Development of the gastric morphology and fornical bacterial/epithelial association in the white-tailed rat *Mystromys albicaudatus* (Smith 1834)

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The growth and development of the stomach, gastric epithelia and associated microflora of *M. albicaudatus* have been quantified and a chronology of morphological, histological and ultrastructural events documented. In neonates, a monogastric stomach was present with distinct separation of glandular (antral) and cornified (fornical) regions. Development of the fornix ventricularis and 'grenzfalte' characterized the transitional period and gave rise to the bilocular condition. An early autochthonous microflora of facultative cocci and cocco-bacilli became established. In the infantile phase, fornical papillae developed and provided microhabitats for colonization by symbiotic anaerobic bacilli. Cocci and cocco-bacilli remained attached to (or successive populations colonized) the folded fornical epithelium and pregastric pouch. All common bacteria , were attached to the gastric epithelium by a capsular (slime) layer in palisade formation. The synchrony of events in gastric development (appearance of papillae, ingestion of solid food and the colonization of papillae by bacilli), the numerical abundance of papillae bacilli, and the absence of epithelial damage, suggest that the bacilli are autochthonous, symbiotic and aid the digestive processes of *M. albicaudatus*.

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Die groei en ontwikkeling van die maag, maag-epiteel en geassosieerde mikroflora van *M. albicaudatus* is gekwantifiseer en die chronologie van morfologiese, histologiese en ultrastrukturele gebeurtenisse is gedokumenteer. In pasgeborenes was 'n monogastriese maag aanwesig met duidelike skeiding van klieragtige (antrum) en verhoringde (fornix) gebiede. Ontwikkeling van die fornix ventricularis en 'grenzfalte' het die oorgangstydperk gekenmerk en het gelei tot die tweekamertoestand. 'n Vroeë outochtoniese mikroflora van fakultatiewe kokke en kokko-basille het gevestig geraak. In die infantiele fase het fornix-papille ontwikkel en mikrohabitatte voorsien vir kolonisasie deur simbiotiese anaërobe basille. Kokke en kokko-basille het vasgeheg gebly aan die gevoude fornixepiteel en die pregastriese sak (of opeenvolgende bevolkings het dit gekoloniseer). Alle gewone bakterieë was deur middel van 'n kapsulêre (slym) laag in palissade-formasie aan die maagepiteel geheg. Die sinchronisme van gebeure in maagontwikkeling (verskyning van papille, inname van vaste voedsel en kolonisasie van papille deur basille), die groot hoeveelheid basilli aan die papille en die afwesigheid van skade aan die epiteel dui daarop dat die basille outochtonies en simbioties is en bydra tot die verteringsproses van *M. albicaudatus*.

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The gastric morphology of adult *Mystromys albicaudatus* has been described (Maddock & Perrin 1981). The papillated bilocular hemiglandular stomach comprises a glandular antrum and a keratinized pars oesophagea divided into a papillated fornix and non-papillated pregastric pouch (PGP); cardiac, fundic and pyloric glands are present in the antrum (Maddock & Perrin 1981). Gastric papillae have been recorded in only four rodent species; *Myospalax myospalax* (Carleton 1973), *Cricetomys gambianus* (Caiman, Quenum, Kerrest & Goueffon 1960), *Tachyoryctes splendens* (Rahm 1976, 1980) and *Mystromys albicaudatus* (Perrin & Curtis 1980) and their precise adaptive functions are unknown. Maddock & Perrin (1981) proposed that the papillae of *M. albicaudatus* increase surface area for bacterial attachment thus facilitating a symbiotic relationship between the rodent and the gastric micro-organisms.

To further the earlier morphological description of this rat's stomach, development of gastric features was observed in rats from birth to 80 days of age. Previous post-natal studies of this rodent were restricted to gross physical and behavioural investigations (Meester & Hallett 1970; Hallett & Meester 1971). In this more circumscribed and detailed examination, gastric development was studied at gross, light and electron microscope levels.

During the study various factors which may be responsible for stimulating papillary development in the juvenile stomach were examined, and a morphological investigation of the association between the autochthonous bacteria (in juvenile and adult white-tailed rats) and the fornical epithelium was initiated. In addition to contributing to a clearer understanding of the gastric morphology of the white-tailed rat it was considered imperative to understand these associations before beginning a study of the role of bacteria in digestion in this rodent.

Materials and Methods

Development of the gastric morphology

Breeding colonies of *Mystromys albicaudatus* were established and data obtained from 34 animals (14 litters) between 0 and 80 days of age. Stomachs (with and without contents) were weighed before fixation in Bouin's and stomach weight, expressed as a percentage of body weight, was used as a relative measure of gastric development.

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Procurement and preparation of tissues for histological and scanning electron microscopical examination were done according to the techniques used by Maddock & Perrin (1981). Since the stomach wall stretched with degree of filling, absolute thickness was of questionable value. Therefore, in the histological sections, the number of cell layers in each stratum was counted and used as a measure of epithelial thickness (Sakata & Tamate 1976). However, the keratinized papillae were not tensile and papillary stratum corneum thickness was determined by direct measurement. The number of (developing) papillae per mm transect of fornical epithelium was counted and although distribution was not regular, the results were expressed as the mean number of papillae per cm^2 of fornical epithelium. Incremental (relative) growth of the papillae was calculated from the formula:

$$\frac{A - B}{t}$$

where A and B represent the two quantitative values separated by time t in days.

Weaning was defined as the earliest age at which 50% of the juveniles could survive without parental care. Young from subsequent litters were removed from their mothers at progressively earlier ages and survival was noted in solitary juveniles provided with food and water *ad libitum*.

The gastric nomenclature adopted for adult *M. albicaudatus* (Maddock & Perrin 1981) was used to describe juvenile morphology; two additional definitions were necessary to distinguish juvenile and adult features. They were:

(i) Papillary bud — a bulbous projection of the corpal stratum germinativum layer into the lamina propria. The stratum corneum of the papillary bud (PB) extends into the gastric lumen (except during the first week of life). The PB's are the gastric papillary precursors and resemble epithelial pegs found between connective tissue papillae of cheek epithelium (Landay & Schroeder 1977) and the epidermis (Ham 1969). A PB is recognized as a papilla when it reaches 10% of the mean adult papillary length.

(ii) Interpapillary epithelium — the keratinized, stratified squamous epithelium between the PB. It is the forerunner of adult folded fornical epithelium (FFE ≡ FCE Maddock & Perrin 1981) but does not assume the highly folded condition until the third or fourth week after birth.

Bacterial/epithelial associations

Adult *M. albicaudatus* were used to ensure the presence of an indigenous alimentary flora. Surface examination of the non-glandular epithelium and determination of bacterial/epithelial associations were facilitated by treating rats with different doses of the general antibiotic, oxytetracycline. Depending on dosage, the drug rendered the rodents' gastro-intestinal tract germ-free (sterile) or largely reduced the gut flora (specially treated rats, STR). Conventional animals (normal gastro-intestinal flora) were also used in this comparative study.

Pairs of conventional rats were housed in an animal room in plastic cages with straw bedding. Tap water and commercial rat pellets were supplied *ad libitum*.

Specially treated rats (STR) were housed individually in elevated stainless steel cages so that faeces and urine collected outside the cage. The cage was kept in a laboratory free from other animals and was entered only once a day for cleaning and feeding purposes. No bedding was supplied and the animal was administered 50 mg of oxytetracycline orally per day for five days. Tap water and commercial pellets were supplied *ad libitum*.

Sterile rats were housed and treated in the same way as STR with the following exceptions. The cage was placed in a sterile plastic container with a glass cover which was removed once a day to administer 100 mg of oxytetracycline orally, and for cleaning and feeding purposes. Water was acidified to 0,001 M with HCl and rat pellets were autoclaved. Both were supplied *ad libitum* throughout the five-day treatment.

