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The postnatal development of the alimentary canal in the opossum

II. Stomach*

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INTRODUCTION

The gestation period of the opossum is remarkably short, lasting only 12½ days. The newborn opossum crawls unaided from the birth canal to the maternal marsupium and attaches to one of thirteen teats. Here, within the protection of the marsupium, postnatal development of the opossum continues. Milk soon enters the stomach and is clearly visible through the anterior abdominal wall. Heuser (1921) examined the stomach just prior to and 3 days after birth and noted that the gastric mucosa of the pouch-young opossum is comprised primarily of columnar cells. The present study reports the appearance of the gastric mucosa at birth and details the extensive development and differentiation that occur during the postnatal period.

MATERIALS AND METHODS

Eighty opossums (*Didelphis virginiana*) were used in the study. Pouch-young opossums were divided into the following ten groups according to their snout–rump lengths (SRL): 1·5 (newborn, less than 24 hours old), 2·0, 2·5 (9 days old), 3·5, 4·5, 5·5, 8·0, 10·0, 13·0, 14·0, 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 were removed, primarily from the body of the stomach but, in addition, at all stages some blocks were also taken from the pyloric region. All tissues were 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 a pH of 1·0 and of 2·5, and aldehyde fuchsin.

Additional blocks of tissue were fixed for 4 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 osmicated in 1·0 % osmium tetroxide at 0 °C for 2 hours. The specimens were processed routinely, infiltrated with and embedded in Epon 812. Thick sections were cut at 0·5–3 μm and stained with toluidine blue. For electron microscopy thin sections were mounted on uncoated grids and stained with uranyl acetate and lead citrate.

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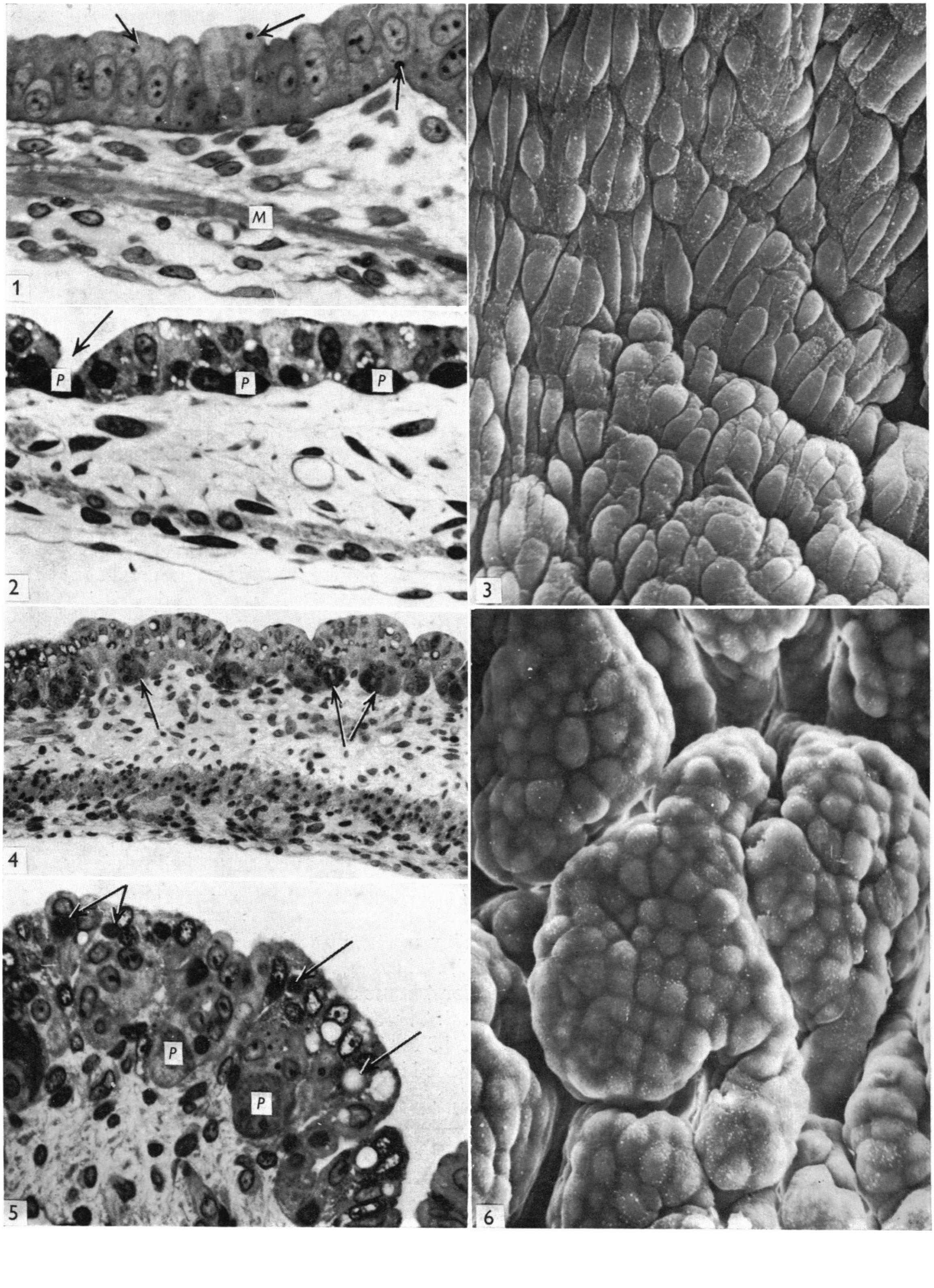
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The sections were examined in a RCA EMU-3F electron microscope operated at 50 kv.

