

Intestinal mucosal morphometry and ileal epithelial renewal in conventional and germ-free rats fed an amylo maize starch diet

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Summary — Intestinal mucosal morphometry and ileal epithelial renewal were studied in conventional (CV) and germ-free (GF) rats fed either poorly digestible amylo maize or normal maize starch diets. Intestinal morphometry and position of labelled enterocytes were studied at various times after tritiated thymidine injection. With amylo maize starch diet, no difference was observed in the size of crypts (C), villi (V) and C + V between duodenum and jejunum both in CV and GF rats. In the ileum, however, values were significantly lower than those in the duodenum and jejunum. Furthermore, the presence of the microbial flora led to higher values when compared with GF values. Despite the morphological modifications in the ileum, no significant difference was detected in the labelled cell positions and epithelial renewal time between CV and GF values. This suggests that the resistant part of amylo maize starch was responsible for the modification in mucosal morphometry and the longer ileal epithelium renewal time in CV rats which then becomes similar to that in GF rats.

bacteria / intestinal renewal / rat / starch

Résumé — Effets d'un amidon d'amylo maïs sur la morphométrie de la muqueuse intestinale et le renouvellement de l'épithélium iléal chez le rat axénique et le rat holoxénique. La morphométrie de la muqueuse intestinale et le renouvellement de l'épithélium iléal ont été étudiés chez des rats axéniques et des rats holoxéniques recevant un aliment contenant soit de l'amidon normal, soit de l'amidon d'amylo maïs faiblement digestible. Avec l'amidon d'amylo maïs, aucune différence n'est observée pour la taille des cryptes et des villosités entre le duodénum et le jéjunum tant chez les rats axéniques que chez les rats holoxéniques; dans l'iléon cependant, ces valeurs restent plus faibles que dans le duodénum et le jéjunum avec des valeurs plus élevées en présence de la flore bactérienne. Malgré ces différences morphologiques, aucune différence n'existe dans l'iléon pour la position des cellules marquées et le temps de renouvellement entre rats axéniques et rats holoxéniques. Cela suggère que la fraction de l'amylo maïs résistante à la digestion est responsable des mo-

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difications morphologiques et du ralentissement considérable du renouvellement de l'épithélium iléal chez les rats holoxéniques qui devient alors semblable à celui des rats axéniques.

bactérie / renouvellement intestinal / amidon / rat

INTRODUCTION

The presence of microflora in the digestive tract of conventional (CV) rats modifies the morphometry and epithelial renewal of the intestinal mucosa. Thus, villi are significantly longer in the ileum of CV rats than in germ-free (GF) rats, and epithelial renewal is faster in CV than in GF rats (Guenet *et al*, 1970; Meslin *et al*, 1974) or mice (Mastromarino and Wilson, 1976).

Incorporation of lactose in the diet lengthens ileal epithelial renewal time in CV rats so that it becomes similar to that observed in GF rats (Meslin *et al*, 1981).

Dietary fibers have been implicated in the effects of diets on the epithelial mucosa either because of a possible mechanical action on the intestine (Komai and Kimura, 1980; Ecknauer *et al*, 1981; Southon *et al*, 1985; Dirks and Freeman, 1987) or because they reach the large intestine and are fermented by the microbial flora, lower caecal pH and produce short-chain fatty acids (SCFA) (Sakata, 1987). Other plant polysaccharides, *eg* resistant starch, could also contribute to this effect. It has been recently demonstrated that an amylo maize starch diet brought about a 2-fold increase in the bile acid pool in the small intestine of CV and GF rats and stimulated ileal bile acid absorption (Sacquet *et al*, 1983; Riottot and Sacquet, 1985). This type of starch is only partially hydrolyzed by endogenous digestive enzymes in CV and GF rats (Andrieux *et al*, 1989). The resistant fraction of starch reaches the large intestine and is fermented by the microflora in CV rats (Andrieux and Sacquet, 1986).

Other mechanisms have been evoked to explain the stimulatory action of the bacterial flora upon epithelial proliferation including: i), the qualitative and quantitative variations of bile acids which remain conjugated in GF rats (Meslin *et al*, 1978), and are only half as abundant in the small intestine of CV as in GF rats (Riottot *et al*, 1980); and ii), neurohumoral secretions (Tutton, 1973).

The aim of this study was to investigate the effects of amylo maize starch, which is poorly digestible but fermentable by the bacterial flora, compared with normal maize starch, which is highly digestible, on mucosal morphometry and ileal epithelial renewal in the small intestine. In order to dissociate the effects of maize composition and the effects of the bacterial flora, we compared GF and CV rats.

