0014-4754/96/070664-07$1.50 + 0.20

The diet and gut microflora influence the distribution of enteroendocrine cells in the rat intestine

R. Sharma* and U. Schumacher

Human Morphology, University of Southampton, Southampton SO16 7PX (United Kingdom), Fax +44 1 703 594433
Received 2 November 1995; received after revision 25 January 1996; accepted 21 February 1996

Abstract. Several functions of the gut are locally influenced by peptides and biogenic amines released from enteroendocrine cells. The aim of the present study was to assess whether the luminal stimulus of diet or microbial flora or diet-microbial interactions have an influence on the distribution of enteroendocrine cells along the crypt-surface axes of the small and large intestine. The effects of diet and indigenous flora were investigated by comparing the numbers of argyrophil and serotonin immunoreactive cells in the jejunum and colon of germ free and conventional rats fed either a purified diet containing fine ingredients or a commercial diet containing crude fibre of cereal origin. The effects of human flora were analysed in germ-free rats inoculated with human faecal organisms. 1. Feeding the commercial diet reduced the number of argyrophil endocrine cells in the jejunum and serotonin immunoreactive cells in the colon of germ-free animals but increased the serotonin immunoreactive cells in the colon of conventional animals. 2. The rat flora increased the serotonin immunoreactive cells in the colon of animals fed a commercial diet and decreased in those fed a purified diet. 3. Inoculation of human flora increased the numbers of serotonin immunoreactive cells both in the jejunum and colon. The results provide evidence that the dietary changes and diet-microbial interactions can affect the regional number of enteroendocrine cells.
Key words. Jejunum; colon; diet-microflora interactions; gastroenteric peptides; serotonin; human flora.

Enteroendocrine cells are scattered in the epithelium of the mammalian gastrointestinal tract, and each individual cell type is capable of producing and secreting a specific biologically active peptide and/or amine[1,2]. Studies using immunohistochemical methods have identified at least 16 different subpopulations of enteroendocrine cells based on the nature of their stored peptides and biogenic amines and each maintains a distinctive distribution along the gastrointestinal tract from stomach to the anal canal[3]. Since most of the enteroendocrine cells in the intestinal tract are of 'open type'[4] and reach the gut lumen by apical processes, it has been suggested that these cells may respond to intraluminal stimuli such as pH and nutrients[3]. A few studies are available in which the in vivo release of gastroenteric peptides has been shown to be influenced by the ingestion of proteins and lipids[5,6] and by the presence of the conventional flora in the intestinal lumen[7]. Investigations using in vivo techniques, however, must be correlated with analysis of changes in gastroenteric peptides in situ.

Much of the research on the effects of luminal stimuli on intestinal mucosa has been concerned with the modification of the diet and bacterial flora and there is evidence that the diets containing fibre[8,9] and the presence of flora in the intestinal lumen[10,11] stimulate the cell turnover rates of the intestinal crypts and alter the composition of intestinal mucins. However, the precise mechanism by which these changes occur in the gastrointestinal mucosa is not known and whether the activity of enteroendocrine cells is influenced by the interactions between the diet and gut microflora in the intestinal lumen remains to be investigated.

Investigating the in situ changes in endocrine cell population in response to luminal stimuli has been limited by a lack of an appropriate model system. The use of germ-free rats fed different diets and harbouring either a conventional flora or a flora of human origin in our laboratory has provided means for characterising the changes in the synthesis and secretion of intestinal mucins[11]. Because the composition of the indigenous gut microflora is characteristic of each species, the validity of transposing data from animals to man is open to question. In an attempt to simulate the human condition more closely in rats we included a group of animals born germ-free and inoculated after weaning with a human microflora. The present study utilises this experimental model to evaluate the effects of diet and microflora on distribution of enteroendocrine cells along the gastrointestinal tract. Since enteroendocrine cells contain biogenic amines in addition to their peptides, we focused our investigation on quantification of

* Corresponding author.

Table 1. Composition of diets fed to rats.

