Differences in intestinal protein synthesis and cellular proliferation in well-nourished rats consuming conventional laboratory diets

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1. Male Wistar rats (100 g) were given a commercial pellet feed or a semi-synthetic diet *ad lib.* Although the pellet-fed group grew slightly faster than the other group during the early part of the feeding period, there was no significant difference between the final weights of the groups.

2. The fractional rates of protein synthesis in jejunum, proximal ileum and liver were measured by a technique based on the determination of L-[4−^{3}H]phenylalanine incorporation over a short time period. Protein synthesis was higher in both jejunum and ileum of the pellet-fed rats compared with those eating the semi-synthetic diet, but there was no difference between the rates of protein synthesis measured in the livers of the groups.

3. The rate of mucosal cell division was significantly faster in the ileal mucosa of the pellet-fed group compared with the other group, and there were significant differences in some aspects of mucosal morphology.

4. The maintenance of higher rates of cell turnover and protein synthesis in animals given a commercial pellet feed is unexplained, but it may be related to the presence of non-absorbable polysaccharides or other complex plant materials in the pellet feed.

The mucosa of the small intestine is characterized by rapid cell proliferation and has a rate of protein synthesis which is very high in comparison with other tissues, accounting for as much as 14% of total body protein synthesis in the rat (McNurlan *et al.* 1979). It is also well established that the mucosa is very sensitive to reduced nutrient intake, showing reduced rates of cell proliferation (Brown *et al.* 1963) and a loss of mucosal mass, associated with a decline in protein synthesis (McNurlan *et al.* 1979). It seems probable that the high rate of protein synthesis in the mucosa is accounted for by rapid cell division, and that changes in one are linked to changes in the other. Alternatively the synthesis and replacement of protein in developing and mature cells, together with the production of mucus and other secretory proteins, might account for a large proportion of the protein synthesized in the gut.

It has become clear recently that even in well-nourished animals, mucosal growth is enhanced by the inclusion in the diet of vegetable materials rich in complex polysaccharides (Younosjai *et al.* 1978), and both cell proliferation and the morphology of the villi are influenced by non-starch polysaccharides (Ecknauer *et al.* 1981; Tasman-Jones *et al.* 1982). Differences in activities of mucosal glycosyl transferases have also been detected in animals receiving a commercial diet or an otherwise nutritionally similar semi-synthetic diet devoid of non-starch polysaccharides (Biol *et al.* 1984).

As the result of preliminary work which showed that rats, well-nourished on a semi-synthetic diet or a commercial pellet diet, had rates of mucosal cell division that differed, the present study was undertaken to determine whether such changes are associated with significant alterations in the rate of mucosal protein synthesis.

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Table 1. *Composition of the diets (g/kg diet)*

Semi-synthetic diet		Commercial pellet diet‡	
Casein	168	Protein	192
Sucrose	326	Free sugar	37
Starch	326	Starch	475
Maize oil	80	Fat	37
Cellulose	40	Cellulose	24
Minerals*	40	Non-cellulosic polysaccharide	75
Vitamin mix†	20	Total dietary fibre	99
		Moisture	92
		Ash	55

* Contained (g/kg diet): $CaHPO_4$ 13·00, $CaCO_3$ 8·20, KCl 7·03, Na_2HPO_4 7·40, $MgSO_4.H_2O$ 4·00, $MnSO_4.H_2O$ 0·18, $ZnCO_3$ 0·10, $FeSO_4.7H_2O$ 0·144, $CuSO_4$ 0·015, KIO_3 0·001.

† Contained (mg/kg diet): nicotinic acid 60, cyanocobalamin in mannitol 50, calcium-D-pantothenate 40, thiamin hydrochloride 10, riboflavin 10, pteroylmonoglutamic acid 5, D-biotin 1, menadione 1, Rovimix E-25 (Roche) 300, Rovimix A-500 (Roche) 25, Rovimix A-500/D3 (Roche) 15, choline bitartrate 1800.