Tissues for scanning electron microscopy were prepared 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. The 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.

Calibrated pH papers (Micro Essential Laboratories, Brooklyn, New York) were used to determine the pH of stomach contents.

Quantitative studies of the gastric mucosa were made on the 1·5, 3·5, 5·5, 8·0, 10·0, 13·0 and 22·0 cm stages, using sections of Epon-embedded material. Three sections from each of three animals were used at each stage. The depth of the mucosa and of its constituent parts was determined from five random measurements from each section, using a filar micrometer. The number of mitotic cells in the lining epithelium, and the numbers of parietal cells, chief cells and mitotic figures in the glandular epithelium were calculated per mm^2 of tissue. The areas were estimated throughout the entire section by means of a calibrated net micrometer.

RESULTS

Light microscopy

The gastric mucosa of the newborn opossum (1·5 cm) has a simple columnar epithelium (Fig. 1). The epithelium, which lies on a distinct basement membrane, contains scattered lipid droplets of varying size (Figs. 1 and 2). Parietal cells within the epithelium usually lie adjacent to the underlying basement membrane and stain intensely with toluidine blue (Fig. 2). Parietal cells also, in part, line the lumen of the

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Fig. 1. A section through the wall of the stomach. The epithelium is columnar in type and exhibits small scattered lipid droplets (arrows). Myoblasts of the muscularis externa (*M*) form only a thin delicate layer. Newborn (1·5 cm). Epon 812-toluidine blue. ×400.