MATERIAL AND METHODS

Two groups of 8 male inbred F344 4-month-old rats were used; they weighed (mean \pm SEM) 320 ± 5 g (CV rats) and 284 ± 8 g (GF rats). For one month before the end of the experiment they received a standard semi-synthetic diet containing normal maize starch 60 g, casein 20 g, maize oil 9 g, cellulose 5 g, minerals and vitamin mixture 4.5 g (compositions previously described; Andrieux and Sacquet, 1986). Normal maize starch contained (g/kg) : amylose 300 and amylopectin 700.

Two other groups (15 rats each) of the same strain and age were also used. They weighed 280 ± 8 g (CV rats), and 300 ± 6 g (GF rats). They also received an experimental diet for 1 month. This diet had the same composition as that described above except that it contained amylo maize starch 60 g instead of normal maize

starch. The amylo maize starch contained (g/kg) amylose 700 and amylopectin 300 (Eurylon®; Roquette Frères, Lestrem, France).

The diets were sterilized by gamma irradiation (40 kGy) in vacuum-sealed plastic bags. Diets and water were provided *ad libitum*.

Temperature and relative humidity of the animal room were controlled (21 ± 2 °C, $60 \pm 5\%$ respectively). The lighting schedule was also controlled (12 h light/dark). Rats were placed on wire mesh-bottomed cages and germ-free rats were maintained in vinyl isolators.

Tritiated thymidine (CEA, Saclay, France, SA 37 GBq/mM) was injected intraperitoneally (37 kBq/g body weight) to rats under light ether anaesthesia. In the normal maize-fed group, 2 CV and 2 GF rats were killed by an overdose of sodium pentobarbitone (Sanofi, 60 mg/kg ip) 1, 8, 27 and 41 h respectively after the label injection. In each CV and GF amylo maize-fed group, 5 rats were killed 1, 27 and 41 h respectively after the label injection.

Detailed methods for labelling with tritiated thymidine, fixation and sampling of small intestine, histoautoradiography and quantification of histological slides have already been published (Guenet *et al*, 1970).

Villi length and depth of the crypts of Lieberkühn were recorded with an ocular micrometer in the duodenum (5 cm from the pylorus and before Treiz's ligament), jejunum (middle of the small intestine) and ileum (5 cm before the caecum) on at least 20 well-oriented crypt-villus units per rat and expressed in μm . As differences between CV and GF amylo maize-fed rats appeared in the ileum, at various times after the tritiated thymidine injection the number of enterocytes were counted in thin longitudinal section from the base of the crypt to the top of the villus, and 3 compartments were distinguished crypt, villus and the compartment extending from the base of the crypt to the farthest labelled cells. For simplicity, this compartment was called the compartment of labelled cells. Cellular production per column over a 24-h period was computed from the changes in the labelled enterocytes at various time intervals.

It has been generally demonstrated that there is a linear relationship for labelled epithelial cells migration *versus* time when labelled cell positions are expressed in epithelial cell number; thus, knowing the number of epithelial cells to be re-

placed on the villus, the time needed for the labelled enterocytes to reach the top of the villi can be calculated and this gives an estimation for the ileal epithelial renewal time. Equation of linear regression was calculated from labelled epithelial cell positions *versus* time and provided a calibration curve. Epithelial renewal time was calculated from the total number of cells to be replaced on the villus by the inverse regression (straight line case) method (Drapper and Smith 1981) and expressed as mean \pm confidence interval.

Statistical analysis

Values are expressed as means \pm SEM. For each variable, comparison between the 3 anatomical sites was made by variance analysis, and for each intestinal site, comparison between CV and GF rats was made by Student's *t*-test. Differences were considered significant at $P < 0.05$.

RESULTS

Morphometry

Depth of crypts, length of villi and the size of crypt + villus in the duodenum, jejunum and ileum are reported in figure 1 for normal maize-fed rats and in figure 2 for amylo maize-fed rats. In both CV and GF normal maize-fed rats (fig 1), a decreasing villus size gradient was observed from the duodenum to the ileum (CV villi duodenum 370 ± 14 μm , jejunum 312 ± 24 μm , ileum 218 ± 14 μm ; GF villi duodenum 400 ± 20 μm , jejunum 280 ± 15 μm , ileum 143 ± 7 μm). In both CV and GF amylo maize-fed rats (fig 2), the depth of crypts was similar in the duodenum, jejunum or ileum (135 ± 8 μm). Villus length did not differ between duodenum and jejunum (445 ± 10 μm) but was smaller in the ileum. For these variables, no difference was observed between CV and GF rats in the duodenum and jejunum. In the ileum, however, crypt depth, villus length and crypt + villus size