‡ Commercially-pelleted stock cubes (CRM, Labsure); analysis performed in laboratory as follows: protein, Kjeldahl nitrogen × 6·25; free sugars, hot ethanol extract measured as sucrose (Roe, 1955); starch, hydrolysis with D-amyloglucosidase (*EC* 3.2.1.3), hot ethanol extract, measured as glucose (Roe, 1955); fat, extracted using dichloromethane–methanol (9:1, v/v); cellulose and non-cellulosic polysaccharide, (Englyst *et al.* 1982); moisture, freeze-dried; ash, dry-ashed in muffle furnace at 525° for 48 h.

MATERIALS AND METHODS

Animals

Male Wistar rats (approximately 110 g) were randomly divided into two groups of ten, and caged in pairs in a room at 21° having a 12 h light–12 h dark cycle. The first group received a commercial pellet diet (Labsure CRM; Christopher Hill Group, Poole, Dorset) and the second group a powdered semi-synthetic diet prepared in the laboratory. Dietary compositions are given in Table 1. Food and water were available *ad lib.*, food intakes were measured daily and body-weights were recorded twice weekly.

Fractional rates of protein synthesis

After 28 d the fractional rates of protein synthesis in small intestinal mucosa and liver were determined by the technique of Reeds *et al.* (1982), with minor modifications. The experiments were conducted between 10·00 and 12·30 hours.

Rats received an intraperitoneal injection of L-[4−^{3}H]phenylalanine solution in water (50 μCi/ml; 150 μmol/ml) and were killed by cervical dislocation at 5 and 15 min after injection. The abdominal cavity was opened and flushed with 75 ml ice-cold saline solution (9 g sodium chloride/l) to remove unabsorbed radioisotope. The liver was rapidly excised, rinsed with ice-cold saline, blotted dry, weighed and frozen in liquid nitrogen within 1 min of death. When deep-frozen, the whole liver was pulverized using a pestle and mortar pre-cooled with liquid N_2. The entire small intestine, also removed within 1 min of death, was flushed with ice-cold saline to remove food debris and extended on the bench with minimal stretching. The duodenum and distal ileum were discarded by removal of the first one-twelfth and final three-twelfths of the length of the small intestine; the jejunum and proximal ileum were obtained by equal division of the remainder. Both lengths of intestine were slit open and the mucosa was scraped from the underlying muscle layer with microscope slides on a cooled glass plate.

Tissue samples were placed in pre-cooled homogenizing tubes, weighed and acidified with cold perchloric acid (40 g/l; 6·0 ml/0·5 g tissue) and centrifuged. The supernatant fraction was neutralized with potassium hydroxide (200 g/l) and the pellets were washed three times with $HClO_4$ (40 g/l; 10 ml), resuspended in sodium hydroxide (0·3 M; 8 ml) and incubated at 37° for 1 h. The protein was re-precipitated with $HClO_4$ (200 g/l; 2 ml), collected by centrifugation and washed with $HClO_4$ (40 g/l; 5 ml) to give a protein pellet which was then hydrolysed in hydrochloric acid (6 M; 5 ml) for 24 h at 110° and dried in a vortex evaporator. The dry residue was washed four times with water (1 ml) and resuspended in sodium citrate (0·5 M; 3 ml; pH 6·3).

The specific activity of free phenylalanine in the supernatant fraction and the protein hydrolysate was measured after the enzymic conversion of phenylalanine to β-phenythylamine (Garlick *et al.* 1980). The activity of β-[^{3}H]phenythylamine was measured by liquid scintillation counting using Scintran Cocktail T (BDH Ltd, Poole, Dorset) in a Philips PW4700 liquid-scintillation spectrometer.

