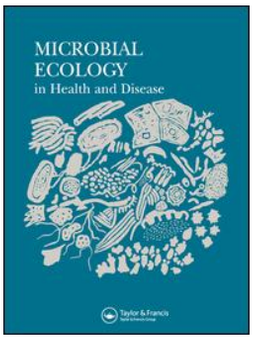

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Gender, Age and Microbial Status Influence upon Intestinal Cell Kinetics in a Compartmentalized Manner: An Experimental Study in Germfree and Conventional Rats

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The aim of this study was to investigate the cell kinetics and epithelial structure in the intestinal tract in cohorts of young and old germfree (GF) and conventional AGUS rats of both sexes. The young rats were 35 days old and the old rats were 190 ± 60 days old. All rats were fed the same diet and water *ad libitum*. At the beginning of the experiments, the rats were starved for 2.5 h. Thereafter, all animals were given vincristine 1 mg/kg intraperitoneally (i.p.) 4 h before they were sacrificed. Specimens from eight parts of the intestine were investigated. The cell kinetic parameters determined in the small intestine were percentage of cells in mitosis, growth fraction (GrF), total number of cells in the crypts and villi, depth of the crypts, height of villi and the crypt/villus (C/V) ratio. In the colon, the percentage of cells in mitosis, GrF and the total number of cells in the crypts, as well as depth of the crypts, were determined. Taken together, the effect of gender was most pronounced in the upper part of the small intestine, the effect of age was most pronounced in the lower small intestine, whereas the effect of microbial status was relatively more pronounced in the duodenum and in the large intestine. Thus, these factors are acting in a compartmentalized manner in the intestinal tract. In conclusion, gender, age and microbial status have to be taken into account when the compounds and conditions influencing the intestinal morphometric parameters are studied. *Key words*: cell kinetics, gastrointestinal tract, germfree, gender, age, normal flora.

INTRODUCTION

Throughout life, all conventional macroorganisms are exposed to many different strains of microbes. In principle, this exposure might end in three different ways, i.e. the microbes might eradicate the host, the host might eradicate the microbes, or they might stay together and crosstalk with each other (1). In studies of these crosstalks, the microflora-associated characteristics (MAC)/germfree animal characteristics (GAC) concept has been shown to be of considerable value (2).

A MAC is defined as the recording of any anatomical structure, physiological, biochemical or immunological function in a macroorganism that has been influenced by the microflora. When functionally active microorganisms are absent, as in germfree (GF) animals or newborns or sometimes in relation to the ingestion of antibiotics, these structures and functions are defined as GACs.

The MAC/GAC concept has been applied to a long series of structures and functions in different organisms under physiological and pathophysiological conditions (for review see (1–3)). The gastrointestinal tract (GIT) is the site in which most interactions have been studied. Morphological differences were the first to be described between GF animals and their conventional (Conv) counterparts (4), followed by other differences (3).

The cell renewal system in the GIT involves proliferation of undifferentiated epithelial cells, followed by differentiation and migration from the site of production to the functional site and elimination from the mucosa. In Conv mice, the normal rate of cell turnover has been calculated to be about 10^8 cells/day (5). It is assumed that the renewal process is a major defence mechanism and it has been claimed that this is down-regulated when microbes are absent (4).

However, in previous studies on GF and Conv rats, conflicting results have been presented regarding intestinal morphological and cellular parameters. Discrepancies have been found in the length of villi, the depth of the crypts, as well as in the rate of mitosis (4, 6–8). In addition to a

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microbial influence, the possibility exists that other factors, e.g. gender, age and nutrition, may also influence structures and functions in the GIT. As will be discussed later, information about age and gender, as well as other parameters, vary considerably in previous studies. Therefore, the aim of the present study was to investigate whether—and to what extent—gender, age and microbial status influence intestinal cell kinetics in GF and Conv rats, after a defined period of fasting.

MATERIALS AND METHODS

Animals

Altogether, 23 GF and 24 Conv AGUS rats (9), gender- and age-matched, were divided into groups of six animals with the exception that the group of old male GF rats (M old GF) comprised just five rats. The young rats were 35 days old and the old rats were 190 ± 60 days. All animals were fed a steam-sterilized standard rat chow (R36, Lactamin, Vadstena, Sweden) and had free access to water. Artificial light was available between 6 a.m. and 6 p.m.; temperature was maintained at 24 ± 2°C and humidity at 55 ± 10%. The GF animals were kept in lightweight stainless steel isolators and controlled weekly for GF status (10). Food was withdrawn 2.5 h before initiation of the experiment.

The experimental design was approved by the Local Ethical Committee for Animal Research (Stockholm Nord, Sweden).

Study design

Vincristine (Oncovin, Lilly S.A. Fegersheim, France), 1 mg/kg, was injected intraperitoneally (i.p.) (11) starting at 10 a.m. and with an interval of 12 min between each animal. All rats were subjected to laparotomy following an injection of mebumal (25 mg/kg) i.p., exactly 4 h after vincristine injection.

