J. Anat. (1977), **123**, 1, pp. 21–45
With 37 figures
Printed in Great Britian

The postnatal development of the alimentary canal in the opossum

III. Small intestine and colon*

WILLIAM J. KRAUSE, J. HARRY CUTTS AND C. ROLAND LEESON

Department of Anatomy, University of Missouri, Columbia, Missouri 65201

(*Accepted* 2 *November* 1975)

INTRODUCTION

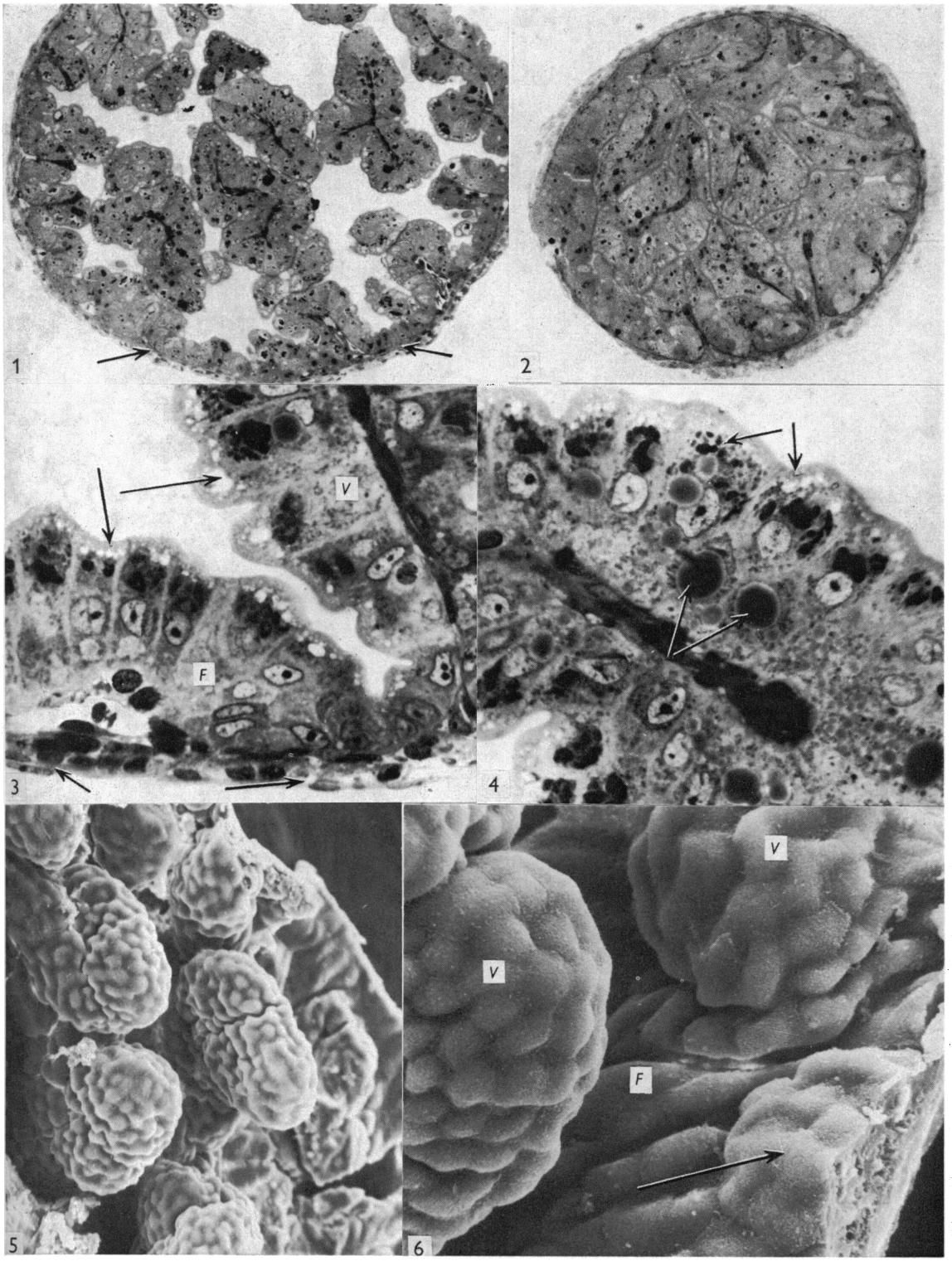
The epithelium of the distal small intestine in a number of fetal and suckling eutherian mammals is capable of sequestering macromolecular protein from the intestinal lumen (Clark, 1959; Clarke & Hardy, 1971; Anderson, 1963; Graney, 1968; Morris, 1968; Williams & Beck, 1969; Staley, Jones & Marshall, 1968; Cornell & Padykula, 1969; Kraehenbuhl & Campiche, 1969; Hugon, 1970; Staley, Corley, Bush & Jones, 1972; Lev & Orlic, 1973; Orlic & Lev, 1973). This process is of considerable importance since it is one of the mechanisms by which passive immunity is passed from mother to offspring. Morphologically the intestinal epithelial cells of these suckling animals differ markedly from those of the adult, showing extensive apical complexes of anastomosing tubules and/or vesicles and large supranuclear vacuoles. Absorbed materials are found initially in the apical network of tubules and then in the large supranuclear vacuoles (Clark, 1959; Graney, 1968; Kraehenbuhl & Campiche, 1969). Similar morphological observations have been made on the epithelial cells of the small intestine of a suckling prototherian, the echidna (Krause, 1972), and large apical inclusions have been noted also in the apices of intestinal epithelial cells of a metatherian, the newborn opossum (Heuser, 1920; Krause & Leeson, 1969).

The following study presents the normal postnatal development of the small and large intestines of the opossum, *Didelphis virginiana*. Emphasis has been placed on the changes that occur in the mucosa during this extended period of development.

MATERIALS AND METHODS

One hundred and five opossums (*Didelphis virginiana*) were used in the study. Pouch-young opossums were divided into the following groups according to their snout–rump lengths (SRL): 1·5 (newborn, less than 24 hours old), 2·5 (nine days old), 3·0, 3·5, 4·5, 5·5, 6·0, 7·0, 8·0,10·0, 11·5, 13·0, 15·0, 19·0, 20·0, 22·0 and 28·0 cm. Four adults also were used. The animals were killed by decapitation and as quickly as possible blocks of tissue, primarily from the proximal and distal segments of the

* Supported by grant 6515 Penrose Fund from the American Philosophical Society, and by University Research Council Grant NSF1074 for general research support.


1
2
V
F
3
4
5
V
V
F
6

small intestine as well as from the colon, were removed and placed in Bouin's solution or in 10% buffered neutral formalin. The tissues were processed routinely, embedded in paraffin, sectioned at about 7 μm and the following staining procedures employed: haematoxylin and eosin, Masson's trichrome, van Gieson, toluidine blue, periodic acid-Schiff (PAS) before and after treatment with saliva, 0·1 and 1·0% alcian blue at both pH 1·0 and pH 2·5, 0·1% alcian blue combined with periodic acid-Schiff at both pH 1·0 and 2·5 (Mowry, 1956; 1960; Lev & Spicer, 1964), and aldehyde fuchsin.

Additional blocks of tissue were fixed for four hours at 0 °C in 3·5% glutaraldehyde buffered in 0·1 M phosphate to a pH of 7·4. The tissues were washed in buffer and post-osmicated in 1% osmium tetroxide at 0 °C for two hours. The specimens were processed routinely, infiltrated with and embedded in Epon 812. Thick sctions of the material were cut at 0·5–3·0 μm and stained with toluidine blue. For electron microscopy thin sections of this material were mounted on uncoated grids and stained with uranyl acetate and lead citrate. The sections were examined in a RCA EMU-3F electron microscope operated at 50 kv.

Tissues for scanning electron microscopy were fixed as for transmission electron microscopy. The tissues were then dehydrated in alcohol and transferred to amyl acetate prior to critical point drying with liquid CO_2. Dried tissues were placed on spinner stubs and coated with a gold-palladium alloy to a depth of 20 nm in a vacuum evaporator. Specimens were viewed in a Cambridge Stereoscan Mark II electron microscope.

Quantitative studies of the small intestine were made at the 1·5, 2·5, 3·5, 5·5, 8·0 13·0 and 22·0 cm stages, and included measurement of the depth of the mucosal epithelium, the depth of the crypts and the lengths of the intestinal villi. All measurements were made by means of a filar micrometer on three sections for each of three animals at each stage. Epithelium and crypts for measurement were selected at

---

Fig. 1. A section taken from the duodenal region shows scattered, long villi within a central patent duodenal lumen. The muscularis externa is extremely thin and is represented only by a few scattered myoblasts. Interposed between the intestinal epithelium and the surrounding muscularis externa in a well developed capillary bed (arrows). Epon 812 section. Toluidine blue. × 100. Newborn opossum (1·5 cm).

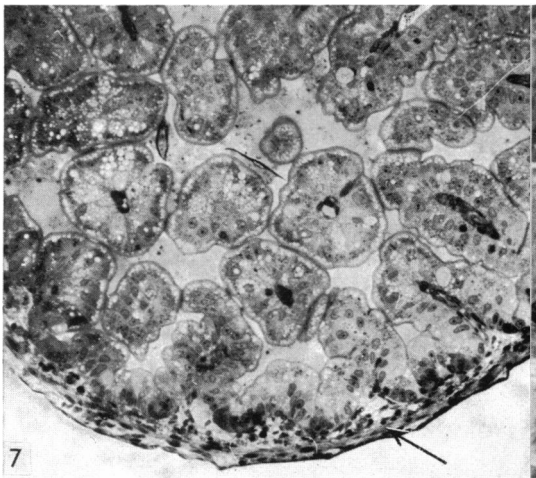
Fig. 2. A segment taken from the ileal region illustrates that the cross sectional diameter of the small intestine is much less in this region than in the duodenal area. Villi fill the intestinal lumen. Epon 812 section. Toluidine blue. × 100. Newborn opossum (1·5 cm).

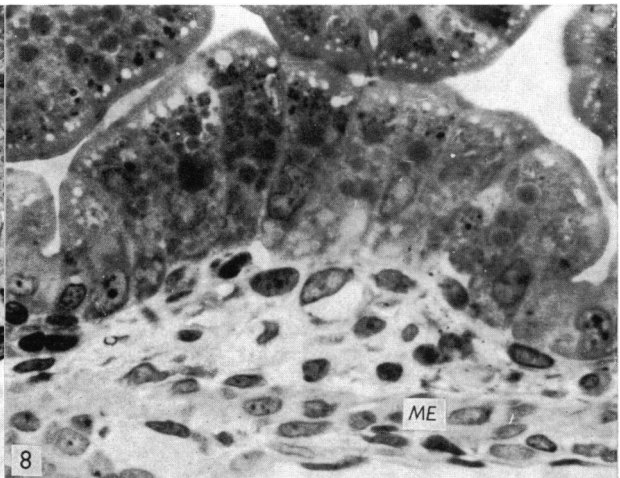
Fig. 3. A portion of the intestinal wall illustrates the thin muscularis externa and the surrounding mesothelial cells of the serosa (small arrows). The intestinal epithelial cells lining the intestinal floor (*F*) and adjacent villus (*V*) show an apical endocytic complex (large arrows), as well as absorbed material. Epon 812 section. Toluidine blue. × 375. Newborn opossum (1·5 cm).

Fig. 4. Intestinal epithelial cells of a villus. Irregular, dense complexes of absorbed material (small arrows) are found between the apical endocytic complex and the intestinal cell nuclei. Lipid droplets (large arrows) generally are found concentrated between cell nuclei and the basal plasmalemma. Epon 812 section. Toluidine blue. × 400. Newborn opossum (1·5 cm).

