rates alter considerably in the young animal, with the most pronounced changes being seen at weaning (Al-Nafussi & Wright, 1982). Some workers have reported reduced proliferation in old animals (Lesher, Fry & Kohn, 1961; Fry, Lesher & Kohn, 1962), whilst other groups have found no change in cell production with age (Ecknauer, Vadakel & Wepler, 1982). Increased proliferation of cells in aged animals has also been described (Holt & Yeh, 1988, 1989; Holt, Yeh & Kotler, 1988; Thompson *et al.* 1988).

The number of villi may reach a maximum value in the young adult rat (Clarke, 1972) and then decreases slightly, while the number of crypts increases. This has been associated with a large increase in crypt/villus ratio which occurs at weaning (St Clair & Osborne, 1985) as a consequence of increased crypt fission. Crypt fission is, nevertheless, still seen in adults (Cheng & Bjerknes, 1986), which could explain increased crypt/villus ratios reported in old age (Altman & Enesco, 1967).

The effect of age on the villi has also produced conflicting results, Villus size may increase with age (Meshkinpour, Smith & Hollander, 1981; Holt, Pascal & Kotler, 1984) or may not (Corazza, Frazzoni, Gatto & Gasbarrini, 1986; Clarke, 1977) and reports of decreased digestive function have been published (Bowman & Rosenberg, 1983; Navab & Winter, 1988; Holt, Tierney & Kotler, 1985). Some of this discord could be attributed to regional differences within the small intestine, but there is some evidence for the various regions of the small intestine behaving in similar manner, at least during development (Mayhew & Carson, 1989).

We have recently demonstrated that intestinal absorption and crypt cell production rates can be measured concurrently (Goodlad, Plumb & Wright, 1987, 1988). In this paper we describe the application of these techniques to an investigation of the effects of development and age on the rat gut.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats 3 to 121 weeks old were fed a standard pelleted diet *ad libitum* (Labshure PRD, Poole, Dorset). They were kept in wire-bottomed cages, 4 to a cage, and water was available *ad libitum*.

Methods

All rats were injected with vincristine sulphate (1 mg kg^{-1} intraperitoneally; Tillots Laboratories, Henlow, Bedfordshire, UK) at 09.00 hours, anaesthetised at various times after injection and the intestine prepared for the study of water absorption. Rats were only killed after the luminally perfused (and nourished) segment was placed in the gut bath. The remaining sections of intestine and the colon were removed, measured and samples of the proximal and distal small intestine and mid-colon fixed. While the first rat gut was being perfused, the second rat was prepared for the second gut bath. Each gut was perfused for 25–30 minutes, after which the perfused portion was measured and then dried at 60 °C for 2 days.

Crypt cell production rate

Intestine samples were fixed in Carnoy's fluid and stored in 70% (v/v) ethanol. They were later stained with the Feulgen reaction and the crypts displayed by microdissection. The number of arrested metaphases in 10 intestinal crypts or 20 colonic crypts was counted and the mean values plotted against time since injection. The slope of the line was then fitted by linear regression to give the crypt cell production rate (CCPR) and its standard error (Goodlad & Wright, 1982).

Morphometry

The serosa and muscle layers were carefully dissected away from the ileal mucosa of Feulgen reaction-stained tissue and the number of crypt necks or villus bases seen in five eyepiece ocular grids per animal recorded (each grid was 0·2304 mm^2). Differential focus was utilised so that only the crypt diameters were visible when the crypt number was quantified and only the villus bases and intervillous regions were visible when villus number was scored. The resultant measure of crypt or villi per unit per area would thus reflect the inverse crypt or villus base area. Crypt depth and villus length were measured in several visual fields of well-orientated haematoxylin and eosin stained sections.