Preparation of specimens

Two centimeters of tissue samples were taken from the duodenum and jejunum at 15 and 40 cm distal to the pyloric region (Jej-1, Jej-2) and from the ileum, at 5 cm proximal to the ileo–cecal junction. Samples of 1 cm were taken from the base of the cecum and from the colon 2–3, 5–6 and 8–9 cm from the ileo–cecal junction (C-1, C-2, C-3). Each specimen was placed on a micropore filter (0.2 ωm; Schleicher & Scuell, Dassel, Germany), cut open along its longitudinal axis to obtain a good orientation of crypts and villi, and fixed for 3 h in Carnoy's solution (60% methanol, 30% chloroform and 10% acetic acid). Thereafter, all specimens were kept in 70% ethanol for at least 20 h before they were paraffin-embedded. Three-micrometer thick sections were taken from each specimen 54 ωm apart from each other and stained with hematoxylin and eosin.

Microscopic evaluation

All sections were coded and examined in a blind fashion under light microscope (× 200 Leica DM LS, Germany).

Cumulative mitotic index (MI). The total number of mitotic cells and cell nuclei was counted in the left column of 30 well-oriented consecutive crypts in all sections for estimation of MI. MI is the percentage of cells in the metaphase or mitotic phases prior to metaphase (12). It is calculated using the formula $MI = Nm/Nt \times 100$, where Nm is the number of mitotic cells and Nt is the total number of cells in the left column of the crypt.

Growth fraction (GrF). The GrF is the portion of the cell population that is actively engaged in proliferation. It can be estimated from the frequency distribution of the mitotic figures along the crypt column (7, 12, 13) after estimation of the cut-off position (14) in which cells begin to decycle and leave the proliferative compartment. The positions of the mitotic figures were estimated in 30 well-oriented consecutive crypts, counting from the base of the crypts to the crypt–villus junctions. To maximize the mitotic accumulation, the rats were killed 4 h after injection of the metaphase blocker. To correct for differences due to crypts of unequal size, a normalized crypt was used (13, 14). The data of distribution of mitotic cells were submitted to linear regression analysis using computerized graphic software (Cricket Graph). The cut-off position was estimated by the intersection of the half peak position with the regression curve (12, 14). The GrF is the ratio of cut-off position to the number of cells in the crypt column.

Number of epithelial cells. The total number of cells was counted in a similar manner to MI in 30 well-oriented consecutive crypts in the small intestine and colon and in the villi of the small intestine, respectively.

Depth of crypts, height of villi and crypt/villus (C/V) ratio. In the small intestine the depth of 20 well-oriented crypts was measured from the base of the crypt to the crypt–villus junction, and the heights of 20 villi were measured from the crypt–villus junction to the tip of the villi using a micrometer in the ocular eye-piece (magnification × 100). In a similar way, the depth of colonic crypts was measured from the base of the crypt to the flat margin of the colonic mucosa. Furthermore, in the small intestine the total number of crypts and villi present in 1 mm of mucosa was counted using a micrometer placed in the ocular eye-piece (magnification × 100) to estimate the C/V ratio.

Statistical analysis

Results are given as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to evaluate the differences between the groups. The Kolmogrov–Smirnov one-sample test was used to check for normal distribution. The significance level was $p < 0.05$.

RESULTS

All animals looked healthy throughout the experiment and no macroscopic signs of disease were observed at autopsy. The microscopic examinations created a vast amount of data. Therefore, only significant differences will be commented on and the comments are related to relevant counterparts (gender, age, microbial status).

Gender influence

Mitotic index (MI) (Table I). The MI was increased in the small intestine (except the duodenum) and in the large intestine (except the cecum) in young male GF rats (M young GF), and in the duodenum in old male Conv rats (M old Conv) compared with their female counterparts.

Growth fraction (GrF) (Table II). The GrF of M young GF in the Jej-1, Jej-2 and cecum and of M old GF in the ileum was increased compared with their female counterparts.

Number of crypt cells and depth of the crypts (Fig. 1a, b; Table III). The number of crypt cells in C-2 was higher in M young GF, and in Jej-2 and C-2 in M old GF. The crypts were deeper throughout the small intestine in M young GF, in C-1 in M young Conv, in Jej-2 of M old GF, and in the duodenum, Jej-2 and C-3 in F old Conv.

Number of villus cells and height of the villi (Fig. 2a, b; Table III). The number of villus cells and the height of the villi in M young GF at the Jej-1 was higher than in corresponding females. The number of villus cells in M old Conv in Jej-1 was increased. Villi were taller in F young Conv in Jej-2 and in F old Conv in the duodenum and Jej-2.

Crypt/villus (C/V) ratio (Fig. 2c; Table III). The C/V ratio in M young GF in the duodenum was increased.

