Chapter 1

INTRODUCTION

1.1 The mammalian digestive tract

The digestive tract or alimentary canal in mammals is essentially a long tortuous tube of varying width, extending from mouth to anus. Conventionally, the tract is divided into fore-, mid-, and hindgut. The foregut includes the mouth, pharynx, oesophagus and stomach; the midgut the small intestine, and the hindgut the large intestine (Young, 1970).

The functions in one region of the digestive tract frequently lead to functional changes in following regions, so that a regular sequence of digestive processes is maintained. Certain large glands adjacent to the tract (salivary glands, liver, gall bladder and pancreas) are connected with it by ducts, and form a second component of the digestive system.

The internal structure of the digestive tract conforms to a basic histological pattern. Four major layers are usually present. From the lumen to the outer surface these are: (1) the mucous membrane (mucosa) consisting of a epithelial lining, a supporting lamina propria and a layer of smooth muscle, the muscularis mucosae; (2) the submucosa; (3) the muscularis externa; and (4) the serosa. The size and complexity of these layers may vary considerably along the length of the tract and can be related to the altered functions of the different regions such as the oesophagus, stomach and intestine. Probably the most variable is the mucous membrane, the epithelium of which may be thrown into numerous outgrowths and/or crypts.

Embryologically, the mammalian digestive tract is first formed from yolk sac endoderm which later differentiates into the varied cell types and epithelial

structures of the inner lining of the mucous membrane except for mouth and anus ectoderm. Around the early endoderm is located visceral (splanchnic) mesoderm, which gives rise subsequently to the connective and muscular tissues of the tract.

Although mammals have acquired the ability to obtain nutriment of different kinds from a wide range of habitats, the anatomical pattern of their digestive system shows relatively few special features. The differences between the digestive tracts of mammals are mainly seen in the structures involved in obtaining food and in the occurrence of modified regions of the tract for cultivation of symbiotic micro-organisms that play a key role in cellulose breakdown. Investigators in the first half of the nineteenth century observed that, like the eutherians (the so-called "true" mammals), the marsupials included carnivorous, omnivorous and herbivorous species. It was also obvious that the dietary array in marsupials was associated with some anatomical differences. In the latter part of the nineteenth century and early part of this century, it became clear that the basic histological and embryological features of eutherians and marsupials were similar.

1.2 The adult marsupial digestive tract

Diagrams of representative digestive tracts of marsupial carnivores, omnivores and herbivores are shown in Figure 1. It is sometimes difficult, however, to define the exact limits of each category (Hume, 1982).

Carnivores (notably the dasyurids and thylacinids in Australia and many didelphids and caenolestids in South America) show some variation in the anatomy of their digestive tracts, but they are relatively unspecialised and approach what may be termed a generalised mammalian plan. The oesophagus has a short abdominal region; the stomach is small to medium-sized; the small and large intestines are relatively short; the caecum, if present, is small and the colon is

Figure 1   Representative digestive tracts of marsupials (after Hume, 1982)

       carnivore (Dasyurus maculatus)
       omnivore (Didelphis marsupialis)
   herbivore (Macropus giganteus)

CARNIVORE

Stomach

Oesophagus

Small intestine

Colon

20 cm

OMNIVORE

Stomach

Colon

Oesophagus

Caecum

Small
intestine

5 cm

HERBIVORE

Hindstomach

Sacciform
forestomach

Pylorus

Duodenum

Small
intestine

Tubiform
forestomach

Caecum

Distal colon

Proximal colon

20cm
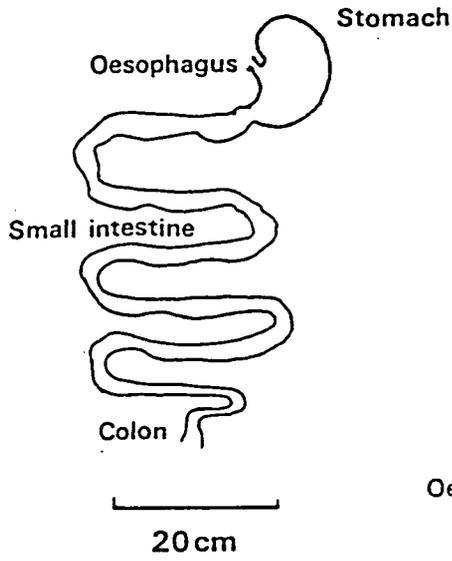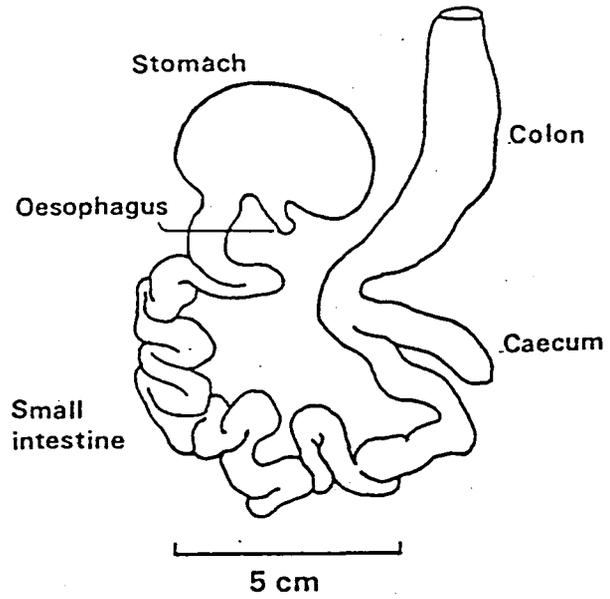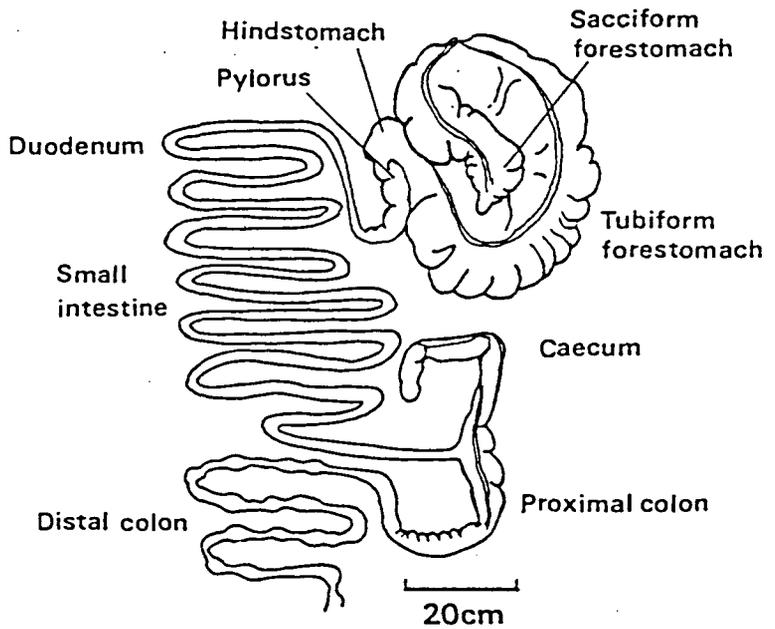
usually similar in diameter to the small intestine (Hume, 1982). The microanatomy of the digestive tract of marsupial carnivores has not been extensively studied. Indeed, a recent symposium on Australian carnivore marsupials (see Archer, 1982) did not include a paper on any aspect of the structure and function of the digestive system.

Marsupial omnivores are broadly represented taxonomically and the digestive tract is similar morphologically to that of carnivores. Part of the diet of omnivores is plant material and microbial digestion occurs in the hindgut, primarily in the caecum. Omnivorous marsupials thus often have a larger caecum and a larger and longer colon than the carnivores. The most thoroughly studied omnivore digestive systems are those of the North American opossums, Caenolestes obscurus (Osgood, 1921), Marmosa robinsoni (Barnes, 1977) and Didelphis virginiana (Krause, Cutts and co-workers in a series of reports on microanatomy extending over the last 15 years).

It is amongst the Australian diprotodont herbivores that the marsupial digestive tract shows considerable morphological and functional specialisation. Most of the adaptations are associated with microbial fermentation since marsupial herbivores, like their eutherian counterparts, are unable to synthesise the enzymes necessary to hydrolyse cellulose. Herbivore evolution in both eutherians and marsupials depended on the development of a symbiotic relationship between the mammalian "host" species and micro-organisms capable of fermenting the cellulose of plants.

Based on the region of the gut where microbial fermentation occurs, eutherian and marsupial herbivores can be divided into two groups, namely, hindgut and foregut fermenters. The discrimination is not absolute but in species where both types occur, one of them predominates. In eutherians, hindgut fermentation is concentrated in a large caecum (as in the horse and rabbit). Foregut fermentation is exemplified by the ruminants.

In the marsupials, hindgut fermenters include the non-macropodid herbivores, for example, the wombats (Family Vombatidae), koalas (Family Phascolarctidae), most of the gliders (Family Petauridae) and possums (Family Phalangeridae). The most thoroughly examined hindgut fermenter is the Australian brush possum, Trichosurus vulpecula. The stomach of the brush possum is relatively small and lacks a gastric gland-free cardiac stomach typical of foregut fermenters; the caecum is large and acts as a fermenting chamber but fermentation of cellulose in this species may be secondary to hydrolysis of starch in meeting its carbohydrate requirements (Tyndale-Biscoe, 1973).

The macropodids (kangaroos and wallabies) are of particular relevance to this study and are accordingly reviewed in more detail. Renewed interest in the morphology, and particularly the physiology of the macropodid gut, is reflected in the number of important papers that have appeared in the last decade. The general morphology of the kangaroo digestive tract is shown in Figure 1.

Kangaroos and wallabies have a long intra-abdominal oesophagus (Richardson, 1980; Hume and Dellow, 1980). According to Richardson (1980), such a long oesophagus perhaps allows unhampered movement by the heavy stomach during postural changes and locomotory activity. Examination of the oesophagus in a wide range of species led Obendorf (1984) to classify them into four types based on the surface morphology of the oesophageal lining: (I) Smooth lining with small and simple longitudinal folds, includes species of Dendrolagus, Dorcopsis, Lagorchestes, Petrogale, Peradorcus, Setonix and Thylogale; (II) Smooth lining with large, pleated longitudinal folds, includes Macropus antilopinus, bernadus, fuliginosus, giganteus, robustus and rufus; (III) Entire oesophagus lined with finger-like papillae, includes Macropus agilis, dorsalis, eugenii, parma, parryi, irma and Wallabia bicolor; (IV) Oesophagus proximal to diaphragm lined with longitudinal leaf-like laminae, distal to diaphragm lined with fine finger-like papillae, Macropus rufogriseus.

The epithelial lining of the oesophagus consists of keratinized stratified squamous cells and no oesophageal glands are present. The degree of keratinization of the epithelial layer of the oesophagus is closely related to feeding habit (Obendorf, 1984).

Early in the nineteenth century, anatomists such as Home and Owen (see Tyndale-Biscoe, 1973) described the similarities between the stomach of kangaroos and ruminants. Histological observations of the kangaroo stomach were first reported in the second half of the nineteenth century (Shäfer and Williams, 1876) and again emphasised ruminant similarities. It was not until the middle of this century (Moir et al., 1956) that macropodid stomach physiology was shown to be similar to that of ruminants. It should be pointed out, however, that despite descriptions of digestive physiology of macropodids as "ruminant-like", Hume (1982) pointed to numerous physiological features of the macropodid stomach (and intestine) which are somewhat different from those of the Ruminantia.

The stomach in kangaroos and wallabies is large, such that when full it can weigh up to 15 per cent of total body weight. It is a long, tubular organ which is deeply sacculated along the greater curvature. Regions of the stomach have been delimited in various ways. Richardson (1980) referred to three regions based on gastric flexure giving proximal, middle and distal compartments. Langer et al. (1980) provided a useful subdivision into three regions based on morphology – the sacciform forestomach (the cranial end which bends sharply ventrad and forms a blind sac); the tubiform forestomach (the main tubular section); and the hindstomach (the caudal end composed of the gastric pouch and pylorus). On the external surface of the sacciform and tubiform regions of the forestomach are located three bands of muscle called taenia. Functionally, the sacciform and tubiform regions of the forestomach are where microbial digestion of food takes place, while in the hindstomach hydrochoric acid secretion and peptic digestion occurs.

One further criterion has been used to divide the adult stomach into regions, namely, the structure of the mucosal epithelium (Gemmell and Engelhardt, 1977). Four regions are recognised – the oesophageal and cardiac regions (forestomach) and the fundic and pyloric regions (hindstomach). These four regions have proved to be particularly useful as markers for description of stomach development.

The oesophageal region contains the gastric sulcus which extends from the oesophageal opening along the lesser curvature of the forestomach and is lined with non-glandular stratified squamous epithelium. It is well developed in several species of Macropus (including M. eugenii), reduced in ontogeny in M. robustus and absent in Thylogale thetis (Langer et al., 1980). The function of this region is to direct the passage of non-fibrous food caudally. The cardiac region is lined with simple columnar epithelium and is involved in mucus secretion. The fundic region or gastric pouch has four epithelial cell types: surface cells, mucous neck cells, parietal cells and chief cells. Both the surface epithelial cells and mucous neck cells produce mucin, the parietal cells secrete hydrochloric acid and the chief cells produce the enzymes of gastric secretion or pepsinogen (Gemmell and Engelhardt, 1977). Finally, the pyloric region is lined with columnar epithelial cells which produce mucin.

The morphology of the macropodid intestine conforms with that described by Richardson and Wyburn (1980) for Macropus eugenii. Most of the small intestine is loosely coiled caudal to the sacciform forestomach. The duodenum is short. The large intestine has a simple caecum which is cylindrical in shape, has no taenia or sacculation, and is tapered slightly towards a blind end. The body and the apex of the caecum are mobile. Although the caecum and the proximal colon of all macropods appear to be areas of microbial fermentation, they are not well developed (Hume and Dellow, 1980). The colon joins the rectum which terminates

at the anus in the cloaca. A description of the histology of the macropodid intestine has hitherto been lacking.

1.3 Background to the study

One of the unique features of the marsupials is their pattern of development. The gestation period is short and much of morphogenesis is compressed into a narrow developmental "window" in the second half of uterine life. At birth, weight of the young is extremely low in relation to maternal body weight and many organ systems are in a relatively undifferentiated state. The organisation of the digestive tract at birth and during early stages of extrauterine life poses many questions about the structure and function of tissues and organs, but many of these questions remain open.

Compared with the large amount of information available on the structural and functional development of the digestive tract of eutherians (see Koldovský, 1969; Henning, 1973; Grand et al., 1976; Henning, 1981, for reviews), data for marsupials are meagre. With a single exception, literature on any one species is diffuse and the observations are more or less fragmentary. The exception is the omnivorous North American opossum, Didelphis virginiana. For more than a decade, Krause and Cutts and their co-workers have examined the development of the digestive system of D. virginiana in some detail in late embryonic and postnatal stages. A series of papers on their light and electron microscope observations built on earlier observations by Heuser (1921), provides a marsupial model not only for comparisons with eutherians but also for comparative studies on Australian species.

The development of the digestive tract of Australian marsupials is known in fragmentary form for several species, mainly in relation to the macropodid stomach. Griffiths and Barton (1966) described the structure and peptic activity

in pouch young of the red kangaroo Macropus rufus. In the newborn the primordium of the spiral groove was present but in young, up to 200 days, the secretory cells of the fundic and cardiac regions consisted of one cell type only, which presumably secreted both hydrochloric acid and pepsin. However, Langer (1979) found in two species of Thylogale at 156 days or at weaning, the parietal cells of the fundic region were structurally differentiated.

The general aim of this investigation was to provide a histological and cytological account of the early development of the digestive system in a single species of macropodid (the tammar wallaby). The emphasis has been placed on the various regions of the digestive (gastrointestinal) tract. As noted above, a comprehensive description of digestive system development in an Australian marsupial has yet to be reported. This study is thus an attempt to fill, in part at least, an obvious lack in the marsupial literature using transmission and scanning electron microscopy as well as light microscopy.

The period of development selected for examination was the phase of ontogeny when the bulk of morphogenesis and differentiation occurs. Previous observations made in the School of Biological Sciences at Macquarie University indicated that these foundation events are encompassed by the period from approximately one week before birth to six weeks after birth. The material described here represents this particular window of development.

Birth occurs during the selected developmental window. The transition from an intrauterine to an extrauterine environment ("extrauterine gestation" of Sharman, 1975) involves a battery of rapid changes in the young. The newborn thus represents an important stage in this study, although possible correlations between structural and functional adaptations were sought throughout the six-week postnatal period.

1.4 The study animal

Using the classification of Kirsch (1977), the taxonomic position of M. eugenii within the marsupials is as follows:

Superorder   : Marsupialia

Order   : Diprotodonta

Superfamily   : Phalangeroidea

Family   : Macropodidae

Subfamily   : Macropodinae

Genus   : Macropus

Species   : Macropus eugenii   (Desmarest, 1817)

Macropus eugenii is a small wallaby with a total body weight ranging from 4 to 6 Kg in females and 6 to 10 Kg in males. It is a greyish-coloured animal, variably rufous on the shoulders, and grey-buff on the belly. It is a browsing animal, typically in low scrub where it moves rapidly by hopping low to the ground with the forelimbs held out characteristically away from the body.

The abundance and distribution of M. eugenii have been severely reduced by the clearing of land for agriculture. It occurs today in allopatric populations in South Australia (Eyre Peninsula, Kangaroo Island, Flinders Island and Greenly Island) and in Western Australia (from Geraldton to Hopetoun, Houtman's Abrolhos, Garden Island and Middle Island). It is known by different common names including the tammar wallaby, the dama wallaby and the Kangaroo Island wallaby. Rumour has it that the South Australian and Western Australian populations are different species but until this question is resolved the widely-used name of tammar is adopted.

The breeding season in the wild and in captivity is from late January to early July. Early embryos (blastocysts) may be maintained in a quiescent state throughout the non-breeding season and resume development in early January.

Mating occurs within hours of birth and the resulting embryo is carried in embryonic diapause in the uterus so long as a suckling young is present. The newborn is at a similar stage of anatomical and physiological development to other marsupials. The fore-limbs and shoulder regions are well developed and the digits are armed with sharp, recurved claws. The senses of sight, balance and hearing are undeveloped. There are very conspicuous subcutaneous blood vessels and a moist skin, and respiration across the skin is important (Tyndale-Biscoe, 1973; Lillegraven, 1976).

The tammar has become an important research animal. It is relatively small, easy to handle and sufficiently robust to withstand repeated catching, handling and experimental manipulation. It also breeds readily in captivity. Because of its status as a research animal, an understanding of its early development assumes particular importance.

Chapter 2

MATERIALS AND METHODS

2.1 Animals

2.1.1 Maintenance of adults

The breeding colony of tammar wallabies used in this study was derived from an original stock taken on Kangaroo Island, South Australia. Animals were maintained in the fauna park at Macquarie University, Sydney, usually in groups consisting of 12 mature females and 2 mature males, in outside enclosures of about 50 x 20 metres. A diet of grass clippings and low protein kangaroo cubes (Doust and Rabbidge Pty Ltd) was supplemented on occasions with lucerne hay.

2.1.2 Embryo and pouch young age determination

Late embryos of known gestational age (based on days after removal of a suckling young already in the pouch) were carefully removed from the uterus. Head length and crown-rump length measurements were recorded for each so that embryo age could be estimated from the growth curves of Renfree and Tyndale-Biscoe (1973). Comparison of ages determined from known gestational age with those determined from growth curves showed them to be closely similar in each case. Following convention, the ages of embryos are given as days from removal of pouch young (RPY).

Daily pouch inspections of animals approaching the expected time of birth established the birth date of any newly found young. Prediction of the day of birth was possible using various criteria. From an observed mating or from the presence of a vaginal plug, the expected day of birth could be calculated from the

known gestation period (28.3 $\pm$ 0.1 days) as well as from the day of removal of pouch young - RPY-(27.5$\pm$ 0.7 days) (Berger, 1970). The time of birth could also be predicted, but with less precision, from the appearance of the inner surface of the pouch. Most of the time the pouch lining is composed of a brown to black flaky material which is usually lost shortly before birth. The day of birth was designated Day 0 (= newborn) and all ages of pouch young are given as days post partum. Sometimes the date of birth could not be determined directly and age was estimated from pouch young growth curves (Berger, 1970).

2.1.3 Embryo and pouch young numbers

Under normal circumstances only one young is produced each year. However, additional embryos and pouch young were obtained by removing pouch young and thereby activating the corpus luteum which induces the quiescent blastocyst to resume development. A total of 29 embryos and pouch young obtained during the 1983, 1984 and 1985 breeding seasons (see Table 1) were examined by light and/or electron microscopy or for dissection.

2.2 Histological and cytological techniques

2.2.1 Light microscopy

Five pouch young from newborn to 40 days were dissected to remove the stomach for morphological drawings. Four late embryos aged from 21 to 25 days (RPY) and eight pouch young from newborn to 40 days were examined histologically and/or histochemically (Table 1). Embryos and the newborn were fixed in Bodian's fluid (90 parts 80% ethyl alcohol: 5 parts glacial acetic acid : 5 parts formalin), paraffin wax embedded, serial sectioned at 7 μm and stained with Harris' haematoxylin and eosin. In older pouch young the entire gut was removed and serially sectioned.

Table 1: Details of embryos and pouch young examined

Specimen No.[1]	Age in Days[2]	Examination[3]	Comments[4]
83/99	21 p.c.	LM	entire embryo
83/112	23 p.c.	LM	entire embryo
83/115	23 p.c.	LM	entire embryo
83/400	25 p.c.	LM	entire embryo
83/45	0	LM	
83/32	0	LM	
83/56	0	LM	entire young
83/400	0	LM	
83/41	0	MA	stomach morphology
83/36	0	TEM	
85/56	0	TEM	
85/71	1	SEM	
84/321	5	LM	
84/56	5	MA	stomach morphology
84/321	10	LM	
84/71	10	MA	stomach morphology
85/421	10	TEM	
85/326	10	SEM	stomach only
84/71	20	LM	
84/56	20	MA	stomach morphology
84/34	20	SEM	
85/71	20	TEM	
85/206	20	SEM	
83/288	40	TEM	oesophagus only
84/288	40	LM	
84/326	40	MA	stomach morphology
85/26	40	SEM	
85/26	40	SEM	oesophagus only
85/25	40	TEM	

1: year taken and mother's number
2: p.c. = embryo age post coitum, remainder pouch young age post partum
3: LM = light microscopy, TEM = transmission electron microscopy,
   SEM = scanning electron microscopy, MA = macroscopic examination
4: no comment = various regions of tract examined

As well as the standard haematoxylin and eosin staining for nuclei and cytoplasm, a variety of histochemical procedures were followed to determine the location of specific substances or organelles. These included: periodic acid – Schiff (PAS), for glycogen and other carbohydrates; PAS/haematoxylin/fast green, for carbohydrates, nuclei and cytoplasm; PAS/haematoxylin/orange G, for carbohydrates, nuclei and acidophilic cytoplasm of parietal cells; and Mallory Triple stain for connective tissue, muscle and nuclei.

All sections were examined with an Olympus Vanox microscope and selected sections photographed with an Olympus PM-10-A automatic camera using PAN F 35 mm film.

