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THE GASTRIC MORPHOLOGY OF THE WHITE-TAILED RAT *MYSTROMYS ALBICAUDATUS* (A. SMITH 1834) AND PRELIMINARY INVESTIGATIONS OF ITS DIGESTIVE PROCESSES.

by

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FRONTSPIECE: the white-tailed rat, Mystromys albicaudatus.

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LIST OF ABBREVIATIONS USED IN THIS THESIS.

APB.....Anaerobic papillae bacilli

BHI.....Brain heart infusion

COB.....Cytoplasmic oval bodies

DB.....Dense band

ERP.....Endoplasmic reticulum

FCE.....Folded corpal epithelium

GI.....Gastro-intestinal

H and E.....Hematoxylin and eosin

KG.....Keratohyalin granules

KG protein.....Keratohyalin granule protein

KH.....Keratohyalin

MCG.....Membrane coating granules

PAS.....Periodic acid Schiff

PB.....Papillae buds

PGF.....Pregastric fermentation

PGP.....Pregastric pouch

RER.....Rough endoplasmic reticulum

SEM.....Scanning electron microscopy

STR.....Specially treated rat

TEM.....Transmission electron microscopy

VFA.....Volatile fatty acids

CONTENTS

GENERAL INTRODUCTION.

Rodents have a world wide distribution and occupy numerous habitats (Walker 1975). Comparative gastric morphological studies of a number of myomorph genera from different zoogeographical regions have indicated great structural diversity: in that completely glandular, single-chambered stomachs (Vorontsov 1962; Perrin & Curtis 1980); single-chambered stomachs with varying proportions of keratinised and glandular epithelia (Vorontsov 1962; Dearden 1969; Carleton 1973; Perrin & Curtis 1980); and more complex, two-chambered stomachs with reduced glandular epithelium restricted to a disc-like area on the greater curvature (Vorontsov 1962; Dearden 1969; Carleton 1973; Luthje 1976), have been described. Although these forms were recognised last century (Retzius 1841 and Toepfer 1891 op. cit. Bensley 1905) it was only during this decade that a descriptive, gastric terminology was developed (Carleton 1973; Perrin & Curtis 1980). These three stomach types were named unilocular glandular (Perrin & Curtis 1980), unilocular hemiglandular and bilocular discoglandular (Carleton 1973) respectively. In a survey of new world cricetids, Carleton (1973) suggested that the complex stomach evolved from the unilocular hemiglandular type. If Carleton's ideas (1973) are broadened to include all myomorph rodents, the continuum can be seen to range from the unilocular glandular to the bilocular discoglandular type (Perrin & Curtis 1980).

Evolution from a simple to a complex stomach type has been associated with climatic changes during the Miocene (Moir 1968; Vorontsov 1962). During this epoch large areas of forest were replaced by savannah and steppe vegetation (Moir 1968) causing many

rodents to change their 'forest' diet (omnivory, Landry 1970; insectivory/granivory, Vorontso. 1962) to a herbivorous diet typical of savannah species. This change from protein-rich food to herbivory was facilitated by various behavioural, physiological and anatomical adaptations including stomach sacculation and an increase in keratinised relative to glandular gastric epithelium (Vorontsov 1962); that is, modifications seen in the bilocular discoglandular stomach. Three theories attempt to explain the evolution of this complex stomach (Bensley 1905; Vorontsov 1962; Carleton 1973).

Before reviewing these hypotheses, it is important to consider how increasing gastric complexity is associated with such dietary changes. Herbivory involves ingestion of cellulose (the fibrous component of plants) which, although abundant and a potentially important food resource, is not directly available to most animals because they lack cellulases (Parra 1978). A number of bacteria and protozoans digest cellulose and dense populations of these micro-organisms occur in expanded portions of the herbivore alimentary tract where conditions are ideal for microbial growth (Moir 1968). In this symbiotic association the micro-organisms obtain energy by fermentation of the hosts' digesta (particularly cellulose and related plant polymers) while the host absorbs and incorporates into its metabolism the waste products of fermentation in the form of volatile fatty acids (VFA's) (Moen 1973). Since a herbivorous diet has low energy per unit mass, herbivores must ingest large volumes of this food to satisfy their energy requirements (Parra 1978). The spacious microbial fermentation chambers (in a pregastric, caecal or colonic position) allow for storage and slow digestion of high roughage diets (Parra 1978). As a result of these adaptations much of the herbivores' energy can be derived from a cellulose diet.

Fermentation in the foregut is not unique to ruminants (Moir 1968; Bauchop 1977) and since the pioneering work of Moir, Somers & Waring (1956) ruminant-like digestive traits (including pregastric fermentation - PGF), have been found in a number of mammals from different taxa (Appendix 1; Moir 1968; Bauchop 1977). Pregastric and hindgut fermentations are therefore alternative strategies which can be adopted by many mammalian herbivores although ruminants maximise the former strategy (Parra 1978). Thus, increase in rodent stomach complexity may well represent a development towards foregut fermentation in response to the relative increase in fibrous food during the Miocene epoch (Vorontsov 1962).

In 1905, Bensley suggested that the unilocular hemiglandular and bilocular discoglandular conditions were generated by encroachment of keratinised epithelia into the glandular regions of the stomach. Such changes were facilitated primarily by ingestion of large volumes of abrasive food, associated with increased herbivory, which damaged the mucosal layer, resulting in atrophication of the of the fundic glands, and their replacement by keratinised epithelia (Bensley 1905). Cardiac glands, considered intermediate in this process, arose from the loss of parietal and chief cells while the stratified squamous epithelium resulted from mechanical degradation of the cardiac glands (Bensley 1905). In addition to abrasion, over-distention of the stomach and increased retention of large volumes of food with low digestibility, also contributed to increasing the area of keratinised epithelia.

Bensley (1905) proposed that the development from a single-chambered to a sacculated stomach increased storage area for bulky food and possibly prolonged salivary amylase activity in the non-

glandular chamber. Increased maceration and bacterial and salivary enzymatic digestion in this region would sufficiently reduce food consistency so that abrasion of the glandular epithelium would be minimised. Sacculation therefore prevented further glandular loss by abrasion.

The second theory explaining gastric modification in rodents was proposed in the early sixties when a study of the alimentary system of the Muroidea was published (Vorontsov 1962). This author, like Bensley (1905), believed a dietary change to herbivory was reflected by increased complexity of rodent stomachs. A number of anatomical modifications of the alimentary tract associated with this change were suggested (Vorontsov 1961, 1962).

1. To mechanically break down a roughage diet the lower jaw movement developed from a grasping to a grinding action, the masticatory musculature was strengthened and the tuberculous molars became laminated and flattened, converting from a brachydontic to a hypsodontic condition.

2. There was an increase in total gut length, and areas for fermentation (either in the foregut or hindgut) developed: a decrease in glandular relative to non-glandular gastric epithelium and gastric sacculation provided a forestomach fermentation chamber analagous to a rumino-reticulum; or the relative length of the small intestine decreased while there was an increase in relative size, absorptive area and complexity of the caecum and colon providing an area for hindgut fermentation.

Such adaptations enabled rodents t o utilise a cellulose-rich diet. Vorontsov (1962) recognised a basic morphological similarity between the bilocular discoglandular rodent stomach and that of the ruminant. He believed that the complex condition indicated ruminant-like pregastric fermentation in the rodent (animals with simple stomachs possessed caecal or colonic fermentation). This idea was appealing, particularly during the era when pregastric fermentation was being demonstrated in many non-ruminants (Moir 1968).

In 1973 Carleton published the results of a comparative study of new world cricetid gastric morphology in which he questioned Vorontsov's reasoning (1962). Carleton disagreed that the gastric similarities between rodents and ruminants were sufficient to suggest similarity of function and he mentioned a number of major differences between the stomachs of these orders (Carleton 1973). A prime objection was that food habits often did not correlate with Vorontsov's functional interpretation of gastric structure. For example, some rodents with bilocular discoglandular stomachs, which according to Vorontsov (1962) should indicate herbivory, select a concentrate diet (Carleton 1973).

Carleton (1973) pointed ou' that not only cellulose but also starch and glycogen were important in mammalian nutrition. As an alternative to the pregastric fermentation theory he developed Bensley's suggestion, (1905) that sacculation of the stomach provided an area of neutral pH, allowing prolonged salivary amylolysis of the starch and glycogen components of the diet (Carleton 1973). (Usually gastric acidity denatures salivary amylase reducing its effectiveness but in the sacculated stomach

this problem can be overcome.) The prolonged salivary amylase theory (Carleton 1973) implied that rodents with complex stomachs had probably retained the 'primitive' seed/insect-diet. This hypothesis therefore differed from those of Bensley (1905) and Vorontsov (1962) who suggested that dietary change was the main stimulus for development of a complex stomach.

To date no specific studies have been completed that support or reject either Vorontsov's (1962) or Carleton's (1973) hypotheses. However, few authors accept Bensley's (1905) interpretations (Horner, Taylor & Padykula 1965) which have not received favour since it was found that herbivores with completely glandular stomachs (for example, the beaver Castor canadensis; Currier, Kitts & Cowan 1960) eat abrasive foods (Carleton 1973). Although it may be energetically uneconomical for small herbivores (less than ten kg) to have foregut fermentation (Janis 1976; Parra 1978) there are exceptions to this rule (Parra 1978) and therefore Vorontsov's hypothesis (1962) cannot be rejected out of hand. Neither can Carleton's ideas (1973) as numerous authors have suggested the advantages of a pregastric amylolytic resevoir (Bensley 1905; Krishnamurti, Kitts & Smith 1974).

Prolonged salivary amylase activity and pregastric fermentation are thus the current theories explaining evolution from unilocular glandular to bilocular discoglandular stomachs. Certainly these hypotheses are speculative and warrant more detailed investigation particularly in microbiological and biochemical directions. But these ideas do offer plausible explanations for the development of the complex stomach which can be used as starting points for further research. Unfortunately work in this field has been limited to a

few rodents having simple stomachs (the laboratory rat Rattus norvegicus and laboratory mouse Mus musculus; Peters & Gaertner 1973; Peters 1973; Kunstyr, Peters & Gaertner 1976; Gaertner & Pfaff 1979 and the golden hamster Mesocricetus auratus; Matsumoto 1955; Hoover, Mannings & Sheerin 1969; Banta, Warner & Robertson 1975, Ehle & Warner 1978) and there has been no advancement of these ideas since their conception in the early sixties and seventies. A number of detailed studies on single species, with varying degrees of gastric complexity, are required so that these theories can be tested, elaborated or rejected as necessary.

The majority of myomorph rodents have a gastric morphology conforming to Carleton's (1973) modified evolutionary series but a few show atypical adaptations, the most interesting being those exhibited by the mole-rat Myospalax myospalax, the African mole-rat Tachyoryctes splendens, the giant rat Cricetomys gambianus and the white-tailed rat Mystromys albicaudatus. These rodents have sacculated stomachs and possess numerous papillae in the proximal, non-glandular chamber (corpus) which house large bacterial populations (Carleton 1973; Rahm 1976, 1980; Caiman, Quenum, Kerrest & Goueffon 1960; Maddock & Perrin 1981; Appendix 6). Such adaptations are unique; they do not occur in any ruminant or ruminant-like mammals nor can they be readily explained in terms of Bensley's (1905), Vorontsov's (1962) or Carleton's (1973) theories although the presence of forestomach papillae and bacteria suggest pregastric fermentation.

Nothing is known about digestive function in these four rodents and an investigation of their stomachs and feeding habits is required. Since M. albicaudatus is easy to breed (Hall, Persing,

White & Ricketts 1967) and was readily available, it was chosen for study. The morphology of the stomach and its bacteria are reported in the first section of this thesis. Also included in this section is a description of gastric development and microbial succession from birth to adulthood. In Section II results of a number of biochemical analyses, feeding experiments and measurements of the gut are discussed to supplement interpretation of the morphological results. Vorontsov's (1962) and Carleton's (1973) theories are considered in relation to M. albicaudatus's adaptations. The thesis is concluded with considerations of gastric evolutionary development in this rodent (Section III).

Many mistakes were made in the earlier general studies (Bensley 1905; Vorontsov 1962; Carleton 1973) when contentious theories were raised and numerous questions posed. However, this work laid the foundations for a new field of biology, the importance of which has only recently been realised (Moir 1968; Bauchop 1977; McBee 1977; Montgomery 1978). The present study of M. albicaudatus is more detailed and as a result, it is hoped that new ideas on the evolution and function of rodent alimentary adaptations will be stimulated. It is also anticipated that this thesis will initiate more detailed investigation into the feeding and digestive biology of M. albicaudatus, in addition to similar work on other myomorph rodents.

TAXONOMY.

The white-tailed rat was first described as Otomys albicaudatus by Sir Andrew Smith (1834) who found it in the Albany district of the Eastern Cape Province (Roberts 1951; De Graaff 1981). Wagner, in 1841, described a similar rat with white feet in the Grahamstown area and named it Mystromys albipes. The present name, Mystromys albicaudatus was given to the species in 1953 (Ellerman, Morrison-Scott & Hayman 1953) although two subspecies, a larger, darker M. a. fumosus and M. a. albicaudatus, were descibed in 1905 (Thomas & Schwann 1905) and 1939 (Allen 1939) respectively. M. albicaudatus is considered the only southern African representative of the subfamily Cricetinae (Dean 1978; De Graaff 1981) and its full classification is presented in Table 1.

Table 1. Classification of the white-tailed rat, Mystromys albicaudatus. (after De Graaff 1981).

Order	Rodentia	(Bowdich 1821)
Suborder	Myomorpha	(Brandt 1855)
Superfamily	Muroidea	(Miller & Gidley 1918)
Family	Cricetidae	(Rochebrune 1883)
Subfamily	Cricetinae	(Murray 1866)
Genus	Mystromys	(Wagner 1841)
Species	albicaudatus	(A. Smith 1834)

DISTRIBUTION AND STATUS.

M. albicaudatus is found in South Africa in the montane and highveld grasslands of southern Transvaal, Natal, Orange Free State and Eastern Province (Davis 1962). There is a relict population in the South Western Cape biotic zone (Davis 1962).

During the Pleistocene epoch the Cricetinae were more common than the Murinae, a position that is reversed today (Davis 1962). In the Kromdraai B collection from Sterkfontein 287 cricetines of two species were found and described versus 27 murines of five species (Davis 1962). One of these cricetines is now extinct but the other, Mystromys hausleitneri, differs little from the present M. albicaudatus and is considered a chronospecies Mystromys hausleitneri-albicaudatus (Davis 1962). The abundance of these two subfamilies in the 'rodent breccia' of Sterkfontein, and the fact that there are no fossil murids from the Tertiary in Africa (Misonne 1969), suggests that the Cricetidae were first established in South Africa (Kingdon 1974) and the Murinae are an invading group, originating from an Asian cricetodont stock before the Pleistocene epoch (Misonne 1969).

Today the Murinae are well established in South Africa and the Cricetinae are in a retrogressive position, resorting to specialisation in order to survive in competition with the Murinae (Misonne 1969). This is emphasised by the fact that M. albicaudatus has recently been classified as an endangered species (Dean 1978).

SECTION I

THE MORPHOLOGY OF THE STOMACH AND RELATED FEATURES OF *M. ALBICAUDATUS*.

CHAPTER ONE.

GROSS, HISTOLOGICAL, HISTOCHEMICAL AND ULTRASTRUCTURAL STUDIES OF THE STOMACH.

INTRODUCTION.

Ultrastructural studies of rodent stomachs are limited (Sakata & Tamate 1976) and the electron microscope has more often been used in medical studies of the oral cavity of the laboratory rat, R. norvegicus (Squier 1968; Hayward, Hamilton & Hackeman 1973). In contrast, a number of gross and histological examinations of myomorph gastric structure have been published (Vorontsov 1962; Dearden 1966, 1969; Carleton 1973; Luthje 1976; Perrin & Curtis 1980) and a useful descriptive nomenclature for gastric form has developed (Carleton 1973; Perrin & Curtis 1980).

These studies have provided an insight into the diversity of gastric form in the suborder, Myomorpha. Microtine rodents have the most advanced gastric morphology as the majority have bilocular discoglandular stomachs, and none have the simple unilocular glandular type (Vorontsov 1962). Cricetids show interesting gastric modifications (for example gastric papillae) and display a full evolutionary range, from many with the unilocular hemiglandular type, to a few showing the complex form (Vorontsov 1962; Carleton 1973). Murids have a varied stomach morphology: unilocular glandular and unilocular hemiglandular forms are common but not the more complex type (Vorontsov 1962; Luthje 1976).

Two different hypotheses, foregut fermentation (Vorontsov 1962) and prolonged salivary amylase activity (Carleton 1973) have been

proposed to explain this diversity. To examine M. albicaudatus in relation to these theories, a detailed idea of the rodents' gastric morphology was required. A microscopical examination of the white-tailed rat was therefore initiated to determine its gastric anatomy, histology and ultrastructure in detail, prior to feeding and enzyme experiments.

MATERIALS AND METHODS.

Adult M. albicaudatus were killed by chloroform anaesthesia and placed on ice to retard autolysis. Stomachs, including approximately 10 mm of the oesophagus and duodenum, were removed, cleaned of gut contents and placed in either Bouin's fixative or 10 % formalin.

A dissecting microscope was used to examine gross morphology of longitudinally bisected stomachs and photographs were taken with a Nikon F2 camera and 55 mm lens. Stomach dimensions were measured with calipers, and micro-anatomical features with a Leitz micrometer. A punch was used to obtain discs of papillated epithelium of 13 mm diameter and papillae on the discs were counted. The approximate increase in corpal surface area, afforded by the papillae, was determined by panometric means.

Paraffin embedded material was processed and sections, 7 um thick, were cut. Frozen sections fixed in Bouin's were sectioned on a freezing microtome. General purpose tissue stains as well as specific histochemical stains were used when examining the gastric epithelium (Table 1,1). Light micrographs were taken through a Wild M 400 Photomacroskop or a Vanox microscope fitted with an Olympus C 35 camera.

Table 1,1. Stains used when examining the gastric histology of M. albicaudatus.

Stain.	Tissue stained.	Reference.
Hematoxylin & eosin	General purpose, nuclear material	1
Hematoxylin alum	Nuclear material	2
Toluidene blue	General purpose, mucin	1
Feulgen	Nuclear material	1
Oil red O	Lipid	1
Sudan black B	Lipid	3
Periodic acid Schiff	Mucopolysaccharides	1
Aldehyde fuchsin	Sulphur groups	1
Ferric ferricyanide	Reducing substances	1
Aniline blue, Orange G (Azan)	Keratin	4

1. Humason 1967
2. Luna 1968
3. Sumner & Sumner 1969
4. Ayoub & Skhlar 1963

Additional blocks of tissue were fixed in 5 % cold buffered glutaraldehyde for a minimum of 12 h. Tissue used for scanning electron microscopy (SEM) was critical point dried (Anderson 1951), coated with gold palladium and examined with a JEOL JSM/VS scanning electron microscope. Secondary fixation and embedding of the tissues used for transmission electron microscopy (TEM) followed the procedure

of Cross (1979). Sections were cut on an LKB mark 3 ultramicrotome and stained with uranyl acetate and lead citrate (Cross 1979). A Hitachi HV/11B transmission electron microscope was used to examine the sections.

To facilitate scanning electron microscope examination of the corpal epithelium surface, conventional (normal gastric flora), sterile (bacteria-free stomach) and specially treated rats, STR, (reduced gastric flora) were used (Table 1,2; see also pages 88 & 89).

Table 1,2. Antibiotic (oxytetracycline; Rio Ethicals (Pty.) Ltd., Jhb.) treatment of the alimentary flora of M. albicaudatus.

Rat treatment.	Food and water.
Conventional rats.	Tap water, pelleted food.
Sterile rats.	100 mg oral oxytetracycline per day for 5 days. Pellets and water were autoclaved.
Specially treated rats (STR).	50 mg oral oxytetracycline per day for 5 days. Tap water, pelleted food.

RESULTS.

Gross Morphology.

The stomach of M. albicaudatus was markedly sacculated (Fig. 1,1 a & b) and differed from Carleton's (1973) simple and complex types (Fig. 1,2) and the completely glandular type (Perrin & Curtis 1980; Vorontsov 1962). The proximal, non-glandular pars oesophagea, consisted of a papillated corpus and a non-papillated pregastric pouch (PGP) (Fig. 1,1). The glandular antrum was separated from the pars oesophagea by a bordering fold of tissue, the grenzfalte (Fig. 1,1).

The oesophagus entered the mid-dorsal region of the corpus at right angles. Superior to the gastro-oesophageal junction, the corpus extended cranially, forming the fornix ventricularis (Fig. 1,1). Inferior to this junction and at right angles to it, the lesser curvature extended to the antrum where it folded back on itself forming the cardiac pouch and eventually moving cranially to the pylorus (Fig. 1,1). This fold was called the incisura angularis (corpo-pyloric fold; Dearden 1960). The outer antral and PGP serosal layers were attached throughout the length of the fold (Fig. 1,1) thus differing slightly from Carleton's description of the incisura angularis (Carleton 1973).

Corpal papillae, a few of which were bifurcate, originated immediately distal to the gastro-oesophageal junction and were irregularly orientated in the corpus (Fig. 1,1). (Papillae measurements are summarised in Table 1,3). The non-papillated PGP formed a constriction between the corpus and the antrum (Fig. 1,1).

Figure 1 a. Photograph of a bisected stomach of M. albicaudatus illustrating gross morphology. To avoid confusion, anatomical terms used in this thesis are defined as follows:-

C = CORPUS - proximal region of stomach including the fornix ventricularis.

P = PREGASTRIC POUCH - non-glandular epithelium separating the corpus and antrum.

A = ANTRUM - distal, glandular region of stomach.

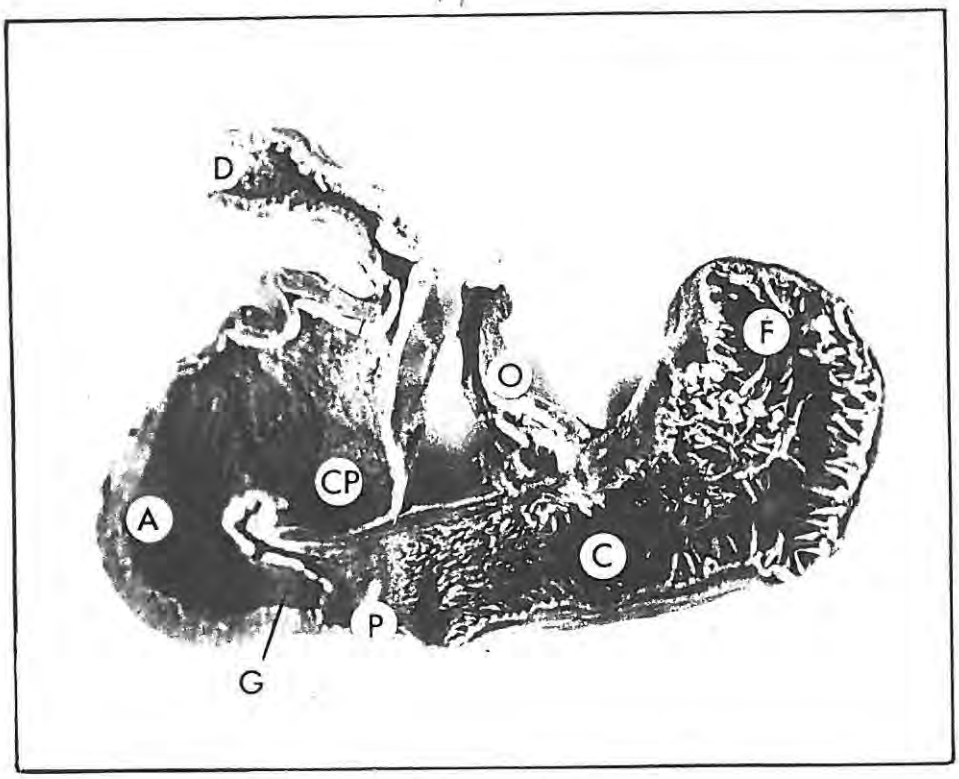
F = FORNIX VENTRICULARIS - diver iculum of the corpus extending craniad beyond the gastro-oesophageal junction.

G = GRENZFALTE - bordering fold of tissue separating the glandular and non-glandular epithelia.

I = INCISURA ANGULARIS - prominent angle on the lesser curvature of the stomach.

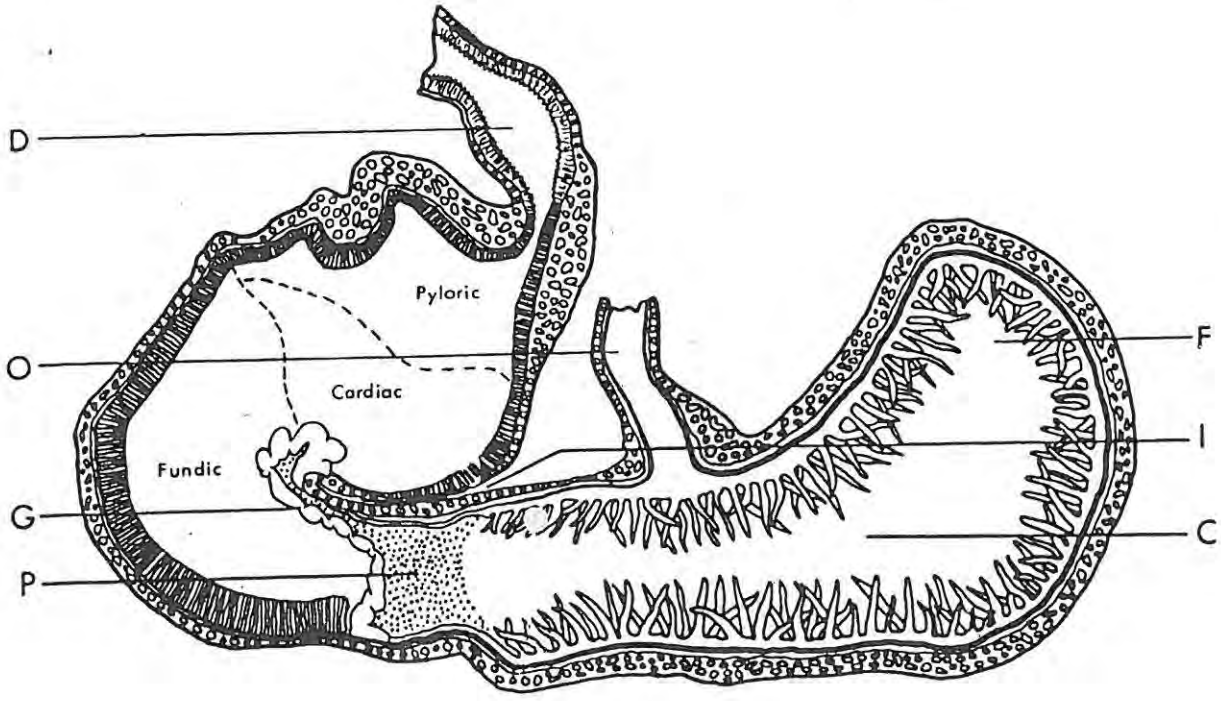
O = oesophagus; D = duodenum; CP = cardiac pouch; Fu, Py and Ca = fundic, pyloric and cardiac regions of the antrum respectively.

Figure 1 b. Semi-diagrammatic drawing of photograph in Fig. 1 a (same lettering).



1 cm

Figure 1,1 a



Glandular tissue

Transition area

Muscularis externa

Non-papillated epithelium

Papillated epithelium

Duodenal epithelium

Figure 1,1 b

Figure 2. A generalised diagram of the three stomach types (modified after Carleton 1973). (a) unilocular hemiglandular, (b) papillated bilocular hemiglandular (M. albicaudatus) and (c) bilocular discoglandular. Lettering as in Fig. 1 a. L = glandular epithelium; N = non-glandular epithelium.

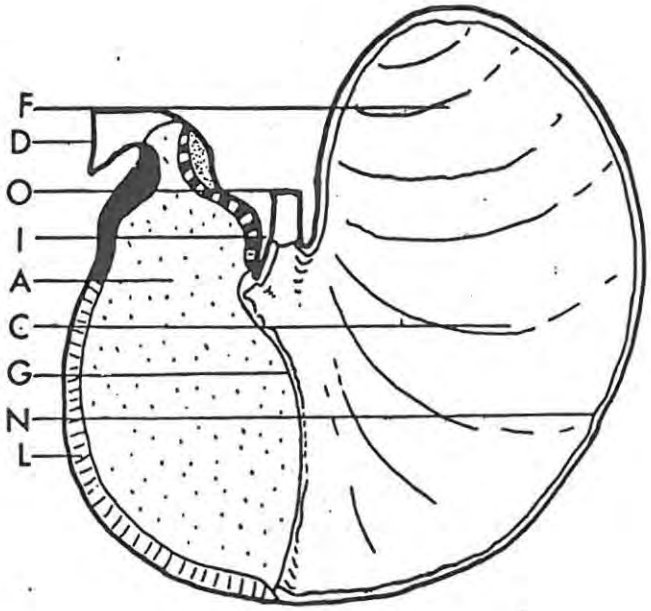


Figure 1,2 a

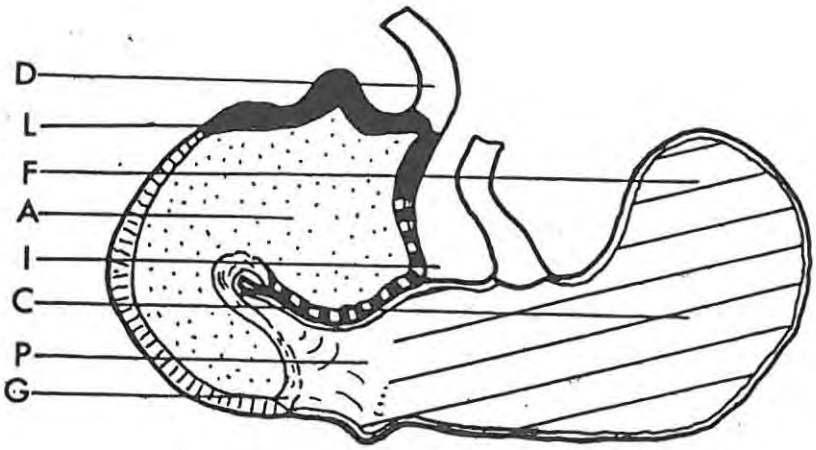


Figure 1,2 b

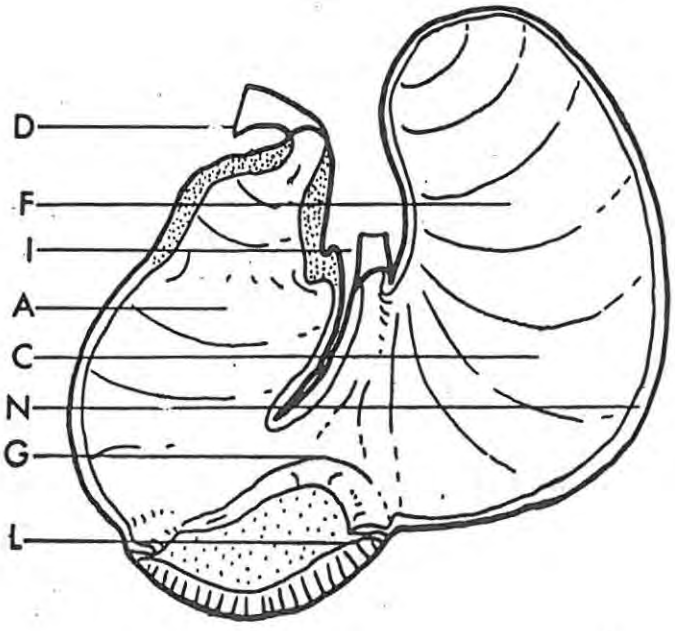


Figure 1,2 c

Table 1,3. Papillary measurements from the corpus of M. albicaudatus.

Papillae.		Surface area increase.
Density (n* = 1 300) (n = 10)	Length (n* = 124) (n = 10)	5 times.
550/cm^2 ± 115	1,8 mm ± 0,45	

n* = number of samples examined.
n = number of animals examined.

The antrum was a glandular sac originating immediately distal to the grenzfalte. This fold extended from the extremity of the angle of the incisura angularis to a point on the greater curvature of the stomach (Fig. 1,1). The asymmetrical U-shape of the antrum (the larger, distal, section of which leads directly to the duodenum) was due to the incisura angularis (Fig. 1,1).

Histology.

With the exception of the epithelium, a typical mammalian stomach tissue plan (Dearden 1966, 1969; Madge 1975) was seen in the white-tailed rat. A thin serosa (conspicuous in the gastro-oesophageal region) and muscularis externa comprising two smooth muscle layers, inner circular (stratum circulare) and outer longitudinal (stratum longitudinale), were present (Plate 1,1). Generally the muscularis externa was thicker in the corpus (where it supported a keratinised, papillated epithelium) than in the PGP or antrum. The stratum circulare was approximately four times thicker than the longitudinal layer in the antrum, twice as thick in the corpus; in the fornix

Plate 1,1. A micrograph of the gastric tissue layers in the region of the corpus. Connective tissue stains blue and keratin deep red; it is not possible to clearly distinguish the muscularis mucosa between the lamina propria and deeper submucosal connective tissue layers. S = serosa, OLM = outer longitudinal muscle layer, ICM = inner circular muscle layer, E = epithelium, P = papilla.

Plate 1,2. The inner circular smooth muscle layer (ICM) thickens to form the pyloric sphincter. Pyloric mucosa (Py) changes to duodenal mucosa (Do) immediately distal to the sphinter. The muscularis mucosa (Mm) is conspicuous.

Plate 1,3. The oesophageal sphincter is formed by a mixture of thickened, inner circular oesophageal striated (StM) and gastric circular smooth muscle (SmM) fibres on the rodent's sinistral side (right hand side in photograph). The folded stratified squamous epithelium of the oesophagus (OE) and the gastric papillated epithelium (E) are indicated.

Plate 1,4. Tissue layers in the PGP. Note the lamina propria (Lp), the relatively thick muscularis mucosa (Mm) and blood vessels (Bv) in the submucosa (Sb). The four epithelial layers are also indicated (strata basale (sb), spinosum (ss), granulosum (sg) and corneum (sc). B = bacteria, ICM = inner circular muscle and OLM = outer longitudinal muscle layers.

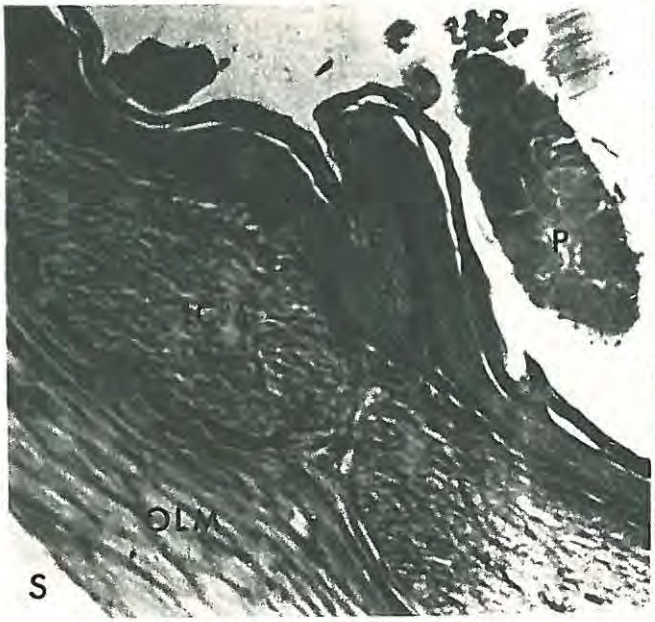


Plate 1,1 50 µm

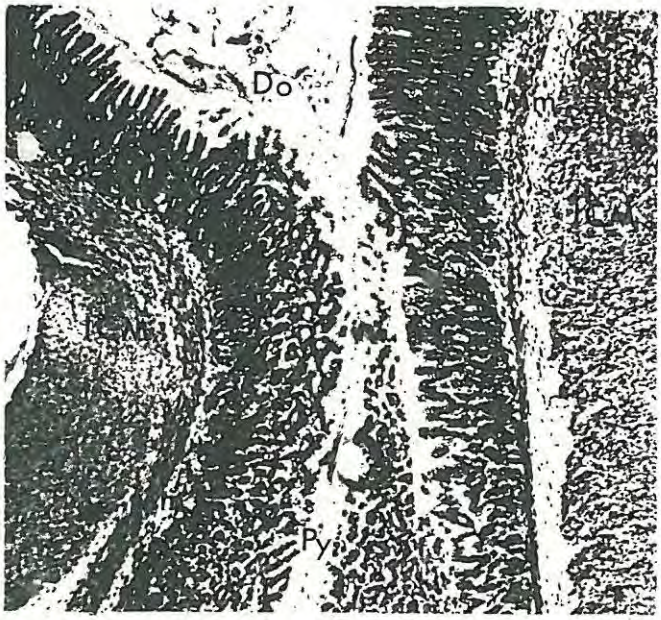


Plate 1,2 0,2 mm

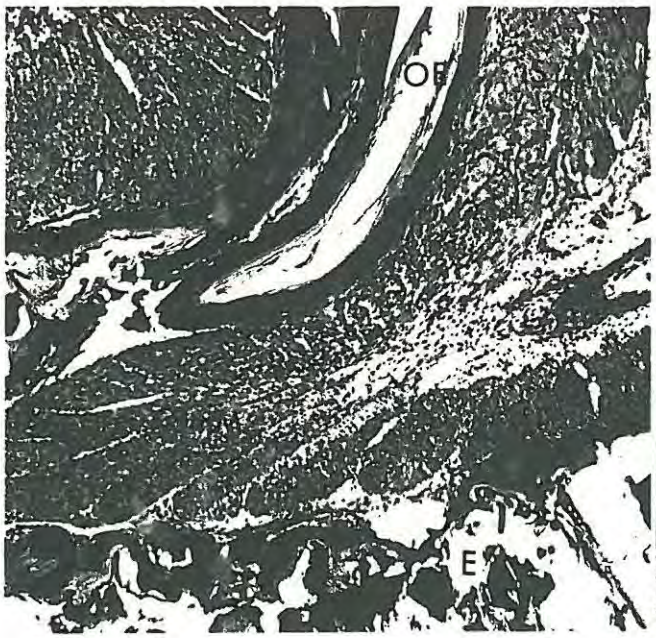


Plate 1,3 0,2 mm

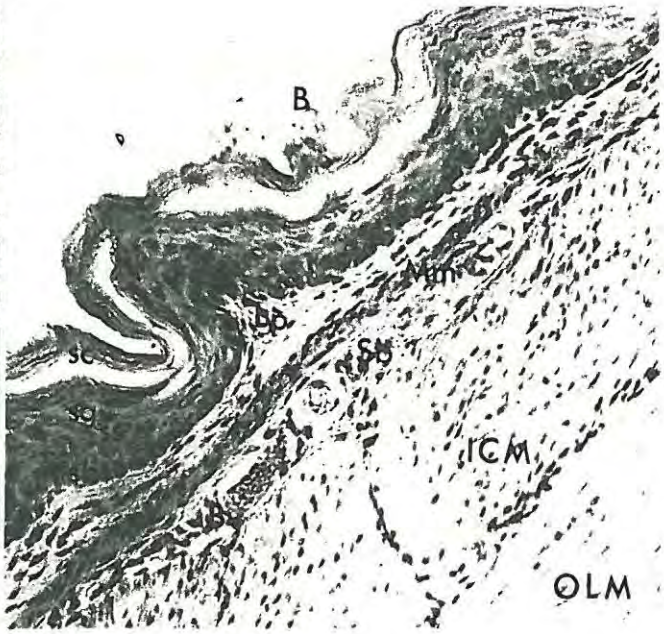


Plate 1,4 50 µm

ventricularis the layers had equal thickness. The stratum circulare was exceptionally well developed at the pyloric (Plate 1,2) and gastro-oesophageal (Plate 1,3) junctions where it formed the pyloric and oesophageal sphincters respectively.

The oesophageal muscularis externa consisted of striated muscle (Plate 1,3), the outer longitudinal layer continued from the dextral side of the oesophagus to the incisura angularis. Longitudinal muscle on the left of the oesophagus penetrated the outer smooth muscle layer of the stomach so that a transition region of both smooth and striated fibres occurred at the gastro-oesophageal junction (Plate 1,3). The oesophageal inner circular striated muscle layer changed to gastric smooth muscle on both sides of the oesophageal sphincter (Plate 1,3).

A submucosa of loose connective tissue with nerve fibres and numerous blood vessels maintained a constant thickness in the corpus and PGP (Plate 1,4) but was often absent from the antrum where the muscularis externa and mucosa were closely attached (Plate 1,5). The corpal muscularis mucosa was continuous with that of the oesophagus but was incomplete, being represented by an indistinct longitudinal, smooth muscle layer (particularly evident in the fornix ventricularis). Inner circular and outer longitudinal smooth muscle fibres occurred in the muscularis mucosa in the antrum, except along the greater curvature where longitudinal muscle predominated; few transverse fibres occurred in the middle of this layer at regular intervals.

Fine reticular connective tissue and elastin fibres constituted the lamina propria which had an irregular thickness in both the corpus (due to the folded epithelium) and in the antrum (where it extended

Plate 1,5. A section through the base of the cardiac glands which consist of one cell type. Note the proximity of the muscularis mucosa (Mm) and externa (ICM) causing exclusion of the submucosa.

Plate 1,6. Section through the cardiac region showing the lamina propria (blue stain) extending between, and supporting the glandular epithelium. M = muscle layers.

Plate 1,7 (a). A longitudinal section through the corpal epithelium showing the differences between the adjacent FCE and papillary epithelium (PE). The difference in thickness between the malpighean layers (Mp), horny layers (H) and bacterial covering (B) of the two epithelia is evident. Bacteria penetrate the papillary epithelium but only attach to the outermost layer of the FCE. Note the complete keratinisation of the papilla (red stain).

Plate 1,7 (b). A higher power micrograph of the junction between the FCE and a papilla. The horny cells are orientated parallel to the epithelium in the FCE but have an irregular orientation in FCE and the papilla. The horny cells are orientated parallel to the epithelium in the FCE but have an irregular orientation in the papillae. (Lettering as in plate 1,7 a)

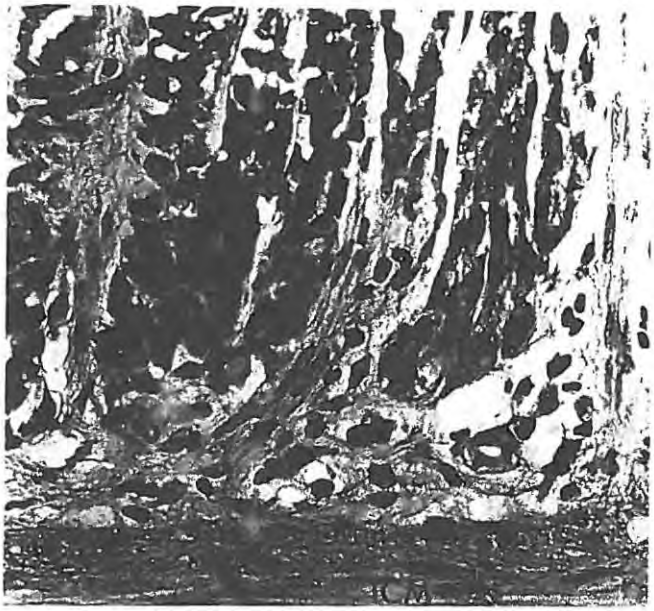


Plate 1,5 — 20 µm

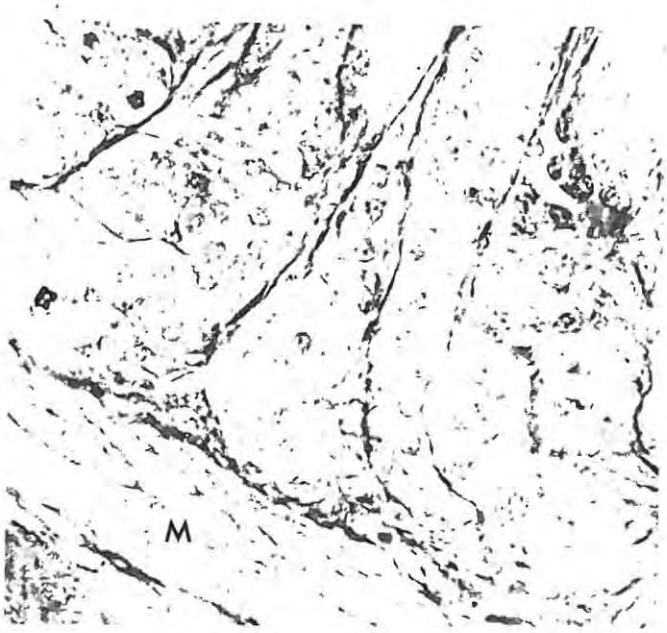


Plate 1,6 — 25 µm

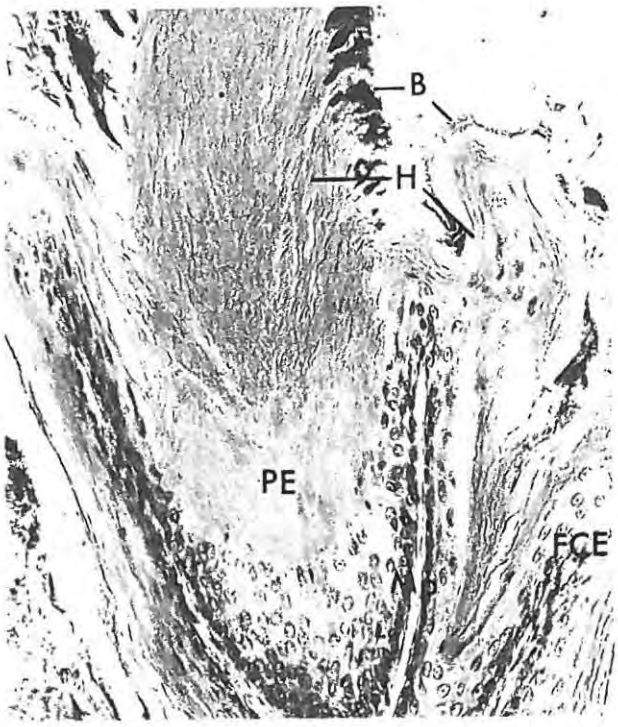


Plate 1,7 a — 50 µm

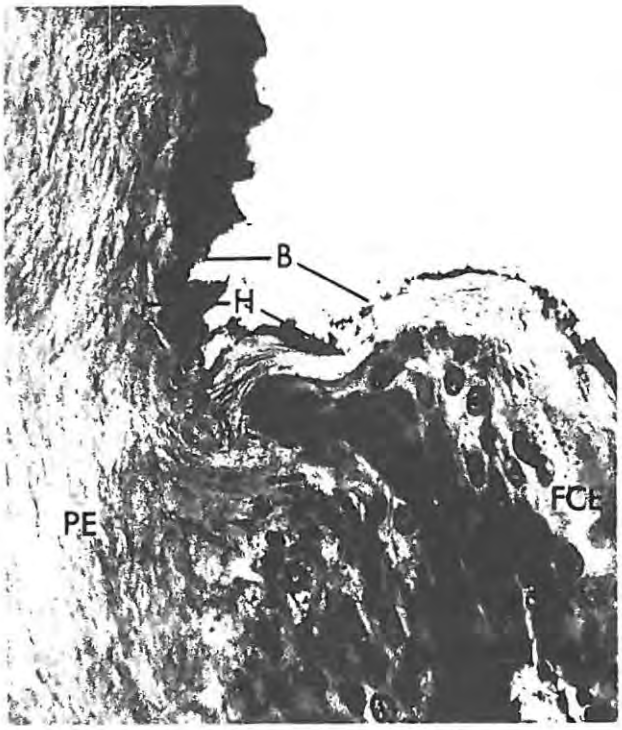


Plate 1,7 b — 25 µm

between the glandular tissue; Plates 1,5 & 1,6). In the antrum, the lamina propria also contained smooth muscle fibres from the underlying muscularis mucosa. Interlocking connective tissue and epithelial papillae, termed epithelial pegs by Hyden & Sperber (1965), were absent.

The pars oesophagea was lined by stratified, squamous epithelium. Keratinisation had taken place through keratohyalin thus forming "soft" keratin; all the layers typical of the mammalian epidermis (Jarrett 1973), with the exception of the stratum lucidum, were well represented in these regions (Plates 1,4; 1,7; 1,8 & 1,9). A folded corpal epithelium (FCE) lined the corpus, and between the folds were keratinised papillae (Plate 1,7 a & b) which differed from those of the rumen by lacking a connective tissue core, or swollen cells in the superficial layers (Hofmann 1973). These papillae increased the corpal surface area approximately five fold (Table 1,3). There was a difference in cell size between the malpighean layers of the papillary epithelium and the FCE; cells of the FCE were approximately twice the size of those in the papillary epithelium (Plates 1,8 & 1,9). The spinous and granular cells of the FCE were parallel to the basement membrane (Plate 1,9), those of the papillary epithelium were often perpendicular and formed long columns of cells (Plate 1,8). The oesophagus resembled the FCE and consisted of stratified squamous epithelia which was highly folded to facilitate distention during the passage of food.

The stratum corneum constituted the main part of the papillae (Plate 1,7 a) and there was an extensive stratum spinosum (Plate 1,8) compared to the FCE (Plate 1,9) and PGP (Plate 1,4). Masses of symbiotic bacteria penetrated the papillae horny layer forming

Plate 1,8. Section through the papillary epithelium showing the four epithelial strata; basale (sb), spinosum (ss), granulosum (sg) and corneum (sc). The stratum basale is two layers thick and there is a thick stratum spinosum and corneum. Cells of the malpighean layers are arranged in long perpendicular rows, often at 90° to the basal membrane. The papillae cells are approximately half the size of the FCE cells shown in Plate 1,9. Note the keratohyalin granules (KG).

Plate 1,9. Section through the FCE showing the four epithelial strata. The basal layer of the FCE is one cell thick and the other strata are thinner than those of the papillae shown in Plate 1,8. FCE cells are larger than papillae cells and are parallel to the basal membrane. Note the keratohyalin granules (KG). (Lettering as in Plate 1,8.)

Plate 1,10. The junction between the papillated corpus and the non-papillated PGP. The bacterial covering of the FCE and PGP are similar. Larger numbers of micro-organisms aggregate at the base of epithelial folds. P = papilla, B = bacteria, M = muscle layers.

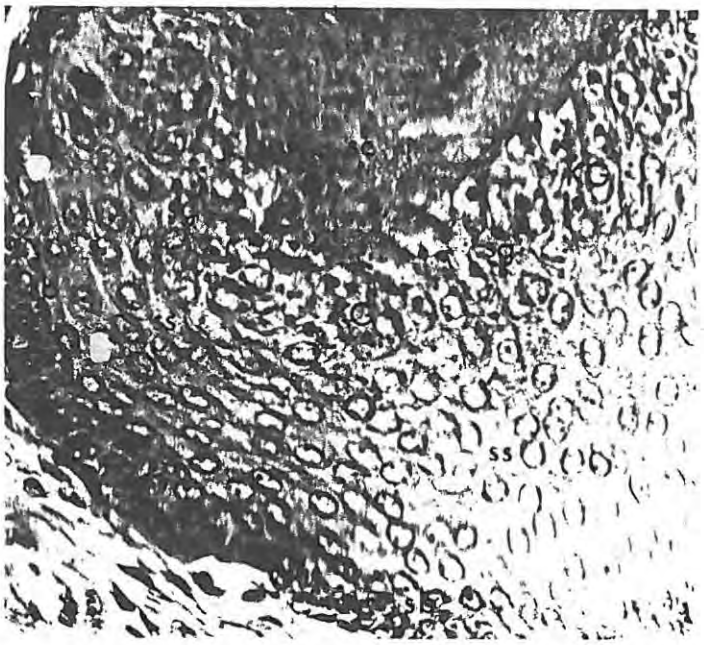


Plate 1,8 — 25 µm

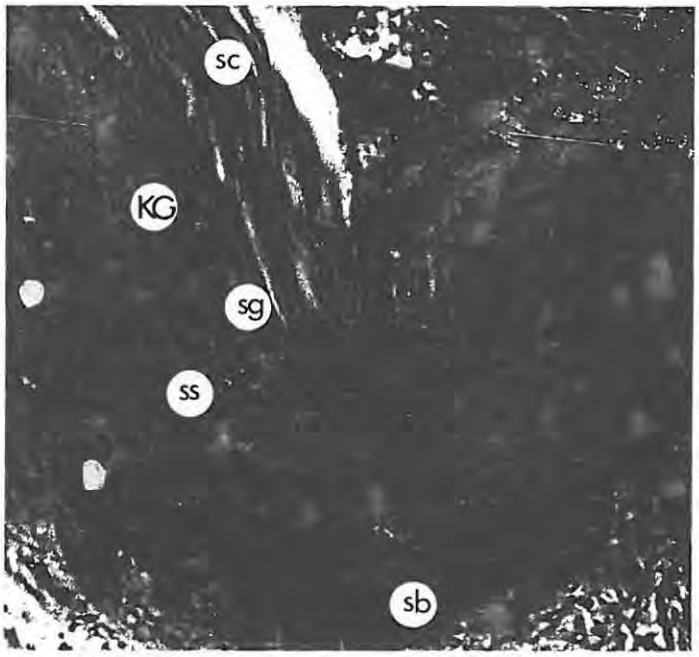


Plate 1,9 — 10 µm

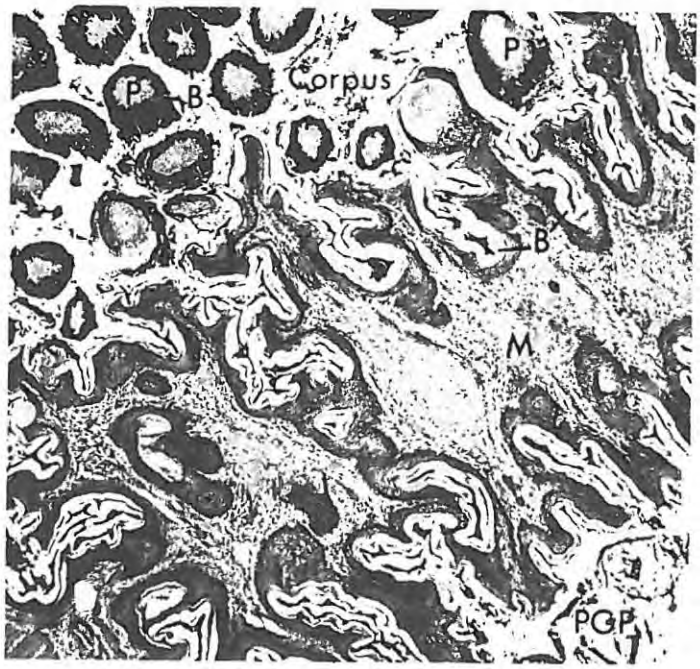


Plate 1,10 — 0,2 mm

numerous microhabitats throughout the papillae length (Plate 1,7 a). This was not seen in the adjacent FCE (plate 1,7 a & b) or PGP (Plate 1,4) where bacteria were fewer and formed a thin surface layer.