Table I

MI in different compartments of male (M) and female (F), young and old, germfree (GF) and conventional (Conv) rats

Groups	Compartments*							
	Duodenum	Jej-1	Jej-2	Ileum	Cecum	C-1	C-2	C-3
M young GF	27.3 ± 2.4	31.4 ± 1.7^{1}	$33.0 \pm 2.2^{1,3}$	$32.7 \pm 3.7^{1,3}$	15.9 ± 2.6^{2}	$12.7 \pm 3.9^{1,2}$	11.5 ± 2.7^{1}	$13.9 \pm 1.7^{1,2}$
F young GF	18.8 ± 11.1	20.6 ± 11.4^{1}	20.6 ± 12.0^{1}	21.1 ± 12.3^{1}	13.4 ± 3.1	6.8 ± 4.5^{1}	6.4 ± 3.5^{1}	5.6 ± 4.5^{1}
M young Conv	20.3 ± 11.2	21.1 ± 12.8	21.8 ± 11.6^{3}	20.7 ± 11.5^{3}	15.7 ± 1.4^{2}	9.0 ± 4.4	8.6 ± 4.5	9.5 ± 4.8
F young Conv	18.9 ± 11.8	20.9 ± 11.6	19.8 ± 11.9	19.3 ± 10.4	13.3 ± 2.3	9.3 ± 4.9	6.4 ± 4.1	6.4 ± 4.8
M old GF	22.4 ± 9.5	25.6 ± 11.6	23.6 ± 10.3	23.4 ± 10.7	12.1 ± 2.5^{2}	$5.7 \pm 3.1^{2,3}$	8.1 ± 3.0	7.1 ± 2.2^{2}
F old GF	20.7 ± 12.4	20.0 ± 11.8	19.1 ± 12.1	19.7 ± 11.9	13.5 ± 3.0	6.2 ± 4.3	6.5 ± 2.6	6.6 ± 4.8
M old Conv	29.1 ± 3.5^{1}	26.0 ± 2.4	26.2 ± 3.0	20.3 ± 3.7	9.5 ± 2.5^{2}	10.5 ± 1.9^{3}	7.1 ± 2.5	8.6 ± 1.8
F old Conv	25.1 ± 1.3^{1}	26.5 ± 3.8	26.5 ± 2.8	23.6 ± 3.2	11.6 ± 1.9	8.4 ± 1.6	8.7 ± 1.7	10.1 ± 1.8

* Jej-1 is 15 cm distal to the pyloric region; Jej-2 is 40 cm distal to the pyloric region; Ileum: 5 cm proximal to ileo–cecal junction, C-1 is colon at the 2–3 cm from the cecum, C-2 is colon at the 5–6 cm from the cecum, and C-3 is colon at the 8–9 cm from the cecum. Results are given as mean $\pm$ SD[1,2,3]. $p<0.05$ for differences from counterpart.
[1] Male and female; [2] young and old; [3] GF and Conv.

Table II

GrF in different compartments of male (M) and female (F), young and old, germfree (GF) and conventional (Conv) rats

Groups	Compartments*							
	Duodenum	Jej-1	Jej-2	Ileum	Cecum	C-1	C-2	C-3
M young GF	0.69 ± 0.1	$0.71 \pm 0.0^{1,3}$	0.74 ± 0.1^{1}	0.69 ± 0.0^{2}	0.54 ± 0.0^{1}	0.58 ± 0.1	0.55 ± 0.1	0.52 ± 0.1^{2}
F young GF	0.58 ± 0.1	0.64 ± 0.0^{1}	$0.62 \pm 0.1^{1,2}$	0.64 ± 0.1^{3}	$0.46 \pm 0.1^{1,3}$	0.56 ± 0.1	0.52 ± 0.0^{2}	0.50 ± 0.0^{2}
M young Conv	0.64 ± 0.0^{2}	$0.64 \pm 0.0^{2,3}$	0.64 ± 0.1^{2}	0.68 ± 0.1	0.52 ± 0.0	0.60 ± 0.0	0.58 ± 0.1	0.55 ± 0.0^{2}
F young Conv	0.66 ± 0.0^{2}	0.67 ± 0.1^{2}	0.68 ± 0.1^{2}	0.72 ± 0.0^{3}	0.53 ± 0.0^{3}	0.66 ± 0.1	0.58 ± 0.1	0.57 ± 0.1
M old GF	0.80 ± 0.1	0.75 ± 0.0	0.80 ± 0.0	$0.79 \pm 0.1^{1,2}$	0.60 ± 0.1	0.65 ± 0.1	0.61 ± 0.1	0.64 ± 0.1^{2}
F old GF	0.71 ± 0.2	0.70 ± 0.1	0.74 ± 0.1^{2}	0.68 ± 0.1^{1}	0.53 ± 0.0	0.62 ± 0.1	0.65 ± 0.1^{2}	0.60 ± 0.1^{2}
M old Conv	0.82 ± 0.0^{2}	0.77 ± 0.1^{2}	0.79 ± 0.1^{2}	0.70 ± 0.1	0.57 ± 0.1	0.68 ± 0.1	0.59 ± 0.1	0.70 ± 0.1^{2}
F old Conv	0.79 ± 0.1^{2}	0.78 ± 0.0^{2}	0.79 ± 0.0^{2}	0.73 ± 0.1	0.58 ± 0.1	0.63 ± 0.0	0.64 ± 0.1	0.64 ± 0.1

* Jej-1 is 15 cm distal to the pyloric region; Jej-2 is 40 cm distal to the pyloric region; Ileum: 5 cm proximal to the ileo–cecal junction, C-1 is colon at the level 2–3 cm from the cecum, C-2 is colon at the level 5–6 cm from the cecum, and C-3 is colon at the level 8–9 cm from the cecum.
Results are given as mean $\pm$ SD[1,2,3]. $p<0.05$ for differences from counterpart.
[1] Male and female; [2] young and old; [3] GF and Conv.