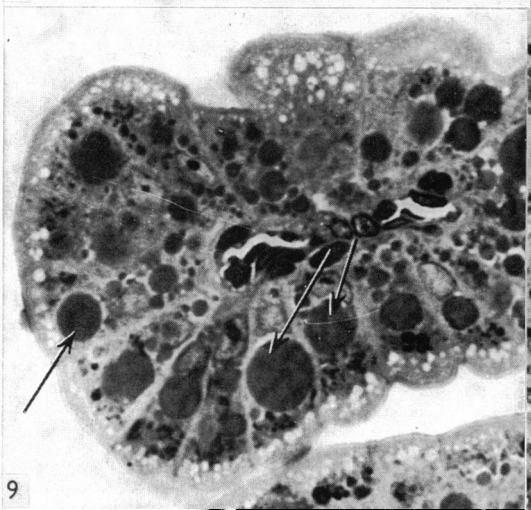
Fig. 5. A scanning electron micrograph of a portion of the duodenum shows the scattered villi and the intervening intestinal floor. × 200. Newborn opossum (1·5 cm).

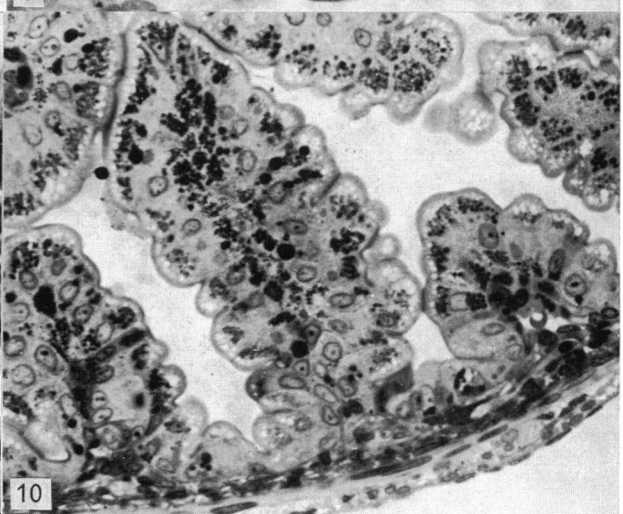
Fig. 6. The details of the apices of intestinal epithelial cells on two villi (*V*) and the intestinal floor (*F*). What appears to be the initial outgrowth of a forming villus is shown at lower right (arrow). × 500. Newborn opossum (1·5 cm).

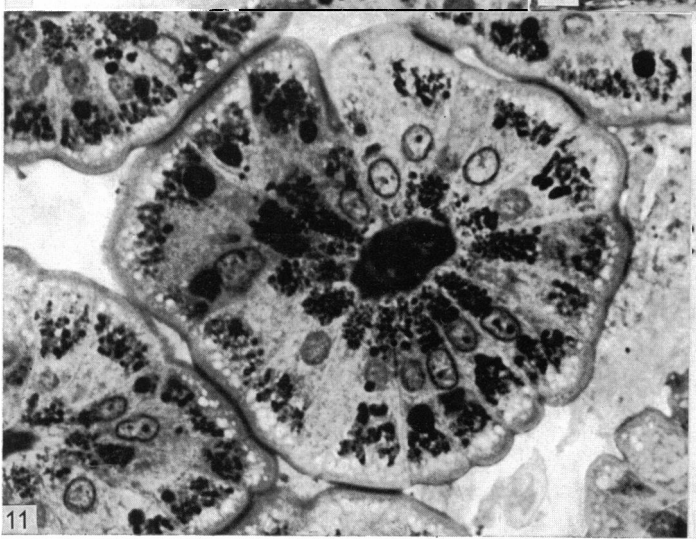

7

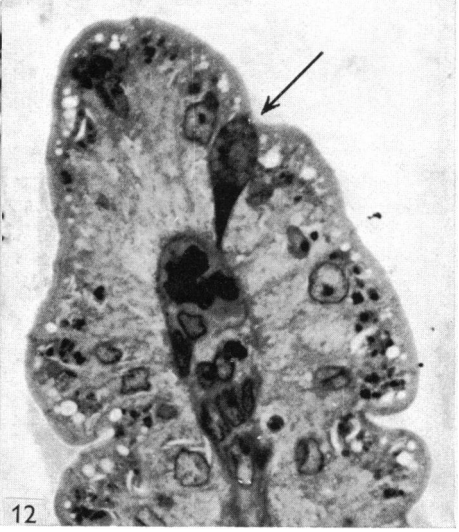


8


9


10


11


12

random. The lengths of the villi represent the lengths of the longest villi as determined by inspection of the sections. Paneth and goblet cells were counted in random fields and are reported as the number of cells per 1000 epithelial cells. The irregular nature of the sections precluded determination of the area. The locations of Paneth cells were studied also and are reported as percentages of Paneth cells found on the upper parts of the villi, at the bases of villi, and in the crypts.

The developing colon was assessed at the same stages and in a similar fashion. The depths of the mucosal epithelium and of the crypts were determined from random measurements. The number of mitotic cells and goblet cells were counted per 1000 epithelial cells.

RESULTS

Light and scanning electron microscopy

Small intestine

In the newborn opossum (1·5 cm), the small intestine is not uniform in diameter throughout its length: in the mid-duodenal region it is wide and long villi project into a distinct lumen (Fig. 1). In the distal segment of the small intestine, however, the diameter is much smaller and stubby villi occlude the narrow lumen (Fig. 2). In the duodenum, the outer layers of the wall are represented only by a single layer of myoblasts and a scant, delicate connective tissue (Figs. 1–3). An extensive capillary bed lies between the mucosa and the muscularis externa in this region. The intestinal epithelium covering both the intestinal floor and villi shows an extensive apical endocytic complex, numerous dense irregular aggregates of material in the supranuclear regions, and large lipid droplets (Figs. 3 and 4). A delicate connective tissue containing vessels comprises the core of developed villi. When the proximal small intestine is viewed from the mucosal surface, the intestinal floor is readily observed between scattered villi (Figs. 5 and 6). Small elevations are often observed projecting from the intestinal floor (Figs. 3 and 6), and are thought to represent the initial evaginations of developing villi.

Fig. 7. The duodenum of the 2·5 cm opossum (9 days) shows an increased number of villi and continued development of the muscularis externa (arrow). Epon 812 section. Toluidine blue. × 100.

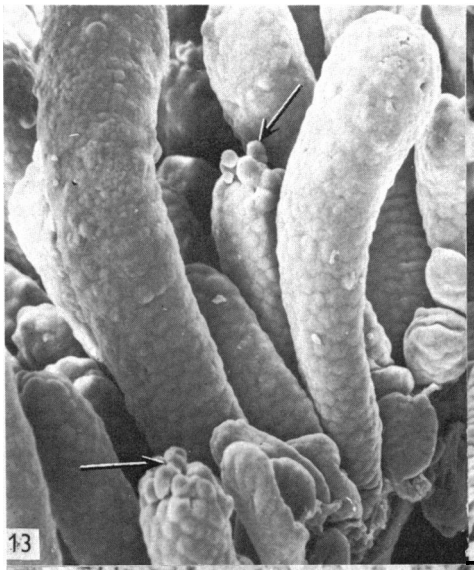
Fig. 8. A segment taken through the wall of the small intestine shows in greater detail the forming muscularis externa (*ME*) and the active intestinal epithelium of the intestinal floor. What may be the initial stage in the formation of a villus occupies the centre of the field of view. 2·5 cm opossum. Epon 812 section. Toluidine blue. × 400.

Fig. 9. A portion of a duodenal villus from the 2·5 cm opossum illustrates the continued intense activity of the intestinal epithelium and the large size of lipid droplets (arrows). Epon 812 section. Toluidine blue. × 375.

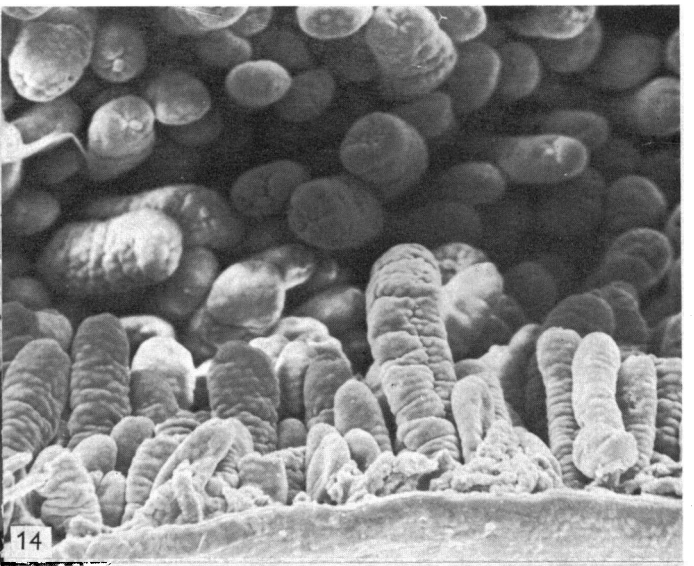
Fig. 10. The muscularis externa of the 3·0 cm opossum (*ca.* 13 days) shows both circular and longitudinal layers. Villi vary in height and appear more closely packed than in previous stages. Epon 812 section. Toluidine blue. × 250.

Fig. 11. A cross section of an intestinal villus illustrates in greater detail the activity of the intestinal epithelium. The apical endocytic complex remains a prominent feature and absorbed materials fill the supra- and subnuclear cytoplasm. 3·0 cm opossum. Epon 812 section. Toluidine blue. × 400.

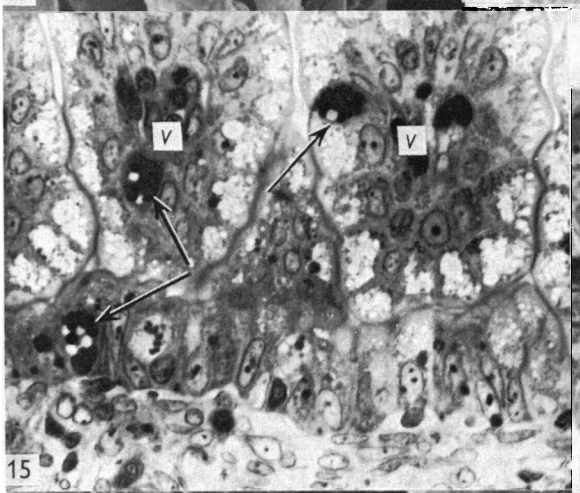
Fig. 12. The apex of an intestinal villus taken from a 5·5 cm opossum (*ca.* 32 days). The endocytic complex remains a prominent feature of the intestinal cells, although it is not as well developed as in earlier stages. A goblet cell also is shown (arrow). Epon 812 section. Toluidine blue. × 375.