Protein synthesis (K_s; %/d) was calculated from:

$$K_s = \frac{S_b \times 100 \times 1440}{S_a \times t},$$

where t is the incorporation time (15 min) and S_b is the specific radioactivity of phenylalanine in tissue protein (McNurlan *et al.* 1979). S_a, the mean specific activity of the free phenylalanine between 0 and t, was estimated from the values obtained for groups of five animals killed at 5 and 15 min. K_s was determined for individual animals in the 15-min group by multiplying each free amino acid specific activity at 15 min (S_{a15}) by the value S_a/S_{a15}, obtained from the group mean (Garlick *et al.* 1980). The time interval used in the calculation accounted for the period between killing the animal and cooling the tissue.

Mucosal morphology and cell production rate

The rate of production of mucosal epithelial cells was estimated by a modification of the metaphase-arrest technique (Wimber & Lamerton, 1963). Estimates of villous height and the ratio, crypt:villus were obtained from the same tissue samples (Clarke, 1970). Two groups of ten animals were maintained on the experimental diets, as described, for 28 d. On successive mornings the animals in each group were given an intra-peritoneal injection of vincristine sulphate (Sigma, London) in distilled water (1 mg/kg body-weight), and were killed at successive 12-min intervals. The entire small intestine was removed, flushed with saline, everted and extended on the bench with minimal stretching.

The proximal twelfth (duodenum) of the total length was discarded, and the next 100 mm of jejunum removed to fixative (ethanol–acetic acid; 75:25, v/v). A 100 mm sample of proximal ileum was obtained from the mid-intestine by cutting 50 mm either side of the mid-point.

Sub-samples of the fixed intestine (5–10 mm) were passed through ethanol–water (50:50, v/v; 10 min) and distilled water (10 min) and stained in bulk by the Feulgen reaction. Subsequent manipulations were carried out in acetic acid (45:55, v/v). The total number of blocked metaphases was counted in micro-dissected crypts (ten per site) which were gently squashed beneath a coverslip and examined under the compound microscope. The cell birth-rate per crypt was determined by plotting the mean number of blocked metaphases *v.* time after injection, and estimating the slope by linear regression. One animal in each group was excluded from the analysis because of the presence of anaphase nuclei in the crypts, indicating incomplete metaphase arrest.

Estimates of the number of crypts and villi per mm^2 serosal surface were obtained by means of a dissecting microscope fitted with a calibrated eyepiece. An estimate of villous height was obtained from the mean for ten micro-dissected villi.

Table 2. *Body-weight, food intake, length of small intestine and mucosal weight for rats fed on the commercial pellet or semi-synthetic diet for 28 d*

(Values are means with their standard errors for ten rats)

Dietary group†...	Commercial pellet		Semi-synthetic	
	Mean	SE	Mean	SE
Final body-wt (g)	297	5	279	9
Body-wt gain (g)	189	4	172	9
Average food intake (g)	621	14	545**	17
Length of small intestine (mm)	111	2	100***	2
Fresh wt of scraped mucosa:				
Jejunum (g)	1·52	0·07	1·29*	0·06
Wt/unit length (mg/mm)	4·09	0·13	3·86	0·16
Proximal ileum (g)	1·60	0·05	1·32***	0·05
Wt/unit length (mg/mm)	4·32	0·10	3·97	0·14

Mean values were significantly different: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details, see Table 1 and p. 88.

Statistical methods

The rate of cell division in the mucosa (cell divisions per crypt per h) was estimated by deriving the slope of blocked metaphases *v.* time from injection to death, by the method of least squares. The standard error for the estimate of the slope, and the significance of differences between slopes were calculated in the conventional way. The significance of differences between means was estimated by Student's *t* test for paired or unpaired comparisons.

RESULTS

Growth and food intake

The mean values for body-weight gain and food intake over the 28 d feeding period are shown in Table 2. The rats fed on the semi-synthetic diet consumed an average of 12% less food over this period than the commercial-pellet-fed rats and also gained weight at a slightly slower rate, although this was only significant during the early part of the feeding period (Fig. 1).