WATER ABSORPTION

The rat was anaesthetised with ether and canulae inserted into the small intestine just below the ligament of Treitz and 5 cm above the ileocaecal valve. The intestine was then rinsed with warm, gassed saline and perfused with a segmented flow of perfusion medium and warm, moist gas. The medium was a modified Krebs–Henseleit bicarbonate solution (pH 7·4) equilibrated with the gas mix and containing 28 mmol/l of glucose and 141 nmol/ml of phenol red. The gas mix was 95% O_2 and 5% CO_2. A modified Starling resistance was fitted to the outflow to inflate the intestine to a

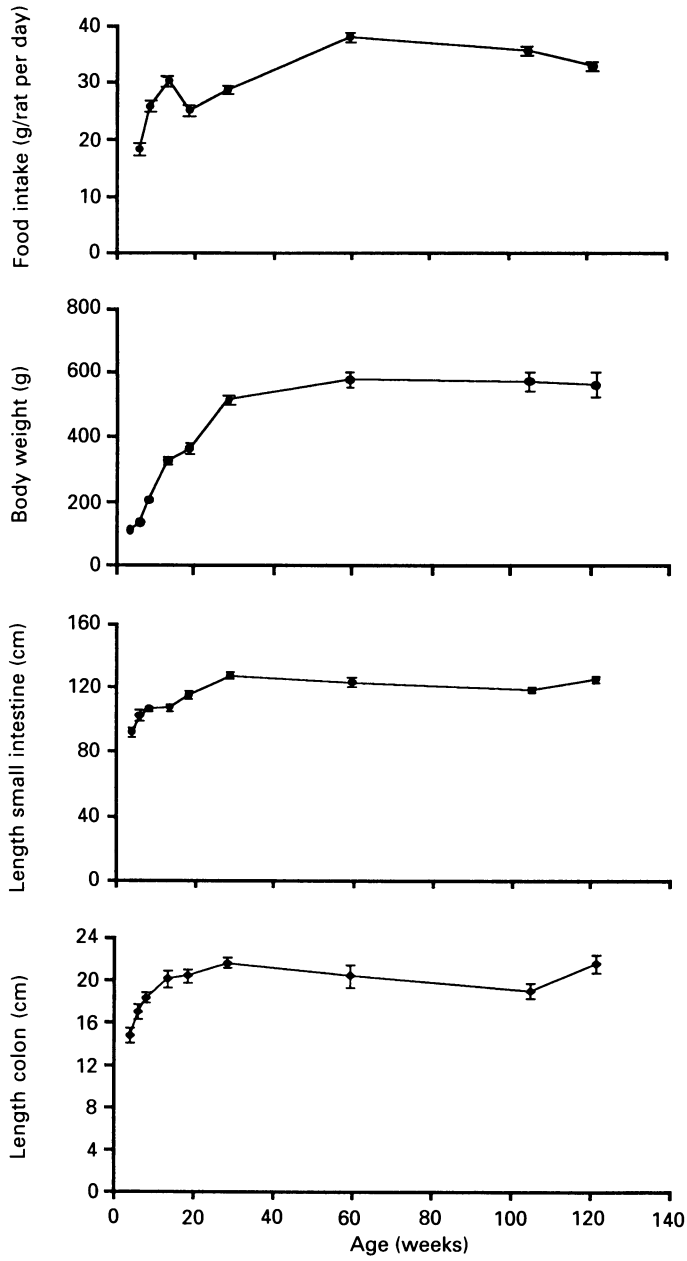

Fig. 1. The effects of rat age on food intake, body weight and intestinal lengths.

pressure of 40 mmHg. When the perfusion medium had reached the distal cannula the mesentery was carefully stripped off and the segment suspended in a gut bath. Warm moist gas was blown into the bath to keep the serosal surface moist and to push out the absorbed fluid, which was then collected over 5 minute periods by a fraction collector. The first fraction was discarded and the next three fractions were weighed to calculate mean water absorption.