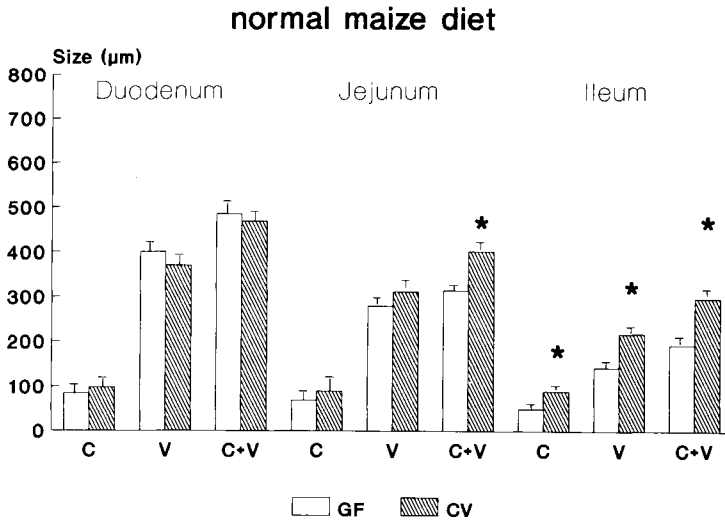


Fig 1. Length of crypts (C), villi (V) and C + V in the duodenum, jejunum and ileum of germ-free (GF) and conventional (CV) rats fed normal maize. Mean values with their SE (vertical bars). * $P < 0.05$ between CV and GF (Student's *t*-test).

were significantly greater ($P < 0.001$) in CV rats when compared with GF animals (CV crypts $136 \pm 7 \mu\text{m}$, villi $254 \pm 8 \mu\text{m}$; GF crypts $108 \pm 7 \mu\text{m}$, villi $215 \pm 10 \mu\text{m}$).

Ileal cell number

In normal maize-fed rats ileal crypt cell number was higher in CV than in GF rats ($P < 0.05$) (table I). Epithelial cell number was not significantly different in ileal crypts of CV and GF amylo maize-fed rats but was greater for the ileal villi of CV rats than for GF animals (table II).

Ileal cell migration

The migration of labelled epithelial cells in the ileum of CV and GF normal maize-fed

Table I. Epithelial cell number in longitudinal sections of crypts, villi and crypts + villi in the ileum of GF and CV rats fed normal maize.

	n	Crypts	Villi	Crypts + villi
GF	8	21.1 ± 0.3	47.8 ± 0.9	69.2 ± 0.9
CV	8	$23.8 \pm 0.6^*$	47.5 ± 2	71.3 ± 1.8

Means \pm SEM for *n* GF (germ-free) and CV (conventional) animals. * $P < 0.05$ between CV and GF.

rats is shown in figure 3. Ileal renewal time, defined by the intersection of labelled cell migration lines and the *y*-values corresponding to the total number of epithelial cells, was 50 ± 1.2 h and 72.5 ± 1.5 h respectively for CV and GF normal maize-fed rats.

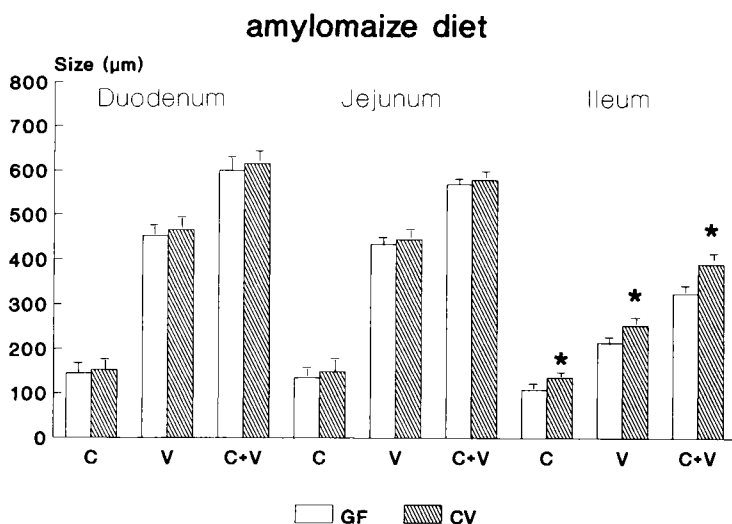


Fig 2. Length of crypts (C), villi (V), and C + V in the duodenum, jejunum and ileum of germ-free (GF) and conventional (CV) rats fed amylomaize. Mean values with their SE (vertical bars). * $P < 0.05$ between CV and GF (Student's *t*-test).