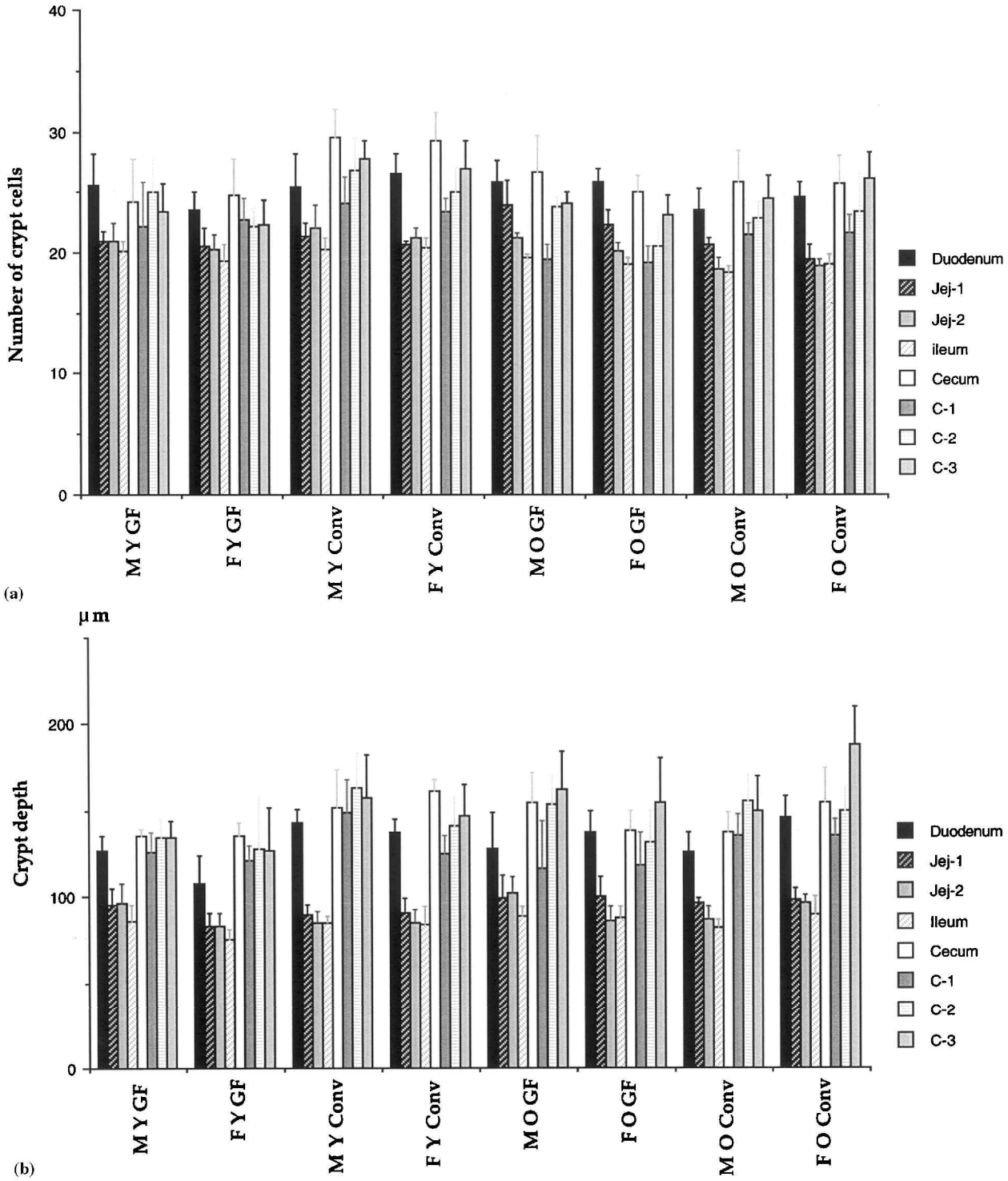

Fig. 1. Total number of crypt cells and crypt depth in the different parts of the small and large intestine of the male (M) and female (F), young (Y) and old (O), germfree (GF) and conventional (Conv) rats.

Age influence

Mitotic index (MI) (Table I). The MI in M young GF in the cecum and C-1, C-3, and in M young Conv in the cecum was increased compared with the older ones.

Growth Fraction (GrF) (Table II). The crypts were enlarged in M old GF in the ileum and C-3, in F old GF in Jej-2 as well as in C-2 and C-3, in M old Conv small intestine (except the ileum) and C-3, and in F old Conv small intestine (except the ileum) compared with the younger ones.