2.2.2 Electron microscopy

Twelve pouch young from newborn to 40 days were collected and dissected out to remove the digestive tract plus associated organs, in whole or in part, for electron microscopy. For Transmission Electron Microscopy (TEM), small pieces of tissues ($\pm$ $0.5mm^2$) were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for two hours. The tissues were washed in three changes of buffer for 5 minutes each and then post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2) for one hour. After the specimens were washed with distilled water (3 changes), they were block-stained with 2% aqueous uranyl acetate for half an hour, dehydrated in graded alcohol and then infiltrated and embedded in Spurr's resin. "Thick" sections (1 μm) were cut with a Mod. 1140/Autocut microtome, stained with 1% methylene blue or toludine blue and observed under an Olympus BH-2 light microscope. Thin sections were cut with an OMU2 ultra-microtome, stained with 0.5% uranyl acetate followed by Reynold's lead citrate, examined with a JEM 100CX transmission electron microscope and photographed with Kodak Electron Film 4489.

For Scanning Electron Microscopy (SEM), the stomach and the short lengths of oesophagus, and small and large intestine were removed and cut up into segments (0.5 - 2.0 cm). They were then repeatedly flushed with physiological saline solution via a syringe and fine gauge (No. 27) needle to remove lumen contents, slit open longitudinally and pinned to plates of wax with the internal surface uppermost. The mucosal surface of the specimen was once again flushed with physiological saline from a syringe to remove as much of the adhering mucus as possible. Specimens were then fixed in 2% glutaraldehyde with 0.1 M phosphate buffer (pH 7.2) for 24 hours at room temperature, washed in three changes of buffer for 20 minutes each and post-fixed in 1% osmium-tetroxide in 0.1 M phosphate buffer for one hour. They were washed once again in three changes of buffer and dehydrated in a graded acetone series to 100%. The samples were then transferred to a critical point dryer in 100% acetone and cooled to 10°C. They were then dried using liquid CO_2 in a Sorvall unit. Dried tissues were mounted on aluminium stubs with silver conductive paint and sputter-coated with gold in a Polaron E 5000 sputterer coating unit. Observations were made in a JSM-840 Scanning Electron Microscope accelerated at 15-25 KV and photographs taken using Ilford FP4 film.

Chapter 3

DEVELOPMENT OF THE BUCCAL CAVITY AND OESOPHAGUS

3.1 Results

3.1.1 Buccal cavity

Observations on the buccal cavity were restricted to light microscopy on the newborn and transmission and scanning electron microscopy on 40 day pouch young. The tongue was regarded as the organ of special interest in the context of the digestive system; examination of sectioned material of buccal cavities of pouch young older than newborn was of limited value because of section damage due to cranial ossification.

In the newborn, the outer surface of each lip was covered with keratinised epidermal cells (Figure 3), while the inner margins of the lips were thickened and much less keratinised. The upper and lower lips were fused and the resulting lip closure formed a rigid, rounded, terminal oral aperture or shield. The mucosal epithelium lining the entire buccal cavity was squamous and stratified. In the region of the pharyngeal hard palate, some keratinisation had taken place while in other pharyngeal regions such as the nasal and laryngeal surfaces, the epithelium had not begun specialisation. Both the soft palate and the epiglottis were well developed.

The tongue of the newborn after fixation and excision and viewed under a stereomicroscope was spatulate in shape and approximately 3.5 mm in length. A deep, dorsal, oval groove (2.5 mm long by 2.0 mm wide) was present anteriorly. In transverse section, the tongue groove was more or less "V"-shaped (Figure 2), but in some specimens, fixed at different times after removal from the nipple, the groove was shallower.

The musculature of the tongue was very well developed. It was composed of numerous bundles of striated muscle fibres that crossed each other (Figures 2 and 3). Stratified squamous epithelium covered the free surfaces of the tongue; the upper surface was thick and keratinised while the lower surface was thin and smooth. The upper surface epithelium seen in the transmission electron microscope showed desquamation of flattened dense cells in the stratum corneum. Below the stratum corneum, cells showed the initial stages of separation into strata but retained some contact through persisting intercellular bridges (Figure 4). Under the stereomicroscope, three small rounded projections could be seen on the upper surface near the posterior margin of the tongue. A large pointed epiglottis projected forwards from immediately behind the tongue.

By 40 days the tongue was about 9.5 mm long. The groove was shallower, as seen in the scanning electron microscope (Figure 5), measuring about 5.5 mm in length by 4.5 mm in width. Near the posterior margin of the tongue, three rounded bodies, forming a triangle with the apex directed backwards, were more prominent than in the newborn. These three circumvallate papillae were not fully differentiated since no furrow surrounded them. Towards the anterior margin of the tongue groove highly keratinised conical papillae (Figure 6) were tightly packed. A few small, scattered, rounded bodies were found near the lateral margins of the tongue, probably representing early stages in the development of fungiform papillae. Filliform papillae, common in the adult tongue, were not observed.

Immature salivary gland tissue was present in light microscope sections of the ventro-lateral regions in the posterior part of the head. The scattered tissue occurred in a connective tissue field. It consisted mainly of ducts lined by low cuboidal epithelium but some small alveloli contained pyramid-shaped cells surrounding a small central lumen.

3.1.2 Oesophagus

Light microscope observations were made on late embryos and pouch young up to 40 days, with the latter also being used for scanning and transmission electron microscopy.

The embryonic oesophagus was a cylindrical tube made up of an inner lining of stratified columnar epithelium surrounded by layers of mesenchyme cells without a distinct outer boundary. In 21 day (Figure 7) and 23 day embryos, the epithelial lining was mainly composed of two layers of columnar cells, each cell containing an oval interphase nucleus or mitotic figure located towards the basal end. Surrounding the epithelium there was a relatively broad region of small, slightly condensed mesenchyme cells. In the 25 day embryo (Figure 8), the epithelium was irregularly two or three cells deep. Outside the epithelium the mesenchyme cells were loosely arranged and delineated externally by a thin layer of small, fine myoblasts.

In the newborn (Figure 9), the oesophagus varied slightly in shape throughout its length, being circular in cross-section in the proximal and middle regions and oval towards the distal end. In sectioned material, the epithelial lining was thrown into weak mucosal folds and was still only two to three cells deep. The cytoplasm above the nucleus in most cells in the luminal layer gave a weakly-positive PAS reaction, as did the basement membrane on the opposite surface of the epithelium.

In the proximal region of the newborn oesophagus (Figure 10), restricted areas or "patches" of ciliated columnar cells were observed in favourable, conventionally-stained paraffin sections. In both the proximal region and distally towards the entry to the stomach, the epithelium was irregular in form and numerous "cavities" or intercellular vacuoles were present (Figure 9). Some of these vacuoles were relatively large and had a very thin epithelial partition

separating them from the oesophageal lumen. The mesenchyme layer surrounding the epithelium was supplied with several relatively large capillaries containing nucleated blood cells. The number of capillaries was greatest near the proximal end of the oesophagus and decreased posteriorly. The muscularis muscosae was absent and the muscularis externa was represented by two layers of myoblasts which stained more intensely with haematoxylin and eosin than in the late embryo. A thin layer of mesenchyme, the tunica adventitia, joined the outer surface of the oesophagus to surrounding structures.

At 5 days (Figure 11), the external morphology of the oesophagus was unchanged. Histologically, the increased size and thickness of the epithelium accompanied slight changes in structure. The luminal layer was composed of tall, columnar cells with larger, rounded to oval nuclei located basally. Patches of ciliated cells were present. Most of the cells, especially those with an expanded apical end were packed with PAS-positive granules and were distinguishable for the first time as goblet cells. The basal layer of epithelial cells consisted of low, columnar cells with small, oval, darkly-stained nuclei. Mucosal folding was more prominent than in the newborn but intercellular vacuolation of the epithelium was less obvious. Outside the epithelium, the mesenchyme layer was thicker and its blood capillaries smaller and fewer than in the newborn. The inner ring of surrounding myoblasts was clearly defined as the circular layer of the muscularis externa but the longitudinal muscle layer was not evident. Outside the muscularis externa the adventitia had increased in thickness.

From 10 days (and on to 40 days), the oesophagus was oval in cross-section throughout its length before fixation but often rounded up after treatment. Apart from an increase in the number of areas of columnar cells giving a PAS-positive reaction, the epithelium was histologically similar to that of 5 day specimens. The muscularis externa was also the same as at 5 days but the mesenchyme layer was thicker and, for the first time, some cells had differentiated into typical

fibroblasts. In the distal region, small mucosal folds were present between the larger ones seen in earlier stages. In the distal region alone, the adventitia surface was covered by a single layer of squamous mesothelial cells forming a distinct serosa.

In the 20 day pouch young (Figure 12), the diameter of the oesophagus was nearly twice that of the newborn. The main differences from earlier stages were as follows:

(1) in the luminal layer of the epithelium, areas of tall columnar cells with large, oval nuclei were interspersed with low columnar cells; (2) the epithelium as a whole appeared more stratified; (3) goblet cells could no longer be clearly distinguished from other PAS-positive cells; (4) mucosal folds were more prominent; (5) the mesenchyme between the epithelium and the muscularis externa had increased in thickness and in the number of fibroblasts; (6) the inner circular layer of the muscularis externa, consisting of two rows of muscle fibres in previous stages, was increased to three rows; (7) a thin, inconspicuous layer of longitudinal muscle cells was present outside the circular layer of muscularis externa.

By 40 days the oesophagus had increased considerably in size. In diameter it was more than twice as large as equivalent regions in the newborn. Together with overall increase in size, the epithelium was somewhat different in appearance from that of earlier stages. As well as an increase in thickness, the transformation of the stratified columnar epithelium into stratified squamous epithelium had commenced towards the luminal surface as in the oesophageal region of the stomach (see Figure 21). Cuboidal or polygonal cells with small, darkly-stained and mostly rounded nuclei were orientated with their long axes parallel to the epithelium surface. A thin coat of PAS-positive material was present at the luminal surface and in many of the most superficial cells but

distinct goblet cells could no longer be identified. Patches of ciliated cells remained, and using Nomarski interference optics, enlarged microvilli could also be identified among the cilia. Adjacent to the superficial cells the columnar cells were weakly PAS-positive but towards the opposite (basal) surface they were not.

Up to and including 40 day pouch young, a muscularis mucosae was absent so there was no clear demarcation between the lamina propria and the submucosa. The connective tissue of the two regions was much the same. On the other hand, the muscularis externa showed continued development, particularly in the distal region. Both the inner circular and outer longitudinal muscle layers were approximately 40 μm thick. The outer adventitia of the oesophagus was similar to that at 20 days.

Scanning electron microscope examination of the internal surface of the oesophagus at 40 days showed that the luminal surface was characterised by deep longitudinal folds of the mucosa. At higher magnification, three types of cells were observed at the epithelial surface (Figures 15 and 16). One type was more or less polygonal in surface view and possessed a dense covering of microvilli. Another type had relatively long and thick cilia distributed over the free surface. The ciliary nature of these organelles was confirmed from transmission electron microscopy which showed the typical 9 + 2 microtubule configuration. Interspersed between the cilia were usually large, stubby microvilli. A third kind of cell was larger, angular in outline, ruffled or corrugated over its surface and covered with a packed layer of smaller microvilli.

The proportions of the three cell types changed throughout the length of the oesophagus at 40 days. In the proximal region (Figure 13), the ciliated cells were in greatest abundance and declined sharply further back such that towards the middle region they were infrequent. Towards the distal (stomach) end, ciliated cells were absent but increasing numbers of corrugated cells were present.

Figures 2 to 6

Figure 2   Transverse section of head of newborn in nasal region, showing a deep groove in the anterior end of the tongue. (H and E stain; scale = 0.5 mm).

Figure 3   Longitudinal section of head of newborn in nasal region, showing well developed tongue musculature and thickened epidermis of oral shield. (Mallory triple stain; scale = 0.5 mm).

Figure 4   Section of upper surface of tongue of newborn, showing stratum corneum and stratum spinosum. Flattening and detachment of keratinised cells present towards upper right. (TEM x 6,730).

Figure 5   Upper surface of excised tongue of 40 day pouch young. (SEM x 10).

Figure 6   Well keratinised conical papillae near tip of tongue of 40 day pouch young. (SEM x 75).

cp = conical papillae;   hp = hard palate;   ib = intercellular bridge;   nu = nucleus;   os = ossification;   t = tongue;
tg = tongue groove;   vp = circumvallate papillae.
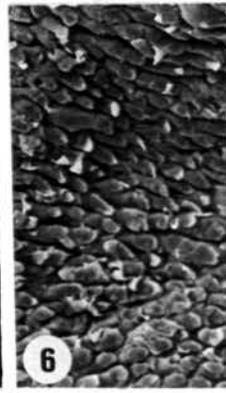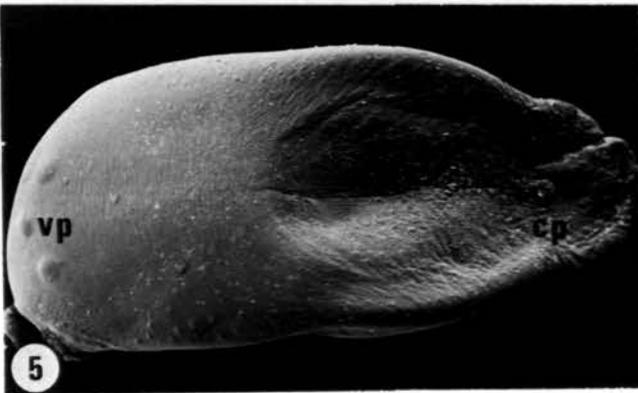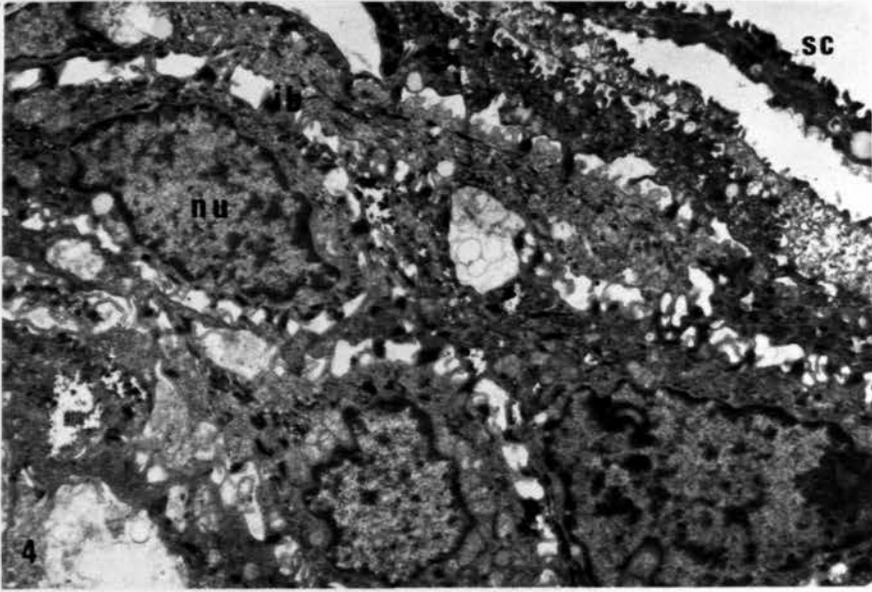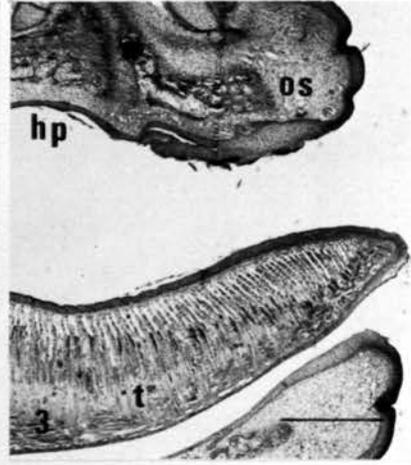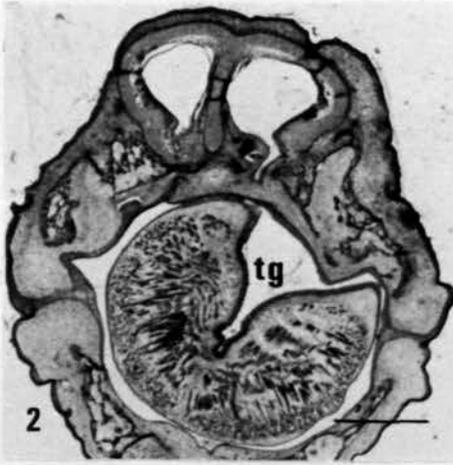
Figures 7 to 9

Figure 7   Transverse section of oesophagus of 21 day embryo. Mesenchyme surrounding stratified columnar epithelium has not differentiated. (H and E stain; scale = 50 µm).

Figure 8   Transverse section of oesophagus of 25 day embryo. A ring of myoblasts is present, indicating early differentiation of muscularis externa (arrow). The epithelium is thickened and there are numerous mitotic divisions. (H and E stain; scale = 50 µm).

Figure 9   Transverse section of oesophagus of newborn, showing intercellular vacuolations within the epithelium and extensive blood capillaries in the connective tissue below the epithelium. (H and E stain; scale = 50 µm).

bc = blood capillary; iv = intercellular vacuole; l = lumen; md = mitotic division; me = muscularis externa.

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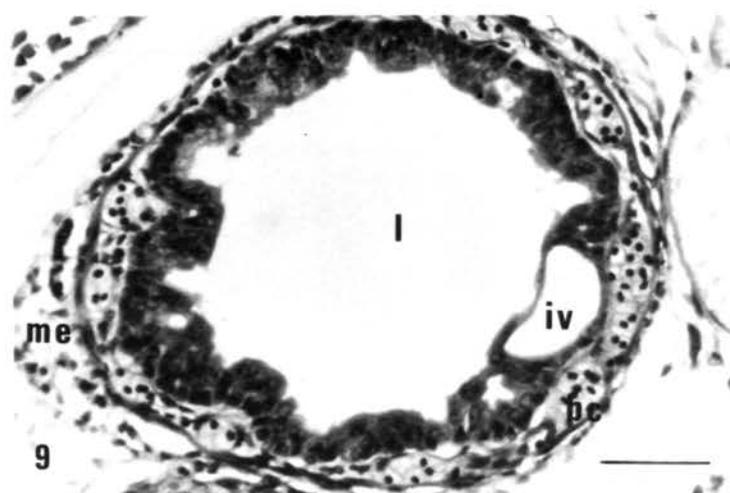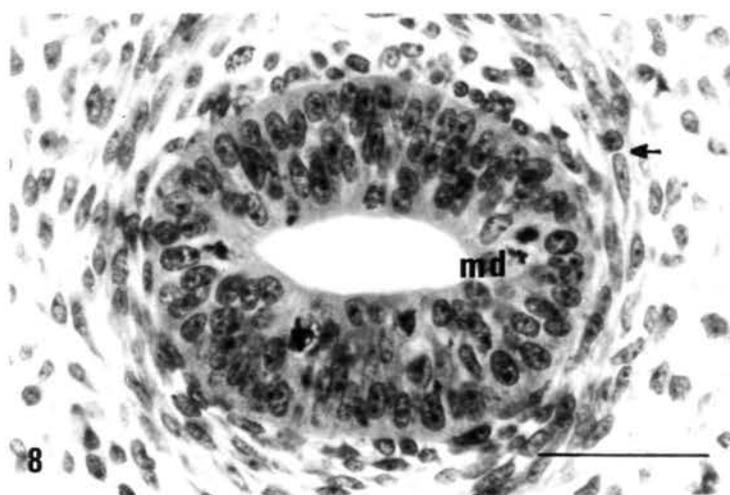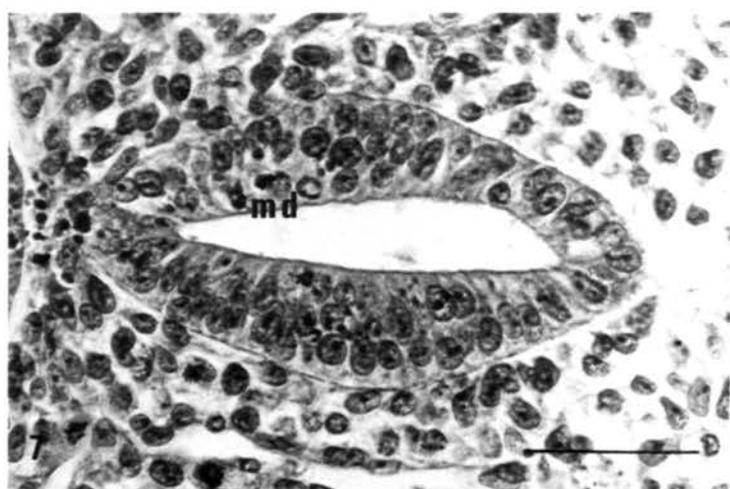
Figures 10 to 12

Figure 10   Ciliated epithelial cells lining oesophageal lumen. Anterior end of oesophagus of newborn. (H and E stain; scale = 10 µm).

Figure 11   Transverse section of part of oesophagus of 5 day pouch young showing numerous goblet cells in the luminal layer of the epithelium. (PAS/H/Fast green stain; scale = 50 µm).

Figure 12   Transverse section of part of oesophagus of 20 day pouch young, with increased stratification of epithelium and layers of muscularis externa. (H and E stain; scale = 50 µm).

c = cilia; gc = goblet cell; me = muscularis externa; st = stratified columnar epithelium.
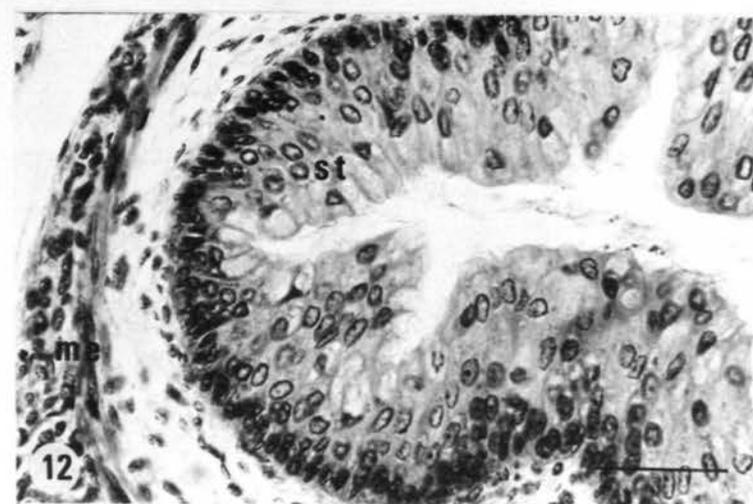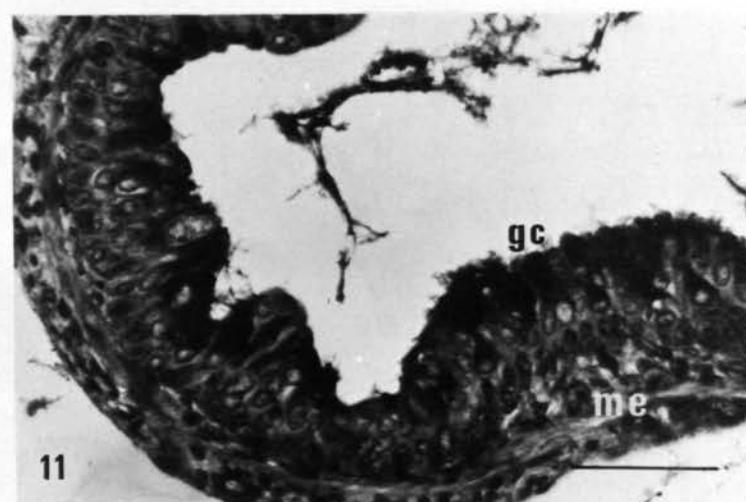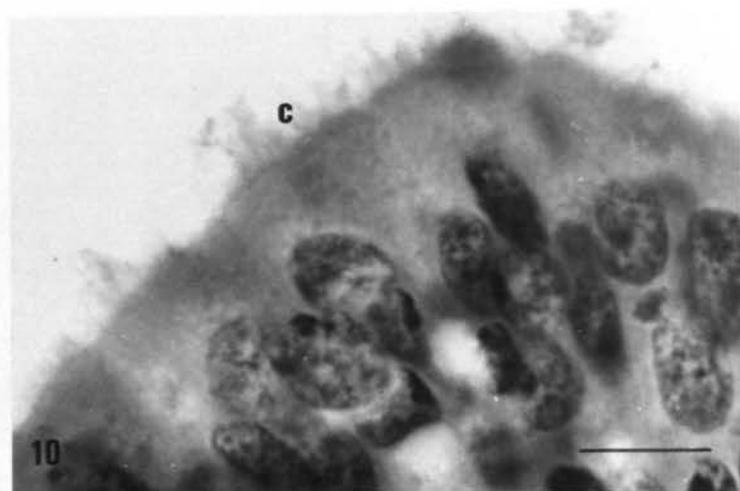
Figures 13 to 16

Figure 13   Upper surface of epithelial lining in proximal region of oesophagus of 40 day pouch young. Long, densely-packed ciliated cells are predominant in this area. (SEM x 1,070).