The junction between the corpus and PGP was marked by the termination of the papillated epithelium (Plate 1,10). PGP epithelium was folded and had a thicker malpighean layer and more distinct granular layer than the FCE but was otherwise histologically similar (Plates 1,4 & 1,10). Bacterial colonisation of the PGP was also similar to that of the FCE (Plates 1,4 & 1,7 a & b). The grenzfalte, separating the glandular and non-glandular areas, was a keratinised flap of tissue with a muscularis mucosa core (Plate 1,11). This flap appeared to be continuous with the thickened glandular epithelium on the distal side; in other words keratinised on the side of the PGP and glandular on the opposite side. This was not the case as the fold was separate from the glandular epithelium and, as in the meadow mouse Microtus pennsylvanicus (Golley 1960), was keratinised on both sides (Plate 1,11).

Distal to the grenzfalte was the antrum, histologically divisible into cardiac, pyloric and fundic regions with short transition areas between each (Fig. 1,1 b). Along the lesser curvature, extending from the grenzfalte, was the cardiac pouch (Plates 1,1 a & 1,12) with short, branched glands which increased in length towards the pylorus (Plate 1,13). Mucus-secreting glands with wide foveolae occurred in the pyloric region (Plate 1,13) and chief (or peptic or zymogen) cells were found at the base of the gland in the pyloric/fundic transition area Long tubular, fundic glands with narrow foveolae lined the greater curvature and extended to the grenzfalte (Plate 1,14). These glands presented a typical mammalian cytology: cuboidal neck cells, followed by mucous neck cells, chief cells at the base with parietal

Plate 1,11. The keratinised grenzfalte (G) (with a muscularis mucosal core) separating the PGP and antrum (A), extends from the greater curvature of the stomach (GC) to the incisura angularis (IA). F = fundic epithelium.

Plate 1,12. Branched cardiac glands in the cardiac pouch (Plate 1,1 a) supported by the lamina propria, have an uneven surface topography indicated in this longitudinal section. Mucus cells predominate in these glands.

Plate 1,13. Longitudinal section through the mucus cells of the pyloric region. Note the wide foveolae of these glands.

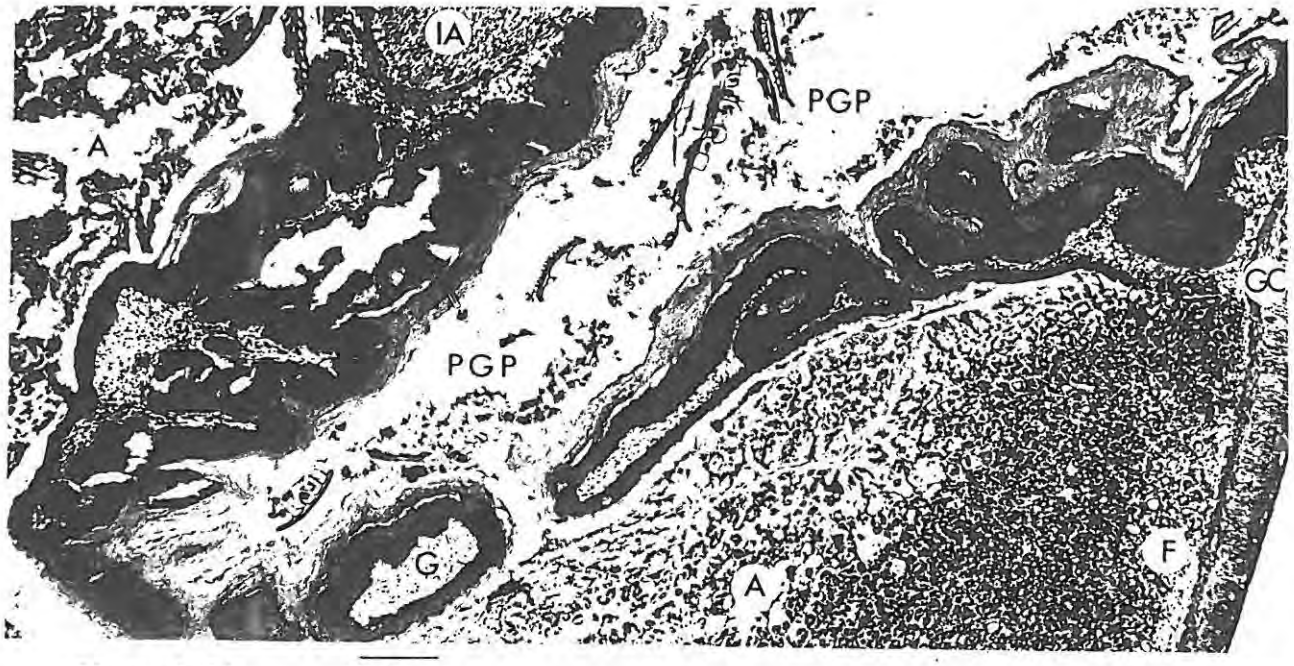


Plate 1,11 0,2 mm

Plate 1,12 50 µm

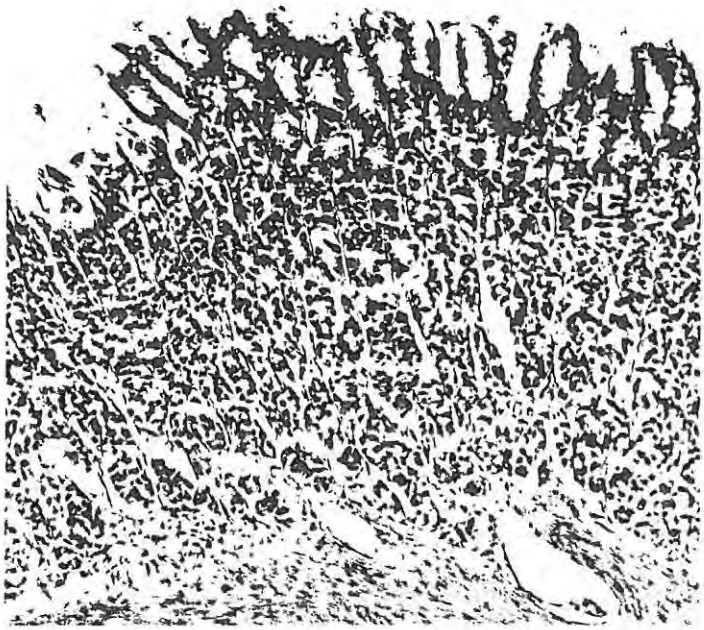


Plate 1, 13 0,1 mm

(or oxyntic) cells throughout the gland but mainly in the lower regions (Plate 1,14).

The pyloric glandular epithelium was replaced by duodenal mucosa immediately distal to the pyloric sphincter (Plate 1,2). The superior duodenum was ascending (Plate 1,1; see Dearden 1969) but there was no evidence of a prepyloric pouch on the lesser curvature of the stomach (Dearden 1969). The thickened inner circular muscle layer, comprising the sphincter, continued along the greater and lesser curvatures into the antrum (Fig. 1,1), ensuring a powerful closing mechanism.

Histochemistry.

Hematoxylin and eosin (H & E) and toluidene blue stains demonstrated a lack of nuclei in the stratum corneum but detected the presence of keratohyalin granules in the stratum granulosum (Plates 1,8 & 1,9). These features indicated complete keratinisation and formation of "soft" keratin. Sections staine with Azan stain (Table 1,1) also indicated the presence of keratin (Plates 1,15 a & b). Lipid droplets were not revealed in the corpal epithelium although oil red O and Sudan black stains were used. Tissues increased in stain intensity towards the stratum corneum suggesting an increase in lipid content.

The slight PAS positive reaction in the corpal basal and spinous cell cytoplasm was probably indicative of glycogen, important in the keratinisation process. The lamina propria and the bacteria were strongly PAS positive. Gastric mucin was present in the lumens of the cardiac (Plate 1,16) and pyloric glands but only in the foveolae of the fundic glands. No PAS reaction or toluidene blue metachromasia occurred in the corpus indicating an absence of mucus in the pars oesophagea.

Plate 1,14. Longitudinal section through the fundic glands. Three regions, characterised by diffe-ent cells, are present; A = surface mucus and mucus neck cells, B = basophilic chief cells, C = acidophilic parietal and basophilic chief cells. L = lumen.

Plate 1,15 a & b. The PGP (a), FCE and papillary (b) epithelia stained for keratin (Azan stain). The deep red colour indicates the keratinised stratum corneum and the fact that the papillae consist mainly of keratin. Connective tissue is deep blue in colour.

Plate 1,16. A longitudinal section through the cardiac region showing the occurrence of mucus (purple) in the lumen of the glands. PAS stain.

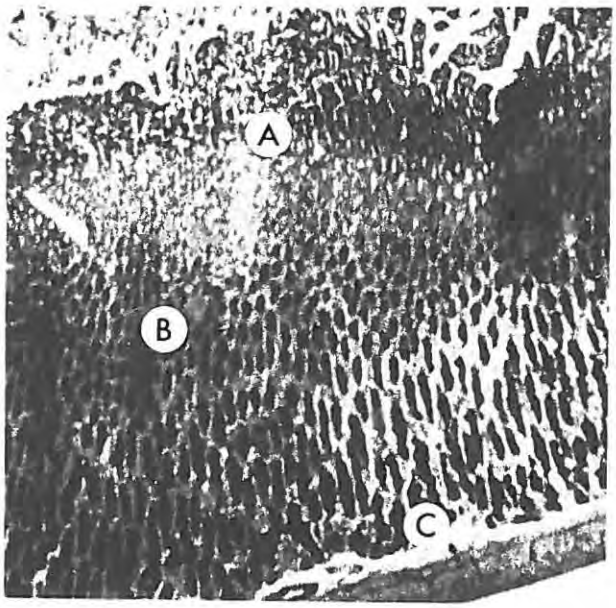


Plate 1,14 5 µm

Plate 1,16 50 µm

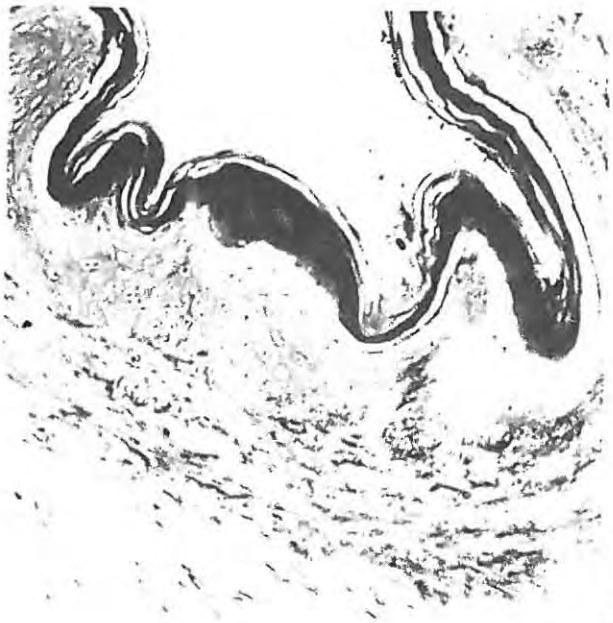


Plate 1,15 a 50 µm

Plate 1,15 b 50 µm

The presence of sulphur groups in the superficial epithelial layers of the pars oesophagea was confirmed by increased intensity of the aldehyde fuchsin (potassium permanganate) and ferric ferricyanide stains towards the stratum corneum. The stratum corneum stained positively with both stains.

Scanning electron microscopy (SEM).

During the SEM examination, gastric tissue from conventional, sterile and specially treated rats (STR) was used. The papillae of sterile rats were examined because numerous bacteria in conventional animals completely obscured surface detail (Plates 1,17 to 1,20). The FCE and PGP epithelium was visible in conventional rats because of fewer bacteria in these regions (Plates 1,21 & 1,22).

A major feature of the pars oesophagea, clearly demonstrated by SEM, was the difference in size among desquamating cells; PGP (Plate 1,21) and FCE (Plate 1,22) cells were similar in size but were larger than papillae cells (Plate 1,23). Similar size discrepancies were seen in histological sections (Plates 1,8 & 1,9). The FCE surrounded the papillae base where there was an abrupt decrease in size from the FCE to the papillae cells (Plate 1,23).

Differences were noted among the three rat types (conventional, sterile and STR). Surface cells of the sterile rats (Plate 1,20) were more regularly orientated (particularly in the FCE) than those from rats with a bacterial flora (Plate 1,19) suggesting that the bacteria may influence desquamation (see Abrams, Bauer & Sprinz 1963). STR occupied an intermediate position with compact epithelium interspersed with irregularly orientated squames in the FCE (Plate 1,24). STR

Plate 1,17. A low power scanning electron micrograph of a papilla from a conventional rat. Numerous attached bacteria give the papilla a 'fuzzy' appearance.

Plate 1,18. A papilla at the same magnification as that shown in Plate 1,17 after the rat was treated with antibiotic. The desquamating surface cells of the papilla are visible in this micrograph.

Plate 1,19. The papilla in plate 1,17 (ie from a conventional rat) shown at higher magnification. Rod-shaped bacteria are seen almost completely obscuring the epithelial surface cells.

Plate 1,20. The papilla in Plate 1,18 (ie from a sterile rat) shown at higher magnification. Regions (microhabitats) for bacterial attachment are evident. Note the relatively regular orientation of the desquamating cells.

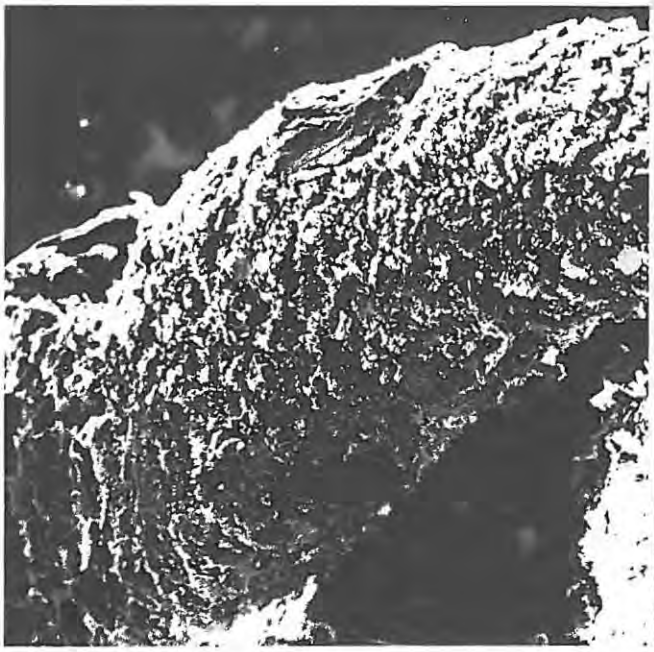


Plate 1,17 0,1 mm

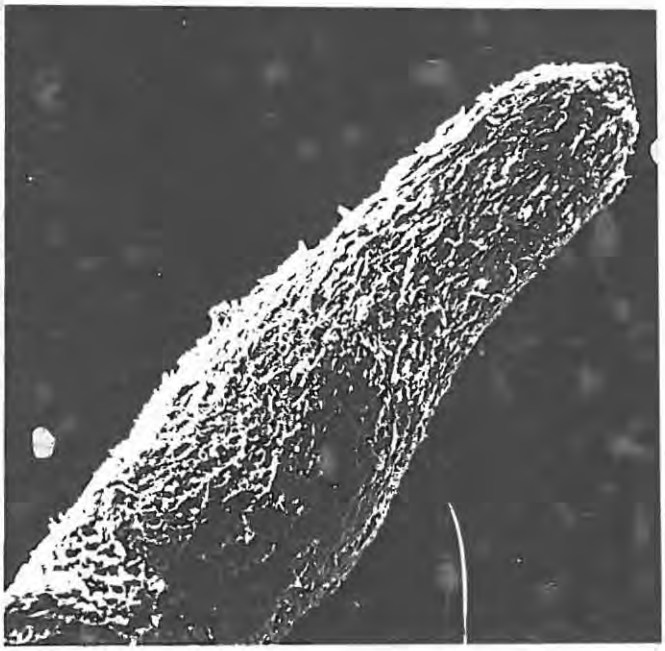


Plate 1,18 0,1 mm

Plate 1,19 25 μm

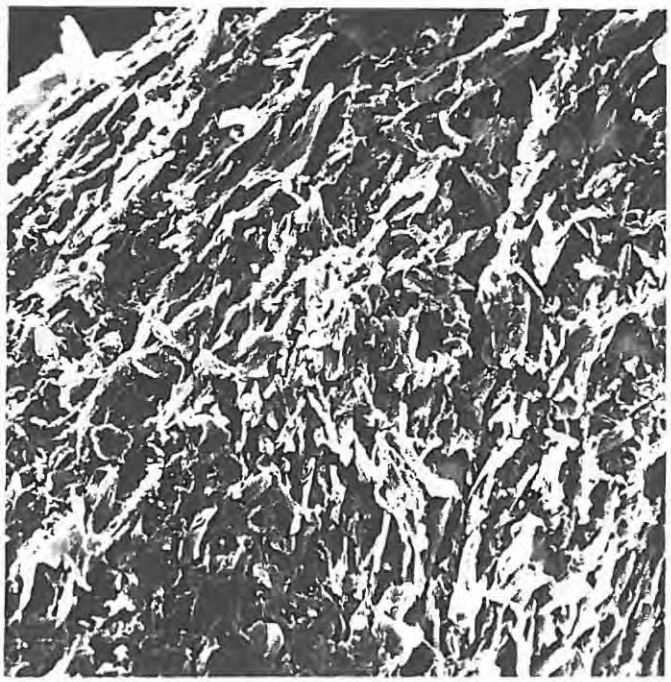


Plate 1,20 25 μm

Plate 1,21. A micrograph of the PGP epithelium from a conventional rat. Considerably less bacteria occur on this epithelium compared to the papillary epithelium (Plates 1,17 & 1,19). Note the absence of microhabitats and that the PGP desquamating cells are similar in size to those seen in the FCE (Plate 1,22).

Plate 1,22. A micrograph of the FCE epithelium from a conventional rat. Bacterial colonisation of the FCE resembles that of the PGP, ie fewer bacteria than on the papillary epithelium. FCE and PGP desquamating cells are of similar size.

Plate 1,23. A micrograph of a papillae base illustrating the size difference between the FCE and papillary cells (PE) (papillary cells are smaller and more numerous). FCE cells form a collar (arrow) around the papilla. Specially treated rat.

Plate 1,24. The FCE surface from a STR. Most of the epithelium is irregularly orientated but some regions of regular, compact epithelia occur. Note the smaller papillae cells in the upper right.

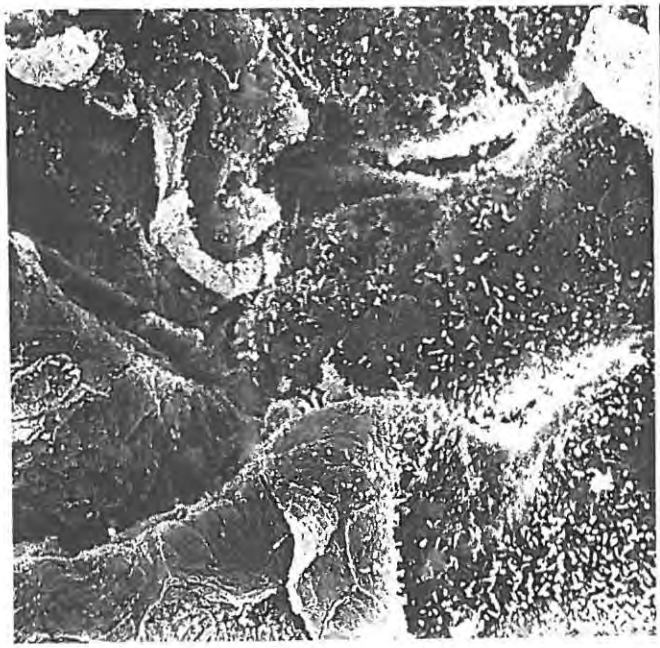


Plate 1,21 15 μm

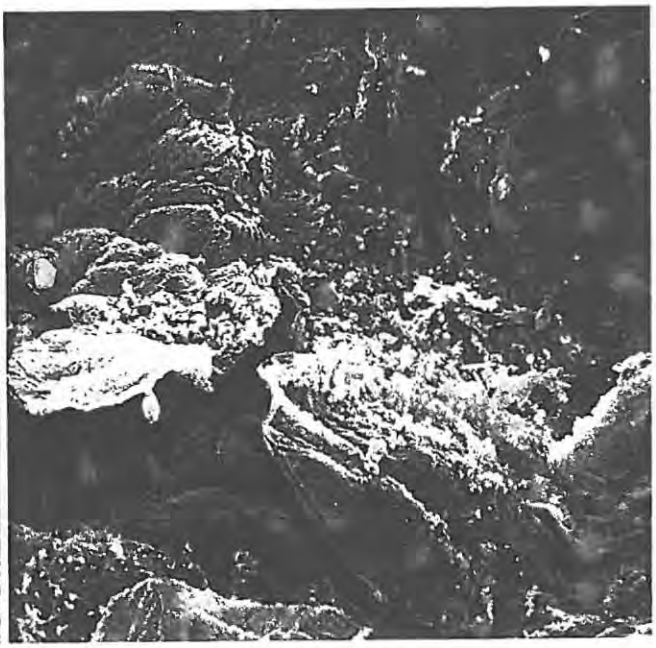


Plate 1,22 10 μm

Plate 1,23 25 μm

Plate 1,24 0,1 mm

papillae squames were also irregularly orientated (Plate 1,23). The PGP epithelium from conventional animals was similar to the FCE of STR.

The thick layers of desquamating papillary cells formed microhabitats for bacteria (Plate 1,25). This type of bacterial colonisation was peculiar to the papillae and afforded a large area for microbial colonisation. Similar microhabitats were absent from the FCE and PGP epithelium were fewer bacteria were found (Plates 1,21 & 1,22).

Foveolae of gastric glands were visible in the antrum (Plate 1,26) unless covered by mucus (Plate 1,27). Few structures, resembling isolated cocci, were seen in the antrum (Plate 1,28) but other micro-organisms, for example yeasts and protozoa, were absent. This may be due to the fact that these organisms are often free-living and thus would be lost during SEM preparation. Microvilli covered the surface epithelial cells in the cardiac (Plate 1,26) and pyloric regions.

Transmission electron microscopy (TEM).

TEM studies of the pars oesophagea confirmed the light microscope findings and a normal sequence of keratinising epithelium, similar to other tissues (Adams 1976; Fraser, MacRae & Rogers 1972), was seen (Plate 1,29).

Stratum basale.

The lamina propria, rich in collagen and fibroblast cells (Plate 1,30) occurred below the basal membrane which was separated from the thin, convoluted plasma membrane by an electron translucent space

Plate 1,25. A microhabitat between the desquamating cells (S) of a papilla from a STR. In conventional rats these desquamating cells are covered by bacteria. The great increase in surface area for bacterial attachment, afforded by microhabitats on the papilla surface, is apparent. Note the bacilli and cocco-bacilli.

Plate 1,26. The foveolum (F) of a cardiac gland showing surface mucus cell microvilli (Mi).

Plate 1,27. The surface of the cardiac region (Ca) partially covered by mucus secretions (Mu).

Plate 1,28. The pyloric region. Glands are completely obscured by mucus and only the positions of the foveolae are indicated by holes in the mucus. Occassional oval bodies (O) (of various sizes) and few bacteria (B), are present on the glandular surface but are not attached to the mucus.

Plate 1,25 5 μm

Plate 1,26 5 μm

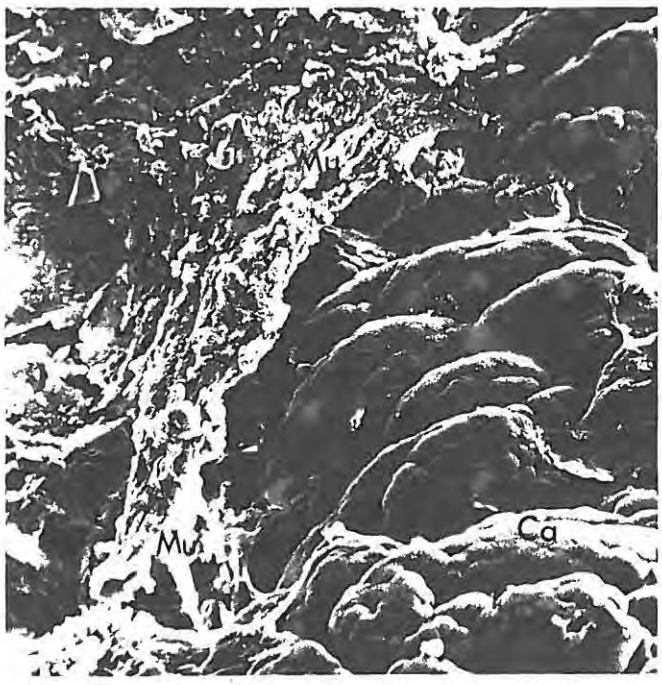


Plate 1,27 25 μm

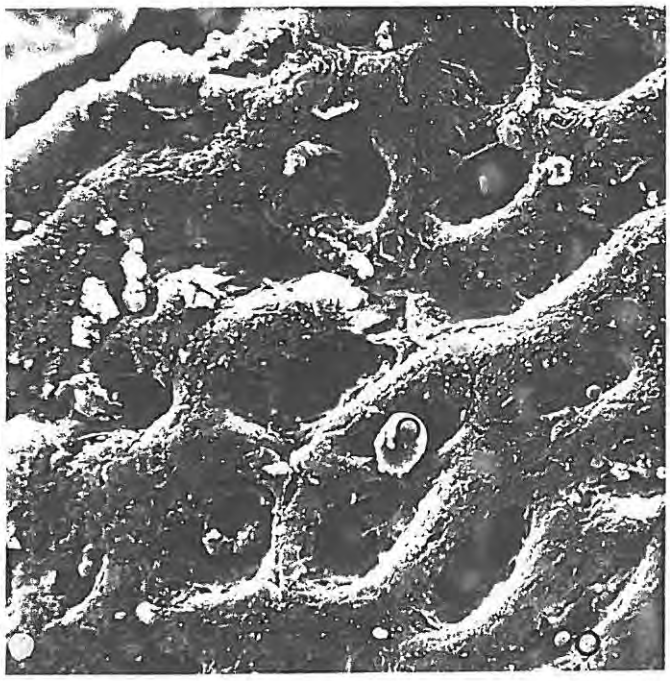


Plate 1,28 25 μm

Plate 1,29. A transmission electron survey micrograph through the FCE. The lamina propria (Lp) and four epithelial layers; strata basale (SB), spinosum (SS), granulosum (SG) and corneum (SC) are indicated. The cells flatten, increase in size and lose cytoplasmic organelles from the deep to the superficial epithelial layers. Tonofilaments (T) become apparent and COB appear in the upper spinous region. KG, characteristic of the stratum granulosum vary in size. The superficial malpighean layers are dominated by free ribosomes, KG and tonofilaments, most other organelles have degenerated. The horny cells consist of keratin fibrils in an electron dense matrix forming the keratin complex. The thick plasma membrane has numerous processes that interdigitate with other cells and maintain intercellular cohesion.

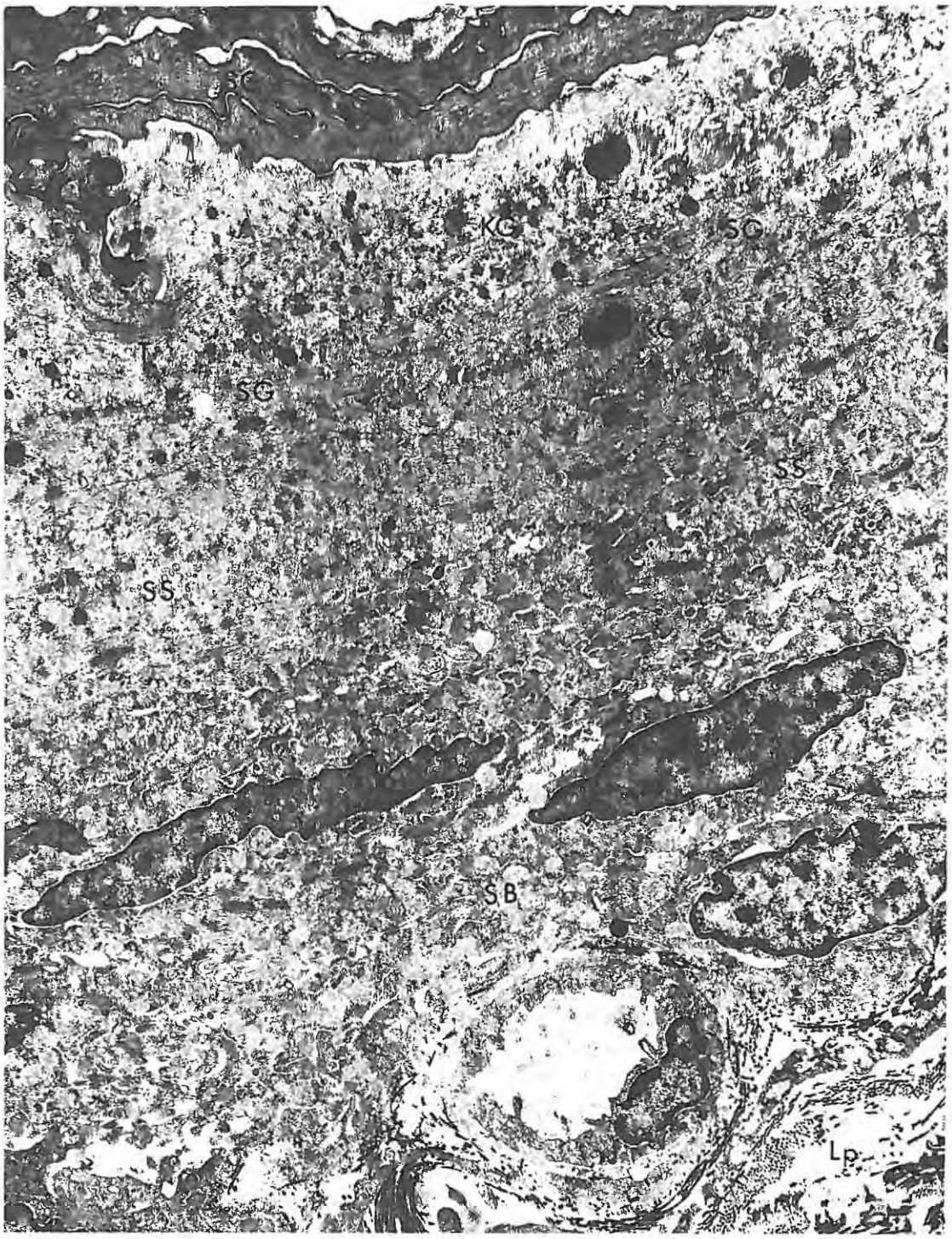


Plate 1,29 1 μm

(Plate 1,33). Mitochondria, with distinct membranes and cristae, were more abundant in the papillary basal cells than in the tonofilament-rich PGP or FCE (Plates 1,31; 1,32 & 1,33). Numerous microvillii extended from the basal cells into wide intercellular spaces but desmosomes were not common (Plates 1,31; 1,32 & 1,33). Free ribosomes occurred in the cytoplasm but golgi bodies and rough endoplasmic reticulae were rare (Plates 1,31; 1,32 & 1,33).

Stratum spinosum.

This layer, extensive .n the papillary epithelium, was characterised by invaginated cell borders and numerous desmosomes which conformed to the typical desmosome description (Skerrow & Matoltsy 1974; Staehelin 1974; Matoltsy 1975). The desmosomes consisted of a dense central or mid line in the intercellular space flanked by a narrow, low density region. A dense layer representing the outer leaflet of the plasma membrane and a thick, dense intracellular plaque formed a conspicuous structure of alternating light and dark lines (Fig. 1,3 a & b). A light zone, the electron lucent layer, approximately 165 A wide, on the cellular side of the plaque was bordered by a dense band (DB; Fig. 1,3 a & b). The plasma membranes were approximately 200 A apart and the whole structure measured about 850 A from DB to DB. Tonofilaments often associated with these desmosomes, the structures being refered to as desmosome complexes.

An increase in free ribosomes and free tonofilaments occurred in the spinous layer and cytoplasmic oval bodies (COB) appeared (Plate 1,34), some with a laminated internal structure (Plate 1,37) but most having a uniform, low electron dense interior (Plate 1,34). Larger,

Plate 1,30. Collagen and elastin fibres, blood vessels and fibroblast cells comprise the loose connective tissue of the lamina propria which underlies the gastric epithelium. This section through the corpus, shows collagen fibres in TS (Cft) and LS (Cfl) and an oblique section through a blood vessel (Bv). Elastin fibres are not visible as they are difficult to demonstrate without using s ecial techniques.

Plate 1,31. The multi-layered stratum basale of the papillary epithelium. The cells are cuboidal with a large nucleus (N). Many mitochondria (Mt) occupy t..e cytoplasm around the nucleus and free ribosome-like bodies are abundant. Tonofilaments, rough endoplasmic reticulum and golgi bodies are rarely seen. The thin plasma membrane is highly convoluted, relatively free of desmosomes and forms numerous microvillous extensions into wide intercellular spaces (I).

Plate 1,32. The stratum basale of the FCE resembles the papillary basal layer but has fewer mitochondria (Mt) than the papillae cells (Plate 1.31). Lettering as in Plate 1,31.

Plate 1,33. A deep basal cell from the PGP showing stratum basale characteristics (see Plate 1,31) in addition to collagen fibres (Cf) from the underlying lamina propria and a hazy electron translucent space between the connective tissue and basal membrane (arrow). Mitochondria (Mt) are found in numbers approximating those in the FC⁻ (Plate 1,32). Lettering as in Plate 1,31.

Plate 1,30 3 μm

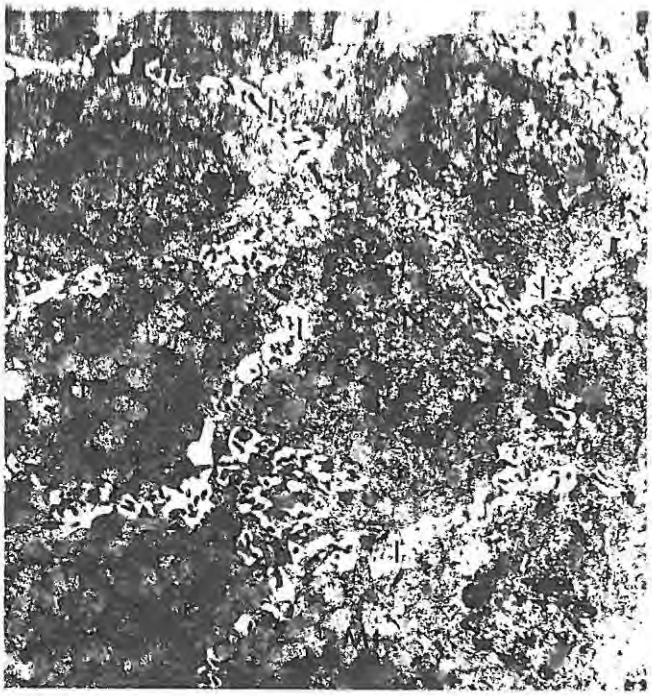


Plate 1,31 2 μm

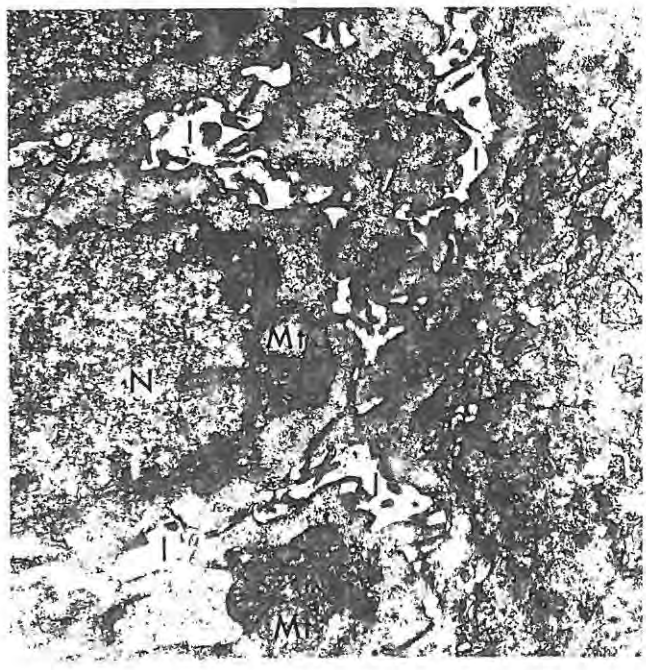


Plate 1,32 1 μm

Plate 1,33 1 μm

Fig. 1,3. A high power micrograph of a desmosome from the stratum spinosum (a). Desmosomes associate with tonofilaments and form the desmosome complex. Details of this complex are indicated in Figure 1,3 b: a fine, dense mid-line (Ml); thin, dense plasma membrane leaflets (Ol); an opaque intracellular plaque (Ip) and an electron-lucent layer (Ell) bordered bordered by a dense band (DB) (Matoltsy 1970). At the cell junction the leaflets are about 200 A apart while the whole structure is approximately 860 A wide from DB to DB. Fig. 1.3 a is not to scale.

0,3 µm

Figure 1,3 a

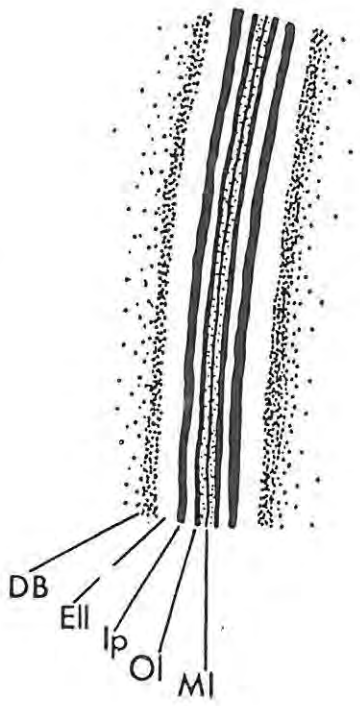


Figure 1,3 b

Plate 1,34. Upper spinous cells from the FCE. KG from the granular layer are visible at the top left. There is an increase in tonofilaments (T) and pale COB (some of which are large); other cellular organelles have begun degenerating and are absent from this section although final degradation processes are often not complete until the later stages of differentiation. D = desmosomes; SS = stratum spinosum; SG = stratum granulosum.

Plate 1,35. The junction between the granular (SG) and horny (SC) cells of the FCE. Large aggregations of KG are rare. This micrograph shows dispersal of a KG aggregation in the lower stratum corneum into fine, electron dense particles (arrows). Note the thick plasma membrane of the horny cells. D = desmosome.

Plate 1,36. A micrograph showing dispersing heterogenous KG in the upper granular layers of the FCE (arrow). Notice the appearance of COB along the cell borders and in some cases in the intercellular spaces (COB). The lack of cytoplasmic organelles is evident in the stratum granulosum as is the abundance of tonofilaments (T) (some closely associated with the KG) which form part of the densely packed keratin layer. Note the thick plasma membrane of the horny cells. D = desmosome; SG = stratum granulosum; SC = stratum corneum.

Plate 1,37. The junction between the spinous (SS) and granular (SG) layers. A spinous cell with degenerating mitochondria (Mt), small electron opaque particles (possibly ribosomes) and laminated COB (arrow) is visible in the lower left. Homogeneous intranuclear and intracytoplasmic KG, the latter surrounded by electron opaque particles and bundles of tonofilaments (T), characterise the granular layer. N = nucleus.

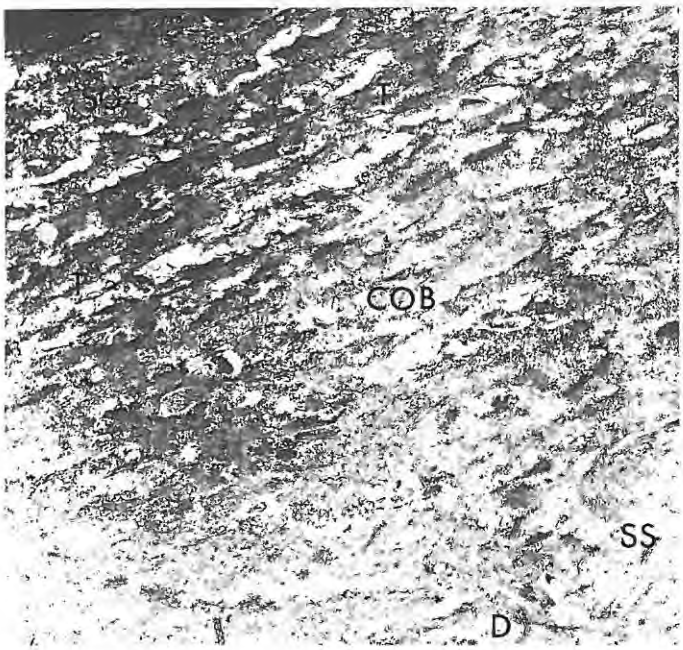


Plate 1,34 1 μm

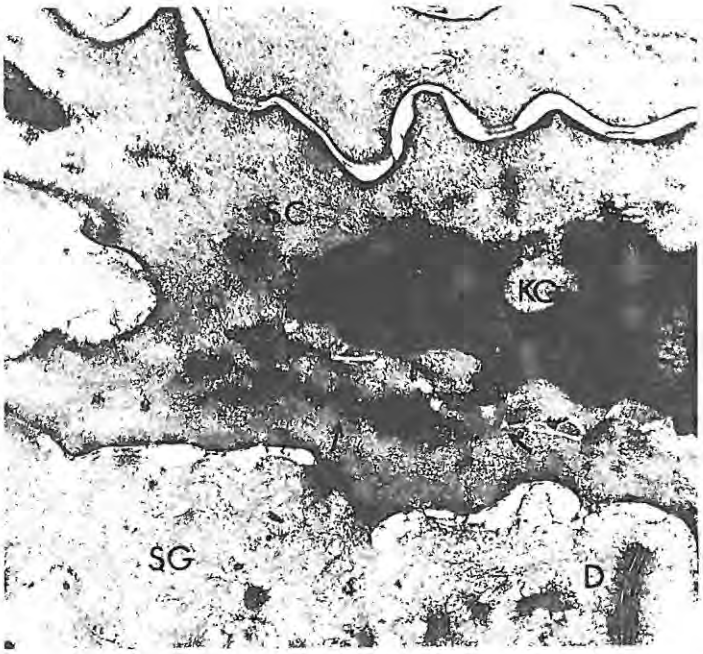


Plate 1,35 0,4 μm

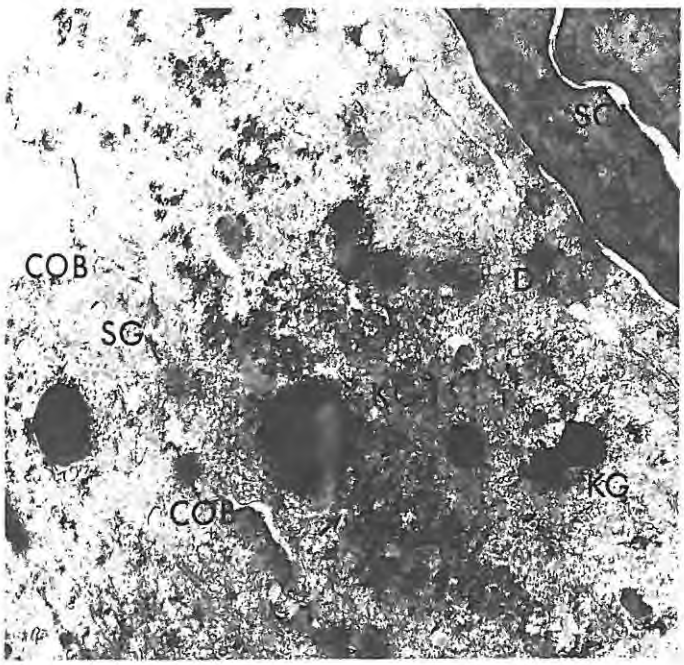


Plate 1,36 0,5 μm

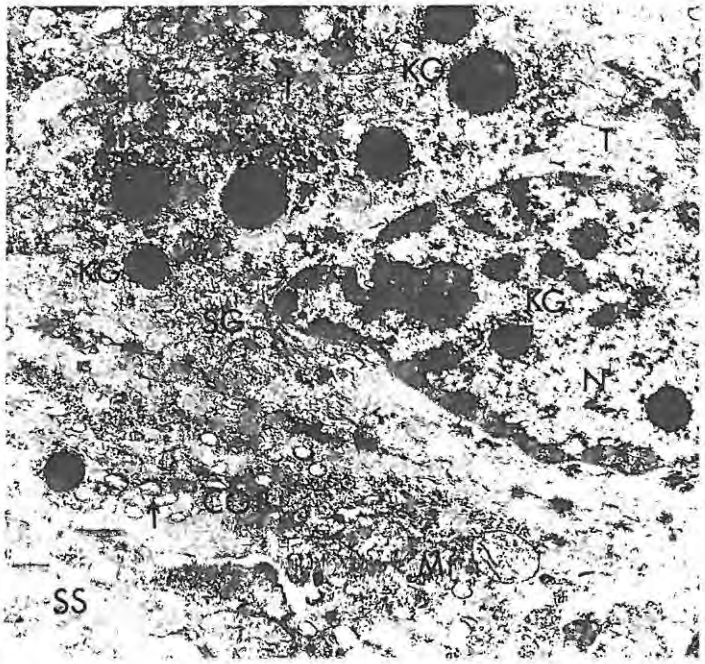


Plate 1,37 0,8 μm

electron translucent bodies were also seen in the FCE epithelium (Plate 1,34). In the superficial regions, cellular flattening, parallel to the basal membrane occurred and a slight decrease in the number of organelles was noted (Plate 1,29).

Stratum granulosum.

The diagnostic feature of the granular layer was the electron dense keratohyalin granules (KG) (Plates 1,36 & 1,37) which generally increased in size towards the horny layer but showed a large size variation (Plate 1,29). Large aggregations of keratohyalin (KH) were not common and the granules dispersed into fine particles in the upper layers (Plates 1,35 & 1,36). Intranuclear and intracytoplasmic KG were morphologically variable (Plate 1,37); heterogenous granules (with electron dense structures in a lighter matrix) were seen in the papillary and FCE cells (Plate 1,36) and resembled composite granules (Jessen 1970). Homogenous electron dense granules were also present (Plate 1,37). Granules with a low density core and electron dense periphery were noted in the FCE (Plate 1,38).

KG were associated with tonofilaments and surrounded by electron dense particles (Plate 1,39). In opportune thin sections the fine substructure of the granules resembled the small particles around the periphery (Plate 1,39). Similar particles, that may have been ribosomes, were distributed throughout the cytoplasm (Plate 1,37).

In the superficial region of the granular layer were various cytoplasmic oval bodies (COB), more numerous than in the spinous region. The COB often aggregated at the plasma membrane and were seen

Plate 1,38. Unusual KG in the FCE with a comparatively pale core and electron opaque periphery are seen in the upper granular layers. The stratum corneum (SC) is on the top left. Degenerating mitochondria (Mt) and homogenous COB are visible. SG = stratum granulosum.

Plate 1,39. A high power micrograph showing the KG substructure which resembles an aggregation of the small, electron opaque cytoplasmic particles. The close association between tonofilaments (T) and KG is seen in this micrograph.

Plate 1,40. Superficial granular cells, the horny layer is visible at the top left (SC). A KG is seen in the dilated cisternae (arrows) of an endoplasmic reticulum which possesses a darker matrix than the surrounding cytoplasm.

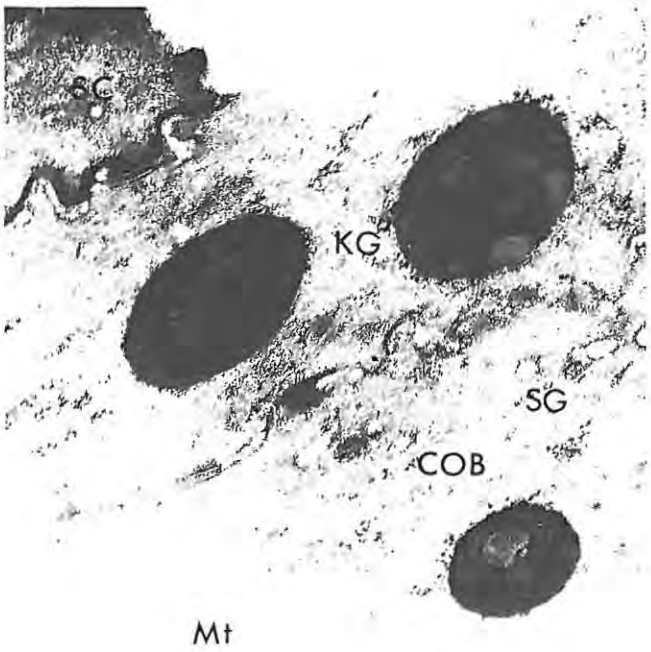


Plate 1,38 0,3 µm

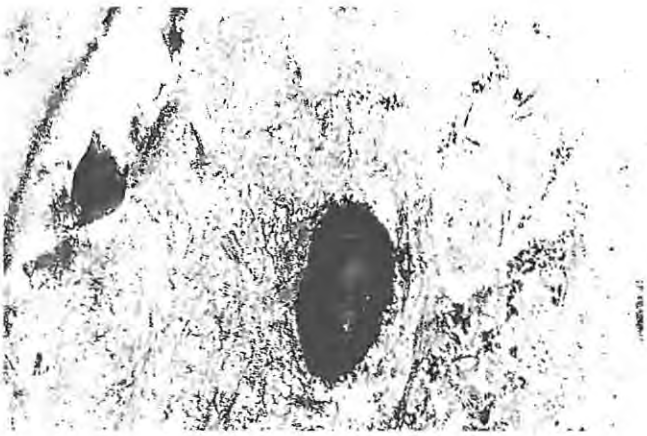


Plate 1,39 0,3 µm

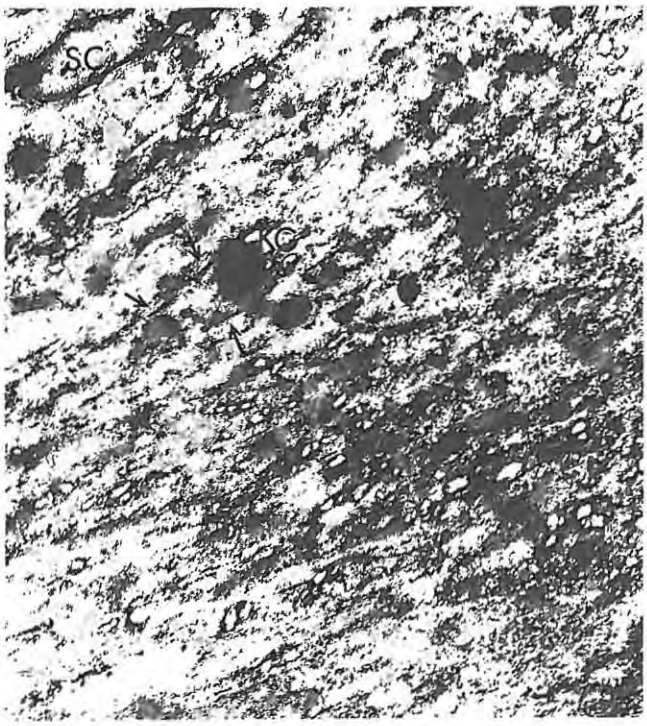


Plate 1,40 0,5 µm

in the intercellular spaces (Plate 1,36). Selective organelle degeneration (Lavker & Matoltsy 1970) was advanced (Plate 1,29) and few degenerating nuclei and mitochondria remained (Plates 1,29 & 1,38). Superficial cells were flattened and the cytoplasm consisted of dispersing KG, tonofilaments and few COB (Plate 1,29).

At high magnification, structures resembling dilated rough endoplasmic reticulum (RER) cisternae (see Lavker & Matoltsy 1970) were present in the pars oesophageal granular cells (Plate 1,40). Between the dilated cisternae was a less dense material, conceivably endoplasmic reticulum protein described by Lavker & Matoltsy (1970; Plate 1,40).

Stratum corneum.

A thickening of the horny cell's plasma membrane marked the junction between the stratum granulosum and the stratum corneum (Plates 1,29; 1,35 & 1,36). Few desmosomes, hemidesmosomes, degenerating nuclei and KG in various stages of dispersion were visible in the lowest horny cell layer (Plate 1,35). These organelles were absent from the higher levels and intercellular attachment was mediated by squamosomes (see Allen & Potten 1974) and interdigitating cell borders (Plates 1,41 & 1,42). The cytoplasm of the stratum corneum consisted of a filament-amorphous matrix and the cell was surrounded by a thickened envelope (Plates 1,41 & 1,42); resembling a B-type cell (Brody 1970).

At the papillary periphery, spaces between desquamating cells widened and bacterial colonisation of these spaces corresponded to the microhabitats seen in the scanning electron micrograph (Plate 1,25). Attachment of bacteria to the corpal epithelium is discussed in Chapter 3.

Plate 1,41. A section through the horny layer of a papilla showing the filamentous-amorphous matrix. Note the fewer desmosome-like junctions and numerous intercellular processes formed by loss of cytoplasmic water and subsequent cell shrinkage. The cells are devoid of organelles and contain a fibril-rich keratin complex bounded by a thick cell membrane. Q = squamosomes.

Plate 1,42. Superficial cells of the PGP horny layer showing the highly interdigitated cell borders maintaining cell cohesion (except the outermost cell) and the filamentous matrix of the cells. Fibres in the penultimate layer are orientated at 90 degrees to the angle of section. B = bacterium.