Purified diet (P)[a]	
Component	concentration (g/kg)
Casein	250
Maize starch	380
Potato starch	100
Sucrose	50
Cellulose powder	80
Soya bean oil	60
Mineral mix	60
Vitamin mix	20
Commercial diet (C)[b]	
Cereal products (barley, maize, wheat and wheatfeed)	775
Extracted soya bean meal	107
Fish meal	98
Mineral and vitamin supplement	20

[a]Kindly provided by Dr Yoshio Saito, Calpis Intestinal Flora Laboratory, Kanagawa (Japan).
[b]Calculated crude fibre content 4.1%.

these cells by using the Grimelius argyrophilic method and immunohistochemistry using antiserum against serotonin. The Grimelius technique stains most endocrine cell types except the peptide YY cells and somatostatin and cholecystokinin producing cells[12]. Serotonin is not localised in all enteroendocrine cells and is present in non-peptide producing endocrine cells[13].

Materials and methods

Animals, association of intestinal microflora and diets. A total of twenty five male Lister hooded rats was used for the study. The germ-free animals (n = 10) were weaned on the appropriate sterile diet when 3 weeks old and maintained in plastic isolators. The conventional controls (n = 10) were born and maintained in the open laboratory. Another five germ-free rats were inoculated one week after weaning (i.e. at 4 weeks of age) with a suspension of human faecal organisms by oral intubation (HFA) which is the standard procedure for colonising rat gut with human flora[14]. They were also maintained in an isolator. All rats were killed at 9 weeks of age.

Two nutritionally adequate diets, which differed markedly in constituents and consequently in texture, were used (table 1). Five germ-free and five conventional animals were fed on a commercial rat diet (diet GR3; Special Diets Service, Witham, Essex); the fibre content of this diet was derived from coarsely milled cereal products. Five germ-free rats and five conventional rats were maintained on a diet prepared in the laboratory from purified ingredients which contained 8% powdered cellulose as the source of fibre (table 1). Both diets were sterilised by gamma-irradiation at 50 kGy. The five HFA rats received the purified diet; isolator space was inadequate to accommodate a similar group given the commercial diet.

Tissue preparation and staining procedure. Animals were killed at 9 weeks of age and samples from the mid-region of the small intestine (jejunum) and proximal large intestine (colon, 2 cm from the ileocecal junction) were fixed in 10% buffered formalin and embedded in paraffin wax. Serial 5 µm sections were stained with the Grimelius silver nitrate technique[15] for the study of argyrophilia of endocrine cells. For immunocytochemical demonstration of serotonin the sections were stained by the avidin-biotin-complex (ABC) procedure[16] using primary antiserum goat antiserotonin conjugated to bovine serum albumin (Incstar, Wokingham, Berkshire, UK). Sections were dewaxed and placed in 100% ethanol, and endogenous peroxidase was blocked using 1.6% hydrogen peroxide in methanol. After rehydration, sections were washed in 0.15 M Tris-buffered saline, pH 7.3 (PBS) and incubated for 20 min in 10% nonimmune rabbit serum. Incubation with the primary antiserum (1:1000) in PBS/0.3% Triton x-100 containing 1% normal rabbit serum was carried out overnight at 4 °C in a moist chamber. This was followed by a brief wash in PBS, and incubation for 30 min in the secondary antiserum (biotinylated rabbit anti-goat immunoglobulin, 1:200). After a further wash in PBS, sections were treated with ABC complex from Peroxidase standard PK-4000 (Vector Laboratories, Peterborough, UK) for 30–90 min, washed again in PBS, and then incubated in diaminobenzidine tetrahydrochloride (DAB) in Tris HCl buffer pH 7.3 with 0.001% hydrogen peroxide for 5–10 min. The controls included: 1. Use of nonimmune goat serum in place of the primary antiserum. 2. Pretreatment of antiserum with Serotonin/BSA conjugate. Sections were lightly counterstained with Harris haematoxylin, mounted in DPX and examined by light microscopy.