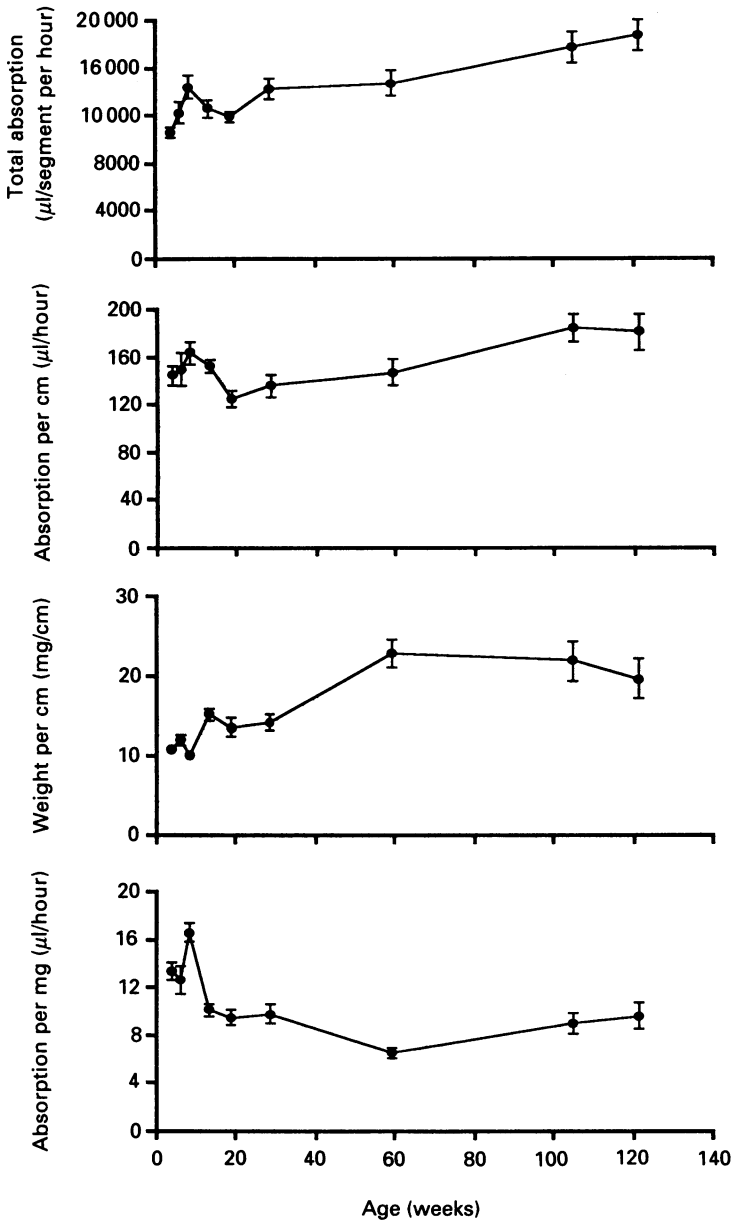

Fig. 2. Total absorption, absorption per cm, weight per cm gut and absorption per mg gut.

STATISTICS

Results are presented as the mean ± standard error of the mean. Metaphase arrest lines were fitted by least squares linear regression.

RESULTS

Food intake, body weight and intestinal and colonic length all increased with age, reaching a plateau at around 30 weeks (Fig. 1). Total absorption increased rapidly at