Plate 1,41 2 μm

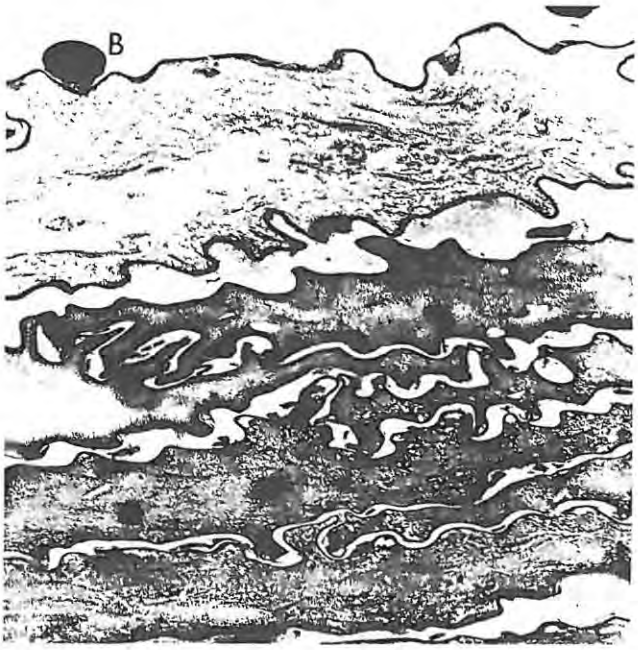


Plate 1,42 1 μm

Plate 1,43. A section from a chief cell from a fundic gland. Features typical of these cells are apparent: Z = zymogen granules, ER = endoplasmic reticulum, N = nucleus.

Plate 1,44. A parietal cell from a fundic gland. Note the caniliculus with microvilli (V) around the nucleus (N) and the abundance of mitochondria (Mt). Z = zymogen granules from an adjacent chief cell.

Plate 1,45. An enterochromaffin cell surrounded by chief cells (CC). Note the electron dense granules and the light cytoplasm of the endocrine cell. N = nucleus.

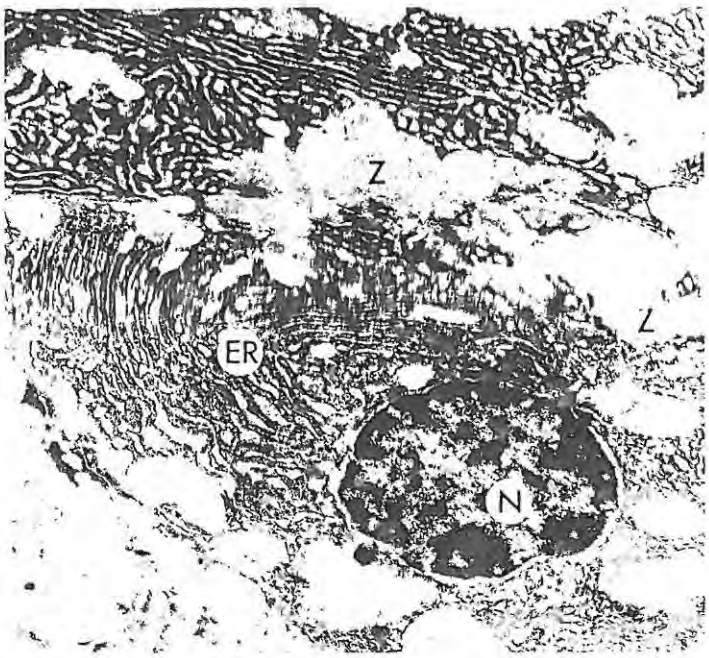


Plate 1,43 — 1 μm

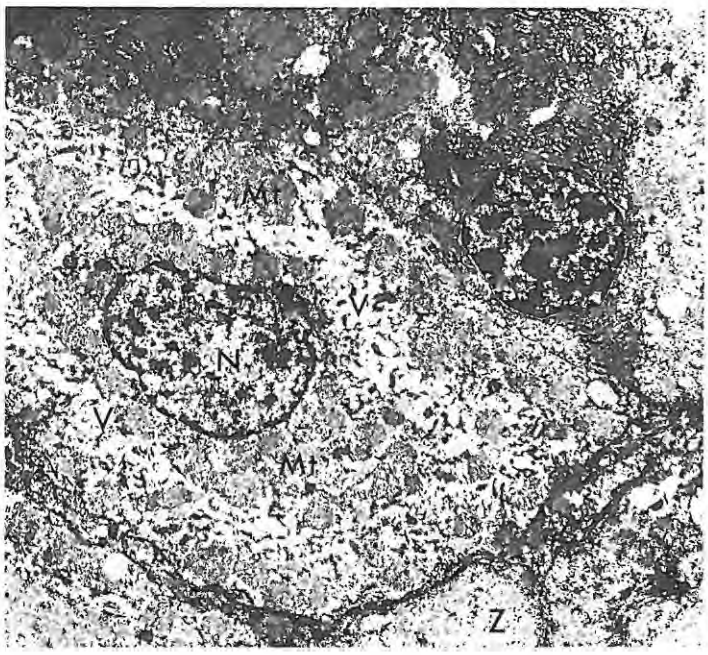


Plate 1,44 — 2 μm

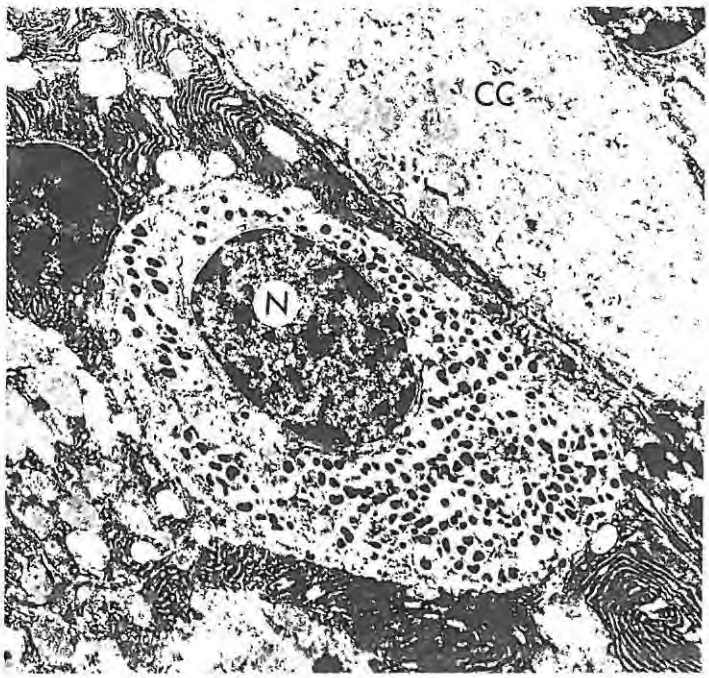


Plate 1,45 — 2 μm

TEM observations confirmed the presence of surface epithelial cells with microvilli, chief cel's (Plate 1,43) and parietal cells (Plate 1,44). Enterochromaffin cells were present in the fundic region (Plate 1,45).

DISCUSSION.

Gross morphology and histology.

The corpus of M. albicaudatus has a non-glandular, stratified squamous epithelium modified to form keratinised papillae, while the antrum possesses cardiac, fundic and pyloric glands. Compared with the simple and advanced stomach types (Carleton 1973), M. albicaudatus has both primitive (hemiglandular) and advanced (bilocular) characteristics. The term papillated bilocular hemiglandular is adopted for such a stomach which parallels that of C. gambianus (Caiman et al. 1960). This name suggests that the stomach is transitional between the unilocular hemiglandular and bilocular discoglandular conditions, a conclusion reached by Perrin & Curtis (1980).

Musculature at the gastro-oesophageal junction in M. albicaudatus superficially resembles that of certain microtine rodents, as a layer of longitudinal striated muscle continues from the oesophagus to the incisura angularis or corpo-pyloric fold (Dearden 1966). In microtines the striated and smooth muscle in the oesophageal sphincter and adjacent corpo-pyloric fold contributes to an effective closing mechanism which is increased by gastro-oesophageal pediculated flaps (Dearden 1966). Closing of the gastro-oesophageal junction in M. albicaudatus is mediated by the oesophageal sphincteric muscles only.

The incisura angularis, which is not adjacent to the sphincter, is not directly involved in this closing mechanism. Instead, the outer longitudinal striated muscle layer forms part of a complex muscle system linking oesophageal sphincteri, action with constriction of the PGP. To explain the function of this system it is proposed that during feeding the oesophageal sphincter is relaxed and food enters the corpus. At the same time striated and smooth muscle fibres in the incisura angularis contract preventing food from entering the antrum. Only when food in the corpus has been subject to particular digestive functions, (see Section II), do these muscles dilate and open the constriction between the corpus and antrum.

Generally the outer tissue layers do not deviate from the normal gastric tissue plan (Dearden 1966, 1969; Madge 1975) except in the relative thickness of the muscle layers in different regions of the stomach. Peters and Gaertner (1973) found that the forestomach of R. norvegicus and M. musculus was thin and elastic while the glandular stomach was thick-walled and muscular. The lack of forestomach musculature means that mechanical preparation of food in the rodent stomach cannot be similar to that of ruminants (Krzywanek 1927). M. albicaudatus however, has a thicker muscularis externa in the corpus than in the antrum suggesting that the mechanical preparation of food in the forestomach is more important in the white-tailed rat than in the rodents examined by Peters and Gaertner (1976).

The characteristic gastric feature of M. albicaudatus is the completely keratinised corpal papillae covered with bacteria. These papillae cannot absorb any digestion products that may be formed in the corpus but rather increase surface area for attachment by large numbers of bacteria (Chap. 3). The stratified squamous FCE and PGP epithelium resemble the non-glandular gastric epithelium present in many myomorphs (Dearden 1969; Sakata & Tamate 1976; Luthje 1976). Here the stratum corneum is thinner (compared to the papillae) and

consequently the capillaries in the lamina propria are in closer contact with the corpal contents. Absorption of gastric digestion products may occur through the FCE and PGP epithelium although their surface area is probably too small for a significant uptake.

The antrum with cardiac, pyloric and extensive fundic glands is indicative of much protein digestion by M. albicaudatus. It is improbable that this rodent utilises bacterial protein since relatively few of the attached bacteria would be expected to pass to the antrum; certainly not enough to warrant a large area for protein digestion. (It has been shown that R. rattus and M. musculus do not obtain protein from a gastric bacterial source either, Gaertner & Pfaff 1979). Alternatively, M. albicaudatus may have a sufficiently protein-rich diet to necessitate the large area (antrum) for protein digestion.

Ultrastructure.

The typical ultrastructural features of keratinising epithelia (Fraser et al. 1972; Spearman 1973) are present in the pars oesophagea of M. albicaudatus. Epithelial cells (keratocytes, Fraser & MacRae 1978), generated in the stratum basale, undergo progressive and functional death as they pass th. ough the mid epithelial layers to form the dead, stratum corneum (Ugel 1971; Jarrett 1973; Spearman 1973). During cell differentiation protein-rich keratogenic structures (cytoplasmic oval bodies - COB, keratohyalin granules - KG and tonofilaments), synthesised in the cytoplasm, increase in number at the expense of other organelles (for example nucleus, mitochondria, rough endoplasmic reticulum - RER) which are degraded by lysosomal hydrolytic enzymes (Jarrett 1973; Spearman 1977). In the upper malpighean layers cytoplasmic organelles are degraded and extruded

into the intercellular spaces with cytoplasmic water (Spearman 1977; Fraser & MacRae 1978). Consolidation of the cell containing keratogenic proteins (involving the formation of disulphide linkages) produces a filamentous amorphous-matrix complex (keratin complex) bound by a thickened plasma membrane from which surface cells are sloughed (Matoltsy 1975; Fraser & Macrae 1978). The keratogenic structures (COB, KG and tonofilaments) play a major role in these drastic morphological and chemical changes; each structure will be discussed independantly and current views on their functions related to this study.

Keratohyalin granules (KG).

A conspicuous feature of the gastric keratinising epithelium of M. albicaudatus is the electron dense KG in the stratum granulosum. Much controversy has centred around KG in all epithelial types but recent sophisticated extraction and purification techniques and detailed electron microscope studies have led to a better understanding of these granules (Jessen 1970; Ugel 1971; Fukuyama & Epstein 1973; Matoltsy 1975). Many authors have demonstrated the morphological diversity of KG (Farbman 1966; Matoltsy & Matoltsy 1970; Jessen 1970; Fukuyama & Epstein 1973; Jessen, Peters & Hall 1974, 1976) and a number of different granules are present in the gastric epithelium of the white-tailed rat. This diversity can be explained by variation in the KG constituents (for example sulphur, phosphorus) in the same or different epithelia (Dubrul 1972; Fukuyama & Epstein 1973; Jessen et al. 1976) while the chemical and morphological differences may reflect different functions of the KG (Jessen et al. 1976). KG may also exhibit regional variations resulting from differences in the transformation rate of components into their keratin form (Fukuyama & Epstein 1973). The heterogeneity of KG in M.

albicaudatus is probably due to some of these factors. Chemical variation (and therefore morphological variation) may be attributed to the slightly different functions of the FCE, PGP and papillary epithelia.

The early views concerning the origin of KG were based on morphological evidence only. Oemke & Petry (1964) and Sognnaes & Albright (1956), noted intranuclear and intranucleolar KG and propounded that KG originated in these organelles. Although internuclear KG are common (Jessen 1970) the idea of a nuclear origin is no longer accepted (Squier 1968). Another view was that the small particles around the KG were ribosomes synthesising keratohyalin (Rhodin & Reith 1962; Farbman 1966; Squier 1968) but subsequent studies have shown that these particles are either inactive or are not ribosomes at all (Fukuyama & Epstein 1967 op. cit. Ugel 1971).

It is believed that KG form by the aggregation or polymerisation of small particles (ribonucleoproteins, Ugel 1971) synthesised in the cytoplasm of differentiating epithelial cells (Matoltsy & Matoltsy 1970; Jarrett 1973; Spearman 1977). Disulphide bonds between the protein sub-units maintain the KG in an aggregated state (Matoltsy & Matoltsy 1970; Matoltsy 1975) and these granules may coalesce forming large units in the transformational cells (cells between the granular and horny layers; Lavker & Matoltsy 1970). However, transformation cells are not seen in this study probably as a result of different keratinisation processes between the gastric epithelia of M. albicaudatus and the epithelium studied by Matoltsy and co-workers.

As a result of ribonuclease activity (and other unknown mechanisms) in the region of the granular/horny cell junction, the large KG disperse (Ugel 1971) and release proteins which form the interfibrillar matrix of the horny cell (Fraser & MacRae 1978). It has been shown by Lavker & Matoltsy (1970) that another protein, endoplasmic reticulum protein (ERP), combines with the dispersing KG protein prior to the formation of the horny cell matrix in the rumen. ERP is present in the gastric epithelium of M. albicaudatus but may be peculiar to gastric mucosal tissue as no ERP was found in human buccal epithelium (Landay & Schroeder 1977) and has not been reported in other epithelia.

A complication was introduced by Jessen (1970) who identified two KG with different functions in the same epithelium. One type of granule formed the horny cell matrix and the other, which occurred along the cell periphery, caused plasma membrane thickening (Jessen et al. 1974). The morphological gastric study of the white-tailed rat is consistent with recent ideas that KG are important in the formation of the horny cell matrix but do not support the claim that certain KG cause cell membrane changes.

Tonofilaments.

Tonofilaments are abundant in keratinising epithelia (Meyer & Schroeder 1975) and are involved with KG in the formation of the horny layer (Matoltsy 1975). Drockmans and co-workers demonstrated by biochemical analyses that there are two types of epithelial tonofilaments; 'free' cytoplasmic tonofilaments and those associated with the desmosome complex (Drochmans, Freudenstein, Wanson, Laurent, Keenan, Stadler, Leloup, & Franke 1978). Free tonofilaments,

synthesised in the suprabasal cells, often appear in bundles (Fukuyama, Murozuka, Caldwell & Epstein 1978) and consist of a fibrous protein, pre-keratin, which gives the keratin molecule its alpha helical structure (Matoltsy 1975). Polypeptide chains in prekeratin and in horny cell keratin fibres are similar (Matoltsy 1975) suggesting that tonofilaments are involved in the formation of the fibrous component of the filamentous amorphous-matrix complex (Jessen et al. 1976; Fukuyama et al. 1978; Fraser & MacRae 1978).

Tonofilaments of the desmosome complex have a different function and they form a cytoskeletal network (stabilised by desmosomal attachments, Matoltsy 1975; Drochmans et al. 1978). The resulting cellular stability and flexibility allows dispersal of mechanical stress across the whole tissue rather than on individual cells (Staehelin 1974; Matoltsy 1975; Fukuyama et al. 1978). This function is expected to be important in the stomach (due to gastric churning) where mechanical stresses are high. Epithelial stresses may account for the numerous tonofilaments in the gastric epithelium of M. albicaudatus and in the rumen (Lavker & Matoltsy 1970).

The desmosome complex is broken down with the other organelles in the stratum granulosum and the tonofilaments disperse. Fukuyama and co-workers claim that these tonofilaments form keratin fibrils in the horny layer (Fukuyama et al. 1978) although biochemists do not support this idea (Spearman 1977). Breakdown of the intercellular junctions causes a loss of intercellular cohesion resulting desquamation of the surface cells (Staehelin 1974). The lower cells of stratum corneum are held in place by an intercellular cementing substance and irregular cell borders (Allen & Potten 1974). The cohesiveness of the

thick stratum corneum in M. albicaudatus can be explained by the highly interdigitating nature of the horny cells compared with the PGP or FCE cells.

Cytoplasmic oval bodies (COB).

The small oval bodies of the mid-epithelial layers have been given various names by different authors. Many names are synonyms for a particular organelle with similar functions but some oval bodies are functionally different from others. For this reason the term cytoplasmic oval bodies (COB) is used here as it groups these structures on the basis of morphological similarities only and does not accord them a specific function. The possible functions of the various COB in M. albicaudatus will be briefly considered. It must, however, be emphasised that there is considerable dispute concerning the identification and function of these organelles.

On the basis of positive PAS reactions (Lavker 1969; Lavker, Chalupa & Opliger 1969) and electron micrograph studies (Lavker, Chalupa & Dickey 1969) COB in rumen epithelium were identified as mucus granules. The negative PAS reaction in the corpus of M. albicaudatus indicates that mucus or lipid granules (which give a PAS positive reaction) are absent from this region.

Lysosomes have been found in sheep ruminal epithelium (Lavker & Matoltsy 1970; Landay & Schroeder 1977) and hydrolytic enzymes, originating from lysosomes, may be involved in cellular degeneration preceding keratinisation (Jarrett & Spearman 1964; Lavker & Matoltsy 1970; Meyer & Schroeder 1975). No typical lysosome-like bodies were seen in M. albicaudatus but it is possible that during cellular degeneration some of the numerous COB seen in the rat's gastric

epithelium are lysosomes. ERP is released by the action of hydrolytic enzymes in the stratum granulosum (Lavker & Matoltsy 1970) and since this protein is present in the corpal epithelium of the white-tailed rat, it is likely that lysosomes are involved in its release.

The thick horny cell membrane is marked in the corpus of M. albicaudatus although it is unknown what causes the membrane changes. An early idea was that electron dense membrane-coating granules (MCG) attach to the granular cell membrane in the sheep rumen and extrude their contents into the intercellular space (Matoltsy & Parakkal 1965). Membrane thickening is facilitated by deposition of this keratinolytic-resistant material on the outer cell surface (Farbman 1964; Matoltsy & Parakkal 1965). Electron translucent and a few laminated COB attach to the plasma membrane of the granular cells in the white-tailed rat but it is impossible to detect extrusion of any material into the intercellular space and the role of these bodies in the rat is unknown. If they are involved in cell membrane thickening, their presence in the most superficial granular cells of the rat, although contrary to previous reports (Farbman 1964; Matoltsy & Parakkal 1965) is consistant with the fact that cell membrane changes occur at a later stage of differentiation in M. albicaudatus than in the sheep rumen (Lavker & Matoltsy 1970).

Recently, it was reported that KG fuse with the plasma membrane and cause membrane thickening (Jessen et al. 1974, 1976). Although there was no evidence of this in M. albicaudatus, because the KG dispersed in the upper layers, it is possible that KG protein is incorporated into the cell membrane and contributes to the formation of the horny cell envelope.

A wide variety of epithelia undergo keratinisation and details of the differentiation process often vary in different tissues (Spearman 1977; Fraser et al. 1972). To avoid confusion a summary of the sequence of keratinisation of the gastric epithelium of M. albicaudatus is presented below.

Tonofilaments are the first keratogenic structures to appear and they occur in small numbers in the basal cells. COB occur in the spinous cells and KG in the granular layer; all structures increase in number toward the mid-granular layer. A concomitant decrease in cytoplasmic organelles occurs although there is no evidence of lysosomes in this región. This is unexpected but some of the numerous COB may release hydrolytic enzymes which would degrade the organelles. In the stratum granulosum small electron dense particles aggregate to form KG which do not coalesce but disperse and mix with ERP in the region of the granular/horny cell junction where there are numerous tonofilaments. Cell membrane changes in this region may be caused by COB which deposit material onto the outer plasma membrane. Horny cells of the pars oesophagea contain fibrils in a random orientation embedded in an amorphous matrix; other organelles are absent. In the light of recent evidence it is tempting to believe that tonofilaments and KG in the gastric epithelium of the rat form the keratin complex but this cannot be stated with certainty until detailed biochemical studies are completed.

The clear epithelial stratification and presence of KG in the stratum granulosum of the gastric epithelium of the white-tailed rat is indicative of "soft" keratinisation. The corpal papillae show a different type of keratinisation to the FCE and PGP epithelium and the thick papillary stratum corneum resembles the pathological epidermal

condition commonly called hyperkeratosis (Jarrett 1973). However, this condition is not pathological in M. albicaudatus but is a normal, physiological adaptation whereby symbiotic bacteria are provided with ecological niches. The term physiological hyperkeratosis is thus suggested for this type of keratinisation and orthokeratin is suggested for the soft keratin of the FCE and PGP.

Epithelial growth factors.

Factors controlling the growth of the physiological hyperkeratotic (papillary) epithelium must differ from those controlling PGP and FCE epithelium growth as these epithelia have strikingly different thicknesses. Since epithelial thickness is influenced by mitotic rate (Bullough 1975), it is reasonable to expect that mitotic rate differs between these epithelia. Mitosis in mammalian tissue is controlled by the interaction of two chemical messengers; a tissue specific 'chalone' and a short range mesenchymal factor (Bullough 1975). The mesenchymal factor stimulates mitosis in cells immediately adjacent to the connective tissue (basal cells) but is inhibited by the long range chalone. In the epidermis, after mitosis in the basal layer, daughter cells either enter post-mitosis by migrating into the upper cell layers or continue mitotic division in the stratum basale (Bullough 1975). Normally the chalone is inhibitory, few mitotic divisions occur and half the daughter cells continue dividing in the mitotic cycle and half become post-mitotic (Fig. 1,4). During the post-mitotic phase the chalone also slows the aging process (keratinisation) by inhibiting post-mitotic transitional phases (Fig. 1,4 (1)).

At low chalone concentration (eg. during epidermal damage) mitosis is not inhibited and stimulation from the mesenchymal factor

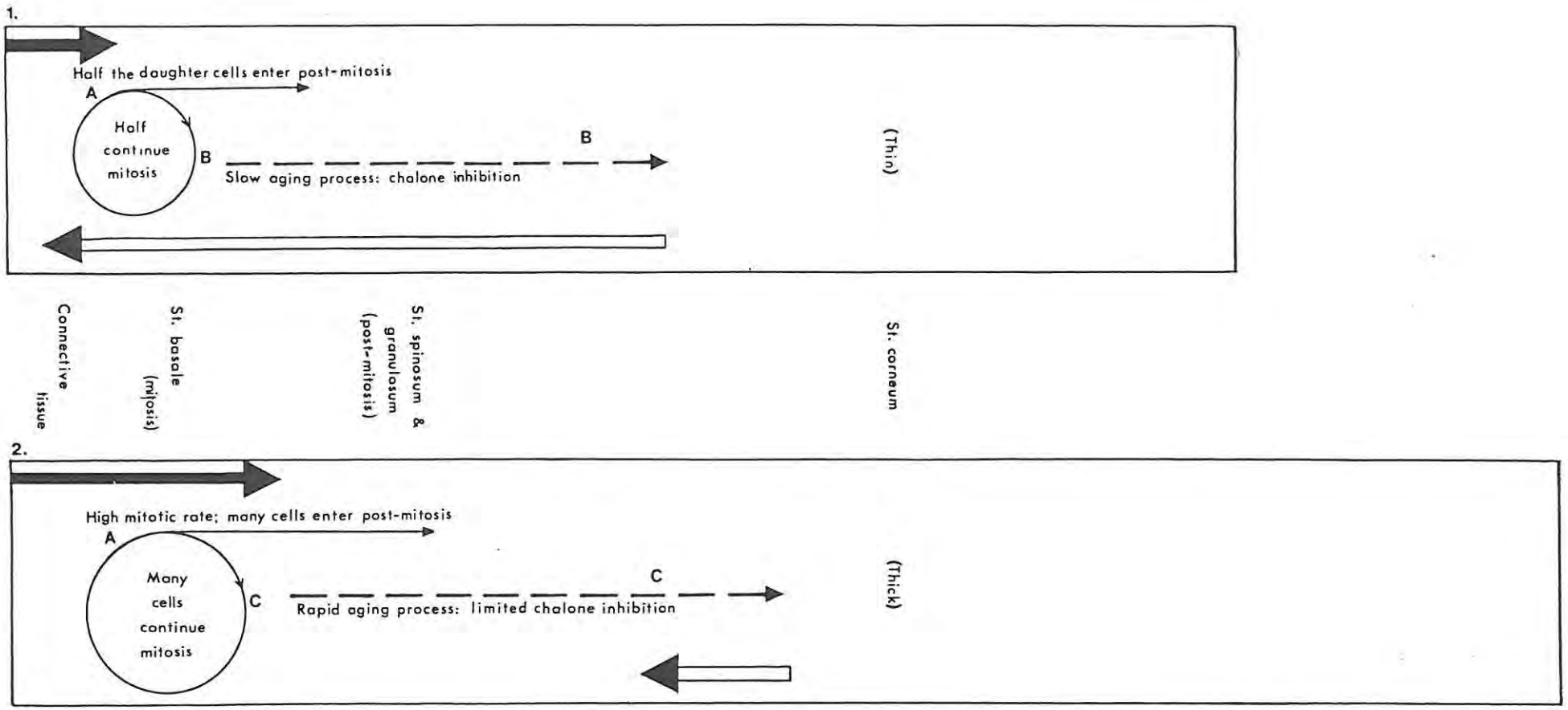


Figure 1,4. Diagram showing action of chalone (⟸) and mesenchyme (⟹) factors in controlling mitosis and epithelium thickness. 1. Epithelium with normal chalone concentration. 2. Epithelium with low chalone concentration - normal papillary epithelium of M. albicaudatus.

A. Decision to enter post-mitotic phase or continue mitosis; determined by chalone concentration.
B. Large chalone inhibitory influence.
C. Small chalone inhibitory influence.

is effective, more cells become post-mitotic (as a result of cell crowding and lateral cell pressure in the stratum basale) than under conditions of normal chalone concentration. The large number of cells in the post-mitotic stage are not inhibited by the chalone and aging (keratinisation) proceeds rapidly (Fig. 4,1 (2); Bullough 1975).

The epidermis exists in two states; phase 1 (characterised by a high chalone effectiveness causing low mitotic rate and slow post-mitotic aging, the epithelium/connective tissue junction is not folded and changes in mitotic rate do not effect epidermal thickness; Fig. 1,4 (1)) and phase 2 (with low chalone effectiveness and therefore a high mitotic rate and faster post-mitotic aging, a folded epithelial/connective tissue junction and/or a double basal layer and changes in mitotic rate cause changes in epidermal thickness; Fig. 1,4 (1)) (Bullough 1975). As all mammalian tissue types so far examined contain tissue specific chalones which interact with mesenchymal factors to control tissue thickness (Bullough 1975) it is not unreasonable to propose that gastric epithelium thickness in M. albicaudatus is controlled by similar chemical messengers. The thickened papillary epithelium with a double or triple basal layer parallels phase 2 type epidermis and, as a result of low chalone effectiveness, is expected to have a high mitotic rate causing increased epithelium thickening. The low chalone concentration would allow the mesenchymal factor to exert its stimulatory influence on the basal (and possibly suprabasal) cells. Thus, many cells will be undergoing mitosis and numerous cells will enter the post-mitotic phase, causing an increase in epidermal thickness (Fig. 1,4 (2)). The thick mid-epithelial layers may be a result of the high mitotic rate and subsequent production of numerous post-mitotic cells. In contrast, the FCE and PGP epithelium, parallels the phase 1 type

epidermis with high chalone effectiveness resulting in a low mitotic rate and thin epithelium. The folded epithelial connective tissue junction is probably an adaptation for distention of the stomach during feeding rather than a result of the mitotic rate.

The idea that a low chalone concentration is responsible for increased papillary epithelium thickness is appealing. However, it is likely that a combination of factors are involved in papillary hyperdevelopment. Autochthonous gut bacteria affect the epithelium with which they are in contact (Coates & Fuller 1977) by accelerating intestine epithelial desquamation and cell renewal rates (Abrams et al. 1963). In view of this, (Abrams et al. 1963) it might be expected that the large number of bacteria between the surface cells of the papillae (compared to the rest of the pars oesophagea) increase papillary desquamation and hence cell renewal rates. Areas having different concentrations may have a different magnitude of bacterial influences on the epithelial cells (Abrams et al. 1963). Thus, the fewer bacteria in the PGP and FCE will have less influence on the rate of epithelial desquamation. If the papillae of the white-tailed rat are indeed similar to the phase 2 type epidermis (Bullough 1975), increased cell renewal, assisted by the bacteria, will generate a thick epithelium. This idea is supported by the findings that sterile rats, unlike their conventional counterparts, have a regularly orientated epithelium (a similar observation was reported by Abrams et al. 1963) and that papillary cells are about half the size of the FCE and PGP cells. The irregular orientation may be a result of faster desquamation while small cell size may be related to rapid cell renewal rates.

In conclusion, the non-glandular corpus of M. albicaudatus is keratinised; FCE, papillary and PGP epithelia are present, each with different mitotic rates and thicknesses. The papillae are considered to play an intrinsic role in the corpus but owing to the absence of vascularisation and their keratinised form, they are not absorptive like rumen papillae (Hyden & Sperber 1965; Lavker, Chalupa & Dickey 1969; Henrikson 1970). Instead the papillae may be important in maximising surface area for bacterial attachment (Maddock & Perrin 1981). The concept will be explored further in Chapter 3 and Section II.

CHAPTER TWO.

DEVELOPMENT OF THE GASTRIC MORPHOLOGY.

INTRODUCTION.

The gastric morphology of adult M. albicaudatus has been described (Maddock & Perrin 1981; Chap. 1). The papillated bilocular hemiglandular stomach comprises a glandular antrum and keratinised pars oesophagea which is divided into a papillated corpus and non-papillated pregastric pouch (PGP); cardiac, pyloric and fundic glands are present in the antrum (Maddock & Perrin 1981; Chap. 1). Gastric papillae have been recorded in only four rodent species, M. myospalax (Carleton 1973), C. gambianus (Caiman et al. 1960), T. splendens (Rahm 1976, 1980) and M. 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 facilitating a symbiotic relationship between the rodent and the micro-organisms.

Prior to this study there had been no investigation of the gastric morphology of M. albicaudatus, and to complete the morphological desciption (Chap. 1), 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) but in this examination gastric development was studied at gross, light and scanning electron microscope levels.

Due to its papillated corpus the stomach of M. albicaudatus superficially resembles a rumen. Ruminant gastric development

(papillae in particular) is stimulated by absorption of VFA's, principally acetic, propionic and butyric (Brownlee 1956; Tamate, McGilliard, Jacobson & Getty 1964; Richard & Ternouth 1965). VFA's are liberated during microbial fermentation of plant material in the rumino-reticulum (Flatt, Warner & Loosli 1958) and are absorbed through the stomach wall (Barcroft, McAnally & Phillipson 1944), the absorptive surface area of which is greatly increased by the papillae (Hofmann 1973).

The stomach of the white-tailed rat differs in structural detail from that of the ruminant and its gastric papillae do not function in absorption (Maddock & Perrin 1981). It is therefore probable that factors other than VFA absorption are involved in stimulating corpal development in this rodent. Thus, corpal microbial succession in juvenile M. albicaudatus, weaning age and gastric development were considered together and possible stimuli for gastric development were examined. The appearance of a particular gastric flora (bacilli) and ingestion of solid food between 14 and 17 days corresponds to the transition from papillae buds to adult-like papillae. Because of this temporal synchrony, solid food and the presence of bacilli are considered to play a role in stimulating papillae growth.

MATERIALS AND METHODS.

Breeding colonies of M. albicaudatus were established in the laboratory and data were obtained from 34 animals (14 litters) between 0 and 80 days of age. Cages were checked twice daily for births and young were assumed to have been born on the day when first seen (day zero). A Sartorius overhead balance was used to weigh the rats prior to sacrifice. Stomachs, with and without gastric contents, were weighed before fixation; stomach weight, expressed as a percentage of body weight, was used as a relative measure of gastric development.

Procurement and preparation of the tissue for microscopical examination was done according to the techniques used by Maddock & Perrin (1981; Chap. 1). A dissecting microscope was used to examine gross morphology and photographs were taken with a Nikon F2 camera. Sections, 7 um thick, stained with hematoxylin and eosin (Humason 1967) were viewed and photographed with a Vanox microscope fitted with an Olympus C 35 camera. Micro-anatomical features were measured with a Leitz micrometer eyepiece. Scanning electron microscope material was viewed and photographed with a JEOL JSM/VS scanning electron microscope.

Since the stomach wall stretched with degree of filling, absolute thickness was difficult to measure accurately and therefore of questionable value. The number of cell layers in each stratum was thus used as a measure of epithelial thickness (Sakata & Tamate 1979). However, the keratinised papillae were not tensile and papillary stratum corneum thickness was determined by direct measurement. The number of papillae per mm transect of corpal epithelium was counted with the aid of a light microscope. Although distribution of the papillae in the corpus was not regular, these results were converted to the mean number of papillae per cm^2 of corpal epithelium.

The Students' *t* test with 95 % (0,05) probability was used to determine significant differences between means. Incremental growth was used as a relative measure of growth over a period of time and was calculated from the formula:

$$\text{Incremental growth} = \frac{A - B}{t}$$

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 juvenile rats provided with food and water *ad libitum*.

The gastric nomenclature adopted for adult *M. albicaudatus* (Maddock & Perrin 1981; Chap. 1) was used to describe juvenile morphology; two additional definitions were necessary to distinguish juvenile and adult features. They are:

1. Papillae bud - a bulbous projection of the corpal malpighean layer into the lamina propria. The stratum corneum of the papillae bud (PB) is thicker than that of the interpapillary layer (*qv*) and extends into the gastric lumen (except during the first week of life). The PB are the gastric papillae precursors and resemble the epithelial pegs found between connective tissue papillae of cheek epithelium (Landay & Schroeder 1977) and the epidermis (Ham 1969). A PB is recognised as a papilla when it reaches 10 % of the mean adult papillae length.

2. Interpapillary epithelium - the keratinised, stratified squamous epithelium between the PB. It is the forerunner of adult folded corpal epithelium (FCE) but does not assume the highly folded condition until the third or fourth week after birth.

RESULTS.

Gross morphology and histology.

Post-natal corpal changes (0 - 80 days) were separated into four developmental periods; neo-natal (0 - 7 days), transitional (7 - 16 days), infantile (17 - 25 days) and post-weaning (26 days and older), on the basis of anatomical features and the appearance of adult characteristics (Table 2,1).

Neo-natal period (0 - 7 days).

Stomach shape at birth differed from the adult bilocular form (Chap. 1; Table 2,1; Plate 2,1); the large fornix ventricularis, elongated corpus and sacculations of the bilocular hemiglandular stomach (Fig. 1,1) were absent. However, this monogastric condition was lost towards the end of the first week of life and the stomach began to aquire the adult form.

Distinct glandular (antral) and non-glandular (pars oesophageal) regions were visible at birth, and PB occurred in the corpus (Fig. 2,1; Table 2,1). Significant differences existed between the number of cell layers in the buds and interpapillary epithelium ($\underline{P} < 0,05$; Fig. 2,2), and between the thickness of the papillary stratum corneum at birth and at 7 days ($\underline{P} < 0,001$; Fig. 2,3) suggesting rapid growth of the PB. The corpal epithelium was completely keratinised at birth and thus differed from the newborn mouse oesophagus which is mucus-secreting and has nucleated squamous cells (Parakkal 1967).

Plate 2,1. The bisected stomach of a two day old rat. The PGP constriction and grenzfalte are absent and the stomach has a monogastric shape. PB are not visible. A = antrum, C = corpus, O = oesophagus. Scale bar in millimeters.

Plate 2,2. The bisected stomach of a fifteen day old rat. PB are visible in the corpus (C) and the grenzfalte (G) is seen between the antrum (A) and corpus (C). Scale bar in millimeters. Lettering as in Plates 2,1 and 2,2.

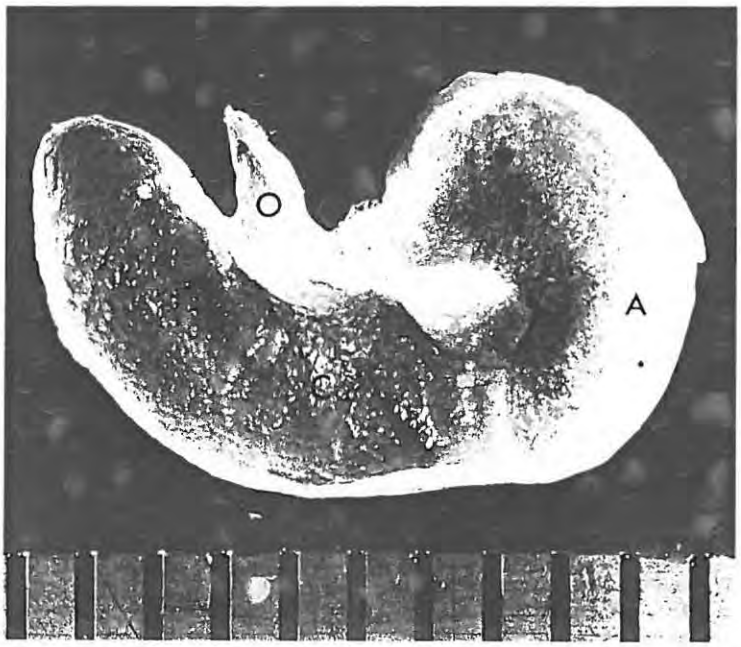


Plate 2,1

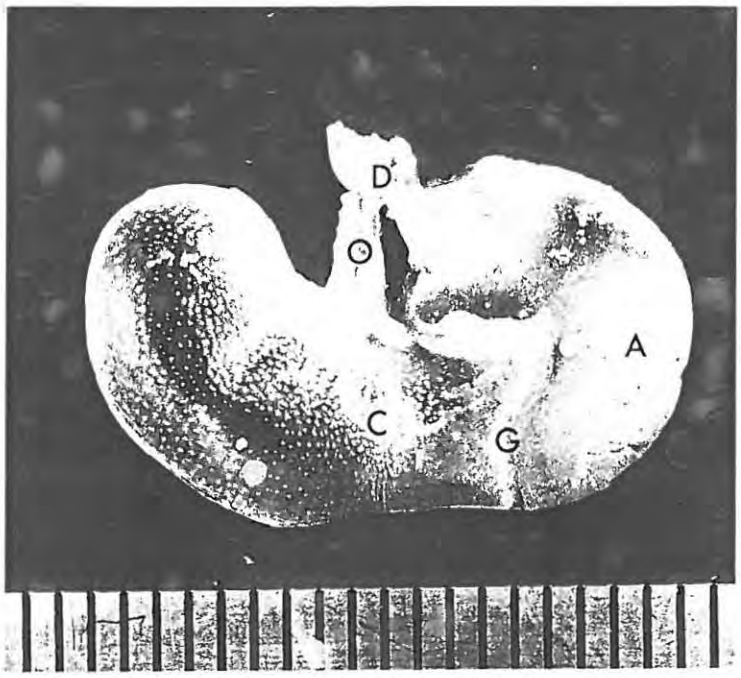


Plate 2,2

Table.2,1. Summary of the major macro- and micro-scopic developmental changes in the stomach of M. albicaudatus.

Gross observations.	Histological observations.
Neo-natal period (0 - 7 days).	
Unilocular stomach, glandular & non-glandular regions present.	Tissue layers (except epithelium) resemble adult.
No corpal papillae, PGP, fornix ventricularis or grenzfalte.	Corpus has level topography & consists of 4 strata.
Tissues translucent.	PB, numerous near oesophageal region, do not affect topography.
Milk present.	Few attached bacteria.
	Cardiac & pyloric glands present; fundus lacks parietal & chief cells.
Transitional period (7 - 16 days).	
Bilocular shape.	Corpal epithelium undulatory.
Small epithelial protrusions in the corpus.	PB occur between small folds.
Corpus and PGP indistinct.	PB stratum corneum thickens.
Grenzfalte & fornix ventricularis appear.	Parietal & chief cells present in the fundus.
Milk present.	
Infantile period (17 - 25 days).	
Papillae appear; corpus is thus distinguished from the PGP.	Papillae mainly in gastro-oesophageal region.
Tissues translucent.	Papillae have thick bacterial layer similar to adult condition.
Milk present at 17 days.	
Weaned between 20 & 25 days.	
Post-weaning period (older than 25 days).	
Gastric tissue opaque; antrum thick, corpus thinner.	FCE developed.
Stomach divisions visible with the naked eye.	Gradual increase in papillary stratum corneum causes increase in papillae length.
Solid food in stomach.	

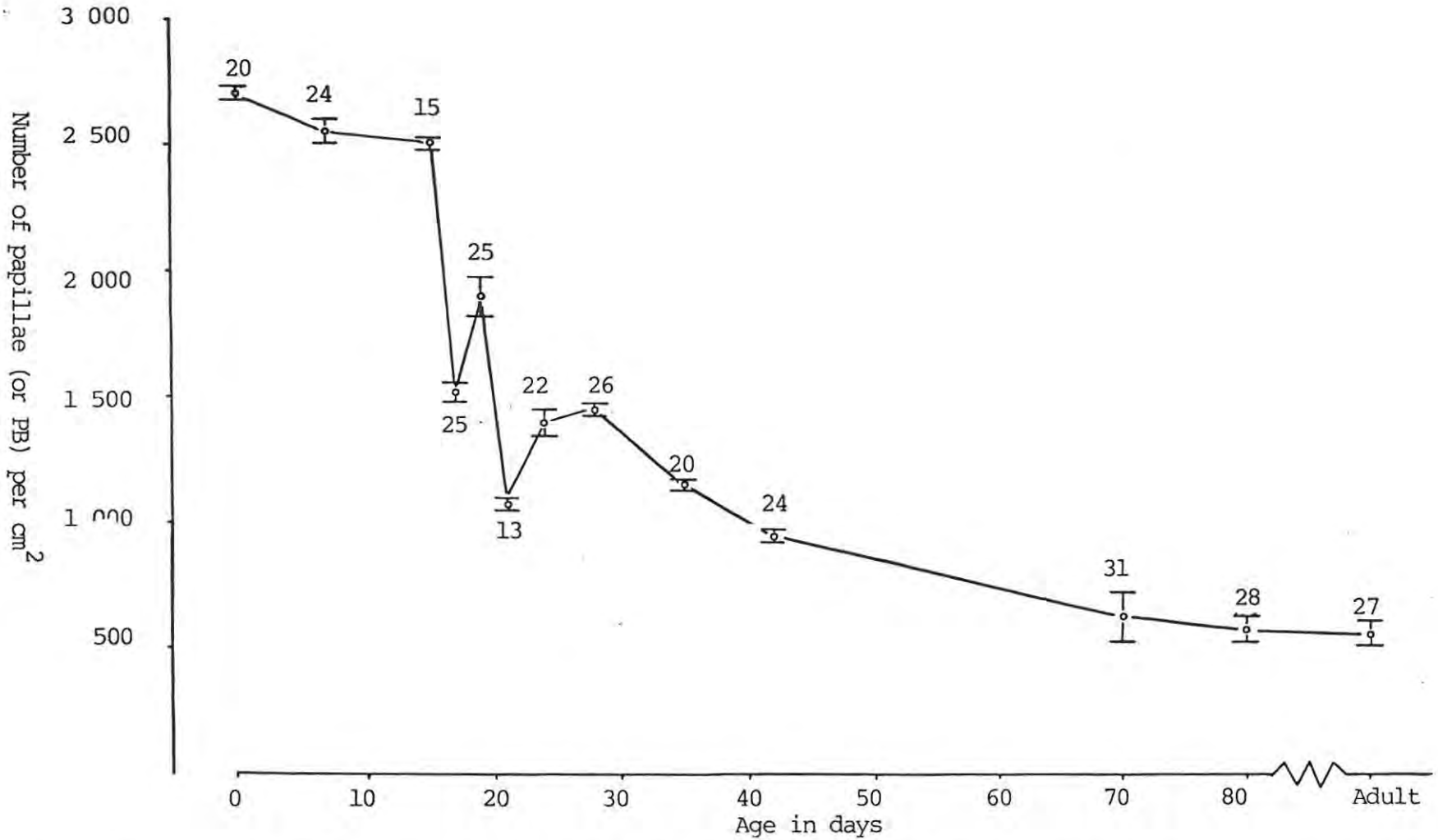


Figure 2,1. Number of papillae (or PB) per cm^2 of corpal epithelium in M. albicaudatus. Vertical bars = one standard deviation. Sample size indicated.

Figure 2,2. Post-natal development of the gastric papillary and interpapillary epithelia of M. albicaudatus showing the mean number of cell layers per epithelial stratum.
∘ = Stratum basale; • = Stratum spinosum; × = Stratum granulosum; ▲ = Stratum corneum. Vertical bars = one standard deviation. Sample size = 20.

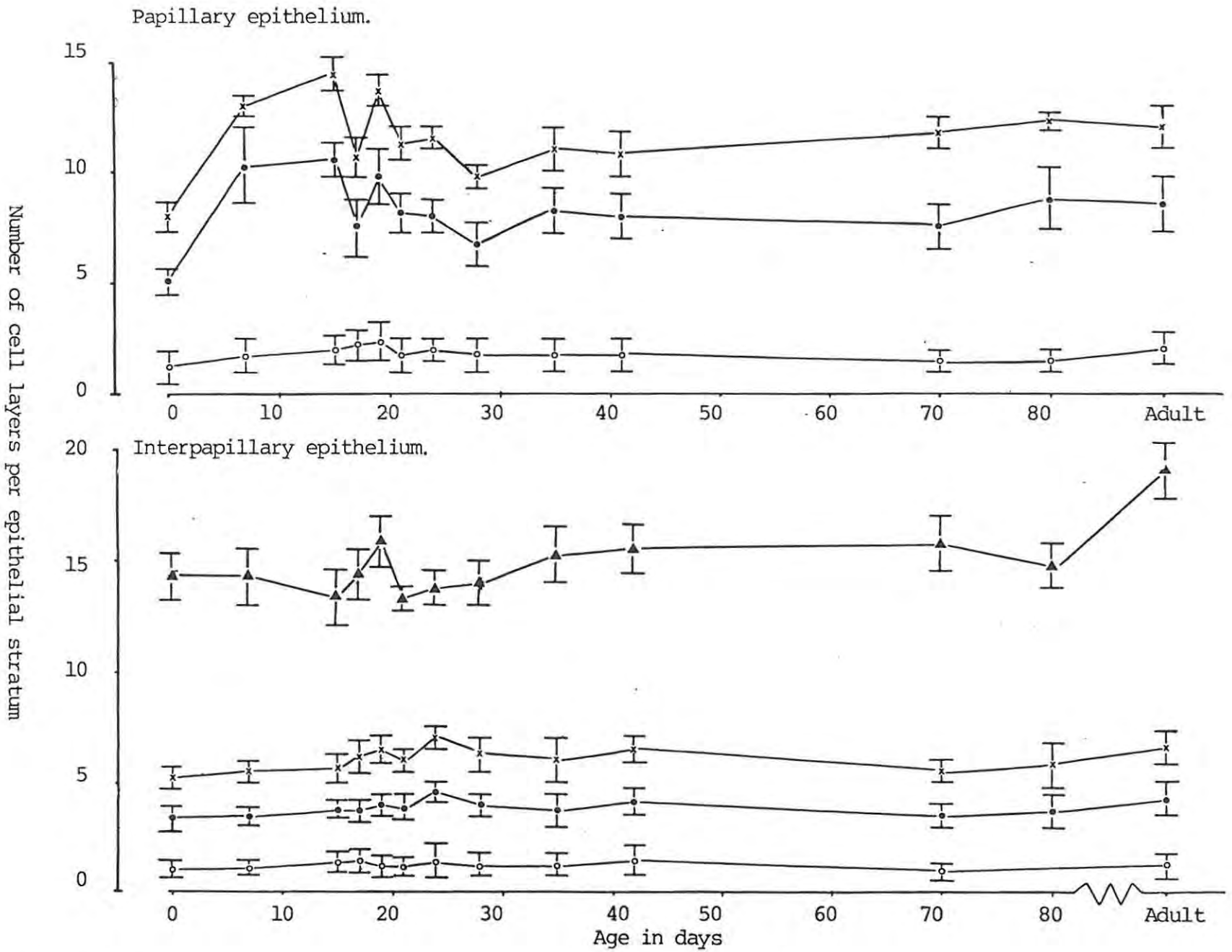

Papillary epithelium.
15
10
5
0
0
10
20
30
40
50
60
70
80
Adult
Interpapillary epithelium.
20
15
10
5
0
0
10
20
30
40
50
60
70
80
Adult
Age in days
Number of cell layers per epithelial stratum

Figure 2,2.

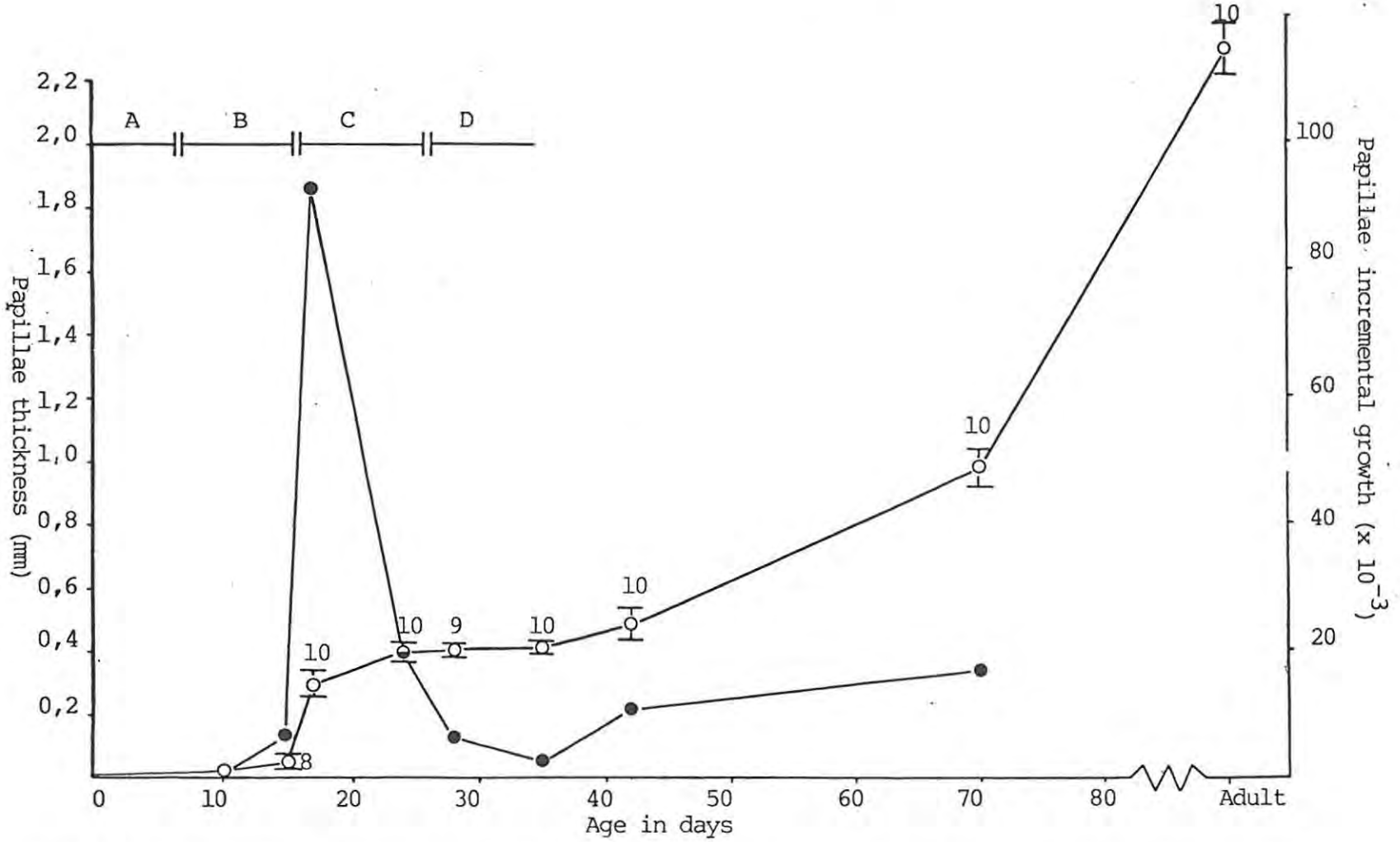


Figure 2,3. Absolute increase (o) and incremental growth (●) of papillary stratum corneum with age in M. albicaudatus. Vertical bars = one standard deviation. A = neo-natal; B = transitional; C = infantile; D = post-weaning periods.

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.

Transitional period (7 - 16 days).

During this period development of a fornix ventricularis resulted in elongation of the corpus. Also evident at this age was the grenzfalte and constriction between tne glandular and non-glandular regions, causing sacculation of the stomach (Table 2,1). At the end of this period the stomach had aquired a bilocular shape but lacked papillae and the complete gastric divisions of the adult (Plate 2,2).

The number of PB in the corpus was similar to that at birth ($\underline{P} > 0,05$; Fig. 2,1) but the malpighean layers in the PB were significantly thicker than in the interpapillary epithelium ($\underline{P} < 0,005$ Fig. 2,2) due to an increase in the spinous and horny cell layers. The thickness of the PB horny lay r increased significantly during the transitional period ($\underline{P} < 0,001$; Fig. 2,3) and a high incremental growth occurred between the ages of 10 and 15 days ($7,2 \times 10^{-3}$; Table 2,2). The thick stratum spinosum of the PB often consisted of cells, perpendicular to the basal membrane, extending in rows to the stratum granulosum; granular cells were parallel to the basal membrane. This was a feature of the papillae in all subsequent ages (including the adult; Chap. 1). The differences between the PB and interpapillary epithelia are suggestive of slightly different keratinisation procedures (Chap. 1).

Table 2,2. Increase in thickness of papillary stratum corneum occuring with age.