Figure 14   Upper surface of epithelial lining of oesophagus of 40 day pouch young posterior to region shown in Figure 13 (above). Ciliated cells are less frequent. (SEM x 1,150).

Figure 15   Ciliated cell from oesophagus of 40 day pouch young, enlarged from lower left of Figure 14 (above). Long cilia project into lumen between enlarged microvilli. (SEM x 7,070).

Figure 16   Upper surface of epithelial lining in distal region of oesophagus of 40 day pouch young. All cells have microvilli and are rounded or polygonal in shape. Large polygonal cells are corrugated. Ciliated cells are absent. (SEM x 2,235).

c = cilia;   cc = corrugated cell;   mv = microvilli.
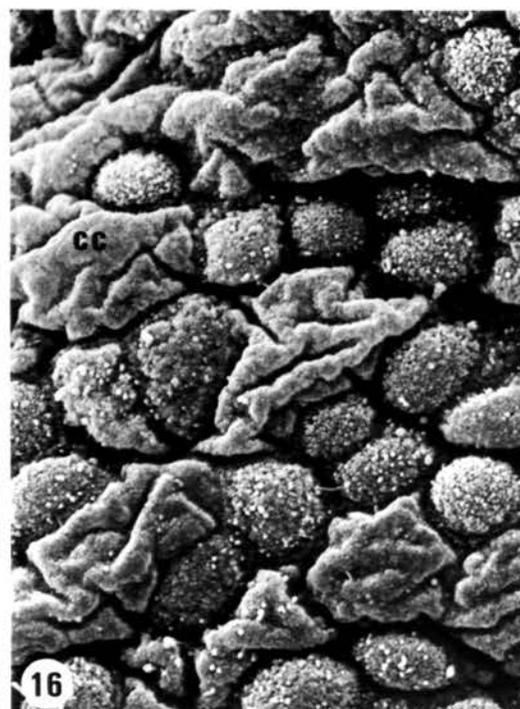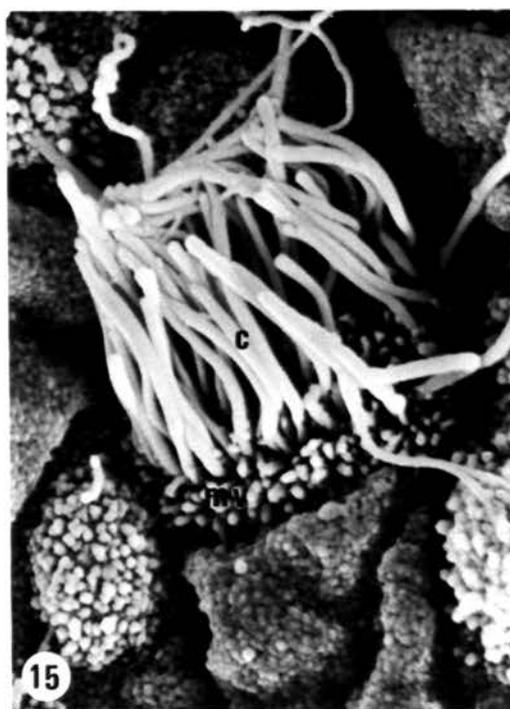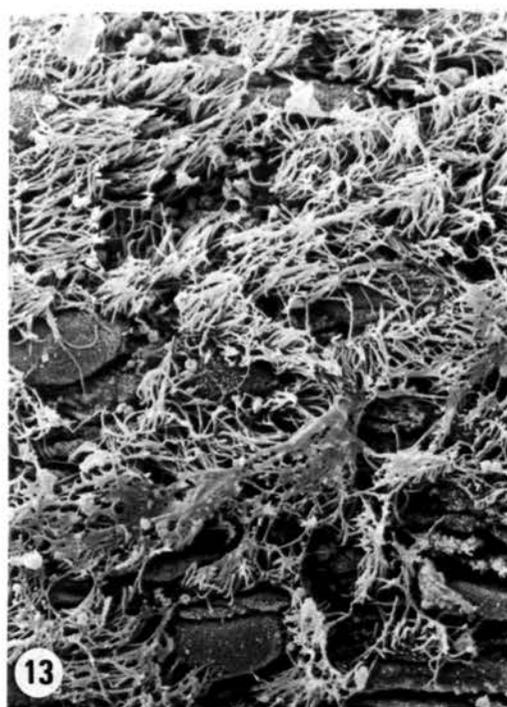
3.2 Discussion

In mammals, the squamous epithelium of the mouth and oesophagus is continuous with the epithelium covering the body surfaces. However, as pointed out by Balinsky (1960), the epithelium which covers the external surfaces and lines the mouth is embryologically different from the lining of the oesphagus. The stratified squamous epithelium of the epidermis and oral mucosa is ectodermal in origin in mammals while that of the oesophagus is derived from endoderm (Parakkal, 1967).

The small rounded mouth and fused lips of the newborn are well adapted for sucking onto the nipple and for retaining the expanded nipple inside the buccal cavity. Other features also promote attachment to, and retention on the nipple, such as the relatively extensive buccal cavity and the muscular, grooved tongue which Hill and Hill (1955) suggested was capable of a suction pump-like action. The milk passing to the oesophagus is prevented from entering the glottis partly by the architecture of the tongue. Although the anterior region is semi-tubular in form because of the groove, the posterior region is flattened, leaving a narrow passage between it and the ventral surface of the hard palate. The well developed epiglottis extends anteriorly and dorsally so that the glottis opens into the nasopharynx. In this way, the young can respire while suckling.

The development of the various types of tongue papillae is far from complete in early pouch young. The tongue of adult marsupials has been extensively investigated (Poulton, 1883; Sonntag, 1924; Kubota et al., 1963; Krause and Cutts, 1982). All species examined possess keratinised filiform, fungiform and foliate papillae widely distributed over the upper surface and three larger circumvallate papillae in the rear which along with the fungiform type contain taste buds. The developmental histology of the marsupial tongue has not, however, been described. It is clear that papillar differentiation is incomplete and

keratinisation weak in early pouch young of the tammar; indeed, not all types of papillae are represented at this stage.

The limited observations on the tammar presented here also show that a patch of conical papillae near the tip of the tongue in early pouch young may be unusual in macropodids. A comparable patch has so far been reported only for three species of didelphids (Krasue and Cutts, 1982). It would be of interest to determine whether this patch is retained in the adult tammar tongue.

The developmental changes in the epithelium lining the mammalian oesophagus are unique. The general pattern of histogenesis is that a multilayered columnar epithelium is transformed on its outer surface into a ciliated epithelium and is later replaced by stratified squamous epithelium. In some mammals typical ciliated cells are retained throughout embryonic development while in others (such as the rat) the transient cilia have been described as atypical in shape and lack the characteristic basal body (Ševčenko and Vacek, 1973). There is also great variation in the extent of conification of the stratified squamous epithelium later in development.

Amongst marsupials, the only species for which detailed histological observations have been made on the developing oesophagus is the North American opossum (D. virginiana) (Krause et al., 1976). At birth, the differentiation of the oesophagus of the tammar resembles that of the opossum in many respects. Given the relatively short gestation periods in marsupials, it is not unexpected that the level of organization in the newborn marsupial is less advanced than in eutherians, even in such short gestational eutherians as rodents. Basically, in both marsupials examined, the oesophagus at birth consists of an epithelium two or three cells deep surrounded by a delicate mesenchyme outside of which is a fine muscularis externa of only a few loosely-knit myoblasts.

Despite the general similarities, however, the tammar oesophagus at birth

shows some differences from that of the opossum. This again might be anticipated in view of the gestational period of the opossum (12.5 days) being less than half the length of that of the tammar (28 days). For example, the newborn opossum appears to lack the intercellular vacuolation of the epithelium which is an obvious feature of the tammar. It seems plausible to suggest that this vacuolation is a mechanism which allows for rapid expansion of the epithelium and its absence in the opossum may simply be a reflection of a slower rate of increase in diameter of the oesophageal lumen. Similar vacuolation has been reported in the embryonic oesophagus of the hedgehog, pig, rat, rabbit and human (Johns, 1952; Grand et al., 1976). Long ago, Johnson (1910) noted that such vacuoles in human embryos would disappear by breaking into the lumen and cause reduced thickness of the epithelium and increased size of the lumen.

No reference is made by Krause et al. (1976) to the presence of ciliated cells in the newborn opossum oesophagus. This difference compared with the tammar is short-lived however, since by 10 days after birth ciliated cells were found between flattened superficial cells which line the opossum oesophagus. In additon to cilia, the opossum cells (like those in the tammar) often show numerous elongated microvilli. Although cilia apparently develop later in the opossum oesophagus they persist into the adult stage, unlike the tammar. The persistence of cilia in the oesophagus of the opossum (in the depths of the transverse folds in the distal parts of the organ) is regarded as unique among mammals (Krause et al., 1976). Some time after 40 days in the tammar the cilia are lost since, according to Obendorf (1984), the entire adult oesophagus is lined with finger-like papillae, but no indication of these structures was evident at 40 days.

Development of the oesophageal epithelium of the tammar differs from that of the opossum in that the tammar never develops oesophageal glands. The oesophageal glands of the opossum begin as solid outgrowths from the base of the oesophageal epithelium at about 20 days after birth and continue their

development during the rest of postnatal life. Oesophageal glands in other mammals such as the pig and man (Johnson, 1910; Mottet, 1970) usually develop from similar outgrowths of the epithelium in the prenatal period and consist of mucous and serous cell types. The tammar, along with more than 30 other macropodid species studied by Obendorf (1984) lacks oesophageal glands and thus their mucus contribution to food entering the stomach. Possibly the enlarged and active salivary glands of macropodids more than compensate for the deficiency in oesophageal glands. A similar teleological explanation may account for the fleeting appearance of distinct goblet cells during oesophageal development in the tammar.

Another feature of the tammar which distinguishes it from the opossum is the failure of the muscularis mucosae to develop during early development. In most adult macropodids a prominent muscularis mucosae is present, consisting of smooth muscle fibres but in a few wallaby species, including the tammar, no muscularis mucosae is present in the proximal half of the oesophagus while the distal half contains a novel skeletal muscle layer (Obendorf, 1984). These skeletal muscle elements presumably develop later than 40 days after birth in the tammar.

Chapter 4

DEVELOPMENT OF THE STOMACH

4.1 Results

4.1.1 General anatomy

Extensive morphological changes took place in the stomach during late embryonic development and early pouch life. In Figure 17 major changes in size and shape are shown in line diagrams from camera lucida drawings, representing various ages from newborn to 40 days. In late embryos the stomach was small, tubiform in shape, and smooth in outline. Shortly after birth it was already considerably wider than the oesophagus and slightly curved, establishing clearly the greater and lesser curvatures. Rapid increase in size and degree of curvature took place so that in 20 day pouch young it was turned ventrally into a single-twist coil. By 40 days the coiling was even more pronounced and the enlarged greater curvature, which was smooth in the previous stages, was sacculated in appearance.

Also included in Figure 17 is a schematic distribution of the types of mucosal epithelium lining the various surfaces of the stomach. On this basis, four regions of the stomach are recognised, namely, the oesophageal, cardiac, fundic and pyloric regions. Delimitations of these regions were arrived at from reconstructions of paraffin-sectioned material.

Histological differentiation of the epithelium of the various regions of the stomach showed even more spectacular variety than morphological change and was already in train in the newborn. As late as 25 days' gestation (i.e. 3 days before birth) the stomach was lined with columnar epithelium of two to three layers of cells. The luminal surface was smooth, and the basal surface rested on a distinct basement membrane which was surrounded by dense mesenchyme. Three

Figure 17   Dorsal and ventral views of the developing stomach of pouch
young: (i) newborn, (ii) 5 days, (iii) 10 days; (following
page) (iv) 20 days, (v) 40 days.

hatched = oesophageal region; clear = cardiac region;
heavy stipple = fundic region; light stipple = pyloric region.

DORSAL
VIEW

VENTRAL
VIEW

(i)
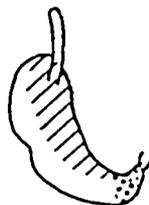
(ii)
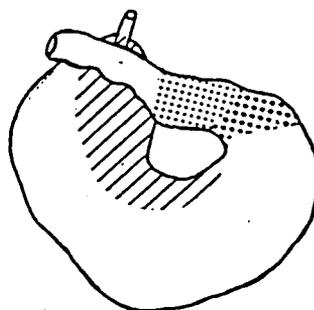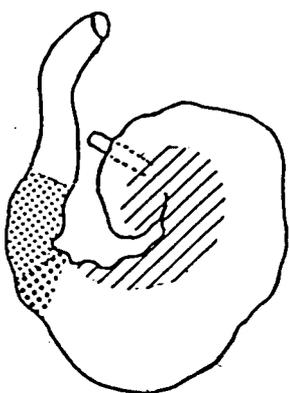
(iii)
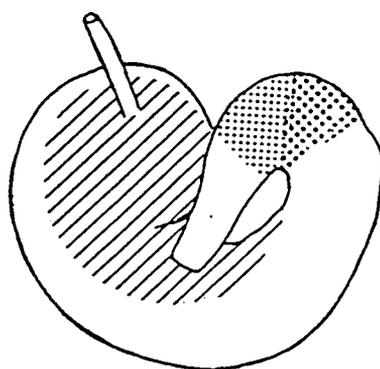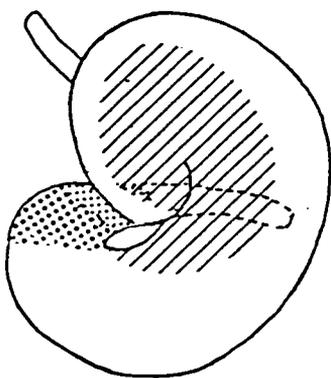
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DORSAL
VIEW

VENTRAL
VIEW

(iv)
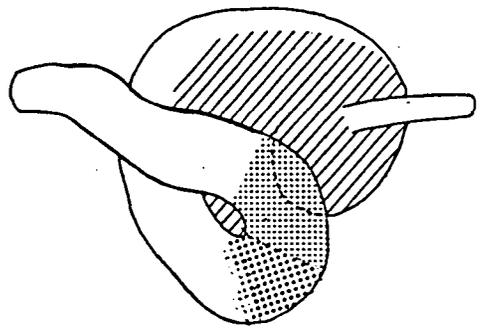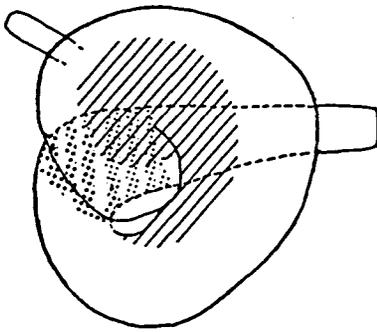
(v)
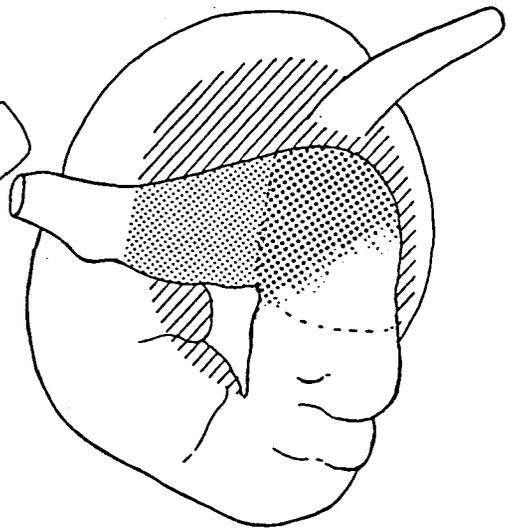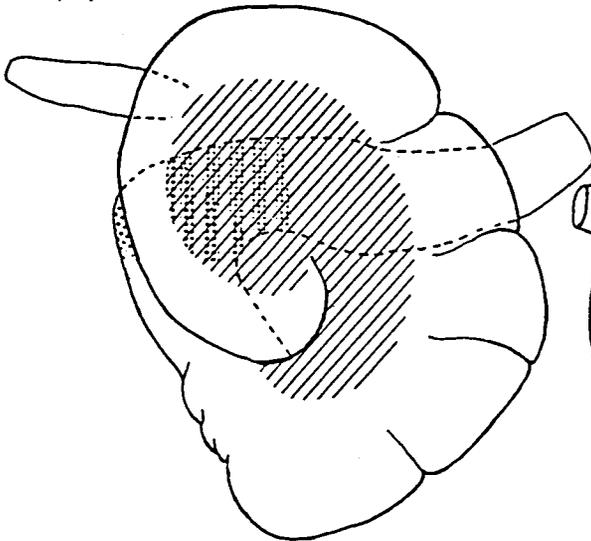
5 mm

days later at birth, the luminal surface of the stomach was thrown into numerous irregular folds (Figure 18). Along the lesser curvature, the stomach wall was thicker and the mucosal folds larger and deeper than those along the greater curvature.

For a description of the developmental changes in the mucosal epithelium of the stomach, the four regions mentioned earlier, i.e. the oesophageal, cardiac, fundic and pyloric regions will be treated in that order. This division was recognised by Gemmell and Engelhardt (1977) and was preferred to others employed by workers using criteria more relevant to the adult stomach.

4.1.2 Oesophageal region

In the newborn, the oesophageal region (Figure 17) already possessed a distinct groove in the mucosal stratified columnar epithelium called the gastric sulcus (Figures 18 and 19). Both light and scanning electron microscopy revealed two lateral folds formed as protrusions of the mucosa into the lumen along the lesser curvature. These folds represented the lips of the gastric sulcus. The basal layer of cells in the gastric sulcus and lips was composed of closely-packed, low columnar cells, with oval nuclei basally. The luminal layer consisted of tall columnar cells with oval or rounded nuclei, also located basally. Light staining of PAS-positive material was found at the luminal surface. The sulcus extended from the entrance of the oesophagus towards the posterior end of the lesser curvature. The groove was shallower and the lips thinner towards the pyloric end (Figure 19). The epithelium peripheral to the sulcus and lips was very similar to that of the distal oesophagus.

At five days the gastric sulcus was larger and deeper than in the newborn. The surface of the entire oesophageal region, including the sulcus, was no longer smooth but showed numerous small crevices in the thickened epithelium. Cells of

the luminal layer were more strongly stained with PAS. Intercellular vacuolations of the epithelium were still present and at 10 days were more numerous, particularly in the region outside the gastric lips. Under the scanning electron microscope the surface epithelial cells were polygonal in shape and densely covered with microvilli.

Further increase in the intensity of PAS staining of mucus could be seen in luminal epithelial cells at 20 days. Most of the cells had somewhat compressed nuclei towards the basal end while the expanded apical end was well supplied with PAS-positive mucus. In some of the cells, mucus could be seen protruding or already extruded from the plasma membrane into the stomach lumen. Occasional groups of luminal cells were flattened with their long axes parallel to the epithelial surface.

The main differences between the oesophageal region of the 40 day pouch young and earlier stages were the structural organization of a squamous epithelium (Figure 21), the increased number of PAS-positive cells and a more developed gastric sulcus. At the periphery of the oesophageal region there were numerous intercellular vacuoles. The thickness of the epithelium and its stratified nature decreased further away from the sulcus and finally graded into the simple columnar epithelium of the surrounding cardiac region. Mucosal folds were still present lining the floor of the gastric sulcus in scanning electron micrographs (Figure 20) but the sulcus surface was slightly flattened compared with earlier stages.

4.1.3 Cardiac region

The cardiac region occupied much of the inner surface of the greater curvature of the stomach (Figure 17). In the newborn, the luminal surface of the epithelium was undulating with shallow pits between the ridges. The epithelial

cells covering the free surface of the mucosa and lining the gastric pits were uniform in structure. They were of the simple columnar type, with an oval nucleus situated in the middle of each cell and the luminal surface covered with a few short microvilli (Figure 22). The cytoplasm contained dispersed free ribosomes, a few rounded to elongated mitochondria mostly in the apical region, and rough endoplasmic reticulum in the supranuclear region.

At the base of some of the gastric pits, occasional cells close to the basement membrane were different from the rest and probably represented early stages in the differentiation of parietal cells. Under the light microscope (Figure 25) they were rounded with large, centrally-located nuclei and cytoplasm that stained intensely with eosin, orange G and methylene blue. Although the cell bodies were located near the basement membrane, it was clear from transmission electron micrographs that each cell extended to the free surface of the pit (Figure 23). The free surface was irregular in shape and covered with slender, smooth-surfaced microvilli.

Mature parietal cells possess secretory canaliculi which extend into the cell from the free surface. They were not observed in the newborn but were observed in older pouch young in cells of similar structure and identical location. Accordingly, these cells are regarded as presumptive, immature parietal cells. The cell membranes on the lateral and basal surfaces were frequently infolded. Rounded to elongate mitochondria were concentrated in the apical region. The cytoplasm was rich in free ribosomes and rough endoplasmic reticulum with the latter especially located basally and laterally.

In the 5 day pouch young, the depth of the pits and the number of young parietal cells had increased. The wedge-shaped cells were more clearly defined between the bases of the other "undifferentiated" epithelial cells. From light microscope observations, the surface epithelial cells lining the cardiac region in

10 day pouch young gave a slight PAS-positive reaction. Vacuoles were present above the nuclei, an observation confirmed by the occurrence of small membrane-bound, granule-rich vesicles in transmission electron micrographs. Other conspicuous non membrane-bound bodies containing material of light electron density (probably lipid) were found in the supranuclear cytoplasm. However, the most important observation at this stage was that a few cells regarded as putative parietal cells were found to contain small secretory canaliculi covered with sparse, relatively long microvilli.