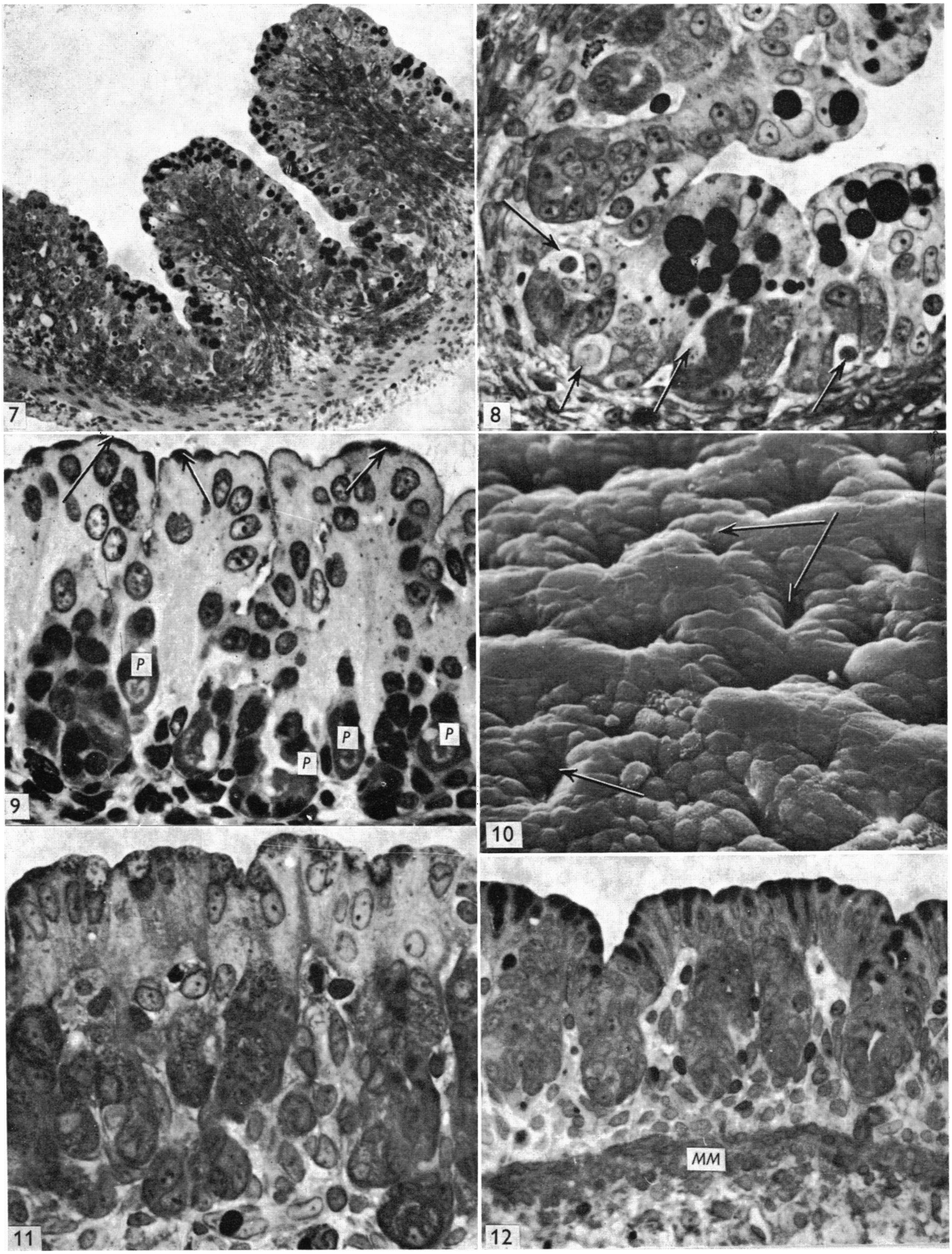
Fig. 2. A section from the newborn stomach, approximately 12 hours older than the specimen shown in Fig. 1. Scattered parietal cells (*P*) lie near the basement membrane of gastric lining epithelium. The epithelium shows a slight increase in lipid droplets. Parietal cells also are found at the bases of epithelial invaginations (arrow). What appear to be mucin granules, which stain densely, are present in the apices of the columnar lining cells. Epon 812-toluidine blue. ×350.

Fig. 3. A scanning electron micrograph of the gastric surface from a newborn opossum. The surface appears flat and shows few mucosal folds. Microvilli appear concentrated at cell boundaries. ×800.

Fig. 4. A section through the gastric wall of the 9 day old opossum (2·5 cm) shows definite gastric glands (arrows) and continued development of the muscularis externa. Epon 812-toluidine blue. ×150.

Fig. 5. A high power photomicrograph of the gastric mucosa illustrates in greater detail the developing gastric glands. Parietal cells (*P*) are found at the bases of the developing glands and the columnar lining cells still contain large lipid droplets (arrows). 2·5 cm opossum. Epon 812-toluidine blue. ×350.