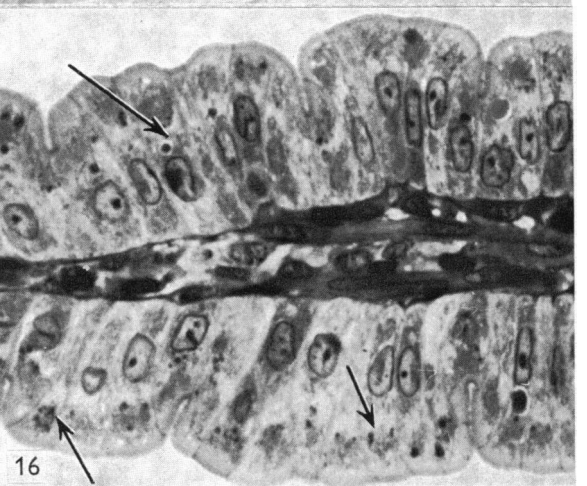
13



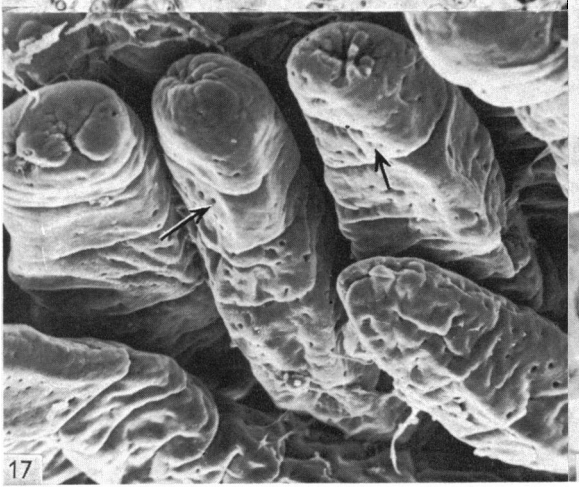
14



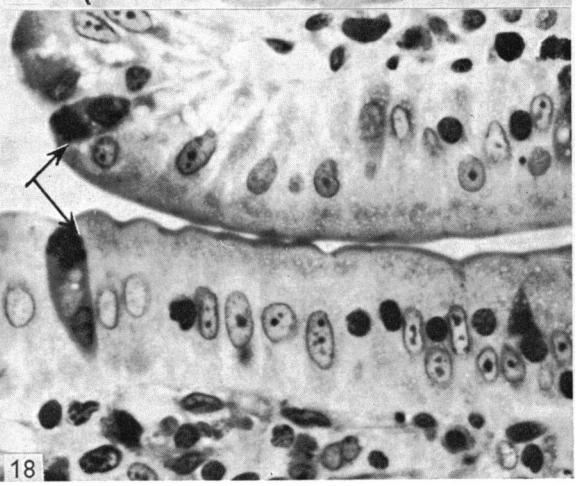
15



16



17



18

Villi increase in both size and number by 2·5 cm (9 days) (Fig. 7). The muscularis externa and submucosa show further development and the intestinal epithelium continues to show an extensive apical endocytic complex as well as numerous inclusions (Figs. 7 and 8). What appear to be forming villi continue to develop from the intestinal floor, which is comprised only of a narrow zone of connective tissue containing vessels and covered by an active intestinal epithelium (Fig. 8). Intestinal epithelial cells that cover the villi now show large accumulations of lipid, in addition to an active apical endocytic complex. Much of the lipid is in the form of large droplets that often measure 18 μm or more in diameter and are located in the supranuclear region (Fig. 9). Numerous smaller lipid droplets also are observed within the subnuclear cytoplasm, often lying immediately adjacent to the basal plasmalemma of the intestinal epithelial cells.

Villi show continued growth and differentiation in the 3·0 cm opossum (*ca.* 13 days postnatal)* resulting in a decrease in the area that is occupied by the primitive intestinal floor (Fig. 10). The muscularis externa shows definite inner circular and outer longitudinal layers of smooth muscle. The intestinal epithelium that covers the villi and the intestinal floor continues to show an active apical endocytic complex (Figs. 10 and 11). Numerous dense inclusions occupy major areas of both the supranuclear and subnuclear cytoplasm (Fig. 11). The large collections of supranuclear lipid observed previously appear diminished by the 3·5 cm stage but the epithelial cells continue to show an abundance of small lipid droplets. The intestinal epithelium shows absorptive activity through the 5·5 cm stage, with the majority of inclusions now restricted to the apical and supranuclear cytoplasm (Fig. 12). Occasional goblet cells (Fig. 12) and Paneth cells also are found scattered within the epithelium covering villi at this stage: a few of these cell types were noted at the base of villi and on the intestinal floor as early as 3·0 cm (*ca.* 13 days).

Villi continue to show marked differences with regard to height and to the state of differentiation in the small intestine of the 6 cm and 7 cm opossum (Figs. 13 and 14). Some villi may appear tall and club-like, others short and finger-like: these differences are thought to represent different stages of development. Large blebs are often

* Approximate age determinations are based on results presented by Moore & Bodian (1940) and Reynolds (1942).

Fig. 13. Ileal intestinal villi of the 6 cm opossum (*ca.* 37 days) vary considerably in length. Many appear elongate and club-like. Large blebs are often observed on the apices of villi at this stage of development (arrows). × 175.

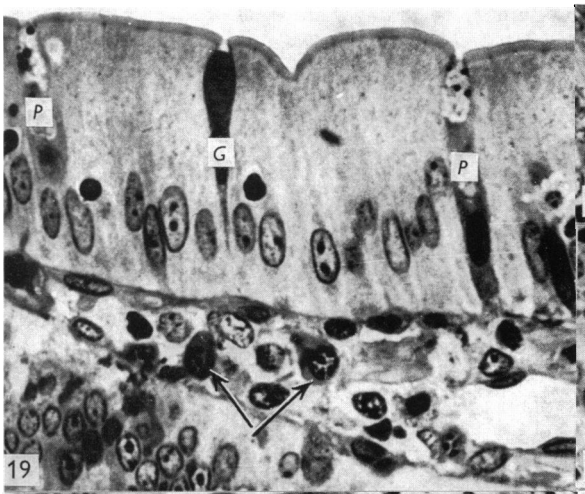
Fig. 14. Villi of the 7 cm opossum (*ca.* 44 days) appear more uniform in shape as compared with earlier stages. However, considerable variation in height continues to be apparent. × 100.

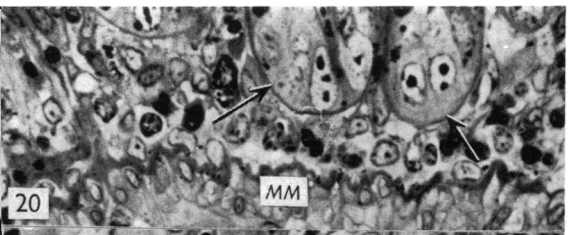
Fig. 15. Intestinal epithelial cells lining villi (*V*) and the intestinal floor of the 8 cm opossum (*ca.* 50 days) continue to show vacuolization. Paneth cells (arrows), with granules of varying stainability, are also shown. Epon 812 section. Toluidine blue. × 375.

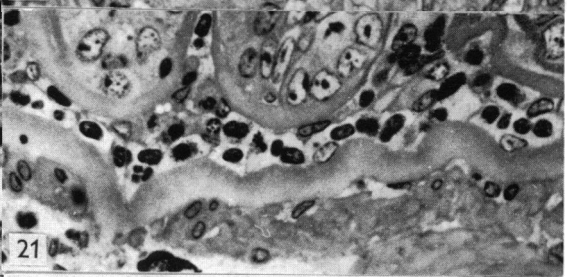
Fig. 16. A portion of an intestinal villus taken from a 13 cm opossum (*ca.* 75 days). The intestinal epithelial cells now show only scattered elements of the endocytic complex and a few inclusions (arrows). Epon 812 section. Toluidine blue. × 375.

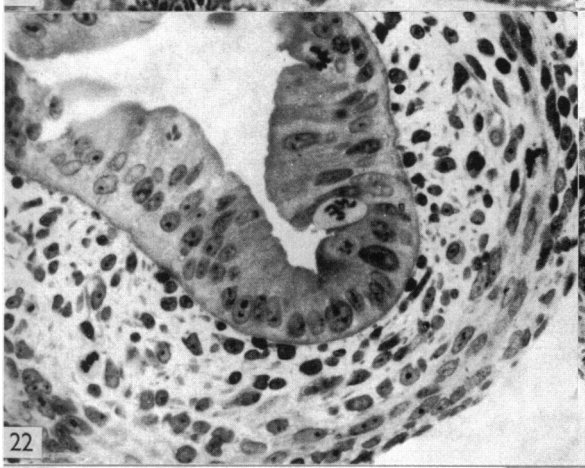
Fig. 17. Duodenal villi of the 13 cm opossum (*ca.* 75 days) appear large, finger-like and of a uniform height. The apices of goblet cells appear as depressions within the coat of microvilli (arrows). × 175.

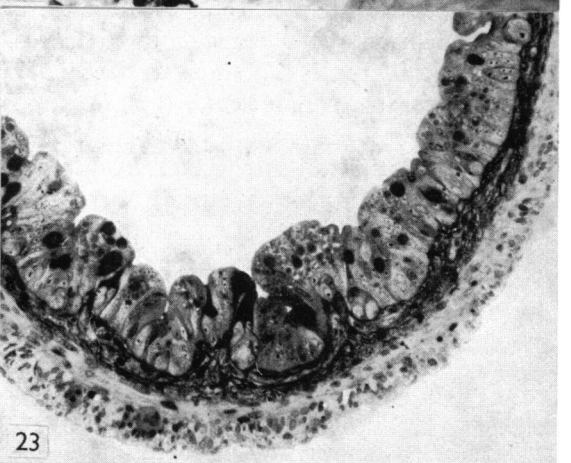
Fig. 18. Paneth cells of the opossum are found lining intestinal villi and are often observed near the apex (arrows). 15 cm opossum (*ca.* 95 days). Epon 812 section. Toluidine blue. × 375.

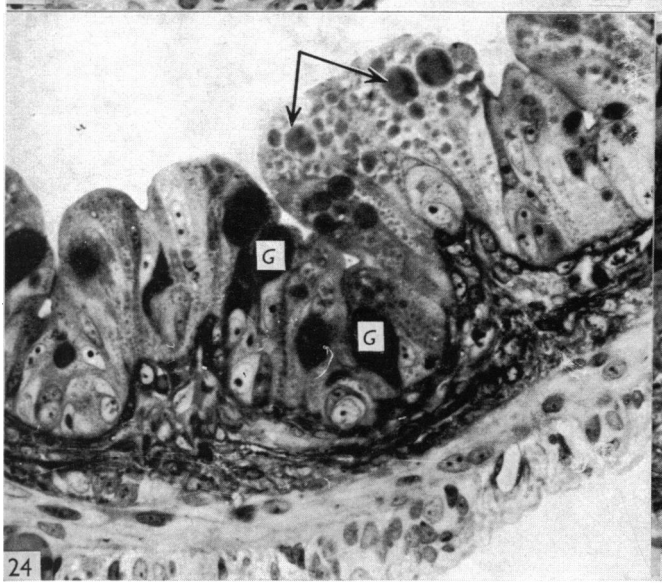

P
G
P
19

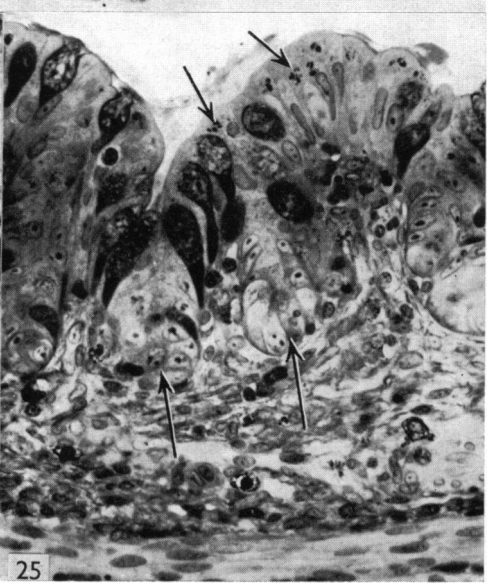

MM
20


21


22


23


G
G
24


25

observed at the apices of villi at this particular stage of development and may represent sloughing cells (Fig. 13). Intestinal epithelial cells continue to show a vacuolated apical endocytic complex through the 8 cm stage of development (*ca.* 50 days postnatal) (Fig. 15). This activity appears to be more marked in epithelial cells covering villi than in those at the bases of villi, where less vacuolation is observed. The cells show a reduction in the number of inclusions present, although lipid droplets continue to be frequently observed. Connective tissue elements now comprise a larger portion of the intestinal wall and of the cores of villi. Paneth cells are present in increased numbers on villi and show marked differences in the stainability of individual granules (Fig. 15).