At 20 days, many surface cells were more intensely PAS-positive. In transmission electron micrographs there was a marked increase also in the number of vesicles with secretory granules towards the apical surface. Parietal cells were now readily distinguishable with light and electron microscopy. In transmission electron micrographs of parietal cells (Figure 24), the cytoplasm was extremely well supplied with mitochondria, the secretory canaliculi were much expanded and the long microvilli lining them were more numerous. Adjacent parietal cells were often at quite different stages of differentiation. In 40 day pouch young (Figure 26), the free surface epithelial cells were morphologically similar to earlier stages. Cytological differences included increased numbers of PAS-positive cells (seen as secretory granules in plastic-embedded sections) and numerous lipid-like bodies in supra- and subnuclear regions. Gastric pits were deeper and parietal cells more numerous. They were found not only at the bottom of the pits but also along the sides between the PAS-positive cells.

4.1.4 Fundic region

In the newborn, no distinctive boundary was discernible between the cardiac and fundic regions since the lining of both regions appeared the same, namely, simple columnar epithelial cells with centrally-located nuclei arranged as an undulating layer with shallow pits. In fact, the development of the fundic

region up to 20 days was much the same as that of the cardiac region as indicated by the graded stippling in Figure 17.

In the 20 day and 40 day pouch young, while much of the fine structure of the various cell types in the fundic epithelium resembled the cardiac region, marked differences in the organization of the epithelium were evident. For example, the entire mucosa was distinctly folded and variable in height and the gastric glands were relatively long (Figure 27) and gastric pit openings distinct (Figure 28). The cells in the neck of the developing gastric glands were similar to the surface epithelial cells, except for a reduction of membrane-bound vesicles in the apical cytoplasm and the presence of short irregular microvilli on the free surface. These cells probably represented differentiating mucous neck cells in the developing gastric glands.

Below the neck in the gastric glands there were two types of cells. One type, still undifferentiated, was distributed amongst the parietal cells. The parietal cells were similar to those in the cardiac region. The nuclei were indented, mitochondria were present in large numbers, endocytotic vesicles occurred immediately below the surface microvilli, interdigitation of adjacent cell membranes was common and secretory canaliculi with their lining microvilli were densely packed in the basal cytoplasm. All the above features of fundic region cells at 20 and 40 days are shown in Figures 31 to 35.

4.1.5 Pyloric region

Unlike the cardiac-fundic boundary, the fundic-pyloric boundary was clearly defined from the beginning of pouch life. In the newborn, the mucosa of the pyloric region was already thrown into folds and deep pits (Figure 29). This region could be characterised not only by having a thick, folded mucosal wall but also by possessing a simple epithelium of columnar cells with nuclei flattened against the base.

Like other regions of the stomach, mucous secreting surface epithelial cells became more prominent, and their intracellular vacuoles more numerous, during development up to 40 days.

Ultrastructurally the mucous cells lining the developing pyloric glands in 40 day pouch young were identical with the mucous neck cells of the fundic glands. The surface epithelial cells in the pyloric region were similar to those in the cardiac and fundic regions. Compared with mucous gland cells, they were taller and contained more dense secreting granules.

Mucosal folding and pyloric gland deepening characterised development at 40 days. Scanning electron micrographs showed clearly the extent of the folding (Figure 30). In addition to mucous secreting cells deep in the glands, the epithelium also contained undifferentiated cells and solitary endocrine cells whose cytoplasm was generally less dense than that of surrounding cells but contained numerous dense secretory granules.

4.1.6 Outer layers of the stomach

The remaining layers of the stomach wall showed a greater degree of developmental uniformity than the epithelial lining.

At birth, no muscularis mucosae was present between the lamina propria and the submucosa. The other muscular layer of the stomach wall, the muscularis externa, had developed but its differentiation was far from complete. It consisted of a circular layer of smooth muscle three cells in depth with elements of the myenteric plexus arranged around the outside. The circular layer was not enlarged at the distal end of the pyloric region to form a pyloric sphincter but it was more clearly delineated than in other regions of the stomach. The outermost layer of the stomach, the serosa, was represented by a thin layer of connective tissue bounded by mesothelium. At five days the muscularis mucosae had still not

appeared, while the muscularis externa had continued to develop with a new outer thinner layer of longitudinal smooth muscle cells arranged outside the myenteric plexus.

In the older stages examined, the muscularis mucosae was not identified until 40 days and then only as a ring of scattered myoblasts. On the other hand, the muscularis externa continued to grow and develop. The inner circular muscle layer remained thicker than the outer longitudinal layer and the interposed myenteric plexus had expanded. The serosa appeared to be unchanged at 40 days.

The pyloric region at 40 days was the most thick-walled region of the stomach, with the lamina propria, submucosa, and muscularis externa all thicker than in other parts. The circular muscle layer of the muscularis externa at the distal end was sufficiently robust to be regarded as pyloric sphincter (Figure 38).

Figures 18 to 21

Figure 18   Transverse section of stomach of newborn with a prominent gastric sulcus. (H and E stain; scale = 0.25 mm).

Figure 19   Inner surface of stomach of newborn. The gastric sulcus is clearly defined. (SEM x 20).

Figure 20   Gastric sulcus of stomach of 40 day pouch young is larger and deeper than in younger stages. Undulating surface of sulcus is clearly different from that of sulcus lips. (SEM x 50).

Figure 21   Longitudinal section of wall of gastric sulcus of 40 day pouch young, showing early stage in transformation of stratified columnar epithelium to stratified squamous epithelium. Flattening of epithelial cells can be seen in the luminal layer. (H and E stain; scale = 50 µm).

cl = circular layer of muscularis externa; gs = gastric sulcus; l = lumen; ll = longitudinal layer of muscularis externa; sl = sulcus lip; st = stratified columnar epithelium.
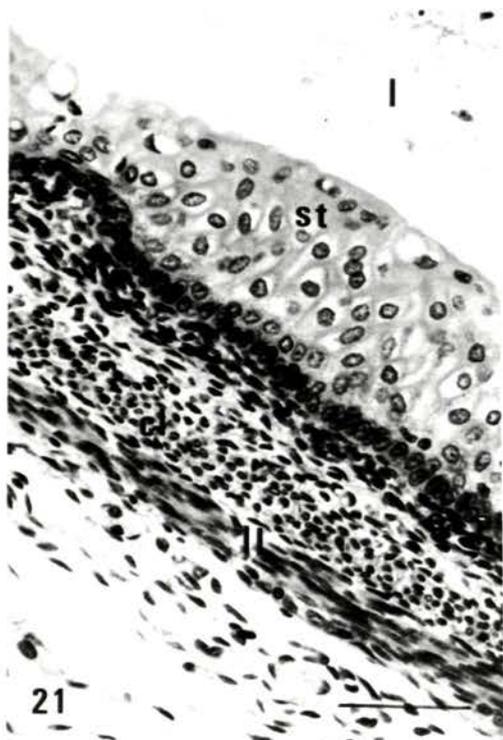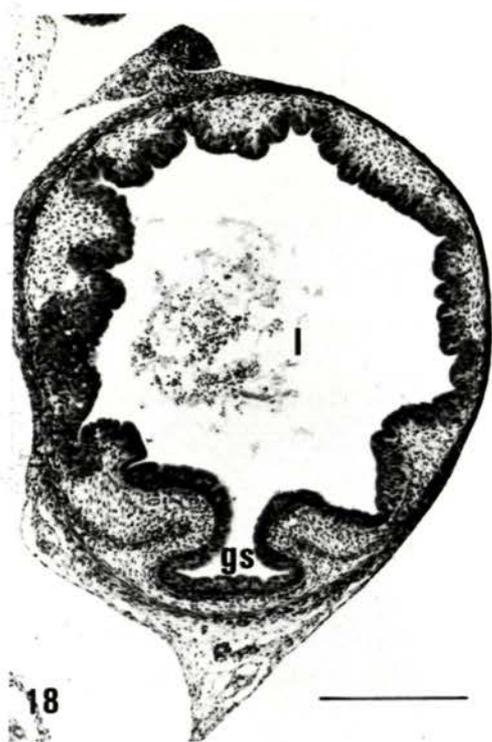
Figures 22 to 24

Figure 22   Surface epithelial cells lining cardiac region of newborn
stomach. Cells are of simple columnar type with occasional
microvilli on luminal surface. Mitochondria are mainly in
the apical cytoplasm. (TEM x 6,800).

Figure 23   Early development of parietal cell at base of an invagination
of cardiac stomach epithelium of newborn. Free surface of
parietal cell is covered with slender, densely-packed
microvilli. Mitochondria are present mainly in the apical
cytoplasm. (TEM x 6,800).

Figure 24   Parietal cells in cardiac region of stomach of 20 day pouch
young, showing different stages of development at base of a
gastric pit. Older stages of developing parietal cells exhibit
more numerous mitochondria and well developed secretory
canaliculi. (TEM x 5,100).


bm = basement membrane;   er = rough endoplasmic
reticulum; l = lumen; m = mitochondria; mv = microvilli;
nu = nucleus; sc = secretory canaliculi.

22

23

24
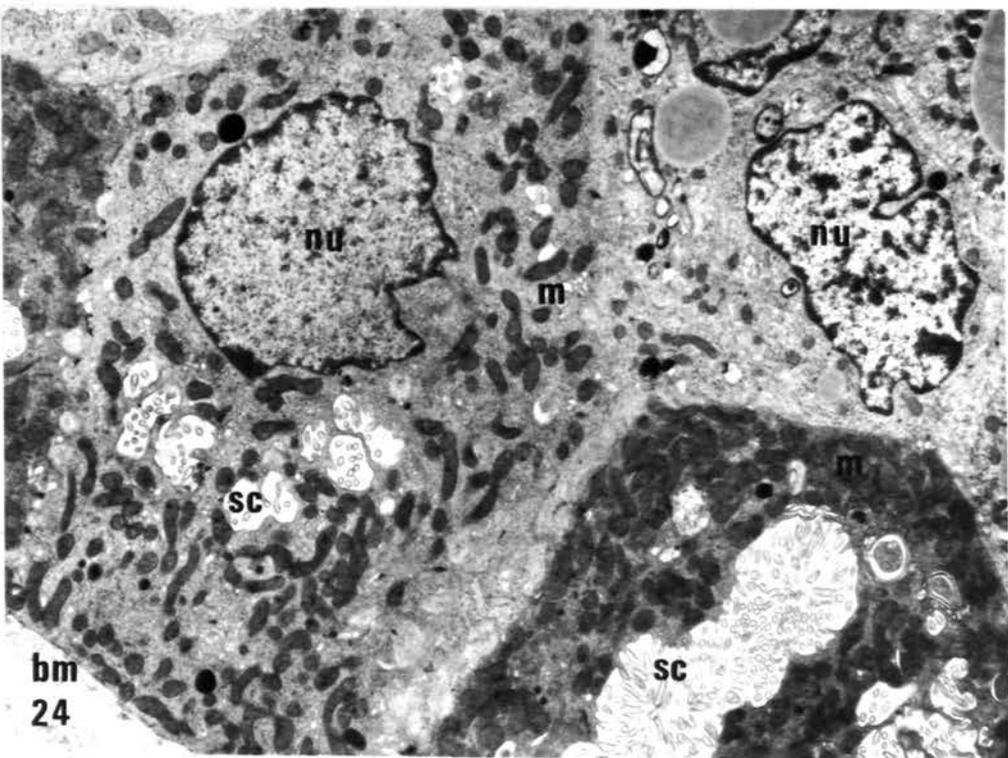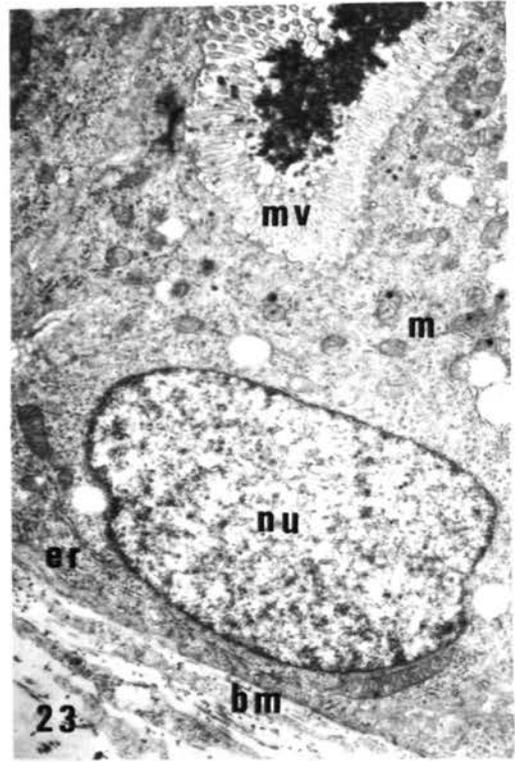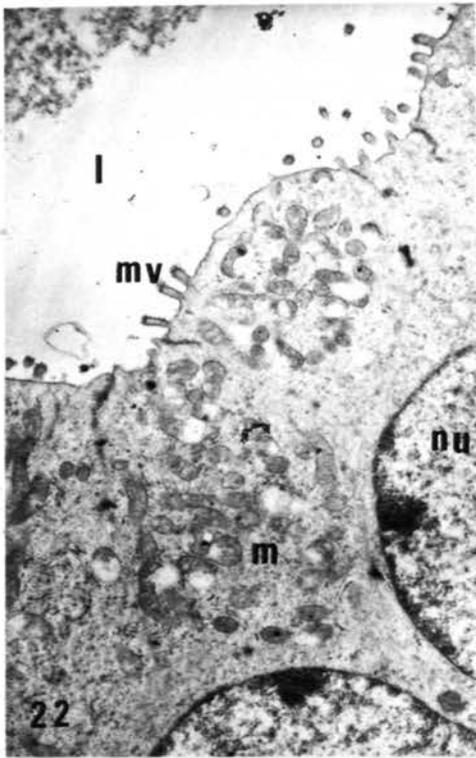
Figures 25 to 30

Figure 25   Transverse section of epithelial cells lining cardiac region of stomach of newborn, showing undulating epithelium with shallow gastric pit between ridges. A parietal cell is present in basal region among undifferentiated epithelial cells. (Plastic embedding; methylene blue stain; scale = 20 μm).

Figure 26   Transverse section of cardiac stomach of 40 day pouch young. Many parietal cells are present in gastric pits. Numerous secretory granules are located in surface epithelium and lipid bodies are coumon in supra- and sub-nuclear regions. (Plastic embedding; methylene blue stain; scale = 50 μm).

Figure 27   Longitudinal section of fundic glands in stomach of 20 day pouch young. Gastric glands elongated and parietal cells (arrows) occur on basal and lateral margins of pits. (H and E stain; scale 50 μm).

Figure 28   Fundic stomach of 20 day pouch young. A few small gastric pit openings (arrows) present between epithelial cells. (SEM x 840).

Figure 29   Transverse section of pyloric stomach of newborn, showing extensive folding of mucosa. (H and E stain; scale 50 μm).

Figure 30   Upper folded surface of pyloric mucosa of 40 day pouch young. (SEM x 67).

gp = gastric pit; lp = lipid body; me = muscularis externa; pc = parietal cell; sg = secretory granules.

25

26

gp

lh

sg

pc

me

27

28

29

30
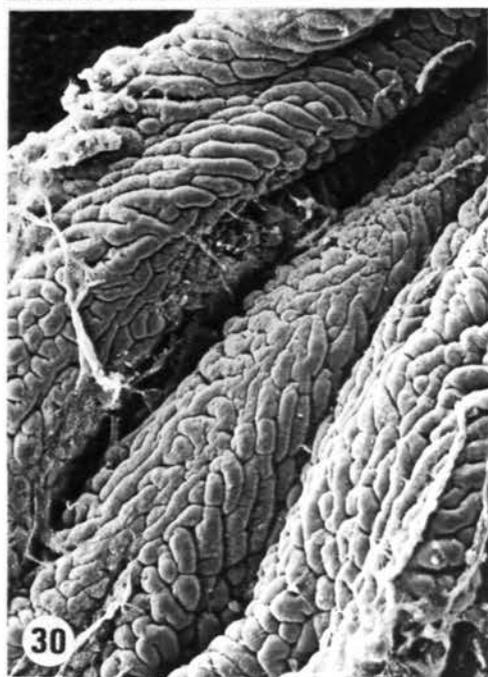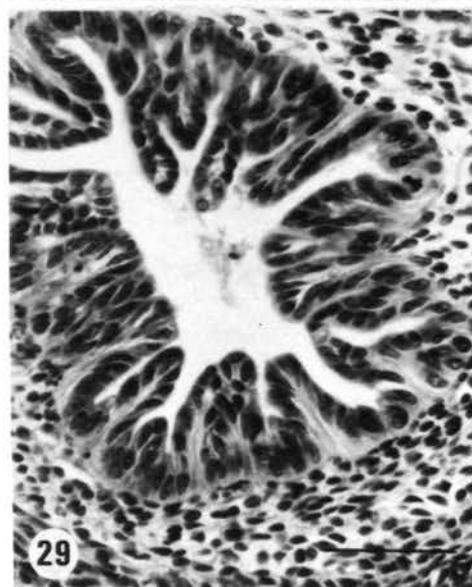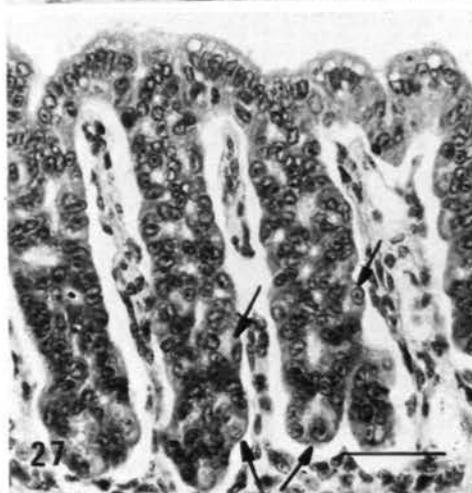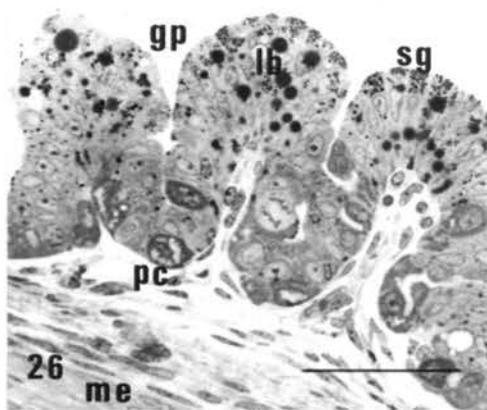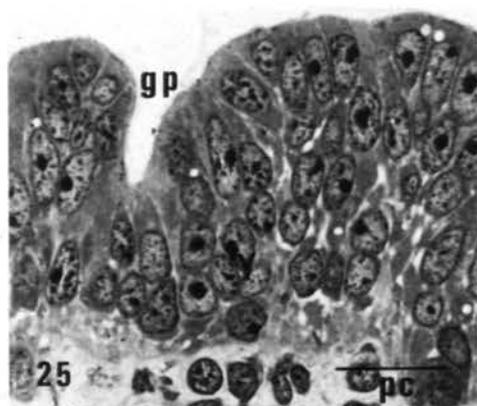
Figures 31–33

Figure 31   Apical surface of developing parietal cell from fundic region of 40 day pouch young. Young parietal cells have indented nuclei, many mitochondria and vesicles below microvilli. (TEM x 1,040).

Figure 32   Basal portion of parietal cell from fundic stomach of 20 day pouch young. Note extensive secretory canaliculi lined with abundant microvilli and numerous vesicles, along with mitochondria. (TEM x 7,650).

Figure 33   Basal portion of parietal cell from fundic region of 40 day pouch young, with well developed secretory canaliculus with microvilli. (TEM x 6,680).

m = mitochondria; mv = microvilli; nu = nucleus;
sc = secretory canaliculus; ve = vesicle

mv

ve

m

nu

31

32

ve

mv

m

33

mv   sc

m
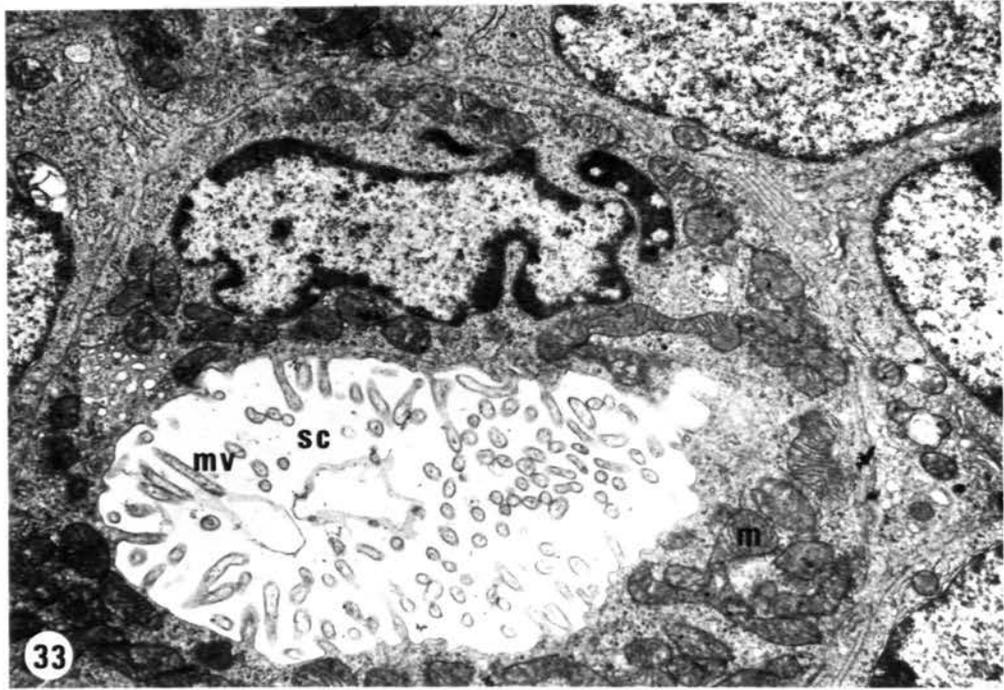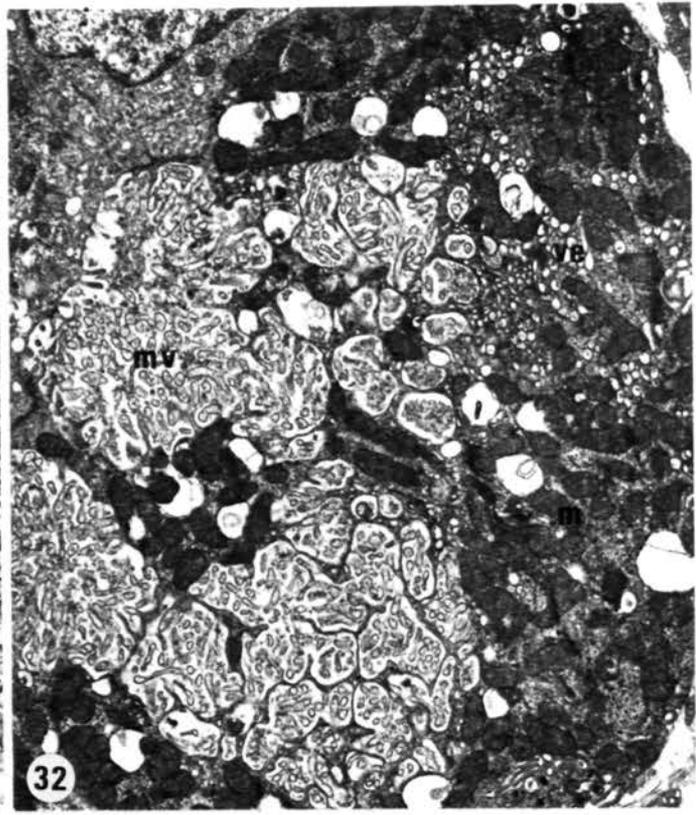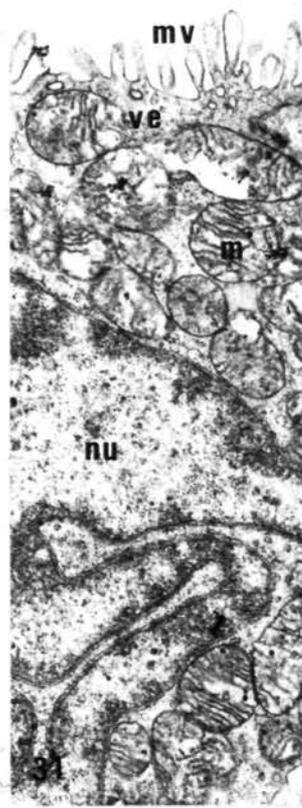
Figures 34 and 35

Figure 34   Surface epithelial cells of fundic stomach of 40 day pouch young. Secretory granules of varying density aggregated just below apical surface. Large lipid bodies in supra-and subnuclear cytoplasm. Interdigitation of lateral cell membranes in perinuclear region. (TEM x 5,965).