Fig. 6. The mucosal surface of the 9 day old opossum shows considerable infolding. The apices of the lining cells appear rounded and are not as elongated as those present in the newborn (Fig. 3). ×800.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stomach. On occasion, clefts are observed within the lining epithelium and these usually have parietal cells at their bases (Fig. 2). Such clefts may represent the initial formation of gastric glands. The muscularis externa consists of only a few myoblasts near the external surface of the stomach wall (Figs. 1 and 2). When viewed from the mucosal surface, the stomach interior is flat and the apices of the epithelial cells are rounded or elongate (Fig. 3). Microvilli are concentrated at the epithelial cell junctions. The stomach of the 9 day old opossum (2·5 cm) shows definite, but short, gastric glands that contain parietal cells (Figs. 4 and 5). The muscularis externa shows considerable development as compared to the newborn opossum and exhibits a definite organization into layers (Fig. 4). Lining epithelial cells contain an increasing number of large lipid droplets (Fig. 5). The mucosal surface shows considerable folding at this stage of development (Figs. 5 and 6) and, when viewed from the luminal surface, the apices of the lining epithelial cells appear rounded, with scattered microvilli (Fig. 6). The 3·5 cm opossum (*ca.* 17 days postnatal)* exhibits continued folding of the gastric mucosal surface (Fig. 7). The gastric glands now contain parietal cells, endocrine cells and undifferentiated cells (Fig. 8). The surface lining epithelium shows a marked increase in lipid droplets, some of which measure 15 μm in diameter (Figs. 7 and 8). By 4·5 cm (*ca.* 20 days) such droplets are not as frequent in the gastric lining epithelium as in previous stages (Fig. 9). Small clusters of mucin granules are concentrated in the apices of cells of the surface lining epithelium. The gastric glands show continued development but consist only of the cell types noted in earlier stages (Fig. 9). When viewed from the mucosal surface, the orifices of the gastric pits (foveolae) are readily observed in the 5·5 cm (*ca.* 27 days) opossum (Fig. 10). The developing gastric glands show a considerable increase both in depth and in the number of parietal cells by the 8 cm stage (*ca.* 50 days). The apices of the surface lining cells throughout the stomach at this stage also show a

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

Fig. 7. The gastric mucosa of the 3·5 cm (*ca.* 17 days) opossum shows continued development of the gastric glands. The surface epithelium exhibits a considerable increase in 'lipid' droplets as compared to younger stages. Epon 812-toluidine blue. × 100.