Period (days).	mm.	n.	% increase.	Incremental growth ($X\ 10^{-3}$).
-	0,012 ± 2,8 ($x\ 10^{-3}$)	10	-	-
0 - 7	0,017 ± 2,2	"	10	41,7	0,7
7 - 10	0,022 ± 1,9	"	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	-

At 7 days of age parietal and chief cells were apparent in the fundus (Plate 2,3), the epithelium of which had increased in thickness from birth.

Infantile period (17 - 25 days).

The infantile period ranged fror the earliest at which solid food was sampled (16 days, Meester & Hallett 1970) to the latest recorded weaning age (25 days; Table 2,3). There were two obvious subdivisions in this period: pre-weaning (16 - 20 days) and weaning (20 - 25 days).

Table 2,3. Post-natal development (in days) of certain features associated with weaning in M. 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

Between 15 and 17 days the PB stratum corneum had an incremental growth of 93,5 X 10^{-3}, the highest in the study (Fig. 2,3; Table 2,2). As a result of this rapid growth the PB reached 10 % of the adult length, and with the appearance of the papillae the distinction between the PGP and corpus became clear. Consequently the juvenile stomach resembled that of the adult in all respects except relative mass (Fig. 2,4) and papillae length (Fig. 2,3). Concomitant with the development of the papillae was their colonisation by numerous bacteria so that (although shorter) the papillae resembled those of the adult (Chap. 1).

Although juveniles were suckled in their fourth week, they survived independantly between the ages of 20 and 25 days (Table 2,3). This was considered as the weaning period but no major morphological changes occurred during these 6 days (Plate 2,4).

Post-weaning period (26 days and older).

Juveniles entering this period could survive independantly (Table 2,3) and had a gastric morphology similar to adults (Table 2,1; Plate 2,5); their gastric physiology and microbial ecology probably resembled the adult too.

Plate 2,3. Section through the lower fundic region of the stomach of a seven day old rat. Absent at birth, a few light staining parietal (Pa) and dark staining chief (Ch) cells have developed during the first week of life. Ct = connective tissue, M = muscle layers.

Plate 2,4. The bisected stomach of a 25 day old rat. Papillae (P) are present in the elongated corpus (C) and the PGP and grenzfalte (G) are obvious. Adult features have formed (see Fig. 1,1). Scale bar in millimeters. Lettering as in Plates 2,1 and 2,2.

Plate 2,5. The bisected stomach of a 42 day old rat. Gastric divisions of the adult (thick walled antrum (A); papillated corpus (C) and PGP constriction) can be seen clearly; the papillated bilocular hemiglandular condition is evident. Papillae, however, have not yet reached the adult length.

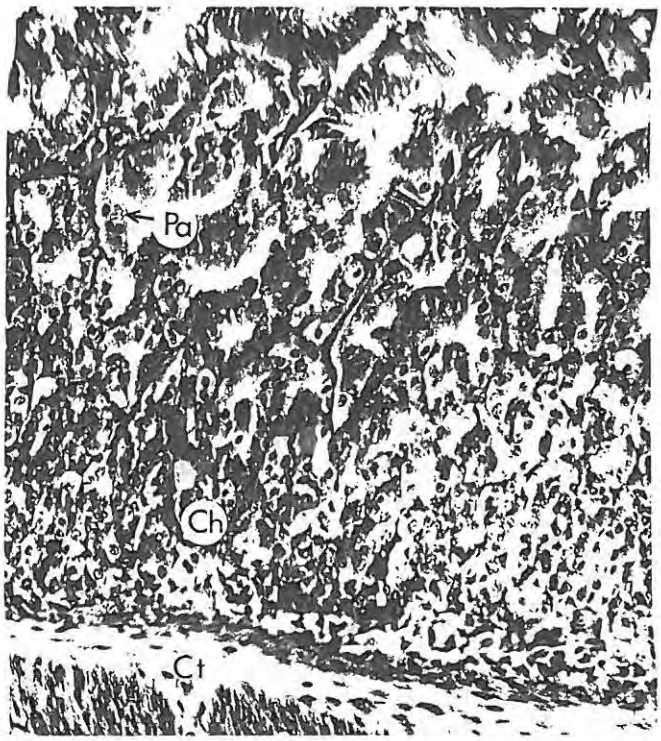


Plate 2,3 30 µm

Plate 2,4

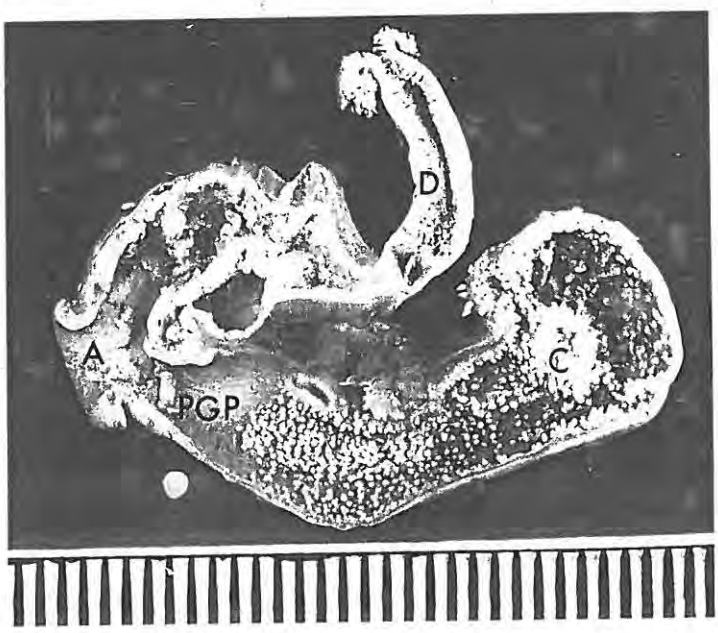


Plate 2,5

No major macro- or microscopic changes occurred during post-weaning (Table 2,1). Incremental papillary growth, although considerably less than in the previous period, was still high (Fig. 2,3; Table 2,2); absolute papillary growth was gradual and the papillae increased from 0,38 mm at 16 days to almost 1 mm at 70 days (Fig. 2,3; Table 2,2).

General observations.

The marked changes in relative stomach weight of M. albicaudatus did not correspond to the developmental divisions (Fig. 2,4). Rats between the ages of 7 and 19 days had a constant, mean value of 1,6 % (1,6 ± 0,13;) but this increased from 1,5 % prior to weaning to a peak of 4,0 % at 51 days (Fig. 2,4). Thereafter, relative stomach weight decreased and the 70 day old rat stomach reached the adult value of about 3 % of total body weight (Fig. 2,4). The rapid increase in stomach weight is due to the ingestion of solid food. Ingestion of solid food results 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 increase proportionately.

The relative abundance of papillae declined steadily from birth to adulthood (Fig. 2,1). 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 (Figs. 2,1 & 2,3).

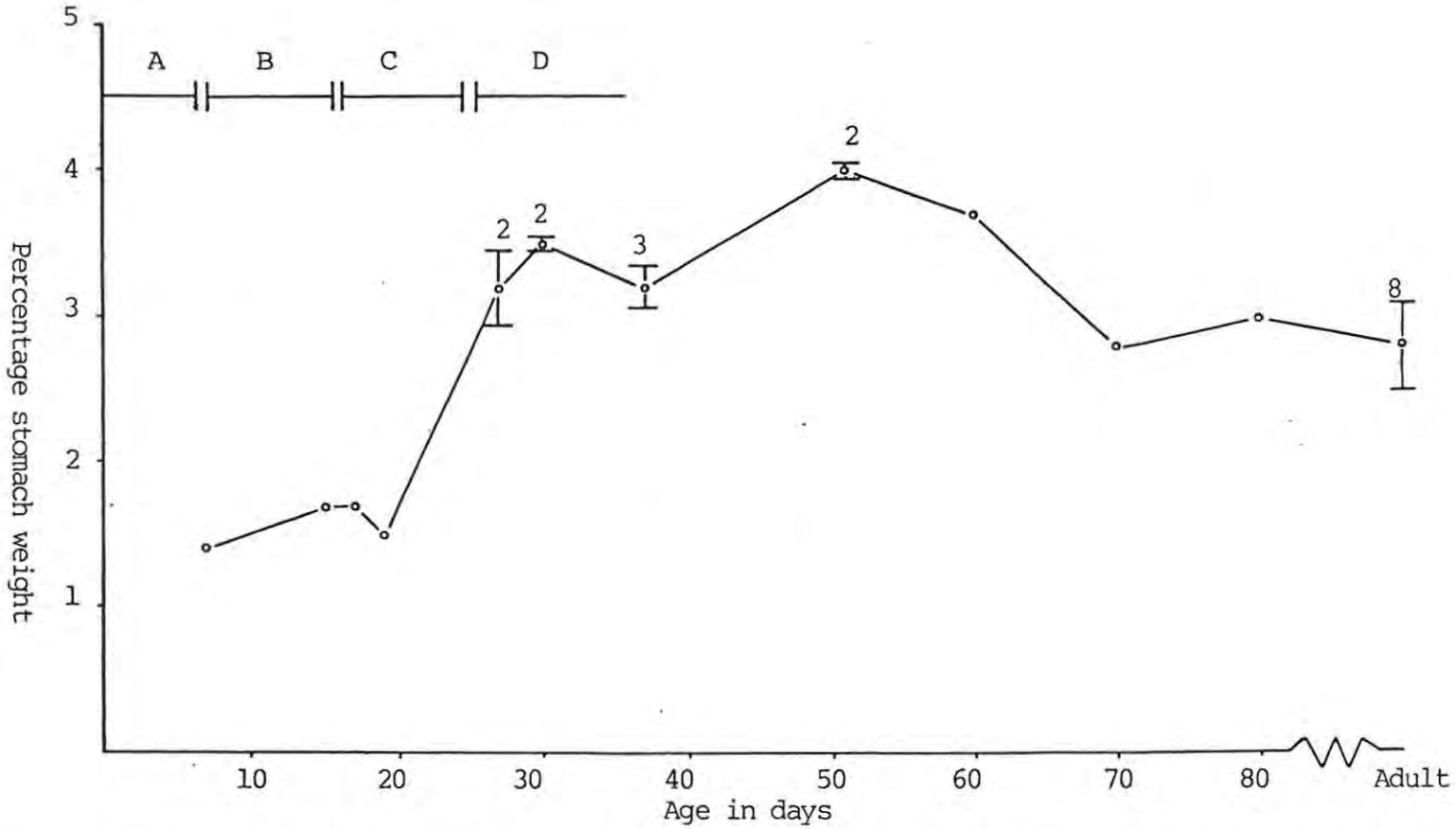


Figure 2,4. Post-natal development of the stomach of M. albicaudatus. Empty stomach weight expressed as percentage of body weight. Vertical bars = one standard deviation. Sample size indicated. Lettering as in Fig. 2,3.

The number of cell layers in the papillary malpighean epithelium increased rapidly from birth to a maximum at 15 days, then declined through the weaning period (Fig. 2,2). There was no significant difference between the number of adult papillary malpighean cell layers and the number immediately post-weaning ($P > 0,4$). A slight increase in interpapillary malpighean cell number occurred at 19 days and both papillary and FCE epithelia showed growth after 80 days (Figs. 2,2 & 2,3).

Scanning electron microscopy (SEM).

Papillae were not seen in the corpus of M. albicaudatus during the first 14 days (Plate 2,6) but PB were evident at 15 days (Plate 2,7). The cell size of the papillae-free epithelium of the young rat (Plate 2,6) resembled that of the adult FCE and PGP (Plates 1,21; 1,22 & 1,24) although microbial colonisation of the developing stomach was sparse compared with the adult FCE. Groups of cocci, either single or in branched chains covered parts of the epithelium in a monolayer (Plates 2,8 & 2,9) but bacteria-free zones separated these clusters of bacteria (Plate 2,8). Rod-shaped bacteria were absent. Singular, round bodies, devoid of detail, were seen on the epithelium surface (Plate 2,10). These could be fungal spores and were the only other 'micro-organisms' in the stomach.

Changes in the corpal epithelium (Table 2,1) were also seen in the SEM micrographs of the 15 and 17 day old rats. In contrast to the PB present at 15 days (Plate 2,7), short stumpy papillae had appeared in the older rats (Plate 2,11). Bacilli, similar to those in the adult, attached to these papillae (Plate 2,12) while cocco-bacilli and cocci, seen in the corpus at 15 days and earlier, were restricted to the interpapillary epithelium (Plate 2,13). The simultaneous

Plate 2,6. A low power scanning electron micrograph of the corpal surface of a 10 day old rat. Although papillae buds (PB) are visible in light micrograph sections they did not alter the epithelial surface topography. The corpal epithelium of the 10 day rat resembles the adult FCE (Plate 1,22).

Plate 2,7. PB seen on the surface topography of the 15 day old rat; the buds are small and closely packed having a density of 2 520/cm^2.

Plate 2,8. The corpal epithelium of a 10 day old rat illustrating presence and distribution of bacteria which occur in groups separated by bacteria-free zones (Bf).

Plate 2,9. A high power micrograph of the predominant micro-organisms on the 10 day corpal surface Cocci, single and in chains, are present but bacilli are rarely seen.

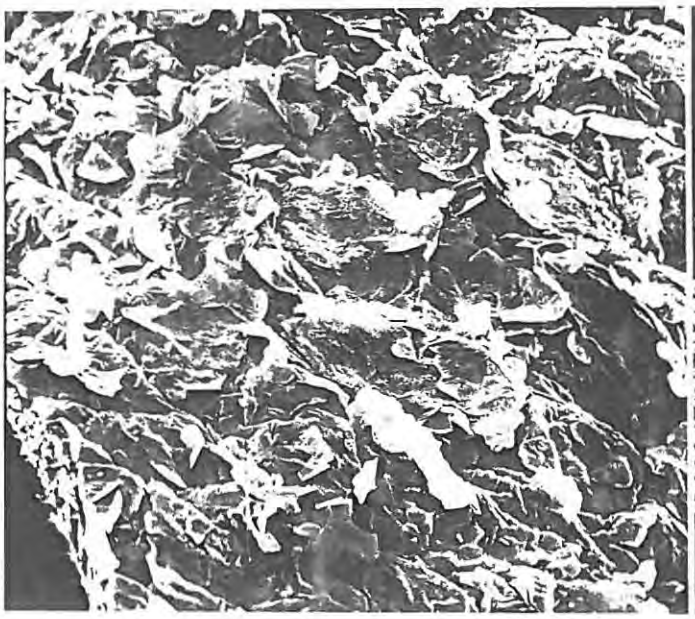


Plate 2,6 0,1 µm

Plate 2,7 0,1 µm

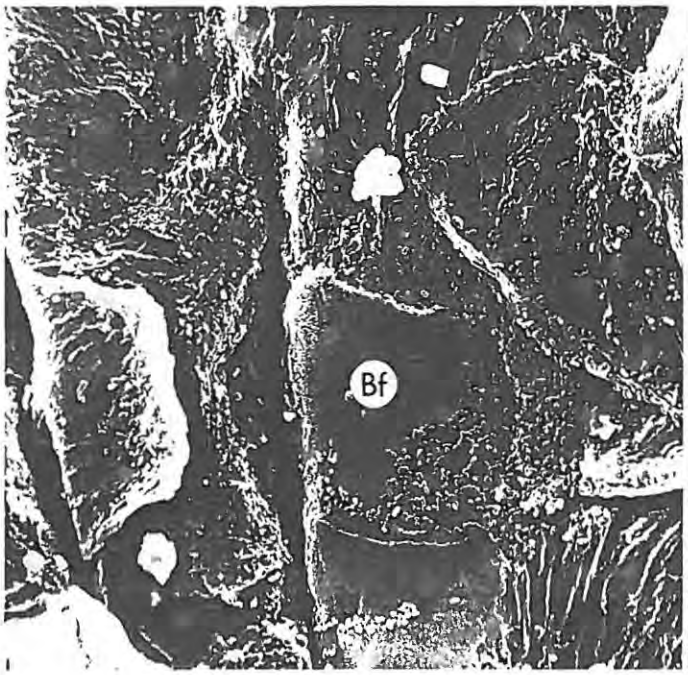


Plate 2,8 15 µm

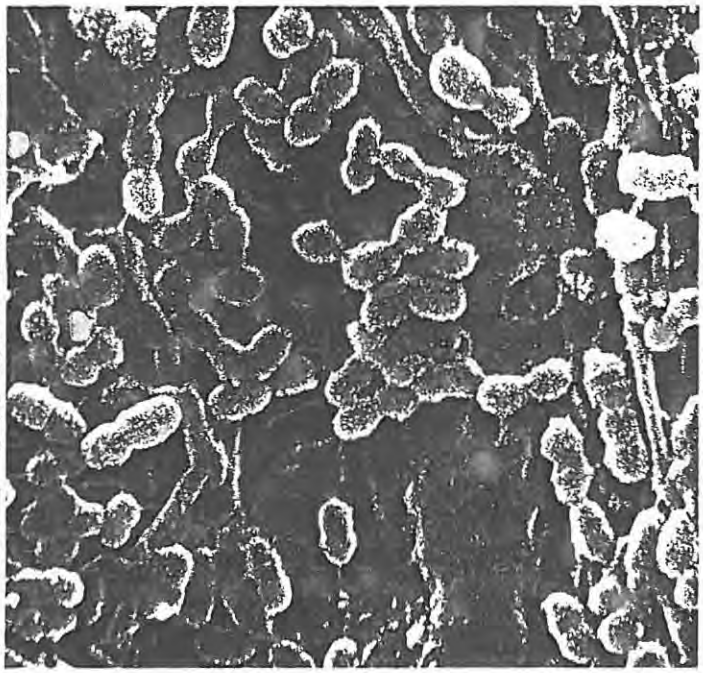


Plate 2,9 1 µm

Plate 2,10. Oval bodies (arrows) are seen on the corpus of the 10 day old rat. An idea of the body's size can be obtained by comparison with the bacteria in Plate 2,8 which is magnified 870 times. The 365 times magnification of this plate is too low to reveal bacterial cells.

Plate 2,11. PB in the 15 day old rat stomach (Plate 2,7) developed into papillae by 17 days and the interpapillary (IP) and papillary (PE) epithelia are also discernable at 17 days of age.

Plate 2,12. The surface of a papilla from a 17 day old rat. Once the papillae had formed (ie reached 10 % of adult length), large numbers of bacilli appeared in the stomach and attached to the papillary epithelium.

Plate 2,13. The FCE surface of a 17 day old rat. Although bacilli colonised the corpus of the 17 day old rat, the microbial flora of the interpapillary epithelium (cocco-bacilli) was similar to that of younger animals (Plates 2,8 & 2,9). The bacteria were however, more abundant in the older rat and were densely packed in many areas.

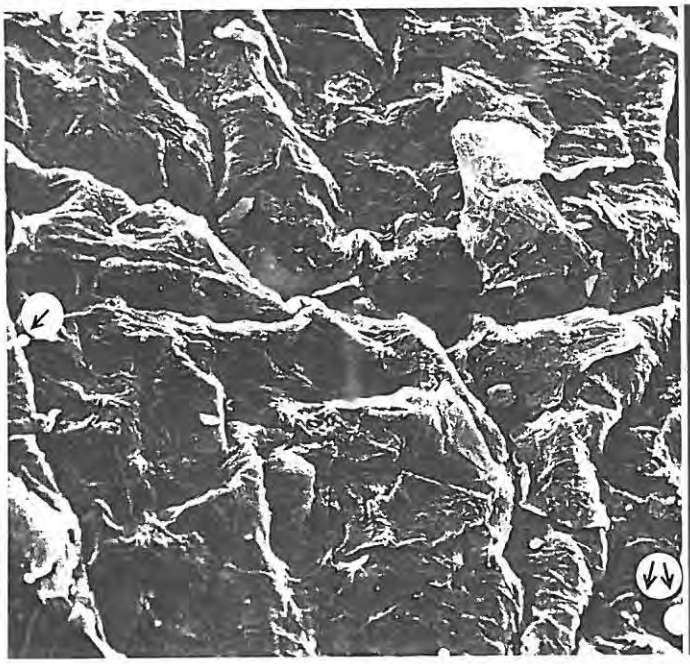


Plate 2,10 25 µm

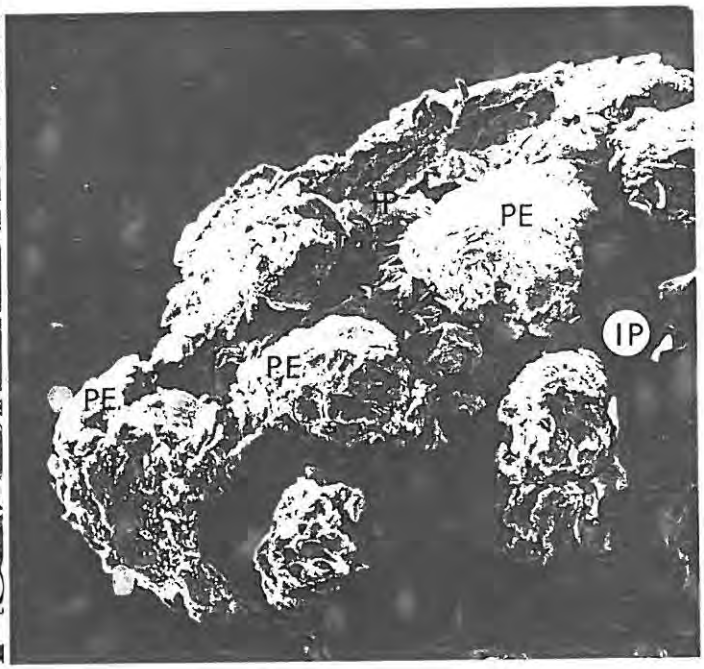


Plate 2,11 0,1 µm

Plate 2,12 2 µm

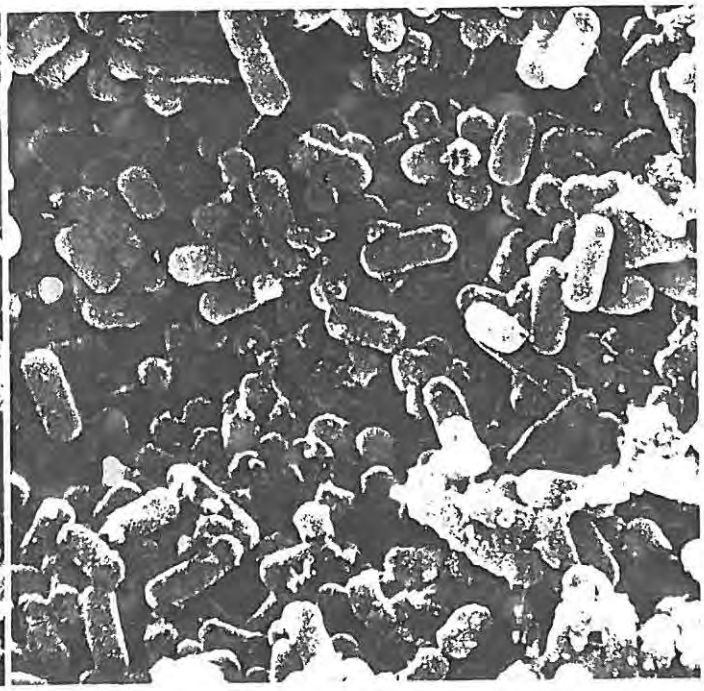


Plate 2,13 1 µm

appearance of the papillae and the bacilli suggests that these bacteria are specific to the papillae (the papillary microhabitats, Chaps. 1 & 3). Bacteria-free areas were present in the corpus but the cocci no longer occurred in monolayers (Plate 2,13) indicating an increase in the number of organisms as the autochthonous flora was established (Chap. 3). Fungal spores (?), similar to those in the 10 day old corpus, were more numerous in the 17 day old rat.

The corpal epithelia of 17 and 19 day old rats were similar; papillae did not show a great increase in length, regional separation of morphologically different bacteria (bacilli and cocci) was again noticable (Plates 2,12 & 2,13) and bacteria-free areas were present in the interpapillary region (Plate 2,14).

Rats older than 24 days had adult corpal features in that the FCE and papillary epithelium were distinct (Plate 2,15) and the papillae differed from the adult in length only (Plate 2,16). Habitat preference among the bacteria was maintained (Plates 2,17 & 2,18) and bacilli, characteristic of adult papillae, were abundant (Plate 2,19). Bacteria-free areas were fewer, smaller and restricted to the FCE (Plate 2,20).

Plate 2,14. Bacteria-free (Bf) areas between groups of cocci and cocco-bacilli on the interpapillary epithelium of a 19 day old rat. Bacilli are absent from these interpapillary regions.

Plate 2,15. 42 day old rat corpus. This micrograph of a papilla base reveals the clear difference between the FCE and papillary epithelium (PE) particularly the difference in bacterial cover and cell size of the two regions. Bacteria-free areas are few on the FCE at this age.

Plate 2,16. Papillae of the 42 day old rat are similar to the adult although shorter (See Plates 2,7 & 2,9). The different epithelia (FCE and papillary - PE) are also well shown in this micrograph.

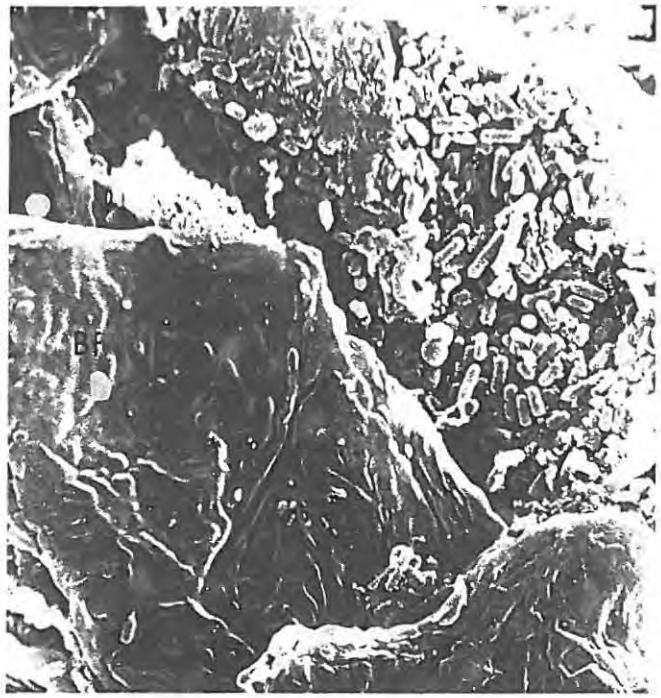


Plate 2,14 — 3 µm

Plate 2,15 — 20 µm

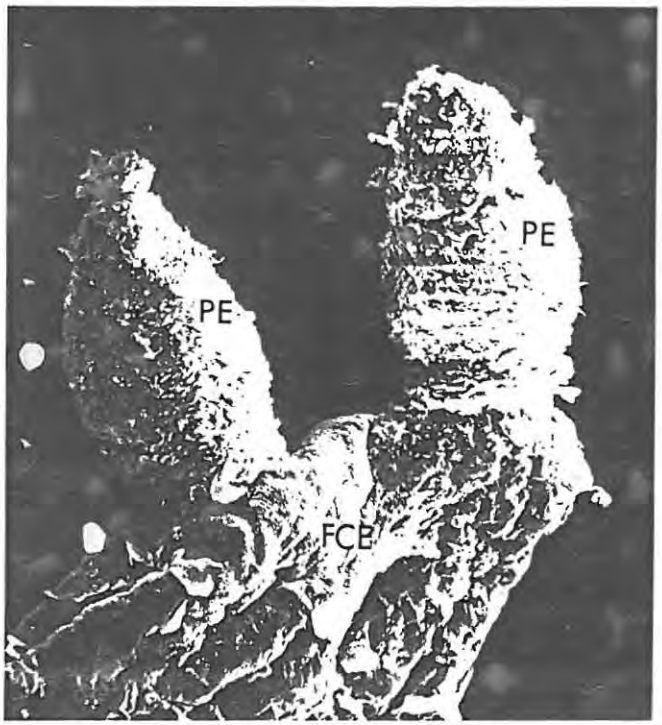


Plate 2,16 — 0,1 µm

Plate 2,17. The FCE of a 28 day old rat. Cocco-bacilli and cocci predominate on the FCE but rod-like bacteria are absent (see Plate 2,18).

Plate 2,18. The papillary surface of a 28 day old rat. Rod-shaped bacteria occupy the surface while cocci or cocco-bacilli are rare (see Plate 2,17).

Plate 2,19. A transverse section through a 35 day old papilla showing the mode of bacilli (B) attachment to the epithelium. Note the large numbers of bacteria and their 90° orientation to the papilla surface. Note too, the honey-comb appearance of the papilla.

Plate 2,20. The interpapillary epithelium from a 42 day old rat showing the groups of cocci separated by bacteria-free zones (Bf).

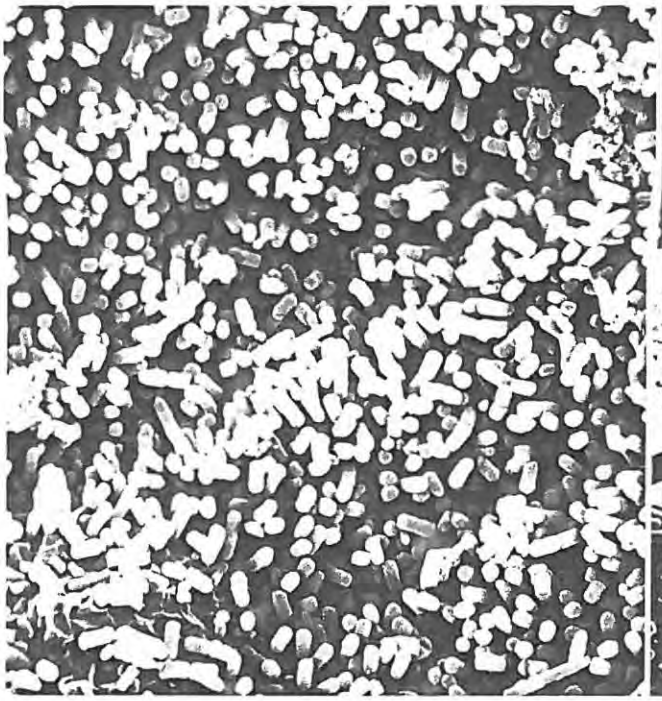


Plate 2, 17 3 μm

Plate 2,18 3 μm

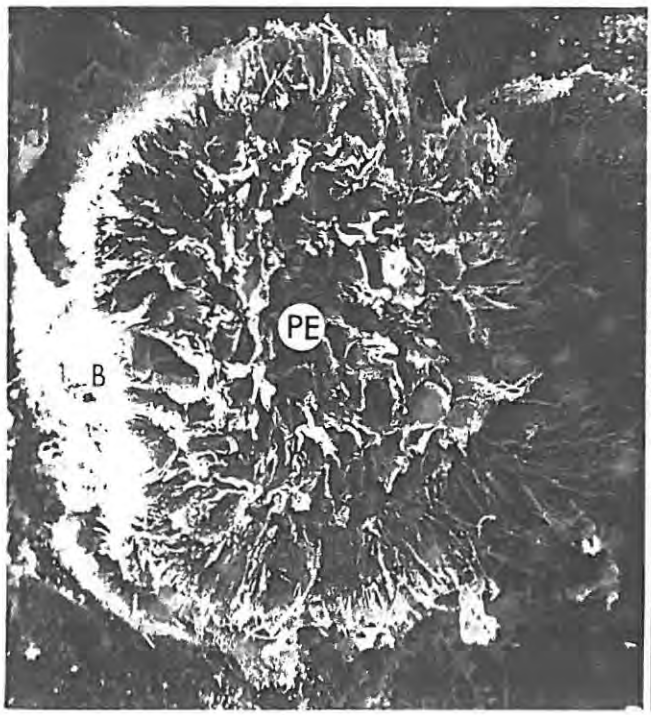


Plate 2,19 20 μm

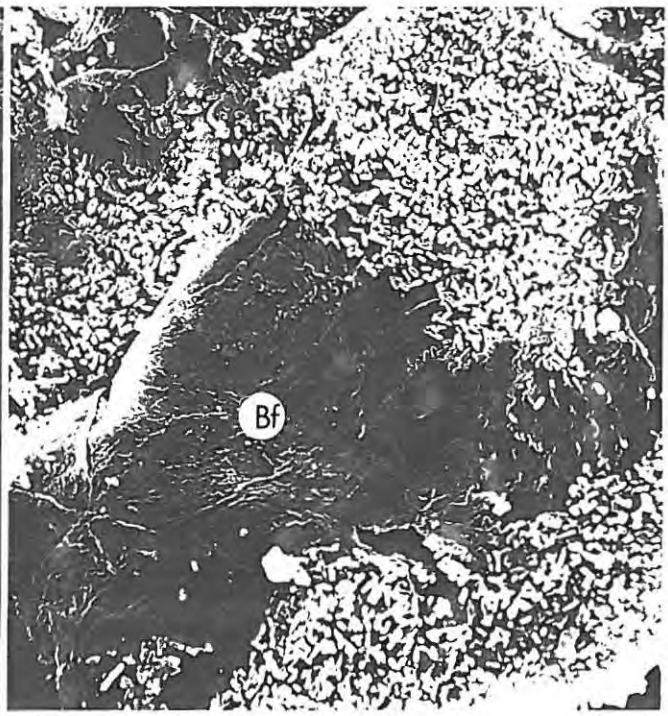


Plate 2,20 10 μm

DISCUSSION.

The four stages recognised during post-natal gastric morphogenesis in M. albicaudatus assists in understanding various mechanisms and processes of stomach development. The sequence of gastric changes during the first 25 days of the rats' life are summarised in Figure 2,5. Some of these features occur simultaneously and others occur sequentially (Fig. 2,5) suggesting that gastric development is controlled by certain allogenic factors during early 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 early gastric development is stimulated by external factors such as ingestion of solid foods, faeces or roughage and it is probable that development is innate (Fig. 2,5). Gastric development is determined by innate growth in ruminants, different diets merely enhance this potential (Warner & Flatt 1965).

Microbial as well as morphological gastric development occurs and a microbial flora is established in the gut during the first few days of life. A variety of microbes invade the gut after birth, many are transitory, but a few favour the gastric conditions characteristic of a milk diet and colonise the stomach. These are mainly facultative anaerobes (Schaedler, Dubos & Costello 1965; Savage, Dubos & Schaedler 1968). Cocco-bacilli and cocci, abundant in the corpus of the rat in early life, are representative of this early autochthonous flora (Fig. 2,5).

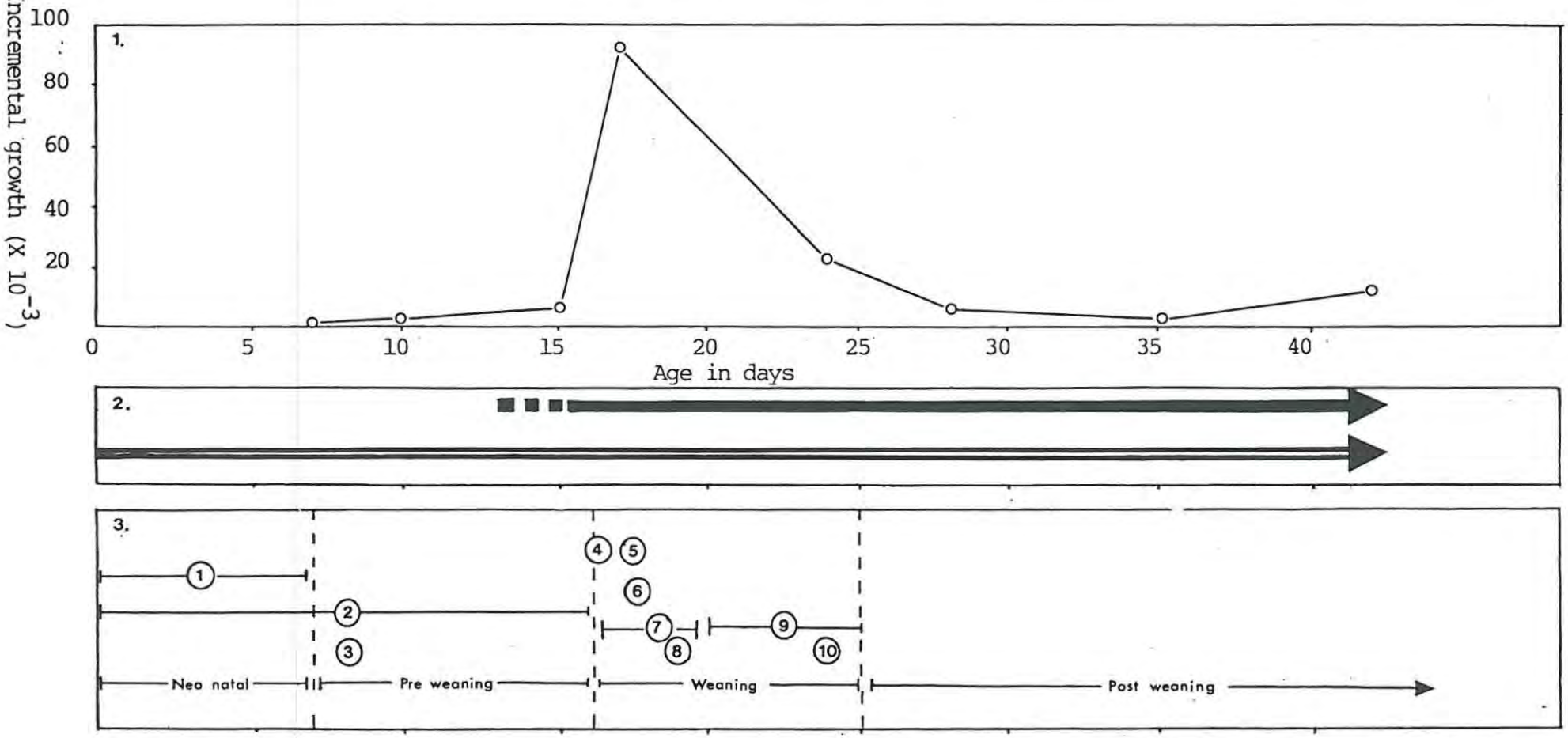


Figure 2,5. Diagrams showing increase in papillae thickness with age (1), proposed periods of influence on papillae growth by external stimuli (▬▬) and innate growth potential (═══)(2), and sequence of events associated with gastric development in M. albicaudatus.

1. Development of fundic glands.
2. Facultative anaerobes establish an autochthonous flora in stomach.
3. Unilocular shape develops into bilocular shape.
4. Ingestion of solid food.
5. Stomach conditions change.
6. Bacilli colonise 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.

A noticable feature of corpal development in M. albicaudatus is the rapid growth of PB (and papillae), first apparent between 10 and 15 days. Since it is unlikely that external factors stimulate early development, increase in papillary length is presumably genetically controlled. However, it seems improbable that the exceptional growth between 15 and 17 days is due solely to an innate mechanism. It is more likely that growth is stimulated by a number of allogenic factors during this period. An example of external development is found in the rumen where hay- and grain-rich diets increase growth rate and papillary development but a milk only diet results in a slower rate of rumen development (Warner & Flatt 1965).

Determination of papillary stimulatory growth factors in the white-tailed rat is beyond the scope of this study but the coincident appearance (after the bilocular stomach is acquired and prior to weaning) of bacilli, the sampling of solid food and the development of PB into papillae (Fig. 2,5) appears significant and strongly suggests that these factors influence papillary growth. The synchrony and interrelationships of these factors are considered below.

Solid food, first sampled at 16 days of age (Hallett & Meester 1971; Fig. 2,5) is a major event in the development of the stomach since anaerobic bacilli are introduced into the gut via the food (see Schaedler et al. 1965; Savage et al. 1968). (Bacteria may also be introduced into the gut if the young eat adult faeces; a common practice among rodents, Ewer 1968). There is evidence that the presence of these bacilli strongly influence papillae growth. The new diet (solid food) causes changes in gastric conditions which may become unfavourable for the juvenile rat's facultative microbial community but suit the anaerobic bacilli introduced with the solid

food. These bacilli are specific to papillary micro-habitats. At the time of ingestion of solid food, PB develop into papillae which become colonised by bacilli (Fig. 2,5) owing to competitive exclusion of other micro-organisms. The anaerobic papillae bacilli (APB) do not occupy the FCE which is colonised by morphologically different bacteria. (These cocco-bacilli and cocci may represent part of the early facultative community or they may be newly introduced bacteria which are particularly suited to the new gastric conditions).

The sequence of events between 15 and 17 days of age is of major significance. Innate potential alone is responsible for gastric development during the first two weeks of life, during which time papillae growth is rapid as a result of a low chalone concentration in the epithelium (Chap. 1). Rapid papillary growth ensures that the bacilli are provided with specific papillary microhabitats when they enter the stomach with solid food at about 16 days of age. It might be expected that once bacterial colonisation of the papillae occurred, papillae growth rate would slow. But this does not happen and it is probable that ingestion of solid food and the presence of the bacilli and bacterial products are further stimuli for papillae growth.

The high rate of papillae growth could be due to the effect of the rapid colonisation by the APB. It is known that autochthonous bacteria increase the desquamation rate and hence mitotic rate of intestinal epithelia (Abrams et al. 1963; Coates & Fuller 1977) and it is suggested that colonisation of the papillae by bacteria causes the already high papillary growth rate to increase. However, the FCE also achieves maximum thickness during the appearance of the APB. Increased FCE growth cannot be explained by bacterial stimulation only (bacilli attach to the papillae only) therefore another stimulus must be

involved. A possible factor is the abrasion of solid food on the FCE which will also affect the papillae. Abrasion increases the desquamation rate and as a result the mitotic rate increases (see Abrams et al. 1963) causing an increase in papillae growth.

The role of chemical stimuli on gastric development cannot be discussed in detail here although one point is considered. A major stimulatory force for papillary growth in ruminants is the presence of VFA's in the rumino-reticulum (Brownlee 1956; Richard & Ternouth 1965). VFA's are produced by numerous symbiotic bacteria which ferment the ingesta and the large numbers of bacteria in the corpus of the white-tailed rat may produce VFA's. Although the morphology of the rat stomach indicates that absorption through the papillae is minimal (Maddock & Perrin 1981; Chap. 1), absorption may occur through the thinner FCE. Given these conditions, VFA's could influence gastric growth in a manner similar to that described for the ruminant (Brownlee 1956; Richard & Ternouth 1965).

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. The main feature of development is the high papillae growth rate which is probably due to a number of factors; the effects of mechanical abrasion and bacterial influence on desquamation and hence mitotic rates, chemical stimulation and a high innate growth potential due to a low chalone concentration. Innate rapid papillae growth during the first two weeks of life provides the APB with specific habitats. Rapid papillae growth thereafter allows the juvenile stomach to become functionally and physiologically similar to the adult. Symbiosis is only possible if there is a large bacterial population (Maddock & Perrin 1981). Since bacteria attach

to the papillae, their number is dependant on papillae length. The juvenile stomach will be able to function like that of an adult (with full symbiosis) when the bacterial number reaches adult proportions; hence rapid papillae growth.

Although these ideas are speculative, the simultaneous appearance of papillae, APB and the ingestion of solid food is surely more than coincidental. Detailed investigation of these factors should be the starting points for further study.

CHAPTER THREE.

BACTERIAL MORPHOLOGY AND MICROBIOLOGY OF THE CORPUS.

INTRODUCTION.

It is well known that indigenous micro-organisms colonise the proximal as well as the distal regions of the gastro-intestinal tract of mammalian herbivores (Schaedler et al. 1965; Savage et al. 1968; Hungate 1968; McBee 1977). It has also become clear that these microbes exert a marked influence on host physiology and trophic metabolism (Dubos & Schaedler 1962; Dubos, Costello, Schaedler & Hoet 1965). The most well known and best understood of such symbiotic associations is that in the ruminant rumino-reticulum (Hungate 1968) although similar microbial fermentative processes occur in the stomach of certain non-ruminants (Appendix 1), these animals with pregastric fermentation are refered to as PGF mammals (Moir 1965, 1968). In non-ruminant, non-PGF animals such as rodents (McBee 1977) and equids (Janis 1976; Bell 1971) fermentation occurs in the caecum and/or colon.

It must be realised that host/microbe relationships are not only limited to feeding but may also include immunological associations. Interaction between gastro-intestinal micro-organisms (Savage 1969; Morotomi, Watanabe, Suegara, Kawai & Mutai 1975) results in less well adapted microbes being excluded from a habitat by well adapted species, thus regulating microbial localisation in the gut and maintaining stability (Savage 1970). Meynell (1963) demonstrated that indigenous micro-organisms may release waste products (VFA's), toxic to pathogenic invaders, thereby inhibiting their colonisation of the gut and preventing disease in the host. A different type of

immunological function occurs in herbivores with a complex stomach and diverse gastric flora (Freeland & Janzen 1974). Some plants have defensive chemicals called secondary compounds that are injurious to herbivores; bacteria in the stomach may detoxify these chemicals enabling the host to sample new, potentially harmful, foods without suffering ill effects (Freeland & Janzen 1974).

Despite the possibility that host/microbe immunological reactions (Meynell 1963; Freeland & Janzen 1974) may be of primary importance in the biology of M. albicaudatus, they are not the main concern of this investigation. The aim of the study was to examine gastric bacterial/epithelial associations in the white-tailed rat thus complementing previous microscope studies (Chaps. 1 & 2) and to contribute to a better understanding of gastric function in the rat. Emphasis was placed on the papillary bacteria and a case for non-fermentative symbiosis was presented (cf Moir 1968).

MATERIALS AND METHODS.

Adult M. albicaudatus were used in this study to ensure the presence of an indigenous alimentary flora. Surface examination of the non-glandular epithelium and determination of bacterial/epithelial associations was facilitated by treating rats with different doses of a general antibiotic, oxytetracycline (Rio Ethicals (Pty.) Ltd., Jhb.). Depending on the quantity, 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.

Treatment and housing of rats.

1. Conventional rats were housed in an animal room in plastic cages with straw bedding; one adult male and female per cage. Tap water and commercial rat pellets were supplied ad libitum.

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

3. 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 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.

STR and sterile rats were killed with chloroform anaesthesia on the sixth day of treatment and tissues were prepared for microscopy according to the techniques outlined in Chapter 1. Procedure for examination of tissues and photographic techniques and equipment have been described (Chap. 1; Maddock & Perrin 1981). Sections for light microscopy were stained with Periodic acid Schiff (Humason 1967), hematoxylin and eosin (Humason 1967) and modified azan (Ayoub & Skhlar 1963) stains. Malachite green was used as a spore stain for bacteria (Cruickshank, Duguid, Marmion & Swain 1975).

Three dimensional views of the papillae surface were obtained by stereophotography. Specimens prepared for scanning electron microscopy (Chap. 1), 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. (Use of the stereoscope is explained in Appendix 3.)

A superficial examination of the gastric microbiology of M. albicaudatus was implemented. Removal of allochthonous organisms from the rat gut, to facilitate autochthonous flora studies, was achieved by treating rats as in 3 (above) but without using antibiotic. The rats were killed and their stomachs removed. After the oesophagus and PGP were tied off, the stomachs were rinsed in 70 % alcohol and placed in a sterile petri-dish under a flow of oxygen-free gas (95 % hydrogen and 5 % carbon dioxide). Half the corpal contents and papillae were placed in separate anaerobic tubes filled with sterile saline. (Anaerobic conditions were ma.ntained under the gas flow and by gassing the tubes when they were open). The remaining corpal contents and papillae were similarly treated but under aerobic conditions.

The contents of the four tubes were mixed on a vortex mixer and used to innoculate aerobic and anaerobic brain-heart infusion (BHI) and meat extract broths which were incubated overnight at 35° C. Thereafter the eight broths were serially diluted and pure cultures obtained by streaking the inocula onto BHI plates under aerobic and anaerobic conditions. Anaerobic plates were incubated in Gas Pak anaerobic jars. Pure cultures were identified with the API 20 anaerobic system (Analytab Products Inc., New York) in addition to standard identification tests (Holderman & Moore 1972).

RESULTS.

Histology of bacterial/epithelial associations.

Corpal epithelia from conventional, STR and sterile rats was examined. Conventional rats possessed a thin layer of bacteria on the FCE and PGP epithelium (Chap. 1; Plates 1,4 & 1,7) but larger numbers of micro-organisms were found at the base of epithelial folds, particularly in the FCE (Plate 3,1). Bacteria-free zones (Chap. 2) were rather more common in the PGP. In contrast, considerably larger populations of bacteria colonised the papillae (Chap. 1) and were more densely packed than those of the PGP or FCE (Plates 3,1 & 3,2). In hematoxylin and eosin treated sections from conventional rats the papillae core stained lightly, the irregular orientation of the horny layers was distinguishable and bacteria penetrated the stratum corneum for a considerable distance (Plate 3,3) so that in section the papillae had a thick, dense outer bacterial zone (Plates 3,1 & 3,2). At high magnification this zone was seen to consist of pockets of bacteria or microhabitats (Plate 3,3) but even at this magnification the exact association between the host and microbes was difficult to assess.

An interesting observation was made when papillae from sterile rats were examined. The dark bacterial zone (Plates 3,1 & 3,2), was reduced to a thin, diffuse layer (Plate 3,4) and the core of the sterile papillae had a honeycomb structure markedly different from the more tightly packed papillae layers of conventional rats (Plates 3,1; 3,2 & 3,3). The honeycombs were less marked at the base (Plate 3,4) but conspicuous at the edges and tips of the papillae (Plate 3,4). STR had an 'intermediate' condition (Plate 3,5) and papillae from these rats had a thin, dark outer layer of bacteria while the internal honeycomb structure was less evident than in sterile papillae (Plate

Plate 3,1. Longitudinal, light micrograph section through the corpal epithelium. The thin bacterial covering of the FCE contrasts with the large numbers of bacteria on the papillae (PE). There is, however, an increase in number of bacteria in the folds of the FCE (arrow). The dark bacterial zone (B) is indicated.

Plate 3,2. Hematoxylin and eosin stained longitudinal section through the corpus showing the dense, dark bacterial zone (B) on the papilla (PE) periphery. The papillae stratum corneum (SC) stains lightly in comparison and consists of densely packed, but irregularly orientated, layers of dead cells. The thin bacterial covering of the FCE is evident.

Plate 3,3. A high power micrograph of the distal part of a papilla. Pockets or microhabitats (M) of bacteria (B) are visible amongst the papilla horny cells (PE) indicating the extent of bacterial penetration of the epithelium.

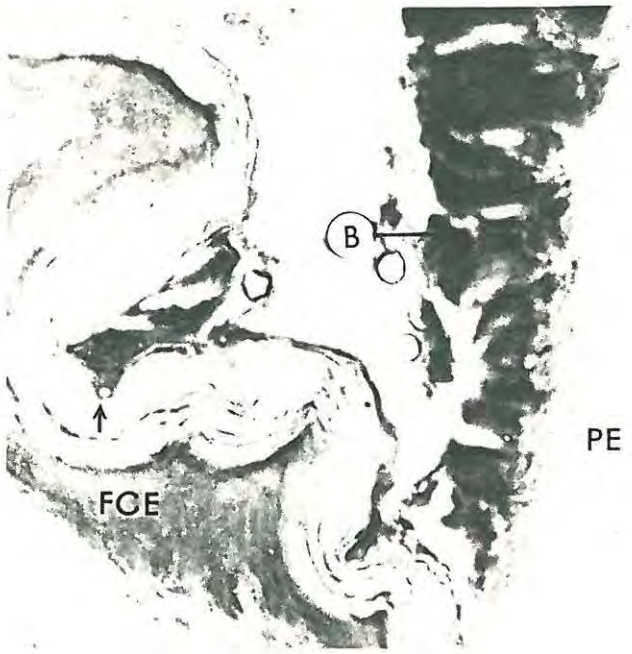


Plate 3,1 — 50 µm

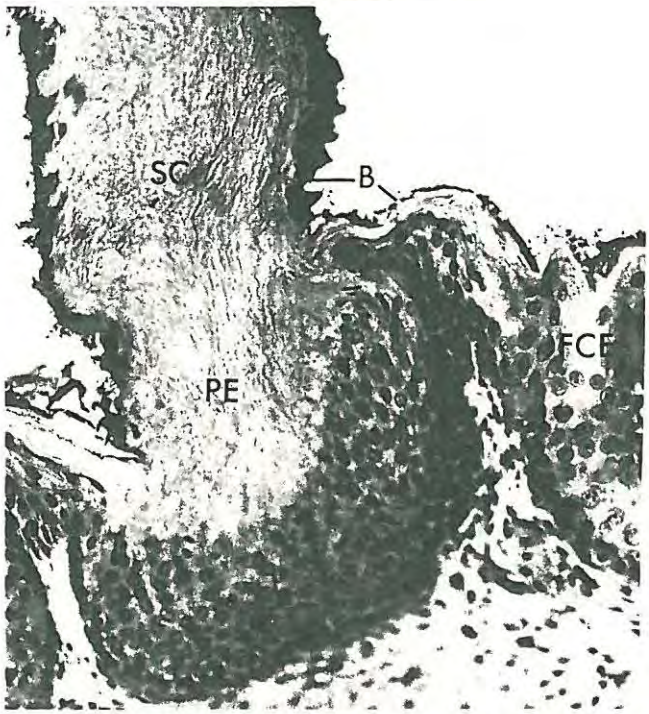


Plate 3,2 — 50 µm

Plate 3,3 — 0,4 mm

Plate 3,4. Papillae from a sterile rat. The bacterial zone is greatly reduced (RB) and the horny cells are less densely packed than 'conventional' papillae (Plates 3,1 & 3,2) forming a honeycomb-like structure. This honeycomb-structure predominates on the papillae periphery and horny layers at the papillae base are more densely packed.

Plate 3,5. A section of the corpus from a STR. The intermediate characters of this animal-type are evident; the bacterial zone (B) and the limited extent of the honeycomb structure. (See Plates 3,2 & 3,4).