Quantitative and statistical methods. The expression of argyrophilic reaction and serotonin was correlated with the position of cells occupied along the crypt-to-surface epithelial axis. Therefore the crypts and villi of jejunum and colon were divided in three domains: the lower half of the crypt, the upper half of the crypt and the surface epithelial cells. The numbers of Grimelius-positive and serotonin-immunoreactive endocrine cells per unit length of mucosa (4 mm), delineated by an eyepiece graticule, were counted with an eyepiece of magnification × 10 and an objective lens of magnification × 20 in sections of the jejunum and colon from each of the animals. Cells were randomly selected from 10–15 visual fields from each rat showing the longitudinally oriented villi and crypts along the entire length of the mucosa. A similar method to express the number of enteroendocrine cells per unit length of mucosa has been used by previous authors[17,18]. The results are ex-

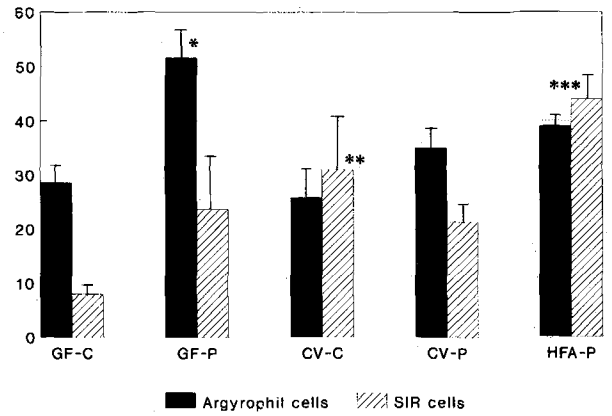

Figure 1. Effects of diet and microflora on the total number of enteroendocrine cells in the jejunum. Values are expressed as mean ± SE per experimental group. For abbreviations of experimental groups see 'Materials and methods'.
*Significantly different at $p < 0.02$ from the corresponding value for germ-free rats fed the commercial diet, **significantly different at $p < 0.05$ from corresponding value for germ-free rats fed the commercial diet, ***significantly different at $p < 0.01$ from the corresponding value for conventional rats fed the purified diet.

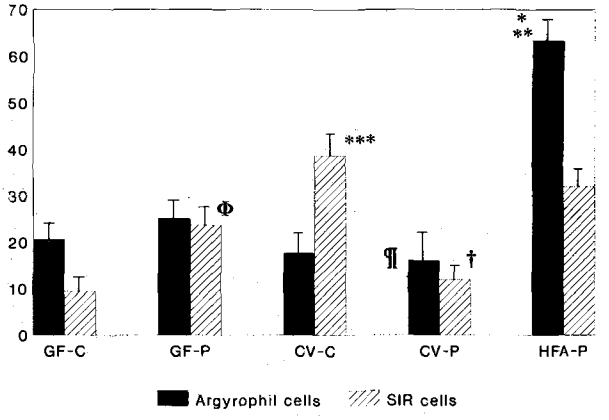

Figure 2. Effects of diet and microflora on the total number of enteroendocrine cells in the colon. Values are expressed as mean ± SE per experimental group. For abbreviations of experimental groups see 'Materials and methods'.
*Significantly different at $p < 0.005$ from the corresponding value for germ-free rats fed the purified diet, **significantly different at $p < 0.001$ from the corresponding value for conventional rats fed the purified diet, ***significantly different at $p < 0.001$ from the corresponding value for germ-free rats fed the commercial diet, Φsignificantly different at $p < 0.01$ from the corresponding value for germ-free rats fed the commercial diet, ¶at $p < 0.05$ from the corresponding value for conventional rats fed the commercial diet, †significantly different at $p < 0.05$ from the corresponding value for germ-free rats fed the purified diet, §significantly different at $p < 0.01$ from the corresponding value for conventional rats fed the purified diet.

pressed as mean ± standard error of the mean. Students t test for unpaired data was used to evaluate differences between groups. Differences were considered significant at the $p < 0.05$ level.

Results

The number of argyrophil endocrine and serotonin immunoreactive (SIR) cells in the surface epithelium, lower crypt, and upper crypt of the jejunum are shown in tables 2 and 4 and those of the colon in tables 3 and 5. Figures 1 and 2 show the total number of argyrophil and SIR cells in the jejunum and colon.

Effects of diet. The influence of diet was assessed by comparing the germ-free and conventional rats receiving either the commercial or the purified diet.