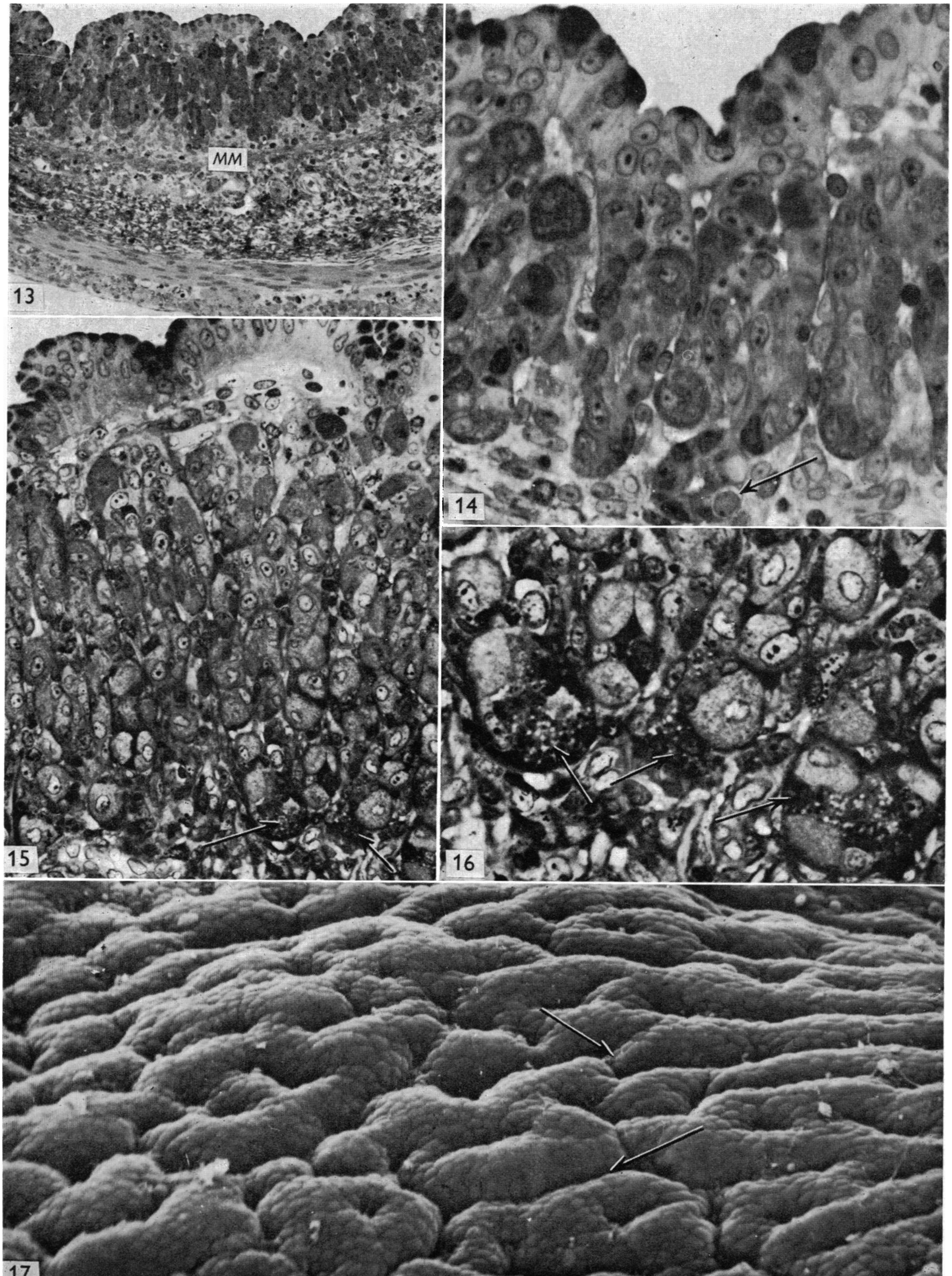
Fig. 8. Increased magnification of the developing gastric glands shows, in addition to parietal cells, numerous light staining cells, thought to be endocrine cells (arrows). 3·5 cm opossum. Epon 812-toluidine blue. × 375.

Fig. 9. The gastric glands of the 4·5 cm opossum (*ca.* 20 days) show continued development. Parietal cells (*P*) remain confined to the bases of the developing glands. Surface cells show few lipid droplets and increased numbers of mucin granules (arrows). Epon 812-toluidine blue. × 425.

Fig. 10. A scanning electron micrograph of the gastric surface from a 5·5 cm opossum (*ca.* 27 days). Gastric pits (arrows) are readily observed. × 500.

Fig. 11. The gastric glands of the 8 cm opossum (*ca.* 50 days) show an increase in depth and in the number of parietal cells. Surface cells also show an increase in the number of mucin granules present. Epon 812-toluidine blue. × 400.

Fig. 12. A section taken near the pyloric region of the stomach illustrates the increase of mucin granules in surface cells. The muscularis mucosae (*MM*) is prominent by this stage of development. 8 cm opossum (*ca.* 50 days). Epon 812-toluidine blue. × 280.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considerable increase in the concentration of mucin granules (Figs. 11 and 12). The muscularis mucosae, particularly in the pyloric region, is well developed (Fig. 12). The prominent muscularis mucosae of the pyloric region is a feature of all subsequent stages, including the adult.