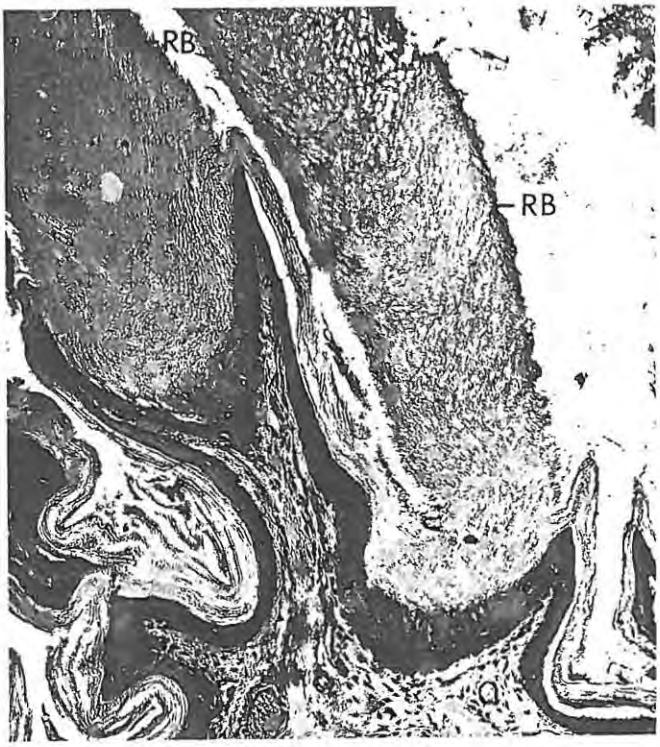


Plate 3,4 0,1 mm

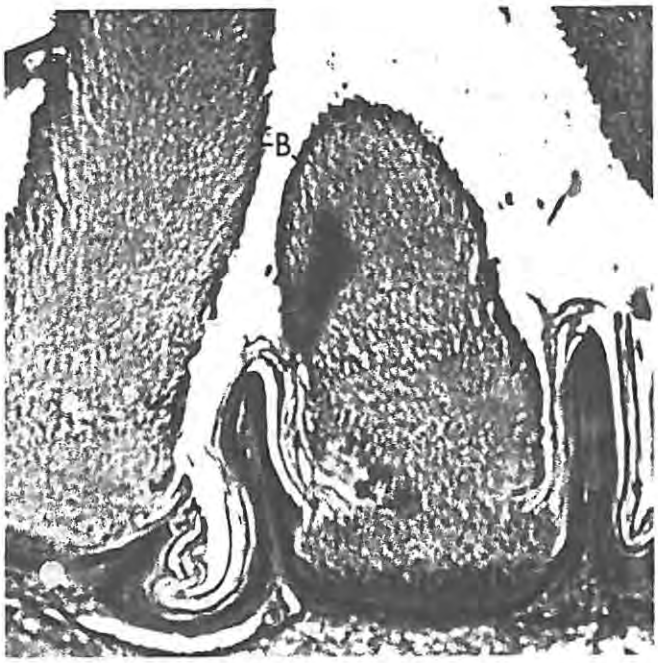


Plate 3,5 0,1 mm

3,4). No morphological differences were noted among the FCE and PGP epithelia from differently treated rats.

Ultrastructure of bacterial/epithelial associations.

For a clear understanding of the association between bacteria and papillae a three dimensional, highly magnified view of the papillae surface was necessary. These requirements were met by collating scanning and transmission electron micrographs and by using SEM stereopairs. Papillae from conventional rats were completely obscured by bacteria and food when viewed with SEM (Plates 1,17 & 1,19) but when tissues from STR (for SEM) and from conventional rats (for TEM) were used, the nature of the association became apparant.

Papillae microhabitats, infered from high magnification light micrographs (Plates 1,7 & 3,3), were in fact intercellular spaces between desquamating surface cells (Plate 3,6). Many intercellular spaces were too narrow for colonisation but all large spaces were occupied by colonies of bacteria (Plate 3,6) suggesting that availability of microhabitats was an important factor limiting bacterial number.

Scanning electron micrographs demonstrated the complete, bacteria-filled microhabitats among thin, irregular desquamating papillae cells (Plate 3,7). Plate 3,7 is a scanning electron micrograph of a microhabitat similar to those shown in section in Plate 3,6 and three dimensional views of the papillary bacterial/epithelial associations is shown in Plates 3,8 (a) and (b). Particularly evident in these stereopairs is the parallel alignment of the bacteria (Plate 3,8 a) and uneven topography resulting from desquamation of the epithelial cells (Plate 3,8 b). Microhabitats are

Plate 3,6. Transmission electron micrograph of a papilla periphery from conventional rats. Bacterial colonisation of the spaces between the horny cells (M) is seen although some intercellular spaces are narrow and consequently bacteria-free (arrow). Longitudinal (LS) and transverse sections (TS) of these microhabitats are seen in this micrograph. B = bacteria, L = lumen.

Plate 3,7. A scanning electron micrograph of a microhabitat from a STR. Large numbers of bacteria are orientated parallel to the microhabitat length. S = desquamating cells that would be covered with bacteria in conventional rats. Some of the bacteria have truncated ends while others taper gently to a point.

Plate 3,6 — 2 μm

Plate 3,7 — 5 μm

Plate 3,8 a. Vertical stereoscopic view of a microhabitat on a papilla from a conventional rat. Because the desquamating cells are colonised by bacilli, the papillary epithelium is not visible. Note the parallel alignment of the bacteria.

96a

Plate 3,8 a

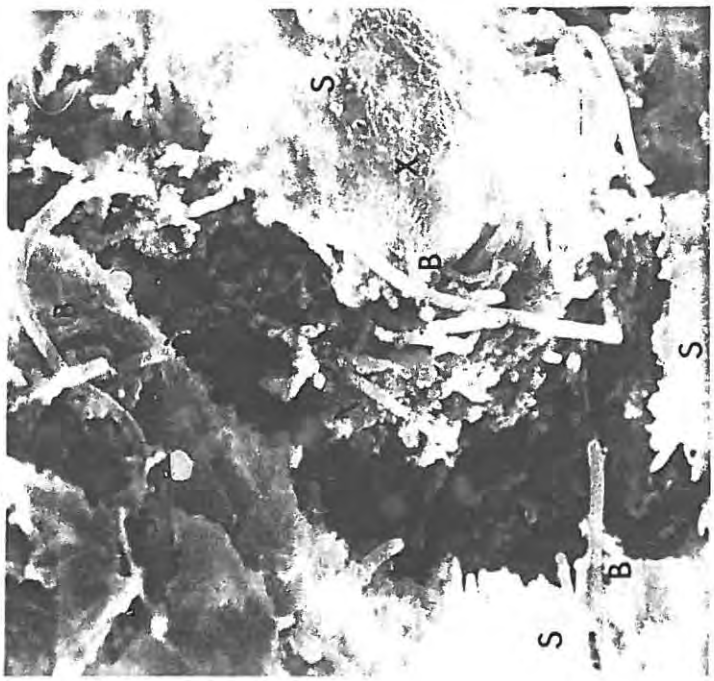
3 µm

Plate 3,8 b. Stereoscopic view of the surface of a papilla from a STR. The superficial desquamating cells (S) are free of bacteria but the deeper regions are still colonised by some bacilli (B). Microhabitats extending beneath the desquamating cells on the right of the stereopairs are indicated (arrows).

97a

Plate 3,8 b

4 μm



seen between these cells (Plates 3,8 a & b). It must be remembered that Plate 3,8 (b) is from a STR. In conventional rats microhabitats are concealed beneath micro-organisms attached to superficial epithelial cells (Plates 1,17; 1,19 & 3,8 a). Because of this thick bacterial covering it is probable that the micro-environment in these deeper regions differs from lumen or surface conditions. This idea was supported by the finding that some bacteria persisted in the microhabitats of rats treated with antibiotics (Plate 3,13).

The papillae were covered by rod-shaped bacteria (Plates 3,8 a & 3,9) orientated roughly parallel to the length of the microhabitat (Plates 3,7 & 3,10). The bacteria lacked special attachment appendages (for example pili and filaments) and were attached end-on to the epithelium by means of a thickened microbial capsular layer (Plate 3,14). No inflammation, damage or penetration of the epithelium was caused by the bacteria.

Compared to the papillae, the FCE and PGP presented different epithelial surfaces for bacterial colonisation and microhabitats resembling those on the papillae were absent. As a consequence, relatively fewer, morphologically different bacteria occurred on the outermost FCE and PGP horny layers. The bacteria attached (by means of a thickened capsule) to small depressions on the epithelium surface (Plates 3,15 & 3,16). Some invaginations almost enveloped the bacteria (Plate 3,16) but others were rather shallow (Plate 3,15). However, this difference may have been due to the angle of section and not representative of the mode of attachment.

Plate 3,9. A high power micrograph of the bacilli that colonise the papillae. These bacilli occur singly and some cocci (C) are visible amongst the rods.

Plate 3,10. A SEM transverse section through a papilla from a conventional rat showing dense populations of bacilli (B), their penetration and orientation 90° to the papilla surface (PE).

Plate 3,11. A scanning electron micrograph of rod-shaped bacteria that are predominant on the papillae surface although some cocci (C) are present among the bacilli (B). These bacilli have truncated ends.

Plate 3,12. Bacilli on the papillae surface. Two morphologically different bacilli-types are noted; one with gently tapering ends, the other with truncated ends (arrows).

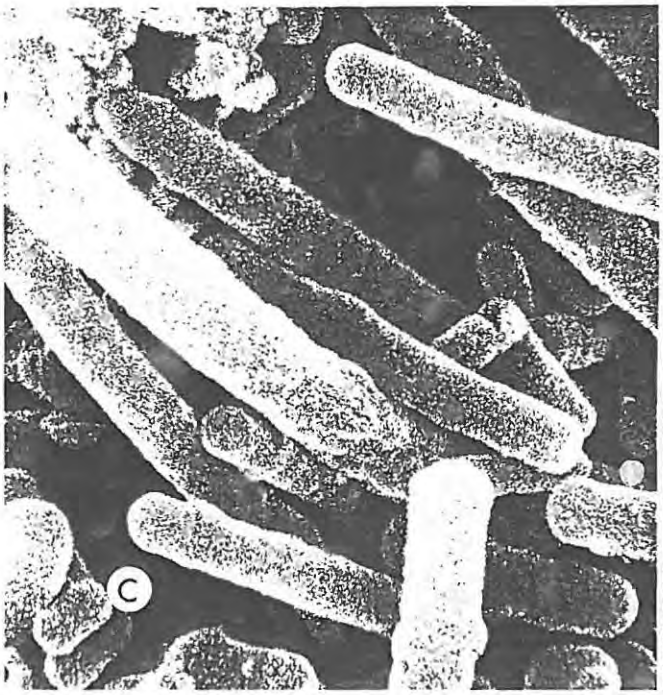


Plate 3,9 0,5 μm

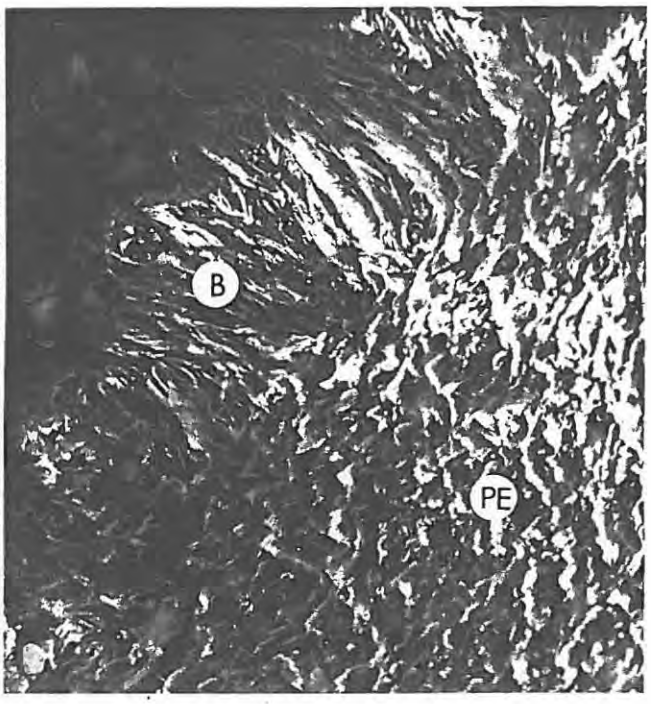


Plate 3,10 20 μm

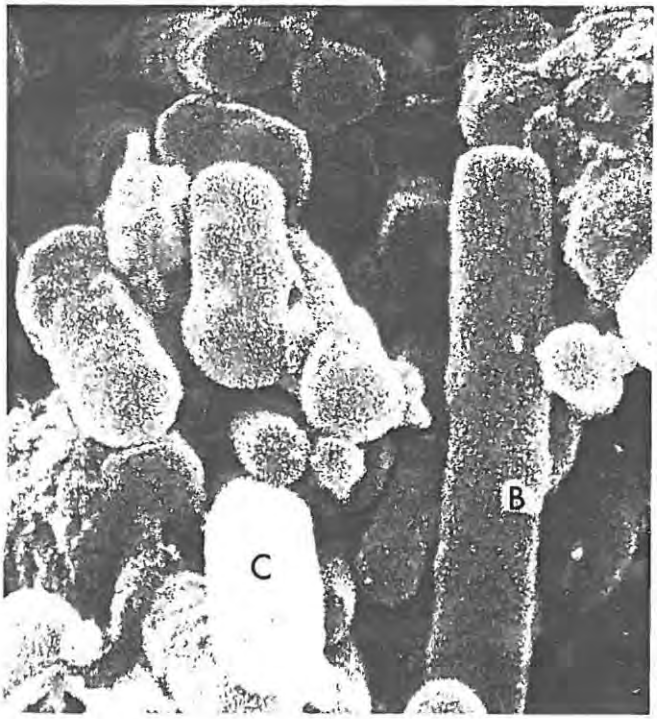


Plate 3,11 0,4 μm

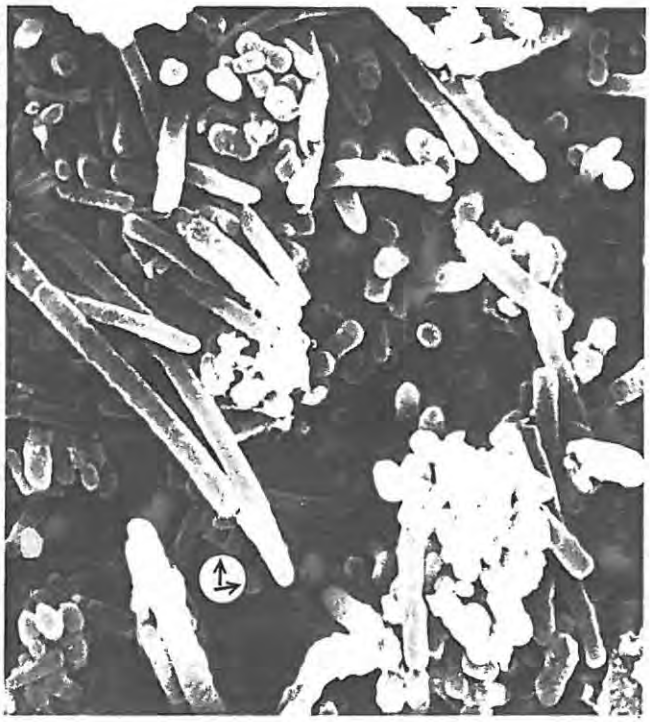


Plate 3,12 2 μm

Plate 3,13. Transmission electron micrograph of the papillae from a sterile rat. Comparison with Plate 3,7 reveals that treatment with oxytetracycline removed most bacteria (B) although some remain in the deeper microhabitats.

Plate 3,14. A high power micrograph of a dividing bacterium attached to a desquamating papillary cell (S). 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).

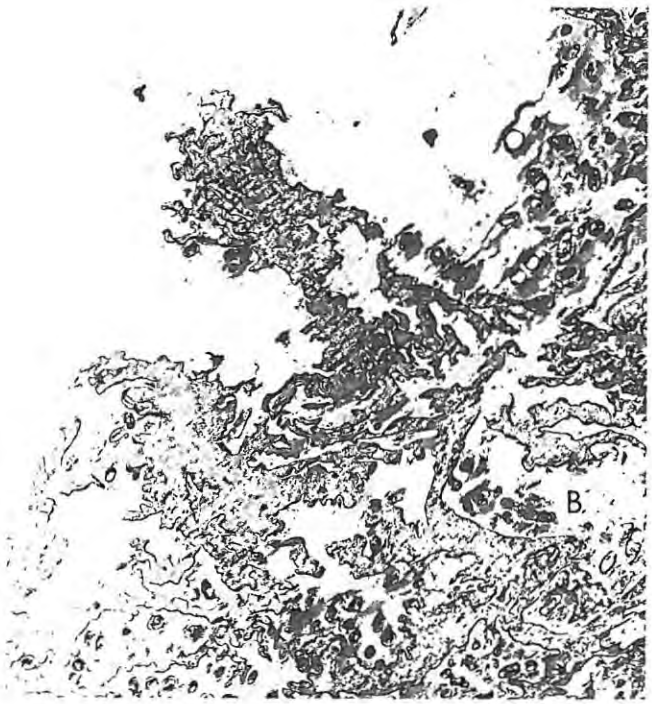


Plate 3,13 — 3 µm

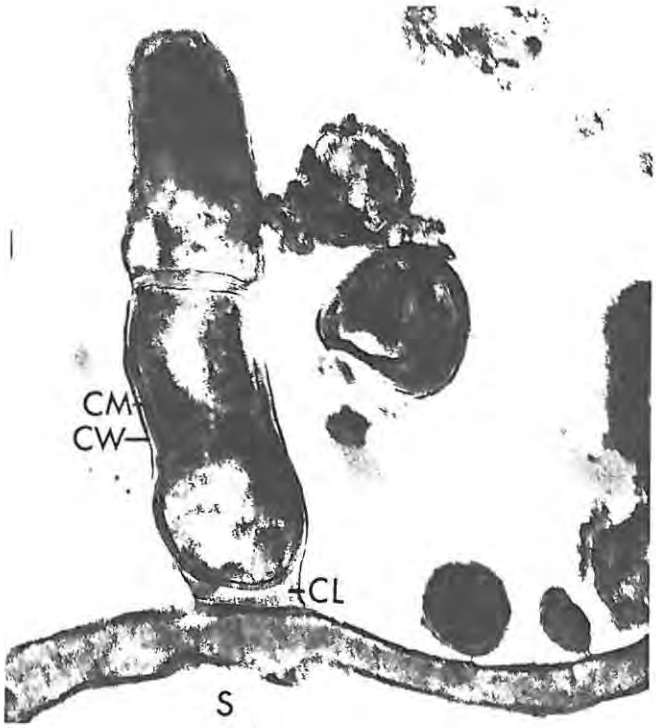


Plate 3,14 — 0,3 µm

Bacterial morphology.

The habitat separation of morphologically different bacteria noted in post-weaning M. albicaudatus (Chap. 2) was seen in the adult. Apart from a few cocci, presumably dislocated from elsewhere in the system or transient in the gut, non-segmented bacilli dominated the papillae surface and underlying microhabitats (Plates 3,7; 3,9 & 3,10). The bacilli were morphologically similar except some had sharply truncated ends while others tapered gently (Plates 3,7; 3,11 & 3,12) which may indicate different species. The bacilli occurred singly and were approximately 6,5 um by 0,7 um in size (Plates 3,9 & 3,14). There was no evidence of endospores in TEM sections (Plate 3,14) or in isolated bacteria stained with malachite green but bacteria in situ were seen in various stages of vegetative division (Plate 3,14). Flagellae were not present and cytoplasmic detail of the bacteria was not visible in TEM sections.

A triple layered cell membrane consisting of electron dense outer layers enclosing a lighter inner zone was resolved with TEM and was about 90 A thick (Plate 3,14). A thicker, single layered, electron translucent cell wall (approximately 280 A thick) was seen in these sections but this observation was not clear and an additional thin, electron dense layer may have been present (Plate 3,14). The capsule or slime layer that surrounded the bacterium thickened three to five times at the site of epithelial attachment (Plate 3,14).

Bacteria in the FCE and PGP occurred in distinct colonies separated from each other by bacteria-free zones. Short rods (coccobacilli) about 1,0 um by 0,4 um in size attached to the epithelium in pallisade formation and constituted the majority of the FCE and PGP microbiota (Plates 3,15; 3,16 & 3,17). Bacilli were rarely seen and

Plate 3,15. Bacterial attachment to the FCE in conventional rats. One bacterium has just completed vegetative division (arrow) while another is undergoing division (D). Details of cell wall and cell membrane morphology are visible as well as the hazy capsular layer (CL) which mediates bacterial attachment. Most bacteria occur in shallow depressions (Dp).

Plate 3,16. Bacterial attachment to the FCE in conventional rats showing their presence in deep depressions (Dp) in the keratinised cells. The double layered cell wall is visible. CL = capsular layer, B = bacteria.

Plate 3,17. Scanning electron micrograph of the cocci and coccobacilli that colonise the FCE. Bacilli are uncommon; those seen in this micrograph have probably been dislocated from the papillae.

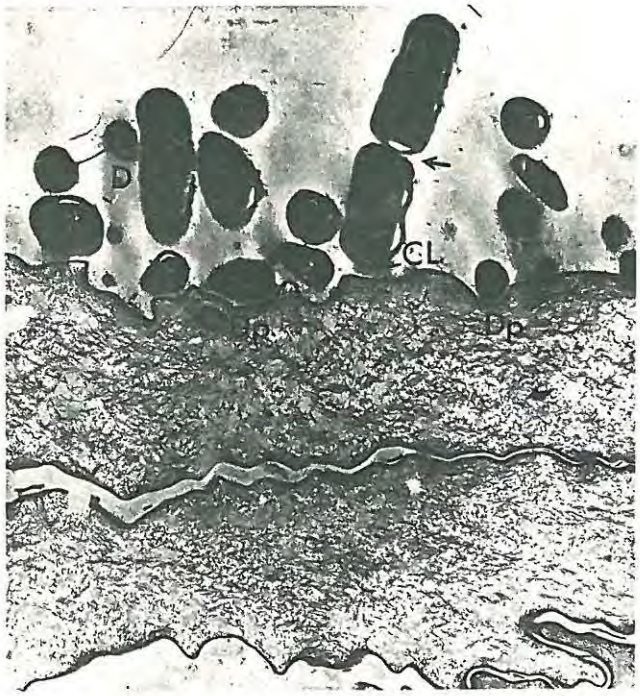


Plate 3,15 0,6 µm

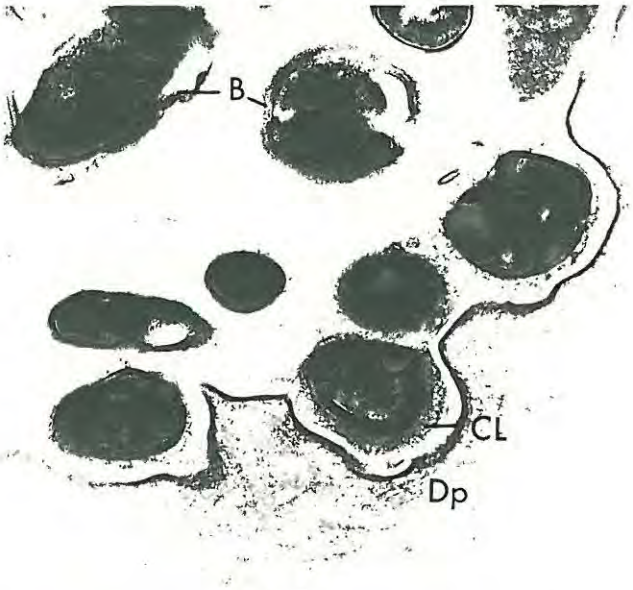


Plate 3,16 0,3 µm

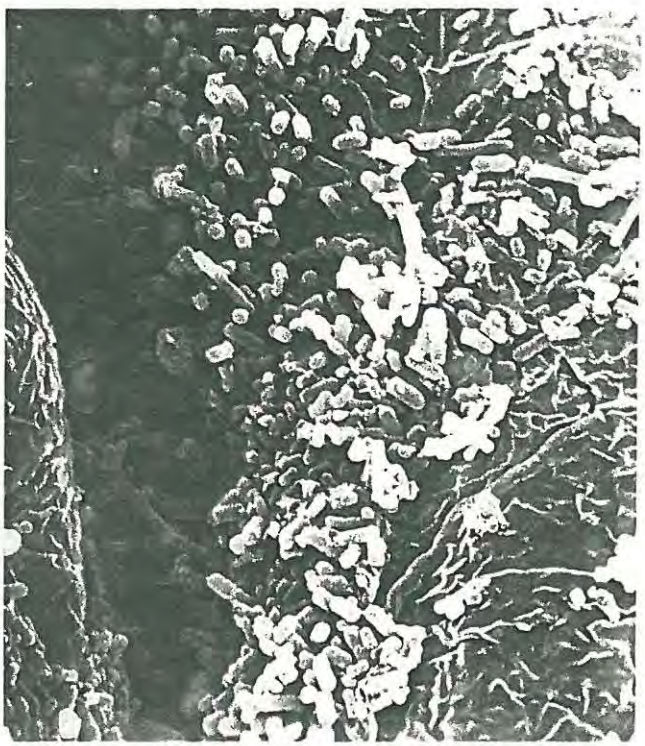


Plate 3,17 3 µm

were not attached to these epithelia. No flagellae or endospores were present but various stages of veg tative division were seen in TEM sections (Plate 3,15). The cell wall comprised two layers; an electron translucent inner zone which was about three to four times thicker than the dense outer layer (Plate 3,16). The total cell wall thickness was about 150 A. A thin hazy capsule mediated bacterial attachment to the epithelium (Plate 3,15 & 3,16).

Bacteriology.

While obtaining pure cultures, smears of the inocula were made and examined with a light microscope. Examination of the material

Table 3,1. A summary of some of the characteristics of the three bacteria identified in the corpus of M. albicaudatus. Tests follow the procedures outlined in Cowan & Steel (1966).

	1.	2.	3.
Morphology.	cocco-bacilli. prs, short chains.	short rods. single (swarms).	short rods. single & prs.
Gram stain.	(+)	(-)	(-)
Spores.	(-)	(-)	(-)
Motility.	non-motile.	motile.	motile.
Air tolerance.	facultative.	facultative.	facultative.
McConkey's agar.	growth.		growth.
Haemolytic prop.	(-)		(-)
Citrate use.	(-)		(+)
Catalase prod.	(+)	(+)	(+)
Glucose.	fermentation.		
Glucose.	no oxidation.		

1. Streptococcus.
2. P. vulgaris.
3. Ps. flourescens.

cultured in broths revealed oval cocci, some of which occurred in long chains, and short rods (cocco-bacilli). Rod-shaped bacteria (from the papillae) were uncommon and never seen in subsequent smears. Pure cultures of three micro-organisms were obtained; Proteus vulgaris, Psuedomonas flourescens and Streptococcus (Table 3,1). All were facultative anaerobes and no obligate anaerobes were identified.

DISCUSSION.

In a recent monograph on microbial ecology, Alexander (1971) suggested that the broad ecological concepts (for example, niche, habitat, competition, communities) are equally applicable to large scale ecosystems, and micro-ecosystems such as the gastro-intestinal (GI) tract. In the mammalian gut numerous micro-organisms interact with various environmental factors to produce a stable ecosystem which has been likened to a flowing stream (Savage 1977 a). Allogenic and autogenic successions (forces exerted by the environment and environmental changes due to microbial presence respectively) determine the size and composition of the GI microbiota (Savage 1977 a; Clarke 1977 b) which is separated into autochthonous and allochthonous microbes (Dubos et al. 1965; Savage 1977 a). Dubos and co-authors' definitions of autochthonous and allochthonous micro-organisms (Dubos et al. 1965) do not accord with recent concepts of microbial ecology (Alexander 1971) and Savage's interpretations (Savage 1977 a) are used here. Autochthonous (indigenous) micro-organisms are those that multiply and maintain climax communities in specific GI habitats in which they have a definitive niche; in other

words, they colonise the niche natively (Savage 1977 a & b). Allochthonous microbes, on the other hand, merely pass through a microhabitat (in which they have no niche) and consequently do not usually reach high populations (Savage 1977 a). They are derived from ingested material, the respiratory tract, higher regions of the GI canal and in the case of coprophagus hosts, from the lower GI tract as well (Savage 1977 a). These species can be indigenous in one GI habitat but if removed may become allochthonous in another (Savage 1977 a).

All niches in the GI tract are occupied by an autochthonous community forming a stable ecosystem (Alexander 1971). If stability is disrupted (for example by starvation or infection) indigenous microbes are eliminated and their vacated niche annexed by an allochthonous species until stability returns (Savage in press, 1977a; Tannock & Savage 1974). Even in a stable system some allochthonous microbes may be abundant making it difficult to distinguish them from the indigenous micro-biota (Alexander 1971; Savage 1977 a & b). Such a distinction is necessary, particularly when determining the microbial composition and its influence on the host's biology (Dubos et al. 1965; Gordon & Pesti 1971; Savage 1972, 1977 b). To this end certain criteria for autochthony have been suggested (Table 3,2).

Table 3,2. Criteria of autochthony for micro-organisms in the GI tract (modified after Savage 1977 b).

1. Can grow anaerobically.
2. Always present in adults.
3. Colonise the GI tract during succession in infants and reach and maintain stable climax communities throughout the life of healthy adults.
4. Always colonise certain areas of the tract.
5. May associate intimately with the epithelium or multiply at a rate to avoid being washed from their habitat (Clarke 1977 b; Savage 1977 a).
6. Can utilise nutrients normally entering the habitat and tolerate environmental extremes.
7. Can compete successfully with other organisms (Clarke 1977 b).

Gastric bacteria.

Bacilli that colonise the gastric papillae of M. albicaudatus conform well to the autochthonous criteria (Table 3,2) and are considered indigenous, occupying a niche that necessitates an intimate microbe/epithelium association. However, this conclusion is based on microscope evidence only and before all criteria (Table 3,2) are satisfied with certainty, identification of these bacilli in M. albicaudatus of different ages and from different colonies is required.

The inability to culture the papillary bacilli indicates a degree of specificity in their requirements. Bacilli were seen in broths innoculated directly with papillae but they died, possibly because exacting nutritional and environmental requirements were not satisfied. Not least among these requirements may be a need for anaerobiosis. Although anaerobic isolation and culture techniques were used as far as possible, exposure to some oxygen was inevitable when the stomach was opened. But only strict anaerobes would be deleteriously affected by such low oxygen concentrations and these micro-organisms are therefore thought to be fastidious anaerobes. For this reason, and the fact that anaerobes can outnumber aerobes by 1 000 to 1 in the GI tract (Savage et al. 1968; Clarke 1977 a), almost certainly other anaerobes (possibly indigenous) escaped culture too.

Streptococcus is morphologically similar to some bacteria that colonise the FCE and PGP epithelium. Micro-organisms of this genus comprise a major group of facultative anaerobes in the mammalian gut (Smith 1965; Hungate 1966) and are common in the non-glandular stomach of laboratory rodents (R. norvegicus and M. musculus, Schaedler et al. 1965; Kunstyr 1974; Savage & Blumershine 1974) where they are believed to be indigenous (Dubos et al. 1965; Suegara, Morotomi, Watanabe, Kawai & Mutai 1975). Because of their gastric ubiquity, autochthony and their resemblance to FCE and PGP bacteria it is likely that Streptococci occur on the FCE and PGP epithelium and may be indigenous.

Lactobacilli attach to the gastric stratified squamous epithelia of rodents (Schaedler et al. 1965; Savage 1972, 1979; Savage & Blumershine 1974; Kunstyr 1974; and many others) and are considered indigenous (Dubos et al. 1965; Savage et al. 1968; Morotomi et al. 1975; Wesney & Tannock 1979). This microbe also attaches to the

stratified squamous alimentary epithelia of many vertebrates (Smith 1965; Tannock & Smith 1970; Wesney & Tannock 1979). Their wide distribution led to the suggestion that Lactobacilli are present in many animals with similar GI epithelia (Savage et al. 1968) particularly herbivores since this microbe is abundant in carbohydrate-rich environments (Clarke 1977 b). For these reasons it is likely that Lactobacilli occur in the corpus of M. albicaudatus despite the inability to identify the genus in culture. It is unclear why Lactobacillus was not cultured but these micro-organisms may be strict anaerobes needing specific culture media, BHI being inadequate for their requirements.

Proteus vulgaris is an intestinal inhabitant (Buchanan & Gibbons 1974) and can be expected in the stomach of coprophagus animals such as M. albicaudatus. Pseudomonas flourescens is found in tap water (Savage in press) but would have been killed by acidification of the drinking water and was probably introduced to the stomach of M. albicaudatus by contamination despite efforts to reduce this possibility. Both micro-organisms are common in the environment (soil and water; Buchanan & Gibbons 1974) and allochthonous in the GI tract of R. rattus (Dubos et al. 1965; Savage 1970) suggesting that they were introduced to the gut by contamination and are allochthonous in the stomach of the white-tailed rat.

The autochthonous bacteria (Streptococcus, Lactobacillus and almost certainly other micro-organisms that were not cultured) that cover areas of the FCE and PGP are considered inessential for gastric digestion in M. albicaudatus. A variety of microbes pass through the stomach (Savage 1977 a) and it is inconceivable that the epithelium will not be colonised by bacteria suited to the gastric conditions. The host need not benefit directly from the association, and provided

the microbes cause no damage, a stable relationship can develop. It is difficult to imagine any trophic advantage(s) for the host in such an association, whereas micro-organisms presumably benefit from the constant gastic conditions and nutrient supply.

Of course, the indigenous FCE and PGP bacteria will indirectly affect the host in immunological and/or other ways (Meynell 1963; Savage 1970; Freeland Janzen 1974) and as such can be considered autochthonous. However, these bacteria contrast with the papillae micro-organisms which are believed to directly aid the host in digestion (Section II).

Bacterial attachment.

The gastric microbiota associates intimately with the epithelium, an important criterion for autochthony in the stomach (Table 3,2; Savage in press, 1977 a), the advantages of which have been stressed by Chong & Tannock (1978) and Savage & Blumershine (1974). Attachment is desirable as peristalsis (Coates & Fuller 1977) and ingesta flow hampers bacterial colonisation of certain habitats. Microbe/epithelial associations in alimentary tracts vary (Savage 1979) but corpal bacteria in M. albicaudatus adhere directly to the epithelium by their capsular layer. The effects of attachment on the epithelium are limited to a slight depression on the mucosal cells. But as similar depressions are present in the lower bacteria-free, horny layers, microbial action cannot be the primary cause of these indentations and it is probable that the microbes select the depressions which afford greater surface area for adherence.

Bacterial attachment in M. albicaudatus resembles that of Lactobacillus which attach to the non-glandular regions of mammalian stomachs (Smith 1965) and to the crop of chickens (Wesney & Tannock 1979). Although the exact mechanism of attachment is unknown (Suegara et al. 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) although recent investigators disagree (see Savage 1970).

It is interesting to note that, as well as having a similar type of epithelial attachment, the corpal bacteria in M. albicaudatus (i.e. FCE and papillary bacteria) attach end-on or in pallisade formation. Similar occurrences were seen in the non-glandular stomach of the laboratory rat (Brownlee & Moss 1961) and the laboratory mouse (Savage & Blumershine 1974) and the latter workers suggested that it allows for bacterial contact over a larger surface area facilitating transfer of metabolites. At least two morphologically different bacilli inhabit the papillae of the white-tailed rat and although their interdependance is unknown, the parallel alignment resulting from end-on attachment provides excellent opportunity for intercellular metabolite transfer. Pallisade attacnment also allows for an increase in the number of bacteria per unit area able to contact the epithelium (Savage & Blumershine 1974). This may be important in M. albicaudatus and will be elaborated later.

Microhabitats.

Although all the corpal bacteria of M. albicaudatus have similar modes of attachment, papillae bacilli are unique in occupying microhabitats between desquamating papillary cells. Formation of these cavernous intercellular spaces could be a normal consequence of

desquamating epithelia modified by bacterial, and possibly other influences. The microhabitats are not produced by desquamation alone as many deeper habitats occur where desquamation is not advanced and intercellular spaces are mostly narrow. These intercellular spaces are colonised and modified (enlarged) by bacilli as the horny cells begin to lose cohesion and microhabitats are formed. This is a continuous process and as surface cells, microhabitats and bacteria are lost, new bacilli colonise the deeper intercellular spaces where cell cohesion is decreasing. It is unknown if there are any factors governing selective colonisation of particular intercellular spaces.

The theory that bacteria modify intercellular spaces to form microhabitats explains papillae cell dynamics and the low desquamation rate in germ-free animals (Abrams et al. 1963). The presence of microhabitats decreases intercellular cohesion causing increased desquamation rate. Since desquamation rate and mitotic rate are related (Abrams et al. 1963) this could be the mechanism whereby the bacteria maintain a high papillae cell turnover (Chap. 2). Rapid desquamation rate results in cells being sloughed off before large microhabitats are formed. Large microhabitats with many bacteria may result in over-exploitation of the micro-environment, upsetting stability. Therefore, the bacteria influence their environment by helping to stimulate cell turnover. Rapid cell turnover will create a habitat, suitable for the microbiota but within the limitations of the host (no overpopulation of bacteria).

Bacilli are limited to the papillary microhabitats of M. albicaudatus and consequently are only found in the stomach once papillae appear (Chap. 2). The microhabitats are therefore considered essential for bacterial survival. These habitats have several

advantages including protection from ingesta flow. As a result of this protection the bacteria need not have high multiplication rates to off-set bacteria carried down the gut (and therefore lost from the habitat) before they can attach to the epithelium (see Clarke 1977 b). After vegetative division an unattached bacterium, sheltered from ingesta flow, has a good chance of attachment before being washed away.

A second advantage is that conditions in the microhabitats are probably unique and provide optimal conditions for multiplication of these bacilli. (Bacterial waste products will not build up if they are absorbed by another species of bacteria; see Savage & Blumershine 1974). As noted in STR, drastic changes in corpal conditions do not immediately affect bacilli in the deeper regions of the microhabitats. Thus, a major function of these regions could be defense against adverse gastric conditions; a nucleus bacterial population remaining in the microhabitats so that the papillae can be recolonised when conditions return to normal.

Symbiosis.

It has been suggested that the papillary bacilli are symbiotic (Maddock & Perrin 1981). Dubos et al. (1965) recognised two types of host/microbe relationship based on micro-organism population dynamics. A 'symbiotic' relationship occurs when the microbes reach and maintain climax communities in the habitat while an 'infective' relationship results in the microbes illiciting an immunological response from the host and being eliminated (Dubos et al. 1965). The high population levels and exclusive attachment of bacilli to the papillae of M. albicaudatus, the lack of epithelial damage and the intimate association between the papillae and microbes in healthy rats

suggests a symbiotic (mutually beneficial) association. The evolution of corpal papillae cannot readily be explained by assuming the bacteria to be parasitic or detrimental to the host (Maddock & Perrin 1981) and it is possible that evolution of the papillae has partially been determined by the autochthonous flora prevailing during evolutionary development (see Dubos et al. 1965). If this is true, it is almost certain that a symbiotic association has evolved between M. albicaudatus and the autochthonous bacilli.

From microscope studies (Chap. I) it appears that the papillae serve to increase surface area for the bacilli. Attachment sites for bacteria are also increased by the nature of the microhabitats and finally by the end-on bacterial mode of attachment. Symbiosis is believed to result only if bacterial populations on the papillae are at a high level (Maddock & Perrin 1981); such high populations are certainly maintained by the mode of attachment and morphological features of the papillae which increase corpal surface area five fold.

Benefit for the microbes is likely to be a constant environment and energy supply although the relationship may be more subtle. Only once food tests and digestibility trials are completed can an evaluation of the hosts' possible advantages from the relationship be made (Section II). A criterion for autochthony is anaerobiosis (Table 3,2; Savage 1977 a) and microbes must obtain energy without using oxygen as a terminal electron acceptor, a process that is often achieved by fermentation (Savage 1977 a). A wide range of organic compounds is available for fermentation in herbivore diets and the relatively constant high temperature of the mammalian gut facilitates a high fermentation activity (Clarke 1977 b).

Many herbivorous mammals have a portion of their gut modified to accomodate microbes which ferment dietary components (often indigestable by the host, for example cellulose) and in so doing release waste products that are utilised by the host (Elsden, Hitchcock, Marshall & Phillipson 1946; Johnson & McBee 1967; Hungate 1968; McBee 1971, 1977). The microbes benefit from a constant environment and food supply while the host obtains energy from otherwise unavailable sources. There are a number of difficulties associated with accepting the theory of pregastric fermentation in small mammals (see Parra 1978) and the theory of prolonged salivary amylase activity (Carleton 1973) may be more applicable to the white-tailed rat. Both these hypotheses will be critically examined in Section II.

SYNOPSIS OF SECTION I.

M. albicaudatus has a papillated bilocular hemiglandular stomach where a bacteria-rich corpus is separated from a glandular antrum by a non-glandular chamber (PGP) and a bordering fold of tissue (grenzfalte) (Chap. 1). The full complement of gastric glands (and large area of fundic epithelium) in the antrum (Chap. 1) is symptomatic of protein digestion although Perrin & Curtis (1980) suggested that protein intake has been reduced in this rodent. The corpal papillae are not absorptive but serve as attachment sites for autochthonous bacteria (Chap. 3). Papillae may also delay food passage, an advantage as ingesta retention in the corpus will increase digestive action in this region. Gastric digesta flow is probably controlled by the PGP/gastro-oesophageal muscle system (Chap. 1).

The synchrony of events in the gastric development of the juvenile rat (appearance of papillae, ingestion of solid food and the colonisation of the papillae by bacilli; Chap. 2) suggest that papillary microbes (APB) aid M. albicaudatus's digestive processes and are autochthonous. The presence of papillae, the fact that the bacteria do not damage the epithelium and that they occur in large numbers in healthy M. albicaudatus is symptomatic of symbiosis (Chap. 3).

An analysis of the complete alimentary tract is required for an appraisal of adaptations towards herbivory (Vorontsov 1962), therefore relevant measurements of the alimentary tract of M. albicaudatus (Perrin & Curtis 1980), not collected in the present study, are summarised in Appendix 2. This morphological data must be examined in

terms of Vorontsov's (1962) and Carleton's (1973) theories and the possible function(s) of the stomach of the white-tailed rat considered. These hypotheses can then be confirmed or rejected by further experimentation and ultimately a definite statement can be made about digestion in this rodent.

Gastric sacculation, corpal papillae and large bacterial populations in the foregut of M. albicaudatus are suggestive of pregastric fermentation (see Moir 1965, 1968). If fermentation contributes to the energy requirements of the white-tailed rat, an area of the gut must be adapted for this purpose (McBee 1977). The small caecum and lack of caecal spiral loops (Perrin & Curtis 1980) are not indicative of a large hindgut fermentation (see Vorontsov 1962) but the corpus and pregastric pouch, superficially similar to the ruminant rumino-reticulum, could harbour an active fermentative flora. If it can be shown that the white-tailed rat is evolving towards herbivory, the concept of extensive gastric fermentation in M. albicaudatus may be unique among rodents.

There are however, problems with accepting the occurrence of gastric fermentation in this rat. Many of the rodents' corpal bacilli occur in deep papillary 'microhabitats' (Chap. 3) and therefore do not come into contact with the ingesta. Although the papillae increase surface area for bacterial attachment, it is this very attachment that limits bacterial influence by preventing complete mixing of the microbes and solid food. Bacterial action is thus limited to the periphery of the chyme and many dietary components will pass through the stomach undigested by the microbes. This situation contrasts with rumino-reticula or caecal processes where fluid ingesta is mixed with numerous free-living bacteria which colonise the food surface or

attain preferential positions with relation to the food fibres undergoing microbial digestion (Hungate 1968). In the rumen, large quantities of food are digested releasing VFA's which are absorbed via numerous papillae and used by the host (Hungate 1968; McBee 1971). Possible absorptive sites in M. albicaudatus are limited to the small FCE and PGP areas.

Secondly, energy losses associated with pregastric fermentation make it advantageous for most small herbivores to utilise hindgut fermentation (see Parra 1978). Fermentation in the corpus of the white-tailed rat cannot be seen to benefit the host sufficiently to account for the complex papillary/bacilli associations and it is suggested that if pregastric fermentation does occur in this rodent, it is of minor symbiotic importance.

Carleton's hypothesis (1973) of high salivary amylase activity facilitating starch breakdown in the forestomach of rodents, may be applied to M. albicaudatus. As a result of gastric sacculation, corpal pH could approach neutrality, so that salivary amylase would not be denatured by acidic gastric secretions and the period of starch digestion would be prolonged. Although appealing, this theory cannot explain the role of the bacteria in the corpus, which appear important in gastric function in the white-tailed rat (Chap. 3).

After a brief analysis of the gross morphological features of the alimentary tract of M. albicaudatus, Perrin & Curtis (1980) concluded that the rodent is not a specialised feeder but has characteristics intermediate between the 'primitive', concentrate- and 'advanced', roughage-type diets. This conclusion is paradoxical as it does not account for the corpal papillae and gastric sacculation which almost

certainly are 'advanced' traits. Thus, gastric morphology of M. albicaudatus supports neither Vorontsov's (1962) nor Carleton's (1973) hypotheses and a modification of these ideas, or a new theory, is necessary to explain gastric function and evolution in the white-tailed rat. As it is clear that further understanding of gastric function cannot be elucidated on the basis of morphological data only, biochemical tests and feeding trials are used in Section II to aid the functional interpretation of the morphological data of the white-tailed rat.

SECTION II.

THE FUNCTION OF THE CORPUS OF *M. ALBICAUDATUS*.

INTRODUCTION TO SECTION II.

The aim of this section is to investigate the feeding habits and digestive processes of M. albicaudatus so that this information can be collated with gastric morphology and an overview of the feeding biology of the rodent obtained. Such an overview cannot be determined simply from an examination of morphological adaptations of the gut which, at best, are only indicative of broad dietary trends (Vorontsov 1962; Carleton 1973; Eisenberg 1978). Although generalisations are convenient when considering trends shown by rodent families, they cannot be accurately applied to single species. Various examples from the literature illustrate this point. The grasshopper mouse Onychomys torridus longicaudus and the genus Peromyscus have bilocular discoglandular stomachs (Horner et al. 1965; Vorontsov 1962) which, according to Vorontsov (1962), are indicative of herbivory. But these rodents select a granivorous and/or insectivorous diet (Horner et al. 1965; Hamilton 1941; Whitaker 1966) illustrating that interpretation of gut structure alone can lead to incorrect conclusions regarding feeding habits. It is therefore essential to relate gut structure with accurate observations of the animals' dietary habits.

However, the paucity of data on the natural foods of African rodents has limited correlations between diet and gut morphology (Perrin & Curtis 1980). Unfounded, broad categorisation of rodents into herbivores, insectivores and granivores (see Landry 1970) is uninformative and imprecise. To increase accuracy in assessing the diet and digestive processes, feeding habits must be quantified. Nevertheless, correlation between diet and morphology may still fail to explain digestive processes in the rodent stomach. For example, M. pennsylvanicus has no morphological gastro-intestinal adaptations which alone can account for its highly efficient digestion of

herbaceous food; this efficiency is believed to result from symbiotic caecal bacteria (Golley 1960). Feeding experiments on C. canadensis revealed that cellulose digestion by caecal symbionts contributed 18 % of the beavers' energy needs (Currier et al. 1960) and it is possible that similar processes occur in the vlei rat, Otomys irroratus (Perrin 1980 a & b). Extensive VFA production in the gut of a number of non-ruminants (Elsden et al. 1946) indicates the major role of microbial fermentation in herbivores. The import nce of microbial symbionts in rodent digestion (McBee 1971, 1977) demands that the influence of gut microbes on the ingesta be considered when interpreting gut function.

In this section a series of preliminary observations of gut function in M. albicaudatus are made. These tests are not exhaustive but give a better understanding of digestive function in the rat than was afforded by the morphological investigation alone (Section I) and are intended to function as guide lines for further research.

A number of unrelated mammalian taxa have anatomically complex stomachs (Moir 1968; Bauchop 1977; Appendix 1), which together with other criteria, are often indicative of ruminant-like digestion (Bauchop 1977). The two theories accounting for similar gastric modifications in rodents (Vorontsov 1962; Carleton 1973) have been discussed at length in Section I. Although both ideas are generalisations based purely on morphological data, it would be rewarding to discover if prolonged salivary amylase activity occurs in the stomach of M. albicaudatus or if the corpus represents an adaptation for pregastric fermentation. The morphological investigation of the stomach of M. albicaudatus (Section I) supports neither hypothesis and in this section biochemical tests and feeding experiments are used to further examine the rodents' digestive

functions. The validity of Vorontsov's theory (1962) with respect to the white-tailed rat will be discussed in Chapter four while Carleton's ideas (1973) are tested in Chapter five. Food preferences of the white-tailed rat are also considered in that chapter.

CHAPTER 4.

PREGASTRIC FERMENTATION.

INTRODUCTION.

Herbivorous animals have at least one expanded portion of their alimentary tract which is usually the site of an active microbial fermentation (Johnson & McBee 1967). Volatile fatty acids (VFA's) principally acetic, propionic and butyric, are the main waste products of fermentation that can be utilised by the host (Elsden et al. 1946). Few animals produce cellulases but since many fermentative bacteria are cellulolytic, herbivores with a large fermentation in their alimentary tract are able to digest and utilise structural carbohydrates (cellulose, hemicellulose and related polymers, for example lignin). Fermentative activity can be measured by determining VFA concentration in a portion of the alimentary tract (Parra 1978). High concentrations of these organic acids have been found in the fore- and hindgut of numerous herbivores (Elsden et al. 1946; Bauchop 1977; McBee 1977) and are known to contribute significantly to the energy requirements of various herbivores (Elsden et al. 1946; McBee 1970; Hume 1977; Parra 1978).

In the past two and a half decades a number of ruminant-like animals exhibiting pregastric fermentation (as opposed to caecal or colonic fermentation) have been described (Appendix 1) and indirect evidence suggests that other animals (hyraxes, peccaries, dugongs and manatees) may use similar digestive processes (Bauchop 1977). Adaptations for ruminant-like digestion are discussed by Bauchop (1977) and Parra (1978) and include formation of diverticulae proximal to the glandular, acidic stomach resulting in a volumetric increase in foregut size (Eisenberg 1978). These gastric sacculations have a

relatively high pH allowing colonisation by symbiotic, fermentative microbes (Hungate 1966; Bauchop 1977). Digestive efficiency is increased in these animals by a slow passage of ingesta through the gut (particularly through the regions of fermentation) so that nutrient extraction is maximised (Parra 1978). Low blood glucose levels, low urea excretion and large salivary glands (producing an alkaline, buffered saliva) are also characteristic of ruminants and ruminant-like animals (Moir 1965, 1968; Bauchop 1977).

Caecal fermentation on the other hand, is common amongst rodents (McBee 1970) but Vorontsov (1962) suggested that increasing gastric complexity in this order was associated with an increase in herbivory and trends towards pregastric fermentation. Although many rodents have complex stomachs (Vorontsov 1962; Carleton 1973; Luthje 1976; Dearden 1969; Perrin & Curtis 1980), foregut fermentation in rodents has only rarely been demonstrated. For example, R. norvegicus and M. musculus have a gastric flora capable of splitting carbohydrates (Peters 1973) and M. auratus has pregastric fermentation involved in digestion of forage cell walls and VFA production (Ehle & Warner 1978; Hoover et al. 1969) and can utilise dietary urea for growth (Matsumoto 1955). M. albicaudatus has a complex stomach (Chap. 1; Maddock & Perrin 1981) therefore the possibility of ruminant-like pregastric fermentation (Vorontsov 1962) in this rat was examined.

Detailed morphological evidence (Section I) however, does not support the concept of gastric fermentation in M. albicaudatus and to further qualify this observation a number of analyses and measurements of the white-tailed rats' alimentary tract (particularly the corpus) were made. Features characteristic of ruminant-like digestion (digesta passage rates, pH and relative stomach weight) were measured

and gastric and caecal contents were analysed for VFA's as an indication of fermentation (Parra 1978). The capability of M. albicaudatus to utilise roughage was also examined as it was believed that the ability to survive on a fibrous diet would be indicative of extensive symbiotic, microbial fermentation in the hosts' alimentary tract.

MATERIALS AND METHODS.

Fibre studies.

To test if the corpal microflora of M. albicaudatus functions in fibre digestion, the rodents' ability to survive on high fibre diets was examined. Five diets ranging from 14 % to 40 % (by weight) crude fibre were prepared (Table 4,1). Ingredients 1 to 4 were finely milled and thoroughly mixed with the other components. 1 % methyl cellulose was used as a binder and the food was made into small biscuits (6cm X 6cm X 2cm) and dried in an oven. The crude fibre content (cellulose, hemicellulose, lignin and cutin) of the diets and commercial rat pellets was analysed by the CSIR National Food Research Institute in Pretoria (Table 4,2).

Twelve adult M. albicaudatus and twelve adult R. norvegicus were housed individually in plastic cages with an ad libitum supply of food (diets 1 to 5) and water. A control group of four white-tailed rats were fed rat pellets instead of the prepared diets. Animals were weighed every three days and their ability to utilise fibre in the diets was determined by their weight change during the ten-day experimental period.

Table 4,1. Compositions of diets fed to M. albicaudatus and R. norvegicus in the fibre digestion experiment.

Ingredients (%).	Diet number.				
	1.	2.	3.	4.	5.
Lucerne meal.	-	11,9	36,2	60,9	90,4
Barley grain.	38,0	66,8	48,3	29,3	4,9
Soya meal.	19,0	16,7	12,1	7,3	2,5
Barley flour.	37,4	-	-	-	-
Sunflour oil.	3,5	2,5	1,5	0,5	-
Salt.	0,6	0,6	0,6	0,7	0,7
$CaCO_3$.	0,5	0,4	0,3	0,2	0,1
$CaHPO_4$.	-	-	0,02	0,13	0,23
MgO_2.	0,1	0,1	0,1	-	-

Table 4,2. Percentage crude fibre content of the commercial rat pellets (P) and artificial diets (1 to 5) used in the fibre digestion experiment.

Diet.	Crude fibre.	Hemicellulose.	Cellulose.	Cutin.	Lignin.
1.	14,4	8,0	4,5	0,2	1,5
2.	23,6	15,6	6,2	0,3	2,1
3.	27,4	12,9	11,8	0,4	2,5
4.	30,5	11,7	15,2	0,6	4,1
5.	40,0	9,9	21,7	1,0	6,4
P.	26,6	16,0	6,2	0,6	1,9

pH and stomach weight.

13 adult M. albicaudatus were killed by chloroform anaesthesia and the full and empty stomach weights recorded. Corpal, antral, and caecal contents were removed, diluted 1/9 (v/v) with double distilled water and the pH measured with a Beckman 3500 digital pH meter.

Rate of passage of ingesta.

Rate of passage is defined as the time taken for undigested residues from a given meal to be excreted in the faeces (Balch 1950; Balch & Campling 1965). The stained particle method (Balch 1950; Kobt & Luckey 1972), which is often used to estimate this value in rodents (Kostelecka-Myrcha & Myrcha 1964 b), was used. Commercial rat pellets (the normal laboratory diet of M. albicaudatus) were ground to powder and stained in a 5 % aqueous solution of crystal violet (Kindel 1960) which was boiled for one hour and left overnight. Next morning the food was washed in running water and dried in an oven. 50 g of stained food was mixed with 150 g of rat pellet powder and made into biscuits, 1 % methyl cellulose being used as a binder.

Two separate ingesta passage experiments were carried out. In the first experiment five M. albicaudatus and five R. norvegicus were starved for 34 hours then given the stained diet for twelve hours (0800 to 2000 hours). Time of ingestion (time zero) was taken as 1400 hours. Twelve hours access to the marked food proved too long to accurately quantify the rate of ingesta passage but this technique allowed a comparison of the pattern of excretion of marked particles by M. albicaudatus and R. norvegicus. The difference in mean values between independant groups were compared by the Student's t test.