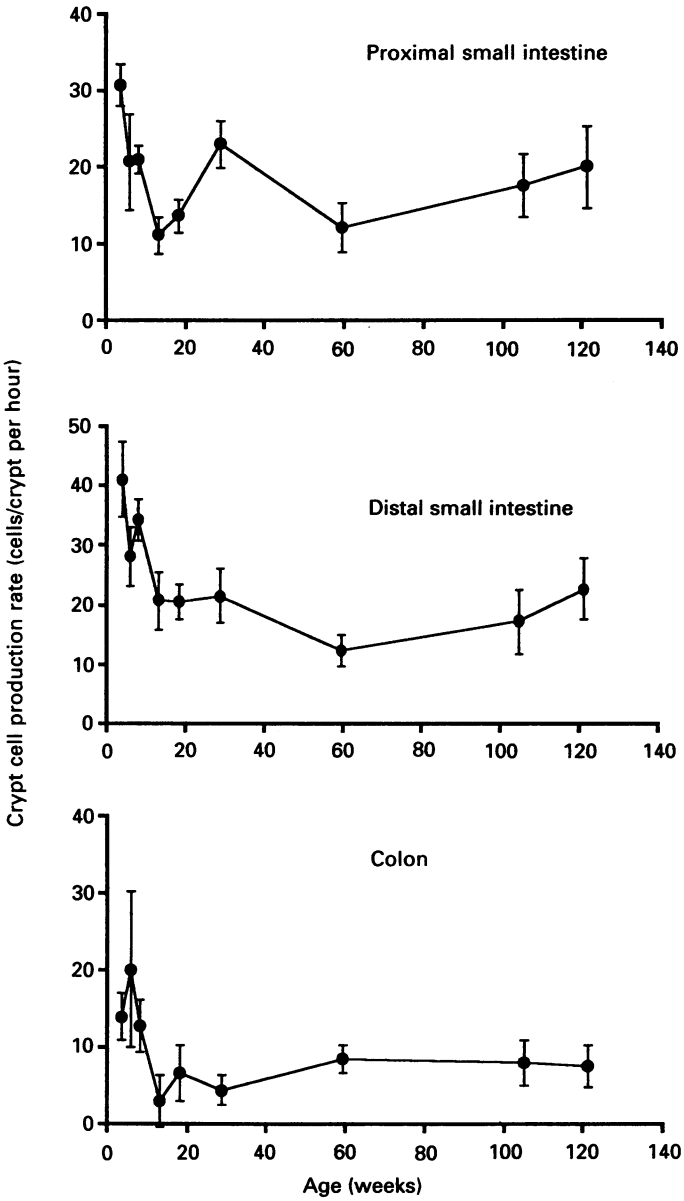

Fig. 3. Intestinal crypt cell production rates in rats of different ages.

first in the young rat, and then more gradually (Fig. 2). The absorption per centimetre of gut showed a similar initial peak, which fell away at 20 weeks, and then rose steadily. There was a marked increase in the weight per cm gut in the older rats, thus the absorption per mg gut decreased considerably after 8 weeks but then remained relatively constant (Fig. 2).

A similar pattern of high initial rates falling after about 8 weeks was seen for the crypt cell production rates, which was most pronounced in the distal small intestine and colon (Fig. 3).

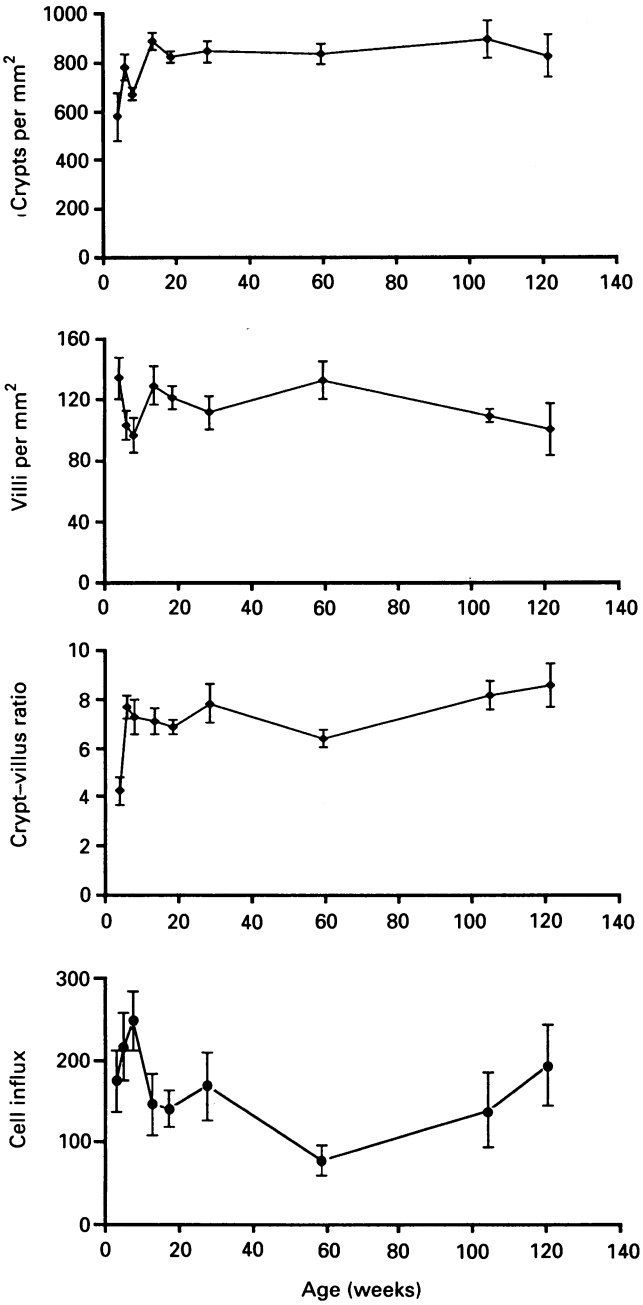

Fig. 4. The number of crypts or villi per area of mucosa and the crypt–villus ratio in the terminal ileum of rats of various ages. Also shown is the net cell influx from the proliferative compartment to the villi (product of the crypt–villus ratio and the crypt cell production rate).