Argyrophil endocrine cells. Jejunum (table 2). The number of argyrophil endocrine cells in the surface epithelium of the jejunum was significantly less in the germ-free rats fed on the commercial diet when compared to those fed on the purified diet. In conventional rats, the number was significantly less in the lower crypt of the jejunum in animals fed the commercial diet as compared to those fed on the purified diet. There were numerous argyrophil endocrine cells in the lower and upper crypt as well as surface epithelium of jejunum in the conventional rats fed the purified diet (fig. 3). The highest total number of argyrophil endocrine cells was found in germ-free animals fed the purified diet (fig. 1).

Colon (table 3). The numbers of argyrophil endocrine cells in the colon of germ free and conventional rats fed on two different diets were similar.

Table 2. Effects of diet and intestinal microflora on number of argyrophil endocrine cells in the jejunum of the rat.

	Commercial diet		Purified diet		
	germ-free rats	conventional flora rats	germ-free rats	conventional flora rats	human flora rats
Surface	13.40 ± 1.9	5.80 ± 1.7[a]	29.00 ± 3.8[a,*]	6.80 ± 1.8	20.80 ± 2.3[d]
Upper crypt	11.8 ± 4.6	16.40 ± 2.6	17.60 ± 2.4	17.20 ± 1.7	18.20 ± 2.1
Lower crypt	3.40 ± 0.9	3.60 ± 2.2	5.00 ± 2.2	11.00 ± 0.8[b,c,e]	0.2 ± 0.2

Values are means ± SE. For feeding regimes and experimental groups see table 1 and 'Materials and methods'.
[a]Significantly different at $p < 0.01$ from corresponding value for germ-free rats fed a commercial diet.
[b]Significantly different at $p < 0.02$ from corresponding value for conventional rats fed a commercial diet.
[c]Significantly different at $p < 0.05$ from corresponding value for germ-free rats fed a purified diet.
[d]Significantly different at $p < 0.002$ from corresponding value for conventional rats fed a purified diet.
[e]Significantly different at $p < 0.002$ from corresponding value for rats inoculated with human faecal flora.
* Significantly different at $p < 0.003$ from corresponding value for conventional rats fed a purified diet.

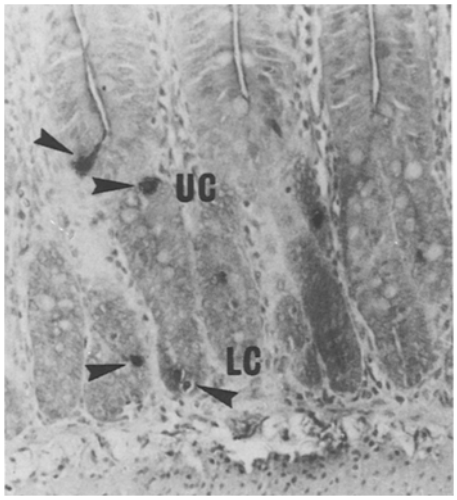

Figure 3. Argyrophil endocrine cells in the jejunum of a conventional rat fed the purified diet. Numerous argyrophil cells (arrows) are seen in the epithelium of the upper (UC) and lower (LC) crypts. Magnification × 265.

SIR cells. Jejunum (table 4). No significant differences in the number of SIR cells were observed in the jejunum between the two germ-free and the two conventional dietary groups.

Colon (table 5). The number of SIR cells was significantly higher in the surface epithelium and lower crypt of germ-free rats fed the purified diet compared to those fed the commercial diet. However, in conventional animals, the number of cells in the upper crypt was significantly higher in the group fed the commercial diet than in their counterparts fed on the purified diet. In germ-free rats fed the purified diet, the total number of SIR cells was significantly higher than in their GF-C counterparts. However, the number of SIR cells was significantly lower in conventional animals fed the purified diet than those fed the commercial diet (fig. 2).

Effects of the microflora and diet-microflora interactions. The effects of conventional flora and the effects of the interactions between the diet and the indigenous flora on the number of enteroendocrine cells were assessed by comparing the data from germ-free and conventional rats receiving the commercial diet and of germ-free and conventional rats receiving the purified diet. The effects of human flora were assessed by comparing germ-free and HFA rats receiving the purified diet. In groups fed on the purified diet, the effects of the two different microfloras were assessed by comparing the data between the conventional rats and rats associated with human flora.