Rats were killed with chloroform anaesthesia. STR and sterile rats were killed on the sixth day of treatment and tissues were prepared for microscopy according to the techniques used by Maddock & Perrin (1981). Malachite green was used as a spore stain for bacteria (Cruickshank, Duguid, Marmion & Swain 1975).

Three-dimensional views of the papillary surface were obtained by stereophotography. Specimens prepared for scanning electron microscopy (SEM) were photographed with a JEOL JSM/VS scanning electron microscope. The specimen was then rotated through 5° and a second photograph taken, thus producing a stereopair.

Results

Development of gastric morphology

Post-natal fornical changes (0 – 80 days) were separated into four developmental periods; neonatal (0 – 7 days), transitional (8 – 16 days), infantile (17 – 25 days) and post-weaning (26 – 80 days) on the basis of anatomical features and the appearance of adult characteristics.

Neonatal period (0 – 7 days)

Stomach shape at birth (Figure 1) differed from the adult bilocular form (Table 1); the fornix ventricularis was small,

Figure 1 The bisected stomach of a two-day-old *M. albicaudatus*. The pregastric pouch (PGP) constriction and grenzfalte are absent and the stomach has a monogastric shape. Papillary buds (PB) are not visible. A = antrum; F = fornix; O = oesophagus. Stomach length = 10 mm.

and sacculations of the bilocular hemiglandular stomach were absent. However, this monogastric condition was lost towards the end of the first week of life and the stomach began to acquire the adult form.

Histologically distinct glandular (antral) and non-glandular (fornical/pars-oesophageal) regions were visible at birth, and PB occurred in the keratinized fornix (2704 ± 25/cm^2 Figure 2). Significant differences existed between the number of cell layers in the papillary buds and interpapillary epithelium ($P < 0,05$ Figure 3) and between the thickness of the papillary stratum corneum at birth and at 7 days ($P < 0,001$ Table 2), suggesting rapid growth of the PB.

The antrum contained pyloric and branched cardiac glands with irregular surface topography. Mucus cells occurred in the fundus but parietal and chief cells were absent.

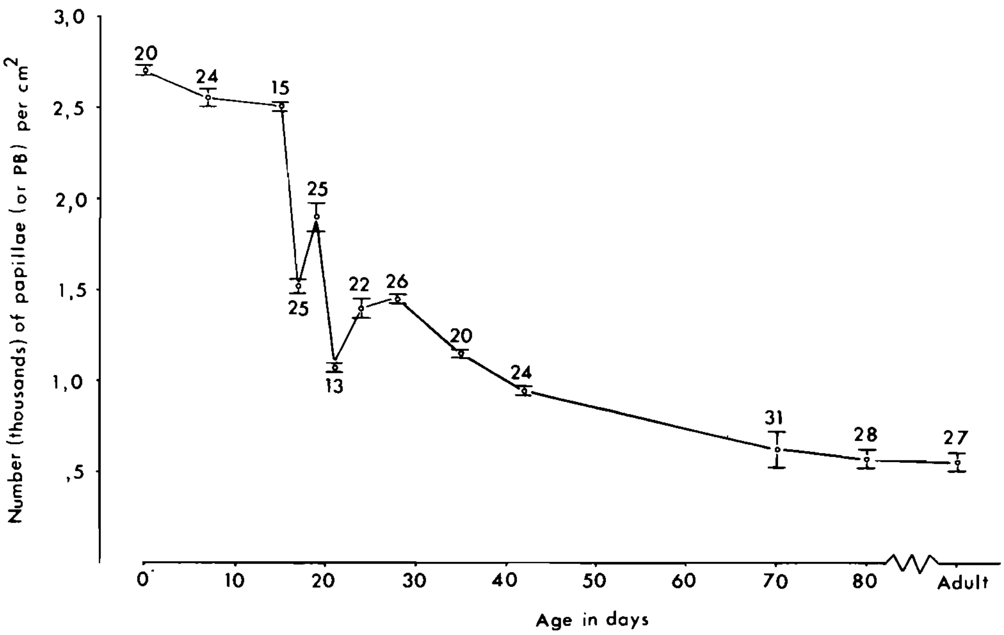

Figure 2 Number of papillae (buds) per cm^2 of fornical epithelium. Vertical bars = one standard deviation; sample sizes are indicated.

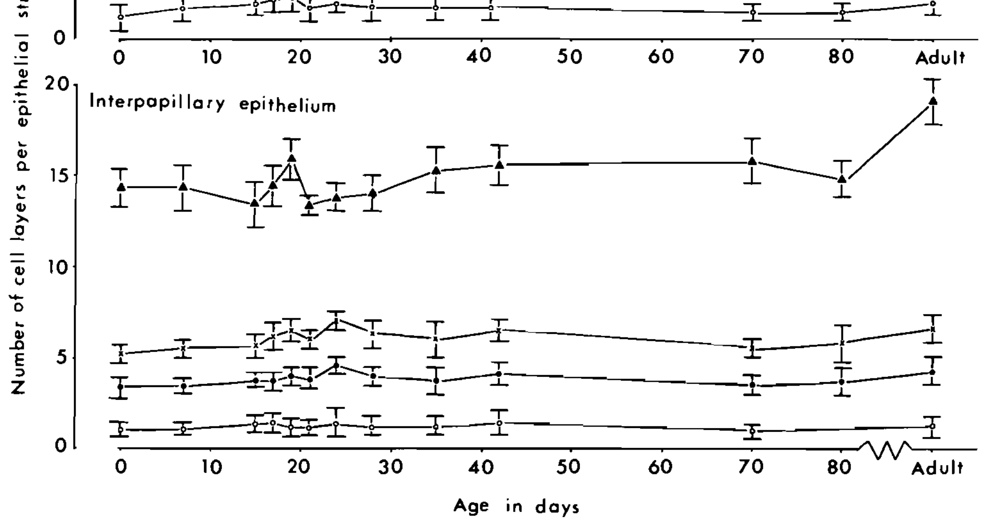

Figure 3 Post-natal development of the gastric papillary and interpapillary epithelia of *M. albicaudatus* showing the mean number of cell layers per epithelial stratum. o = stratum basale; ● = stratum spinosum; x = stratum granulosum; ▲ = stratum corneum. Vertical bars = one standard deviation; sample size = 20.

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Table 1 Summary of the major macro- and microscopic developmental changes in the stomach of *Mystromys albicaudatus*

Gross observations	Histological observations
Neonatal period (0 – 7 days)	
Unilocular stomach, glandular & non-glandular regions present. No fornical papillae, PGP, or grenzfalte. Fornix ventricularis small. Tissues translucent. Milk present.	Tissue layers (except epithelium) resemble adult. Fornix has level topography & consists of four strata. PB, numerous near oesophageal region, do not affect topography. Few attached bacteria. Cardiac & pyloric glands present; fundus lacks parietal & chief cells.
Transitional period (8 – 16 days)	
Bilocular shape. Small epithelial protrusions in fornix. Fornix & PGP indistinct. Grenzfalte appears & fornix ventricularis develops. Milk present.	Fornical epithelium undulatory. PB occur between small folds. PB stratum corneum thickens. Parietal & chief cells present in the fundus.
Infantile period (17 – 25 days)	
Papillae appear; fornix is thus distinguished from the PGP. Tissues translucent. Milk present at 17 days. Weaned between 20 & 25 days.	Papillae mainly in gastro-oesophageal region. Papillae have thick bacterial layers similar to adult condition.
Post-weaning period (older than 25 days)	
Gastric tissue opaque; antrum thick, fornix thinner. Stomach divisions visible with the naked eye. Solid food in stomach.	FFE developed. Gradual increase in papillary stratum corneum causes increase in papillae length.