Table III

MI, GrF, total number of crypt cells (CC), depth of crypts (CL), villus cells (VC), height of the villus (VH) and C/V ratio in the different part of the small and large intestines of the male (M) and female (F), young and old, germfree (GF) and conventional (Conv) rats

	Duo	Jej-1	Jej-2	Ileum		Cecum	C-1	C-2	C-3
MI		D	D D	D D		D	DD	D	DD
GrF		D D	D	d		D			d
CC		d							
CL	Ddd	D	Dd	Dd	M Young GF				
VC	D	DdD	dD			d		D	d
VH	D	D D	D						
C/V	D	d	d	d		d	d	d	d
MI		d	d	d			d	d	d
GrF		d	dd	d		d d		d	d
CC	dd		D						
CL	ddd	dd		dd	F Young GF				
VC	D	dd	dd	d		d	D	d d	d
VH	D	dd	ddd	d					
C/V	d d					d			
MI			d	d		D			
GrF	d	dd	d						d
CC			D	D					
CL	DD	d			M Young Conv				
VC	d	dd	dd			DD	D	D	DD
VH	d	dd	ddd						
C/V							D D	D	
MI									
GrF	d	d	d	D					
CC	DD	D	D	D		D			
CL	D		D		F Young Conv				
VC	d		d			DD	D	D	D
VH	dd	d	Dd						
C/V	D					D	d		d
MI						d	dd		d
GrF				DD					D
CC	D	DD	D D	D					
CL	D		DDD	DD	M Old GF				
VC		DD	D				d	D	
VH		D							
C/V		D	D	D		D			D
MI									
GrF			D	d					
CC	D	D	d D					D	D
CL	D	D	d	D	F Old GF				
VC	D	DD	D	DD			dd	d	d
VH		D	D	D					
C/V				D					d
MI	D					d	D		
GrF	D	D	D						D
CC	d	d	dd	dd					
CL	dd	D	d d	d	M Old Conv				
Vc		DDd	D			d	dD	d	d
VH	d	Dd	dD						
C/V									d
MI	d								
GrF	D	D	D						
CC	d	dd	dd	d					D
CL	D		Dd		F Old Conv				
Vc	d	d d	D	d		d	dD		d
VH	DD	D	DD						
C/V				d					D
									DDD
	GAMs	GAMs	GAMs	GAMs		GAMs	GAMs	GAMs	GAMs
	*5,8,10	6,13,8	8,16,6	3,8,6		*1,5,4	2,4,4	3,2,2	2,6,5

Results are given as means ± SD. D<0.05 for differences from counterpart.
D stands for higher significant differences and d stands for lower significant differences.
** Numbers show some of the differences in the compartmentalized manner. A = age; G = gender; Ms = microbial status.*

Number of crypt cells and depth of the crypts (Fig. 1a, b; Table III). The number of crypt cells was increased throughout Jej-1 in M old GF rats, in the duodenum (vice versa in C-1) in F old GF, in Jej-2 as well as the ileum and large intestine in young male Conv rats (M young Conv), and in the small intestine as well as the cecum and C-1 in young female Conv rats (F young Conv). The crypts were deeper throughout the small intestine (except Jej-1) as well as the proximal and distal part of the large intestine in M old GF, the small intestine (except Jej-2) in old female GF rats (F old GF), the duodenum (vice versa Jej-1) in M young Conv, and the Jej-2 and distal part of the large