Figure 35   Basal region of fundic gland from 40 day pouch young stomach. Several undifferentiated cells are shown together with a young parietal cell (upper right) facing free surface of lumen. (TEM x 5,950).

cm = cell membrane; lb = lipid body; mv = microvilli; nu = nucleus; sg = secretory granules

34

35
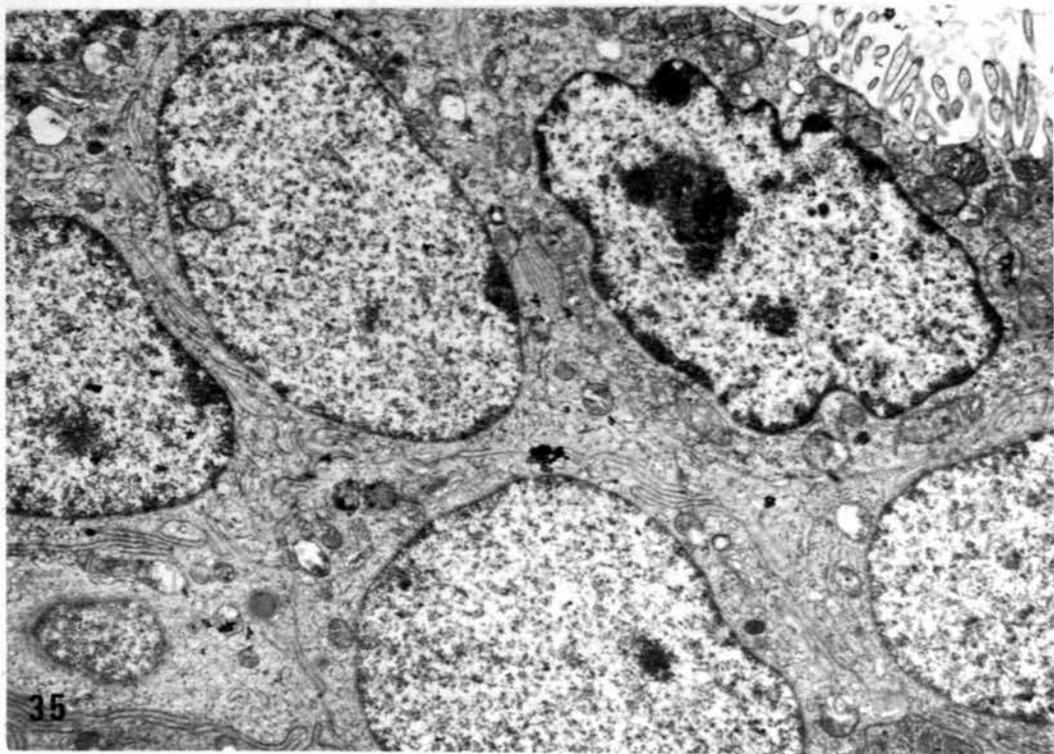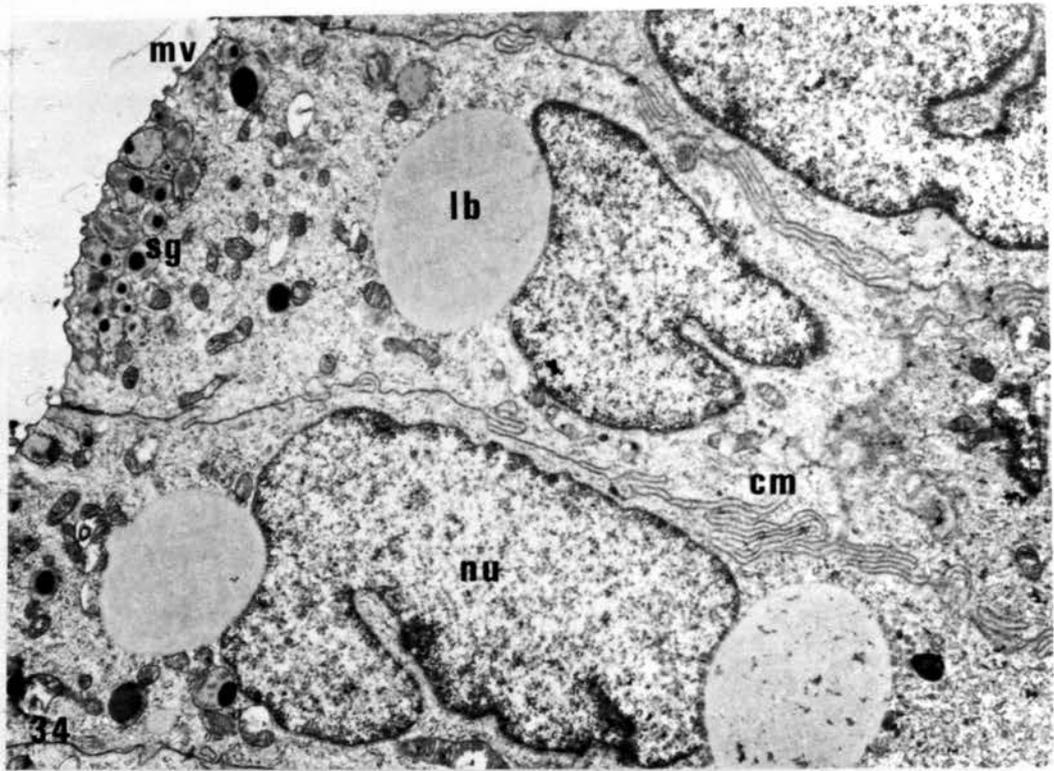
4.2 Discussion

The structure and functions of the adult tammar stomach have been described recently in some detail (Gemmell and Engelhardt, 1977; Langer et al., 1980; Richardson, 1980; Hume, 1982) and the following brief review of the salient features in those descriptions provides a better basis for interpretation of the early developmental events. In the adult tammar stomach, the areas occupied by the various regions are, approximately: oesophageal, 20 per cent; cardiac, 65 per cent; fundic and pyloric regions, 15 per cent. The oesophageal region is lined by cornified, stratified, squamous epithelium. Much of it is occupied by a well developed gastric sulcus which extends from the opening of the oesophagus along the lesser curvature. The sulcus probably plays a role in nutrition by assisting in the caudal movement of liquid digesta to the distal parts of the stomach which may be important in maintaining microbial fermentation rate. The cardiac region makes up the major part of the stomach wall and produces large amounts of mucus. The epithelium, largely contained in cardiac glands, is richly supplied with mucous secreting cells which contain up to three kinds of mucigenous granules. In the fundic region there are mucous secreting cells on the surface and in the neck of the abundant gastric glands with acid (parietal) and enzyme (chief) secreting cells deeper in the glands. The high proportion of parietal cells explains why the pH of the stomach contents in the tammar changes from 7.0 in the cardiac region to as low as 2.0 in the fundic region. The relatively small proportion of chief cells may indicate that pepsin secretion is not as important in the tammar as it is in most other monogastric mammals. The pyloric region possesses a thick, glandular epithelium well supplied with mucigenous cells. All glandular areas of the adult stomach contain various types of endocrine cells but little is known of the interrelationships of their structure and function.

In the newborn tammar, the oesophageal region was already distinct from the remaining regions of the stomach. It was the only region lined with stratified columnar (rather than simple columnar) epithelium and thus resembled the

newborn oesophagus. The gradual transformation into stratified squamous epithelium, which had started by 40 days in the oesophageal region, was synchronous with the same changes in the oesophagus. Another feature of the newborn oesophageal region was the presence of a distinct gutter, the gastric sulcus, with well developed lips. In the newborn of Macropus rufus (the only other macropodid whose early gut development has been described in any detail), the oesophageal region was not so advanced. The epithelium was still simple columnar and the anlage of the gastric sulcus was barely visible (Griffiths and Barton, 1966).

The gastric sulcus has been suggested to function in suckling pouch young of macropodids in a way similar to that of suckling ruminants (Langer et al., 1980). It would thus allow milk to by-pass the cardiac region and pass directly from the oesophagus to the fundic stomach where it could be promptly exposed to proteolytic activity. This proposal may not be tenable, at least in tammar pouch young up to 40 days, for reasons discussed later in relation to the activities of the fundic region.

The early postnatal development of the cardiac and fundic regions was indistinguishable. Both possessed mucous secreting cells and presumptive parietal cells soon after birth. It is not uncommon in mammals for parietal cells to be present early in development of the cardiac stomach and later be lost. Examples include embryos of humans (Salenius, 1962; Nomura, 1966; Grand et al., 1976), rabbits (Hayward, 1967) and pigs (Kirk, 1910), and in newborn rats (Helander, 1969), and opossums (Krause et al., 1976). By 20 days in the tammar, in both cardiac and fundic regions, mature parietal cells with characteristic internal canaliculi and apical tubulovesicular systems were present and were even more prominent at 40 days. Increase in the volume of the tubulovesicular compartment and accumulation of microvilli in canaliculi are thought to be cytological markers of acid production (Leeson, 1974; Ito and Schofield, 1974). It would thus seem that active acid production takes place in the cardiac region of the tammar

before, and probably long after, 40 days. It is not known at what age parietal cells are lost and acid production ends in the tammar cardiac region.

The timing and location of differentiation of parietal cells in the two other macropodids so far studied may be very different from the tammar. In Thylogale thetis, Langer (1979) observed parietal cells with the light microscope at 156 days but not at 146 days of pouch life. They were recorded as being present in the fundic region only. In Macropus rufus, Griffiths and Barton (1966) found no evidence of parietal cells in electron micrographs of "glandular" regions of the stomach of pouch young up to 21 days and they were unable to identify them with the light microscope until 200 days, although stomach contents were shown to be highly acid from a very early age. Griffiths and Barton (1966) did find, however, in the early pouch young examined with the electron microscope that a single type of secretory cell occurred throughout the stomach, containing two kinds of inclusion bodies. One kind was similar to the tubules and vesicles (but not canaliculi) of acid-secreting cells, while the second kind of body was said to be similar in appearance to pepsinogen granules. This remarkable cell type was thought to exhibit the structures and functions of both parietal cells and chief cells and could thus account for peptic activity from a few days after birth. No such cells were observed in tammar pouch young; distinct parietal cells were present at 20 days but chief cells failed to appear by 40 days.

It has been suggested that the gastric sulcus may direct the flow of milk directly from the oesophagus of macropodid early pouch young to the fundic region to enhance proteolytic activity (Langer, 1979). There seems some doubt that this is an important role of the sulcus in either the tammar or the red kangaroo. In the tammar there is no evidence of pepsin-secreting cells in the fundic region (or any other region) of the stomach of early pouch young. On the other hand, in the red kangaroo the whole of the stomach appears to show peptic activity in early stages and in these circumstances the by-pass mechanism would

not increase milk utilization. It may well be more important as a mechanism for the rapid delivery of milk to the small intestine where uptake of maternally-derived immunoglobulins as well as nutrients occurs. Whatever the function of the gastric sulcus in macropodids, it is unlikely to be of profound importance. In the genus Thylogale, T. thetis and T. stigmata lack a gastric sulcus as adults or pouch young whereas it is present in the closely related T. billardieri (Langer, 1979).

The occasional endocrine cell observed in the pyloric glands of 40 day tammars was probably involved in the secretion of the hormone gastrin. In the pyloric mucosa of the postnatal mouse,similar cells are known to produce gastrin which stimulates parietal cells to secrete HCl and induces the growth of the fundic mucosa (Kataoka et al., 1985). A more extensive search of tammar gastric glands in the fundic region is required before the presence or absence of endocrine cells could be established with confidence.

The postnatal development of the stomach mucosa of the opossum, Didelphis virginiana, has been studied in some detail (Krause et al., 1976). While there are general similarities in timing and pattern of differentiation of the stomach in the opossum and the tammar, there are, not surprisingly, differences between the omnivorous didelphid and herbivorous macropodid. For example, the gastric epithelium of the newborn opossum is composed entirely of simple columnar epithelium together with some readily identifiable parietal cells. These cells actually line the lumen and are thus in direct contact with the luminal contents. Endocrine cells are also present in the newborn opossum so it would appear that on cytological grounds the opossum stomach mucosa is more advanced in its development than the tammar. There are no indications of gastric sulcus formation at any stage of opossum development. The major difference in the musculature of the stomach wall is that in the opossum, particularly in the pyloric region, the muscularis mucosae is very well developed.

Chapter 5

DEVELOPMENT OF THE INTESTINE

5.1 Results

5.1.1 Small intestine

In embryos at 21, 23 and 25 days of gestation, the small intestine was uniform in appearance throughout its length. The inner lining was composed of stratified columnar epithelium which rested on a distinct basement membrane (Figure 36). Basally in most cells there was an oval interphase nucleus but mitotic figures were not uncommon. There were no indications of villus formation. The mesenchyme cells surrounding the epithelium were evenly distributed in the 21 day embryo but at 23 and 25 days a slight condensation of cells in the form of a ring indicated the site of differentiation of the muscularis externa.

In pouch young up to 40 days the boundary between the duodenum and the ileum was morphologically indistinct. To ensure that the areas selected were typical of these two regions, histological and cytological descriptions were prepared from standard locations. Duodenal details were recorded from within the U-shaped curvature at the proximal end of small intestine while details of the ileum came from an area towards the junction with the caecum and colon. A distinct jejunum was not observed in pouch young, and even in the adult tammar it is not clearly defined (Richardson and Wyburn, 1980). The description of the development of the small intestine is arranged differently from those of the oesophagus and stomach in that the light microscope, scanning electron microscope and transmission electron microscope observations are

treated in order and each includes a description of the stages from newborn to 40 days.

5.1.1.1 Duodenum

Light microscope observations on the newborn established that relatively small but well organised villi were present. The development of fully formed villi thus takes place in a 3 day period before birth, since in the 25 day embryo they were lacking. A simple columnar epithelium covered both the villi and the intestinal floor and a prominent striated border could be seen at the luminal surface of epithelial cells of the villus, especially towards the tip (Figure 37). Some of the epithelial cells were weakly PAS-positive and probably represented early stages of differentiation of goblet cells. Another prominent feature of epithelial cell cytoplasm was the presence of numerous vacuoles in the apical and supranuclear regions. The duodenal epithelium adjacent to the gastrointestinal junction of the newborn showed invaginations into the surrounding connective tissue clustered in a restricted part of the epithelium when viewed in cross sections of the entire duodenum. These invaginations were precursors of the characteristically duodenal Brunner's glands. The wall of the duodenum outside the epithelium was unexceptional. The narrow core of lamina propria within the villi contained blood capillaries, and further capillaries were present in the connective tissue outside the villar zone. The muscularis mucosae was absent and the muscularis externa consisted of two or three layers of myoblasts. Outside the myoblasts there was a thin layer of connective tissue and a mesothelium forming the serosa.

Several changes were observed under the light microscope in 5 day pouch young. Goblet cells were distributed throughout the duodenal epithelium; vacuolation of the apical and supranuclear cytoplasm of the epithelial cells of the villi was even more prominent; and duct systems associated with the developing

Brunner's glands were composed of weakly PAS-positive low columnar epithelial cells. Also at 5 days the initial differentiation of patches of epithelial cells between the villi had occurred, representing the earliest stage of formation of the Lieberkühn or intestinal glands. The intervillus space above the developing gland is then referred to as the crypt of Lieberkühn. The groups of gland cells stained more darkly with haematoxylin and eosin than those of the villus epithelium and formed shallow invaginations into the surrounding connective tissue. The muscularis externa was now separated into inner circular and outer longitudinal muscle layers with elements of myenteric plexus distributed between the two.

In older pouch young up to 40 days, the size rather than the complexity of most of the tissues and glands increased. Goblet cell number in the epithelium increased as did Brunner's gland number. The latter occupied at least half the circumference of cross sections of the most proximal duodenum at 20 days (see Figure 38) and approximately three-fourths at 40 days. The glands of Lieberkühn were unchanged up to 40 days except for their growth deeper into surrounding connective tissue.

Scanning electron microscopy provided some important additional details concerning villus architecture. In the newborn, the villi in the proximal region of the duodenum were fewer in number and longer than in more distal duodenal regions (Figure 39). They were irregular in shape, with some flattened at their bases and others rounded. Small elevations occasionally seen in the duodenal floor represented the initial development of villi. No patterns, such as alternating rows, were observed in the arrangement of the villi and their surfaces were relatively smooth. Evidence of cell turnover of epithelium was indicated by the presence of bleb-like cells in the process of extrusion. Cells presumably are migrating from crypts in the duodenal floor (Figure 39) on to the surface of a villus to be eventually extruded as bleb-like structures. Cell extrusion was most common at the tips of villi but occurred along the villus shaft and even from the

duodenal floor. In higher power views of apical villus surface (Figures 42 and 43), intact cells were flattened and hexagonal in outline while those undergoing extrusion and cell death were spherical and roughened in appearance. These so-called effete cells were covered with much longer microvilli than the outer surfaces of normal cells. Effete cells just before detachment were held in a shallow depression which remained as a "scar" or crevice at the villus surface after cell loss.

By 20 days the absolute number of villi had increased but there were still fewer proximally than distally along the duodenum. Height and shape of villi varied considerably (Figure 40) and large numbers of effete, detaching cells were present especially over the apical surfaces. In 40 day young, villus number had again increased, villi were more uniform in shape, and the numbers of cells being extruded from the surface of the villi was reduced (Figure 41).

Transmission electron microscope observations provided important details of ultrastructural organisation particularly with respect to the villus epithelium. In the newborn, the apical surface of epithelial cells was covered with a thick compact layer of microvilli surrounded by heavy accumulations of electron-dense material (Figure 45). This compact microvillar margin gives rise to the brush border that was seen at the epithelial surface in light microscope sections. Immediately below the microvillar margin there was a cytoplasmic complex of membrane-bound vesicles and tubules. This tubulovesicular system, part of the endocytic (= endocytotic) complex, has its origins in the pinocytotic activity of the plasma membrane at the bases of the microvilli. The vesicle and tubule components of this anastomosing system close to the luminal surface often contained electron-dense material of the same appearance as that surrounding the microvilli and presumably represented the same material soon after its engulfment. Below the tubulovesicular region were located numerous vacuoles of different size with varying concentrations of dense granular material.

Mitochondria and endoplasmic reticulum were relatively common between the vacuoles. Deeper in the epithelial cells in the supranuclear and perinuclear regions there were larger vacuoles containing light to moderate electron-dense material. In the perinuclear region, smooth, expanded membrane-bound tubules were present as well as rough endoplasmic reticulum. The plasma membranes of adjacent cells were provided with tight and desmosomal junctions and extensive foldings and interdigitations with extensive areas of intercellular space between the foldings.

The fine structure of epithelial cells of the duodenum at 40 days showed considerable differences in comparison with the newborn (Figures 47 and 48). The microvilli were larger and possessed distinct electron-dense thickened apices. Covering the microvillar border was a fuzzy coat or glycocalyx clearly distinguishable in Figure 47, showing its filamentous nature and its association with the microvillus surface membrane, both at the tips and along the lateral surfaces of the microvilli. It is now known that at least some of these fibres represent carbohydrate moieties of glycoprotein digestive enzymes. Stages in the formation of pinocytotic vesicles are more clearly displayed than in the newborn and the vesicular component of the tubulovesicular system is the more prominent. Many other organelles were more prominent than in the newborn and were indicative of high levels of metabolic activity. Mitochondria, Golgi membranes, ribosomes, rough endoplasmic reticulum, large and small vacuoles and anastomosing smooth membrane tubules were all conspicuous. Also in the perinuclear and subnuclear regions of the cytoplasm, bodies made up of aggregations of droplets were a regular component of cells at 40 days.

5.1.1.2 Ileum

Although the boundary between duodenum and ileum was not morphologically distinguishable, there was a steady reduction in the diameter of

the small intestine in a posterior direction so the ileum was smaller in cross section than the duodenum. In general, the ileum had a similar pattern of development to that of the duodenum but certain cytological differences were noted and are emphasised in the brief account given below.

In light microscope sections of the newborn, the ileal villi were rather more blunt and tended to occlude the lumen more than in the duodenum (Figure 49). At 5 days, goblet cells were present between the absorptive columnar cells as in the duodenum at the same age, but they were present in greater numbers in the ileum. Brunner's glands never develop in the ileum. Scanning electron micrographs of the developing ileum were similar to those taken of the duodenal lining.

Ultrastructurally, the tubulovesicular system below the tightly-packed microvilli tended to be more extensive in the newborn ileum (Figure 53) than in the duodenum and also more complex in its anastomosis. The vacuolar component was likewise more extensive in the ileum and the vacuoles appeared to contain more electron dense material. By 10 days these differences were more marked (Figure 54), with pinocytotic activity more prominent, greater vacuole diversity in size and content, including many with fine, flocculent material. Extremely large supranuclear vacuoles were present in absorptive cells towards the apex of villi. The size of these vacuoles gradually decreased toward the base and were absent in the cells of the glands of Lieberkühn. In the basal regions of absorptive cells the plasma membranes of the adjacent cells were separated by large spaces filled with flocculent materials. Goblet cells possessed less compact and thicker microvilli than absorptive cells and contained large granules of mucus.

By 40 days the apical regions of absorptive cells showed variety in the degree of pinocytotic activity. The tubulovesicular system of many cells was by far the most extensive for any age of pouch young in duodenum or ileum (Figure

55). Also, the axial core of microfilaments within the villi could be clearly seen along the extensions into the apical cytoplasm, the whole complex often being referred to as the terminal web. The glycocalyx, forming a fuzzy coat around the villi, was less extensive than in cells of the 40 day duodenum. A few solitary endocrine cells were present in the 40 day ileum. They contained rough endoplasmic reticulum and dense membrane-bound granules occupying much of the cytoplasm and were located on the basal lamina of the ileal epithelium.