Table 2 Increase in thickness of papillary stratum corneum occurring with age

Period (days)	Thickness mm	*n*	Increase %	Incremental growth ($\times 10^{-3}$)
Birth	0,012 ± 2,8($\times 10^{-3}$)	10	–	–
0 – 7	0,017 ± 2,2($\times 10^{-3}$)	10	41,7	0,7
7 – 10	0,022 ± 1,9($\times 10^{-3}$)	7	29,4	1,6
10 – 15	0,058 ± 0,011	8	163,6	7,2
15 – 17	0,245 ± 0,036	10	332,4	93,5
17 – 24	0,389 ± 0,030	10	58,8	20.6
24 – 28	0,414 ± 0,015	9	6,4	6,3
28 – 35	0,437 ± 0,012	10	5,6	3,3
35 – 42	0,497 ± 0,048	10	17.5	10,6
42 – 70	0,990 ± 0,062	10	99,2	17,6
70 –	2,340 ± 0,082	10	136,4	–

Transitional period (8 – 16 days)

During this period development of the fornix ventricularis resulted in elongation of the forestomach. Also evident at this age was the 'grenzfalte' and constriction between the glandular and non-glandular regions, causing sacculation of of the stomach (Table 1). At the end of this period the stomach had acquired a bilocular shape but lacked papillae (Figure 4) and the complete gastric divisions of the adult.

Figure 4 The bisected stomach of a 15-day-old rat. Papillary buds are visible in the fornix (F) and the grenzfalte (G) is seen between the antrum (A) and fornix (F). Stomach length = 18,5 mm; O = oesophagus; D = duodenum.

Figure 5 The fornical epithelium of a 10-day-old rat illustrating presence and distribution of bacteria which occur in groups separated by bacteria-free zones (Bf).

Microbial colonization of the developing stomach was sparse compared with the adult FFE (Maddock & Perrin 1981) and groups of cocci, either single or in branched chains, covered parts of the epithelium (Figure 5).

The number of PB in the fornix was similar to that at birth ($P > 0,05$; Figure 2) but the malpighian layers were significantly thicker in the PB than in the interpapillary epithelium ($P < 0,005$; Figure 3), owing to an increase in the PB spinous and horny cell layers. The thickness of the PB horny layer also increased significantly during the transitional period ($P < 0,001$; Figure 6). A high incremental growth, $7,2 \times 10^{-3}$ (Figure 6) occurred between the ages of 10 and 15 days which increased during the last two days of the transitional period. These differences between the PB and interpapillary epithelia are suggestive of slightly different keratinization procedures.

At eight days of age parietal and chief cells were apparent in the fundus (Figure 7).

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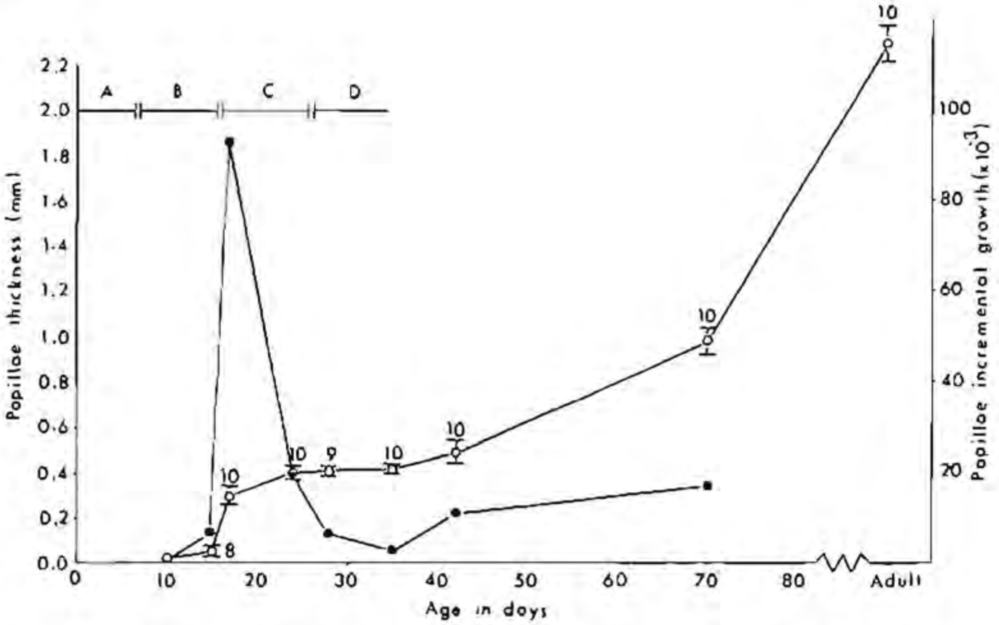

Figure 6 Absolute increase (O) and incremental growth (●) of papillary stratum corneum in *M. albicaudatus*. Vertical bars = one standard deviation; A = neonatal; B = transitional; C = infantile; D = post-weaning periods.

Figure 7 Section through the lower fundic region of the stomach of an eight-day-old rat. A few light staining parietal (Pa) and dark staining chief (Ch) cells have developed during the first week of life.

Table 3 Post-natal development (in days) of certain features associated with weaning in *Mystromys albicaudatus*

	Present study			Meester & Hallett 1970
	Mean	Range	*n*	Range
Eyes open	21,0 ± 1,8	17 – 25	21	16 – 20
Weaned	22,4 ± 1,5	20 – 25	20	32

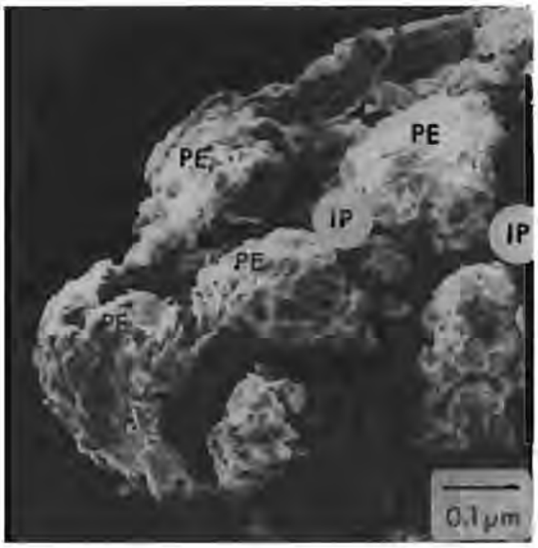

Figure 8 Papillary buds of the 15-day-old rat stomach developed into papillae by 17 days. The interpapillary (IP) and papillary (PE) epithelia were also discernable.

Infantile period (17 – 25 days)

The infantile period ranged from the earliest at which solid food was sampled to the latest recorded weaning age (Table 3). The high incremental growth of the previous period continued and, between 15 and 17 days, the PB stratum corneum had a growth of $93,5 \times 10^{-3}$, the highest during development (Figure 6).

As a result of this rapid growth the PB reached 10% of the adult papillary length, and with the appearance of the papillae (Figure 8), the distinction between the PGP and fornix became clear. Consequently the infantile stomach resembled that of the adult in all aspects except relative mass (Figure 9) and papillary length (Figure 6).