By 13 cm (*ca.* 75 days) there appears to be a marked reduction of the endocytic complex in the intestinal epithelium of a number of the animals examined (Fig. 16). The intestinal epithelium from such animals shows only a few scattered inclusions. In some animals of this stage, however, the epithelium continues to show a well developed endocytic complex and numerous inclusions: in many animals these features persist until the time of weaning (or until solid food is observed within the intestinal tract). When viewed from the mucosal surface, villi of the 13 cm opossum appear more uniform in both height and general conformation (Fig. 17). The intestinal epithelium of the majority of 15 cm opossums examined (*ca.* 95 days) shows only scattered, minute vacuoles in the apical cytoplasm and exhibits a loss of the apical endocytic complex noted in earlier stages. Inclusions are observed only rarely, and lymphoid wandering cells are found within the intestinal epithelium with increased frequency (Fig. 18). Paneth cells are observed frequently on villi, sometimes at their apices (Fig. 18).

The principal cells of the intestinal epithelium of the weaned opossum appear

---

Fig. 19. The intestinal epithelium of the juvenile opossum (22 cm) is tall columnar and contains migrating lymphocytes. A goblet cell (*G*) and two Paneth cells (*P*) are also shown. The core of the villus contains numerous connective tissue cells, including two plasma cells (arrows). Epon 812 section. Toluidine blue. ×450.

Fig. 20. The limiting membranes of the lamina propria taken from the small intestine of the 28 cm opossum (juvenile). One membrane occupies a position on the luminal surface of the muscularis mucosae (*MM*) and the other encompasses the bases of the intestinal glands (crypts of Lieberkühn: arrows). Numerous types of connective tissue cells lie between the membranes. Epon 812 section. Toluidine blue ×375.

Fig. 21. The limiting membranes of the adult opossum are of considerable thickness. Numerous connective tissue cells continue to be sandwiched between the two membranes. Epon 812 section. Toluidine blue. ×375.

Fig. 22. A portion of the newborn (1·5 cm) colon, showing the immature nature of the lining epithelium and the forming muscularis externa. Epon 812 section. Toluidine blue. ×300.

Fig. 23. The colonic lumen of the 9 days old opossum (2·5 cm) shows an increase in diameter. The lining epithelium shows initial stages of infolding and appears to be involved in absorptive activity. The muscularis externa shows definite inner circular and outer longitudinal layers. Epon 812 section. Toluidine blue. ×100.

Fig. 24. Increased magnification of the colonic epithelium of the 2·5 cm opossum (9 days) illustrates lipid droplets (arrows) within the intestinal epithelium. Goblet cells (*G*) also are present. ×350.

Fig. 25. Colonic epithelial cells of the 3·5 cm opossum (*ca.* 17 days) show only scattered amounts of absorptive material (small arrows). Goblet cells are increased in number and intestinal glands are present (large arrows). Epon 812 section. Toluidine blue. ×240.

Table 1. *Quantitative data for developing small intestine*

	Body length (cm)						
	1·5	2·5	3·5	5·5	8·0	13·0	22·0
Depth of epithelium (μm)	30·1± 2·1	28·9± 1·7	32·4± 1·8	31·7± 2·4	31·8± 2·3	30·7± 1·6	29·6± 1·2
Mitoses/1000 ep. cells	7·3± 0·8	5·2± 0·3	2·3± 0·2	0·8± 0·01	0·3± 0·09	0·96±0·18	0·18±0·10
Paneth cells/1000	—	—	3·0± 0·2	5·9± 0·27	17·6± 0·14	39·7± 1·1	53·7± 2·3
Goblet cells/1000	—	—	7·0± 0·8	9·3± 0·16	18·3± 0·72	48·6± 0·94	89·4± 3·6
Depth of crypts (μm)	—	—	—	—	—	58·8±11·2	102·6±13·7
Length of villi (μm)							
proximal	124·6±21·4	152·2±22·8	160·2±19·7	186·6±26·2	213·6±21·4	259·5±28·3	280·5±19·6
distal	91·4± 9·8	103·3±15·2	N.M.	124·7± 7·6	N.M.	168·5±15·3	179·6±21·4

Values are mean ±S.D.

Table 2. *Distribution of Paneth cells*

	Body length (cm)						
	1·5	2·5	3·5	5.5	8·0	13·0	22·0
Total Paneth cells per 1000 epithelial cells	—	—	3·0±0·2	5·9±0·27	17·6±0·14	39·7±0·11	53·7±2·3
% on villi	—	—	83	56	38	20	8
% at base of villi	—	—	17	44	62	26	14
% in crypts	—	—	—	—	—	54	78

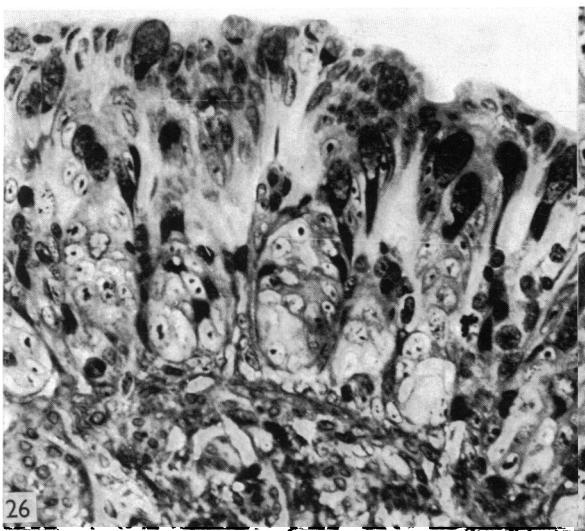
similar to those reported in the majority of mammals (Fig. 19). The intestinal epithelium is comprised principally of tall columnar cells with a prominent striated border, and it contains scattered goblet cells and lymphoid wandering cells. Unlike other species reported to date, Paneth cells are also frequently observed within the epithelium covering villi. The lamina propria and submucosa are well developed and contain numerous connective tissue cells, primarily lymphocytes, plasma cells, mast cells and eosinophils.

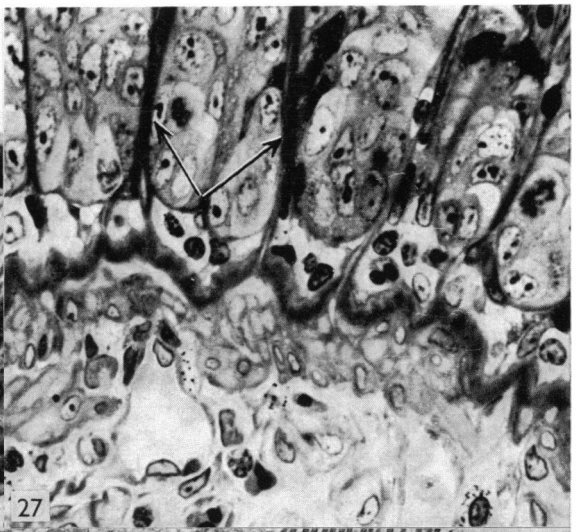
In the juvenile animal (20 cm), two membranes which limit the lamina propria beneath the intestinal glands make their first appearance. By 28 cm these membranes are well established, one covering the luminal side of the muscularis mucosae and the other forming a series of cup-like structures embracing the bases of the intestinal glands (Fig. 20). Sandwiched between these laminae are numerous lymphocytes, plasma cells and eosinophils (Figs. 20 and 21). The laminae show continued development throughout adult life and often attain a depth of 25 μm or more (Fig. 21).

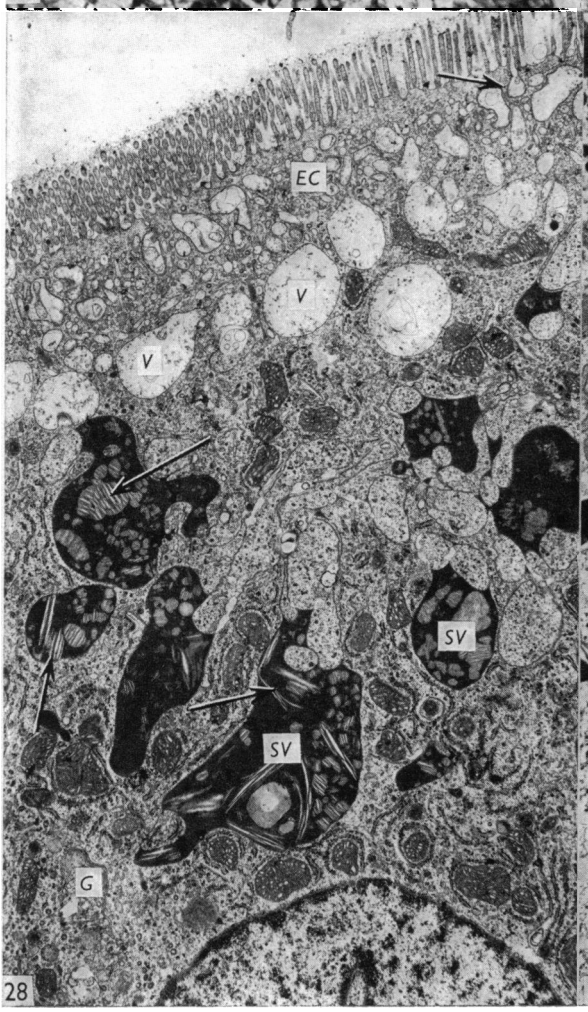
Goblet cells within the intestinal epithelium of pre- and post-weaned opossums stain intensely with PAS, aldehyde fuchsin and alcian blue (at both pH 1·0 and 2·5). Such results indicate that the secretory product, in part at least, is an acidic form of glycoprotein. Paneth cell granules fail to stain with these procedures but do stain pink with eosin and a bright red with Masson's original trichrome.