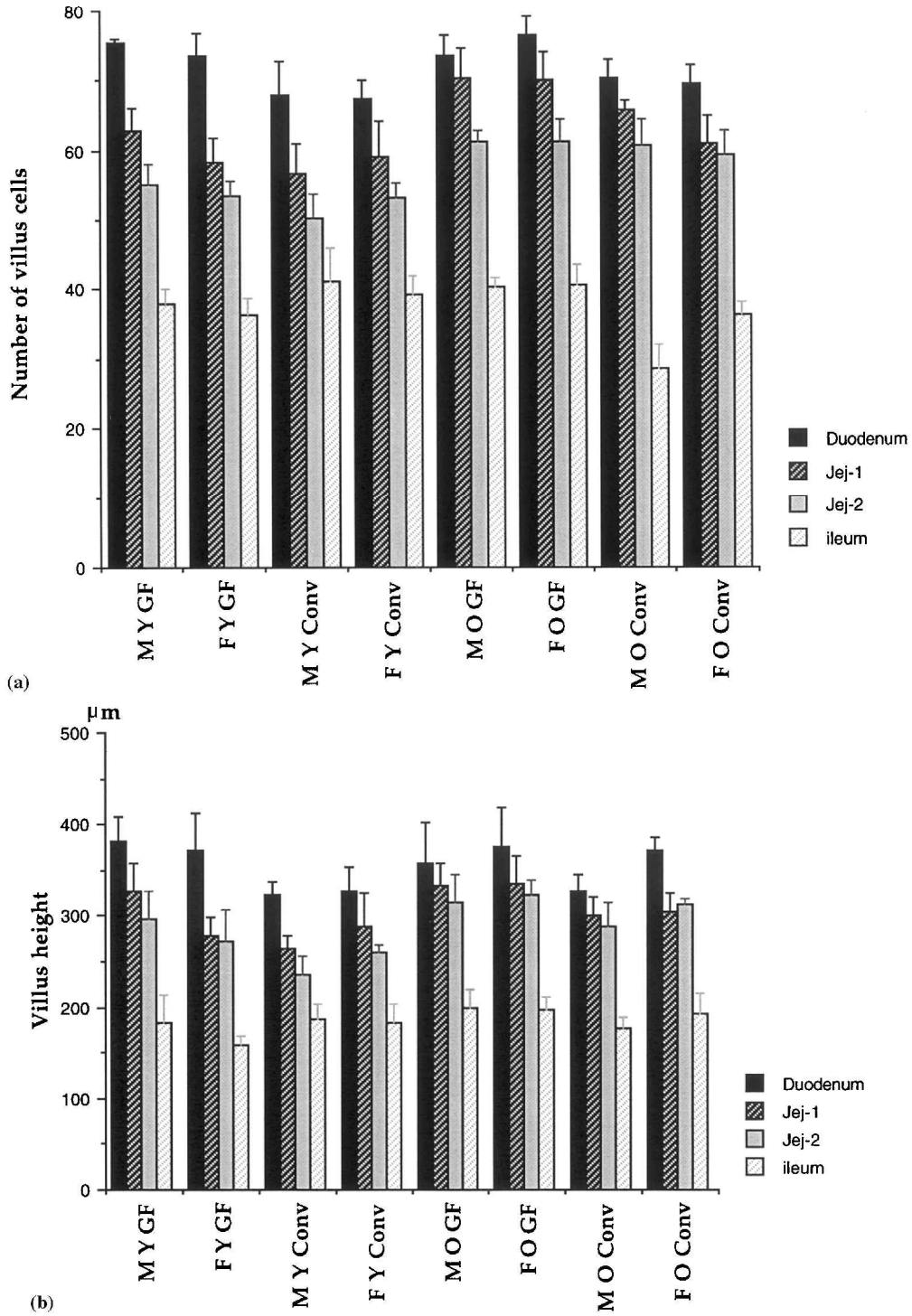

Fig. 2.

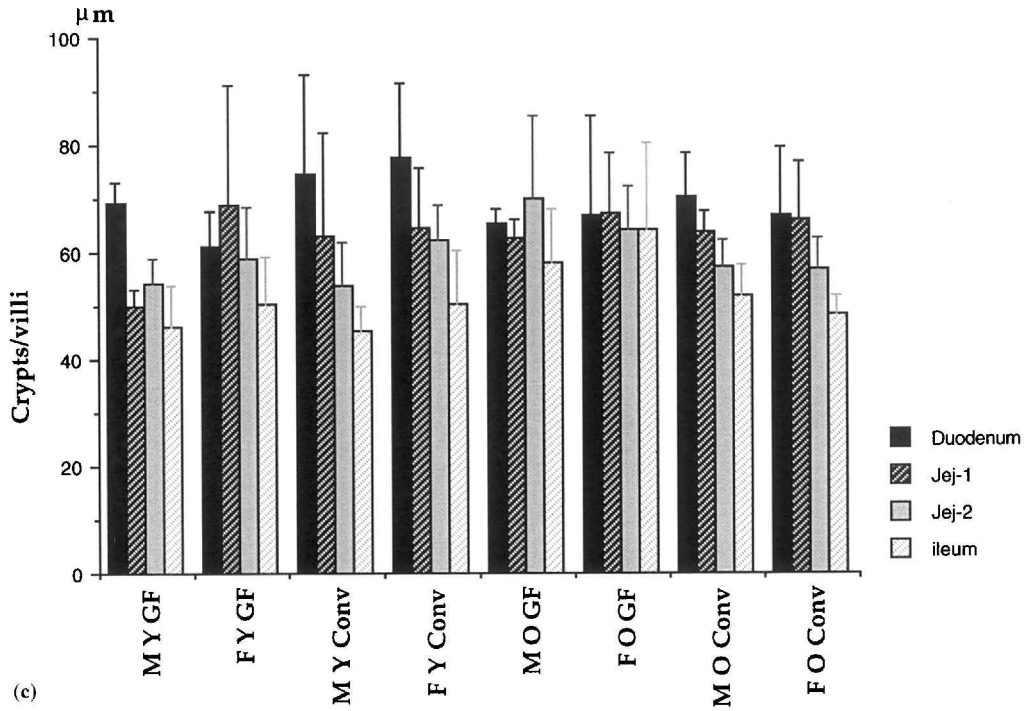

Fig. 2. Villus cells, villus height and C/V ratio in the different parts of the small intestine of the male (M) and female (F), young (Y) and old (O), germfree (GF) and conventional (Conv) rats.

intestine in F young Conv compared with their counterparts.

Number of villus cells and height of the villi (Fig. 2a, b; Table III). The number of villus cells was increased in Jej-1 and Jej-2 in M old GF. The number of villus cells and the height of the villi were increased in F old GF in the small intestine (except the proximal part), and in M old Conv in Jej-1 and Jej-2. Villus cells in old female Conv rats (F old Conv) in Jej-2 and the height of the villi in the small intestine (except the distal part) were increased.

Crypt/villus (C/V) ratio (Fig. 2c; Table III). The C/V ratio in M old GF in the jejunum at all levels and in the ileum was higher.

Microbial influence

Mitotic index (MI) (Table I). The MI in two distal parts of the small intestine in M young GF, and C-1 in M old Conv was increased compared with their counterparts.

Growth fraction (GrF) (Table II). The values of GrF were increased throughout Jej-1 in M young GF, and the distal part of the small intestine and the proximal part of the large intestine in F young Conv.