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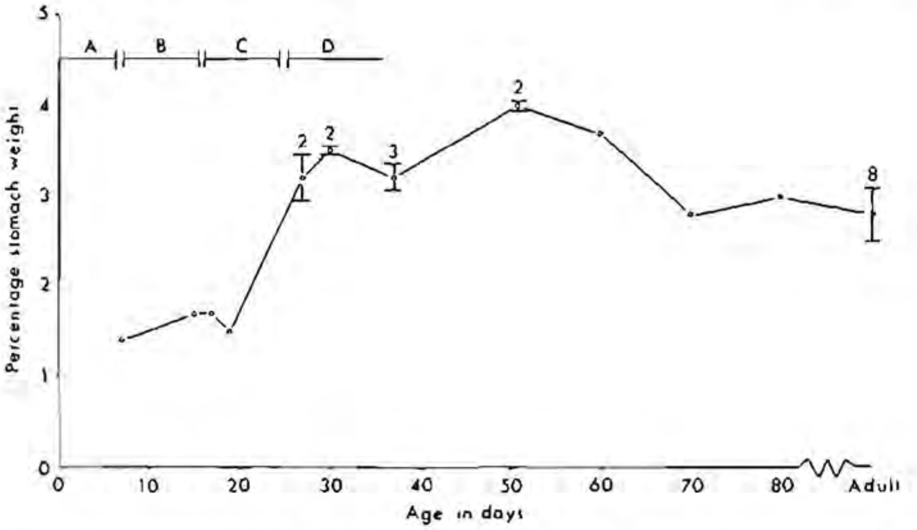

Figure 9 Post-natal development of the stomach of *M. albicaudatus*. Empty stomach weight expressed as percentage of body weight. Vertical bars = one standard deviation; sample sizes indicated. A = neonatal; B = transitional; C = infantile; D = post-weaning periods.

Figure 10 The surface of a papilla from a 17-day-old rat. Once the papillae had formed, large numbers of bacilli appeared in the stomach and attached to the papillary epithelium.

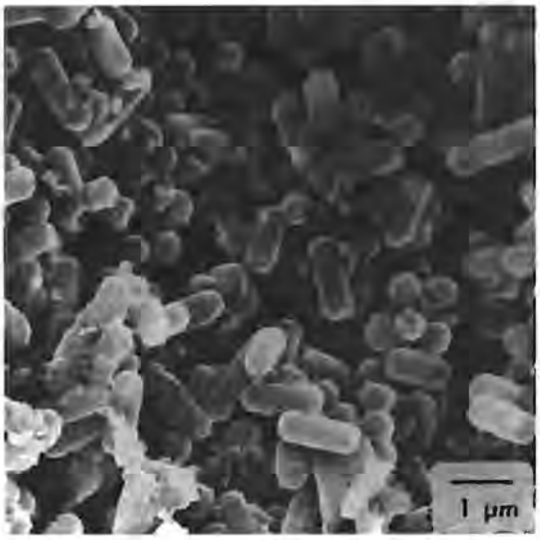

Figure 11 The folded fornical epithelial surface of a 17-day-old rat. Although bacilli colonized the corpus at this age, the microbial flora of the interpapillary epithelium (cocco-bacilli) was similar to that of younger animals. The bacteria were, however, more abundant in the older rat and were densely packed in many areas.

Concomitant with the development of the papillae, was their rapid colonization by numerous bacteria; bacilli, similar to those in the adult, attached to the papillae (Figure 10) while cocci and cocco-bacilli, seen in the fornix of younger rats, were restricted to the interpapillary epithelium (Figure 11). The simultaneous appearance (and persistence) of the papillae and the bacilli suggests that these bacteria are specific to the papillae and to the papillary micro-habitats. Juveniles between the ages of 20 and 25 days showed no major morphological changes during these six days (Figure 12).

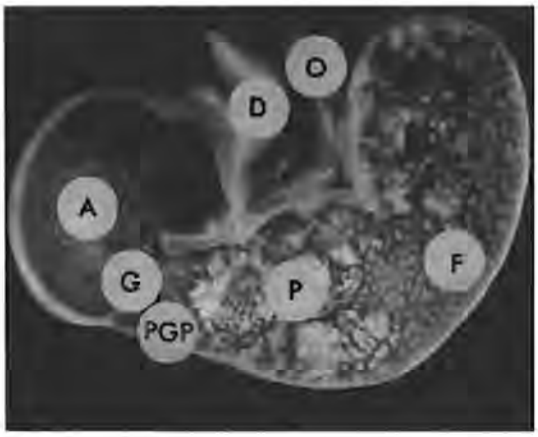

Figure 12 The bisected stomach of a 25-day-old rat. Papillae (P) are present in the elongated fornix (F) and the pre-gastric pouch (PGP) and grenzfalte (G) are obvious. A = antrum; D = duodenum; O = oesophagus. Stomach length = 24 mm.

Post-weaning period (26 – 80 days)

All juveniles entering this period could survive independently (Table 3) and had a gastric morphology similar to the adult

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(Table 1, Figure 13). Their gastric physiology and microbial ecology probably also resembled the adult.

Rats older than 25 days had adult fornical features in that the FFE and papillary epithelia were distinct (Figure 14) and the papillae differed from the adult in length only (Figure 6). Incremental papillary growth, although considerably less than during the previous period, was still high; absolute papillary growth was gradual, the papillae increasing from 0,38 mm at 26 days to almost 1 mm at 70 days (Figure 6). Habitat preferences amongst the bacteria were maintained and bacilli, characteristic of adult papillae, were abundant (Figure 15).

General observations

The marked changes in relative stomach weight of *M. albicaudatus* did not correspond to the developmental divisions (Figure 9). Rats between the ages of 7 and 19 days had a constant stomach weight equal to 1,6% of body weight, but this increased after weaning to a peak of 4,0% at 51 days (Figure 9). Thereafter relative stomach weight decreased and the 70-day-old rat stomach reached the adult value of about 3% of total body weight (Figure 9). The rapid increase in stomach weight was due to the ingestion of solid food, which resulted in an increase in stomach size and gastric musculature out of proportion with body weight increase. After 50 days of age, body and stomach weight increased proportionately.

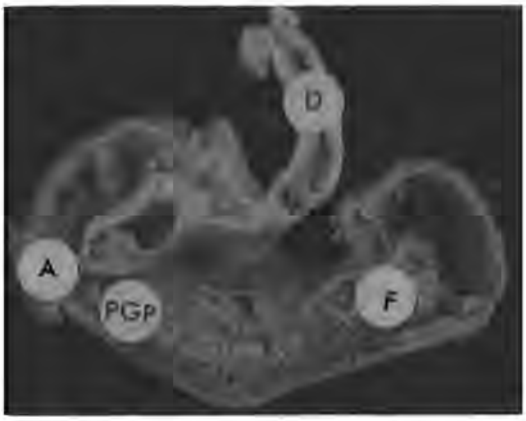

Figure 13 The bisected stomach of a 42-day-old rat. Gastric divisions of the adult (thick-walled antrum (A): papillated fornix (F) and PGP constriction) can be seen clearly; the papillated bilocular hemiglandular condition is evident. Papillae have not reached the adult length. Stomach length = 28 mm.

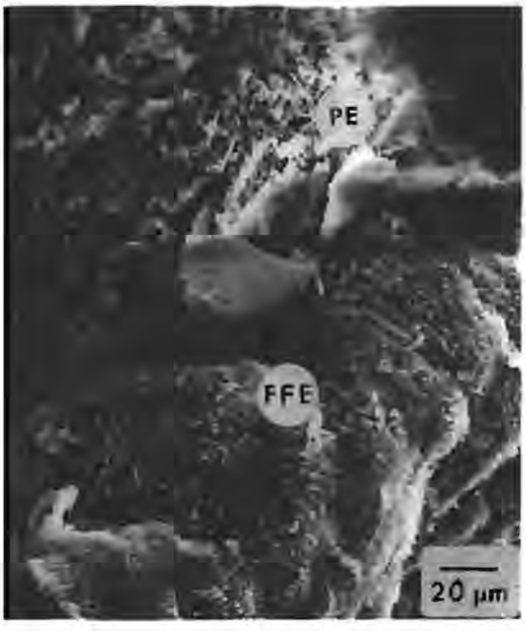

Figure 14 Fornix of a 42-day-old rat. This micrograph of a papilla base reveals the clear difference between the FFE and papillary epithelium (PE) particularly the difference in bacterial cover and cell size of the two regions. Bacteria-free areas are few on the FFE at this age.