Protein synthesis in liver and intestinal mucosa

The small intestines of rats fed on a semi-synthetic diet were significantly shorter than those from the commercial-pellet-fed group and, therefore, total fresh mucosal weight was greater in both jejunum and ileum of pellet-fed animals (Table 3). However, the ratio, small intestinal length:body-weight and mucosal weight per unit length of small intestine were not significantly different between the groups. The livers of rats fed on a semi-synthetic diet were also significantly smaller than those of pellet-fed rats (12·4 (SE 0·7) and 14·7 (SE 0·5) g fresh weight respectively; $P < 0·02$).

The specific radioactivity of free phenylalanine at intervals after injection is shown in Table 4. The specific activity of the free amino acid decreased slightly between 5 and 15 min in all tissues except liver from pellet-fed rats where the values were similar at both time intervals. Values of S_a were calculated on the basis of a linear change with time in the free phenylalanine specific activity, as described by Garlick *et al.* (1980), rather than by the

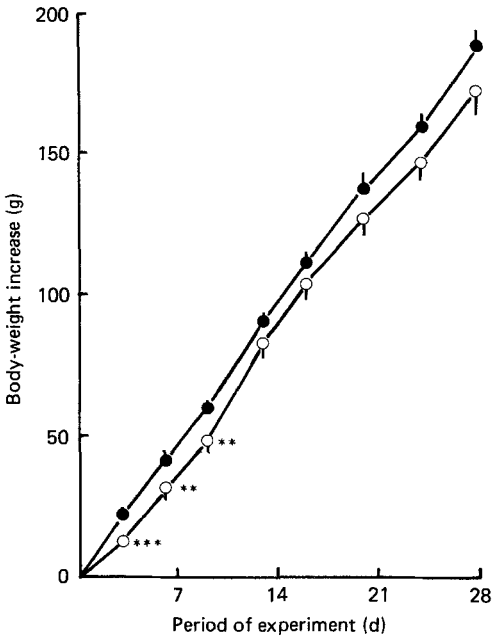

Fig. 1. Increase in body-weight over 28 d for rats fed on a commercial pellet diet (●—●) or a semi-synthetic powdered diet (○—○). Values are means with their standard errors represented by vertical bars. Mean values were statistically significantly different: ** $P < 0.01$, *** $P < 0.001$.

Table 3. *Change with time of specific radioactivity (disintegrations/min per nmol) of free phenylalanine in liver and mucosal tissue from commercial pellet-fed or semi-synthetic-fed rats*

(Values are means with their standard errors for five rats)

Dietary group†...	Commercial pellet				Semi-synthetic			
Time after injection (min)...	5		15		5		15	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Jejunal mucosa	447·6	55·2	445·5	47·5	447·5	19·0	393·4	24·0
Ileal mucosa	453·1	25·6	423·4	20·5	461·9	33·4	407·9	15·8
Liver	381·0	24·5	393·7	26·9	414·5	11·4	403·9	31·7

† For details, see Table 1 and page 88.

method of Reeds *et al.* (1982) which took into account a small rise in specific activity occurring between 5 and 10 min after the injection. It is estimated that in the present study, values obtained by the two methods would differ by less than 3%.

Fractional rates of protein synthesis (%/d) in mucosal scrapes and liver from semi-synthetic-fed and commercial-pellet-fed rats are shown in Table 4. The rate in mucosa from both jejunum and proximal ileum of growing rats fed on the semi-synthetic diet for 28 d was significantly lower (by 22% and 16% respectively) when compared with mucosa from pellet-fed animals. The rate in liver, however, was similar in both groups.

Table 4. *Fractional rates of protein synthesis in liver and mucosal tissue from commercial-pellet-fed and semi-synthetic-fed rats*

(Values are means with their standard errors for five rats)

Dietary group†...	Fractional synthesis rate (%/d)			
	Commercial pellet		Semi-synthetic	
Tissue	Mean	SE	Mean	SE
Jejunal mucosa	105·4	8·4	81·6*	2·3
Ileal mucosa	101·6	5·7	85·2*	2·3
Liver	77·5	5·8	73·2	8·2

Mean values were significantly different: * $P < 0.05$.
† For details, see Table 1 and p. 88.