The number of crypts per unit area increased rapidly with age in the first 13 weeks, indicating that the crypts were getting smaller, whilst the number of villi per unit area decreased from 3 to 8 weeks, and then increased at 12 weeks; the pattern in older rats was less clear, but the villi may have been getting larger with old age.

The changes in crypts per unit area was accompanied by the increase in number of

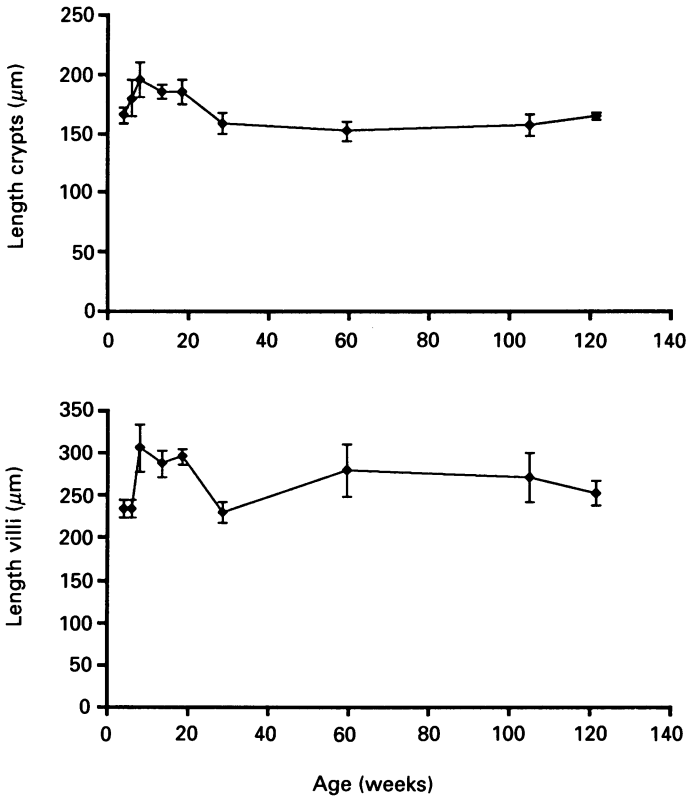

Fig. 5. The variations of crypt and villus length with age in the rat terminal ileum.

crypts associated with each villus (crypt–villus ratio), with the crypt–villus ratio appearing to increase more rapidly (Fig. 4). The product of the crypt–villus ratio and the crypt cell production rate is the net cell influx from the proliferative compartment to the villi. The lowest section of Figure 4 shows that cell influx increased in the young animals, reaching peak values at 8 weeks, and then fell to a relatively constant value which was maintained for Weeks 13, 18 and 28. The value at week 59 seemed lower, and the influx then appeared to increase in the oldest groups. Crypt and villus length also increased in the first 8 weeks, then decreased slightly at 28 weeks before reaching a plateau (Fig. 5).

DISCUSSION

The changes in all parameters measured were most pronounced in the young rats. Crypt cell production was highest in the young rats which had fewer crypts per unit area and a low crypt–villus ratio. Consequently the cell influx into the villous compartment was lower in the youngest age groups, despite CCPR being elevated. It can thus be concluded that a considerable amount of proliferative effort in the young animals is being devoted to cryptogenesis rather than influx to the villous compartment. Less change was seen in the number of villi per unit area. Adult rats had more crypts per unit area (indicating smaller crypt circumferences) and had almost twice as many crypts feeding each villus, but with a lower cell production per crypt. While the crypt or villus number per unit area is not an ideal measure of base area,

it can give a useful approximation, especially as the use of selective focus enables one to study different zones of the mucosa. No villus bases are seen when the crypts are studied; thus the method is probably better for the quantification of crypt diameter than for the villus base, especially as intervillous regions are visible. The method also has the advantage that a considerable number of units can be included in the estimation.