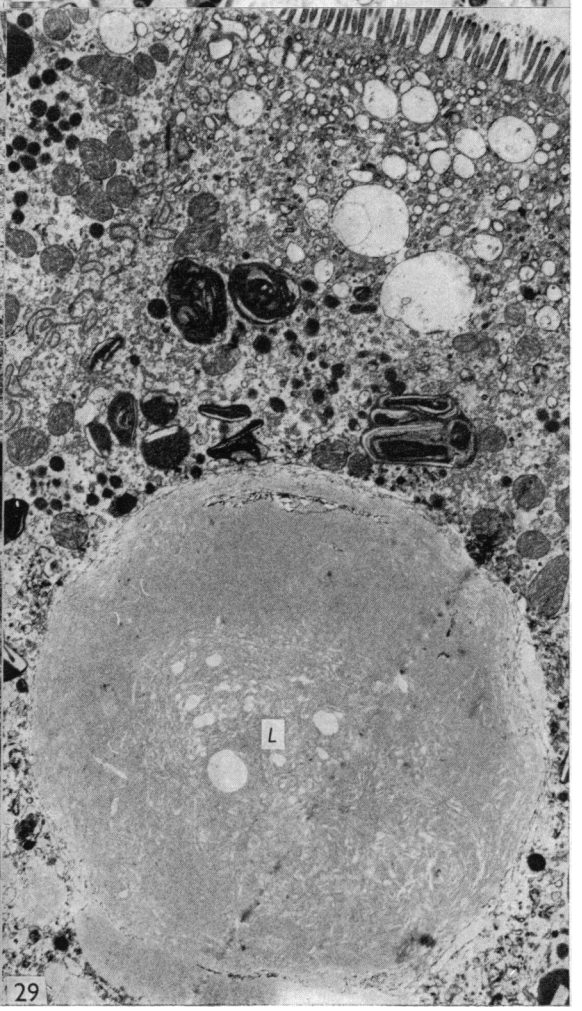
The results of the quantitative survey of the developing small intestine are shown in Table 1. The depth of the mucosal epithelium remains fairly constant throughout the various stages of development. Mitotic figures, prominent at birth, show a continual decline until the 13·0 cm stage, at which time there is a slight peak, coincident with the appearance of well defined crypts. Paneth cells and goblet cells appear first at the 3·5 cm stage and show a progressive increase in their numbers throughout subsequent stages. There is a rather marked difference in the length of the villi in the proximal and distal parts of the intestine: while in both areas the villi show a progressive increase in size, those in the proximal intestine are consistently longer than those of the distal intestine.

Table 2 shows the distribution of the Paneth cells in the intestinal mucosa. These cells first appear at about the 3·5 cm stage and initially are mainly scattered randomly among the surface epithelial cells that cover the villi: only a few are present at the bases of the villi. As the small intestine continues its development, Paneth cells become more concentrated at the bases of the villi and, with the establishment of crypts, are concentrated in these structures. However, a proportion of the Paneth cells is still located along the sides and apices of the villi, even at the 22·0 cm stage.


26


27


EC
V
V
SV
SV
G
28


L
29

Colon

The colon of the newborn opossum (1·5 cm) is surfaced by either a simple or a pseudostratified columnar epithelium and exhibits a lumen of small diameter (Fig. 22). The underlying connective tissue is of considerable depth and the muscularis externa is represented by a layer of developing myoblasts. By 2·5 cm (*ca.* 9 days) the colonic lumen has expanded considerably, the colonic mucosa shows numerous infoldings, and the two layers of the muscularis externa are well developed (Fig. 23). The colonic epithelium contains numerous large lipid droplets and, like that of the small intestine, shows a well developed apical endocytic complex. Numerous goblet cells are observed within the epithelium, which also shows basal invaginations that may represent the initial stages of intestinal gland formation (Fig. 24). By 3·5 cm (*ca.* 17 days), only scattered inclusions are observed in the colonic epithelium, and definite intestinal glands are present (Fig. 25). Goblet cells are found in greater numbers than in younger stages. The apical endocytic complex is not observed in the colonic epithelium at the 4·5 cm stage, and intestinal glands show continued development. The latter are well established by 11·5 cm (*ca.* 67 days) (Fig. 26). As observed in the small intestine, two membranes also develop and limit the lamina propria of the colon in the juvenile animals. As in the small intestine, the membrane on the luminal surface of the muscularis mucosae is most marked. It gives rise to projections that course through the lamina propria and extend between intestinal glands (Fig. 27). The membrane that encompasses the bases of the intestinal glands is poorly developed in the colon and never reaches the depth of its counterpart in the small intestine. As in the small intestine, numerous connective tissue cells are found between the two developing membranes (Fig. 27).

Table 3 shows the quantitative data for the developing colon. The thickness of the mucosal epithelium shows no significant changes throughout the period of postnatal development. Goblet cells are a prominent feature of the epithelial population, even

---

Fig. 26. A segment of colonic mucosa taken from the 11·5 cm opossum (*ca.* 67 days) shows numerous goblet cells and well developed intestinal glands. Epon 812 section. Toluidine blue. ×240.

Fig. 27. The limiting membrane on the luminal surface of the muscularis mucosae is well developed in the colon of the 28 cm opossum. Portions of this membrane extend toward the lumen (arrows) in relation to intestinal glands. The membrane embracing the intestinal glands is less well developed and numerous connective tissue cells lie between the limiting membranes. Epon 812 section. Toluidine blue. ×400.

Fig. 28. The apical and supranuclear region of an intestinal epithelial cell taken from the duodenum of the newborn opossum (1·5 cm). The apical region shows elongate microvilli and the tubulovesicular system of the endocytic complex (*EC*), as well as large underlying vacuoles (*V*). The endocytic complex occasionally shows continuity with the intestinal lumen (small arrow). Mitochondria appear irregular, electron-dense, and have densely packed cristae. The large supranuclear vacuoles (*SV*) are filled with an electron-dense material that contains structures which resemble myelin figures (large arrows). Golgi membranes (*G*) are associated with an amorphous material and numerous small vesicles. Scattered elements of granular endoplasmic reticulum also are evident. ×6000.

Fig. 29. Portions of the apices of two intestinal epithelial cells, one of which contains a large accumulation of lipid (*L*). The endocytic complex, and the underlying vacuoles containing electron-dense material, remain prominent features of the 2·5 cm opossum (9 days). ×5500.

Table 3. *Quantitative data for developing colon*

	Body length (cm)						
	1·5	2·5	3·5	5·5	8·0	13·0	22·0
Depth of epithelium (μm)	33·4± 1·8	33·12± 1·9	31·3 ± 1·4	28·7 ± 1·1	29·2 ± 2·0	29·8 ± 1·6	30·6 ± 2·4
Mitoses/1000	8·4± 0·51	4·2 ± 0·51	1·09± 0·61	0·36± 0·07	0·17± 0·06	0·19± 0·07	0·22± 0·03
Goblet cells/1000	116·0±10·1	208·0 ±18·1	312·0 ±16·4	476·0 ±22·3	516·0 ±29·8	537·0 ±31·2	611·0 ±39·7
Depth of crypts (μm)	—	36·4 ± 1·2	48·3 ± 3·4	91·4 ± 4·1	129·6 ± 6·2	157·3 ± 5·3	166·8 ± 8·1

All values are mean ± S.D.

in the newborn, where they comprise nearly 12% of the surface lining cells. Their numbers increase progressively so that by the 22·0 cm stage goblet cells make up 60% of the mucosal epithelium. Crypts are well-formed structures as early as the 2·5 cm stage, and increase in depth thereafter. Mitotic activity is prominent during the first nine days (2·5 cm) but decreases rapidly in the succeeding stages of development.

Electron microscopy

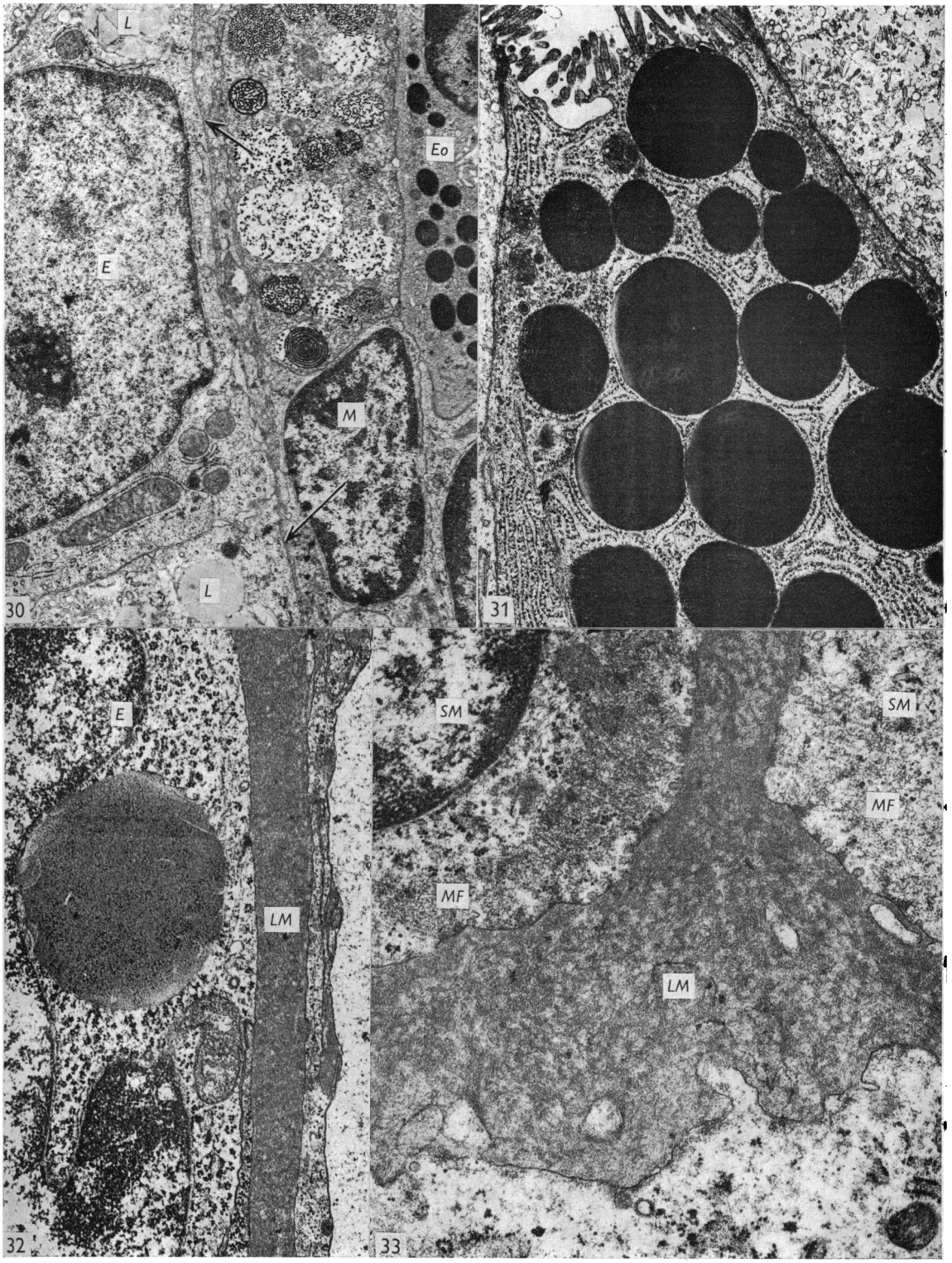
Small intestine

The ultrastructural portion of the present study confirms and extends those observations made by light and scanning electron microscopy. The apical endocytic complex seen in the intestinal epithelial cells of the early postnatal stages consists of numerous vesicles and anastomosing tubules which appear to invaginate into the cytoplasm from areas between microvilli (Fig. 28). The tubulovesicular component of the complex expands into large membrane-bound vacuoles, which appear empty. Immediately subjacent to the empty vacuoles are large irregular complexes of an electron-dense material that contains numerous laminated bodies (Fig. 28). These structures are limited by a membrane and are directly associated with the overlying vacuoles. The cytoplasm shows numerous mitochondria which appear dense and contain numerous cristae. Scattered profiles of granular endoplasmic reticulum and numerous small vesicles containing a light amorphous material also are observed in the supranuclear cytoplasm. Numerous lipid droplets are found scattered throughout the cytoplasm. Endocrine cells containing small membrane-bound, electron-dense granules, and occasional profiles of granular endoplasmic reticulum, are observed in the intestinal epithelium of the newborn and in all subsequent stages examined.