Table II. Epithelial cell number in longitudinal sections of crypts, villi and crypts + villi in the ileum of GF and CV rats fed amylomaize.

	n	Crypts	Villi	Crypts + villi
GF	15	22.4 ± 0.3	49.5 ± 2.1	71.4 ± 2.4
CV	15	23.5 ± 0.6*	57.2 ± 2.5*	78.7 ± 0.8*

Means ± SEM for *n* GF (germ-free) and CV (conventional animals). * $P < 0.05$ between CV and GF.

Migration of labelled enterocytes in the ileum of amylomaize-fed rats (fig 4) was very different from that of normal maize-fed animals. There was no significant difference in the number of labelled cells at 1, 27 and 41 h after tritiated thymidine injection between CV and GF amylomaize-fed

rats. Ileal renewal time was 67.6 ± 0.8 h and 66.2 ± 3.5 h in CV and GF amylomaize-fed rats respectively. These 2 values did not differ significantly.

DISCUSSION

The amylomaize starch diet modified mucosal morphometry both in GF and CV rats; the crypt depth and the villus length were greater than those of GF and CV normal maize-fed rats. The differences in the villus length between duodenum and jejunum in the GF and CV normal maize-fed rats disappeared when they were fed an amylomaize diet.

There was a great difference in the ileal enterocyte migration between GF and CV normal maize-fed rats; with amylomaize diet, ileal enterocyte migration was similar between GF and CV rats.

normal maize diet

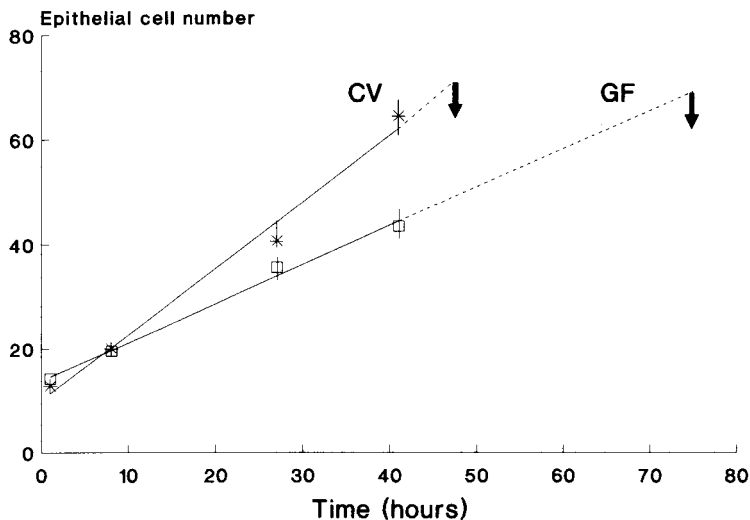


Fig 3. Enterocyte migration in the ileum of germ-free (GF) and conventional (CV) rats fed normal maize. Symbols are means \pm SEM; y = regression line equation. GF $y = 0.77t + 13.3$; $r^2 = 0.94$; cell production/24 h 18.6 ± 0.4 ; renewal time (h) (mean \pm CI) 72.5 ± 1.5 . CV $y = 1.20t + 11.4$; $r^2 = 0.96$; cell production/24 h 29 ± 0.3 ; renewal time (h) (mean \pm CI) 50 ± 1.2 .

The increase in the crypt depth and villus length for the 3 intestinal sites both in GF and CV amylo maize-fed rats suggests that amylo maize starch might have a direct effect on epithelial morphometry independent from that of bacterial flora. This effect on villus length is greater in the jejunum than in the duodenum. Ileal epithelial cell number was not different for GF rats fed either amylo maize starch or normal maize starch, whereas it was greater for CV amylo maize-fed rats than for CV normal maize-fed rats. Thus, amylo maize starch had a trophic effect on ileal villus epithelial cellularity in CV rats.

Ileal epithelial renewal time did not differ in GF rats fed either amylo maize or normal maize starch. In contrast, for CV

rats, amylo maize starch considerably increased ileal epithelial renewal time which became similar to that observed for GF rats. This lengthening of ileal epithelial renewal time, as previously observed for lactose in CV rats (Meslin *et al*, 1981), seems to constitute an indirect effect of amylo maize starch which might be related to fermentation of this starch by bacterial flora.