5.1.2 Large intestine

The large intestine in late embryos and pouch young up to 40 days was smaller in diameter than the small intestine. Beginning in the newborn an outgrowth of the proximal region of large intestine, the caecum, was present at the junction of the ileum and colon. At the distal end of the large intestine the rectum was poorly differentiated from the colon up to 40 days of pouch life.

In late embryos, the large intestine was a simple tube with an inner lining of stratified, columnar epithelium with a relatively high mitotic index, and a surrounding layer of mesenchyme.

5.1.2.1 Caecum

At birth, the caecum was a very small outgrowth of the large intestine. The lumen was small and the pseudostratified columnar epithelium lining the lumen was relatively thick (Figure 57) and actively dividing. A distinct basal membrane was present. The epithelial cells were weakly PAS-positive and contained large, oval nuclei. The outer mesenchyme showed little sign of differentiation under the light microscope except for a delicate layer of myoblasts in the position of the muscularis externa. By 5 days the caecum had changed considerably. It had elongated somewhat and the epithelial cell lining had

differentiated into simple columnar with the cell nuclei located basally. A few goblet cells were found scattered among the epithelial cells. Villi were not present but the mucosa was thrown into folds forming shallow clefts which were the early stages of development of the crypts of Lieberkühn. At the base of the crypts, Lieberkühn or intestinal gland cells had begun to differentiate and appeared to be similar to those in the small intestine of the same age. The muscularis externa consisted of inner (thicker, circular) and outer (thinner, longitudinal) layers of the muscularis externa. At 20 days (Figure 58), goblet cells occurred on the surface of the muscosal folds but more particularly along the crypts, the latter extending down into the lamina propria. There were no changes of importance at 40 days.

Transmission electron microscopy showed that the epithelial cell luminal surface of the newborn caecum (Figure 61) had short, sparse microvilli and a less well developed pinocytotic vesicle layer than in the small intestine. Numerous elongated mitochondria were present in the supranuclear system along with heavy concentrations of ribosomes. Compared with the newborn, the cells of the 40 day caecum (Figure 62) were covered with stout, well-packed microvilli on the luminal surface. The core microfilaments of the villi were not especially clear but their extensions into the apical cytoplasm were sufficiently developed to form a broad region of terminal web, free of vesicles. The latter formed another distinct layer immediately below the web and still deeper in the cytoplasm, larger vacuoles and mitochondria were plentiful. Endocrine cells were occasionally observed near the base of the caecal epithelium and showed no obvious differences in fine structure from the endocrine cells of the small intestine.

5.1.2.2 Colon

In the newborn colon, the mucosal epithelium was stratified columnar as in the late embryos and in the newborn caecum (Figure 59). The circular layer of the

muscularis externa was more obvious than in the newborn caecum and the mesenchyme outside the muscularis externa was slightly denser than that on the inside. Otherwise, in the newborn and up to 40 days (Figure 60), the light microscope details of development of the colon were much the same as for the caecum. The only notable difference between the proximal and distal regions of the colon was in the luminal surface. The distal region in cross section possessed a luminal margin that was stellate in outline rather than the smoothly rounded outline of the proximal colon. It is possible, however, that the region with the stellate margin to the lumen could represent the anterior region of the rectum rather than the distal region of the colon.

Ultrastructurally, the epithelium of the newborn colon had many features in common with the newborn caecum with the notable exception of the microvilli which were much longer and narrower and covered with a wide, filamentous fuzzy coat or glycocalyx (Figure 63). At five days the changes were similar to those of the caecum although the number of goblet cells seemed to be somewhat higher than in the caecum. Later stages were similar to the caecum except that by 40 days (Figure 64) intracellular vacuolation appeared to be much more widespread, which may point to the activity of the colon in lipid uptake.

Figures 36 to 38

Figure 36   Transverse section of small intestine of 25 day embryo. Stratified columnar epithelium of distinct basement membrane surrounded by slightly condensed mesenchyme. (H and E stain; scale = 50 μm).

Figure 37   Transverse section of part of duodenum from newborn showing well developed villi. Simple columnar epithelial cells contain small vacuoles in apical cytoplasm and possess a prominent striated border on free surface. Blood capillaries are common below epithelium. (PAS/H/Fast green stain; scale = 50 μm).

Figure 38   Transverse section through gastro-intestinal junction in 20 day pouch young. Developing Brunner's glands occur near pylorus and duodenal villi are sparse. (H and E stain; scale = 0.5 μm).


be = blood capillary; Bg = Brunner's gland; bm = basement membrane; du = duodenum; l = lumen; mc = mesenchyme; py = pylorus; sb = striated border; va = vacuole; vi = villus.
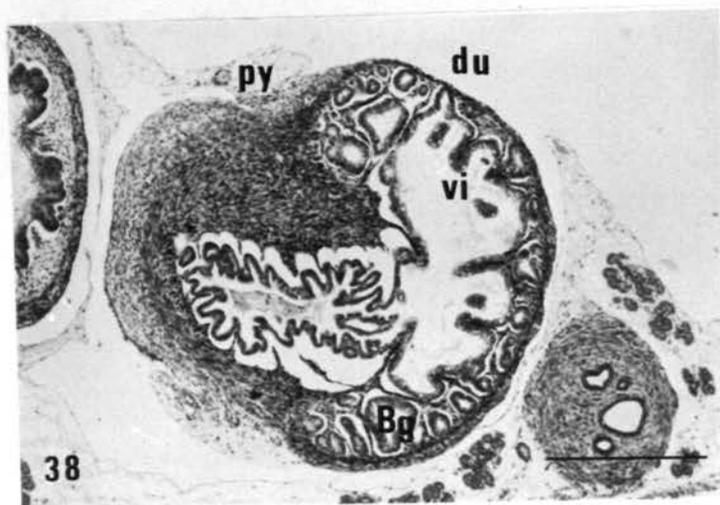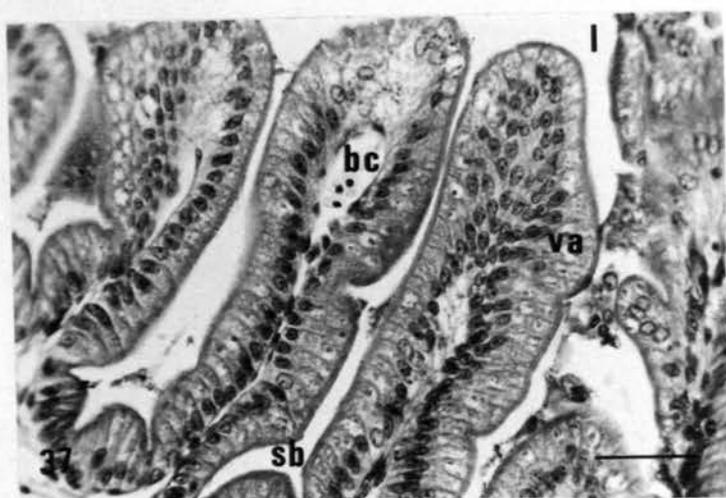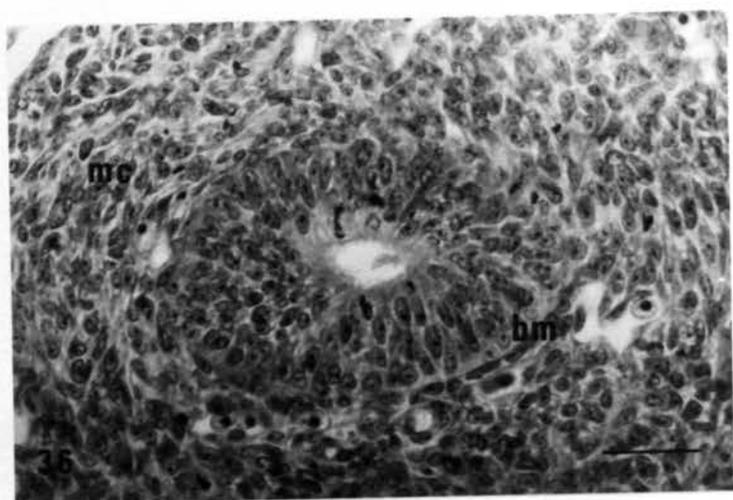
Figures 39 to 41

Figure 39   Newborn duodenum showing sparse villi and occasional crypt openings. Bleb-like cells can be seen on surface of villi and duodenal floor. (SEM x 125).

Figure 40   Tips of duodenal villi of 20 day pouch young with large numbers of bleb-like cells. (SEM x 230).

Figure 41   Tips of duodenal villi of 40 day pouch young. Fewer blebs than in Figure 40 (above). (SEM x 195).

bl = bleb; cr = crypt; if = intestinal floor; vi = villus.
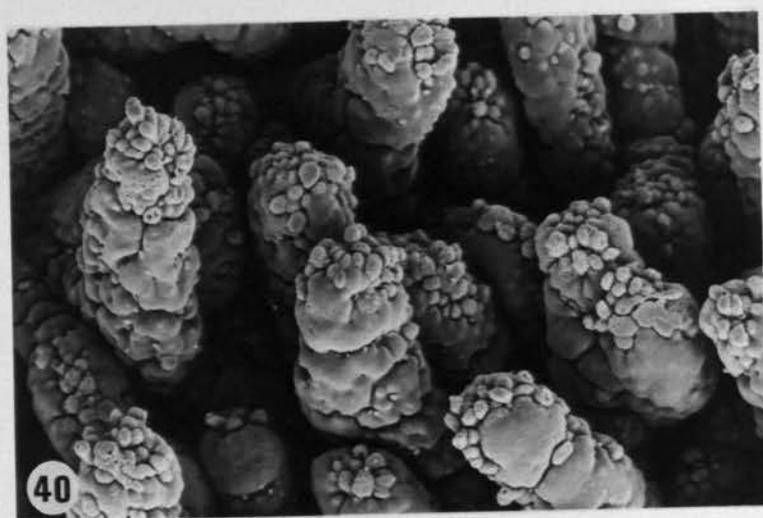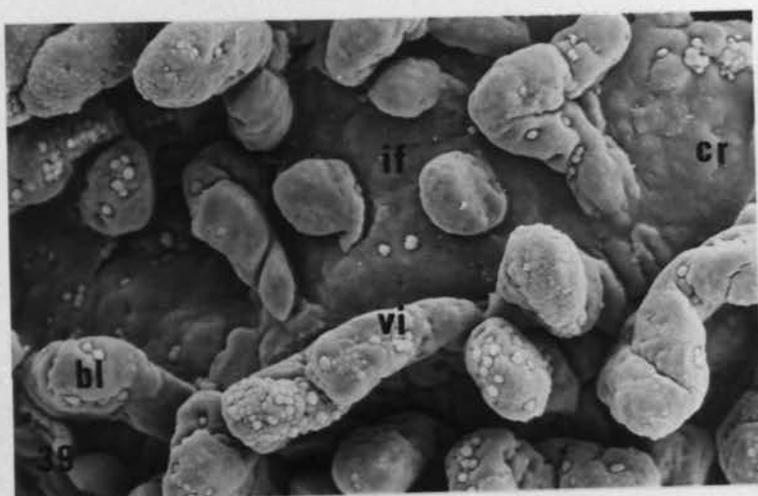
Figures 42 to 44

Figure 42   Villus tips of 40 day pouch young duodenum, showing surface
              of closely packed hexagonal epithelial cells, bleb–like cells
              covered with microvilli and detachment clefts. (SEM x 920).

Figure 43   High power of single bleb at surface of villus of 40 day
              pouch young duodenum.   Rounded cell, with prominent
              microvilli covering surface, late in the process leading to
              detachment. (SEM x 5,080)

Figure 44   High power of epithelial cell surface and fractured face
              showing densely packed microvilli.   40 day pouch young
   duodenum. (SEM x 12,750).

dc = detachment cleft; mv = microvilli.

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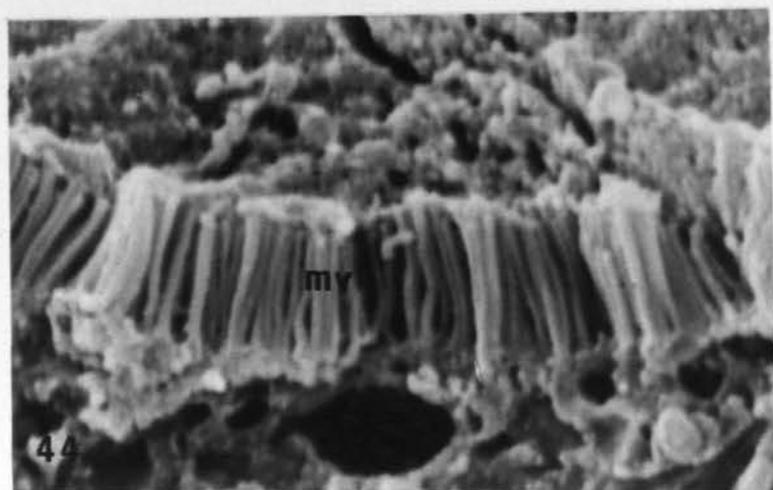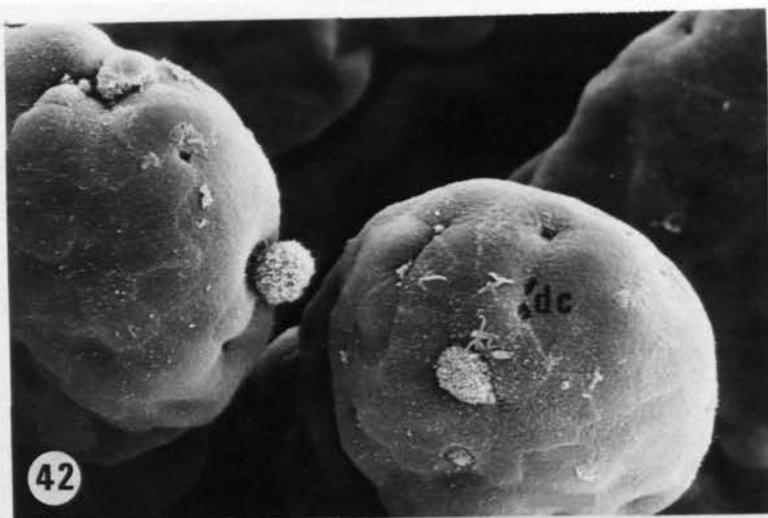
Figures 45 and 46

Figure 45   Apical region of epithelial cell of newborn duodenum. Note network of tubulovesicular system and vacuoles containing varying electron-dense material. Large vacuoles are present in supranuclear area. (TEM x 1,110)

Figure 46   Epithelial cells perinuclear regions of newborn duodenum. A large supranuclear vacuole is present immediately above nucleus on left. Dilation of adjacent plasma membranes and intracellular smooth membrane tubules can be seen. (TEM x 11,180)

mv = microvillus; sm = smooth membrane tubules;
ts = tubulovesicular system; va = vacuole
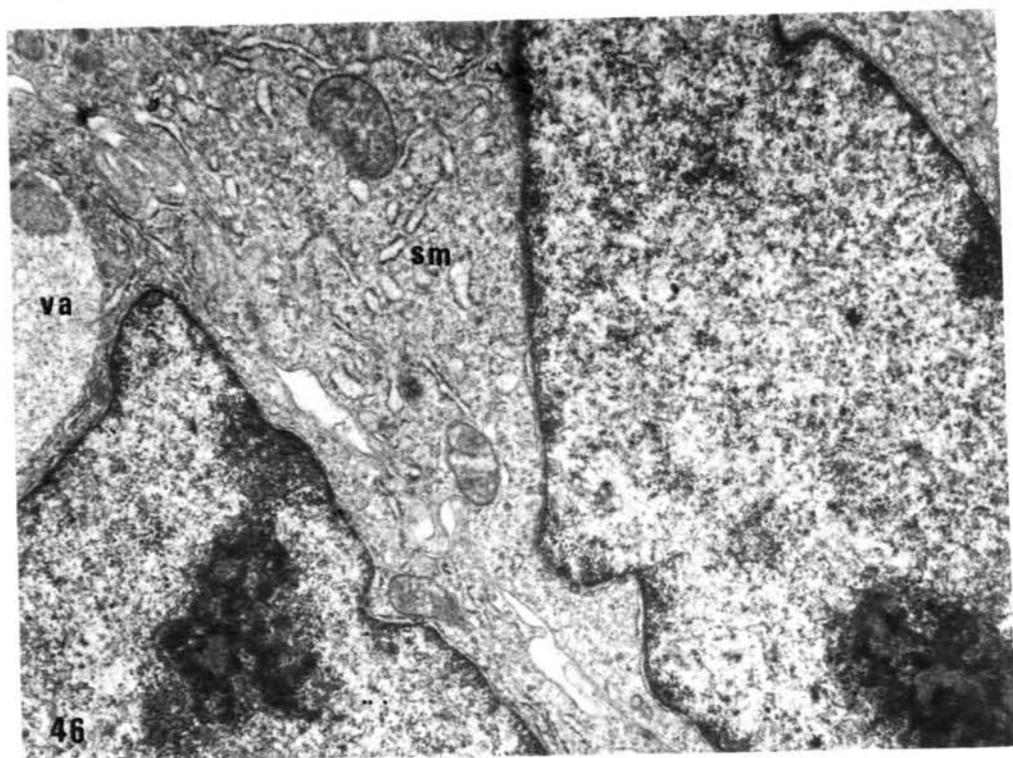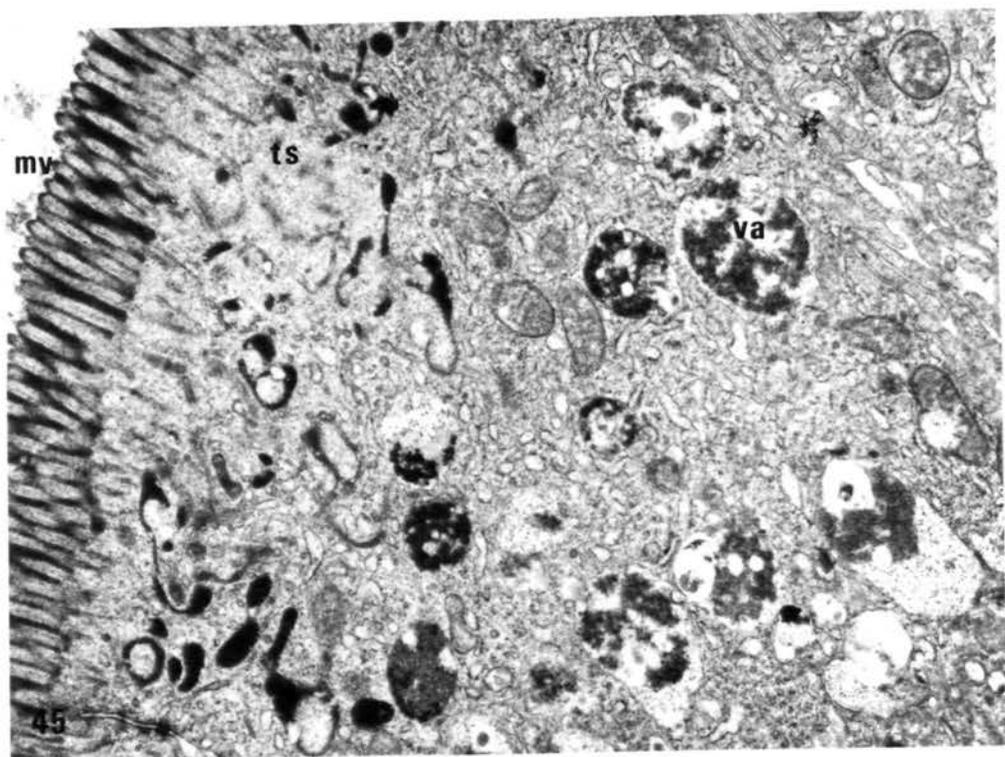
Figures 47 and 48

Figure 47   Apical region of epithelial cell of 40 day pouch young duodenum. Dilation of elements of tubulovesicular system has occurred and several tubules are continuous with intestinal lumen (arrows). (TEM x 21,400).

Figure 48   Perinuclear regions of epithelial cells of 40 day pouch young duodenum. Large aggregations of droplets, enlarged smooth membrane tubules and some rough endoplasmic reticulum can be seen. (TEM x 11,200).

dr = droplet aggregations; er = rough endoplasmic reticulum; fc = fuzzy coat; jc = junctional complex; m = mitochondria; mv = vicrovilli; sm = smooth membrane tubules; ts = tubulovesicular system; va = vacuole
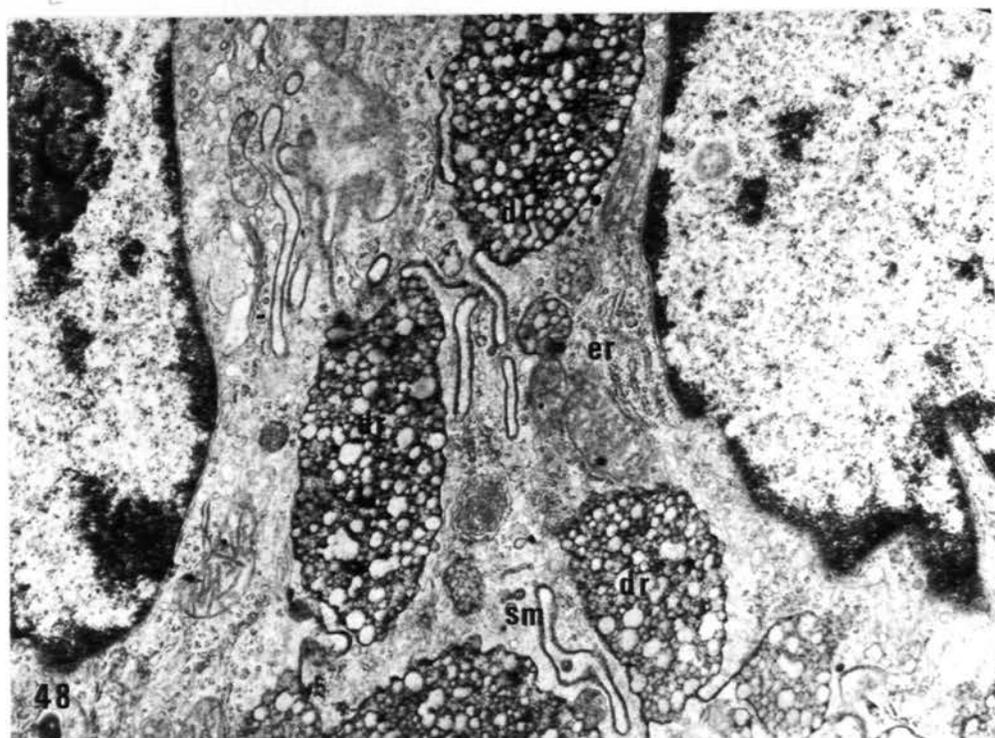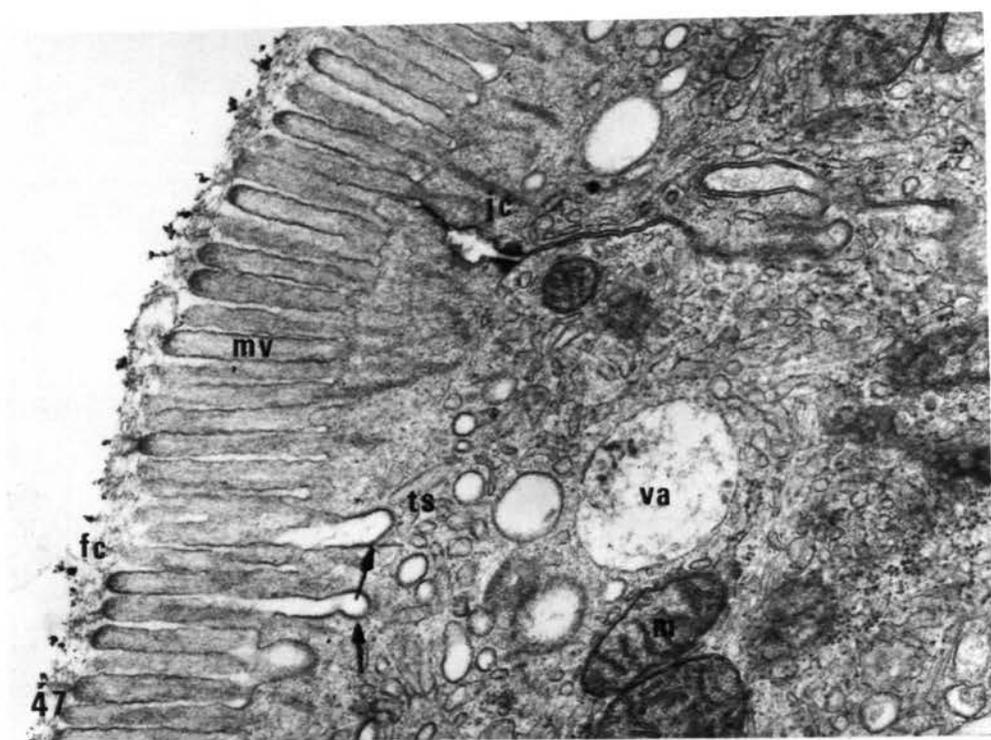
Figures 49 to 52

Figure 49 Transverse section of ileum of newborn. Villi almost occlude lumen. Vacuoles containing small granules present in apical cytoplasm. (Plastic embedding; methylene blue stain; scale = 50 µm).