Table 5. *Mucosal morphology and cell production rate in animals fed on the commercial pellet or semi-synthetic diet*

Dietary group‡...	Commercial pellet		Semi-synthetic	
	Mean	SE	Mean	SE
Villi (/mm²):				
Jejunum	8·2	0·5	8·0	0·3
Ileum	11·3††	0·3	13·4**†††	0·3
Villous height (μm):				
Jejunum	723	24	663*	13
Ileum	517†††	17	636***	11
Crypts (/mm²):				
Jejunum	230	9	247	6
Ileum	198†	5	239***	5
Crypt:villus:				
Jejunum	28·6	1·1	31·1	1
Ileum	17·6†††	0·5	18·0†††	0·3
Crypt-cell production rate (cells/crypt per h):				
Jejunum	22	4	14	2
Ileum	25	5	16*	3
Net villus influx (cells/villus per h):				
Jejunum	628	126	445	62
Ileum	442	96	285	55

Mean values for commercial-pellet-fed group were significantly different from those for semi-synthetic-fed group: * $P < 0.05$, ** $P < 0.02$, *** $P < 0.001$.
Mean values for jejunal site were significantly different from those for ileal site: † $P < 0.05$, †† $P < 0.002$, ††† $P < 0.001$.
‡ For details, see Table 1 and p. 88.

Mucosal morphology and cell production rate

The animals in both groups had leaf-shaped villi similar to those described by other workers (Clarke, 1970). In the commercial-pellet-fed animals, the villi were significantly taller at the jejunal site than in the proximal ileum, but there was no such difference in the other group (Table 5).

At both sites, the crypt cell production rate was lower in the animals fed on the semi-synthetic diet than in the commercial-pellet-fed group, although the difference was not statistically significant at the proximal site. The product of the crypt cell production rate and the ratio, crypt: villus, which gives the total number of new cells per villus per h, is also given in Table 5.

DISCUSSION

The mammalian small intestine undergoes structural and functional adaption in response both to frank malnutrition and to changes in dietary composition (Karasov & Diamond, 1983). When studying the effects of dietary change it is therefore important to ensure that animals are adequately nourished. In some studies rats given elemental or synthetic diets gained weight slowly by comparison with control animals given a commercially prepared, pelleted diet (Tasman-Jones *et al.* 1982; Sircar *et al.* 1983), but there is no evidence that the animals in the present study failed to thrive. Total food consumption was lower with the semi-synthetic diet but, over the 28 d feeding period, there was no apparent difference in the rate of growth for the two groups of animals, except during the first 9 d, when the semi-synthetic-fed animals were presumably adapting to the dietary change. We have no explanation for the decrease in food intake, but it is possible that rats find a powdered diet less palatable than the pellet feed.

The fractional rates of protein synthesis in mucosa from both jejunum and ileum of our commercial-pellet-fed animals were close to those reported by others using similar techniques (McNurlan *et al.* 1979; Garlick *et al.* 1980). However, the rates of protein synthesis at both the sites were significantly lower in rats fed on the semi-synthetic diet. No such differences in protein synthesis in the livers of the two groups were observed, which suggests that the dietary effect may be peculiar to the intestinal mucosa.

The technique employed by ourselves and by previous workers offers no means of subdividing the global estimate of the fractional rate of protein synthesis into components due to cell division, cellular protein turnover and protein secretion. McNurlan *et al.* (1979) observed reduced rates of protein synthesis in the jejunum in rats which had been starved for 2 d, and concluded that the effect could be accounted for largely by a reduction in mucosal cell turnover. There is no direct evidence for this however. In the present study we have observed a slower crypt cell production rate in the animals eating the semi-synthetic diet, and this suggests that slower mucosal cell turnover is at least partly responsible for the lower rate of mucosal protein synthesis.