Number of crypt cells and depth of the crypts (Fig. 1a, b; Table III). The number of crypt cells was higher throughout the proximal and distal part of the large intestine in the M young Conv, and in the duodenum, the cecum as well as C-2 and C-3 in F young Conv. The number of cells in the crypt increased in the small intestine (vice versa in the proximal part of the colon) in M old GF, and Jej-1, Jej-2 (vice versa in the proximal and distal part of the colon) in F old GF. The crypts were deeper in the duodenum, C-1 and C-2 in M young Conv, and in the proximal part of the small and large intestine in F young Conv, in Jej-2, ileum in M old GF, and in the distal part of the colon in F old GF.

Number of villus cells and height of the villi (Fig. 2a, b; Table III). The number of villus cells as well as the height of the villus in the small intestine (except the distal) part, in M young GF, the proximal part of the small intestine in rats F young GF, and Jej-1 in M old GF was increased. The villus cells in F old GF at all levels in the small intestine except Jej-2 was higher.

Crypt/villus (C/V) ratio (Fig. 2c; Table III). The C/V ratio was increased in the duodenum in F young Conv, and in the ileum in F old GF compared with their counterparts.

Variation in mitotic index (MI) (Table IV). In a few animals it was observed that MI could be considerably reduced in some compartments, but was equal to the mean values in other compartments. A typical example is shown in Table IV. Such reductions were observed without regard to different variables under study. As is evident from the table, these different readings occurred in compartments when studying the MI and the values had a considerable influence on the SD.

Table IV

Individual compartmentalised variation in the MI

Group	Duodenum	Jej-1	Jej-2	Ileum	Cecum	C-1	C-2	C-3
M Old GF	7.19	5.14	5.47	4.58	10.57	3.14	2.79	3.27
M Old GF	26.2 ± 4.8	30.7 ± 2.1	28.2 ± 1.7	28.1 ± 2.4	12.4 ± 2.7	6.4 ± 3.1	9.5 ± 0.3	8.1 ± 0.7
M Old GF	22.4 ± 9.5	25.6 ± 11.6	23.6 ± 10.3	23.4 ± 10.7	12.1 ± 2.5	5.7 ± 3.1	8.1 ± 3.0	7.1 ± 2.2

On the first row, the MI of one M old GF. The third row shows mean values ($n = 5$) and the SD of the whole group of M old GF; the second row shows the mean value ± SD where values presented on the first row are subtracted from values on the third row.

DISCUSSION

In principle, intestinal cell kinetic parameters represent a balance between proliferative and exfoliative processes including apoptosis. In fact, proliferation and exfoliation might be influenced by several factors, originating either directly or indirectly from the host, the diet, or the intestinal flora. In short, these very complex interactions can be outlined as follows.

Under physiological conditions, the proliferative processes are influenced by the requirement of an adequate area for absorption of luminal, i.e. mainly diet-derived components and a similar requirement for an adequate replacement area for exfoliated cells. It is generally assumed that the composition, as well as the amount, of diet might have an influence on intestinal cell kinetic parameters. With free access to food, such factors as the level of activity and hormonal status etc. will act on the daily intake.

Exfoliation, which in fact can also be looked on as a major antimicrobial defence mechanism in the gut, might be influenced by 'aggressive factors' deriving from the diet, the flora, and the host itself. The external factors, i.e. those deriving from diet and flora, are supposed to act mainly locally and from the luminal side while host-derived factors may also act from the mucosa side and over wider areas.

In the present study, the major variables are gender, age, and microbial status. When evaluating the present data, it should be kept in mind that conglomerates of proliferative and exfoliative factors may interfere differently in the three settings.

Gender

With one exception (old Conv rats), cell proliferation tends to be higher in male than in female rats. The major factor behind this difference might be the level of androgenic hormones. As early as 1972, Wright et al (12), working with castrated Conv mice, showed that treatment with testosterone increased the MI, as well as the GrF, in the animals. They concluded that 'the action of testosterone on the GrF may constitute an important component of the general mitogenic effect of the hormone on both target and non-target tissues'. It seems reasonable to assume that testosterone has similar effects in other mammals as well. Another important factor might be intake of food. Zylan & Brown (15) showed that 2–3-month-M old Conv ate significantly more than female rats of the same age; a similar higher intake in male rats was reported by Strohmayer & Greenberg (16).

Age

The same two regulatory principles, i.e. the level of androgenic hormones and intake of food (17), may also be at work in the age groups (12, 18–20). In both GF and Conv rats, Wostmann et al. (17) found serum testosterone levels to be twice as high in 4–12-month-old rats than in 19–24-months-old rats. Although our young rats were younger than those investigated by Wostmann et al. (17), it is assumed that our rats were in early puberty, thereby producing high levels of testosterone. It is well known that strain differences might exist. Eckstein et al. (21) showed that the first estrus occurs at 36 days in Wistar rats and we have observed a similar estrus age in our AGUS rats (unpublished results). In another study, Fujita et al. (22) demonstrated a marked decrease in plasma testosterone levels in old male rats. In contrast, adult female rats show low levels of testosterone regardless of age. It is well established that young rats, regardless of sex, are more active than older rats. This activity, in addition to growth itself, increases the demand for food. With free access to food, young animals will eat more.