Argyrophil endocrine cells. Jejunum (table 2). When the germ-free and conventional rats were compared there were significantly less argyrophil endocrine cells in the surface epithelium of jejunum in conventional animals fed both commercial and purified diets. The highest number of argyrophil endocrine cells in the lower crypt of the jejunum was observed in conventional rats fed on the purified diet.

No significant differences in the number of jejunal argyrophil endocrine cells were found between the germ-free and HFA animals fed the purified diet. However, the HFA rats when compared with those harbouring the

Table 3. Effects of diet and intestinal microflora on number of argyrophil endocrine cells in the colon of the rat.

	Commercial diet		Purified diet		
	germ-free rats	conventional flora rats	germ-free rats	conventional flora rats	human flora rats
Surface	13.00 ± 3.3	5.60 ± 1.2	20.40 ± 4.0[a]	6.60 ± 3.3	47.40 ± 6.2[b,c]
Upper crypt	6.40 ± 1.2	9.60 ± 1.2	4.80 ± 1.1	8.20 ± 2.8	15.80 ± 2.9
Lower crypt	1.20 ± 1.3	2.40 ± 0.7	0	1.40 ± 0.7	0.2 ± 0.2

Values are means ± SE. For feeding regimes and experimental groups see table 1 and 'Materials and methods'.
[a]Significantly different at $p < 0.03$ from corresponding value for conventional rats fed a purified diet.
[b]Significantly different at $p < 0.01$ from corresponding value for germ-free rats fed a purified diet.
[c]Significantly different at $p < 0.001$ from corresponding value for conventional rats fed a purified diet.

Table 4. Effects of diet and intestinal microflora on number of serotonin immunoreactive cells in the jejunum of the rat.

	Commercial diet		Purified diet		
	germ-free rats	conventional flora rats	germ-free rats	conventional flora rats	human flora rats
Surface	3.00 ± 0.7	5.00 ± 2	4.00 ± 2.6	5.33 ± 1.2	9.00 ± 1.1
Upper crypt	2.75 ± 0.8	13.33 ± 5.3	10.6 ± 4.6	12.33 ± 0.8	15.33 ± 0.6[b]
Lower crypt	2.25 ± 1	12.67 ± 4.1[a]	9.00 ± 6	3.67 ± 0.3	19.67 ± 3.5*

Values are means ± SE. For feeding regimes and experimental groups see table 1 and 'Materials and methods'.
[a]Significantly different at $p < 0.03$ from corresponding value for germ-free rats fed a commercial diet.
[b]Significantly different at $p < 0.05$ from corresponding value for conventional rats fed a purified diet.
*Significantly different at $p < 0.01$ from corresponding value for conventional rats fed a purified diet.

Table 5. Effects of diet and intestinal microflora on number of serotonin immunoreactive cells in the colon of the rat.

	Commercial diet		Purified diet		
	germ-free rats	conventional flora rats	germ-free rats	conventional flora rats	human flora rats
Surface	7.00 ± 2.8	9.33 ± 3.3	15.00 ± 2.5^{a}	7.67 ± 2.1	17.00 ± 1^{e}
Upper crypt	2.50 ± 0.8	26.00 ± 2.6^{c}	2.67 ± 0.8	2.33 ± 0.8^{b}	$9.33 \pm 0.8^{d,**}$
Lower crypt	0	3.33 ± 2	$6.00 \pm 0.5^{*}$	2.00 ± 0.5^{d}	5.67 ± 1.7

Values are means $\pm$ SE. For feeding regimes and experimental groups see table 1 and 'Materials and methods'.
[a]Significantly different at $p < 0.05$ from corresponding value for germ-free rats fed a commercial diet.
[b]Significantly different at $p < 0.001$ from corresponding value for conventional rats fed a commercial diet.
[c]Significantly different at $p < 0.002$ from corresponding value for germ-free rats fed a commercial diet.
[d]Significantly different at $p < 0.005$ from corresponding value for germ-free rats fed a purified diet.
[e]Significantly different at $p < 0.01$ from corresponding value for conventional rats fed a purified diet.
*Significantly different at $p < 0.001$ from corresponding value for germ-free rats fed a commercial diet.
**Significantly different at $p < 0.01$ from corresponding value for conventional rats fed a purified diet.

conventional flora had significantly reduced argyrophil endocrine cells in the lower crypt but significantly increased the number in the surface epithelium of the jejunum.