The values for villous height and the ratio, crypt: villus in our pellet-fed rats were in good agreement with those reported by Clarke (1970) who also used Wistar rats fed on a commercial diet, and whose histological methods were similar to our own. In our semi-synthetic dietary group, the villi were significantly shorter than those of the pellet-fed group at the jejunal site, but taller at the ileal site. Thus the usual decline in villous height from jejunum to ileum was not apparent (Clarke, 1970; Kapadia & Baker, 1976). A significantly greater weight of fresh mucosa was obtained from both regions of intestine in the pellet-fed animals. However, in view of their slightly greater intestinal length this observation provides no clear-cut evidence for a difference in mucosal mass.

The mechanisms leading to altered mucosal morphology, lower rates of mucosal cell turnover and protein synthesis remain to be established. In a recent report Sircar *et al.* (1983) have shown that giving rats synthetic, liquid or solid diets leads to a marked reduction in DNA synthesis in gastric and colonic mucosa compared with that of rats fed on a commercial pellet feed, although no such effect was observed in jejunum. However, a reduction in cell renewal rate and in villous size in the jejunum of animals given an elemental

diet had previously been reported by the same group (Ecknauer *et al.* 1981). No clear-cut relation between dietary nutrient levels and mucosal growth was established by the latter studies, although it was shown that the addition of bulk in the form of cellulose did little to reverse the effects of the synthetic diets.

Of the many differences between the two diets used in the present study, perhaps the most important is the presence in the commercial pellet feed of a host of complex plant-cell constituents, including a substantial amount of non-cellulosic polysaccharide classed as dietary fibre. The addition of finely divided cellulose to fibre-free diets does not appear to stimulate mucosal cell division to any great extent (Ecknauer *et al.* 1981; I. T. Johnson and J. M. Gee, unpublished results). However, mucosal growth has been shown to be enhanced by the presence of vegetable components, rich in dietary fibre (Younosjai *et al.* 1978). We have no unequivocal evidence for a difference in mucosal growth, as defined by the criteria of Younosjai *et al.* (1978), but it seems likely that the absence of plant cell components is responsible for the lower protein synthesis of the semi-synthetic-diet group in this study. It is possible that the presence of larger particles of plant cell-wall material in the pellet diet may exert a direct mechanical stimulus to the mucosa, leading to increased cellular exfoliation. Alternatively, plant-cell constituents, or perhaps their breakdown products, may exert a biochemical stimulus to cell division.

The wider implications of the present results lie in their probable importance for some aspects of human nutrition. It has frequently been observed that an increase in the quantity of unavailable polysaccharides in the human diet leads to a significant rise in faecal N losses, and it is often suggested that this is derived from endogenous sources (McCance & Walsham, 1948; Southgate & Durnin, 1970; Walker, 1975). Bender & Mohammidiha (1981) have carried this view further and argued that the low apparent digestibility of legume proteins is due to endogenous losses associated with particularly high rates of cell turnover in animals fed on legumes. Fairweather-Tait *et al.* (1983) have confirmed that some increased cell loss occurs in rats fed on cooked kidney beans (*Phaseolus vulgaris*) added to a semi-synthetic diet but, in so far as the two studies are comparable, the effect appears to be similar in magnitude to the result obtained in the present study using the pellet diet.

Increased cell proliferation and loss in the small intestine may provide a source of endogenous N for faecal excretion, although much depends on the extent to which exfoliated mucosal cells are susceptible to rapid digestion and reabsorption. Further work is required to determine which dietary constituents are responsible for the faster cell division and protein synthesis seen in rats consuming the commercial pellet feed and, more generally, to discover to what extent endogenous proteins released in the small intestine are lost into the large bowel, there to become available for bacterial utilization and ultimate faecal excretion.

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