In the second experiment five laboratory-reared M. albicaudatus were again starved for 34 hours but were only given two hours access to the stained food (1700 to 1900 hrs). This enabled more accurate determination of food passage rates in the white-tailed rat. Time zero was 1800 hours and at 1900 hours the marked food was replaced with normal rat pellets.

In both experiments the total faecal excrement was collected one hour after time zero, and at intervals not exceeding 4 hours for the next four days (96 hours). Faeces were dried at 60° C, crushed to powder and the number of stained particles per 0,1 g faeces counted under a dissecting microscope (10 X magnification). (This technique allowed more than one particle count to be made per sample as samples weighing more than 0,1 g were usually collected). The number of stained particles excreted per unit time was expressed as a percentage of the total number of particles excreted over the experimental period. These cumulative total were plotted against time to give excretion curves (Balch 1950; Castle 1956) and the excretion times of 5, 50, 90 and 100 % of the marker and mean retention time of food in the gut (Castle 1956) were calculated from the curves.

When using this technique accurate evaluation of the marker is laborious and problematical (Kobt & Luckey 1972). Stained faecal particles vary in size thus presenting a quantification problem; should two or three small particles be counted as one large particle? Some workers strain the faeces through gauze and only count the remaining large particles (Balch 1950; Castle 1956) whereas others count all the stained particles regardless of size (Kostelecka-Myrcha & Myrcha 1964 a & b). Both methods lead to bias (Balch & Campling 1965), the former to underestimation and the latter to overestimation (Castle 1956).

The small size of rodent faecal particles makes it impracticable to use the sieve method and in this study each stained particle, seen through a dissecting microscope, was counted. Accuracy could be increased by weighing faecal markers, although this method was rejected by Balch & Campling (1965) as being too time consuming. In the case of rodents the small amount of indicator could lead to weighing inaccuracies which would not justify the effort involved. The technique employed in this study is considered the most applicable and practicable method for use with small mammals.

Volatile fatty acid analysis.

The concentration of acetic, propionic and butyric acids in the stomach and caecum of M. albicaudatus was determined by gas chromatography. Adult white-tailed rats were killed with chloroform anaesthesia at 0800 hours: at this hour the gut was full after feeding activity the previous night. Corpal, antral and caecal contents were weighed and the VFA's extracted by ether extraction. 100 ml of this solution was concentrated to five ml using a one metre Vigreux fractionation column.

Samples of five microlitres were injected into a Perkin-Elmer gas chromatograph containing a one metre glass column packed with 10 % Supelco 1200 plus 1 % H_3PO_4 on chromosorb AW 80 - 100 mesh. The injection block was maintained at 220° C, the manifold at 215° C and the column at 95° C. A Hitachi model QPD 54 recorder was used. This technique eluted acetic, propionic and butyric acids in under 10 minutes and the concentrations of the acids were determined by comparison with standard curves obtained from known concentrations of these VFA's.

The relative stomach weights and the gastric and caecal pH's and VFA concentrations of ruminants, non-ruminants and ruminant-like animals were collected from the literature and tabulated. The results were used to compare M. albicaudatus with these other herbivorous groups. Such comparisons indicated whether major ruminant-like trends were being shown by M. albicaudatus.

RESULTS.

Fibre studies.

Fluctuations in the percentage body weight of rats fed the prepared diets or pelleted food are shown in Table 4,3. Under normal conditions (i.e. on a pelleted diet) the relative weight of M. albicaudatus ranged from a loss of 7 % to a gain of about 4 %. When maintained on diet 2, the rodents' weight loss was within the limits of that experienced on the pelleted diet, suggesting that the rats suffered no ill-effects on this 24 % fibre diet (Table 4,3). However, when fed diets containing 27 % (diet 3) or more crude fibre, M. albicaudatus consistently lost weight and died when maintained on diet 5 (40 % fibre). The ability to survive on rat pellets (27 % crude fibre) was probably due to a higher protein content in the pellets than in the diets. The variability of the results and the absence of any measure of precision, prevent definite conclusions. However, a general pattern of increasing weight loss with increasing dietary fibre content, is apparent.

R. norvegicus utilised fibre better than M. albicaudatus in this experiment and showed a weight increase for most diets (Table 4,3). The ability of the laboratory rat to digest roughage is probably due to fermentation in the caecum and colon (Yang, Manoharan & Young 1969) and absorption of the resulting VFA's (Elsden et al. 1946).

Table 4,3. Weight fluctuations (% body weight) of M. albicaudatus and R. norvegicus fed rat pellets (P) and artificial diets (1 - 5). * = animal died.

	M. albicaudatus.		R. norvegicus.	
	Loss.	Gain.	Loss.	Gain.
1.		7,6		3,8
2.	4,4			9,7
		3,7		15,7
		10,0		1,8
3.	21,5		2,1	
	35,9*			11,3
	6,0			13,1
4.	13,1			-
	4,2		8,0	
	8,1			8,4
5.	30,6*		36,0*	
	10,8*			1,8
P.		3,0		
	7,0			
		3,7		
	5,8			

Relative stomach weight.

The gastric and body weights of M. albicaudatus are presented in Table 4,4 and those of other herbivores in Table 4,5. The stomach contents of M. albicaudatus were approximately 3 % of total body weight (Table 4,4). In comparison, animals with foregut fermentation have larger stomachs and their gastric contents range from 6,6 % (the rotnest island quokka, Setonix brachyurus) to 20 % (the king colubus, Colobus polykomos) of body weight; ruminants had intermediate values (8 - 15 %; Table 4,5). Mean relative values for ruminants and ruminant-like animals was about 13 %; higher than that recorded for M. albicaudatus (2,8 %; Table 4,4).

The weight of the full stomach of M. albicaudatus averaged 5,8 % of body weight (Table 4,4) while that of the ruminant-like sloth, Bradypus and kangaroo, Macropus, was about 25 and 15 % of body weight respectively (Table 4,5). These comparisons suggested that M. albicaudatus does not have a capacious stomach required for pregastric fermentation.

Table 4,4. Stomach and stomach content weight of M. albicaudatus (% body weight).

	Body weight.	Stomach wt.		Stomach contents.
		Full.	Empty.	
mean & SD.	89,0 ± 42,6	5,8 ± 1,8	3,0 ± 0,7	2,8 ± 1,6
n.	13	13	13	13
range.	40,0 - 150,9	4,0 - 10,8	2,0 - 4,1	1,2 - 7,1

Table 4,5. Stomach (or caecal) content weight of different herbivores. (% body weight).

Species.	Stomach contents.	Reference.
1. Ruminants (rumino-reticulum).		
Suni	8	Hungate et al. 1959.
Thompson's gazelle	13	"
Zebu	15	"
Sheep	15	"
Cow	14	"
Cow & Sheep	10 - 15	Bauchop 1977.
2. Ruminant-like.		
Camel	10 - 17	Hungate et al. 1959.
Colubus polykomos	10,5 - 20,6	Ohwaki et al. 1974.
Presbytis spp.	17	Bauchop & Martucci'68.
Bradypus spp. (plus stomach)	25	Grasse 1955.
Setonix brachyurus	6,6	Moir et al. 1956.
Macropus (plus stomach)	15	Bauchop 1977.
M. eugenii	6,4	Langer et al. 1980.
M. gigantus	10	"
Thylogale thetis	7,6	"
3. Non-ruminants. (caecum plus contents).		
E. dorsatum	6	Johnson & McBee 1967.
Horse	2,5	Elsden et al. 1946.
Rabbit	7,8	"
R. nove gicus	10,5	"

pH.

The pH of the gastric and caecal contents of M. albicaudatus were similar in all individuals examined (Table 4,6) although no effort was made to standardise stomach fullness (see Kunstyr et al. 1976). Separation between the glandular and non-glandular gastric regions was effective to the extent that the difference in corpal and antral pH was highly significant ($P < 0,001$) although both were acidic (Table 4,6). Caecal contents were neutral (Table 4,6).

Table 4,6. pH values from various regions of the alimentary tract of M. albicaudatus.

	Corpus.	Antrum.	Caecum.
mean and SD.	4,57 ± 0,46	2,67 ± 0,39	6,95 ± 0,09
n.	10	9	5
range.	3,90 - 5,03	2,36 - 3,37	6,83 - 7,09

Table 4,7 illustrates the pH range of gastric and caecal contents for a variety of herbivores including those showing ruminant-like adaptations. In regions of microbial fermentation (forestomach in ruminants and ruminant-like animals; caecum in non-ruminants) the pH was about 6 and resembled caecal pH in the white-tailed rat (Table 4,6) and other hindgut fermenters (Table 4,7). The comparatively lower pH of the forestomach of M. albicaudatus (Table 4,6) suggested that optimal conditions for fermentation was not present in this region of the gut (see Hungate 1966) and consequently maximum fermentation potential is not realised. The glandular stomachs of the herbivores including M. albicaudatus, had similarly acidic conditions (pH about 2,6; Tables 4,6 & 4,7).

Table 4,7. pH values from various regions of the gut of different herbivores. * op. cit. Bauchop 1977; (a) = conventional rat; (b) = germ-free rat.

Species.	Forestom.	Gl. stom.	Caecum.	Reference.
1. Ruminants.				
Cow	5,8 - 6,8			Hungate 1968.
Cow	6,0 - 7,6	1,1 - 1,3		Dukes 1955.
Cow	6,5			Bauchop 1977.
2. Ruminant-like animals.				
Llama	6,4 - 7,0			*Vallenas & Stevens 1971.
Camel	6,4			Williams 1963.
Choloepus spp.	7,0 - 7,7			*Denis et al. 1967.
Bradypus spp.	5,2 - 6,7			"
C. polykomos	5,5 - 7,0			Ohwaki et al. 1974.
Presbytis spp.	5,0 - 6,7			Bauchop & Martucci'68.
H. amphibius	5,0 - 5,7	2,0		Thurston et al. 1968.
B. penicillata	5,8	2,8		Kinnear et al. 1979.
S. brachyurus	7,6	2,4	7,0	Moir et al. 1956.
	5,7	2,4	6,6	
Macropus spp.	4,6 - 8,0	1,8 - 3,0		Bauchop 1977.
3. Non-ruminant animals.				
M. auratus	5,1	2,0	7,0	Hoover et al. 1969.
L. africana	2,0 - 2,3	6,4 - 6,8		Hungate et al. 1959.
"		6,0 - 6,8		Van Hoven et al.1981.
R. norvegicus	2,9 - 4,8	2,3 - 3,0 (a)		Kunstyr et al. 1979.
	3,6 - 5,6	3,3 - 3,6 (b)		"
C. lanigera	3,4			Krishnamurti et al.'74.

Rate of passage of ingesta.

The results of the ingesta passage experiment for M. albicaudatus and R. norvegicus are presented in Table 4,8 and Fig. 4,1. The rates of ingesta passage for M. albicaudatus (38,3 hours) and R. norvegicus (35,0 hours) were similar and the differences were not statistically different ($P > 0,5$). Although rates of passage in these rodents were similar the pattern of excretion differed, and during the first 15 hours R. norvegicus passed food through its gut faster than did

Table 4,8. Retention time 'r' and appearance of stained particles in the faeces of M. albicaudatus and R. norvegicus.

Rat.	5 %	50 %	90 %	100 %	'r'
			M. albicaudatus.		
1.	4,5	16,0	30,0	35,0	18,4
2.	4,0	15,5	22,0	35,0	15,0
3.	6,0	19,0	30,5	48,0	20,4
4.	4,0	17,5	28,0	35,0	18,4
mean.	4,6	17,0	27,6	38,3	18,1
SD.	± 0,9	± 1,6	± 3,9	± 6,5	± 2,2
					C.V % 12,2 %
			R. norvegicus.		
1.	1,5	15,0	29,0	35,0	15,5
2.	2,0	15,0	26,0	35,0	15,3
3.	2,5	15,0	28,0	35,0	20,4
4.	1,5	14,5	28,0	35,0	14,5
mean.	1,9	14,9	27,8	35,0	15,4
SD.	± 0,5	± 0,3	± 1,3	0,0	± 0,7
					C.V % 4,5 %

C.V. % = co-efficient of variation.

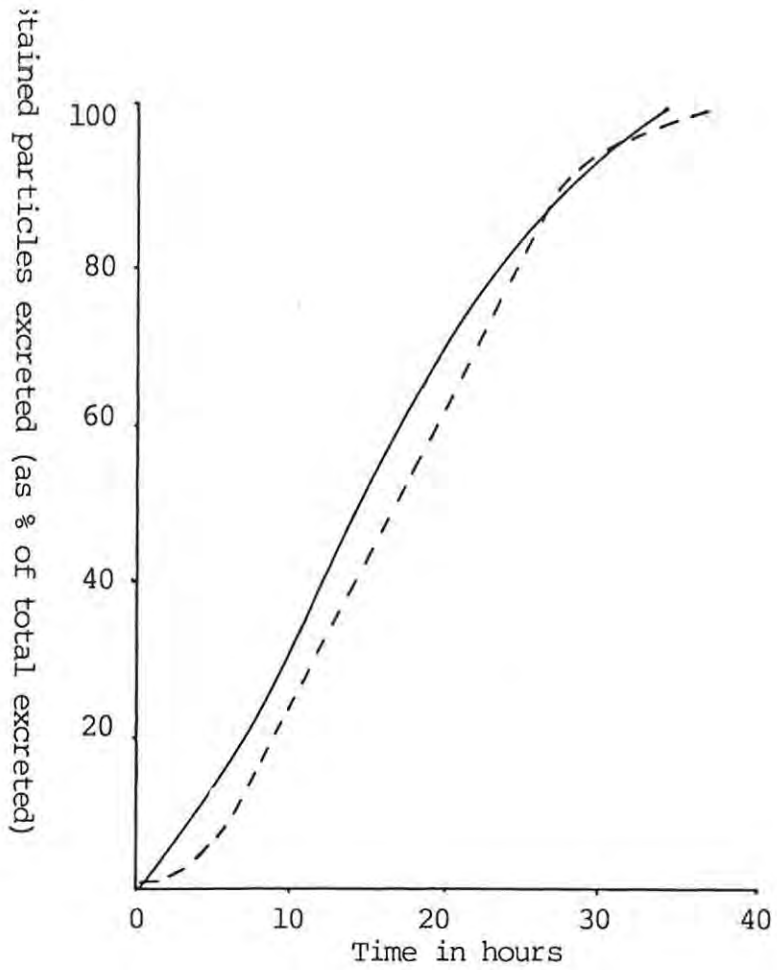


Figure 4,1. Comparison of the excretion curves for stained particles excreted by M. albicaudatus (---) and R. norvegicus (——).

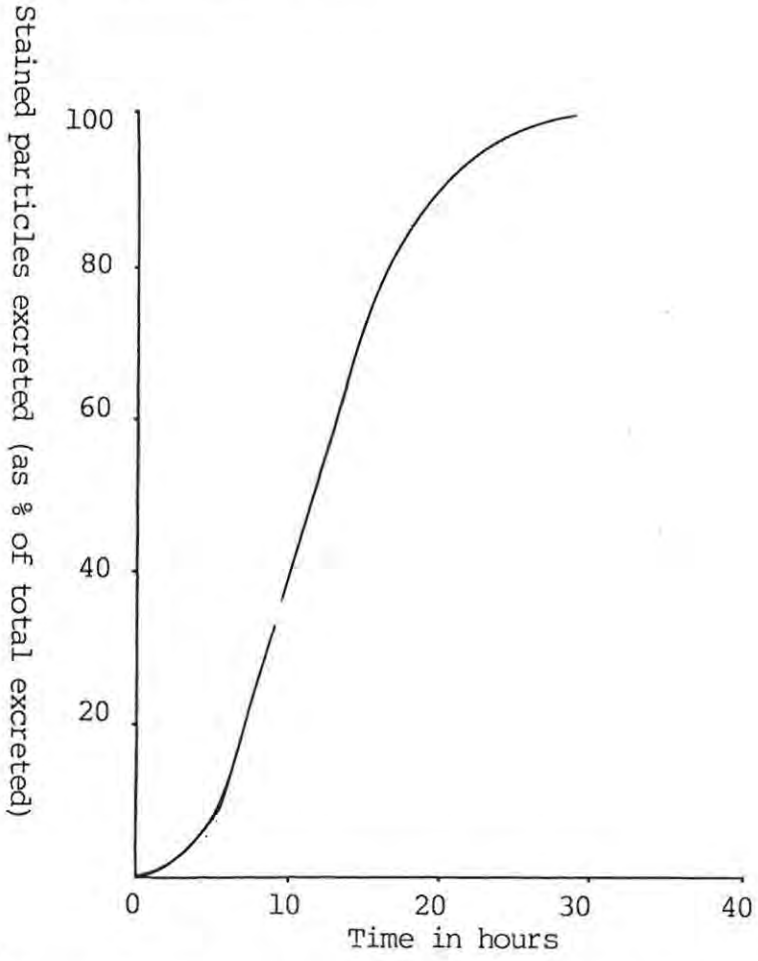


Figure 4,2. Mean excretion curve for stained particles excreted by M. albicaudatus.

M. albicaudatus (Fig. 4,1). On average 5 % of the marker was excreted by M. albicaudatus in 4,6 hours and 50 % in 17,0 hours. These values were significantly different from the 1,9 hours and 14,9 hours taken by R. norvegicus to excrete 5 % and 50 % of the indicator respectively ($P < 0,05$). Thereafter, excretion times were similar for both species and there was no significant difference between 90 % and 100 % excretion times for the rodents ($P > 0,5$).

The average excretion curve for stained particles by M. albicaudatus is presented in Figure 4,2 and the appearance of 5, 50, 90 and 100 % of the marker in the faeces and retention time are presented in Table 4,9. The first appearance of particles in the faeces varied from three hours to 13 hours. The final appearance of the marker was less variable (29 to 37 hours; Table 4,9). The white-tailed rat excreted 50 % of the marker after about 12 hours at a rate of 4,3 % per hour and passed the main mass (75 %) of test food through its gut in 15,8 hours (Fig. 4,2). The final 10 % of the marker was eliminated slowly at approximately 0,7 % per hour and the mean retention time of marked particles in the alimentary tract of this rodent was 12,1 $\pm$ 3,2 hours (Table 4,9).

Consideration of the excretion and retention times of stained particles by M. albicaudatus during the two digesta passage experiments (Tables 4,8 & 4,9) shows the effect of the twelve hour access period to the stained diet (experiment 1). The result of this experiment (twelve hours access) was that food retention in the gut was longer (Table 4,8) than when the rat was given two hours access to the stained food (Table 4,9).

Table 4,9. Retention time 'r' and appearance of stained particles in the faeces of M. albicaudatus. (Time in hours).

Rat.	5 %	50 %	90 %	100 %	'r'
1.	5,0	19,2	25,9	37,5	17,7
2.	3,1	9,0	19,5	37,5	10,3
3.	5,5	11,0	17,8	37,5	11,9
4.	5,1	9,3	14,0	29,0	9,6
5.	3,4	10,0	20,1	29,0	11,0
mean.	4,4	11,7	19,5	34,1	12,1
SD.	± 1,1	± 4,3	± 4,3	± 4,7	± 3,2

Volatile fatty acid analysis.

Results of the VFA determinations in the alimentary tract of M. albicaudatus are presented in Table 4,10). Generally the quantities of acetic, propionic and butyric acids were similar to each other ($P > 0,5$) although the concentration of acetic acid in the caecum and corpus was significantly different ($P < 0,05$).

The gastric and caecal VFA concentration was low in M. albicaudatus (Table 4,10) compared with that recorded in the alimentary tracts of other herbivores known to have an active fermentative flora (Tables 4,11 & 4, 12). This data suggests that extensive fermentation is absent from the gut of the white-tailed rat.

Table 4,10. Volatile fatty acid concentration of the gastric and caecal contents of M. albicaudatus. (mM/g dry ingesta).

Corpus.	Acetic.	Propionic.	Butyric.
mean.	0,031	0,012	0,021
SD.	$4,8 \times 10^{-3}$	$3,4 \times 10^{-3}$	$7,0 \times 10^{-3}$
C.V. %.	1% - 6,9%	8% - 16,5%	12,5% - 16%
Antrum.			
mean.	0,029	0,016	0,026
SD.	0,015	$7,3 \times 10^{-3}$	0,012
C.V. %.	2,8% - 5,9%	4,2% - 10%	4% - 20%
Caecum.			
mean.	0,018	0,012	0,024
SD.	$7,0 \times 10^{-3}$	$3,9 \times 10^{-3}$	$6,8 \times 10^{-3}$
C.V. %.	1,4% - 5,1%	10,7% - 35,6	15,4% - 50%

C.V. %. = coefficient of variation.

Table 4,11. Volatile fatty acid concentration of the gastric contents of various herbivores. (mM/g dry ingesta).

* = op. cit. Bauchop 1977; ** = op. cit. Parra 1978.

Species.	Total VFA conc.	Reference.
Cattle	0,919	**Church 1969.
Sheep	0,892	"
Deer	0,816	**Short 1963.
Presbytis cristatus	0,766	Bauchop & Martucci 1968.
Presbytis entellus	0,883	"
Choloepus spp.	1,156	*Denis et al. 1967.
E. dorsatum	0,089	Johnson & McBee 1967.

Table 4,12. Volatile fatty acid concentration of the caecal contents of various herbivores. (mM/g dry ingesta).

* = op. cit. Yang et al. 1970; ** = op. cit. Parra 1978.

Species.	Acetic.	Propionic.	Butyric.	Total.	Reference.
Guinea pig				0,38	*Hagen & Robinson 1953.
Horse	0,75	0,17	0,05	0,97.	Elsden et al. '46.
Pig	0,40	0,15	0,04	0,59	"
Pig	0,72	0,51	0,10	1,33	*Friend et al. 1963.
E.dorsatum	0,38	0,06	0,07	0,51	Johnson & McBee'67.
Rabbit	0,26	0,03	0,03	0,32	Elsden et al. '46.
Wild rabbit				0,37	**Hemming et al. '72.
Rattus spp.	0,37	0,15	0,14	0,66	Elsden et al. '46.
"	0,50	0,07	,15	0,72	"

DISCUSSION.

Fermentation of fibrous food in the gut of various herbivores contributes significantly to the energy requirements of these mammals (Elsden et al.; McBee 1970; Hume 1977; Parra 1078). However, energy requirements increase relative to decreasing body size (Hungate et al.; Kleiber 1961). Small herbivores (for example, M. albicaudatus) are therefore faced with increased energy requirements but decreased fermentation capacities (Parra 1978). Since the foregut supplies most of the energy requirements of PGF mammals, the total fermentation in small herbivores should be greater, proportional to body weight, than that in large PGF mammals (Hungate et al. 1959). There are a number of ways in which this energy problem can be overcome, for example, selective feeding on easily fermentable food (Bauchop 1978). However, small herbivores would require exceptionally high selectivity of high quality/low fibre food that they may not need fermentative digestion. This high selectivity and the high energy costs of passing food through an additional trophic level (the microbial level) may negate the advantages gained by the foregut fermentation strategy (Janis 1976; Parra 1978).

In non-PGF mammals, food reaching the hindgut is mainly refractive material since the nutritious food has been digested and absorbed in the proximal and midgut regions. Hindgut fermentation is therefore less costly than pregastric fermentation because the nutritious components of the diet are obtained without the need for microbial digestion and only the more refractive material is fermentated. Small herbivores (less than 10 kg) therefore benefit more from hindgut than foregut fermentation (Parra 1978; Van Soest op. cit. Janis 1976).

However, pregastric fermentation in various small herbivores has been recorded (Moir 1968; Hofmann 1973; Bauchop 1977, 1978), thereby contradicting the observations of Parra (1978) and Van Soest (op. cit. Janis 1976). But in many cases there are simple explanations for these observations. For example, arboreal folivores may require gastric fermentation as a detoxicant for plant secondary compounds (Parra 1978) while some PGF mammals (bradypods and macropods) may have low metabolism and hence lower maintenance requirements (Parra 1978). In these animals advantages gained from foregut fermentation outweigh resultant energy losses and greater benefit will be derived from the ruminant-like feeding strategy. If M. albicaudatus does have a similar feeding strategy, the rat must possess some adaptation whereby energy losses associated with foregut fermentation are offset.

Another way in which small ruminant-like mammals can meet increased energy demands is by eating large quantities of food which are passed through the gut rapidly and only the most readily fermentable material is processed (Hungate et al. 1959). Ingesta passage through M. albicaudatus is relatively fast and it is possible that the white-tailed rat may employ this feeding technique. However, the rate of food consumption was not measured in this study and may be worthy of further investigation.

Small mammalian herbivores may also prolong digesta retention in the gut thereby increasing energy yield. Fermentation of fibrous food requires time and to obtain maximum digestion, food retention in the region of microbial fermentation is required (Blaxter 1963 op. cit. Parra 1978). Passage rates however, increase with decreasing body weight in mammals (Mertens 1973 op. cit. Parra 1978) therefore it is expedient to compare passage rates of M. albicaudatus with herbivores of similar size.

The bank vole, Clethrionomys glareolus, has a small intestine longer than the large intestine, while the common vole, Microtus arvalis, has a well developed caecum and hindgut; features indicative of granivory and herbivory respectively (Kostelecka-Myrcha & Myrcha 1964 a). The rate of seed passage through the gut of the granivore, (C. glareolus) is 40,2 hours, significantly different from the 44,3 hour transit time required by M. arvalis (Kostelecka-Myrcha & Myrcha 1964 a). Faster rates in the granivores is a result of their relatively short hindguts and lack of large fermentation areas that delay food passage. The slower rate of passage for M. arvalis is due to retention of food (facilitating fermentation) in the well developed caecum and colon.

M. albicaudatus passes food through its gut ten hours faster than does M. arvalis (Kostelecka-Myrcha & Myrcha 1964 a) and on this basis it is suggested that the white-tailed rat may possess a similar digestive strategy to C. glareolus; maximising seed starch and protein digestion rather than fibre digestion (Kostelecka-Myrcha & Myrcha 1964 a).

In comparison to R. norvegicus, which has an almost constant rate of excretion, M. albicaudatus shows a slow initial excretion of marker although both rodents require a similar time to pass food through their guts. An explanation for this is that food is delayed in a section of alimentary tract of M. albicaudatus but moves rapidly through the rest of the gut. The most likely place for ingesta retention is in the sacculated stomach where the papillae, fornix ventricularis and PGP constriction could facilitate slow passage

rates. Transit through the simple caecum and hindgut is expected to be rapid although the colonic spiral loops (Perrin & Curtis 1980) may delay food. Thus, it is believed that the slow initial excretion rates of the white-tailed rat resu't from food retention in the gastric region (and possibly the colon). Rapid transit through the rest of the alimentary canal accounts for a rate of passage similar to that of the larger R. norvegicus.

Associated with a slow passage rate is an increase in size of the fermentation chamber (Hungate et al. 1959) because increased storage capacity is related to fermentation area (Parra 1978). Since fermentation increases digestion of low protein/high fibre vegetation, large volumes of food must be processed to produce sufficient protein. Fermentation chambers are therefore capacious; PGF mammals have a large foregut and small hindgut while hindgut fermenters have a small foregut but large caecum and/or colon (Parra 1978). A major adaptation for foregut fermentation therefore, is the development of a large, sacculated stomach resulting in increased fermentation area (Bauchop 1977). This adaptation has been demonstrated in all ruminant-like animals (Table 4,5; Bauchop 1977; Moir 1968). M. albicaudatus has an anatomically complex stomach (Chap. 1) but its size is smaller than would be expected if the rodent was utilising pregastric fermentation processes, and small in comparison with other southern African myomorph rodents (Perrin & Curtis 1980). Therefore, neither the rate of ingesta passage nor the stomach size of M. albicaudatus suggests that foregut fermentation contributes significantly to this rodent's energy requirements.

Herbivorous mammals show varying degrees of apparent digestibility for crude fibre, an ability related to (i) alimentary conditions that favour anaerobic bacterial fermentation and (ii) the

efficiency with which end products of fermentation (VFA's) can be absorbed (Fonnesbeck, Harris & Kearl 1974). Thus, sheep with foregut fermentation and coprophagous rabbits, both of whom can absorb the products of fermentation in the small intestine, are able to digest diets containing 55 % crude fibre (Fonnesbeck et al. 1974). Rats and pigs cannot digest such high fibre diets because of low absorption efficiency of the VFA's (Fonnesbeck et al. 1974).

M. albicaudatus looses weight or dies when fed high roughage diets suggesting that either the rodent cannot digest fibre due to the absence of extensive fermentation in the gut, or that absorption of the end products of fermentation is inadequate, or both. If the white-tailed rat possessed pregastric fermentation adaptations, VFA's would be formed proximal to the small intestine and efficient absorption of VFA's would be likely. Since M. albicaudatus cannot utilise high fibre diets and the absorption of fermentation products cannot be limiting (as they would be formed in the corpus, proximal to the main intestinal absorption sites), it is concluded that microbial fermentation in the corpus does not significantly aid this rodent in digestion of structural plant carbohydrates.

However, the pH of the stomach (which is similar to the minimum gastric pH of ruminant-like animals) and the neutral caecal pH, could be indicative of a fermentative microflora (Hungate 1966). But it is likely that the corpal and caecal bacteria also require conditions of almost neutral pH for survival. Thus, pH values alone are not evidence for the presence of cellulolytic bacteria in the gut of the white-tailed rat.

From the preceding discussion, the conclusion that M. albicaudatus does not utilise fermentation products as a major energy source, correlates with the low concentrations of VFA's in the stomach and caecum. Such low concentrations may be indicative of limited fermentative activity in the rodent's alimentary tract. If the corpus had evolved to maximise utilisation of fermentation products, it might be expected that absorption of VFA's would occur in the stomach. However, it appears that these organic acids are not absorbed from the stomach. VFA's cannot be produced in the glandular stomach of M. albicaudatus as there are no bacteria there. Therefore, the similar quantity of VFA's in the corpus and antrum of this rat suggests that gastric VFA absorption does not occur and that these acids are carried from the proximal to the distal stomach by digesta flow. Since gastric absorption of VFA's is unlikely it is probable that the corpus has another function important than fermentation.

However, VFA absorption and utilisation by other corpal bacteria is possible (see Elsden et al. 1946) and it must be recognised that the low concentration of VFA's may be explained by their rapid uptake by bacteria soon after they are formed. Transfer of microbial metabolites in the stomach M. albicaudatus would be facilitated by the parallel alignment of the papillary bacilli (Chap. 3). Despite the possibility of rapid uptake of VFA's by bacteria, the combined results of this chapter strongly suggest that fermentation does not occur in the corpus and that VFA's do not contribute significantly to the energy requirements of the white-tailed rat.

The absence of fermentation in the corpus indicates that the papillary bacteria - APB (which, because they are anaerobes, cannot use O_2 as a terminal electron acceptor), generate energy by a mechanism other than fermentation (Savage 1977 a). As an alternative,

energy can be produced by fixing H_2 with CO_2 to form methane (Scheifinger, Linehan & Wolin 1975) or by using sulphate as a terminal electron acceptor (Doelle 1975 op cit. Savage 1977 a). The small quantity of VFA's in the white-tailed rats' stomach could be produced by the comparatively fewer bacteria colonising the FCE and PGP epithelium (Chap. 3), or by free-living microbes.

In conclusion, the digesta passage rates, gastric size, fibre digestion and analysis of VFA's in the alimentary tract of M. albicaudatus are not indicative of pregastric fermentation. Another explanation for the presence of corpal bacilli and gastric modifications in M. albicaudatus must be sought.

CHAPTER 5.

AMYLASE ACTIVITY IN THE ALIMENTARY TRACT AND FOOD PREFERENCES OF M. ALBICAUDATUS

INTRODUCTION.

There is little evidence to support the concept that the corpus of M. albicaudatus has evolved to accommodate a fermentative, symbiotic microbial flora that contributes to the hosts' energy requirements (Chap. 4). Since the corpal bacilli are not fermentative, their function remains an enigma. Carleton (1973) suggests that rodent gastric sacculation prolongs salivary amylase activity thereby increasing starch digestion, but his hypothesis cannot account for the numerous bacilli in the corpus of M. albicaudatus. Few animals show gastric amylase activity but it has been suggested that some carbohydrases in the mammalian gut have bacterial origins (Krishnamurti et al. 1974; Peters 1973), and this implication may be pertinent to the bacteria-rich corpus of M. albicaudatus. Therefore, alpha amylase activity of the rodents' gut was measured to determine the region of highest enzyme activity and to discover if the corpal bacilli played a role in amylase production.

As fermentation is unlikely in the stomach and the caecum of the white-tailed rat, it is probable that the rodent selects a diet that can be readily digested without the aid of cellulolytic bacteria (i.e. a low fibre diet). Since it is essential to relate gut morphology with feeding habits a knowledge of the food preferences of M. albicaudatus was required: but this rodents' feeding habits are poorly known (De Graaff 1981). Walker (1975) and Roberts (1951) suggested that the white-tailed rat eats seeds and vegetable matter while Perrin & Curtis (1980) on morphological grounds, classified it as a

MATERIALS AND METHODS.

Amylase activity.

Adult M. albicaudatus were killed by chloroform anaesthesia. The corpal, antral, duodenal and caecal contents were removed, diluted 1/9 (v/v) with double distilled water and centrifuged. Enzyme activity (carbohydrate digestion) was determined using the Merckotest for alpha amylase (Merck, Darmstadt, West Germany) and a procedure modified after Street & Close (1956). Further dilution of gut contents was determined by experimentation. Absorption was measured with a Beckman model 25 digital spectrophotometer and enzyme activity expressed in international units; U = micromoles/min at 37° C.

To investigate the contribution by bacilli to the amylase activity of the corpus, the enzyme activity of the corpal contents plus papillae (and hence the bacilli) was measured separately from the enzyme activity of the corpal contents only.

Food preferences.

The feeding trials were designed to indicate the categories of food (Table 5,1) prefered by M. albicaudatus rather than the rodents' preference for individual food items. This rodent is endemic to the Eastern Cape savanna (Davis 1962, De Graaff 1981) and accordingly a selection of plants and insects, available to the rat under natural conditions, was collected from the Albany district. The plants were identified and categorised (Table 5,1) by Mr. A.R.Palmer of the C.D.N.E.C. (Palmer 1981).

In the absence of wild-caught individuals, eight laboratory-reared M. albicaudatus were used. To introduce these rats to a 'natural' diet, they were offered a selection of indigenous plants as

well as rat pellets for five days before the trials. Food preferences were determined using the cafeteria test (Drozdz 1975). A representative species from each food group (Table 5,1) was placed in the cage at the start of each three day trial period, (water and commercial rat pellets were supplied ad libitum) and the percentage of each food item consumed was estimated every 24 hours by comparison with a control quantity. The mean percentage of each food item consumed was calculated for the three day period and food items were then ranked according to the technique of Curtis & Perrin (1979; Table 5,2). The overall rank for each food group was calculated and used as an expression of food preference For convenience, the high concentrate food groups (fruits and seeds of herbs and shrubs; Table 5,1) were considered together (Group 5).

Table 5,1. Food groups comprising the different food categories recognised during the feeding trials.

Food category.	Definition.	Food group.	Group. No.
Graminivore.	Grass eating.	Grass stems and leaves.	1.
Granivore.	Seed eating.	a) grass seeds.	2.
		b) seeds of herbs and shrubs.	5.
Folivore.	Eating leaves and soft stems.	a) herbs.	3.
		b) shrubs.	4.
Frugivore.	Fruit eating.	Fruits of herbs and shrubs.	5.
Insectivore.	Insect eating.	Insects.	6.

Table 5,2. Procedure for ranking individual food items when using the cafeteria food preference test (after Curtis & Perrin 1979).

Class 1. Inedible. None eaten on the first day and less than 30 % taken in total in three days, or 10 % taken on the first day, but thereafter not touched. These species are not likely to be consumed in the wild and would only be selected in cases of extreme food shortage.

Class 2. Unpalatable. 10 - 20 % taken on the first day or 30 - 50 % taken in total over the three day period. This food would only be eaten if species of Classes 3 and 4 were not available.

Class 3. Palatable. 30 - 40 % consumed on the first day or more than 50 % taken in total after three days. These species are less palatable than those of Class 4, but would be likely to form a substantial part of the natural diet.

Class 4. Preferred. 50 % or more consumed on the first day of the test. These species would be taken most readily in the wild.

RESULTS.

Amylase activity.

The amylase activity in the stomach of M. albicaudatus is summarised in Table 5,3. An important finding was the high amylase activity of the corpal contents plus papillae (total amylase activity) compared to that of the corpal contents only (Table 5,3) thus revealing an important role played by the autochthonous gastric bacilli. Since bacterial and salivary amylase are identical (West, Todd, Mason & Van Bruggen 1966) high bacterial amylase in the corpus would aid M. albicaudatus in initial starch (carbohydrate) digestion.

Table 5,3. Alpha amylase activity in the gut of M. albicaudatus. cont. = contents only; cont & pap. = contents and papillae.

	Corpus.		Antrum.	Duodenum.	Caecum.
	cont.	cont. & pap.			
n.	11	7	4	10	4
mean.	4 402	12 692	40	2 989	0
SD.	5 592	6 458	-	3 412	-
SE.	1 686	2 440	-	1 079	-
range.	381 - 16 979	3 598 - 22 995	0 - 40	150 - 8 690	-
C.V. %	127	51	-	114	-

Amylase activity was negligable in the antrum, probably because the acidic conditions (pH 2,7; Table 4,6) denatured the enzyme. Enzyme activity was lower in the duodenum than in the corpus and was non-existent in the caecum (Table 5,3).

A feature of these results was their wide variability indicated by high standard deviations and coefficients of variation which sometimes exceeded 100 % (Table 5,3). This variability could have resulted from differences in degree of individual stomach fullness as amylase activity decreases with fore-tomach emptying (Kunstyr et al. 1976). However, a broad range of enzyme activity was still noted by Kunstyr et al. (1976) despite their efforts to standardise stomach fullness. The large range in enzyme activity in M. albicaudatus might have been reduced if similar standardisation procedures were used although it appears that individuals show much variation in the activity of this enzyme.

Food preferences.

Results of the food preference trials are presented in Table 5,4. Figures 5,1 and 5,2 show the comparative preference by M. albicaudatus for the various food groups and categories respectively. M. albicaudatus showed a clear preference for insects over other food types (Figs. 5,1 & 5,2); the whole insect was often consumed as soon as it was placed in the cage. The rat was attracted by movement and did not attack until the insect moved. Dispatching the prey followed a distinct pattern in both juveniles and adults; the insect was pounced upon, held in the forepaws and eaten head first.

Fruits and seeds (of herbs and shrubs) were also prefered food items (Table 5,4; Fig. 5,1) but grass seeds were not selected by the rodent and were classified as inedible (Table 5,2; Fig 5,2). However, Setaria species grass seeds were an exception and were eaten (Table 5,4). Because the white-tailed rat did not eat grass seeds, the overall granivore category rank (seeds, fruits and grass seeds) decreased from 3,4 for fruits and seeds only, to 3,0 (Table 5,4; Figs.

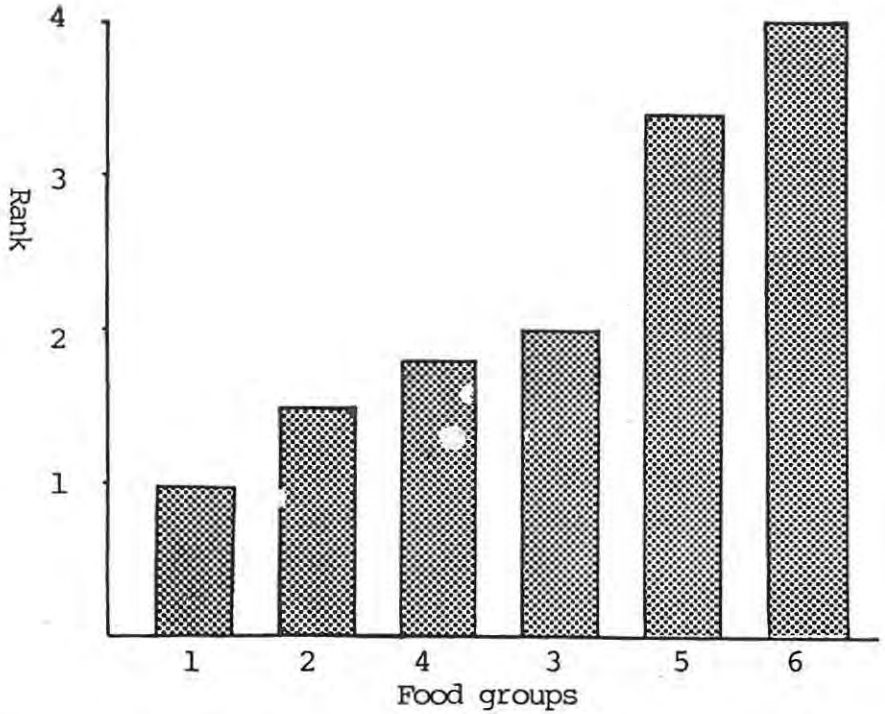


Figure 5,1. Preference shown by M. albicaudatus for the six food groups used in the study. 1. = grasses, 2. = grass seeds, 3. = herbs, 4. = shrubs, 5. = fruits and seeds, 6. = insects.

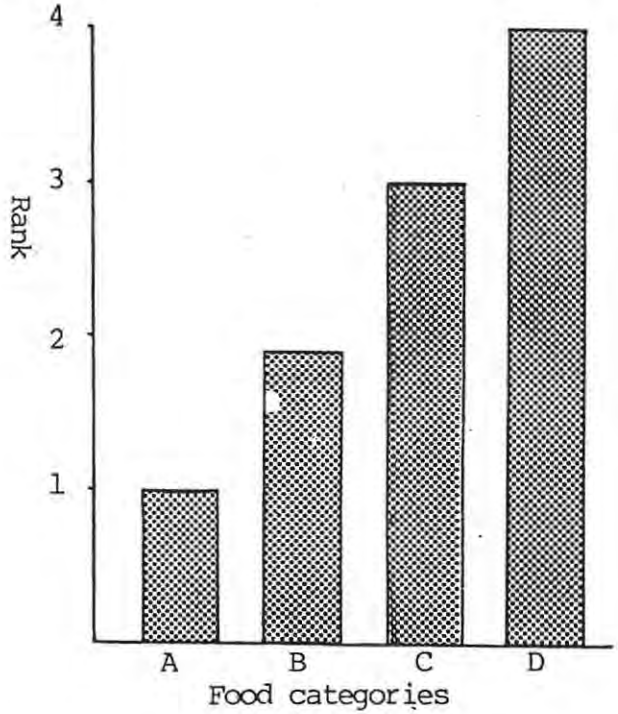


Figure 5,2. Overall categorisation of M. albicaudatus based on the laboratory feeding trials. A = graminivore, B = folivore, C = granivore/frugivore, D = insectivore.

5,1 & 5,2). Nevertheless granivorous foods were considered as palatable/prefered items in the diet of M. albicaudatus (Fig. 5,2), probably forming a major part of the rodents' diet.

The leaves and soft stems of shrubs and herbs were unpalatable to M. albicaudatus (a slight preference was shown for herbs (rank 2,0) over shrubs rank 1,8; Table 5,4). However, certain individual food items in this category were classified as palatable. One shrub, Lycium campanulatum was prefered (Table 5,4) and both bark and leaves were eaten. M. albicaudatus cannot however, be considered a folivore despite its selection of leaves from certain plant species.

Grasses were not eaten by the rat (Table 5,4) and were only used for nesting material. Thus, grasses were considered inedible.

M. albicaudatus selected a diet rich in protein and starch and could therefore be classified as a granivore/insectivore. The fact that it also ate leaves from specific herbs and shrubs has probably lead to the belief that it is omnivorous.

Table 5,4. Food preferences of M. albicaudatus. The mean percentage of each food item consumed on days 1, 2 and 3 of the experiment are recorded. Ranking was established according to Table 5,2; the last column shows the mean rank for each group.

	Day 1	Day 2	Day 3	Rank 1 - 4	Group rank.
GROUP 1. GRASS STEMS AND LEAVES.					
Melica racemosa	2	4	7	1	
Elionurus agenteus	5	7	10	1	
Eragrostis curvula	6	10	12	1	n = 7
Panicum maximum	3	5	15	1	1
Sporobolus spp.	4	11	15	1	
Heteropogon contortus	2	4	8	1	
Setaria spp.	13	17	26	1	
GROUP 2. GRASS SEEDS.					
Cymbopogon spp.	3	10	15	1	
Setaria spp.	32	51	60	3	n = 5
Digitaria spp.	10	15	25	1	2
Elionurus agenteus	1	1	1	1	
Eragrostis curvula	64	83	90	4	
GROUP 3. HERBS.					
Clutia spp.	12	60	76	3	
Helichrysum rosum	0	5	7	1	
Anthrospermum spp.	8	22	31	2	
Crassula lycopodioides	22	34	44	2	n = 8
Stachys aethiopica	3	9	20	1	2
Asparagus africanus	32	51	63	3	
Asparagus africanus	21	33	61	3	
Elytropappus rhinoceratus	9	11	15	1	

Continued overleaf.

	Day 1	Day 2	Day 3	Rank 1 - 4	Group rank.
GROUP 4. SHRUBS.					
Grewia robusta	3	7	18	1	
Portulacaria afra	12	21	36	2	
Diospyros dichrophylla	3	11	23	1	n = 8
Rhigozum obovatum	35	61	72	3	1,8
Lycium campanulatum	69	81	86	4	
Hypoesta verticillata	12	20	23	1	
Rhus spp.	3	3	5	1	
Scutia myrtina	6	10	12	1	
GROUP 5. FRUITS AND SEEDS.					
Maytenus capitata	75	83	87	4	
Asparagus africanus	51	69	74	4	
Grewia robusta	37	46	54	3	n = 6
Acacia karoo	74	85	89	4	3,4
Scutia myrtina	90	90	90	4	
Rhus spp.	74	84	89	4	
GROUP 6. INSECTS.					
Tenebrionid beetles	90	90	90	4	
Tenibrio molitor	51	69	74	4	n = 4
Mimorista pulchellalis	90	90	90	4	4
Periplaneta americana	90	90	90	4	

DISCUSSION.

Amylase activity.

Since the gastric fermentation theory has been rejected as an explanation for the gastric modifications in M. albicaudatus (Chap. 4), the possibility of prolonged gastric salivary amylase activity (Carleton 1973) can now be examined. This theory (Carleton 1973) has appeared more appropriate to M. albicaudatus than Vorontsov's gastric fermentation hypothesis (1962), but the role of the numerous autochthonous corpal bacilli, obviously important in digestion (Chaps. 2 & 3), was problematical, hindering acceptance of the hypothesis. However, in the light of the present findings, a modified form of Carleton's hypothesis (1973) can now be applied to the white-tailed rat.

The sacculated form of the stomach of M. albicaudatus (Chap. 1) allows the development of conditions in the corpus that favour the growth of a specific microflora (Chap. 3). Corpal pH is 4,6 (Table 4,6), possibly too acidic for the growth of fermentative microbes (Hungate 1966) but within the minimum range of amylase activity (Peters & Gaertner 1973). Therefore, amylase-producing bacteria could become established in this region and enter a symbiotic association with the host by aiding in starch digestion. Microbial metabolites in the forestomach of R. norvegicus reduce pH (Peters 1973). This reduced pH lowers gastric amylase activity and thus sterile rats have higher gastric amylase activity than conventional rats (Peters 1973). However, the gastric bacilli in M. albicaudatus are a major source of corpal amylase and it is improbable that their presence would decrease enzyme activity.

An autochthonous microflora (similar to that seen in the adult

rat) colonises the papillae of 16 to 17 day old M. albicaudatus when solid food is first sampled (Fig. 2,5). Papillae increase the area for attachment by the amylase-producing bacilli thereby increasing bacterial numbers in the corpus. High amylase activity in this region undoubtedly results from these considerable bacterial populations. In other words, symbiosis is dependant on large numbers of amylase producing bacteria which in turn are dependant on the increased surface area afforded by the papillae. In the absence of papillae, bacterial numbers and amylase activity would decrease and the host would derive little benefit from the bacterial presence (Maddock & Perrin 1981).

The secure attachment of the bacilli to the papillary epithelium (Chap. 3) imposes serious restraints on applying Vorontsov's gastric fermentation theory (1962) to M. albicaudatus. Because microbial attachment, or preferential alignment to the ingesta, is required during efficient fermentation processes, fermentative micro-organisms are often free-living (Hungate 1968). Thus, a major objection to Vorontsov's theory (1962) is the limited mixing of attached bacilli and food in the corpus of M. albicaudatus. But if the bacteria produce amylase, the enzyme can mix with the ingesta while the bacilli remain attached to the epithelium, in no danger of being washed from the corpus by ingesta flow. Carleton (1973) suggested that increased muscular action occurs in the non-glandular, cornified regions of the rodent stomach resulting in more efficient mixing of food particles. Efficient mixing of corpal amylase and food is likely in the forestomach of M. albicaudatus because of the extensive muscularis externa (Chap. 1). The band of striated muscle extending from the oesophagus to the incusura angularis may aid mixing as peristaltic waves are generated in this region (Carleton 1973; Code & Carlson 1968).

In addition to the high amylase activity in the corpus, a number of conditions in the proximal gut of M. albicaudatus may increase the efficiency of amylolysis. Ingesta passage tests suggest that food is delayed in the stomach (Chap. 4). Slower passage of food in this region means longer periods of amylase digestion resulting in more efficient amylolysis. The acidic gastric conditions (corpus 4,6; antrum 2,7; Table 4,6) may facilitate limited starch digestion in the proximal gut as some starch hydrolysis occurs in the presence of acid (Oser 1965). There is a third factor that may facilitate gastric starch hydrolysis in this rodent. Beta amylase, (found in higher plants; Oser 1965) and alpha amylase which plays an important role in germinating seeds (Fructon & Simmor 1963; Bonner & Varner 1965; West et al. 1966), may be ingested by the rodent. Since M. albicaudatus is granivorous it is likely that this enzyme will occur in the corpus and contribute to starch digestion.

The two amylase forms have complementary functions (West et al. 1966) thus their presence in the corpus would be of great advantage to the rat. The beta form splits the central links of amylopectin releasing end groups while the alpha form splits maltose from these groups (West et al. 1966). Such synergism would cause rapid starch digestion thereby increasing the amount of starch digested per unit time, and thus the energy intake of the rat. The forestomach of R. norvegicus acts as a carbohydrate food store supplementing the liver's energy reserves (Peters & Gaertner 1973; Gaertner & Pfaff 1979). In contrast, the corpus of M. albicaudatus is seen as a region where efficient, rapid digestion of carbohydrate occurs, facilitating the release of much energy which can be stored in the liver after final digestive processes in the small intestine. In this way energy

reserves can be rapidly accumulated, certainly an important feature in an animals whose small size dictates high energy demands (Kleiber 1961). More detailed biochemical investigation of the enzymology in the stomach of M. albicaudatus would be informative.

A major function of the corpal bacilli in M. albicaudatus is therefore, the production of amylase resulting in the formation of a corpal amylolytic reservoir. Contributions of bacterial carbohydrase to aid digestion in the rodent host have been considered (Peters 1973; Carleton 1973; Krishnamurti et al. 1974; Madge 1975) but rarely described; high amylase activity was however, demonstrated in the forestomach of R. norvegicus (Kunstyr 1976). The corpus of M. albicaudatus has a higher amylase activity than that recorded in the laboratory rat (Kunstyr 1976) emphasising the potential of this organ in initiating early carbohydrate (starch and glycogen) digestion.

Pregastric carbohydrate digestion occurs in the corpus of M. albicaudatus but the large glandular antrum, with extensive fundic glands (Chap. 1), is indicative of protein digestion. The diet of M. albicaudatus is rich in carbohydrates, and contains much protein. Presumably the large glandular antrum is required for protein digestion. However, this observation is not in agreement with Perrin & Curtis (1980) who stated that relative intestine lengths, reduction in liver lobes and lack of a gall bladder in the white-tailed rat are indicative of a decrease in protein intake.

In view of the large corpal bacterial population, consideration must be given to the possibility that the white-tailed rat derives energy from digestion of microbial protein. In the ruminant, complex biochemical processes result in the conversion of "waste" urea into

microbial protein (Moir 1968). Daily, more than 50 % of these free-living microbes are digested in the abomasum thus contributing significantly to the protein requirements of the host (Moir 1968). Bacteria are securely attached to the gastric epithelium of M. albicaudatus and only a relatively small number of dead microbes will pass to the antrum and be digested. Recent metabolic studies with R. norvegicus suggest no other protein source but the diet, despite large bacterial populations in the proximal gut (Gaertner & Pfaff 1979). A similar situation is anticipated for M. albicaudatus which has a protein-rich diet and lacks numerous free-living bacteria.

Food preferences.

The value of using the cafeteria test (Chitty 1954) in conjunction with analysis of gut contents (Hansson 1970) for determining the natural food of rodents has been stressed (Drozdz 1966). Generally, correlations between these two methods are good (Drozdz 1966, 1967; Curtis and Perrin 1979; Perrin 1980 a & b) and the cafeteria test may be used with a degree of confidence as an indication of the potential foods of rodents (Drozdz 1966). Gut analysis, on the other hand, is an indication of readily available foods (Drozdz 1966). However, Ferns (1976) found that food prefered by M. agrestis in laboratory tests was rare in the rodents' natural diet demonstrating the difficulties that can arise with the cafeteria method. Ferns' findings (1976) stresses the need for workers to employ more than one method to discover the food habits of rodents.

In the absence of wild M. albicaudatus faecal or gut analyses were impracticable and there was no choice but to use the cafeteria test only. This method can be unsatisfactory as laboratory-reared animals may be reluctant to eat food that they would normally select

in the wild. Despite this disadvantage M. albicaudatus's selection of 'natural' foods during the food test experiment was consistent and agreed with earlier reports (Roberts 1951; Walker 1975; Perrin & Curtis 1980;). The results suggest that M. albicaudatus eats seeds, fruits and insects and are believed to be indicative of the rodents' natural food preferences.

It was unexpected that M. albicaudatus, a savanna grassland species (Davis 1962; De Graaff 1981), rejected grasses in favour of a high energy, concentrate diet (insects, fruits and seeds). These preferences suggest that M. albicaudatus's diet parallels that of the yellow necked field mouse, Apodemus flavicollis (Drozdz 1966, 1967), A. sylvaticus (Hansson 1971) and especially the four striped field mouse, Rhabdomys pumilio (Curtis & Perrin 1979) although further seasonal studies on the diet of the white-tailed rat are required to confirm any similarities. For example, Hansson (1971) and Drozdz (1967) found that the quantity of seeds in the diet of A. sylvaticus and A. flavicollis is related to abundance and that seed deficiencies are compensated for by increased herbage and insect intake resulting in marked seasonal variations in diet. Only seasonal examination of faeces or gut contents from wild M. albicaudatus will determine if the rodent compensates for a scarcity of seeds and fruits by eating more herbage.