By 2·5 cm (*ca.* 9 days) the large membrane-bound lipid droplets occupy a considerable area of the supranuclear cytoplasm (Fig. 29). Numerous small electron-dense vesicles also are noted in the supranuclear cytoplasm. Smaller lipid droplets, which continue to be found throughout the cytoplasm in this and subsequent stages, are often found in relation to the basal cell membrane (Fig. 30).

Paneth cells show an abundance of granular endoplasmic reticulum throughout the cytoplasm, including the apical region (Fig. 31). Mature Paneth cell granules are large and exhibit considerable electron density. In the supranuclear region, forming granules appear directly associated with adjacent Golgi membranes: these granules are limited by a membrane and contain a light amorphous material that varies considerably in electron density. Small transport vesicles also are found between elements of the granular endoplasmic reticulum and nearby Golgi membranes.

The large membranes limiting the lamina propria in the juvenile opossum are morphologically distinct. The membrane encompassing the intestinal glands consists of a fine amorphous material and lies in the region formerly occupied by the basal lamina (Fig. 32). The membrane lying on the luminal side of the muscularis mucosae also consists of amorphous material, but varies in electron density and exhibits a patterned appearance (Fig. 33). Both membranes increase in size during adulthood and the membrane associated with the muscularis mucosae contains a considerable amount of collagen. Similar appearances are found in the limiting membranes of the colon, which differ from those of the small intestine only in their overall depth.


L
Eo
E
M
L
30
31
E
LM
32
SM
SM
MF
MF
LM
33

Colon

The apices of the colonic epithelial cells of the newborn opossum (1·5 cm) show numerous small membrane-bound vesicles that vary considerably in electron density (Fig. 34). Scattered mitochondria, Golgi membranes and numerous free ribosomes are found throughout the cytoplasm, which shows a moderate electron density. Numerous large lipid droplets and a well developed endocytic complex are prominent features of the colonic epithelial cells in the 2·5 cm opossum (Fig. 35). By 4·5 cm (*ca.* 20 days) this complex is no longer found and lipid droplets are not observed in the lining epithelium. Irregular, elongate crystals, first observed at 3·5 cm, are found scattered in the apical and supranuclear cytoplasm of the colonic epithelial cells (Fig. 36). They appear limited by a membrane and show central regions of increased electron density (Fig. 37). The ultrastructural appearances of the colonic epithelium in all subsequent developmental stages, and in the adult, are unchanged from those noted at the 3·5 cm stage.

DISCUSSION

In general, the intestinal tract of the newborn opossum shows a pattern of development similar to that reported in the newborn of other mammalian forms: however, it exhibits some modifications, presumably associated with the remarkably short gestation period of the opossum and to increase the chances of survival immediately after birth. The duodenum shows a well developed lumen with scattered, elongated villi. The distal segments of the small intestine have a diameter only about one third of that of the duodenum. The lumen of the distal small intestine is filled with short immature villi. Throughout the small intestine, the muscularis externa is represented by only a single layer of myoblasts. Interposed between the intestinal lining epithelium and the developing muscularis externa there is a well developed vascular bed that occupies a considerable proportion of the intestinal wall. Additional villi apparently form as a result of evaginations of the epithelium, together with the underlying mesenchyme and vasculature, into the intestinal lumen. Villi are observed at various stages of maturation until just prior to weaning (15 cm; *ca* 95 days). A similar pattern of late villus formation and growth has been reported in other vertebrates, including man (Hilton, 1902; Johnson, 1910; Kammeraad, 1942). Thus,

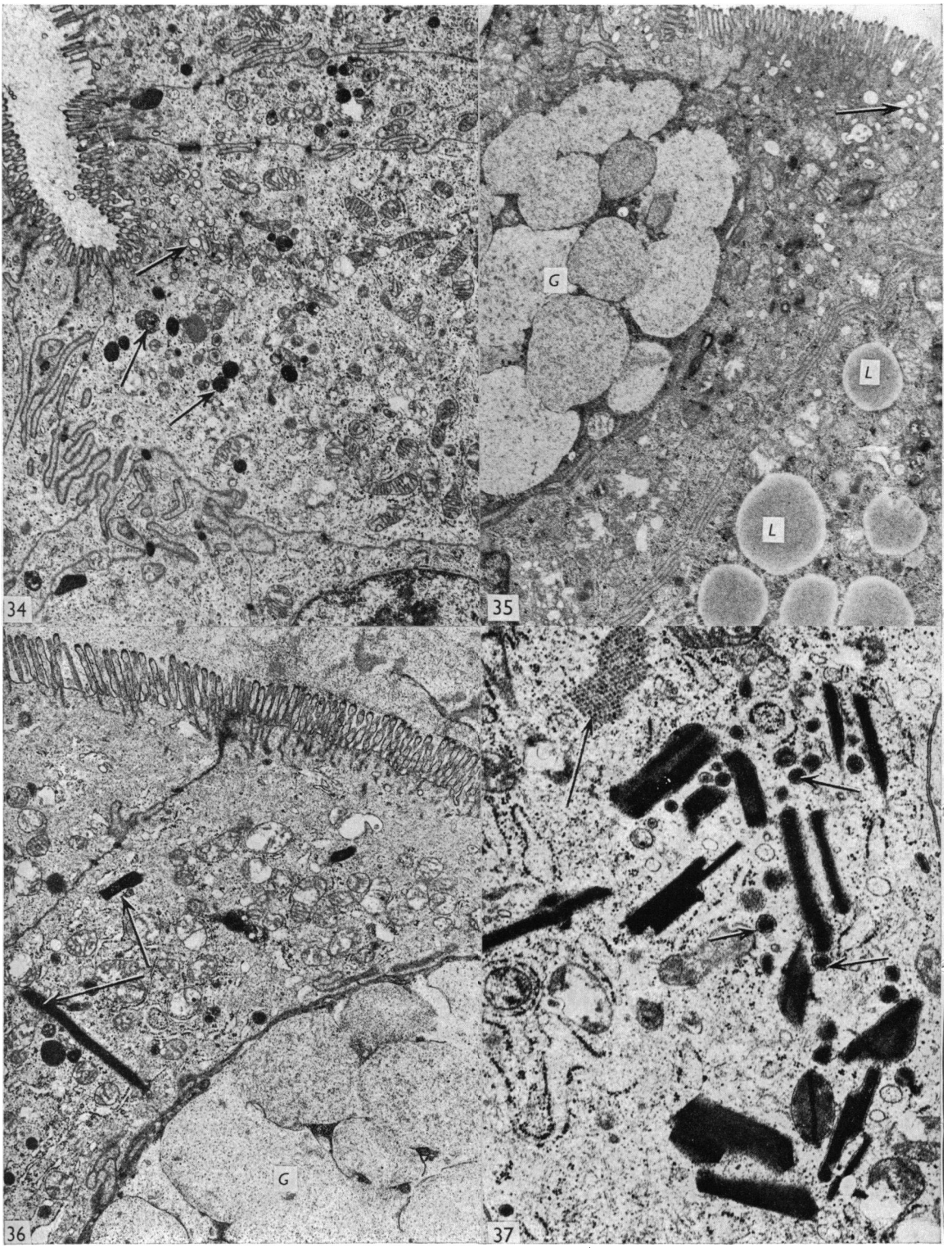
---

Fig. 30. The basal region of intestinal epithelial cells (*E*) from a 8·0 cm opossum (*ca.* 50 days) continues to show scattered lipid droplets (*L*). The epithelium lies on a delicate basal lamina (arrows) at this stage of development. A mast cell (*M*) and a portion of a eosinophilic granulocyte (*Eo*) are also shown. × 5000.

Fig. 31. The apex of a Paneth cell taken from an intestinal villus of a 19 cm opossum (juvenile). The cytoplasm contains large electron-dense granules, associated with numerous profiles of granular endoplasmic reticulum. x10000.

Fig. 32. The base of an intestinal epithelial cell (*E*) and a portion of the limiting membrane (*LM*) encompassing an intestinal gland. The membrane is composed of an amorphous electron-dense material. 28 cm opossum. × 13500.

Fig. 33. A segment of the limiting membrane on the luminal surface of smooth muscle cells (SM) comprising the muscularis mucosae. These cells show pinocytotic vesicles and peripheral accumulations of myofilaments (*MF*). The limiting membrane (*LM*) has a heterogeneous appearance. × 20000.


34
G
L
L
35
G
36
37

the mucosa of the proximal small intestine appears precocious in its morphology in comparison with the stomach of the newborn opossum (Krause, Cutts & Leeson, 1976*b*). The mucosa of the entire small intestine appears to be modified solely for absorption, whereas the remainder of the intestinal wall is very immature in appearance. The stomach of the newborn, which is lined by a columnar or pseudostratified columnar epithelium without gastric glands, does not appear to act as a storage vehicle for ingested milk, but morphologically appears very active in the absorption of lipid during the first three weeks after birth (Krause, Cutts & Leeson, 1976*b*).

Intestinal glands are not present in the newborn opossum and do not develop until relatively late, making their initial appearance at approximately 8·5 cm. The duodenal glands, which are confined to the most proximal portion of the duodenum, begin to develop in the newborn as direct outgrowths from the epithelium of the intestinal floor, and not from the intestinal glands as in other species (Krause & Leeson, 1969). As in other species, the intestinal glands develop as outgrowths between adjacent villi (Hilton, 1902; Kammeraad, 1942) but are shallow in appearance, even in the adult. The late formation of the intestinal glands in the small intestine, together with their extremely short length, may explain why Paneth cells of this particular species are found scattered throughout the intestinal lining epithelium and are often observed at the tips of villi. The location of Paneth cells on villi is quite unlike the localized distribution reported in other vertebrate species, where they are confined to the bases of intestinal glands (Oppel, 1897; Klein, 1906; Castro, Sasso & Saad, 1959; Wheeler & Wheeler, 1964; Krause, 1971). Paneth cells of the opossum show an abundance of granular endoplasmic reticulum and large homogeneous electron-dense granules similar to those reported in the bat (Bloom & Fawcett, 1962), man (Trier, 1963), rat (Behnke & More, 1964) and echidna (Krause, 1971). Histochemical results concerned with the nature of the Paneth cell granules in the opossum were inconclusive since the granules failed to stain with the methods used. These negative results do suggest, however, that the granules contain very little mucin. Paneth cell granules of the mouse (Merzel, 1967; Selzman & Liebelt, 1961) and South American ant bear (Glerean & Castro, 1965) show distinct histochemical features and are composed of a dense protein core surrounded by a halo of acidic mucosubstance. Paneth cell granule synthesis appears similar to that reported in the mouse (Trier, Lorenzsonn & Groehler, 1967). These workers, using H^3-leucine, found the pattern of protein synthesis, intracellular transport, storage and discharge

---

Fig. 34. The apices of colonic epithelial cells from the newborn opossum (1·5 cm) show numerous membrane-bound vesicles (arrows); some of these appear empty, whereas others contain material of varying electron density. × 8000.

Fig. 35. A segment of colonic epithelium from the 2·5 cm opossum showing a goblet cell (*G*) and portions of two epithelial cells. The latter contain tubules of the apical endocytic complex (arrow) and large lipid droplets (*L*). × 10000.

Fig. 36. Scattered crystalline structures (arrows) are observed in the colonic epithelium of the 4·5 cm opossum (*ca.* 20 days). A portion of a goblet cell (*G*) also is shown. × 8000.

Fig. 37. Increased magnification of crystals similar to those shown in Figure 36 reveals that they are limited by a membrane and vary in electron density. The small electron-dense membrane-bound vesicles (small arrows) may represent crystals in cross section. An additional structure with a mosaic pattern is also shown (large arrow). × 15000.

similar to that reported in pancreatic acinar cells (Warshawsky, Leblond & Droz, 1963; Caro & Palade, 1964). Similar morphological observations also have been reported for Paneth cell granule formation in the echidna (Krause, 1971).