As reported in a previous experiment with lactose (Meslin *et al*, 1981), there was no relation between bile acid pool and epithelial renewal because a similar increase in bile acid pool was observed in CV and GF rats fed amylo maize starch (Sacquet *et al*, 1983; Andrieux *et al*, 1989) whereas epithelial renewal was modified only in CV rats.

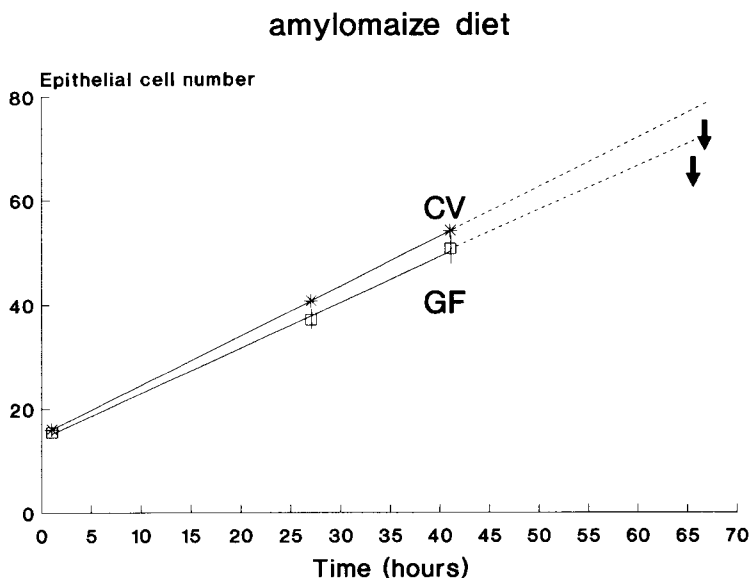


Fig 4. Enterocyte migration in the ileum of germ-free (GF) and conventional (CV) rats fed amylomaize. Symbols are means \pm SEM; $y =$ regression line equation. GF $y = 0.87t + 14.2$; $r^2 = 0.99$; cell production/24 h 23.1 ± 3.2 ; renewal time (h) (mean \pm CI) 66.2 ± 3.5 ; CV $y = 0.95t + 15$; $r^2 = 1$; cell production/24 h 22.8 ± 1.1 ; renewal time (h) (mean \pm CI) 67.6 ± 0.8 .

As for lactose and lactulose, amylomaize starch is poorly digested in adult rats but is partly fermented by the microbial flora in the large intestine (Andrieux *et al*, 1989). This resistance to digestion and/or the associated stimulation of fermentation might be related to the physiological effects of these carbohydrates.

The trophic effect observed on ileal epithelial cell number in CV amylomaize-fed rats does not appear to be related to SCFA production by starch fermentation. SCFAs, especially butyrate, have been reported to have a stimulatory effect on epithelial cell proliferation in the rat intestine and to reduce epithelial renewal time (Sakata, 1987). In amylomaize-fed rats of the same breed, SCFA concentration and particular-

ly butyrate concentration was twice as high as in those fed normal maize starch (Andrieux *et al*, 1989); however, as shown in the present experiment, ileal epithelial renewal time increased in CV amylomaize-fed rats. There is controversy in the literature about whether SCFAs act as a hypertrophic stimulus causing enlargement of gastrointestinal organs or whether they increase epithelial renewal rates (Wyatt *et al*, 1988; Key and Mathers, 1989).

Modifications other than SCFA production have been observed in the large intestine of rats fed amylomaize starch including a significant decrease in bacterial activities of nitro- and azoreductase and β -glucuronidase (Mallett *et al*, 1988) and altered bile acid bacterial transformation (An-

drieux *et al*, 1989). Similar observations have also been reported for various other poorly digestible carbohydrates and have been related to the decrease in caecal pH in CV rats fed these carbohydrates and to a modification of the bacterial population in the large intestine (Andrieux *et al*, 1989). It was also noted that, with amylo maize starch compared with normal starch, caecal size increased 4-fold in CV rats (Sacquet *et al*, 1983).

Our results suggest that poorly digestible carbohydrates are efficient in altering mucosal morphometry between the duodenum and jejunum and in lengthening ileal epithelial renewal time in the CV rats which becomes similar to that in GF rats. It is thus possible by dietary modifications to alter gastrointestinal physiology, not only in the large intestine but also in the ileal part of the small intestine.

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