In this study there is a tendency for an increased GrF in all old groups in all compartments except for one (C-1 in F old Conv), i.e. an upward displacement of the cut-off position. This finding was most pronounced in the small intestine of Conv rats and these alterations in GrF, together with a tendency toward deeper crypts and longer villi in old rats, fits with previous findings of increasing proliferative zone, increased crypt depth, and increased cell numbers in the small intestine of old rats (23), and with an increased intestinal size in old mice (24). However, the mechanisms behind and the impacts of these tendencies are presently not satisfactorily elucidated.

Microbial status

The presence of intestinal flora may, either directly or indirectly, act on the intake of food and the androgen status, i.e. the two major factors influencing intestinal cell kinetics. In an early study by Wostmann et al. (17), it was shown that GF rats on a restricted diet had a serum testosterone level that was 2.5 times higher than in Conv rats. It is well established that the presence of intestinal microbial flora gives rise to metabolites of testosterone, which are not found in GF counterparts (25). Whether these metabolites exert altered androgenic stimuli other than testosterone itself has not been satisfactorily investigated.

The other important factor, i.e. intake of food, differs between GF and Conv rats. GF animals eat more than their Conv counterparts (26). Parts of the diet, especially fibres as well as exfoliated cells, are digested by the flora, and several microbial metabolic products, such as short chain fatty acids and peptides etc., are utilized by the host. The microbial metabolism is most pronounced in the distal part of the GIT. These differences between GF and Conv rats fit with our observations, i.e. there are larger villi in the upper part of the intestine of GF rats and the number of crypt cells is increased in the lower parts of the Conv rats. Interestingly, it has recently been shown that several microbial-derived substances may influence cell proliferation in human intestinal biopsies *in vitro*. Most of the substances expressed a mitogenic effect (27, 28), whereas some other substances such as butyric acid may, under some circumstances, inhibit cell proliferation (29, 30). Ingestion of whole microbial cells may also influence intestinal cell kinetics. Casas et al. (31) and England et al. (32) showed that short-time ingestion of *Lactobacillus reuteri* stimulated the development of longer villi and deeper crypts in the ileum of chicken and turkeys. It seems reasonable to assume that various microbes/microbial products may be at work in a compartmentalized and species-specific manner throughout the GIT. However, the physiological implications of those interactions are virtually unknown.

The aspects of apoptosis were not investigated in the present study. However, it has recently been assumed that apoptosis may have a partial influence on the balance of cell loss in the gut (33). Obviously, this parameter has to be studied under gnotobiotic conditions.

The example of intra-individual variations in the MI warrants some comments (Table IV). In the animals expressing this phenomenon, a normal MI was found in at least one compartment. This demonstrated that a sufficient amount of vincristine had been given. Uribe et al. (34), in an investigation of the proliferative capacity in the stomach and small intestine of Conv rats, found an uneven distribution of mitosis in the entire upper part of the GIT. Interestingly, they found that prostaglandin E_2 reduced

Table V

Experimental conditions in some previous studies of intestinal cell kinetics in rats

Authors	Animal	Age (days)	Gender	Specimens	Fasting time	Start of experiment	GF/Conv	Technique
Gordon & Bruckner-Kardoss (35)	Rats	115–123	Male	Small intestine	Free access	Early p.m.	GF/Conv	No agent
Al-Dewachi et al. (36)	Rats	90	Male	Small intestine	96 h	9.00 a.m.	Conv	Vincristine
Al-Dewachi et al. (37)	Rats	90	Male	Upper jejunum	Unknown	9.00 a.m.	Conv	Vincristine 2.5 h before killing
Cairnie & Bentley (38)	Rats	56	Male	Small intestine	Unknown	9.30 & 10.30 a.m.	Conv	^{3}H-thymidine
Uribe et al. (39)	Rats	Unknown	Unknown	15 cm from pyloric region of jejunum	24 h	8.30 a.m.	GF/Conv	Vincristine (0, 30, 60, 90, 150 min)
Alam et al. (40)	Rats	Unknown	Unknown	Ileum and colon	24 h	8.30 a.m.	GF/Conv	Vincristine (0, 30, 60, 90, 150 min)
Kwo-yih Yeh (41)	Rats	6, 16, 22	Unknown	Duodenum	Free access	7.30 and 8.00 a.m.	Conv	^{3}H-thymidine

and indometacin increased the number of areas with low mitotic scores in a dose-related manner. However, the exact mechanism(s) behind this phenomenon, as well as the possible consequence(s) for the animal, are still far from clear. Prolonged fasting time, which is known to reduce the rate of mitosis, can be ruled out since our animals fasted for periods as short as 2 h before injection of vincristine.

The data given in Table V clearly indicate that the three factors supposed to have marked influences on intestinal cell kinetics, i.e. gender, age, and microbial status, have previously not been simultaneously investigated (35–41). The data also demonstrate variations in other experimental conditions such as fasting time and techniques to demonstrate mitosis. Obviously, previous results should be interpreted with great caution. Our data demonstrate a compartmentalized influence of gender, age, and microbial status on intestinal cell kinetics under standardized experimental conditions utilizing vincristine to arrest mitosis. The importance of well-standardized experimental conditions when studying intestinal cell kinetics is strongly underlined.

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