Colon (table 3). Apart from the highest number of argyrophil endocrine cells observed in the surface epithelium of germ-free animals fed the purified diet there were no other significant differences in the number of colonic argyrophil endocrine cells between the germ-free and the two conventional dietary groups.

The number of argyrophil endocrine cells was similar in the colonic crypts of germ-free and HFA rats but their number was significantly higher in the surface epithelium of the colon in HFA rats (fig. 4) compared with their germ-free and conventional counterparts fed the purified diet. In HFA rats, the total number of argyrophil endocrine cells in the colon was significantly higher when compared with germ-free and conventional rats fed the purified diet (fig. 2).

SIR cells. Jejunum (table 4). The numbers of jejunal SIR cells were similar in germ-free and conventional rats fed the purified diet. However, in rats fed the commercial diet the numbers were significantly higher in the lower crypt (fig. 5) than in the germ-free rats and there was a significantly higher number of total SIR cells in conventional rats as compared to the corresponding germ-free group (fig. 2). Although no significant differences in the number of jejunal SIR cells were found between HFA and germ-free rats, the number of SIR cells in the upper as well as the lower parts of the crypt and the total number of SIR cells was significantly higher in HFA rats compared with their conventional counterparts (figs 1 and 2).

Colon (table 5). In rats fed the commercial diet the number of SIR cells in the upper crypt and the total number (fig. 2) of SIR cells was significantly higher in the colon of the conventional group than in corresponding germ-free rats. However, in rats fed the purified diet

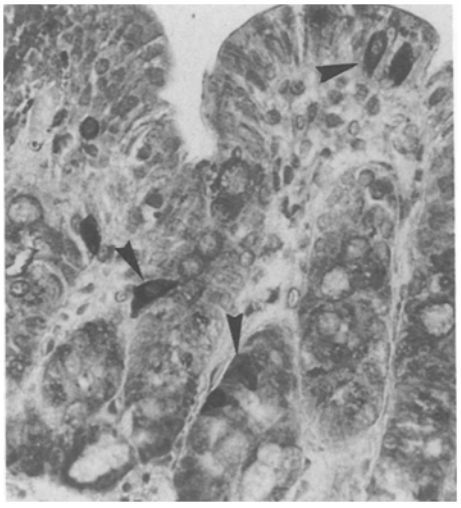

Figure 4. Colonic crypts of a HFA rat fed the purified diet showing argyrophil endocrine cells in the surface epithelium and upper crypts (arrows). Magnification × 265.

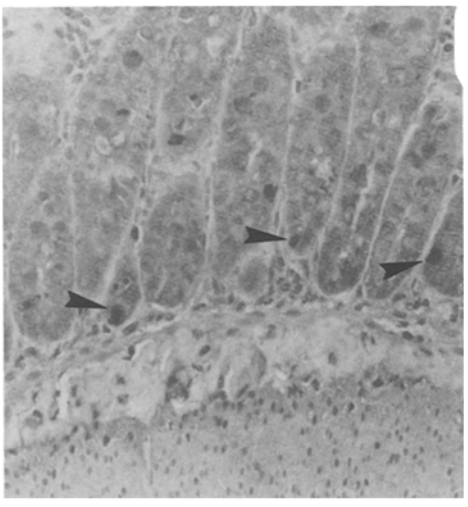

Figure 5. Jejunum of a conventional rat fed the commercial diet. Serotonin immunoreactive cells are seen in the epithelium of the lower (arrows) crypts. Magnification × 265.