Scattered goblet cells are found only in limited numbers in the early postnatal stages and do not comprise a significant population until 15 cm, (*ca.* 95 days). They stain with alcian blue, PAS, and aldehyde fuchsin and are readily distinguished from the Paneth cell population. These results indicate that the secretion of goblet cells is composed of an acidic mucosubstance.

In contrast to the small intestine, the colon shows a prominent goblet cell population by 2·5 cm (9 days) and intestinal gland formation is well established by 4·5 cm (*ca.* 20 days). Hence, with regard to goblet cell population, intestinal gland formation, and development of the muscularis externa, the colon appears accelerated in its development when compared to the small intestine, and does not follow the general pattern of proximal–distal progression of development as reported in other species (Helander, 1973). However, a similar sequence with regard to proliferative activity of the epithelium has been reported in the rat colon (Eastwood & Trier, 1974). The formation of villi in the small intestine does appear to follow a proximal–distal progression, however, with the longer, more mature villi being observed in the duodenum and the shorter less mature variety confined to the distal segments of the small intestine.

The entire mucosa of the stomach, small intestine and colon of the opossum during the first two weeks after birth appears specifically adapted for absorption. The gastric mucosa does not show gastric glands at birth and they are only rudimentary during the next three weeks (Krause, Cutts & Leeson, 1976*b*). Similarly, the mucosa of the oesophagus does not show a true stratified squamous epithelium during this period of postnatal development (Krause, Cutts, & Leeson 1976*a*). The surface lining epithelium of the intestine shows numerous lipid droplets and appears actively involved in the absorption of lipid. Similarly, the lining epithelium of the colon is evidently involved in absorption of lipid (and other materials) during the first two weeks after birth. The intestinal lining cells of the colon also show a well developed apical endocytic complex during this period. As judged from morphological observations, absorptive activity ceases first in the colon and then the stomach in the next week. Absorption in the small intestine remains the dominant morphological feature until just prior to weaning. Gastric absorption of lipids and gastric lipase activity in suckling mammals have been noted in the rat (Helander & Olivecrona, 1970; Engelrud, Olivecrona & Helander, 1971), echidna (Krause, 1972), and opossum (Krause, Cutts & Leeson, 1976*b*). The absorption of lipids by the normal fetal colon also has been reported previously. Garbarasch & von Bülow (1969) observed an increasing amount of lipid in the colonic absorptive cells of the neonatal mouse during the first day after birth. The lipid droplets decrease in size and amount after the end of the suckling period in the mouse, whereas in the opossum they are found only during the second week of suckling. Milk of *Didelphis* in general contains more solids, fat, protein and less carbohydrate than the milk of eutherians (Bergmen & Housley, 1968). Possible differences in milk composition, however, were not determined at varying stages of lactation.

Modification of the small intestine for absorption in the early stages apparently

results in a delay in the appearance of intestinal glands and of normal populations of cell types other than the principal intestinal-lining cell. A vast vascular bed underlies the absorbing epithelium on the intestinal floor and extends up into mature duodenal villi and may increase the absorptive capacity of the small intestine. The intestinal absorbing cells show an active endocytic complex and other morphological features similar to those reported in a variety of eutherian species (Hill & Hardy, 1956; Clark, 1959; Anderson, 1963; Cornell & Padykula, 1969; Wissig & Graney, 1968; Kraehenbuhl & Campiche, 1969; Clark & Hardy, 1970, 1971; Staley, Corley, Bush & Jones, 1972). Unlike the majority of species, however, where absorptive activity is largely restricted to villi of the distal small intestine, the entire small intestine of the opossum shows modifications for absorption. The absorptive activity is not restricted to villi, as in other forms, but occurs in cells lining the intestinal floor. The pathway for lipid absorption in the intestinal tract appears similar to that reported in other species: lipid enters the cisternae of the Golgi complex and endoplasmic reticulum and chylomicra are released between adjacent cell membranes (Friedman & Cardell, 1972*a*; 1972*b*; Sage & Jersild, 1971).

The opossum differs from some suckling species with regard to the large dimensions of the lipid droplets which accumulate in the supranuclear region, particularly during the 3·0 cm and 3·5 cm stages. The nature of the electron-dense material within the large supranuclear vacuoles is unknown, but may represent an area of breakdown of sequestered protein, as reported in other species. Most reports to date indicate that the ileum is the region where macromolecular absorption takes place in the newborn mammal (Clark, 1959; Graney, 1968; Cornell & Padykula, 1969; Kraehenbuhl & Campiche, 1969). However, recent reports by Rodewald (1969, 1970) and Worthington & Graney (1973) have shown that a significant amount of selective transport of exogenous protein occurs in the proximal small intestine.

The intestinal epithelium of the suckling opossum shows an apical complex of anastomosing tubules and vacuoles similar to that reported in several suckling species. A large supranuclear vacuole divides the absorptive cell into apical and basal portions. The supranuclear vacuole is reported to contain hydrolytic enzymes in other species, and is thought to be of considerable importance with regard to breakdown of absorbed materials (Shervey, 1966; Kraehenbuhl & Campiche, 1969; Cornell & Padykula, 1969). The cellular transport of absorbed macromolecules has not only nutritional value, but also immunological relevance, in many species: for example, in ungulates (pig and horse) passive immunity is transmitted largely by intestinal absorption of intact antibodies during suckling. In the rabbit and guinea-pig, however, intestinal absorption and transport of intact antibodies does not occur (Kraehenbuhl & Campiche, 1969). The rat and mouse represent intermediate species where antibodies are transmitted from mother to offspring both across the placenta and by intestinal absorption during suckling. A vesicular mechanism for the transport of intact antibodies has been described by Rodewald (1969; 1970), who used labelled antibody in the proximal small intestine of the rat. Cells of this region lack the large supranuclear vacuoles that characterize the more distal cells, which also have been implicated in selective transport (Clark, 1959; Bamford, 1966; Graney, 1968; Anderson, 1969; Kraehenbuhl & Campiche, 1969). The mechanism by which absorbed material in the distal cells is either directed to the

lysosomal complex of the supranuclear vacuoles to be broken down, or bypasses this complex, as is the case in certain species, is unknown. Cells of both the proximal and distal small intestine of the opossum show large supranuclear vacuoles. Similar observations were noted in the echidna (Krause, 1972). Whether or not the intestinal epithelium of the opossum is involved in the transport of passive immunity is unknown: the very poorly developed placental attachment in the opossum would favour such a hypothesis (McCady, 1938; Sharman, 1961). The delayed differentiation of chief cells in the developing opossum stomach (Krause, Cutts & Leeson, 1976*b*) might be interpreted as additional support for an intestinal route for transfer of immunoglobulins. It is known that three Australian marsupials, the quokka, possum and tammer, derive all maternal immunoglobulins from colostrum and milk in the intestine (Yadav, 1971).

The apical endocytic complex persists until just prior to weaning and, in this respect, the time sequence is similar to that which occurs in the rat. The loss of the absorptive complex occurs first at the base of villi and then progresses toward the villus tip. As this occurs, there is also a progressive loss of the vacuolated cells in a proximal–distal sequence down the intestinal tract. The cessation of uptake of macromolecules by the intestinal epithelium of the suckling rat is not thought to be due to decreased uptake by individual cells, but to a replacement by new cells incapable of this function (Clarke & Hardy, 1969). Rundell & Lecce (1972), however, have reported that the cessation of absorption of macromolecules by the intestinal epithelial cells of the neonatal mouse, rabbit, hamster and guinea-pig occurs before replacement of the initial cell population. These findings indicate that closure is not a direct consequence of turnover of the cell population, and may be more complex. The time of cessation of macromolecular absorption in the small intestinal epithelium of suckling mammals appears to differ with the species, occurring about 1 day in the guinea-pig, 6 days in the hamster, 17 days in the mouse, 18 days in the rat, and 24 days in the rabbit (Brambell, 1958; Lecce, 1966; Morris, 1968; Clarke & Hardy, 1969).

Just after weaning two membranes limiting the lamina propria commence development in the intestinal tract of the opossum (Krause & Leeson, 1969). As the membranes increase in width, numerous connective tissue cells, principally eosinophils, lymphocytes and plasma cells, fill the interstices between membranes. Membranes in the lamina propria of the intestinal tract have been reported in several Australian marsupials (Krause, 1972), as well as in the duckbilled platypus (Krause, 1975). The layer of connective tissue cells between laminae of the intestinal tract of the opossum varies from three to five cells deep, forming a complete sleeve of connective tissue cells that surrounds the entire intestinal tract. It is of interest that only a few Peyer's patches are found in the intestine of juvenile and adult opossums. It may be that the large population of connective tissue cells localized between the membranes of the lamina propria acts as a defensive barrier against the invasion of foreign organisms from the intestinal lumen into the underlying vasculature. A similar hypothesis has been proposed for the cells underlying the gut epithelium of the mouse (Deane, 1964).

SUMMARY

The duodenum of the newborn opossum exhibits a patent lumen containing scattered elongate villi, whereas the distal segments of the small intestine are smaller in diameter and are filled with short immature villi. The muscularis externa through the small intestine consists of a single layer of myoblasts. Interposed between the intestinal lining epithelium and the muscularis externa is an extensive capillary bed that occupies a considerable proportion of the intestinal wall. Additional villi appear to form during the postnatal period as a result of evaginations of the epithelium, together with underlying connective tissue and vasculature, into the intestinal lumen. Intestinal glands are not observed until 8·5 cm, and are shallow in depth even in the adult.

The epithelium of the entire small intestine is modified for absorption until just prior to weaning. The principal intestinal lining cells show an extensive apical endocytic complex, large supranuclear vacuoles and numerous cytoplasmic inclusions. Intestinal epithelial cells of the colon also appear to be modified for absorption during the first two weeks after birth. Although goblet cells and Paneth cells are present during the suckling period, they do not comprise a significant population in the intestinal epithelium until after weaning. In contrast to the small intestine, goblet cells are numerous in the colon by the ninth postnatal day.

The significance of macromolecular absorption and the possibility of passive immunity being transmitted in the opossum during suckling are discussed and related to similar events that occur in the suckling young of several eutherian species. The possible functional significance of two large membranes that develop in the lamina propria of the intestines after weaning also is discussed.

We would like to express our sincere gratitude to Ms D. Sherman, research associate, for her excellent technical assistance and to W. Crawford, F. Sampson, H. Terrill, and C. Caldwell of the Missouri Conservation Commission for the use of materials and facilities in the present study.

REFERENCES

ANDERSON, J. W. (1963). Ultrastructural correlates of protein transport. *Journal of Cell Biology* **19**, 4–5.

ANDERSON, J. W. (1969). The use of anti-horseradish peroxidase (anti-HPO) antiserum in tracing the transport of antibodies through cells. *Anatomical Record* **163**, 147.

BAMFORD, D. K. (1966). Studies *in vitro* of the passage of serum proteins across the intestinal wall of young rats. *Proceedings of the Royal Society* **166**, 30–45.

BEHNKE, O. & MOE, H. (1964). An electron microscopic study of mature and differentiating Paneth cells in the rat, especially of their endoplasmic reticulum and lysosomes. *Journal of Cell Biology* **22**, 633–652.

BERGMEN, H. C. & HOUSLEY, C. (1968). Chemical analyses of American opossum (*Didelphys virginiana*) milk. *Comparative Biochemistry and Physiology* **25**, 213–218.