Figure 50 Transverse section of ileum of 10 day pouch young. Extensive vacuolation of epithelial cells and presence of numerous darkly-staining goblet cells. (Plastic embedding; methylene blue stain; scale 50 µm).

Figure 51 Transverse section of ileum of 10 day pouch young. Development of intestinal glands at base of crypts of Lieberkühn. Myenteric plexus occurs below circular layer of muscularis externa. (H and E stain; scale = 50 µm).

Figure 52 Transverse section through ileo-caecal junction of 20 day pouch young. Villi absent but goblet cells more numerous in caecum than in ileum.
(PAS/H/Fast green stain; scale = 0.25 mm)

ca = caecum; cl = circular layer of muscularis externa; cL = crypt of Lieberkuhn; gc = goblet cell; il = ileum; l = lumen; my = myenteric plexus; va = vacuole.

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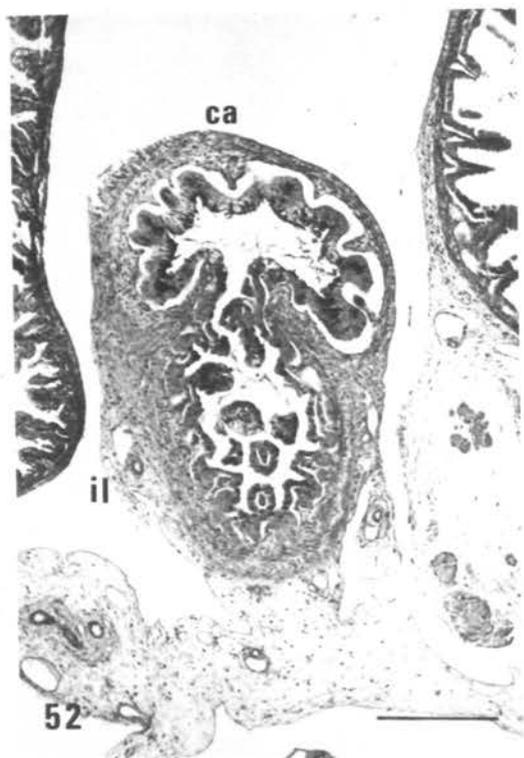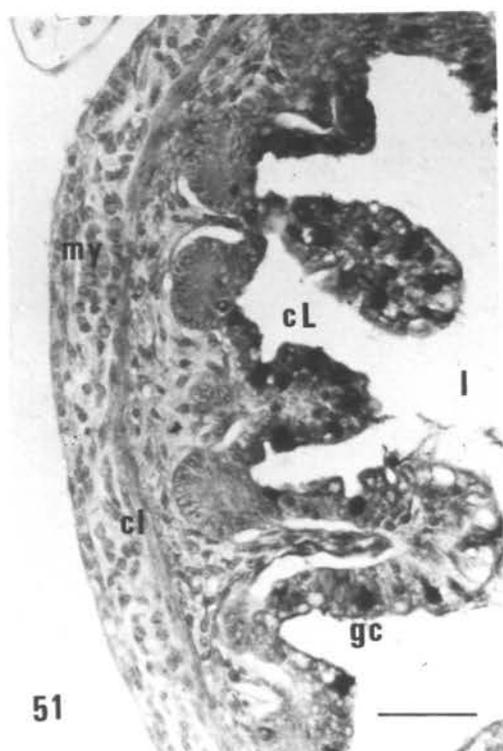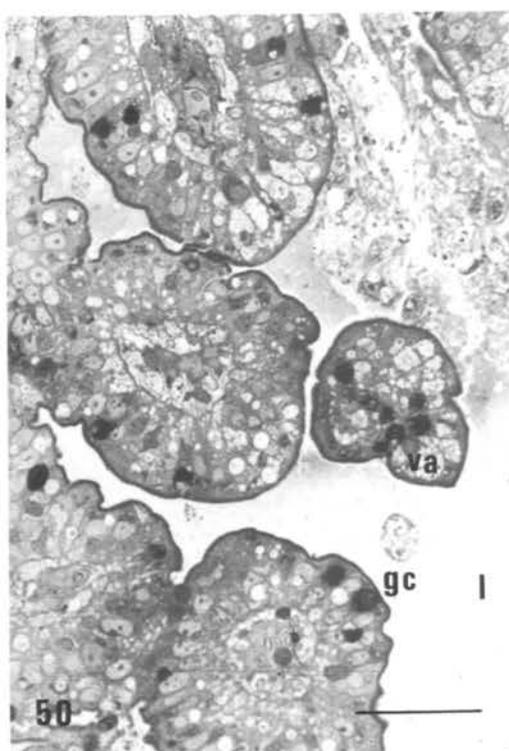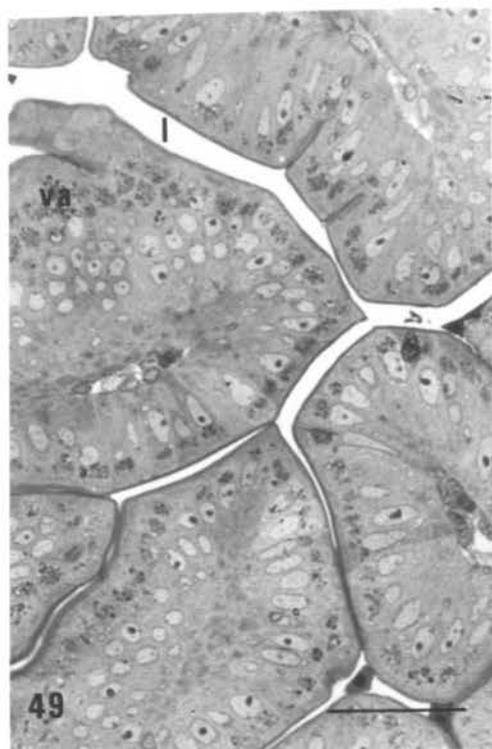
Figures 53 and 54

Figure 53   Epithelial cells of newborn ileum. Well developed tubulo-
             vesicular system below microvilli and numerous vacuoles in
             apical and supranuclear cytoplasm. Both tubulovesicles and
             vacuoles contain electron dense material.

Figure 54   Surface cells of 10 day pouch young ileum. Epithelial cell
             with extensive tubulovesicular system and vacuoles and
             numerous mitochondria. Apical region of goblet cell present
             on right containing large mucus granules and with less
             compact microvilli. (TEM x 7,085).

             gc = goblet cell; ts = tubulovesicular system; va = vacuole.
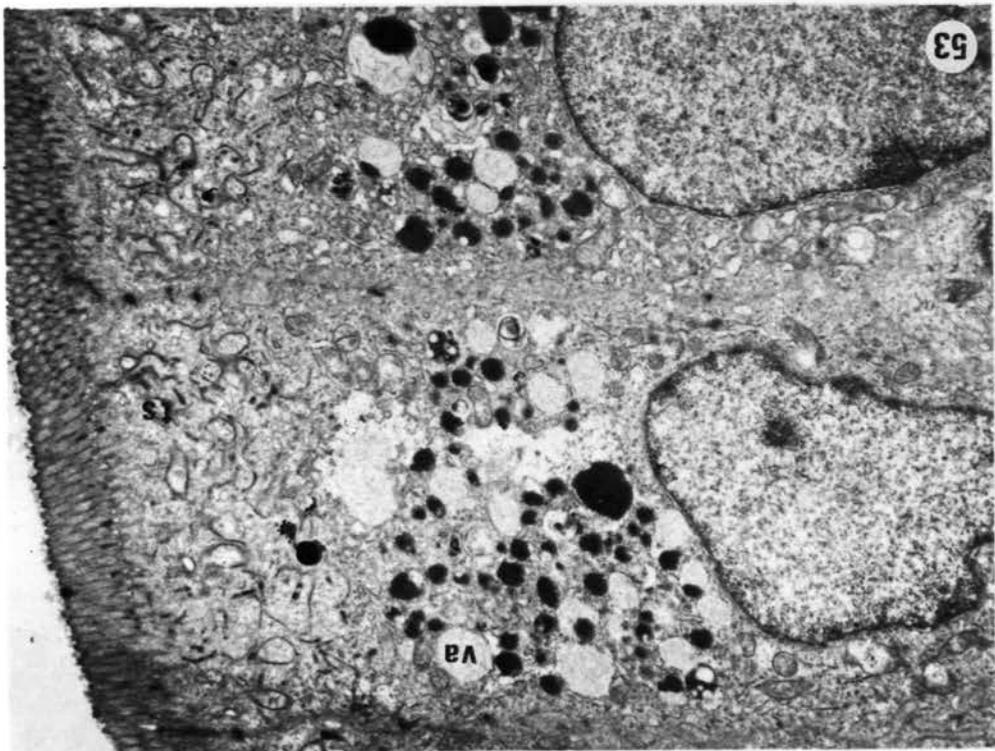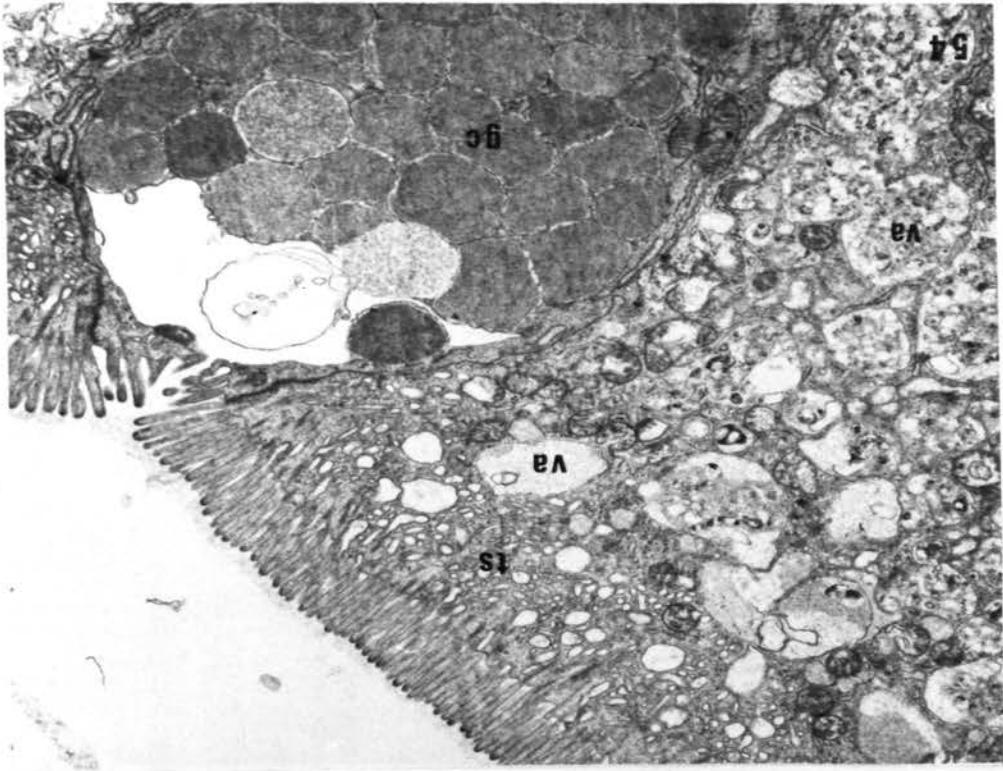
Figures 55 and 56

Figure 55   Apical region of epithelial cell of ileum of 40 day pouch young. Increased branching and dilation of tubulovesicular system and terminal web of microvillar cores are prominent. (TEM x 12,550).

Figure 56   Endocrine cell in basal region of epithelium in 40 day pouch young ileum. Numerous granules and much rough endoplasmic reticulum are present. (TEM x 11,660).

er = rough endoplasmic reticulum; g = granule; nu = nucleus; m = mitochondrion; ts = tubulovesicular system; tw = terminal web.
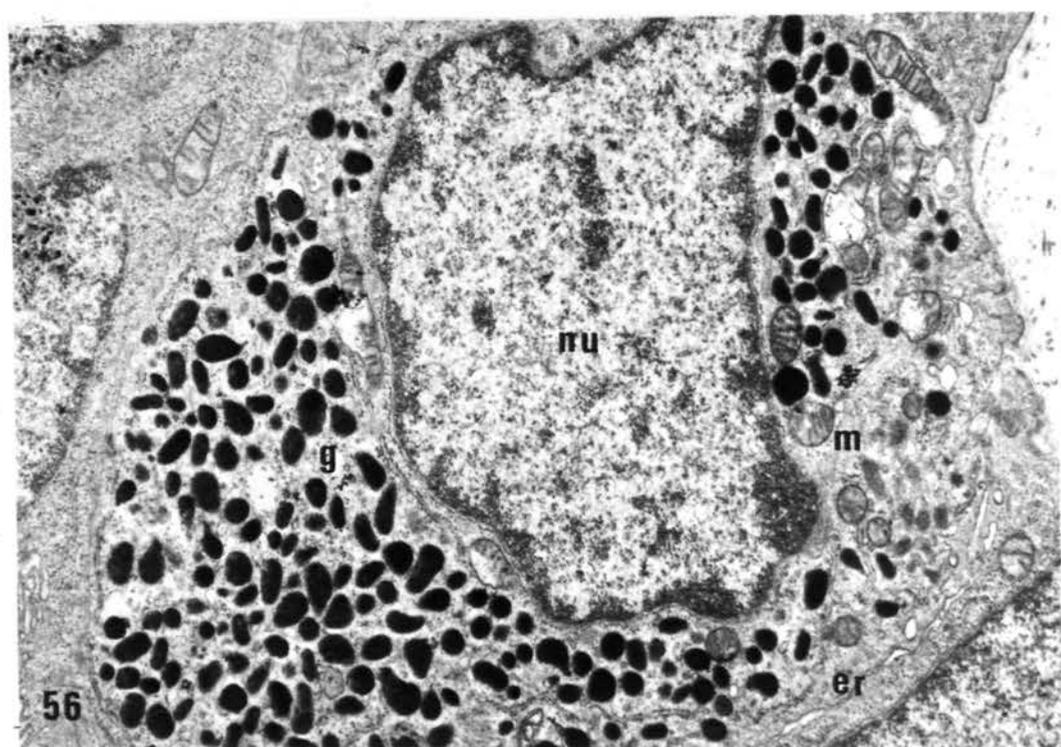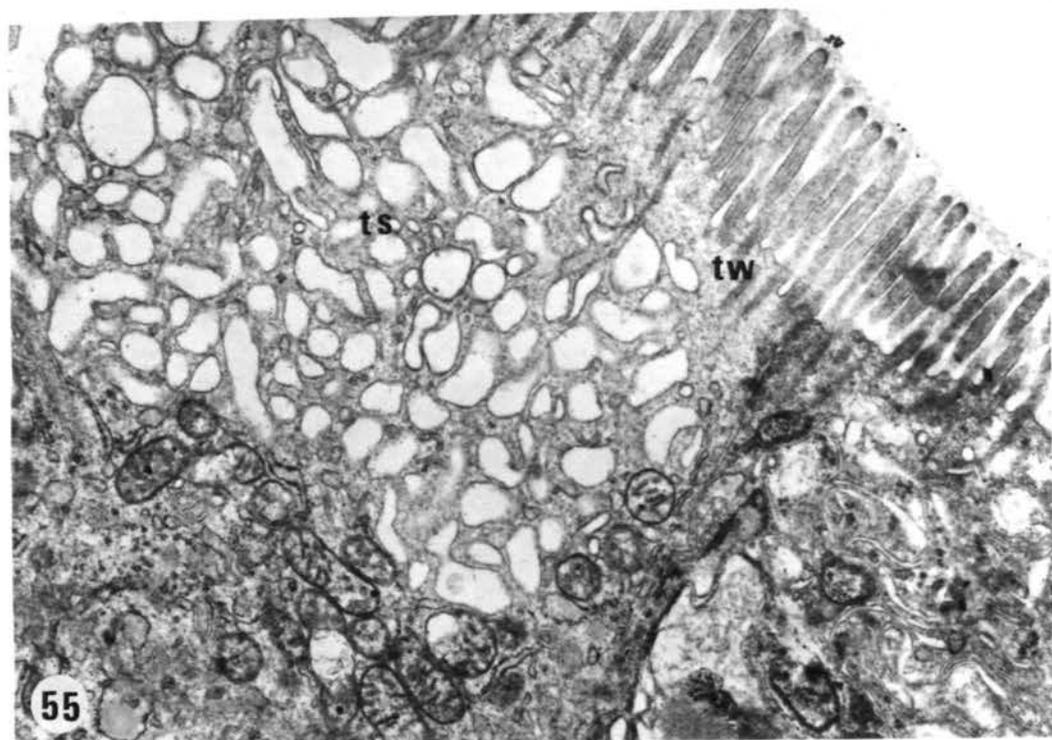
Figures 57 to 60

Figure 57   Transverse section of newborn caecum. Stratified columnar epithelium surrounded by delicated layer of myoblasts. Mitotic divisions common in epithelium. (Plastic embedding; methylene blue stain; scale = 50 μm).

Figure 58   Transverse section of caecum of 20 day pouch young. Well developed crypts with many goblet cells extend down into lamina propria. (H and E stain; scale = 50 μm).

Figure 59   Transverse section of newborn colon. Circular layer of muscularis externa clearly defined around stratified columnar epithelium. (Plastic embedding, methylene blue stain; scale = 50 μm).

Figure 60   Transverse section through colon of 40 day pouch young. Mucosa surface relatively smooth and crypts with numerous goblet cells. (PAS/H/Fast green stain; scale = 50 μm).

cl = circular layer of muscularis externa; cL = crypt of Lieberkuhn; gc = goblet cell; md = mitotic division; me = muscularis externa fully formed.

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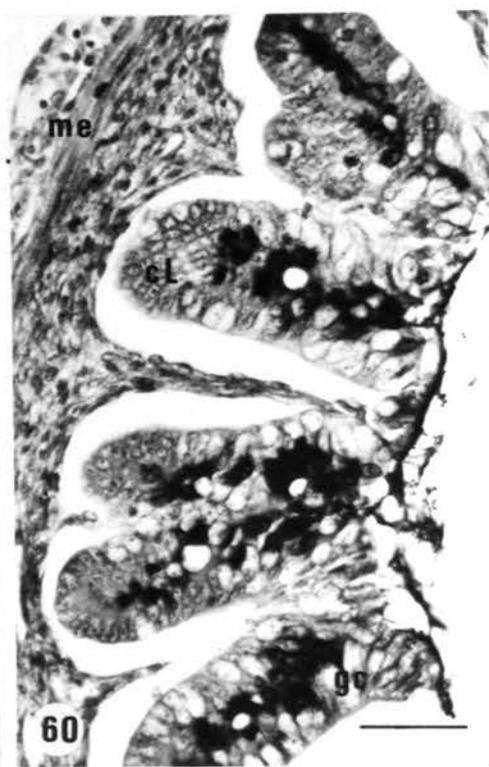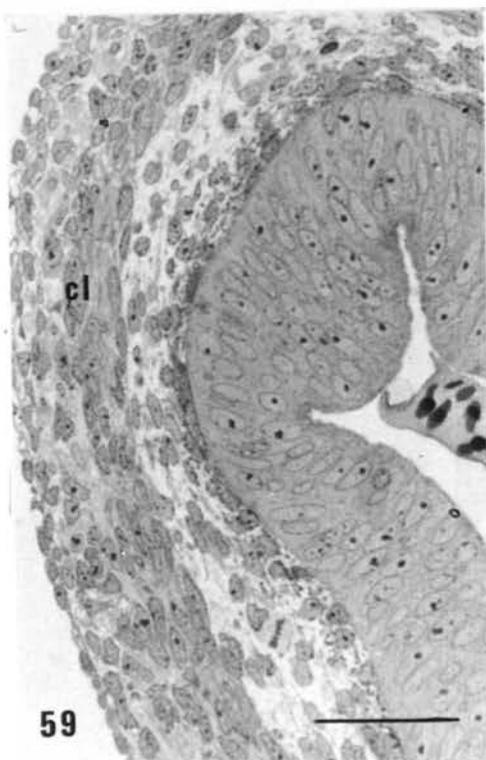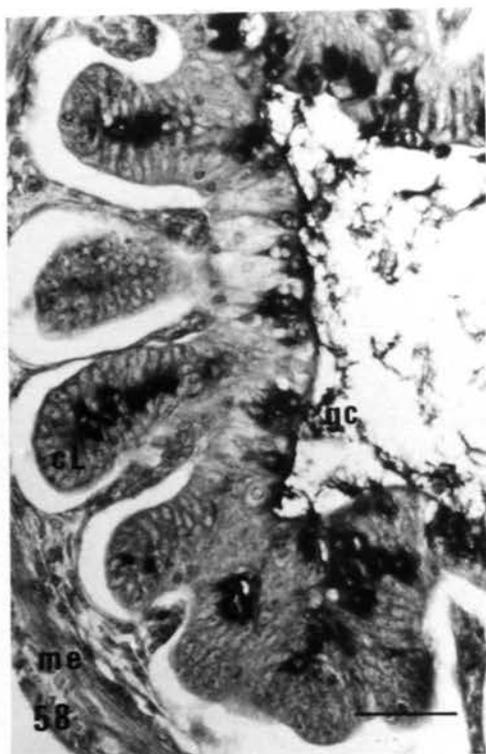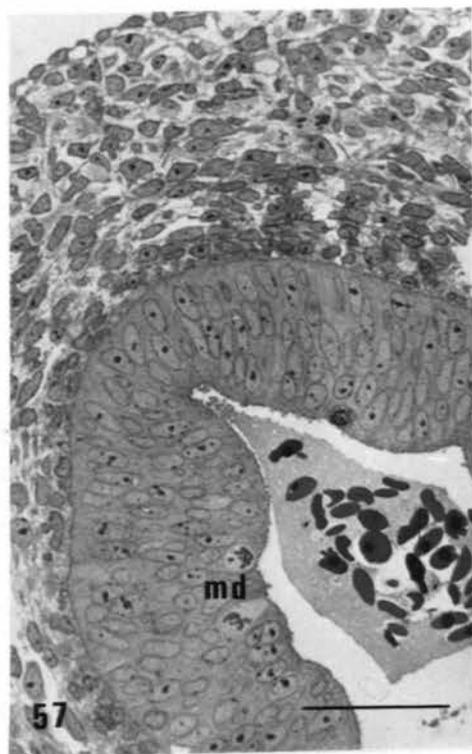
Figures 62 and 62

Figure 61   Epithelial cells of caecum of newborn.   Small vesicles
               present below sparse microvilli and some vacuoles scattered
               in apical and supranuclear cytoplasm.   Mitochondria and
               ribosomes widely distributed. (TEM x 8,500).