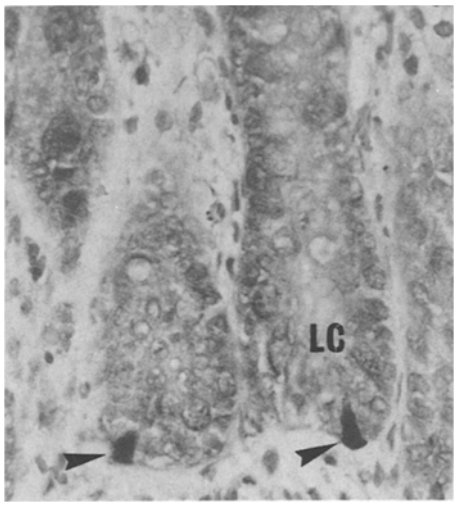

Figure 6. Colonic crypts of a germ-free rat fed the purified diet showing serotonin immunoreactive cells (arrows) in the lower crypt (LC). Magnification × 530.

the number of SIR cells in the lower crypt and the total number of SIR cells was significantly higher in the colon of germ-free rats than in those harbouring a conventional flora (figs 2 and 6). When compared with the germ-free animals the presence of a human flora significantly increased the number of SIR cells in the upper crypt. The number of SIR cells in the surface and upper crypt and the total number of SIR cells were significantly higher in the colon of HFA rats than in the corresponding conventional rats (fig. 2).

Discussion

The enteroendocrine cells provide a source of polypeptide hormones and biogenic amines which are involved in modulation of secretory, absorptive and motor functions of the gastrointestinal tract[12]. Evidence that enteroendocrine cells respond to changes in the luminal environment has emerged from in vitro experiments with nutrients[19] and immunohistochemical studies involving transposition of intestinal segments[20]. The results of in situ analysis of the occurrence and distribution of enteroendocrine cells in different part of the small and large intestine presented in this study indicate for the first time that the number of enteroendocrine cells of the jejunum and proximal colon is also influenced by the dietary composition, presence or absence of microflora and the interactions between the diet and microflora in the intestinal lumen.

The regional differences in the distribution of enteroendocrine cells are well documented[1] and the results of the present study confirm previous observations that enteroendocrine cells are more numerous in the small intestine than in the large intestine[18,21]. In addition to previous studies our results also demonstrate that diet-microbial interactions influence the number and distribution pattern of argyrophil cells and the number and distribution pattern of serotonin immunoreactive cells along the length of the intestinal tract and along the crypt-surface axis. For instance, in response to the commercial diet in the germ-free group there is a decrease in the number of surface and total argyrophil endocrine cells in the jejunum and of serotonin immunoreactive cells in the colon. However, the commercial diet fed to conventional animals results in an increase in the upper crypt and the total number of serotonin immunoreactive cells in the colon. These findings indicate that the interactions between dietary components and microflora regulate the peptide synthesis of enteroendocrine cells in the small intestine and of biogenic amines in the large intestine. In a previous study[11] we found a marked increase in the villus and crypt lengths in the jejunum and a decrease in the length of colonic crypts of germ-free and conventional rats fed the commercial diet. Although the precise regulatory mechanisms by which dietary components modify the cell proliferation of intestinal mucosa are not known, the results presented in this study when correlated with our previous quantitative morphological data, suggest that the mucosal response to diet may be mediated by peptides and biogenic amine synthesised by the enteroendocrine cells. Our observations are consistent with the findings that in rats fed different diets the gastrointestinal peptides such as gastrin, PYY and enteroglucagon play a role in altering the crypt cell proliferation rates[22,23].

A recent study comparing germ-free and conventional animals fed the same diet has shown an increased number of serotonin-containing cells in the distal small intestine and colon of rats harbouring a rat flora[7]. This is in contrast with our findings which show that in conventional animals the number of serotonin immunoreactive cells increase in the jejunum and colon in response to the commercial diet and decrease in response to the purified diet. Our observations therefore indicate that the effects of microflora on the distribution of the enteroendocrine cells depend on the luminal interactions between the flora and dietary constituents and not on the flora alone as recently described[7]. Although the significance of these effects is not yet clear, the possibility exists that diet and flora may influence intestinal motility by modifying the gastroenteric products. The microflora[24] and the release of somatostatin[25] and motilin[26] have been implicated as luminal and systemic factors involved in gastroenteric motility. Although the effect of conventional flora on the number of enteroendocrine cells was dependent on the diet, the difference between the conventional and HFA rats fed the same diet point out the ability of human flora to increase the activity of enteroendocrine cells both in the jejunum and colon. The differences between the effects of

two floras on the distribution of enteroendocrine cells presented in this study therefore emphasise the value of our rat model to determine if different microbial species are involved in the modulation of enteroendocrine cells.