Figure 15 The papillary surface of a 28-day-old rat. Rod-shaped bacilli occupy the surface while cocci or cocco-bacilli are rare.

The density of papillae declined steadily from birth to adulthood (Figure 2). This apparent anomaly was a result of stomach growth; the absolute number of papillae remained constant, but, because of the increase in stomach and papillae size, their relative abundance decreased. The rapid decrease in relative papillae abundance between 15 and 21 days corresponded to rapid papillary growth after 15 days of age (Figures 2 & 6, Table 2).

Bacterial/epithelial associations

For a clear understanding of the gastric bacterial/epithelial association established during the infantile period (17 – 25 days of age) a three-dimensional, highly magnified view of the epithelium was necessary. This requirement was met by collating scanning and transmission electron micrographs and by using SEM stereopairs (although stereopairs are not presented in this paper). The papillary surface from conventional rats was completely obscured by bacteria and food when viewed with a microscope but when examined in conjunction with the tissues from STR and sterile rats, the nature of the association became apparent.

The papillary bacteria occurred in the intercellular space between desquamating cells, here called microhabitats (Figure 16). Some intercellular spaces were too narrow for bacterial colonization but all larger areas contained these microbes (Figure 16). Figure 17 is a scanning electron

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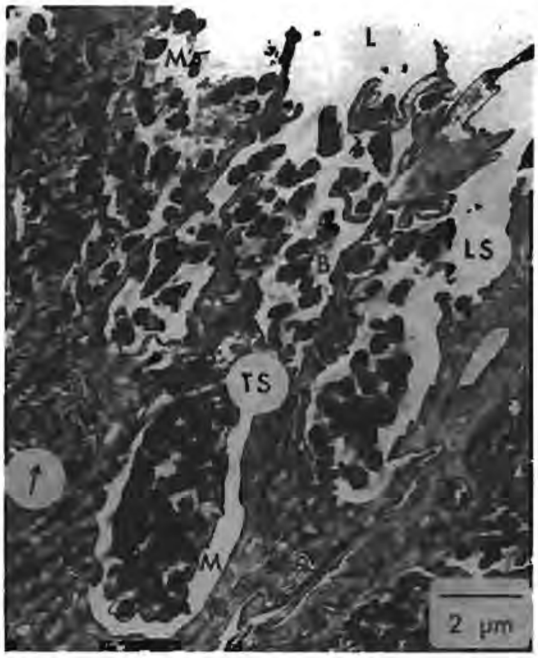

Figure 16 Transmission electron micrograph of the peripheral margin of a papilla of a conventional rat. Bacterial colonization of the spaces between the horny cells (M) is seen although some intercellular spaces are narrow and consequently bacteria-free (↗). Longitudinal (LS) and transverse sections (TS) of these microhabitats are evident. B = bacteria; L = lumen.

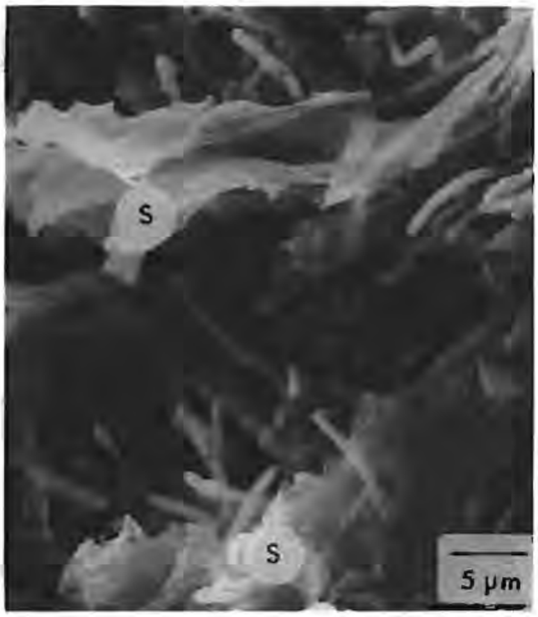

Figure 17 A scanning electron micrograph of a microhabitat from a specially treated rat. Large numbers of bacteria are orientated parallel to the microhabitat lumen. S = desquamating cells that would be covered with bacteria in conventional rats. Some bacteria have truncated ends while others taper gently to a point.

micrograph of a habitat similar to those shown in section in Figure 16 and more clearly reveals the bacteria-filled microhabitats and their relationship to thin, irregular desquamating papillary cells.

Three-dimensional views of the microhabitats showed the association between the bacteria and epithelium. Particularly evident was the parallel alignment of the bacteria (Figure 18) and uneven topography which resulted from the

Figure 18 Vertical view of a microhabitat on a papilla from a conventional rat. Because the desquamating cells are colonized by bacilli, the papillary epithelium is not visible.

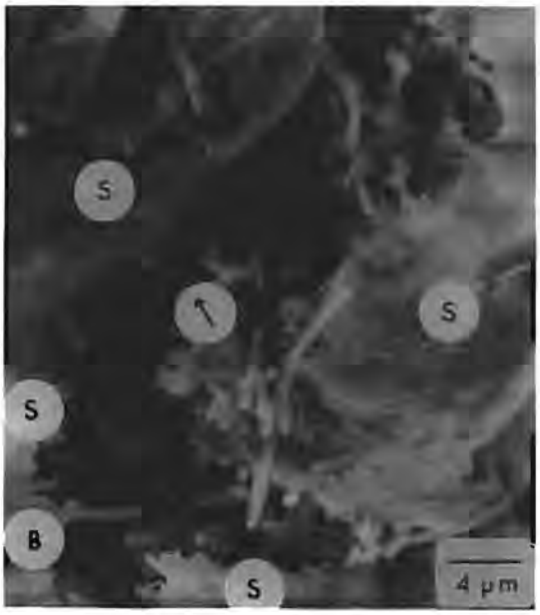

Figure 19 Surface view of a papilla of a specially treated rat. The superficial desquamating cells (S) are free of bacteria but the deeper regions are still colonized by some bacilli (B). Microhabitats extending beneath the desquamating cells are indicated (↗).

sloughing cells (Figure 19). Microhabitats were seen between these cells (Figures 18 & 19). (Note that these micrographs (Figures 17 – 19) were from STR, and in conventional rats the microhabitats are concealed beneath a dense mat of micro-organisms and food.) Because of this thick bacterial covering these micro-environments may differ from lumen or surface conditions. This idea was supported by the finding that some bacteria persisted in the deeper microhabitats of those rats treated with antibiotic (Figure 20).

Habitat separation of bacilli and cocci/cocco-bacilli, noted in juvenile rats, was seen in the adult fornix. Apart from a few cocci, presumably dislocated from elsewhere in

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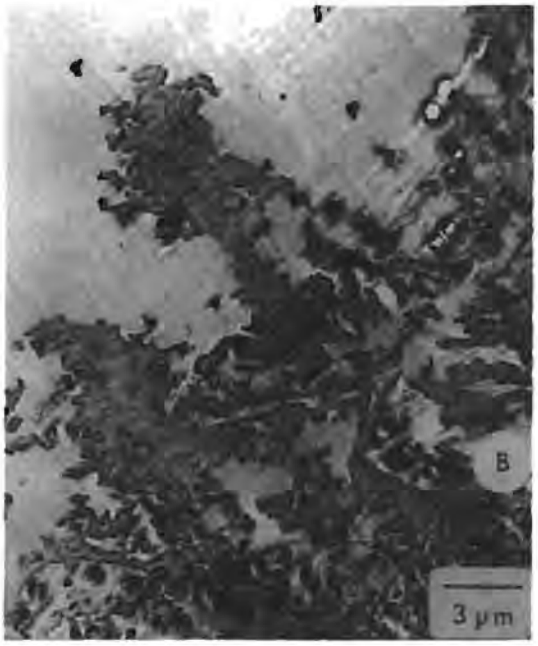

Figure 20 Transmission electron micrograph of the papillae from a sterile rat. Comparison with Figure 16 reveals that treatment with oxytetracycline removed most bacteria (B) although some remain in the deeper microhabitats.