Relationships between rodent gut structure and diet are not as clear-cut as implied by earlier authors (Vorontsov 1962; Carleton 1973). The gut morphology of M. albicaudatus: complex sacculated stomach, corpal papillae, gastric symbionts (Chap. 1 & 3; Maddock & Perrin 1981), simple caecum and colon (Perrin & Curtis 1980) suggests that the corpus and not the caecum initiates and predominates in

fermentation (Maddock & Perrin 1981). This possibility implies, sensu Vorontsov (1962), that M. albicaudatus is better adapted for herbivory than suggested by Perrin & Curtis (1980) despite various unspecialised features of the alimentary tract (Appendix 2).

However, the natural foods prefered by M. albicaudatus, as determined by the cafeteria tests, are rich in concentrates. Fermentative processess are not required to digest insects, fruits and seeds (Chap. 4) and normal mammalian digestive processes should be sufficient to digest this concentrate diet. R. pumilio, whose diet and gut morphology (with the exception of the stomach) are similar to that of M. albicaudatus (Perrin 1980 a & b; Curtis & Perrin 1979), has no specialised fermentation chamber as the stomach and caecum are simple. Perrin & Curtis (1980) suggested that perhaps M. albicaudatus should also be able to digest its food without the need for extensive microbial fermentation. The diet of the white-tailed rat does not suggest the need for elaborate foregut fermentative processes. Neither does its unique gastric morphology concur with the idea that gut specialisations indicate a dietary change from albuminous-(insectivorous, granivorous) to cellular-type feeding (Vorontsov 1962). This conclusion is in agreement with the findings of Chapter 4, and in particular the observation that foregut fermentation is impracticable in most small mammals (Parra 1978).

It is apparent from feeding experiments, measurement of various digestive tract parameters and studies of M. albicaudatus's gut morphology (Section I & II; Perrin & Curtis 1980) that this rodent possesses adaptations enabling it to digest the protein and starch (or

glycogen) components of its insectivorous/granivorous diet efficiently. Therefore, in contrast to Vorontsov's theory (1962), M. albicaudatus has retained a 'primitive' (albuminous) diet but has developed advanced gut characteristics which enable it to increase its digestive efficiency.

Futher studies on the extent of starch digestion and its contribution to the overall nutritional requirements of M. albicaudatus are necessary. During such studies, seasonal variations in the diet of wild white-tailed rats should be considered.

SECTION III.

THEORETICAL CONSIDERATIONS.

CHAPTER 6.

THEORETICAL CONSIDERATIONS.

INTRODUCTION.

In this chapter some of the theoretical aspects arising from the study will be discussed. Many findings reported in Sections I & II are at variance with earlier theories that explain gastric function in complex rodent stomachs (Bensley 1905; Vorontsov 1962; Carleton 1973). Consequently a modified hypothesis which recognises the major role of bacterial amylase in the corpus of M. albicaudatus was formed. A new theory explaining rodent stomach evolution is also required as clearly Vorontsov's implication (1962) that increasing gastric complexity is associated solely with trends towards herbivory is no longer tenable. Therefore, the first part of this chapter reviews gastric evolution in myomorph rodents and a new theory derived from Carleton's evolutionary continuum concept (1973) is proposed. The hypothesis attempts to explicate the present diversity of rodent stomach types, which is not achieved by the earlier theories. The concept is further elucidated by consideration of the possible evolution of M. albicaudatus's papillated bilocular hemiglandular stomach.

Since the 1950's many advances have been made in the field of non-ruminant pregastric fermentation (Vorontsov 1962; Moir 1968; Bauchop 1977; Parra 1978; Kinnear & Main 1979). These findings not only suggest that pregastric fermentation in small mammals is uneconomical (Parra 1978; cf. Vorontsov 1962) but also question the widely held theory explaining the dominance of ruminants and ruminant-like mammals over other non-ruminant herbivores (Janis 1976; Kinnear & Main 1979; Kinnear, Cockson, Christensen & Main 1979). Browsing

perissodactyls were the dominant mammalian herbivores in the Paleocene and Eocene forests about 50 million years ago (Romer 1958). However, during the mid-Tertiary (Oligocene/Miocene epochs, about 25 million years ago), the climate became cooler and more arid which did not favour continued forest growth (Moir 1968; Kinnear & Main 1979). The forests were largely replaced by savannah vegetation (Moir 1968; Kinnear & Main 1979) and a different fauna; artiodactyls radiated and increased in number while perissodactyls underwent a decline (Romer 1958; Moir 1965, 1968). The artiodactyl increase (particularly of ruminants) is interpreted as a result of PGF mammals being more efficient than non-ruminants at digesting the cellulose-rich savannah diet (Moir 1965, 1968; cf. Janis 1976). Similarly, the ruminant-like marsupials increased in number during these epochs (Kinnear & Main 1979) and some myomorph rodents developed complex stomachs (possibly pregastric fermentation) to adapt to this herbivorous niche (Vorontsov 1962). These findings strongly suggest that pregastric fermentation was important in the change of faunal dominance during the mid Tertiary period.

But recent findings do not support the belief that pregastric fermentation of cellulose was the prime factor resulting in the radiation of PGF mammals during these epochs. Many ruminants cannot digest high fibre diets (Hofmann 1973) and those that do select roughage are often less efficient at fibre digestion than non-PGF mammals (Bell 1971; Janis 1976; Kinnear & Main 1979). A similar situation may exist in the macropod marsupials (Hume 1974; Kinnear *et al*. 1979). In the case of myomorph rodents, increased gastric complexity (thought to indicate evolution towards pregastric fermentation, Vorontsov 1962), has occurred in species that select a concentrate diet (Carleton 1973). Finally, energy losses associated

with passing food through an additional (microbial) trophic level often makes hindgut fermentation more economical than foregut fermentation in small mammals (Parra 1978; Kinnear & Main 1979; Chap. 4). Although mammals with pregastric fermentation did assume faunal dominance, there is doubt that this dominance resulted from an ability to digest fibrous food better than non-PGF mammals. What then caused the increase in PGF mammals in the late Tertiary period?

Kinnear & Main (1979) consider PGF mammals to have expanded nutritional niches which gives them an advantage over other herbivores. According to this theory (the expanded nutritional niche hypothesis), PGF mammals eat an unbalanced diet, (with a lack or surplus of essential nutrients) thereby excluding non-PGF herbivores from certain dietary niches. PGF mammals maintain a balanced metabolism and nutrition as essential nutrients lacking in the diet are supplied by the foregut micro-flora. Since other herbivores lack a pregastric flora they must obtain all their nutrients from their food and will be unable to compete with PGF mammals for these unbalanced niches. The expanded niche theory (Kinnear & Main 1979) will be evaluated with reference to animals possessing foregut and hindgut fermentations and a modification of this theory proposed and related to M. albicaudatus. The possibility of this rat possessing an expanded nutritional niche and its ability to efficiently digest starch, are seen as adaptations to offset interfamilial competition.

Gastric evolution in the Myomorpha.

The 'simple' unilocular hemiglandular and 'advanced' bilocular discoglandular rodents stomachs are said to represent end members of an evolutionary continuum (Carleton 1973). Early forest-dwelling,

Tertiary myomorphs ate invertebrates, seeds and fruits (Hershkovitz 1962, op. cit. Baker 1971) or were omnivorous (Landry 1970) and since these rodents selected an easily digestible diet they probably possessed a simple stomach. Carleton's evolutionary continuum concept (1973) was proposed to explain gastric evolution in South American cricetids, but if his theory is modified to include all myomorphs (for example those with a unilocular glandular stomach, Perrin & Curtis 1980) the continuum can be expanded and will range from this simple condition to the bilocular discoglandular type (Chap. 1). An increase in keratinised relative to glandular epithelium is characteristic of the complex rodent stomach (Carleton 1973) but corpal papillae, symbiotic bacteria and other features present in some myomorphs (oesophageal dilations, Vorontsov 1962; Dearden 1966; and corpal diverticula, Perrin pers. comm.) cannot be fitted into this modified continuum. At present there is no theory satisfactorily explaining atypical as well as common gastric modifications in the sub-order. A new concept of stomach evolution in the Myomorpha, which accounts for all features, is therefore proposed.

To include all observed modifications into a concept of gastric evolution, the idea of a branching radiation from an ancestral unilocular glandular (insectivore-like, Myrcha 1966) stomach is suggested. Such a glandular stomach (Perrin & Curtis 1980), with secondary development of non-glandular epithelium and sacculation, has been suggested as ancestral although most authors regard the unilocular hemiglandular condition ancestral (Bensley 1905; Vorontsov 1962; Carleton 1973). However, the latter idea cannot account for the glandular stomach of the mole rat Cryptomys hottentotus or the dormouse Graphiurus murinus (Perrin & Curtis 1980) and it is logical to consider the unilocular glandular condition ancestral.

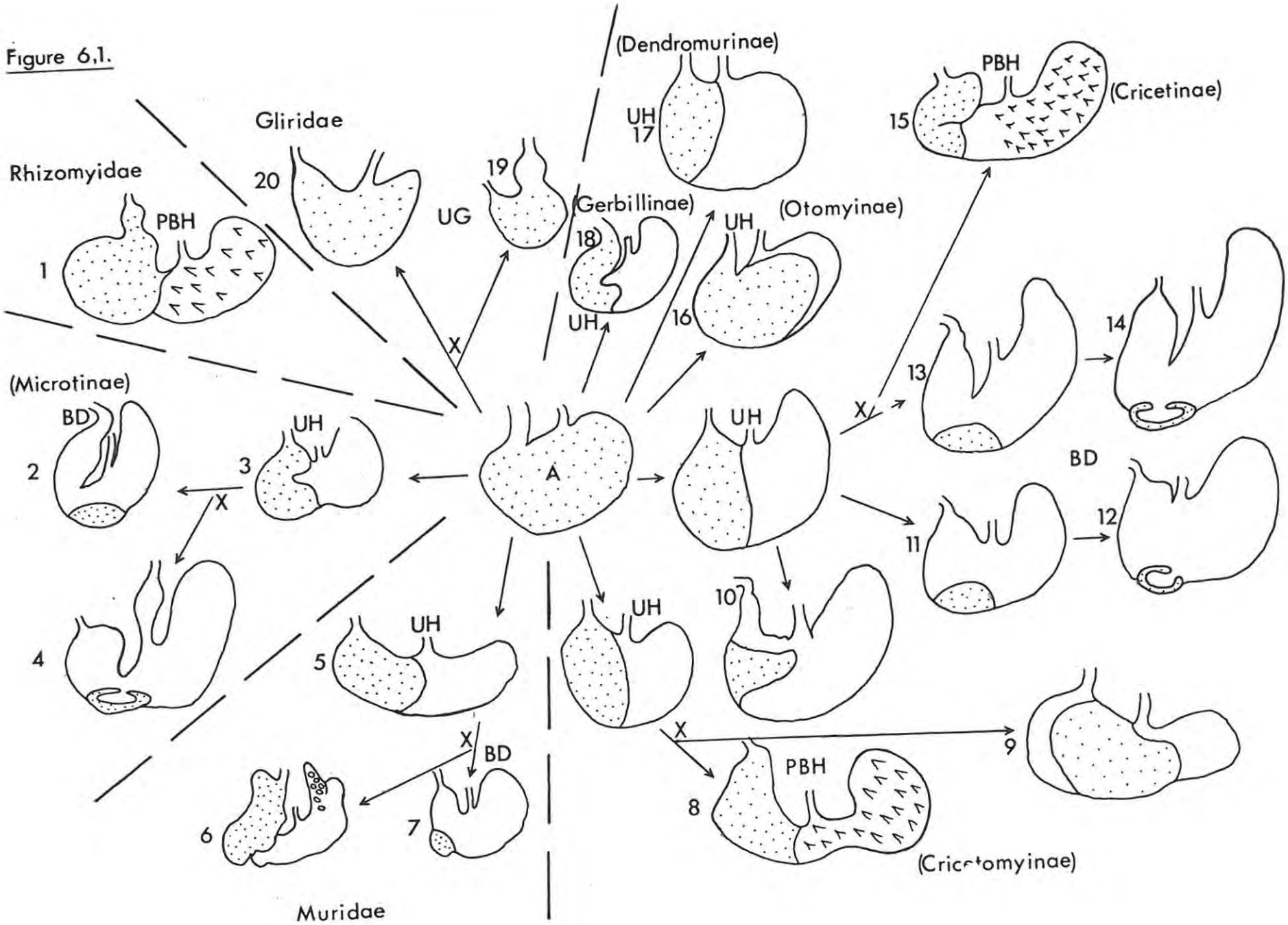
During their evolution rodents have successfully colonised

numerous different habitats and have been confronted with a variety of dietary regimens. Many species may have adapted to the new conditions by gastric modification, thus explaining the wide range of gastric morphologies of extant rodents. Intertaxon similarities probably result from parallel evolution (Bensley 1905; Carleton 1973; Fig. 6,1) although such parallel evolution will not necessarily indicate operation of the same selective forces (Carleton 1973). For example, the development of a bilocular discoglandular from a unilocular glandular stomach is a common phenomenon evident in the new world neotomine-peromyscines, South American cricetids (Carleton 1973) and certain murids and microtines (Vorontsov 1962; Fig. 6,1). It is most likely that different selective forces acted during the gastric evolution in these four families.

Simple glandular stomachs that have changed little from the ancestral type (gerbils and some murids, Vorontsov 1962) can also be incorporated into this radiation and may represent animals that have overcome dietary changes by other adaptations (Vorontsov 1962); for example, caecal fermentation or a.. increase in gut length (Eisenberg 1978). The Gerbillinae probably represent a single evolutionary line with little adaptive radiation of gastric structure (Fig. 6,1) although morphological modifications may have occurred in the hindgut.

Rodents with 'aberrant' stomach morphology (oesophageal dilations, corpal papillae and corpal sacculations) which do not fit into Carleton's original hypothesis (1973) are seen as branching from an established evolutionary line, by employing novel digestive strategies in response to new pressures, as observed in M. albicaudatus (Fig. 6,1).

Figure 6.1.


(Dendromurinae)
UH
17
PBH
(Cricetinae)
15
Gliridae
Rhizomyidae
20
19
UG
(Gerbillinae)
18
(Otomyinae)
UH
16
PBH
1
X
UH
(Microtinae)
BD
2
UH
3
A
UH
X
13
14
BD
X
11
12
4
UH
5
UH
10
X
BD
9
6
7
PBH
8
(Cricetomyinae)
Muridae

It is most unlikely that there is a single causal factor in the evolution of stomach complexity (Perrin & Curtis 1980). The diversity of trophic specialisation in the order suggests a variety of selective pressures. Functional compensation (whereby certain digestive organs intensify their functions to compensate for slower rates of adaptation in others, Vorontsov 1961), and behavioural rather than morphological change, complicates interpretation of selective pressures and resultant morphological adaptations. This prevents formulation of an accurate evolutionary account of gastric development in the Myomorpha. However, the radiation theory is useful because it recognises the diversity of gastric form in the sub-order and the important effects of different selective pressures on morphology. Species with 'aberrant' gastric morphology may readily be included in this theory (Fig. 6,1) but a more factual and accurate hypothesis is the work of palaeontologists.

Gastric evolution in M. albicaudatus.

Stomach modifications of M. albicaudatus are certainly unusual although analogous structures have evolved independently in two or three other species; C. gambianus, T. splendens (Fig. 6,1) and the palaearctic species M. myospalax. By considering the evolution of M. albicaudatus's stomach from a unilocular glandular to papillated bilocular hemiglandular condition, three main morphological changes can be envisaged (Table 6,1).

Table 6,1. Proposed major morphological developments in the stomach of M. albicaudatus.

1. An increase in keratinised relative to glandular epithelium - i.e. from the unilocular glandular to unilocular hemiglandular condition.
2. Gastric sacculation separating the acidic glandular (antrum) from the non-glandular region (corpus) - i.e. from the unilocular hemiglandular to bilocular hemiglandular condition.
3. Papillation of the corpus affording an increase in the area for attachment of symbiotic bacteria - i.e. from the bilocular hemiglandular to papillated bilocular hemiglandular condition.

The development of a bilocular discoglandular stomach has been a general evolutionary trend in many cricetines (Vorontsov 1962; Carleton 1973; Fig. 6,1). Early evolutionary gastric changes in the white-tailed rat (Table 6,1) may have occurred along similar lines although papillation probably occurred as a branch from the bilocular hemiglandular condition (Fig. 6,1). Vorontsov (1962) placed M. albicaudatus's stomach between the unilocular hemiglandular stomach of Calomyscus bailwardi and the partially sacculated stomach of Cricetulus eversmanni, thus implying that the white-tailed rat branched from the main gastric evolutionary line prior to the development of stomach sacculation. The selective pressures causing gastric modifications in the white-tailed rat will probably never be understood, however, the following ideas are offered as a plausible explanation for the development of the papillated bilocular hemiglandular stomach.

It would be energetically economical for a granivore, to store

seeds internally (for example in cheek pouches) rather than make numerous trips from the food source to its nest or to forage continually and be subject to predation. A storage organ would allow the rat to exploit favourable food resources available intermittantly (Golley 1960). In the absence of cheek pouches, sacculation of the stomach of M. albicaudatus may initially have occurred to facilitate food storage (Perrin & Curtis 1980). Seed food storage is a recognised gastric (Peters & Gaertner 1973; Gaertner & Pfaff 1979) and oesophageal function (Golley 1960). Evolution of gastric diverticula are not uncommon and have occurred in a number of unrelated mammalian groups (Moir 1968; Janis 1976), particularly in the gastro-oesophageal region (Moir 1968). M. albicaudatus selects a concentrate diet (seeds and insects) probably similar to that of its early ancestors with completely glandular stomachs. The abrasive effects of a roughage diet may often strongly influence the change from gastric glandular to keratinised epithelium (Bensley 1905; Moir 1968; cf. Carleton 1973). The loss of pyloric (Carleton 1973) or cardiac (Bensley 1905) glands have been associated with generation of the bilocular discoglandular condition in many species and such a stomach possesses mainly fundic glands (Vorontsov 1962; Carleton 1973). However, the full complement of glands (cardiac, fundic and pyloric) in the antrum of M. albicaudatus suggests that major glandular reduction did not occur in this species. It is practicable to view gastric sacculation and increase in keratinised epithelium as synchronous events in the white-tailed rat resulting from the development of a non-glandular (rather than a glandular) diverticulum in the gastro-oesophageal region.

This account of gastric evolution in M. albicaudatus has an advantage in that it does not require an explanation of glandular reduction in the stomach, a feature that is not easily explained in this rodent. (Although this account discredits the idea of absolute glandular reduction, it is obvious that relative glandular reduction has occurred as a result of formation of the keratinised corpus).

A result of sacculation would be separation of the non-glandular and glandular regions, producing a less acidic corpus more suited for microbial growth than the acidic ancestral stomach. Colonisation of the white-tailed rats' forestomach by amylolytic bacteria that could optimally utilise the available nutrients (starch and glycogen of seeds and insects) would not be unexpected. If such bacteria conferred a digestive advantage to the host, increased sacculation (further separating the corpus from the acidic antrum) and development of papillae (allowing an increase in microbial attachment and number) would result in more efficient digestion. Individuals with papillae (and consequently more symbionts) would have a dietary advantage over those lacking papillae. In the presence of competition such individuals (with papillae) would have increased fitness, and a better chance of survival than individuals with less efficient digestive processess. Thus, the latter increase in complexity of M. albicaudatus's stomach and its increased adaptiveness, can be seen to be closely associated with the co-evolution of symbiotic gastric bacteria (see Bauchop 1978).

Expanded nutritional niche.

Not only do the corpal bacteria increase the digestive efficiency of M. albicaudatus but they may also expand its nutritional niche (see Kinnear & Main 1979). Before considering the advantages of an expanded nutritional niche to this rat some irregularities in the theory must be discussed. The processes of hindgut and foregut fermentation are similar (Janis 1976; Parra 1978) and microbially derived nutrients (probably amino acids and/or vitamins) that supplement the diet in PGF mammals can surely be produced by a hindgut as well as a foregut flora. Thus, animals possessing a hindgut flora should also be able to occupy an expanded nutritional niche provided essential organic compounds (amino acids and vitamins) can be absorbed post-caecally (or in the foregut in coprophagous animals). Absorption of VFA's occurs readily in the mammalian hindgut (Barcroft et al. 1944; McBee 1970) and it is possible that amino acid and vitamin absorption will too. PGF mammals did assume faunal dominance during the mid-Tertiary epoch of vegetation change and it is probable that their dominance is related to a dietary advantage over other herbivores. But Kinnear & Main's expanded niche theory (1979) can only explain the rise of PGF mammals if foregut fermentation is shown to be advantageous over hindgut fermentation. A number of authors have shown that hindgut fermenters are often equally or better adapted for fibre digestion (Janis 1976; Kinnear & Main 1979) but within the confines of the expanded nutritional niche theory (Kinnear & Main 1979) there are two situations where pregastric fermentation may be superior (Table 6,2). Only in either of these two situations can the expanded nutritional niche hypothesis explain the radiation of PGF mammals and their dominance over non-PGF species.

Table 6,2. Conditions under which the expanded nutritional niche theory (Kinnear & Main 1979) will give mammals with a gastric flora an advantage over those with a hindgut flora.

1.	If the essential microbial nutrients only become available to the host once the bacteria are digested in the glandular stomach. Excluding coprophagous species, hindgut fermenters appear not to digest microbes in the post-caecal gut and these nutrients will not be available to them (but see Parra 1978). This situation is particularly important if the nutrients include vitamins and proteins as they are contained mainly in microbial cells (Bauchop 1978).
2.	Certain plants may be unavailable to herbivores because they contain toxic secondary compounds. If the pregastric bacteria detoxify these compounds (Freeland & Janzen 1974) the plants and hence their nutrients will become available. Detoxification of secondary compounds in the caecum is impracticable as the toxin will have already passed the major sites of absorption.

The expanded niche hypothesis is reasonable and within certain limits (Table 6,2) offers an acceptable explanation for the radiation of ruminant-like animals. The hypothesis need not be limited to species with a pregastric fermentation but can include any mammal possessing a large symbiotic pregastric flora. M. albicaudatus is not a PGF mammal but its abundant gastric flora may directly or indirectly supply essential organic nutrients lacking in the diet. Thus, it is possible that M. albicaudatus has expanded its nutritional niche. If Kinnear & Main's hypothesis (1979) is applicable to M. albicaudatus,

the rat must derive nutritional benefit from the bacteria according to the limitations discussed above. It is unlikely that much microbial protein digestion occurs in this roden (Chap. 5) and probably neither amino acids nor vitamins will be made available via the digestion of microbes. It is more likely that the corpal bacilli detoxify plant toxins present in the food. If it can be shown that these bacilli act as detoxicants, M. albicaudatus can be considered to have an expanded nutritional niche as the rat will be able to eat food unavailable to individuals with a hindgut microflora.

Throughout this discussion emphasis has been placed on dietary changes or competition for food as primary factors influencing gastric or digestive adaptations. Although these points are important it must be realised that any selective pressure that could be overcome by better food utilisation due to development of a more efficient digestive system could account for gastric evolution in myomorph rodents. Dietary changes alone (for example an increase in herbivory) may not have been the only pressure resulting in gastric modification in M. albicaudatus; adaptation to other environmental conditions may also play a role. This species is a cricetid (De Graaff 1981), a family that generally has smaller litter sizes, gives birth less frequently (Dean 1978) and undergoes smaller population density changes (Perrin & Curtis 1980) in comparison to murids. Cricetids therefore adapt more slowly to environmental changes (Perrin & Curtis 1980). Generally the Cricetidae occupy specialised niches (Misonne 1969) and may live in dry steppes (Gerbillinae) or have adapted to extreme herbivory (Otomyinae) while other cricetids are very large (C. gambianus) or small (M. minatoides) (Perrin & Curtis 1980). Such adaptive specialisation will aid cricetids in competition, particularly with murids which are more generalist feeders and consequently are in a position to more easily adapt to changing

environmental conditions, a factor that has accounted for their radiation in Africa (Perrin & Curtis 1980). It is possible that the digestive modifications described in M. albicaudatus are adaptations whereby this species offsets competition. Niche overlap has been reduced due to gastric modifications and a high digestive efficiency. Competition from granivorous species may be reduced by M. albicaudatus selecting an 'unbalanced' diet which is supplemented by certain nutrients derived from toxic plants. These secondary compounds are detoxified by the rodents' pregastric bacteria. In the absence of similar adaptations and where food is a limiting resource, other small herbivores cannot successfully compete for food with the white-tailed rat.

In conclusion, I will emphasise the important aspects of gastric evolution in M. albicaudatus. It appears that the stomach of M. albicaudatus, after initially following evolution with the main Cricetinae trend (towards a bilocular discoglandular condition), diverged to develop a papillated bilocular hemiglandular stomach. Corpal colonisation by amylolytic bacteria was a comparatively recent development, probably beginning prior to papillation. The advantages of more efficient starch and glycogen digestion have been discussed (Chap. 5) but it is possible that the corpal bacilli perform more than one symbiotic function. Reconsideration of the expanded niche theory (Kinnear & Main 1979) suggests that under certain circumstances (Table 6,2) mammals with an extensive symbiotic pregastric flora, may have a major advantage over mammals with a hindgut flora only. It is suggested that the rodents T. splendens, C. gambianus and M. myospalax and possibly T. paedulcus (Perrin pers. comm.), all of which have extensive pregastric floras, be tentatively included in Kinnear & Main's theory (1979).

If M. albicaudatus benefits from bacterial detoxification of plant secondary compounds it will have an expanded nutritional niche. Such detoxification processess may make essential organic nutrients available to the rat and result in niche expansion. The possibility of an expanded nutritional niche and the efficient utilisation of certain complex carbohydrates by the white-tailed rat as a result of its gastric morphology, are indicative of specialisation.

Specialisations may limit M. albicaudatus's ability to adapt to current changes in habitat due to agricultural encroachment and pest control techniques (Dean 1978) and these man-made catastrophic changes may be responsible for the decline in numbers of the white-tailed rat. However, under 'natural' circumstances, the feeding specialisations could provide an increased fitness for the individual and species thereby aiding in interspecific competition. But it must be remembered that trophic specialisations are only one part of a broader spectrum of adaptive features that ensure survival. When discussing interspecific competition all factors must be considered. However, feeding adaptations in M. albicaudatus can be seen to partially offset the imbalance in Darwinian fitness between this species and the murids in southern Africa.

SUMMARY AND CONCLUSIONS.

Since each chapter contains its own summary of conclusions this resume is purposefully brief. It is divided into three parts; point one reviews the most important findings of the study while points two and three are aimed at aiding further research.

1. The gastric morphology of the white-tailed rat M. albicaudatus was described in detail. The bilocular hemiglandular stomach consists of a papillated corpus, non-glandular PGP and glandular antrum. The antrum contains cardiac, fundic and pyloric glands (suggesting limited glandular reduction during gastric evolution) while the FCE and PGP are lined with orthokeratin. The corpal papillae, which increase surface area for microbial attachment, have undergone a different type of keratinisation called physiological hyperkeratosis.

Streptococci, Lactobacilli and unidentified anaerobic bacilli (which colonise papillary microhabitats) are autochthonous in the stomach of M. albicaudatus but P. vulgaris and Ps. flourescens are probably autochthonous.

Early gastric development is innate but the rapid development of PB into papillae between 15 and 17 days suggests the presence of allogenic growth stimuli: possibly mechanical abrasion by solid food, low chalone concentration in the papillary basal cells and the influence of the APB. Stimulation by VFA presence, however, is unlikely due to the low concentration of these acids in the stomach.

The two current theories explaining gastric modifications in myomorph rodents (Vorontsov 1962; Carleton 1973) were considered in

relation to the adaptations of M. albicaudatus by examining feeding habits, gut biochemistry and digestive strategies of the rodent. A modification of Carleton's prolonged amylase theory (1973) (whereby large numbers of papillary bacilli aid the host in amylose and/or glycogen digestion) best describes the function of the white-tailed rats' corpus. Much protein digestion occurs in the antrum.

In conclusion, a hypothetical radiation theory which emphasises the variety of selective pressures acting during gastric evolution was proposed to explain the diversity of myomorph rodent gastric structure. According to this hypothesis three major events occurred during the gastric evolution of M. albicaudatus from a unilocular glandular stomach; beginning with the development of a gastric food store and sacculation and proceeding to colonisation of the corpus by amylolytic bacteria and corpal papillation, the papillated bilocular hemiglandular stomach evolved. The physiological consequenses of these features may have caused expansion of the nutritional niche of M. albicaudatus as a result of bacterial detoxification of plant secondary compounds. Such adaptations allow this rodent to survive in competition with other small herbivores.

2. The abundance of symbiotic micro-organisms in the corpus of M. albicaudatus distinguishes this species from many other rodents although some microbes are present in the digestive system of all mammals (Eisenberg 1978). Symbionts may however, have different roles including cellulose digestion; utilisation of non-protein nitrogen which can ultimately be digested by the host as microbial protein

(Bauchop 1978); synthesis of B vitamins (Maynard & Loosli 1969), riboflavins and carbohydrases (Krishnamurti et al. 1974; this study) and detoxification of plant secondary compounds (Freeland & Janzen 1974).

In view of the range of symbiotic interactions between mammalian herbivores and microbes it is suggested that non-ruminants with extensive gastric floras (particularly small mammals where foregut fermentation may be uneconomical; Parra 1978), be seen not solely in terms of parallels with ruminants. The possibility of these animals employing a novel herbivorous digestive strategy must also be considered. This is particularly relevant since gut bacteria, because of their large numbers and rapid generation times, have a tremendous capacity to adapt to changing environments (i.e. substrates; Parra 1978). Therefore, depending on gastric conditions and diet, a variety of unique symbiotic interactions between microbe and host may evolve. The study of M. albicaudatus clearly demonstrates this point. Initially the white-tailed rat was considered ruminant-like, its gastric modifications facilitating fibre digestion but further investigation revealed that these modifications enable the rodent to efficiently digest a concentrate diet.

3. Finally I will mention the parts of this study that require further investigation in the hope that work in these specialised fields may arouse the interest of microbiologists and biochemists in particular. Many of these ideas will be applicable, not only to M. albicaudatus, but to any study of rodent gastric function; an important field of research that has not been given sufficient attention in the past.

A major shortcoming of Sections II and III was due to the absence of wild-trapped M. albicaudatus. Because laboratory-reared animals were used, 100 % confidence cannot be placed in the results of the

food preference tests. Therefore, seasonal gut analyses of wild-trapped M. albicaudatus will be of primary importance in any similar study. Such data will indicate the food most favoured in the wild and these items could be analysed for secondary compounds and essential nutrients. The applicability of the nutritional niche theory (Kinnear & Main 1979) could then be examined more closely.

It is important to quantify the extent of protein and starch digestion in the stomach of M. albicaudatus, to relate these results to the abundance of starch and protein in the diet, and to calculate their total contribution to the energy requirements of the rodent.

An important aspect not covered adequately in this study was a detailed examination of the microbiology of the corpus. Isolation and identification of the gastric bacteria, especially the APB, would greatly assist in the understanding of their function in the stomach, particularly if the natural diet is known. The possibility of microbial waste products being utilised by other microbes could then be examined and the means of energy production by the APB determined.

It was shown that the papillae of M. albicaudatus have a rapid growth rate and it was proposed that the APB, low epithelial chalone concentration and mechanical abrasion by food influenced this growth. A more detailed investigation of these 'stimuli' is required.

An examination of urea recycling in this rodent is necessary as nitrogen recycling has been demonstrated in non-ruminant herbivores (see Parra 1978).

Of these five areas of research the first three are most important and only once they have been adequately investigated will an understanding of gastric function in M. albicaudatus be achieved.

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APPENDIX 1.

List of mammals exhibiting pregastric fermentation (modified after Bauchop 1977). ** op. cit. Bauchop 1977; * true ruminants.

Order.	Family.		Reference.
Marsupiala	Macropodidae	Setonix brachyurus	Moir et al. 1956.
		Macropus gigantus	Forbes & Tribe 1970.
		Thylogale thetis	"
		Protemnodon eugenii	Lintern-Moore 1973.
		Megaleia rufa	Hume 1974.
		Macropus robustus	"
		Macropus eugenii	Kinnear & Main 1975.
		Bettongia penicillata	Kinnear et al. 1979.
Primates	Cercopithecidae	Presbytis cristalus	Bauchop & Martucci
		P. entellus	1968.
		Colobus polykomos	Ohwaki et al. 1974.
Edentata	Bradypodidae	Choloepus spp.	**Denis et al. 1967.
		Bradypus spp.	**Toole 1971.
			Marvin & Shook 1963.
Artiodactyla			
	Hippopotamidae	Hippopotamus amphibius	Thurston et al. 1968.
	Camelidae		Hungate et al. 1959.
			Williams 1963.
			Moir 1968.
	Tayassuidae		Langer 1974, 1978.
	Tragulidae		Bauchop 1977.
	Cervidae*		Walker 1975.
	Giraffidae*		"
	Antilocapridae*		"
	Bovidae*		"

APPENDIX 2.

Measurements and features of the alimentary tract of M. albicaudatus that are indicative of herbivory (Perrin & Curtis 1980).
S = specialised, indicating herbivorous adaptations; I = intermediate; P = primitive, indicating ancestral "omnivorous" diet.

Dentition (Molars).	Cusped	I
Ratio of glandular to cornified gastric epithelium.	1,8	S
Stomach wt. (% body wt.).	1,7 - 2,9	I
Caecum wt. (% body wt.).	1,0 ± 0,1	P
Caecal haustra.	0	P
Colonic spiral loops.	0	P
Spiral folds in large intestine.	50	S
No. of liver lobes.	5	S
Gall bladder.	Absent	S
Total length of gut (excl. oes.)	884 ± 146 mm	-
Ratio of hindgut to head/body.	4,9 ± 0,6	P
Ratio of small intestine to colon and caecum.	1,1 ± 0,0	P
Relative length of intestine as % of hindgut length.		
(i) small intestine.	53,1 ± 0,1	I
(ii) caecum.	6,6 ± 0,0	P
(iii) large intestine.	40,3 ± 0,1	I
Overall ranking.		I

APPENDIX 3

Use of the stereoscope.

The stereomicrographs (Plates 3,8 a & b) are set for stereoscopic viewing with a Topcon mirror stereoscope with the eyepieces approximately 20 cm above the photographs. If a different stereoscope is used, place the instrument above the left photograph so that the photograph appears in the centre of the field of view. Move the right photograph (towards or away from the left one) until the X on the right photograph merges into the X of the left photograph. This will produce a stereoscope view.

Note: for smaller stereoscopes the photographs must be moved closer together. For a pocket stereoscope the photographs will overlap.

GASTRIC PAPILLAE OF MYSTROMYS ALBICAUDATUS (SMITH 1834), RODENTIA

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The stomach of the cricetid rodent, Mystromys albicaudatus, is sacculated; a constriction separates the distal glandular region from the proximal keratinised corpus which contains numerous papillae (Fig. 1). These papillae are interesting, not only because they occur in a limited number of rodents (Thallomys paedulcus [1] and Cricetomys gambianus [2]), but also because their function is unexplained.

The papillae are non-vascular and consist entirely of a pseudo-keratinised epithelium (Fig. 2) similiar to parakeratotic epithelia despite the presence of a granular layer (Fig. 3). The usual epithelial layers (strata basale, spinosum, granulosum and corneum) are present (Fig. 3) with the major part of the papillae consisting of a thickened, highly stratified stratum corneum (Fig. 4) suggesting that their primary role is not absorption.

Examination of the stomach of Mystromys albicaudatus reveals that large numbers of bacteria attach to the papillae (Fig. 5) and colonise 'pockets' or microhabitats formed between the desquamating cells of the outer stratum corneum (Fig. 6). In contrast, considerably smaller numbers of bacteria attach to the inter-papillary epithelium and these are believed to be bacteria common to most rodent stomachs (lactobacilli and anaerobic streptococci).

Microscopic examination suggests that the greatly increased surface area afforded by the papillae allows for the attachment of large numbers of autochthonous bacteria. The roles of the bacteria are unknown and studies are underway to identify them, determine their metabolic activities, and thus reveal their possible role in digestion.

Acknowledgements

The advice given by Dr M.R. Perrin and the assistance rendered by Mr A.G. Bruton are much appreciated.

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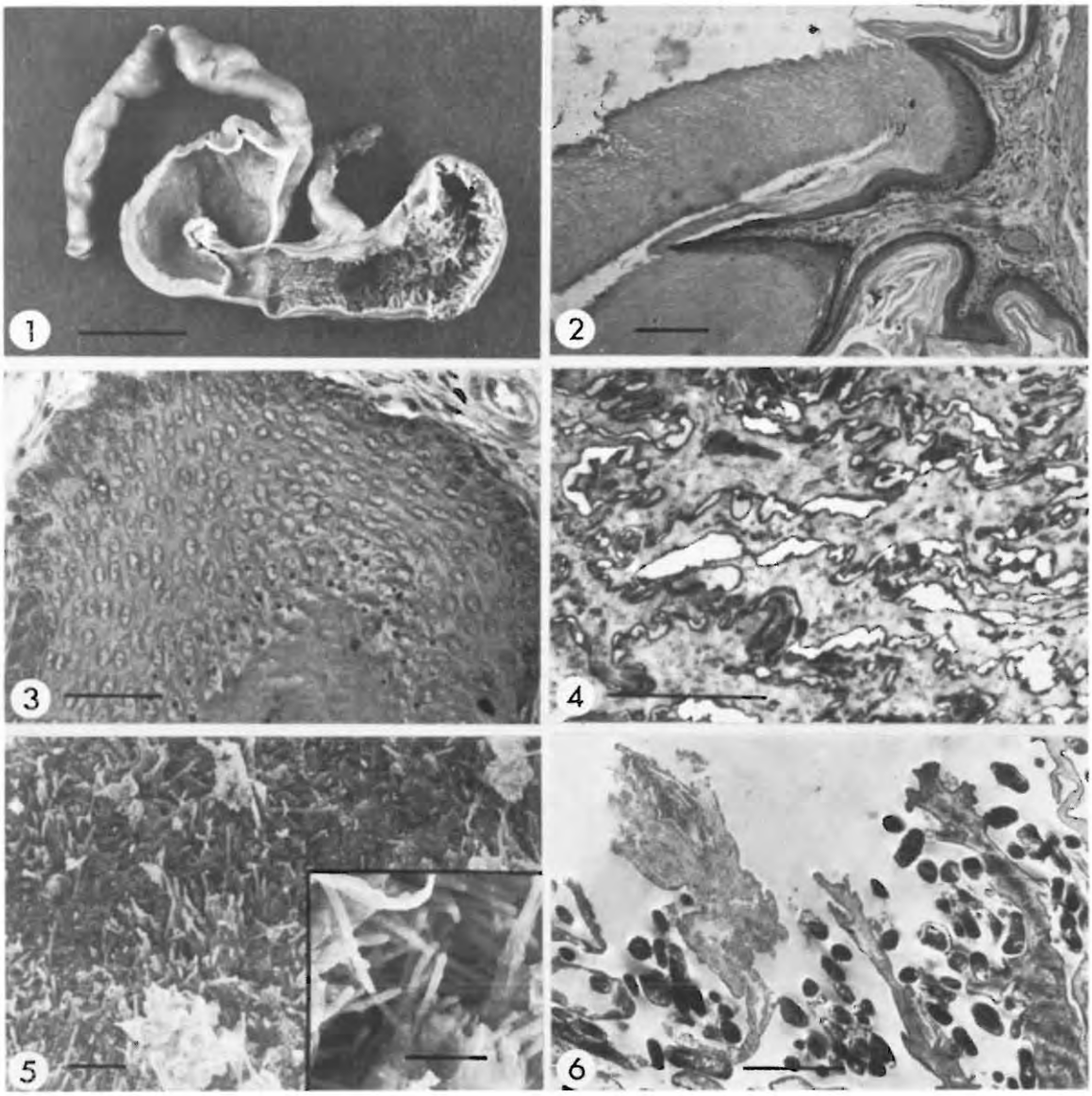

Fig. 1: Bisected stomach of M. albicaudatus bar = 10 mm
Fig. 2: L.s. of papillae and lamina propria (LM) bar = 0,1 mm
Fig. 3: L.s. of base of papilla (LM) bar = 20 µm
Fig. 4: Core of papilla (stratum corneum)(TEM) bar = 5 µm
Fig. 5: Bacteria on papilla surface (SEM) bar = 10 µm (inset = 5 µm)
Fig. 6: Peripheral region of papilla with bacteria (TEM) bar = 2 µm

A microscopical examination of the gastric morphology of the white-tailed rat *Mystromys albicaudatus* (Smith 1834)

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A study of the gastric morphology of *Mystromys albicaudatus* revealed a sacculated stomach with a papillated, keratinized corpus separated from a distal glandular antrum by a pregastric pouch. Gastric morphology of this type is defined as bilocular hemiglandular. Although the forestomach of *M. albicaudatus* bears a superficial resemblance to that of a ruminant, the corpus (and papillae in particular) differ structurally and functionally from the rumen. Whereas rumen papillae are important in absorption, those of *M. albicaudatus* increase surface area for the attachment of symbiotic bacteria.

S. Afr. J. Zool. 1981, 16: 237 – 247

'n Studie van die gastriese morfologie van *Mystromys albicaudatus* het 'n sakvormige maagstruktuur getoon; 'n pregastriese sak het 'n met papillae uitgevoerde gekeratiniseerde corpus geskei van 'n distale kliérryke antrum. Gastriese morfologie van hierdie tipe word gedefinieer as tweesakkig-hemigeklierd (bilocular hemiglandular). Alhoewel die voormaag van *M. albicaudatus* 'n oppervlakkige ooreenkoms toon met dié van 'n herkouer, is die corpus (en die papillae spesifiek) struktureel en funksioneel verskillend van die rumen. Papillae van die rumen is belangrik vir absorpsie terwyl dié van *M. albicaudatus* groter vashegtingsarea bied vir die simbiotiese bakteriese bevolking.

S.-Afr. Tydskr. Dierk. 1981, 16: 237 – 247

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Comparative gross morphological studies of Muroid digestive systems have indicated great structural diversity (Vorontsov 1962; Carleton 1973; Perrin & Curtis 1980). A 'primitive', monogastric stomach and an 'advanced', digastric stomach (with reduction of glandular compared to keratinized epithelium) have been recognized as end points of an evolutionary continuum. Gastric morphology of most rodents examined conforms to this series but a few species show atypical adaptations. For example, *Mystromys albicaudatus* shows the digastric condition but possesses numerous papillae in the proximal, keratinized region of the stomach (Perrin & Curtis 1980); similarly, *Cricetomys gambianus*, has gastric papillae (Caiman, Quenum, Kerrest & Goueffon 1960). The gastric histology and ultrastructure of these species have not been examined and the functions of the papillae are unknown. A microscopical examination of the gastric morphology of *M. albicaudatus* was therefore initiated to determine its histology and ultrastructure, prior to studies of assimilation efficiency and microbial function.

The alimentary tract of *M. albicaudatus* is indicative of a herbivorous/omnivorous diet (Perrin & Curtis 1980). A shift in the diet of certain rodents, from omnivory (Landry 1970) or granivory/insectivory (Vorontsov 1962) to herbivory has been associated with climatic and vegetation changes during the Miocene (Vorontsov 1962; Moir 1968). During this dry period forests were replaced by savannah and steppe vegetation (Moir 1968) causing taxa to adopt herbivorous specializations and thereby reducing interspecific competition. This change was facilitated by various anatomical, physiological and behavioural adaptations (Vorontsov 1962) including sacculation of the stomach, an increase in keratinized epithelium and a decrease in glandular epithelium (Vorontsov 1962; Carleton 1973). Similar modifications in *M. albicaudatus* have been recorded (Perrin & Curtis 1980). In the light of the present detailed study it seems plausible to suggest that such modifications reflect an ability to digest an increasingly herbivorous diet while retaining the ability for proteolytic activity.

Materials and Methods

Adult *M. albicaudatus* were killed by chloroform anaesthesia and placed on ice to retard autolysis. Stomachs, including approximately 1 cm of the oesopha-

gus and duodenum, were removed, cleaned of gut contents and placed in either Bouin's fixative or 10% formalin. The contents of the corpus and antrum were separated, mixed with 20 ml distilled water and the pH measured with a Beckman 3500 digital pH meter.

A dissecting microscope was used to examine gross morphology and photographs were taken with a Nikon F2 camera and 55-mm lens. Stomach dimensions were measured with calipers, and micro-anatomical features with a Leitz micrometer. A punch was used to obtain discs of papillated epithelium of 1,3-cm diameter. Papillae on the discs were counted.

Paraffin embedded material was processed, and sections 7 μm thick were cut. Frozen sections fixed in Bouin's fluid were sectioned on a freezing microtome. General purpose tissue stains were used when examining the gastric epithelium, and specific histochemical stains when examining the non-glandular corpus (Table 1). Photographs were taken through a Wild M 400 Photomacroskop or a Vanox microscope fitted with an Olympus C 35 camera.

Table 1 Stains used when examining the gastric histology of *M. albicaudatus*

Stain	Tissue stained	Reference
Hematoxylin & eosin	General purpose, nuclear material	1
Hematoxylin alum	Nuclear material	2
Toluidene blue	General purpose, mucin	1
Feulgen	Nuclear material	1
Oil red O	Lipid	1
Sudan black B	Lipid	3
Periodic acid Schiff	Mucopolysaccharides	1
Aldehyde fuchsin	Sulphur groups	1
Ferric ferricyanide	Reducing substances	1
Aniline blue, Orange G	Keratin	4

1 Humason 1967
2 Luna 1968
3 Sumner & Sumner 1969
4 Ayoub & Skhlar 1963

Additional blocks of tissue were fixed in 5% cold buffered glutaraldehyde for a minimum of 12 h. Tissue used for scanning electron microscopy (SEM) was critical point dried (Anderson 1951), coated with gold palladium and examined with a JEOL JSM/VS scanning electron microscope. Secondary fixation and embedding of the tissues used for transmission electron microscopy (TEM), followed the procedure of Cross (1979). Sections were cut on an LKB mark 3 ultramicrotome and stained with uranyl acetate and lead citrate (Cross 1979). A Hitachi HU/11B transmission electron microscope was used to examine the sections.

To facilitate surface examination of the corpal epithelium, and to determine bacterial/epithelial associations, conventional (normal gastric flora), sterile (bacteria-free stomach) and specially treated rats (reduced gastric flora) were used (Table 2).

Results

Gross morphology

The stomach of *M. albicaudatus* was markedly sacculated (Figure 1) and differed from Carleton's (1973) 'primitive'

Table 2 Antibiotic treatment of the alimentary flora of *M. albicaudatus*

Rat treatment	Food and water
Conventional	Tap water, pelleted food
Sterile	100 mg oral oxytetracycline per day for 5 days. Pellets and water were autoclaved.
Specially treated	50 mg oral oxytetracycline per day for 5 days. Tap water, pelleted food.

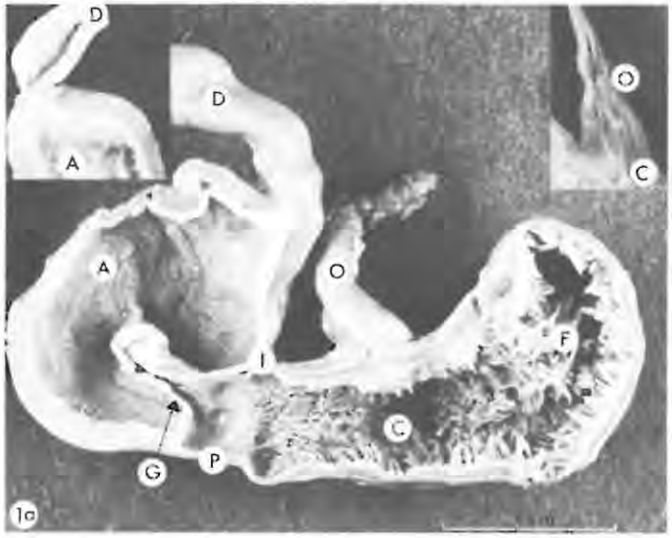

Figure 1a Photograph of a bisected stomach of *M. albicaudatus* illustrating gross morphology. Insets: sections through (right) oesophageal sphincter and (left) duodenal sphincter. To avoid confusion, anatomical terms used in this paper are defined as follows: C = corpus — proximal region of stomach including the fornix ventricularis. P = pre-gastric pouch — non-glandular epithelium separating the corpus and antrum. A = antrum — distal glandular region of stomach. F = fornix ventricularis — diverticulum of the corpus extending craniad beyond the gastro-oesophageal junction. G = grenzfalte — bordering fold of tissue separating the glandular and non-glandular epithelia. I = incisura angularis — prominent angle on the lesser curvature of the stomach. O = oesophagus, D = duodenum, Fu, Py and Ca = fundic, pyloric and cardiac regions of the antrum respectively.

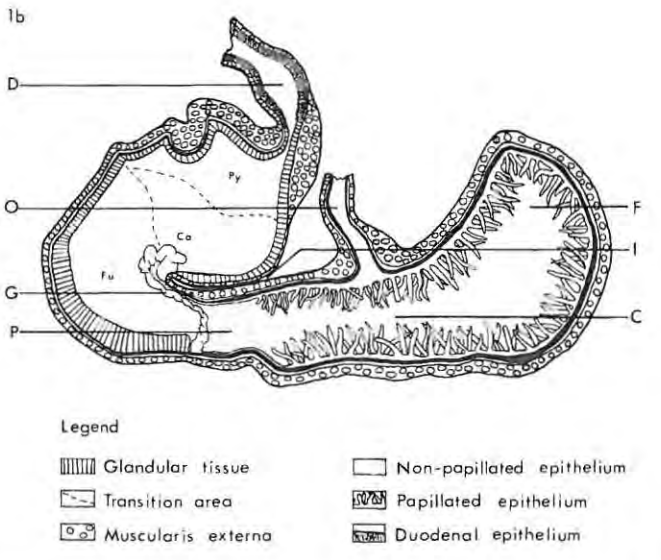

Figure 1b Semi-diagrammatic drawing of photograph in Figure 1a (same lettering).

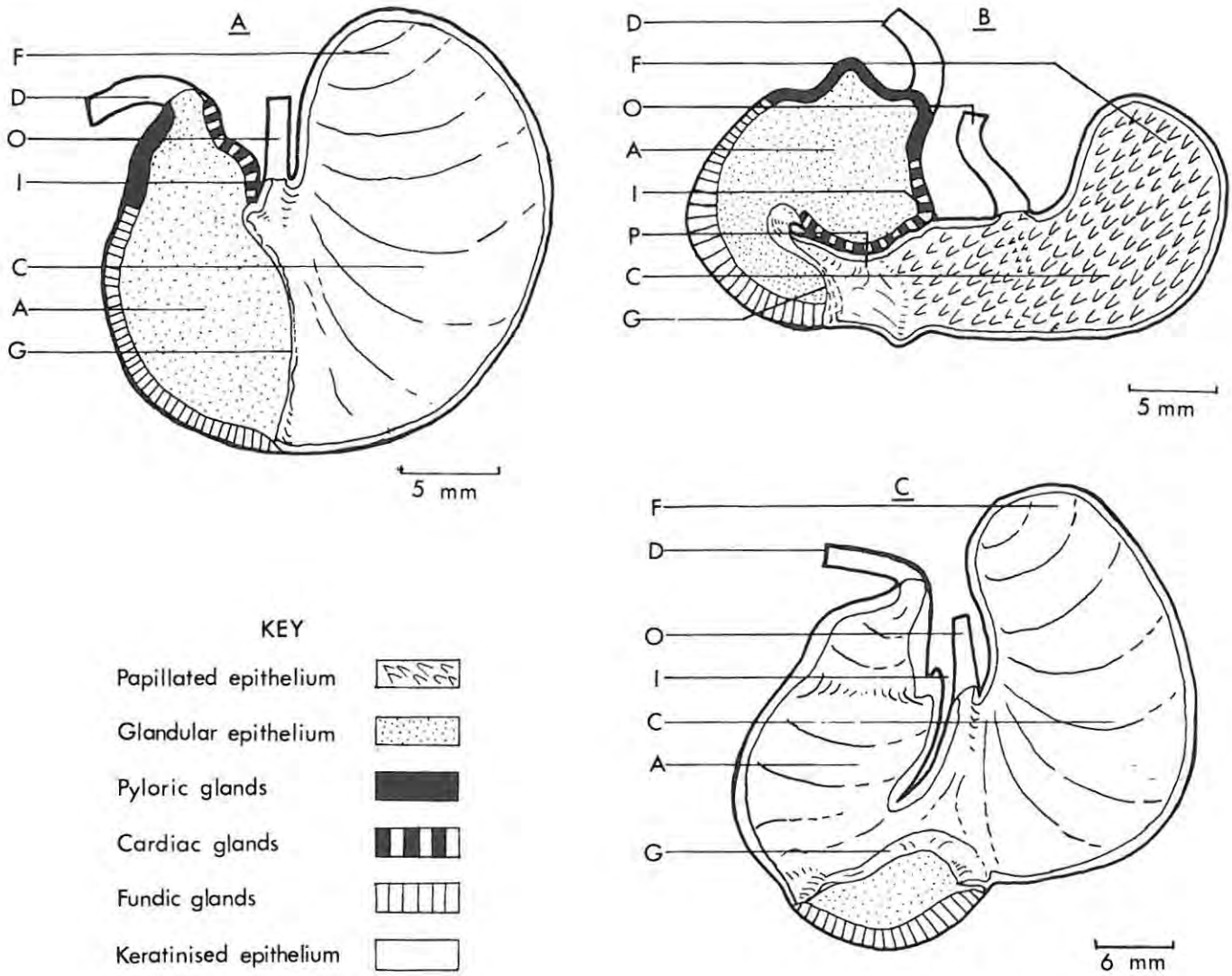

Figure 2 A generalized diagram of the three stomach types (modified after Carleton 1973). (A) unilocular hemiglandular, (B) bilocular hemiglandular (*M. albicaudatus*) and (C) bilocular discoglandular. Lettering as in Figure 1a.

and 'advanced' types (Figure 2). The proximal, non-glandular pars oesophagea consisted of a papillated corpus and a non-papillated pre-gastric pouch (PGP) (Figure 1). The glandular antrum was separated from the pars oesophagea by a bordering fold of tissue, the grenzfalte (Figure 1).