In conclusion, the data presented in this study provide strong evidence for the first time that dietary and microbial luminal factors modify the enteroendocrine cells in the intestinal epithelium. However, the molecular mechanisms which control the differentiation of endocrine cells expressing different gastroenteric peptides remains to be resolved.

Acknowledgements. This work was partly sponsored by the EC-FLAIR Concerted Action Programme No. 9. The authors thank Dr M. E. Coates for conducting the feeding regimes and microbial inoculation of animals.

1 Solcia, E., Capella, C., Buffa, R., Usellini, L., Fiocca, R., and Sessa, F., in: Physiology of the Gastrointestinal Tract, p. 111. Ed. L. R. Johnson. Raven Press, New York 1987.
2 Polak, J. M., in: Handbook of Physiology, p. 79. Ed. S. G. Schultz, American Physiological Society, Bethesda, Maryland 1989.
3 Sundler, F., Ekblad, E., and Håkanson, R., in: Gastrointestinal peptides, p. 1. Ed. D. R. Brown, Springer-Verlag, Berlin 1993.
4 Krause, W. J., Yamada, J., and Cutts, J. H., J. Anat. *140* (1985) 591.
5 Ensinck, J. W., Vogel, R. E., Laschansky, E. C., and Francis, B. H., Gastroenterology *98* (1990) 633.
6 Barber, D. L., Cacace, A. M., Raucci, D. T., and Ganz, M. B., Am. J. Physiol. *261* (1991) G497.
7 Uribe, A., Alam, M., Johansson, O., Midtvedt, T., and Theodorsson, E., Gastroenterology *107* (1994) 1259.
8 Jacobs, L. R., Am. J. Clin. Nutr. *37* (1983) 954.
9 Mathers, J. C., and Kennard, J., Br. J. Nutr. *70* (1993) 567.
10 Meslin, J. C., Andrieux, C., Sakota, T., Beaumatin, P., Bensaada, M., Popot, F., Szylit, O., and Durand, M., Br. J. Nutr. *69* (1993) 902.
11 Sharma, R., Schumacher, U., Ronaasen, V., and Coates, M., Gut *36* (1995) 209.
12 Dayal, Y., in: Endocrine Pathology of the Gut and Pancreas, p. 1. Ed. Y. Dayal. CRC Press, Boca Raton, Florida 1991.
13 Cetin, Y., Histochemistry *93* (1990) 601.
14 Mallett, A. K., Bearne, C. A., Rowland, I. R., Farthing, M. J. B., Cole, C. B., and Fuller, R., J. appl. Bacteriol. *63* (1987) 39.
15 Grimelius, L., Acta Soc. Med. Upsal. *73* (1968) 243.
16 Hsu, S. M., Raine, L., and Fanger, H., J. Histochem. Cytochem. *29* (1981) 577.
17 Sjölund, K., Alumets, R., Berg, N.-O., Håkanson, R., and Sundler, F., Gut *23* (1982) 42.
18 Sjölund, K. G., Sanden, R., Håkanson, R., and Sundler, F., Gastroenterology *85* (1983) 1120.
19 Barber, D. L., in: Gastrointestinal Regulatory Peptides, p. 105. Ed. D. R. Brown. Springer-Verlag, Berlin 1993.
20 Aiken, K. D., Yu, W., Wright, J. R. Jr., and Roth, K. A., Gastroenterology *106* (1994) 1576.
21 Roth, K. A., Hertz, J. M., and Gordon, J. I., J. Cell. Biol. *110* (1990) 1791.
22 Goodlad, R. A., Lenton, W., Ghatei, M. A., Adrian, T. E., Bloom, S. R., and Wright, N. A., Gut *28* (1987) 221.
23 Johnson, I. T., Gee, J. M., and Brown, J. C., Am. J. clin. Nutr. *47* (1988) 1004.
24 Husebye, E., Hellström, P. M., and Midvedt, T., Microecol. Ther. *20* (1990) 384.
25 Efendic, S., and Mattsson, O. E., Acta Radiol. *19* (1978) 348.
26 Strunz, U., Domschke, W., and Domschke, S., Scand. J. Gastroenterol. *11* (1976) 199.