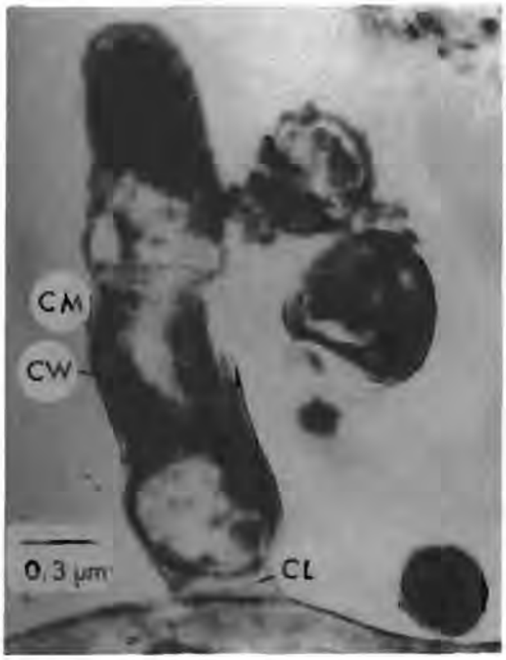

Figure 22 A high power micrograph of a dividing bacterium attached to a desquamating papillary cell. Note the thickened capsular layer (CL) at the site of attachment, the lack of intracellular detail and the conspicuous cell membrane (CM) and cell wall (CW).

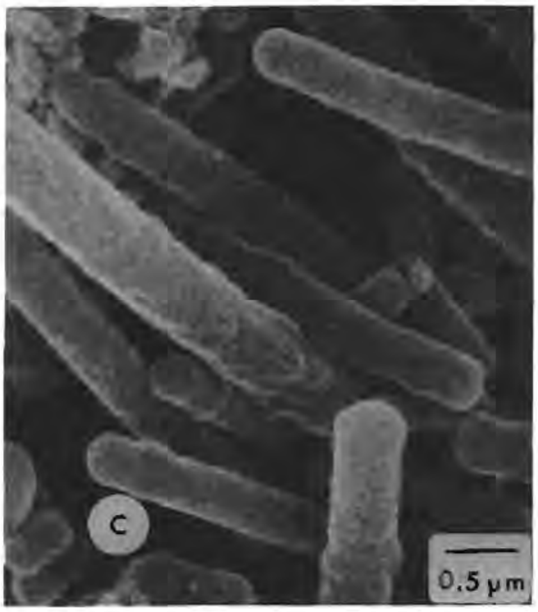

Figure 21 A high power micrograph of the bacilli that colonize the papillae. These bacilli occur singly and some cocci (C) are visible amongst the rods.

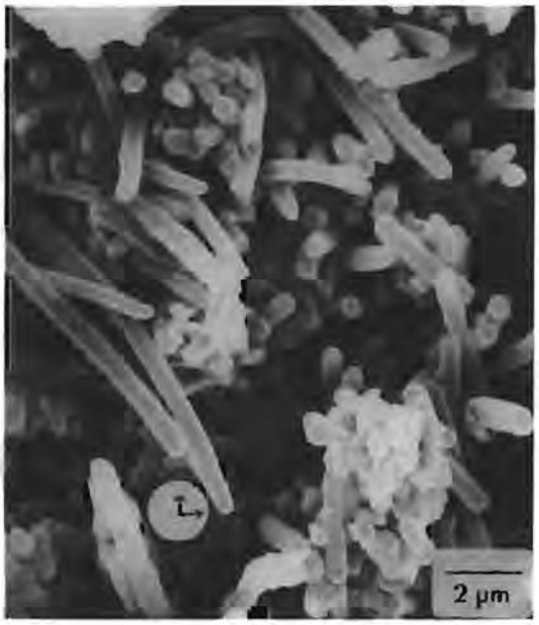

Figure 23 Bacilli on the papillae surface. Two morphologically different bacilli types are noted; one with gently tapering ends, the other with truncated ends (↗).

the system or transient in the gut, non-segmented single bacilli (6,5 μm by 0,7 μm; Figures 21 & 22) dominated the papillary surface and underlying microhabitats (Figures 17 & 21).

The bacilli lacked special appendages (flagellae, pili or filaments) and were attached end-on to the epithelium by means of a thickened microbial capsular layer (Figure 22). No inflammation, damage or penetration of the epithelium was caused by the bacteria. There was no evidence of spores in TEM sections (Figure 22) or in isolated bacteria stained with malachite green, but bacteria *in situ* were seen in various stages of vegetative division (Figure 22).

The bacilli differed in that some had sharply truncated ends while others tapered gently, or had an irregular surface (Figure 23). These differences may be indicative of different species.

A triple-layered cell membrane (about 90 Å thick) consisting of electron-dense outer layers enclosing a lighter inner zone was seen in the high power transmission electron micrographs (Figure 22). A thicker, single-layered, electron translucent cell wall (approximately 280 Å thick) was visible

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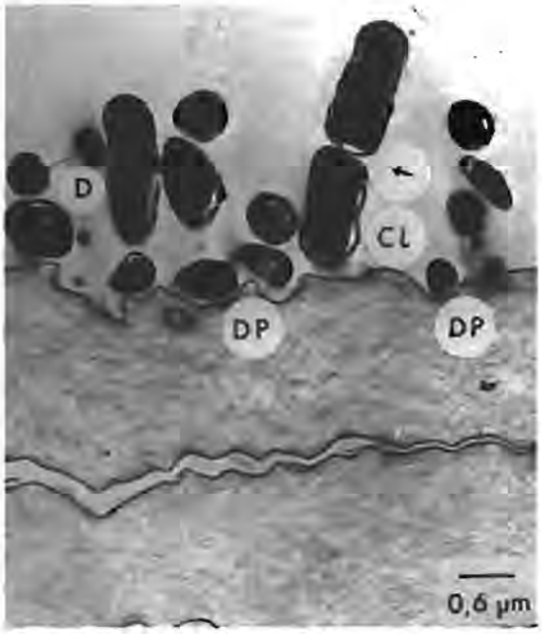

Figure 24 Bacterial attachment to the FFE of a conventional rat. One bacterium has just completed vegetative division (↗) while another is undergoing division (D). Details of cell wall and cell membrane morphology are visible, as is the capsular layer (CL) which mediates bacterial attachment. Most bacteria occur in shallow depressions (Dp).

Figure 25 Scanning electron micrograph of the cocci and cocco-bacilli that colonize the FFE. Bacilli are uncommon; those evident may have been dislocated from the papillae.

although an additional thin, electron-dense layer may have been present (Figure 22). A capsule or slime layer surrounded the bacterium and was considerably thickened (three to five times) at the site of epithelial attachment (Figure 22).

Compared to the papillae, the FFE and PGP presented different epithelial surfaces for bacterial colonization, and microhabitats similar to those on the papillae were absent. Consequently, relatively fewer, morphologically different bacteria (cocci and cocco-bacilli) were present on the outermost FFE and PGP cells. Cocco-bacilli, about 1,0 µm by 0,4 µm in size, attached to the epithelium in palisade formation and constituted the majority of the FFE and PGP microbiota (Figures 24 & 25). Bacilli were rarely seen and did not attach to the epithelia. No appendages or endospores were present but various stages of vegetative division were seen (Figure 24). The cell wall comprised two layers; an electron translucent inner zone which was three to four times thicker than the dense outer layer (Figure 26). The total cell wall thickness was about 150 Å.