The oesophagus entered the mid-dorsal region of the corpus, which was enlarged by a diverticulum of the fornix ventricularis (Figure 1). Papillae, a few of which were bifurcate, were irregularly orientated in the corpus. A constriction (the pre-gastric pouch — PGP) extended from the corpus adjacent to the PGP to the distal side of the grenzfalte (Figure 1).

The antrum was a glandular sac originating immediately distal to the grenzfalte. Its asymmetrical U-shape (the larger, distal, section of which lead directly to the duodenum) was due to an angular notch or incisura angularis (Figure 1). pH readings from the non-glandular and glandular regions, and papillae measurements are summarized in Table 3.

Table 3 pH values from the glandular and non-glandular regions of the stomach of *M. albicaudatus* and corpal papillary measurements

	pH value	Density	Length
Antrum	2,7 (SD = 0,29) (n = 10)	–	–
Corpus	4,6 (SD = 0,46) (n = 10)	–	–
Papillae	–	550/cm^2 (SD = 114) (n^* = 1 300, n = 10)	1,8 mm (SD = 0,45) (n^* = 124, n = 10)

n^* = number of samples examined; n = number of animals examined

Histology

With the exception of the epithelium, a typical mammalian stomach tissue plan (Dearden 1966, 1969; Madge 1975) was seen in *M. albicaudatus*. A thin serosa and muscularis externa comprising two smooth muscle layers, inner circular (stratum circulare) and outer longitudinal (stratum longitudinale), were present. Generally the muscularis externa was thicker in the corpus (where it supported a keratinized, papillated epithelium) than in the PGP or antrum. The stratum circulare was approximately four times thicker than the stratum longitudinale in the antrum, twice as thick in the corpus and in the fornix ventricularis the layers had equal thickness. The stratum circulare was exceptionally well developed at the pyloris and gastro-oesphageal junction where it formed the pyloric and oesophageal sphincters respectively (Figure 1a).

The oesophageal muscularis externa consisted of striated muscle (Figure 3), the outer longitudinal layer continued from the right side of the oesophagus to the incisura angularis. Longitudinal muscle on the left of the oesophagus penetrated the outer smooth muscle layer of the stomach so that a transitional region of both smooth and striated fibres occurred at the gastro-oesophageal junction. The oesophageal inner circular striated muscle layer changed to gastric smooth muscle immediately prior to the oesophageal sphincter (Figure 3).

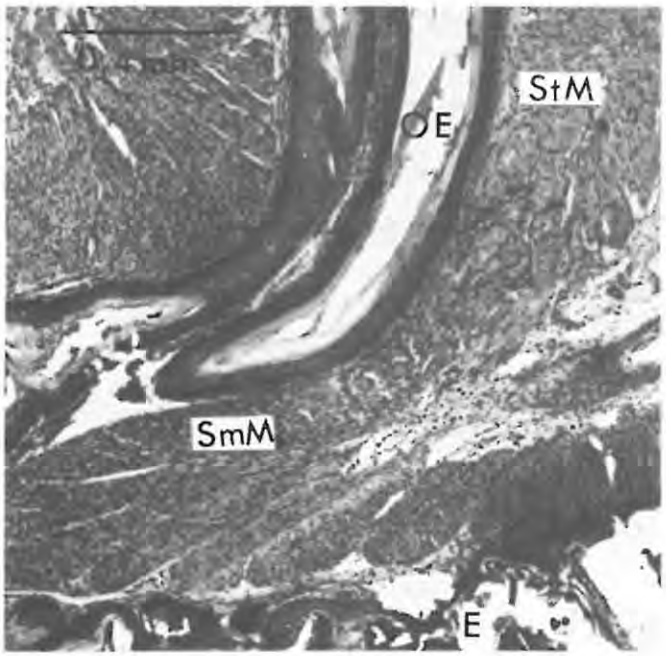

Figure 3 The gastro-oesophageal junction; striated muscle layers (StM) of the oesophageal muscularis externa interdigitate with the gastric smooth muscle layers (SmM). The folded, keratinized oesophageal epithelium (OE) and the corpal papillated epithelium (E) are indicated.

A submucosa of loose connective tissue with nerve fibres and numerous blood vessels maintained a constant thickness in the corpus and PGP but was often absent from the antrum where the muscularis externa and mucosa were closely attached. The corpal muscularis mucosa was continuous with that of the oesophagus but was incomplete, being represented by an indistinct longitudinal, smooth muscle layer (particularly evident in the fornix ventricularis). Inner circular and outer longitudinal smooth muscle fibres occurred in the muscularis mucosa in the antrum, except along the greater curvature where longitudinal muscle predominated. Few transverse fibres occurred in the middle of this layer at regular intervals.

Fine reticular connective tissue and elastin fibres constituted the lamina propria which had an irregular thickness in both the corpus (due to the folded epithelium) and in the antrum (where it extended between the glandular tissue) (Figure 4). In the antrum, the lamina propria also contained smooth muscle fibres from the underlying muscularis mucosa. Interlocking connective tissue and epithelial papillae, termed epithelial pegs by Hyden & Sperber (1965), were absent.

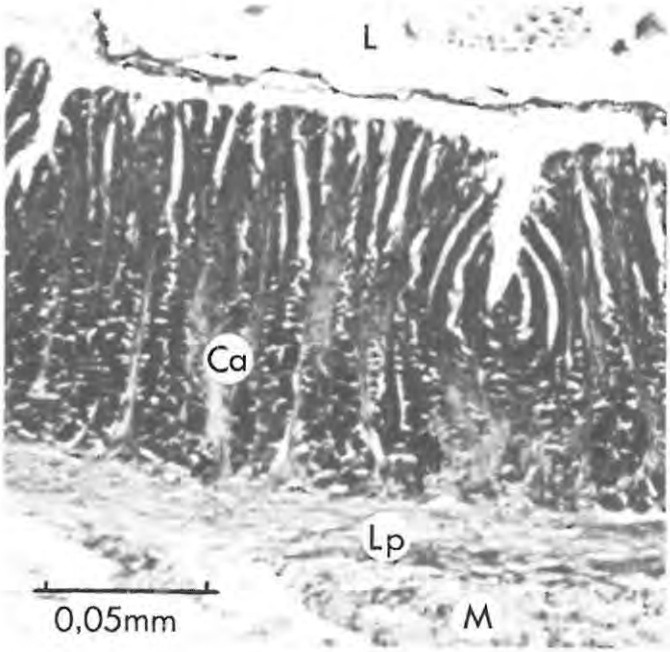

Figure 4 A longitudinal section through the cardiac region of the stomach. The lamina propria (LP) extends between the short, branched cardiac glands (Ca). L = lumen, M = muscle layers.

The pars oesophagea was lined by stratified, squamous epithelia. Keratinization had taken place through keratohyalin thus forming 'soft' keratin and all the layers typical of the mammalian epidermis (Jarrett 1973), with the exception of the stratum lucidum, were well represented in this region. A folded corpal epithelium (FCE) lined the corpus, and between the folds were keratinized papillae (Figure 5) which differed from those of the rumen by lacking a connective tissue core, or swollen cells in the superficial layers (Hofmann 1973). The stratum corneum constituted the main part of the papillae and there was an extensive stratum spinosum compared to the FCE (Figure 5) and PGP (Figure 7). Masses of symbiotic bacteria penetrated the horny layer and formed numerous microhabitats throughout the papillae length (Figure 6). This was not seen in the adjacent FCE where bacteria were fewer and formed a thin surface layer (Figure 5).

The junction between the corpus and PGP was marked by the termination of the papillated epithelium. PGP epithelium was folded and had a thicker malpighean layer

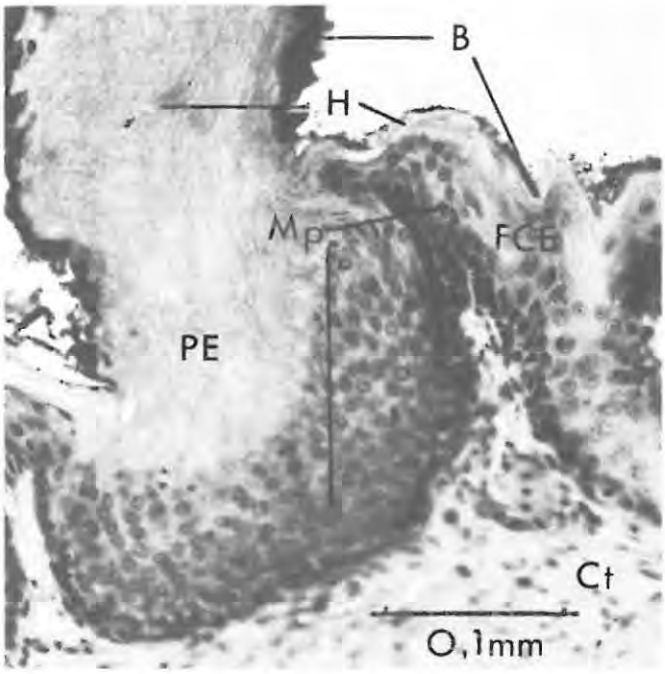

Figure 5 Longitudinal section through the FCE and papillary epithelium (PE). Note the difference in thickness between the malpighean layers (Mp), horny layers (H) and bacterial covering (B) of the FCE and PE regions. Ct = connective tissue.

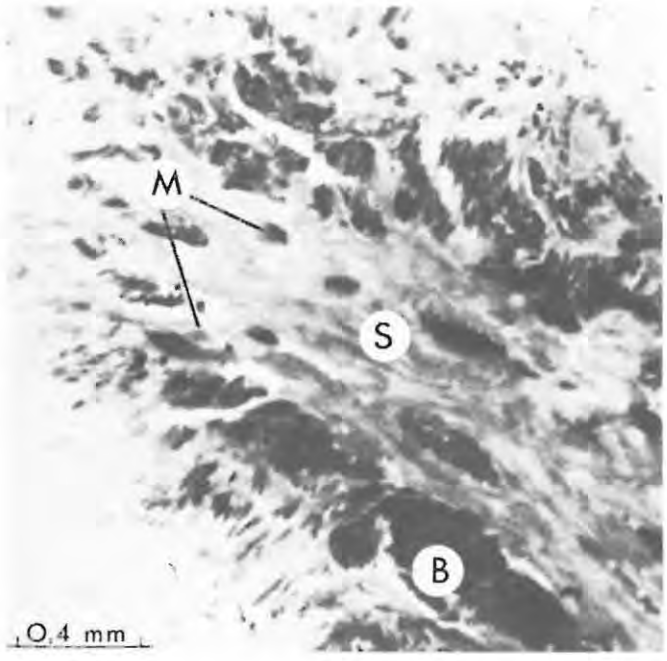

Figure 6 A high power micrograph of a section through the tip of a papilla showing penetration of the keratin by the bacteria (B) and resulting microhabitats (M). S = stratum corneum.

and more distinct granular layer than the FCE but was otherwise histologically similar (Figure 7). Bacterial colonization of the PGP was also similar to that of the FCE. The grenzfalte, separating the glandular and non-glandular areas, was a keratinized flap of tissue with a muscularis mucosa core. This flap appeared to be continuous with the thickened glandular epithelium on the distal side (i.e. keratinized on the side of the PGP and glandular on the opposite side) but this was not the case as keratinization occurred on both sides of the grenzfalte (Figure 8).

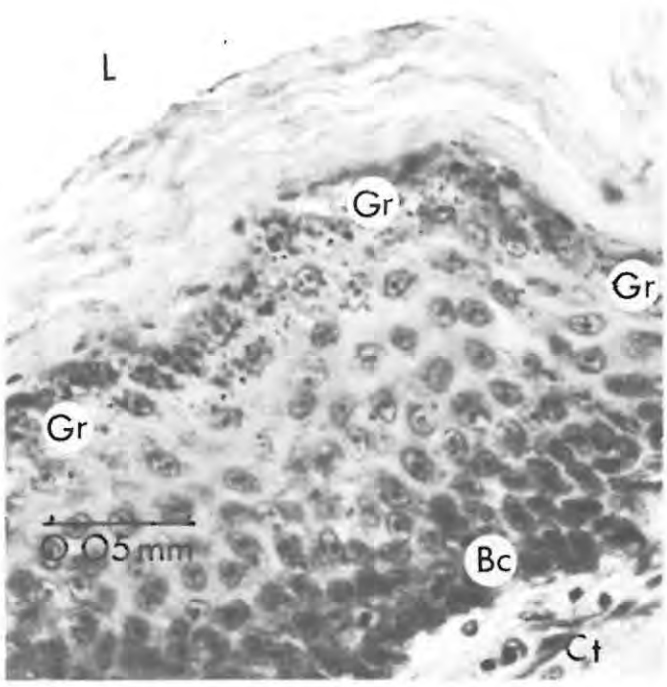

Figure 7 Section through the PGP epithelium showing the distinct granular layer (Gr) and basophilic basal cells (Bc). Ct = connective tissue, L = lumen.

Distal to the grenzfalte was the antrum which was histologically divisible into cardiac, pyloric and fundic regions with short transition areas between each (Figure 1). Along the lesser curvature, extending from the grenzfalte, was the cardiac region (Figures 1 & 4) with short, branched glands which increased in length towards the pylorus (Figure 10). Mucus-secreting glands with wide foveola occurred in the pyloric region and chief cells were found at the base of the gland in the pyloric/fundic transition area. Long tubular, fundic glands with narrow foveolae lined the greater curvature and extended to the grenzfalte (Figures 1, 8 & 9). These glands presented a typical mammalian cytology; that is, cuboidal neck cells above mucous neck cells, followed by chief cells at the base. Parietal cells occurred throughout the gland but predominated in the basal region (Figure 9).

Histochemistry

Hematoxylin and eosin (H & E) and toluidene blue stains demonstrated a lack of nuclei in the stratum corneum but detected the presence of keratohyalin granules in the stratum granulosum. These features indicated complete keratinization and formation of 'soft' keratin. Lipid droplets were not revealed in the corpal epithelium although oil red O and Sudan black stains were used. Tissues increased in stain intensity towards the stratum corneum suggesting an increase in lipid content.

The slight PAS positive reaction in the corpal basal and spinous cell cytoplasm was probably indicative of glycogen, important in the keratinization process. The lamina propria and the bacteria were strongly PAS positive. Gastric mucin was present in the lumens of the cardiac and pyloric glands but only in the fundic gland foveolae. No PAS reaction or toluidene blue metachromasia occurred in the corpus, indicating an absence of mucus in the pars oesophagea.

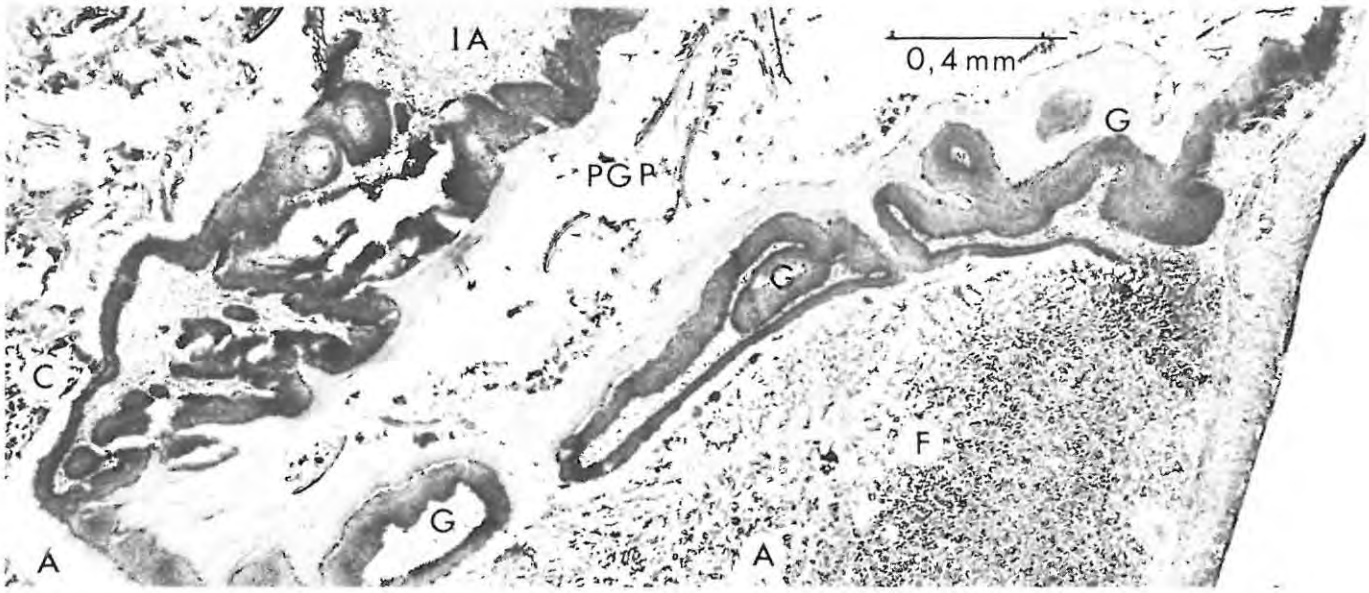

Figure 8 The keratinized grenzfalte (G) separating the PGP and antrum (A), extends from the greater curvature of the stomach to the incisura angularis (IA). F = fundic and C = cardiac epithelia.

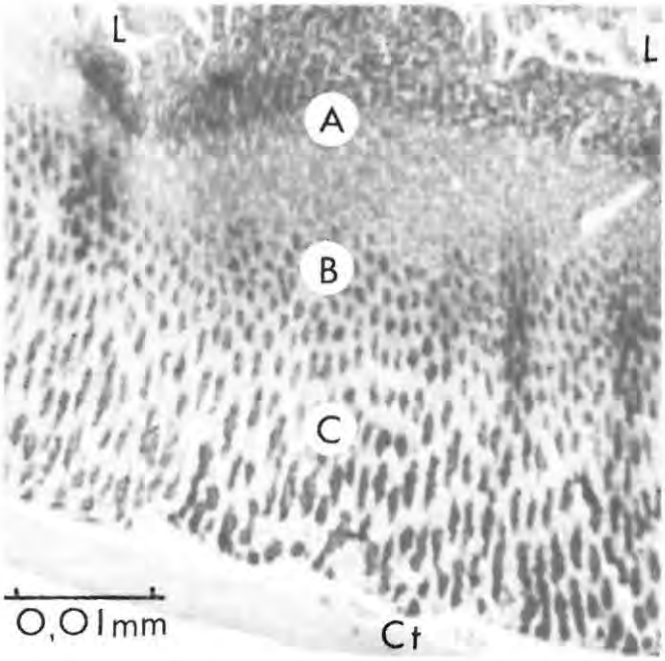

Figure 9 Longitudinal section through the fundic glands. Three regions, characterized by different cells are present; A = surface mucus and mucus neck cells, B = basophilic chief cells, C = acidophilic parietal cells and basophilic chief cells. Ct = connective tissue, L = lumen.

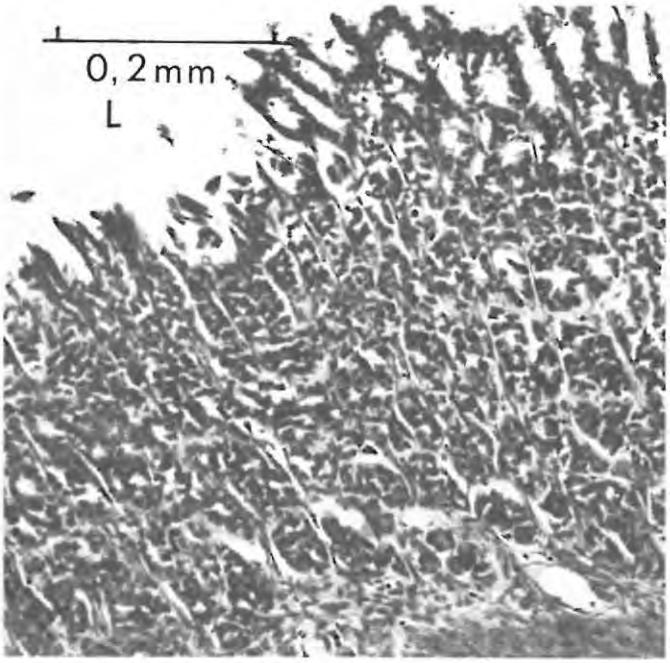

Figure 10 Longitudinal section through the mucus cells of the pyloric region. L = lumen.

The presence of sulphur groups in the superficial epithelial layers of the pars oesophagea was confirmed by increased intensity of the aldehyde fuchsin and ferric ferricyanide stains towards the stratum corneum. The stratum corneum stained positively with both stains.

Scanning electron microscopy (SEM)

Sterile rats were used to examine the papillae because numerous bacteria in conventional rats completely obscured surface detail. The FCE epithelium was visible in conventional rats because of the fewer bacteria in this region. SEM revealed that the desquamating cells of the papillae were smaller than those of the FCE (Figure 11) and that bacteria occurred in areas (microhabitats) between the small squames (Figure 12). Similar bacterial colonization did not occur among the larger FCE squames. Among the irregularly orientated FCE squames there was a compact, regular epithelium (Figure 13) not seen in the papillae. PGP epithelium was seen in conventional rats and was similar to the compact epithelium of the FCE (Figure 13).

Foveolae of the gastric glands were visible in the antrum (Figure 14) unless obscured by mucus. The presence of occasional, isolated bacteria and absence of other micro-organisms, as noted in histological sections, was confirmed. Microvilli covered the surface epithelial cells of the cardiac (Figure 14) and pyloric regions.

Transmission electron microscopy

TEM studies of the pars oesophagea confirmed the light microscope findings and a normal sequence of keratini-

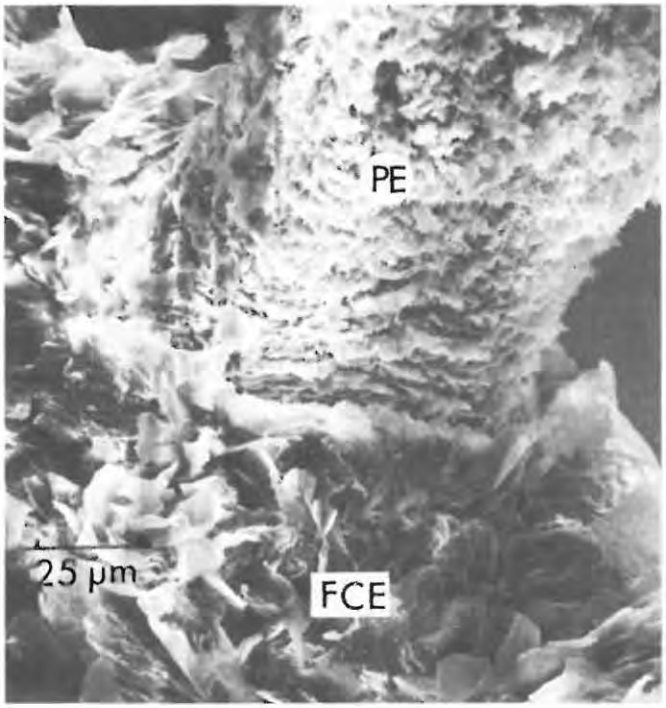

Figure 11 A scanning electron micrograph of the corpus showing the base of a papilla and the FCE. The desquamating epithelial cells of the FCE are larger than the papillary cells (PE). (Specially treated rat.)

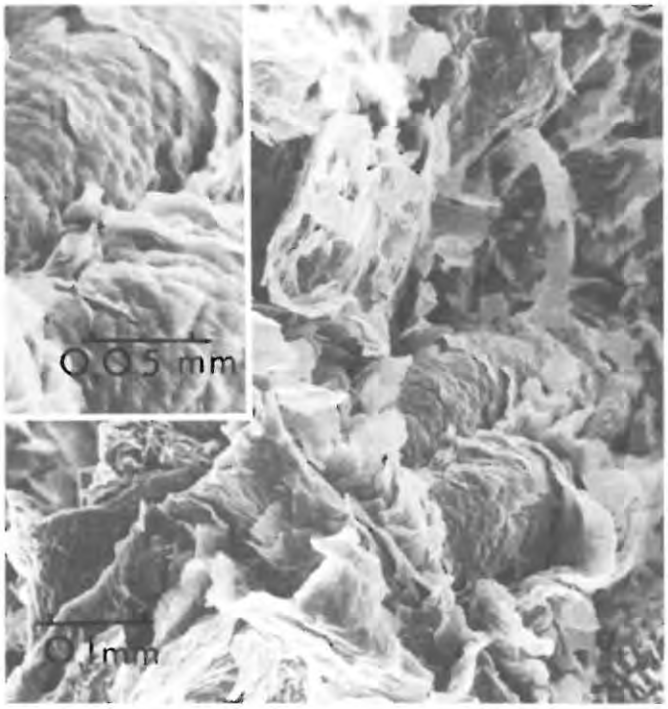

Figure 13 The surface of the FCE from a specially treated rat. The regular compact epithelium is seen left and below centre. The inset shows this area at higher magnification.

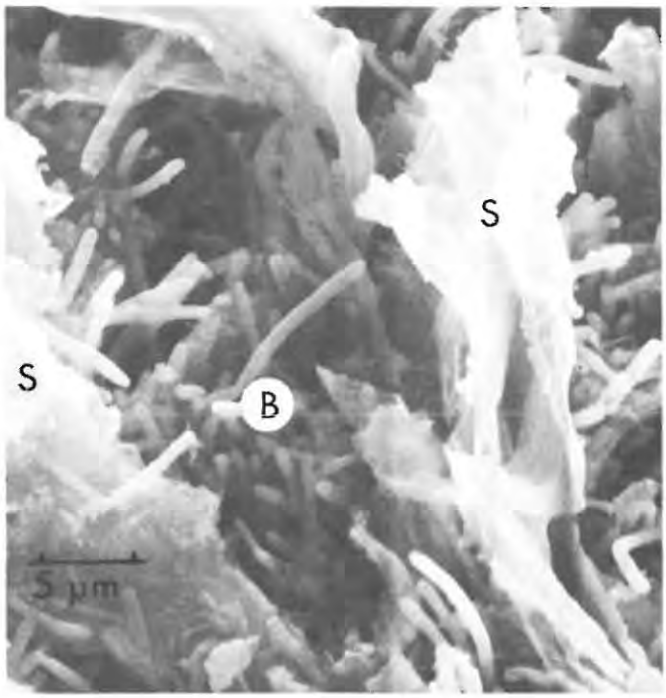

Figure 12 A microhabitat between desquamating cells (S) of a papilla. Note the presence of bacilli and cocco-bacilli (B). (Specially treated rat.)

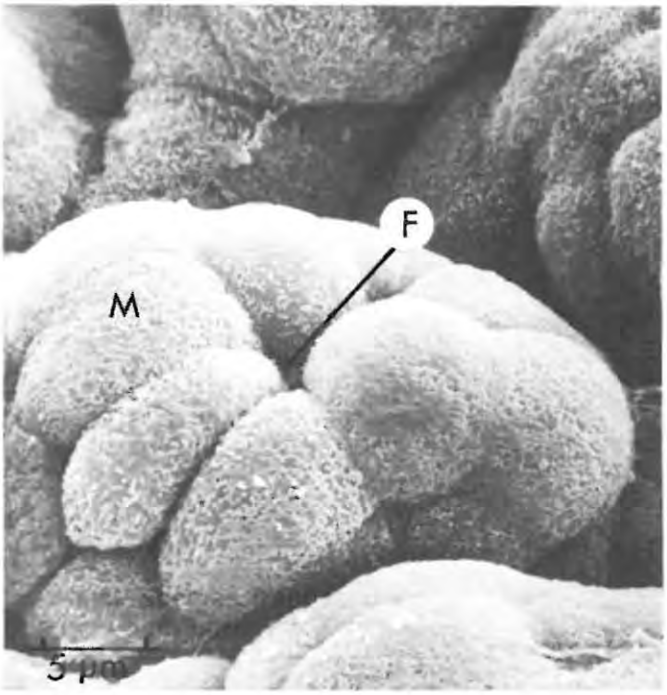

Figure 14 Surface mucus cells with microvilli (M) around the foveolum (F) of a cardiac gland. (Sterile rat.)

zing epithelium was seen although the papillary epithelia, FCE and PGP epithelia had slight ultrastructural differences.

In the stratum basale an electron translucent space was present between the basement and plasma membranes in the pars oesophagea. The papillae, FCE and PGP epithelia had characteristic microvilli extending from the basal cells into wide intercellular spaces. The plasma membrane was thin and highly convoluted (Figure 15). Mitochondria, abundant in the papillary basal cells (Figure 15) were comparatively few in the tonofilament-rich PGP and FCE. Golgi and rough endoplasmic reticuli (RER) were rare.

The stratum spinosum was characterized by an increase in desmosomes and small electron dense granules (probably ribosomes), and tonofilaments and cytoplasmic oval bodies (COB) became apparent (Figure 16). COB were present in all spinous layers and were often membrane-bound with an electron translucent internal structure; few possessed an electron dense or laminated structure. Cellular degeneration and flattening occurred in the superficial regions (Figure 16). This layer was extensive in

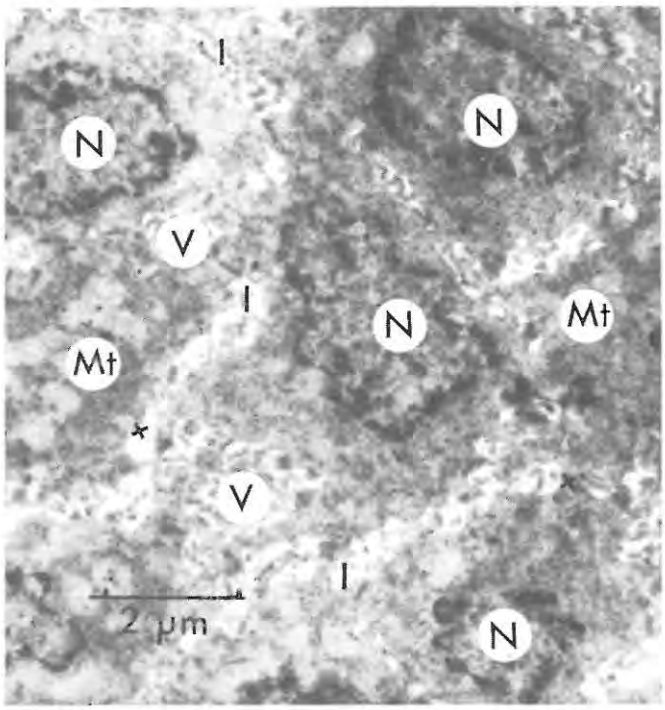

Figure 15 A transmission electron micrograph of the papillary epithelium. Characteristic, wide intercellular spaces (I), microvilli (V) and the thin, convoluted plasma membrane (X) are indicated. The small, opaque granules are ribosomes. Mt = mitochondria.

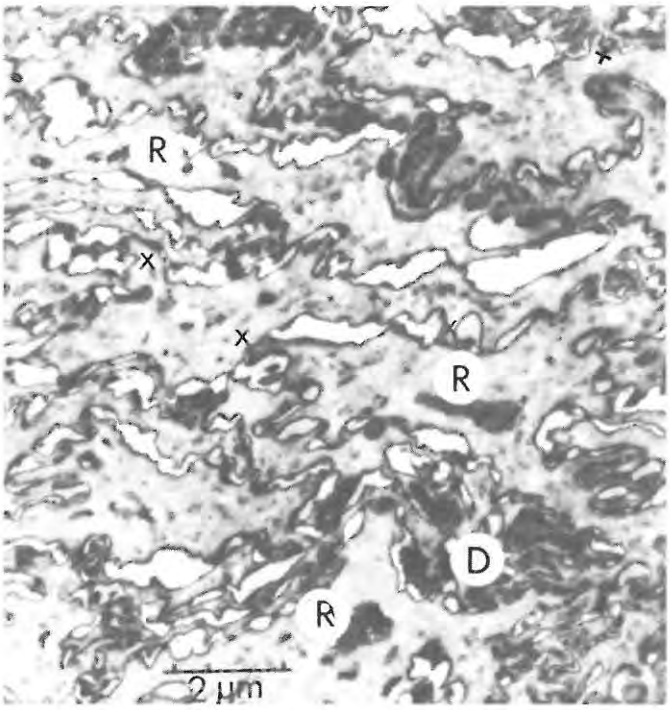

Figure 17 The central area of a papilla. Note the numerous keratin fibrils and cellular interdigitations. R = cellular remnants, D = degenerating desmosomes (squamosomes), X = electron-dense intercellulalr material.

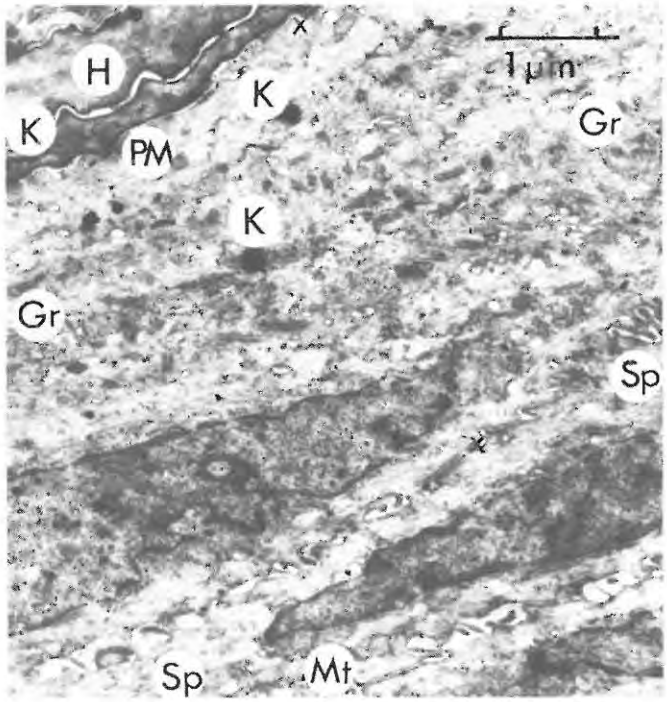

Figure 16 A micrograph showing the strata spinosum (Sp), granulosum (Gr) and corneum (H). Cellular flattening is marked in the upper spinous layers. Fine particle aggregations occur around the keratohyalin granules (K). The thickened plasma membrane (PM) is shown in the stratum corneum. Mt = mitochondria, X = desmosome.

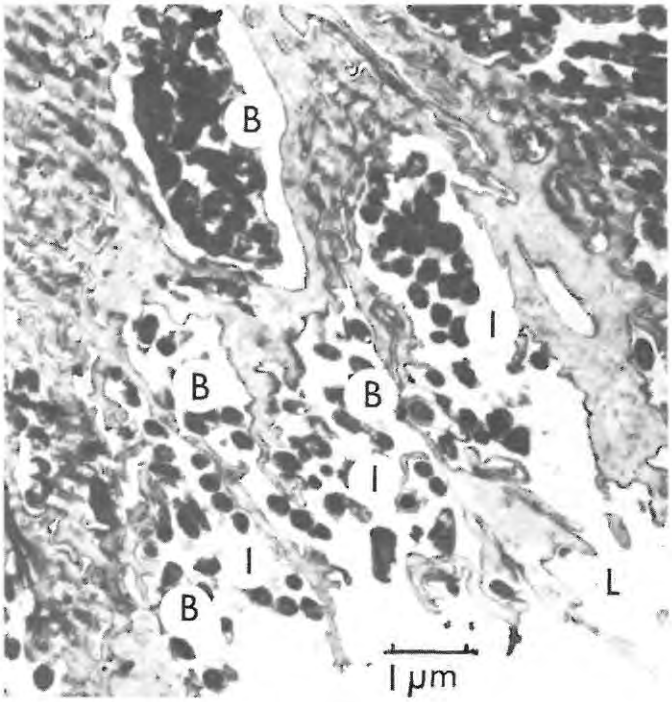

Figure 18 The periphery of a papilla from a specially treated rat. The horny cells are similar to those in Figure 17 although no nuclear remnants are present. Bacteria (B) occur in the widened intercellular spaces (I). L = lumen.

the papillae compared to the stratum spinosum of the PGP and FCE, and spinous cellular changes (common throughout the FCE and PGP layers) were only apparent in the upper papillary stratum spinosum. Cellular degeneration was advanced in the stratum granulosum and the cells contained numerous small, electron-dense granules (which aggregated around the keratohyalin granules — KG) and numerous tonofilaments (Figure 16). Desmosomes and some degenerating nuclei were also present in the superficial layers (Figure 16).

The junction between the strata granulosum and corneum was marked by an abrupt thickening of the horny

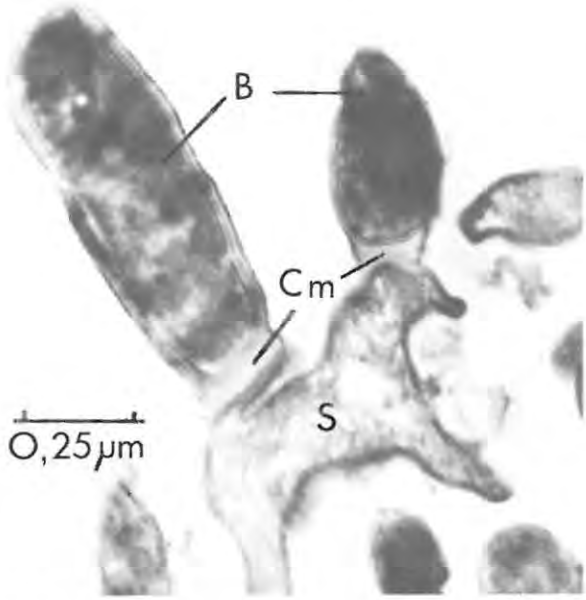

Figure 19 Bacterial attachment to desquamating papillary cells (S). B = bacteria, CM = bacterial capsular membrane.

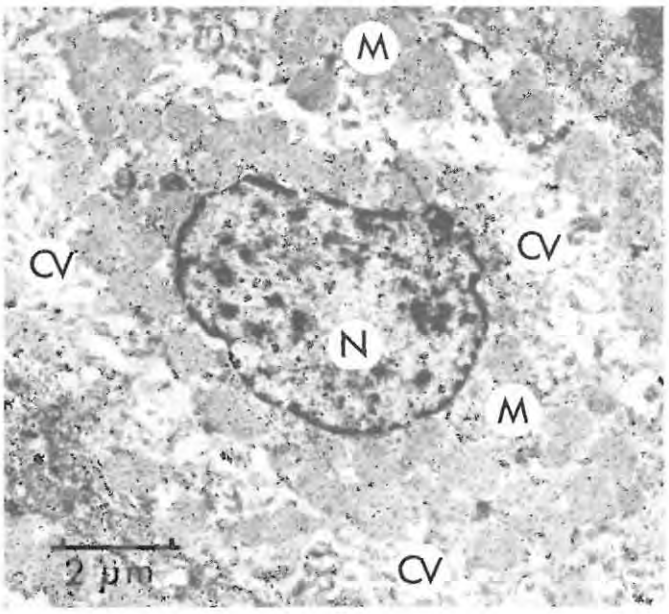

Figure 21 A section through a parietal cell from a fundic gland. Note the canaliculus with microvilli (CV) around the nucleus (N) and the abundance of mitochondria (M).

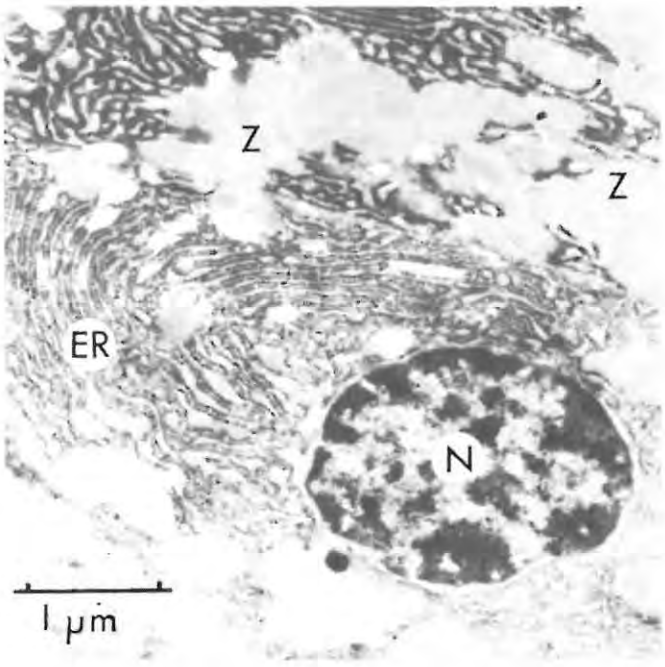

Figure 20 A section through a chief cell from a fundic gland. Features typical of these cells are apparent: Z = zymogen granules, ER = endoplasmic reticulum, N = nucleus.

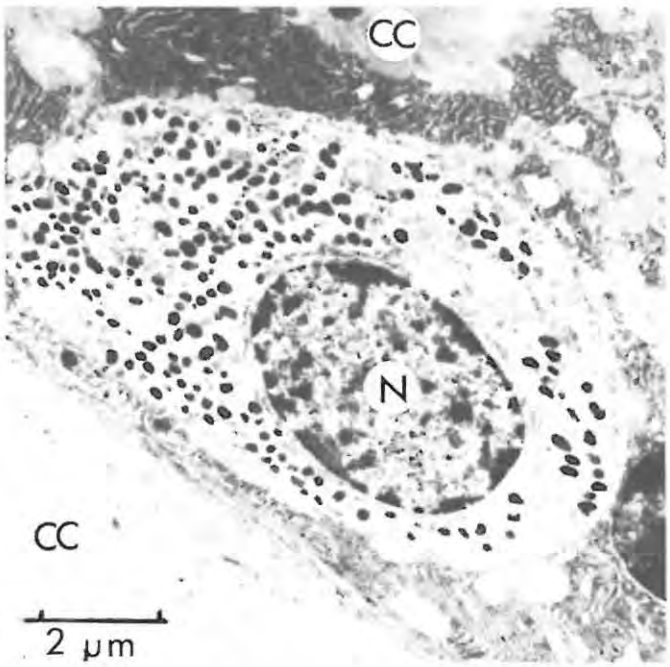

Figure 22 An enterochromaffin cell surrounded by chief cells (CC). Note the electron-dense granules and the light cytoplasm of the endocrine cell. N = nucleus.

cells' plasma membrane (Figure 16). The lower horny cells contained keratin fibrils, KG, and few desmosomes (Figure 16). Other organelles had degenerated. Cells of the mid and superficial stratum corneum contained randomly orientated keratin fibrils in an amorphous matrix and some cellular remnants (Figure 17). Desmosomes had degenerated to form squamosomes (Allen & Potten 1974) and some electron-dense material was present in the intercellular spaces (Figure 17). Desquamating cells showed much interdigitation, particularly in the papillae (Figure 17) and in the papillary periphery the intercellular spaces widened. Bacterial colonization of these wide intercellular spaces corresponded to the microhabitats seen in SEM micrographs (Figures 12 & 18). Some bacteria were attached to the desquamating cells by means of a thickened capsular layer (Figure 19). TEM observations confirmed the presence of surface epithelial cells (with microvilli), chief cells (Figure 20) and parietal cells (Figure 21). Endocrine cells (enterochromaffin cells) were present in the fundic region (Figure 22).

Discussion

The corpus of *M. albicaudatus* has a non-glandular, stratified squamous epithelium modified to form keratinized papillae while the antrum possesses cardiac, pyloric and fundic glands. Carleton (1973) recognized a series of

rodent stomach types from the simple unilocular hemiglandular to the complex bilocular discoglandular type. The gastric morphology of *M. albicaudatus* attains the bilocular condition but shows no overt discoglandular arrangement of the secretory epithelia. The term bilocular hemiglandular is adopted for such a stomach which resembles that of *C. gambianus* (Caiman *et al.* 1960).

Although a discoglandular arrangement is not apparent, *M. albicaudatus* has a papillated bilocular hemiglandular stomach which may be approaching a papillated discoglandular condition. An increase in cardiac, and cardiac/fundic transitional glands (at the expense of pyloric glands, Bensley 1905), restriction of the fundic area and subsequent replacement of cardiac glands by keratinized epithelium generates the modified bilocular discoglandular condition. The position and area occupied by fundic and cardiac glandular epithelia in *M. albicaudatus*, the compensatory increase in the length of the fundic glands (Luthje 1976) and the fact that the grenzfalte is keratinized and not glandular (*cf.* Bensley 1905) is indicative of trends towards the modified digastric stomach type.

Peters & Gärtner (1973) found that *Rattus norvegicus* and *Mus musculus* have a thin, elastic forestomach and a muscular, thick-walled glandular stomach; the lack of forestomach musculature implies that mechanical preparation of food in this organ cannot be similar to that of ruminants (Krzywanek 1927). *M. albicaudatus*, however, has a thicker muscularis externa in the corpus than in the antrum, in addition to a layer of striated muscle extending from the oesophagus to the incisura angularis, suggesting that mechanical preparation of food in the forestomach of *M. albicaudatus* is more important than in the rodents examined by Peters & Gärtner (1973).

Epithelial stratification, with keratohyalin granules in the stratum granulosum, indicates that the pars oesophageal epithelium of *M. albicaudatus* has undergone 'soft' keratinization. Spearman (1977) and Matoltsy (1962) reviewed the process of epidermal keratinization which parallels the keratinization of the non-glandular region of the stomach of *M. albicaudatus*. The papillae show a slightly different type of keratinization to the FCE and PGP epithelium; the thick papillary stratum corneum resembles the pathological epidermal condition termed hyperkeratosis. However, this condition is not pathological in *M. albicaudatus* but is a normal, physiological adaptation whereby symbiotic bacteria are provided with ecological niches. The term physiological hyperkeratosis is thus suggested for this type of keratinization; orthokeratin is suggested for the 'soft' keratin of the FCE and PGP.

The extensive stratum corneum of the papillae is generated by a high mitotic rate in the basal cells. The papillary epithelium, although not possessing epithelial pegs, shows a thickening of the basal layers and may parallel phase two type epidermis which is characterized by a high mitotic rate resulting in a thickened epithelium (Bullough 1975). The abundance of mitochondria in the basal cells may account for the high mitotic rate. (Rumen epithelia also possess numerous basal cell mitochondria, but in contrast to *M. albicaudatus*, these are important in absorption of rumen metabolites.) The FCE and PGP epithelium has a unicellular basal layer and thin epithelium, thus paralleling phase one type epidermis (Bullough 1975).

The precise adaptive functions of the stomach of *M. albicaudatus* are unknown. It has been proposed that sacculation of certain cricetid stomachs permits pH to remain near neutrality in the corpus allowing for continued salivary α-amylase activity (Carleton 1973), which has also been reported for the non-glandular forestomach of the rat (Kunstyr, Peters & Gärtner 1976; Peters & Gärtner 1973). Amylase (optimal pH 7) in this region would, however, be partially denatured by the low pH and would not have optimal activity. The acidity is caused by microbial metabolites, as both pH and amylase activity increase in germ-free rats (Kunstyr *et al.* 1976). Kunstyr *et al.* (1976) therefore considered the forestomach microflora of the rat detrimental to starch digestion. Inner regions of the chyme may, however, have a higher pH than that of the periphery allowing for some degree of amylolysis (Peters & Gärtner 1973). An acidic pH (4,6) occurs in the corpus of *M. albicaudatus* and thus reduction of amylase activity is probable. The acid conditions and presence of numerous bacteria on the corpal papillae suggest that a reservoir with amylolytic activity, is not the primary function of the corpus although it is possible for some starch hydrolysis to occur if the food has a slow passage through the stomach.

The papillae are considered to play an integral role in the corpus but owing to the absence of vascularization and presence of keratinization they are not absorptive, unlike rumen papillae (Hyden & Sperber 1965; Lavker, Chalupa & Dickey 1969; Henrikson 1970). The intimate association between the papillary stratum corneum and numerous bacteria in healthy *M. albicaudatus* suggests a symbiotic relationship. The evolution of the papillae cannot be readily explained by assuming that the bacteria are parasitic or physiologically detrimental to the host. The bacteria on the FCE and PGP epithelium constitute a separate autochthonous flora, characteristic of many rodents (Savage 1977).

An understanding of the mechanisms and processes of this symbiotic relationship will only be gained by studies of digestive physiology and microbial function. It is possible that protein-rich bacteria are digested by *M. albicaudatus*. However, it has been demonstrated that *Rattus norvegicus* and *Mus musculus* do not obtain protein from a gastric bacterial source (Gärtner & Pfaff 1979). Ehle & Warner (1978) suggest that microbial activity in the forestomach of *Mesocricetus auratus*, may assist that in the caecum, and improve forage cell wall digestibility. This process contrasts with Vorontsov's functional compensation theory (1961) but Perrin & Curtis (1980) noted that concurrent morphological modification of forestomach and caecum may be associated with different or complementary digestive processes. The rodent caecum is undoubtedly important for microbial fermentation in many species (McBee 1971). However, the papillated corpus, symbiotic bacteria, stomach sacculation and simple caecum of *M. albicaudatus* suggest that it is the forestomach that initiates and predominates in microbial fermentation processes. Similar conclusions were drawn by Sakata & Tamate (1976) and Hoover, Mannings & Sheerin (1969) when studying *M. auratus*.

In conclusion, the non-glandular corpus of *M. albicau-*

datus is lined with keratinized epithelia, and FCE, papillary and PGP epithelia are present, each with different mitotic rates and thicknesses. The corpal papillae maximize surface area for attachment of symbiotic bacteria, possibly important in carbohydrate fermentation. It is believed that these bacteria, in smaller numbers, would not predispose a dietary advantage to the host; the papillae thus being crucial for the symbiotic relationship. The corpus may have other secondary functions. Ehle & Warner (1978) noted that the effectiveness of forestomach fermentation is decreased by the rapid emptying rate of stomach contents. The papillae of *M. albicaudatus* may slow the rate of gastric ingesta flow causing food to be subjected to microbial activity for a longer period and thus increasing the effectiveness of fermentation. Finally, the findings of Kunstyr *et al.* (1976) with *R. norvegicus* propound that increased starch digestion may occur in the corpus of *M. albicaudatus*. The antrum retains cardiac, fundic and pyloric glands for proteolytic digestion.

M. albicaudatus has developed specialized herbivorous adaptations and retained the ability to digest protein. Specializations of this cricetid are partially offset by the reproductive potential of murids (Perrin 1980). Studies are underway to determine digestive efficiency in relation to the role of the papillary bacteria.

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Notes on the activity patterns of 12 species of southern African rodents and a new design of activity monitor

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The circadian activity patterns of 12 species of southern African rodents are described under controlled laboratory conditions. Activity was measured in a newly described apparatus, in which rodents, traversing infra-red light beams placed across several arenas and nest-boxes, activated a microprocessor which quantified, and regularly printed data on a recorder. Diel patterns of activity were present in all species and most species were nocturnal. Locomotion, and other behaviours, were continuous or discontinuous (phasic) during activity periods. Short-term periods of activity were most obvious in species with a low body mass and were thought to be associated with a feeding rhythm. Continual diel activity with a regular short-term rhythm characterized *Otomys irroratus*, paralleled that of the microtines, and is believed to be necessitated by specific adaptations to herbivory. Crepuscularity in *Rhabdomys pumilio* may be associated with *Hodotermes* predation, while nocturnalism in (arboreal) *Graphiurus murinus* is believed to reduce competition with diurnal granivorous and insectivorous birds. Notes describe the seasonal change in activity of four species; such differences were less marked than those reported for temperate species.

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Die bedrywigheidspatrone per etmaal van 12 spesies van Suider-Afrikaanse knaagdiere onder beheerde laboratorium toestande word beskryf. Die bedrywigheid word gemeet d.m.v. 'n onlangse beskryfde apparaat waarin knaagdiere, sodra hulle oor infra-rooi ligstrale beweeg wat oor verskeie kaste en slaapneste geplaas is, 'n mikroprosesseer-toestel aanskakel wat die data kwantifiseer en dit met gereelde tussenposes d.m.v. 'n opnamedrukker registreer. Dwarsdeur die etmaal is bedrywigheidspatrone by alle spesies waargeneem en die meeste spesies is nagtelik van aard. Beweging en ander gedragspatrone is tydens bedrywigheidstye deurlopend of nie-deurlopend, (d.w.s. dit kom voor in fases). Kortstondige bedrywigheidstye blyk die duidelikste by spesies met lae liggaamsmassa en daar word vermoed dat dit verband hou met die ritme van hul vreetgewoontes. Aanhoudende bedrywigheid met 'n gereelde kortstondige ritme gedurende elke etmaal is 'n kenmerk van *Otomys irroratus*, en loop parallel met die ritme van die genus *Microtus*. Dit word vermoedelik vereis deur spesifieke aanpassings by hul plantvretende aard. Skemertydbedrywigheid by *Rhabdomys pumilio* kan dalk verband hou met die feit dat dit op *Hodotermes* teer, terwyl daar vermoed word dat verhoogde nagtelike bedrywigheid by die boombewoner *Graphiurus murinus* wedywering met diurnale graan- en insekvretende voëls verminder. Die aantekeninge beskryf die seisoenale bedrywigheidswisseling by vier spesies: sodanige wisseling is minder opvallend as die opgetekende wisseling by spesies van gematigde streke.

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The activity patterns of small rodents are governed by several factors, some of them being endogenous, others of an ecological nature (Backlund & Ekeroot 1950). Most rodents display an endogenous circadian rhythm which may be modified by a superimposed short-term feeding rhythm. Activity denotes movement, usually locomotion, although feeding, drinking, elimination and other activities are included (Falls 1968). Ashby (1972) has reviewed some of the patterns of diel activity exhibited in mammals in both the field and laboratory, but it is clear that little is known of the activity phasing of southern African rodents (Choate 1972; Keogh 1973; Nel & Rautenbach 1974; Nel 1975; Christian 1977). The prime objective of this preliminary study was to describe, verify and compare the activity patterns of several species of southern African rodents under controlled laboratory conditions.

The activity monitor described here is capable of sensing the locomotory movements of small mammals, summating the counts of arena, and nest-box (or food-box) activity, and recording data at regular time intervals. Since there are (four) independent arenas, several individuals can be tested simultaneously which permits direct comparison of species, sex, age or social class differences. However, care must be taken to ensure that all subjects of the same species are derived from the same community, unless one is particularly interested in examining shifts in activity phasing caused by niche overlap (different community structure). Results from experimental (e.g. drug-treated) and control (untreated) subjects can be printed sequentially: by extending cables, the responses of subjects to differently simulated environments (temperature, photoperiod) can also be compared.

The microprocessor can be programmed to record counts of activity in any particular area of the arena (e.g. 'wall-walking' versus 'open-field' behaviour) or in nest-boxes and will therefore be particularly useful for studies of exploratory behaviour (Perrin 1971). It has a wide application in small mammal behavioural studies. The results of this study are discussed in relation to habitat and feeding habits, and previous studies.

Materials and Methods

Seven of the 12 species examined (*Graphiurus murinus, Otomys irroratus, Saccostomus campestris, Mus musculus, Praomys natalensis, Aethomys namaquensis* and