BLOOM, W. & FAWCETT, D. W. (1962). *A Textbook of Histology*. 8th edition. Philadelphia: Saunders.

BRAMBELL, F. W. R. (1958). The passive immunity of the young mammal. *Biological Reviews* **33**, 488–531.

CARO, L. G. & PALADE, G. E. (1964). Protein synthesis, storage and discharge in pancreatic exocrine cell: an autoradiographic study. *Journal of Cell Biology* **20**, 473–495.

CASTRO, N. M., SASSO, W. S. & SAAD, F. A. (1959). Preliminary observations of the Paneth cells of the *Tamandua tetradactyla*. *Acta anatomica* **38**, 345–352.

CLARK, S. L. (1959). The ingestion of proteins and colloidal materials by columnar absorptive cells of the small intestine in suckling rats and mice. *Journal of Biophysical and Biochemical Cytology* **5**, 41–50.

CLARKE, R. M. & HARDY, L. N. (1969). An analysis of the mechanism of cessation of uptake of macromolecular substances by the intestine of the young rat ('closure'). *Journal of Physiology* **204**, 127–134.

CLARKE, R. M. & HARDY, R. N. (1970). Structural changes in the small intestine associated with the uptake of polyvinyl pyrrolidone by the young ferret, rabbit, guinea pig, cat, and chicken. *Journal of Physiology* **209**, 669–687.

CLARKE, R. M. & HARDY, R. N. (1971). Histological changes in the small intestine of the young pig and their relation to macromolecular uptake. *Journal of Anatomy* **108**, 63–77.

CORNELL, R. & PADYKULA, H. A. (1969). A cytological study of intestinal absorption in the suckling rat. *American Journal of Anatomy* **125**, 291–316.

DEANE, H. W. (1964). Some electron microscopic observations on the lamina propria of the gut, with comments on the close associations of macrophages, plasma cells, and eosinophils. *Anatomical Record* **149**, 453–474.

EASTWOOD, G. L. & TRIER, J. S. (1974). Epithelial cell proliferation during organogenesis of rat colon. *Anatomical Record* **179**, 303–310.

ENGELRUD, T., OLIVECRONA, T. & HELANDER, H. (1971). Studies on gastric absorption of lipids in the suckling rat. *Scandinavian Journal of Gastroenterology* **6**, 329–333.

FRIEDMAN, H. I. & CARDELL, R. R. (1972*a*). Morphological evidence for the release of chylomicra from intestinal absorptive cells. *Experimental Cell Research* **75**, 57–62.

FRIEDMAN, H. I. & CARDELL, R. R. (1972*b*). Effects of puromycin on the structure of rat intestinal epithelial cells during fat absorption. *Journal of Cell Biology* **52**, 15–40.

GARBARASCH, CH. & VON BÜLOW, F. A. (1969). Histochemical and electron microscopical studies on the epithelium of the fetal colon with special reference to the occurrence of lipids. *Histochemie* **20**, 201–210.

GLEREAN, A. & CASTRO, N. N. (1965). A histochemical study of the Paneth cells of the *Tamandua tetradactyla* (Edentata, Mammalia). *Acta anatomica* **61**, 146–153.

GRANEY, D. O. (1968). The uptake of ferritin by ileal absorptive cells in suckling rats. An electron microscope study. *American Journal of Anatomy* **123**, 227–254.

HELANDER, H. F. (1973). Morphological studies on the development of the rat colonic mucosa. *Acta anatomica* **85**, 153–176.

HELANDER, H. F. & OLIVECRONA, T. (1970). Lipolysis and lipid absorption in the stomach of the suckling rat. *Gastroenterology* **59**, 22–35.

HEUSER, C. H. (1920). The early establishment of the intestinal nutrition in the opossum – the digestive system just before and after birth. *American Journal of Anatomy* **28**, 341–370.

HILL, K. J. & HARDY, W. S. (1956). Histological and histochemical observations on the intestinal cells of lambs and kids absorbing colostrum. *Nature* **178**, 1353–1354.

HILTON, W. A. (1902). The morphology and development of intestinal folds and villi in vertebrates. *American Journal of Anatomy* **1**, 459–504.

HUGON, J. S. (1970). Ultrastructural differentiation and enzymatic localization of phophatases in the developing duodenal epithelium of the mouse. II. The newborn mouse. *Histochemie* **22**, 109–124.

JOHNSON, F. P. (1910). The development of the mucous membrane of the oesophagus, stomach and small intestine in the human embryo. *American Journal of Anatomy* **10**, 521–561.

KAMMERAAD, A. (1942). The development of the gastrointestinal tract of the rat. *Journal of Morphology* **70**, 323–351.

KLEIN, S. (1906). On the nature of the granule cells of Paneth in the intestinal glands of mammals. *American Journal of Anatomy* **5**, 315–330.

KRAEHENBUHL, J. P & CAMPICHE, M. A. (1969). Early stages of intestinal absorption of specific antibodies in the newborn. An ultrastructural, cytochemical and immunological study in the pig, rat and rabbit. *Journal of Cell Biology* **42**, 345–365.

KRAUSE, W. J. (1971). Paneth cells of the echidna (*Tachyglossus aculeatus*). *Acta anatomica* **80**. 435–448.

KRAUSE, W. J. (1972). Light and electron microscopic studies on the gastro-intestinal tract of the suckling echidna (*Tachyglossus aculeatus*). *Anatomical Record* **172**, 603–622.

KRAUSE, W. J. (1972). The distribution of Brunner's glands in 55 marsupial species native to the Australia region. *Acta anatomica* **82**, 17–33.

KRAUSE, W. J. (1975). Intestinal mucosa of the platypus, *Ornithorhynchus anatinus*. *Anatomical Record* **181**, 251–266.

KRAUSE, W. J. & LEESON, C. R. (1969). Limiting membranes of intestinal lamina propria in the opossum. *Journal of Anatomy* **104**, 467–480.

KRAUSE, W. J. & LEESON, C. R. (1969). Studies of Brunners' glands in the opossum. II. Postnatal development. *American Journal of Anatomy* **126**, 275–290.

KRAUSE, W. J., CUTTS, J. H. & LEESON, C. R. (1976*a*). The postnatal development of the alimentary canal in the opossum. I. Oesophagus. *Journal of Anatomy* **122**, 293–314.

KRAUSE, W. J., CUTTS, J. H. & LEESON, C. R. (1976*b*). The postnatal development of the alimentary canal in the opossum. II. Stomach. *Journal of Anatomy* **122**, 499–519.

LECCE, J. G. (1966). Absorption of macromolecules by neonatal intestine. *Biology of the Neonate* **9**, 50–61.

LEV, R. & ORLIC, D. (1973). Uptake of protein in swallowed aminiotic fluid by monkey fetal intestine in utero. *Gastroenterology* **65**, 60–68.

LEV, R. & SPICER, S. S. (1964). Specific staining of sulfate groups with alcian blue at low pH. *Journal of Histochemistry and Cytochemistry* **12**, 309.

MERZEL, J. (1967). Some histophysiological aspects of Paneth's cells of mice as shown by histochemical and radioautographical studies. *Acta anatomica* **66**, 603–630.

MCCADY, E. (1938). The embryology of the opossum. *American Anatomical Memoirs* **16**, Wistar Institute of Anatomy and Biology.

MOORE, C. R. & BODIAN, D. (1940). Opossum pouch young as experimental material. *Anatomical Record* **76**, 319–327.

MORRIS, I. G. (1968). Gamma globulin absorption in the newborn. In *Handbook of Physiology, Volume III.* (ed. C. F. Code), 1491–1512. Baltimore: Williams and Wilkins.

MOWRY, R. W. (1956). Alcian blue technics for the histochemical study of acidic carbohydrates. *Journal of Histochemistry and Cytochemistry* **4**, 407.

MOWRY, R. W. (1960). Revised method of producing improved coloration of acidic polysaccharides with alcian blue 8 GX supplied currently. *Journal of Histochemistry and Cytochemistry* **8**, 323.

ORLIC, D. & LEV, R. (1973). Fetal rat intestinal absorption of horseradish peroxidase from swallowed amniotic fluid. *Journal of Cell Biology* **56**, 106–119.

OPPEL, A. (1897). *Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere. II. Teil: Schlund und Darm.* Jena: Gustav Fischer.

REYNOLDS, H. C. (1942). A contribution to the life history and ecology of the opossum *Didelphis virginiana*, in central Missouri. M.A. Thesis, University of Missouri.

RODEWALD, R. (1969). Route of selective antibody transport in the small intestine of the neonatal rat. *Journal of Cell Biology* **43**, 118A.

RODEWALD, R. (1970). Selective antibody transport in the proximal small intestine of the neonatal rat. *Journal of Cell Biology* **45**, 635–640.

RUNDELL, J. O. & LECCE, J. G. (1972). Independence of intestinal epithelial cell turnover from cessation of absorption of macromolecules (closure) in the neonatal mouse, rabbit, hamster and guinea pig. *Biology of the Neonate* **20**, 51–57.

SAGE, J. A. & JERSILD, R. A. (1971). Comparative distribution of carbohydrates and lipid droplets in the Golgi apparatus of intestinal absorptive cells. *Journal of Cell Biology* **51**, 333–338.

SELZMAN, H. M. & LIEBELT, R. A. (1961). A cytochemical analysis of Paneth cell secretion in the mouse. *Anatomical Record* **140**, 17–22.

SHARMAN, G. B. (1961). The embryonic membranes and placentation in five genera of diprotodont marsupials. *Proceedings of the Zoological Society* **137**, 197–220.

SHERVEY, P. (1966). Observation on the development and histochemistry of the intestinal inclusion bodies of the suckling rat. *Anatomical Record* **154**, 422.

STALEY, T. E., CORLEY, L. D., BUSH, L. J. & JONES, W. W. (1972). The ultrastructure of neonatal calf intestine and absorption of heterologous proteins. *Anatomical Record* **172**, 559–580.

STALEY, T. E., JONES, W. W. & MARSHALL, A. E. (1968). The jejunal absorptive cell of the newborn pig. An electron microscopic study. *Anatomical Record* **161**, 497–516.

TRIER, J. S. (1963). Studies on small intestinal crypt epithelium. I. The fine structure of the crypt epithelium of the proximal small intestine of fasting humans. *Journal of Cell Biology* **18**, 599–620.

TRIER, J. S., LORENZSONN, U. T. & GROEHLER, K. (1967). Pattern of secretion of Paneth cells of the small intestine of mice. *Gastroenterology* **53**, 240–249.

WARSHAWSKY, A., LEBLOND, C. P. & DROZ, B. (1963). Synthesis and migration of proteins in the cells of the exocrine pancreas as revealed by specific activity determination from radioautographs. *Journal of Cell Biology* **16**, 1–28.

WHEELER, E. J. & WHEELER, J. K. (1964). Comparative study of Paneth cells in vertebrates. *Anatomical Record* **148**, 350.

WILLIAMS, R. M. & BECK, F. (1969). A histochemical study of gut maturation. *Journal of Anatomy* **105**, 487–501.

WISSIG, S. L. & GRANEY, D. O. (1968). Membrane modifications in the apical endocytic complex of ileal epithelial cells. *Journal of Cell Biology* **39**, 564–579.

WORTHINGTON, B. B. & GRANEY, D. O. (1973). Uptake of adenovirus by intestinal absorptive cells of the suckling rat. II. The neonatal jejunum. *Anatomical Record* **175**, 63–76.

YADAV, M. (1971). The transmission of antibodies across the gut of pouch-young marsupials. *Immunology* **21**, 839–851.