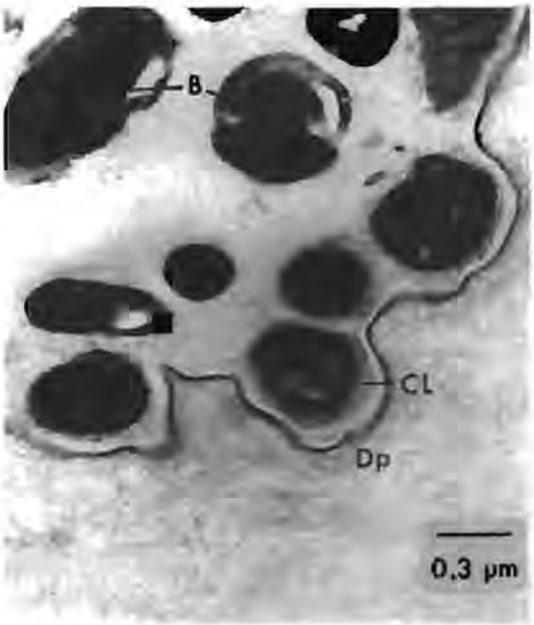

Figure 26 Bacterial attachment to the FFE in conventional rats showing their presence in deep depressions (Dp) in the keratinized cells. The double layered cell wall is visible. CL = capsular layer; B = bacteria.

A thin indistinct capsule mediated bacterial attachment to small depressions on the FFE and PGP epithelial surfaces (Figure 24). Some invaginations almost enveloped the bacteria (Figure 26) while others were shallow (Figure 24). However, this difference may have been exaggerated by the angle of section and not representative of the mode of attachment.

Discussion

The stomach of *M. albicaudatus* develops rapidly from a unilocular condition lacking fundic glands at birth, and most adult characteristics have formed by the beginning of the infantile stage. The sequence of gastric changes during the first 25 days of life is summarized in Figure 27. Some of these features occur simultaneously and others occur sequentially (Figure 27) suggesting that gastric development is controlled by certain allogenic factors during the first weeks of life.

For the first two weeks of life, *M. albicaudatus* remains attached to the mother's nipples (Hallett & Meester 1971). It is therefore unlikely that gastric development during this period is stimulated by external factors such as ingestion of solid food, faeces or roughage and it is probable that control of development is intrinsic (Figure 27).

A noticeable feature of fornical development in the white-tailed rat is the rapid growth of PB (and papillae), first apparent between 10 and 15 days. Since it is unlikely that ex-

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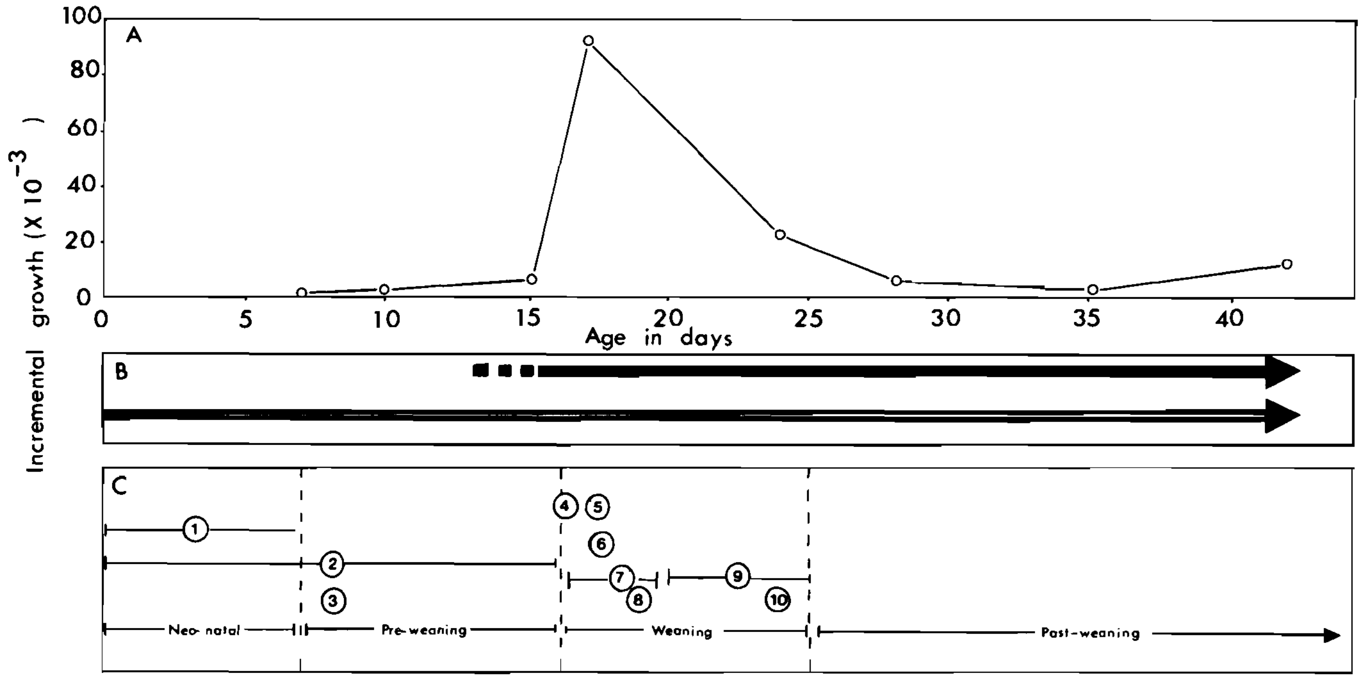

Figure 27 Diagrams showing (A) increase in papillae thickness with age, (B) proposed periods of influence on papillae growth by external stimuli (▬) and innate growth potential (═), and (C) sequence of events associated with gastric development in *M. albicaudatus*. 1. Development of fundic glands. 2. Facultative anaerobes establish an autochthonous gastric flora. 3. Unilocular shape develops into a bilocular shape. 4. Ingestion of solid food. 5. Stomach conditions change. 6. Bacilli colonize the papillae which appear at 15 – 17 days. 7. Anaerobic autochthonous flora established. 8. Regular detachment from mother. 9. Weaning period ($\bar{x}$ = 22,3 days). 10. All adult features present.

ternal factors stimulate early development, this increase in PB length is presumably genetically controlled. However, it seems improbable that the exceptional growth of the PB between 15 and 17 days is due solely to an innate mechanism and it is more likely that growth is stimulated by allogenic factors during this period.

Determination of papillary stimulatory growth factors in *M. albicaudatus* was beyond the scope of this study, but the coincident appearance of bacilli, the sampling of solid food and the period of exceptional PB growth (Figure 27) strongly suggests that these factors influence papillary growth.

Solid food, first sampled at about 16 days of age (Hallet & Meester 1971; Figure 27), is most likely a major factor influencing the development of the stomach since anaerobic bacilli are frequently introduced into the juvenile gut via the food (Schaedler, Dubos & Costello 1965; Savage, Dubos & Schaedler 1968). (Bacteria may also be introduced into the gut if the young eat the faeces of adults; a common practice among rodents: Ewer 1968.) An innate potential alone is responsible for rapid PB growth during the first two weeks of life and ensures that the bacilli are provided with specific papillary microhabitats when they enter the stomach with solid food (or faeces) at about 16 days of age. After bacterial colonization, the papillae growth rate continues at a high level and it is possible that the ingestion of solid food and the presence of bacilli are further stimuli for papillae growth.