The increase in crypt–villus ratio between Weeks 3 and 6 was very rapid, and was probably related to weaning, as reported by St Clair & Osborne (1985). The crypt–villus ratio is usually fairly constant in most models of intestinal adaptation (Wright & Alison, 1984), so that crypt cell production equates to villus influx; however, in the young rats this is obviously not the case, as a considerable amount of the cell proliferation is diverted to cryptogenesis. There are several advantages associated with the CCPR method. Firstly it is a rate method, quantifying the rate of entry of cells into mitosis and, as such, and unlike state methods, cannot be confounded by changes in the duration of the phase of the cell cycle being studied. The second main advantage of the technique is that the denominator (the crypt) is usually a relatively stable unit. There are several cases in the literature where authors have reported no differences in proliferative indices (dividing cells per 100 non-dividing cells) between experimental groups without taking account of the fact that the crypt length (in cells) had also increased; thus, the number of dividing cells per crypt, and consequently the efflux of cells from the crypt, was increased significantly. These problems are avoided when the CCPR method is applied to microdissected crypts; however, the large amount of crypt fission in very young rats means that this method should only be used with caution, unless the crypt–villus ratio is also measured.

The initial peak in absorption was accompanied by an increase in villus size. Cell production and absorption per mg then decreased but, as the animals were still getting heavier, the intestines longer and the weight per cm tissue was also increasing, the total absorption actually increased. The intestine appeared to be less efficient than in the young rats. The increase in food intake more or less parallelled the increase in total absorption and preceded the increase in body weight.

The young gut was, therefore, better able to cope with the demands of the rapid, post-weaning growth. The correlation between food intake and absorption was similar to that observed in a variety of models of intestinal adaptation (Goodlad *et al.* 1987) and confirms the importance of food intake in determining intestinal structure and function. Whether this influence is direct (luminal nutrition) or indirect (moderated via systemic factors) remains to be fully resolved (Wright & Alison, 1984).

While there may have been a slight increase in proliferation in the very old rats, there was no evidence of the marked increases in proliferation suggested by some workers. Intestinal development in the young is presumably the combination of pre-programmed changes with interactions to the dietary and hormonal changes associated with weaning. The question as to whether similar combinations of factors exist in the aged has still to be resolved.

Although it has been said before (Goodlad & Wright, 1982; Wright & Alison, 1984), the investigation of intestinal epithelial cell proliferation and adaptation has often been bedevilled by the use of inappropriate methods. The three-dimensional nature of the intestine means that morphological measures such as villus height or crypt depth, which have been used extensively in previous studies on the effects of ageing, should be used with caution (Clarke, 1973; Hasan & Ferguson, 1981) and need to be supported by other measures. In the present study we have used the absorption assay of Fisher & Gardner (1949) to measure intestinal function, a method that gives a

wholistic measure of total gut function, and circumvents many of the problems associated with poor diffusion through the unstirred layer (Lerner & Miller, 1982) and also avoids those problems caused by warm ischaemia. The absorptive ability of the gut is more than halved by a few minutes intestinal anoxia and severe structural damage to the villi is often seen in everted sac preparations after only 20 minutes perfusion (Plumb,. Burston, Baker & Gardner, 1977).

In studies on the ageing process it is very important to distinguish between the effects of age itself and those of other changes which may be associated with old age. One of the most likely of such confounding factors is the decrease in food intake often found in old age, and food intake is one of the most important determinants of intestinal proliferation and absorption (Goodlad *et al.* 1987).

Although several changes associated with decreased intestinal efficiency in the old have been reported, none of these are unequivocal. Much of this confusion can be attributed to experimental differences, especially the age of the aged group or groups. The variations in the parameters in the adult groups in the present study demonstrate the importance of making the correct choice of the reference point for the 'control' adult values and determining that this reference point is stable.

SUMMARY

Intestinal epithelial morphology, crypt cell production and absorptive function were investigated in 9 groups of rats, aged from 3 to 121 weeks. Absorption per unit length of intestine peaked in the first weeks of life and this was associated with increased villus size, crypt length and absorption per mg tissue weight. Absorption per gut continued to increase throughout life, which could be attributed to a similar increase in intestinal length and weight per cm, despite a decreased absorption per mg in the adult animals. Crypt–villus ratio increased rapidly between 6 and 8 weeks, but then appeared to reach a plateau. Crypt cell production started off at high rate in all sites and then decreased in the young adult. While there was some evidence for a small, non-significant, increase in proliferation in the very old rats, there was no evidence of the marked elevation reported by some workers.

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