Figure 62   Apical region of epithelial cell of caecum of 40 day pouch
               young. Microvilli well developed with prominent associated
               terminal webb.   Numerous vesicles present below web.
               Desmosome complexes and interdigitations of lateral cell
               membranes are clearly seen. (TEM x 17,000).

               cm = cell membrane with interdigitations; dc = desmosome
               complex; m = mitochondria; va = vacuole; ve = vesicle;
               tw = terminal web.

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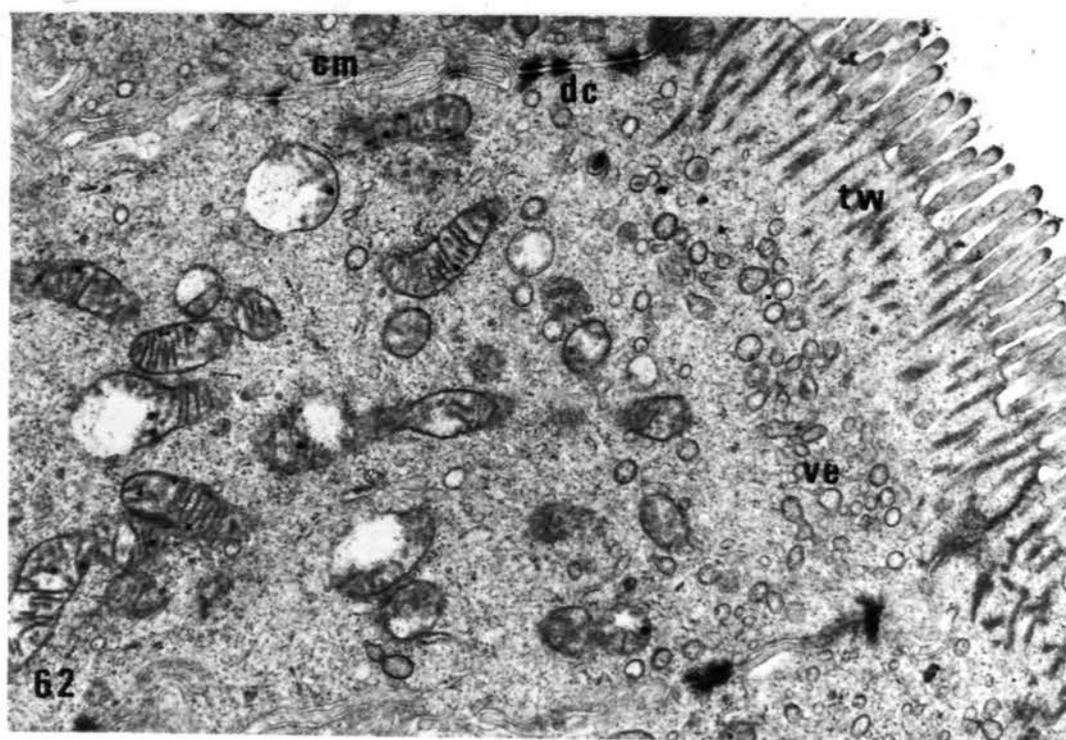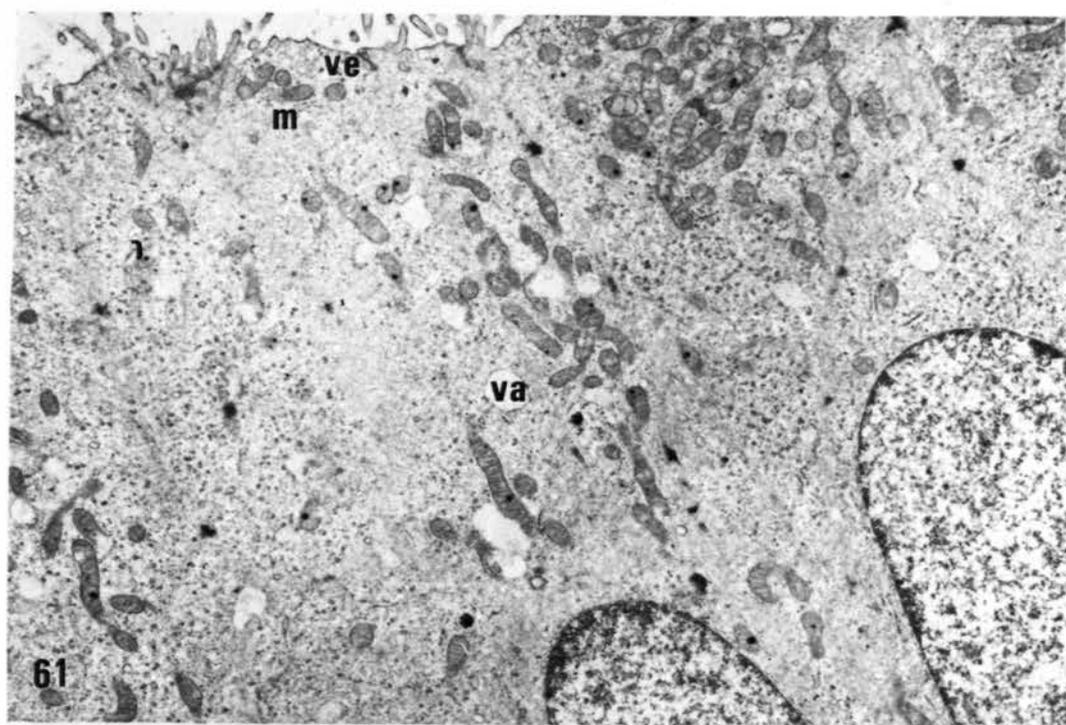
Figures 63 and 64

Figure 63   Apical region of epithelium of newborn colon.   Long
filamentous fuzzy coat covering extended microvilli with
clear terminal web. (TEM x 16,350).

Figure 64   Apical region of 40 day pouch young colon epithelium.
Large numbers of vacuoles of various sizes in both apical
and supranuclear regions. (TEM x 15,700)

fc = fuzzy coat; gc = goblet cell; m = mitochondria;
mv = microvilli; tw = terminal web; va = vacuole.

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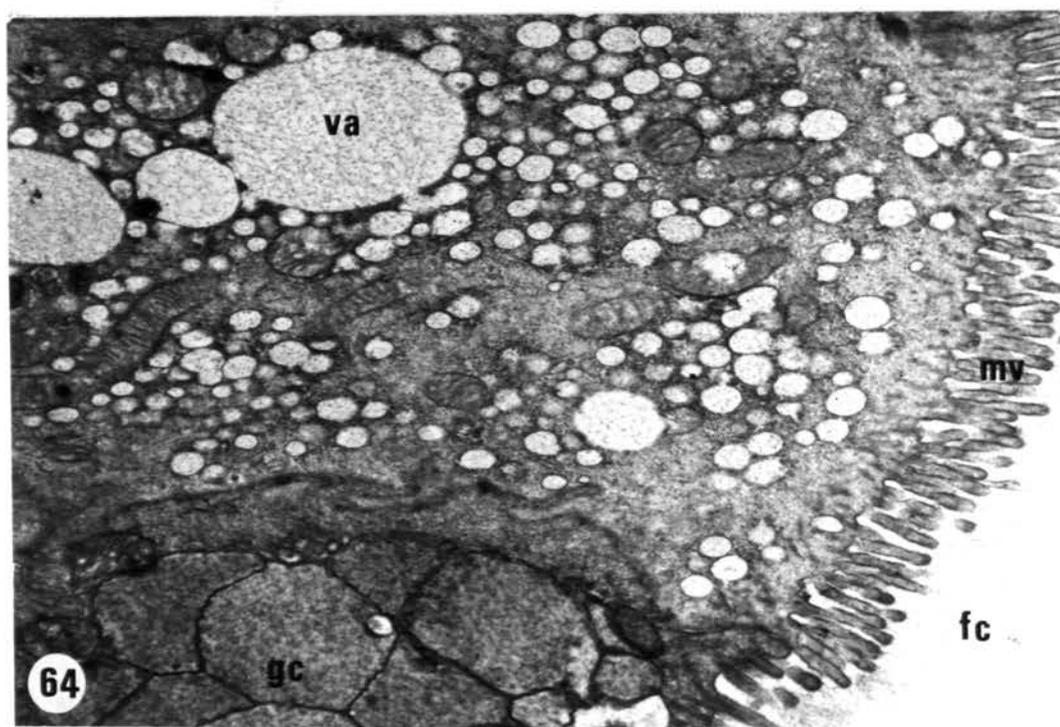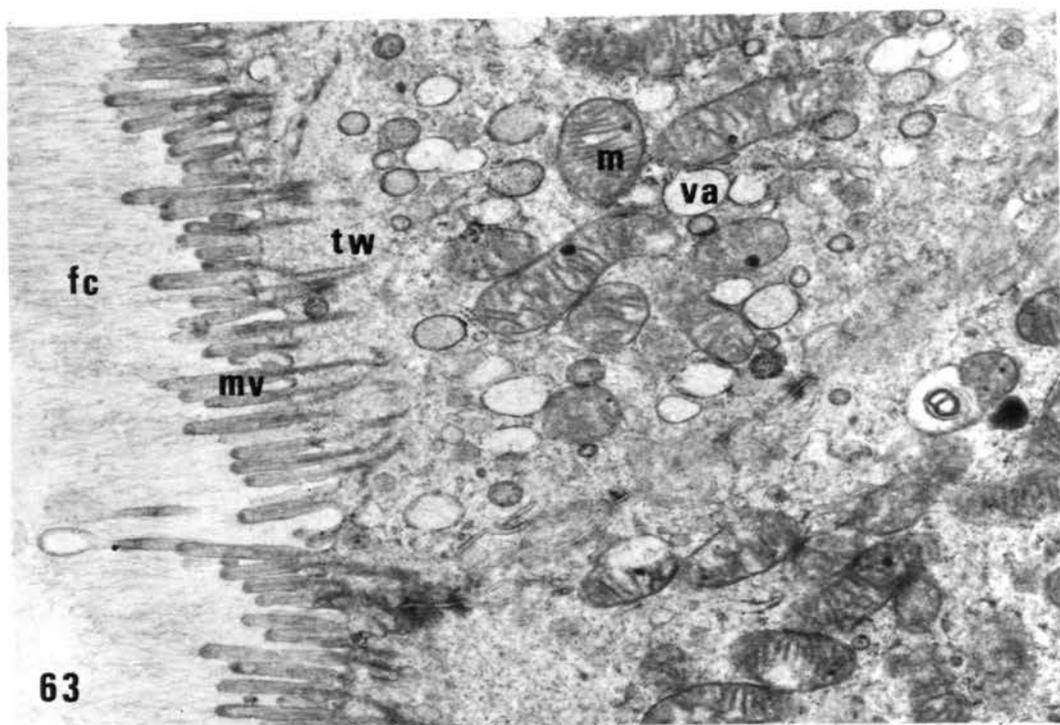
5.2   Discussion

Because of the variation in gestation length, degree of maturity at birth and the period of suckling, mammals show a range of timing of various biological components of intestinal development. On the other hand, birth provides a fixed point in the physiological and morphological development of the intestine since, by this time, the intestine must be sufficiently organised to carry out the digestive and absorptive functions necessary to cope with a neonatal diet of maternal mammary secretions. It is thus a regular feature of all the mammals investigated (including the tammar) that the intestinal mucosa of the neonate has a high level of structural development (Henning and Kretchmer, 1973).

The dominating structural component of the mammalian small intestine is the villus. At the villus surface, covered with columnar epithelial cells, and fringed by a brush border of microvilli, the transfer of molecules from the lumen to the tissues of the offspring takes place. All of these structural relationships are in place in the newborn tammar. They are obviously formed very quickly in the tammar in view of their absence in the embryo three days before birth.

The diet of the suckling mammal contains considerable amounts of protein and the total picture of protein utilization at this stage is quite different from that of the adult. In the adult, extracellular (luminal) digestion is followed by absorption of amino acids and proteins whereas in suckling young, extracellular digestion of protein is minimal and proteins (plus other macromolecules) can be absorbed by pinocytosis as intact molecules in membrane-bound vesicles. Cytological evidence of membrane-bound vesicles in the absorptive cells of duodenum and ileum in tammar pouch young is consistent with such a mechanism of protein uptake. Invaginations of the surface epithelium between the villi (a direct indicator of pinocytotic activity) were rare and ill-defined in the newborn duodenum, more clearly developed at five days and increasingly common and

conspicuous at 40 days. Furthermore, the tubulovesicular system resulting from this pinocytosis changed in appearance and extent during the first 40 days of pouch life, being restricted at birth and extensive later. A similar pattern was observed in the ileum, with a spectacular elaboration of tubules and vesicles in the oldest pouch young.

The steady increase in pinocytotic activity in the small intestine and particularly in the ileum may simply reflect an increasing efficiency in macromolecular uptake and/or an increased volume of milk passing through the small intestine. It would not appear to be related to an increase in total protein concentration in the diet since Green et al. (1980) and Green and Renfree (1982) found that protein concentrations in tammar whole milk and whey increased significantly only much later in lactation. Part of the protein of marsupial milk (probably a very small part according to Deane and Cooper, 1984) is immunoglobulin which is known to be transferred via the villus epithelium to the pouch young circulation (Yadav, 1971). In Didelphis this has commenced within 12 hours of birth (Hindes and Mizell, 1976) and in various species it is known to continue for much of pouch life. No hint of specific antibody uptake could be determined from ultrastructural observations made during this study. In rats, absorption by pinocytosis of intact milk-derived immunoglobins probably occurs in the duodenum. Immunoglobulins and other proteins are also absorbed by pinocytosis in the rat ileum during suckling but they are partially or wholly degraded, probably by lysosomal proteinases such as cathepsins (Davies and Messer, 1984). It would be of considerable interest to explore in the tammar such topics as the regional specificity of uptake of immunoglobulins and the localisation of receptor-mediated endocytosis, using high resolution electron microscopy together with modern molecular probes such as monoclonal antibodies (Quaroni, 1985).

Carbohydrate uptake might also be a factor in the development in early

pouch young of extensive pinocytosis and tubulovesicles in the intestine. Most studies on carbohydrate composition in marsupial milk have examined changes from mid- to late lactation, primarily because of problems associated with collection of enough milk from a small number of animals in the first few days of pouch life. However, Messer et al. (1984) carried out qualitative and quantitative analyses of milk carbohydrates and reported that for the first 72 hours after birth the only carbohydrate found was the disaccharide lactose. The major carbohydrate in the milk of most young eutherians is also lactose but it appears that the mechanism of digestion by eutherian young is different from marsupials. Eutherian young hydrolyse lactose in the brush border using neutral β-galactosidase (lactase). Messer and Czolij (1984) used indigogenic methods to detect enzyme activities in frozen sections of jejunum and ileum of suckling tammar wallabies of unspecified ages. Strong acid β-galactosidase activity was seen within epithelial cells but no neutral β-galactosidase could be detected either at the brush border or intracellularly. This implies that lactose (and other higher oligosaccharides which appear after day 3) must enter the cells prior to their digestion. Messer and Czolij (1984) mention the possibility of entry by pinocytosis and refer to the finding of numerous vesicles in electron micrographs of enterocytes of suckling tammar wallabies. The histochemical test was not recorded as having been applied to duodenal regions so the restriction of activity to the distal region of the intestine requires confirmation.

Pinocytosis thus appears to play a major role in the uptake of milk constituents in the tammar small intestine but it is most unlikely that it is the sole mechanism involved. The glycocalyx remained as a conspicuous filamentous coat around the microvilli in the small intestine of early pouch young and it seems reasonable to suppose that it contributes to the extracellular digestion of macromolecules. In adults, the glycocalyx is rich in digestive enzymes including various phosphatases, glycosidases, peptidases and kinases, some of which could

show activity from early in development. Further indirect evidence of extracellular digestion in the duodenum of early pouch young comes from observations on the developing pancreas. In the newborn, scattered small clusters of pancreatic cells resembling acini were identified in an extensive field of connective tissue. The pancreatic cells frequently contained large numbers of zymogen granules, suggesting that enzyme secretion into the duodenum was likely to take place at a very early age. Several transport mechanisms are known to be involved in the absorption of molecules and ions into intestinal epithelial cells. A recent proposal is that the protein calmodulin acts as a carrier of calcium ions from the actin filaments of the villus core to the myosin filaments of the web (Moog, 1981). The conspicuous cores and web in young tammar intestinal absorptive cells points to an active involvement in molecular transfer as well as the maintenance of the structural integrity of the microvillus.

Absorptive epithelial cells in the tammar small intestine contain other characteristic cytoplasmic inclusions basal to the tubulovesicular system. The large supranuclear vacuole with its fine granular content and a cluster of amorphous flocculent material has also been reported in the suckling rat (Cornell and Padykula, 1969) and echidna (Krause, 1972), and is probably of considerable importance in intracellular breakdown of absorbed materials. The many small adjacent vacuoles of similar constitution probably have a similar function. The relatively large aggregations of homogeneous droplets associated with so-called smooth membrane tubules in the perinuclear region of many cells may be chylomicrons. If so, these accumulations of lipid droplets are occupying intercellular spaces and the smooth membrane tubules represent highly folded plasma membranes of adjacent cells. Krause (1972) observed similar aggregations in the gastrointestinal tract of the echidna. Membrane-bound intracellular lipid appeared to be a much less common type of organelle in the suckling tammar small intestine than in most other suckling mammals examined.

The small intestinal epithelium contains cells that are specialised for secretion as well as for absorption. Most of the secretory cells produce mucin and are enclosed in epithelial pockets forming glands. The earliest glands to develop in the small intestine of the tammar were the duodenal or Brunner's glands, the immediate precursors of which were already present in the newborn and continued to develop up to 40 days. According to Krause (1972), Brunner's glands are present near the pyloric sphincter of all adult mammals and probably secrete an alkaline, mucin-containing fluid which protects the proximal duodenal mucosa from the possible mechanical damage of moving intestinal contents and from ulcerating effects of hydrochloric acid and pepsin secreted by the stomach. Krause (1972) examined Brunner's glands of adults belonging to 14 species of macropodids (not including M. eugenii). They regularly formed a glandular collar immediately distal to the pyloric sphincter and numerous complex ducts emptied on to either the gastric or the duodenal mucosa, depending on the species. In 40 day pouch young the complex duct system had not differentiated so there was no indication of whether secretions would be directed to both gastric and duodenal surfaces. Considerable expansion of the glands must occur after 40 days since the collar arrangement typical of adults was incomplete.

The more distal regions of the duodenum and the full length of the ileum are well supplied with secretory intestinal glands or glands of Lieberkuhn which develop slightly later than Brunner's glands. Paneth cells and argentaffine cells, typically found at the base of the gland in eutherian mammals, were not identified up to 40 days in the tammar but special staining methods were not used and their presence cannot be ruled out.

It has long been known that epithelium lining the villi of the small intestine is continuously renewed by cell division in the crypts of Lieberkuhn and its use in recent years as a model system for the study of cell population kinetics has been widespread (Wright and Irwin, 1982). Cell turnover leads to cell death which is

manifested in the intestine as cell extrusion. The morphological changes during cell extrusion in the tammar are similar to those described and depicted in scanning electron micrographs of cell loss from the intestinal mucosa of mice (Poten and Allen, 1977). In the mouse, cells are lost after separation from their neighbours by intercellular vacuolation, followed by vertical migration (to form bleb-like structures) and eventual detachment. Extrusion of effete cells usually leaves a space at the surface which is soon filled by the lateral movement of the five or six neighbouring cells which "close ranks". The polygonal close packing of the cells may hinder closure, maintaining the space long enough for it to be occasionally fixed histologically. In both mouse and tammar the microvillus covering of cells being extruded is drastically altered compared with surrounding normal cells. The microvilli are reduced in number, increased in size, and more varied in shape.

In the newborn tammar, cell extrusion takes place on the surface of villi and also on the floor of the intestine. That it occurs at all at this stage is unusual when crypts have only just begun to develop. In older pouch young, cell extrusion was most common towards the tips of the villi but occurred to a lesser extent along the shaft. Partridge and Simpson (1980) have suggested that in the mouse, cell death and extrusion may occur anywhere on the villi since there is considerable competition for migration pathways to villous surfaces. Because of their limited life span, cells trapped in such "traffic jams" die and are shed from such positions. Although there was considerable variation in the density and distribution of cells being extruded from tammar villi, there did appear to be a peak in incidence at 20 days.

There are some striking differences between the early development of the small intestine of the tammar and that of the North American opossum (Didelphis virginiana), the only marsupial for which relevant information has been published (Krause and Leeson, 1969; Krause et al., 1977). In the tammar newborn, the villi

in the duodenum and ileum are similarly developed, whereas in the opossum the ileal villi are short and immature. In the tammar, intestinal glands begin to develop at about 5 days while in the opossum they do not make their initial appearance until after 50 days. Furthermore, in the tammar, goblet cells are present at 5 days and increase steadily thereafter, whereas in the opossum goblet cells are found only in limited numbers in the early postnatal stages and do not comprise a "significant" population until around 95 days. Finally, lipid droplets are much less a feature of the supranuclear cytoplasm of the tammar absorptive cells than in the opossum. These features, taken together, indicate that the tammar small intestine is more advanced in its development than the opossum in early postnatal life.

The caecum and colon of the large intestine lack villi but apart from this marked architectural difference the two regions in the tammar do not differ greatly from one another or from the small intestine in their patterns or rates of development. However, goblet cells appeared to be more common at all ages in the colon compared with the caecum and small intestine. Helander (1973) pointed to a general pattern of proximal-distal progression of development in the intestine of mammals. The tammar intestine does not conform to this pattern of development since the duodenum, ileum and colon appear to develop at about the same rate. The opossum also fails to conform to the general pattern but in this case the colon appears to be accelerated in its development compared with the small intestine.

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