The high rate of papillary growth may be due to the presence of the bacilli since it is known that autochtonous bacteria increase the desquamation rate and hence the mitotic rate of intestinal epithelia (Abrams, Bauer & Spintz 1963; Coates & Fuller 1977). It is suggested that by a similar mechanism, the bacilli in *M. albicaudatus* cause the already high papillary growth rate to increase. However, the FFE also achieves maximum thickness during the appearance of the papillary bacilli. Therefore increased FFE growth cannot be explained by bacterial stimulation alone and another stimulus must be involved. A possible factor is the abrasive action of solid food. Abrasion increases the desquamation rate and as a result the mitotic rate (Abrams *et al.* 1963), causing an increase in epithelial growth (of both the FFE and papillae).

The role of chemical stimuli on gastric development cannot be discussed in detail although one aspect must be noted. A major stimulatory force for papillary growth in ruminants is the absorption of volatile fatty acids through papillae in the rumen and reticulum (Brownlee 1956; Richard & Ternouth 1965). However, the gastric papillae of the white-tailed rat do not function in absorption and VFA's are not produced in large quantities in this rodents' fornix (Maddock 1981) thereby preventing the possibility of the mechanism operating.

Thus it is proposed that the crucial event in the gastric development of *M. albicaudatus* is the ingestion of solid food at about 16 days after birth. Ingestion of food introduces papillary bacilli to the stomach. The main feature characterizing development is the high papillary growth rate which probably is due to a number of factors. Mechanical abrasion and the influence of bacteria increase desquamation, and hence, mitotic rates. A high innate growth potential persists throughout life (Figure 27). The rapid innate papillary growth during the first two weeks of life provides the bacilli with specific habitats. Thereafter rapid growth results in an increase in papillary length, number of

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microhabitats and bacilli population density. The simultaneous appearance of papillae, bacilli and the ingestion of solid food is surely more than coincidental.

During gastric morphological development in the white-tailed rat a microbial succession is apparent, initiated by a bacterial flora established in the gut during the first few days of life. A variety of transitory microbes invades the gut after birth but a few favour the gastric conditions characteristic of a milk diet and colonize the stomach. These are largely facultative anaerobes (Schaedler *et al.* 1965; Savage *et al.* 1968). Cocci and cocco-bacilli are representative of this early autochthonous flora (Figure 27). During the third week of life (after the ingestion of solid food) gastric conditions change and become unfavourable for the juvenile rats' facultative microbial community, but evidently suit the bacilli introduced with the solid food. These bacilli are specific to papillary microhabitats and do not occupy the FFE which is colonized by cocci and cocco-bacilli. The FFE microbiota may represent part of the early facultative community or may be newly introduced bacteria particularly suited to the new gastric conditions. Therefore, during the infantile period the young rat acquires a microbial gastric community resembling that of the adult.

In the mammalian gut numerous micro-organisms interact with each other and various environmental factors to produce a stable ecosystem comprising autochthonous and allochthonous microbes (Savage 1977a). Autochthonous microbes are those that multiply and maintain climax communities in specific gastro-intestinal habitats in which they have a definitive niche while allochthonous micro-organisms are ephemeral and do not reach high population densities (Savage 1977a). (Species may be indigenous in one gastro-intestinal habitat but allochthonous in another: Savage 1977a).

All niches in the gastro-intestinal tract are occupied by an autochthonous community forming a stable ecosystem (Alexander 1971). However, even in a stable habitat some allochthonous microbes may be abundant, making it difficult to distinguish them from the indigenous microbiota (Alexander 1971; Savage 1977a,b). A distinction is necessary when determining the influence of the species on the host's biology (Dubos, Schaedler, Costello & Hoet 1965; Gordon & Pesti 1971; Savage 1972, 1977b). To this end certain criteria for autochthony have been suggested (Savage 1977b).

Bacilli that colonize the gastric papillae of *M. albicaudatus* conform well to the autochthonous criteria (Savage 1977b): they are always present in adult hosts, colonize the papillae of 17-day-old juveniles, maintain stable climax communities in adults, always occur on papillae, and it is likely that they efficiently utilize nutrients in the microhabitats, thereby tolerating environmental extremes and competing successfully with other micro-organisms. Papillary bacilli also associate intimately with the epithelium. For these reasons the bacilli are considered indigenous, occupying a niche that necessitates close microbe/epithelium association. However, this conclusion is based on microscopical evidence alone and before all criteria are satisfied, identification of these bacilli in white-tailed rats from different age colonies is required. Nevertheless, evidence points to an autochthonous role for the papillary bacilli and some important comments are now made about the bacterial/epithelial associations in *M. albicaudatus*.

The presence of bacilli in papillary microhabitats is unique; although the mode of attachment is not, and resembles that of *Lactobacillus* in the non-glandular regions of other mammalian stomachs (Smith 1965). Although its exact mechanism of attachment is unknown (Suegara, Morotomi, Watanabe, Kawai & Mutai 1975; Savage 1979) it is believed to be mediated by a macromolecular acidic mucopolysaccharide on the surface of the bacteria (Savage 1970; Savage & Blumershine 1974). It is likely that the capsular layer in the papillary bacteria comprises a similar molecule.

All the fornical bacteria in the white-tailed rat attach to the epithelium in palisade formation. Palisade bacterial attachment has also been noted in the non-glandular stomach of the laboratory rat (Brownlee & Moss 1961) and mouse (Savage & Blumershine 1974). The latter workers suggested that this allows for bacterial contact over a larger surface area facilitating transfer of metabolites. It is possible that more than one species of bacillus occurs on the papillae in *M. albicaudatus* and although their interdependence is unknown, the parallel alignment, resulting from end-on attachment, provides excellent opportunities for interbacterial metabolite transfer. Palisade formation increases the number of bacteria (per unit area) able to contact the epithelium (Savage & Blumershine 1974). If the papillae function to increase the surface area for bacterial attachment (Maddock & Perrin 1981) this mode of attachment will further increase the number of bacilli per unit area.

The bacilli occur in microhabitats which are formed by the desquamating cells of the papillae. Formation of these larger intercellular spaces could be a normal consequence of the desquamating epithelium, accelerated by bacterial influences. The deeper intercellular spaces are narrow and are not colonized by bacteria. However, as the cells assume a more superficial position, desquamation is advanced, cells lose cohesion, and spaces are colonized and enlarged by the bacilli. This is a continuous process and as surface cells, microhabitats and bacteria are lost, bacilli colonize newly-formed deeper intercellular spaces where cell cohesion is decreasing.

The gastric bacilli are confined to papillary microhabitats and only appear in the stomach once the papillae have developed. The habitats are considered essential for bacterial survival and advantageous for protection from ingesta flow. The bacteria need not invest in high multiplication rates to offset bacteria carried down the gut before attachment to the epithelium is achieved (Clarke 1977). After vegetative division, an unattached bacterium sheltered from ingesta flow has a good chance of attachment.

A second advantage is that conditions in the microhabitats are unique and probably provide optimal conditions for the multiplication of bacilli. As noted in sterile rats, drastic changes in fornical conditions, induced by antibiotic treatment, do not immediately affect bacilli in the deeper regions of the microhabitats. Thus a major advantage of these regions might be protection against adverse gastric conditions with nucleus bacterial populations remain-

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ing in microhabitats to permit subsequent recolonization.

The advantages that microhabitats afford the bacilli suggest that the bacilli are non-parasitic. The high population densities and exclusive attachment of bacilli to the papillae, the lack of epithelial damage, the intimate association between papillae and microbes in healthy *M. albicaudatus*, and the conformation of the characteristics of these microbes to the criteria for autochthony (Figure 27) suggest a symbiotic association. The evolution of fornical papillae cannot readily be explained by assuming the bacteria to be detrimental to the host (Maddock & Perrin 1981). It is probable that co-evolution of the papillae and the autochthonous flora has occurred.

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