The stomach of the 60 day old opossum (10 cm) shows continued development of the gastric glands, muscularis mucosae, submucosa and layers of muscularis externa (Figs. 13 and 14). The gastric glands still contain only parietal cells, scattered endocrine cells, and undifferentiated cells (Fig. 14). By the 13 cm stage (*ca.* 75 days) the gastric glands exhibit not only a marked increase in depth but also the initial appearance of chief cells (Figs. 15 and 16). The chief cells are confined to the most basal portions of the gastric glands. They contain zymogen granules which stain either intensely or else not at all with toluidine blue (Fig. 16). Numerous connective tissue cells, primarily eosinophils and mast cells, are observed in relation to the bases of the developing gastric glands. In addition to the orifices of foveolae, the mucosal surface of the 14 cm opossum (*ca.* 80 days) shows numerous irregular infoldings (Fig. 17). The gastric glands of the juvenile opossum (28 cm) contain a large number of parietal cells in the neck and central regions, with a small population of chief cells confined to the bases (Figs. 18 and 19). The chief cells continue to show a heterogeneous population of secretory granules when stained with toluidine blue (Fig. 19). Increased numbers of connective tissue cells lie in the lamina propria adjacent to the bases of the gastric glands. When viewed from the mucosal surface, the orifices of the foveolae in the juvenile opossum are often narrow and irregular (Fig. 20).

Histochemistry

The histochemical portion of the study is confined to animals just before and after weaning. Similar staining reactions are observed in both pre- and post-weaning specimens. Cells of the epithelial component of the gastric mucosa fail to stain with alcian blue either at pH 2·5 or at pH 1·0. The secretory granules of the surface lining epithelium, and the mucous neck cells, stain intensely with PAS before and after digestion with saliva. The remaining cell types fail to stain with PAS. Chief cells, as well as other epithelial components of the mucosa, fail to stain with aldehyde fuchsin. The secretory granules of the chief cells stain a brilliant red when treated with Masson's original trichrome.

Fig. 13. The 10 cm opossum (*ca.* 60 days) shows continued growth of the gastric glands, muscularis mucosae (*MM*), and other elements of the stomach wall. Epon 812-toluidine blue. ×75.

Fig. 14. The gastric glands contain parietal cells, endocrine cells (arrow) and numerous undifferentiated cells. 10 cm opossum. Epon 812-toluidine blue. ×400.

Fig. 15. By 13 cm (*ca.* 75 days), the bases of the gastric glands show occasional chief cells (arrows). The glands have increased considerably in depth and show numerous parietal cells. Epon 812-toluidine blue. ×250.

Fig. 16. Increased magnification of the bases of gastric glands from the 13 cm opossum. The chief cells (arrows) show numerous granules, which either stain intensely or fail to stain. Epon 812-toluidine blue. ×500.

Fig. 17. In addition to gastric pits, the surface of the 14 cm opossum (*ca.* 80 days) shows numerous irregular folds (arrows). ×400.


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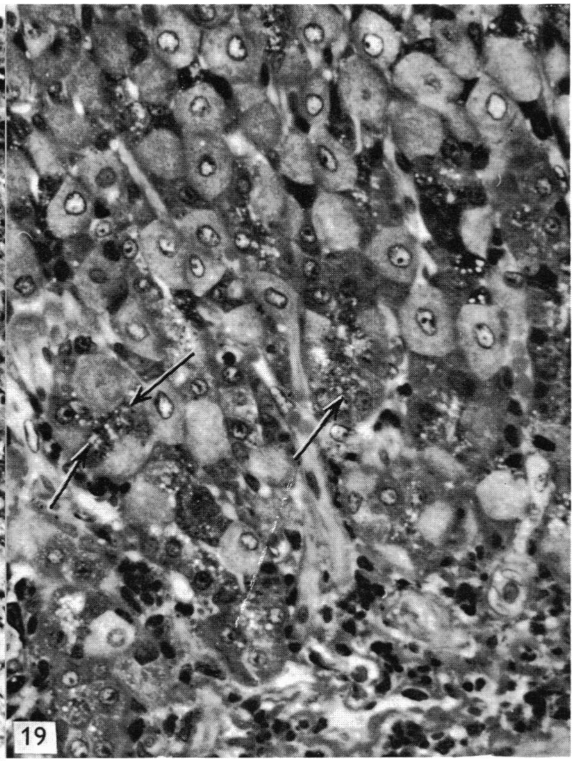
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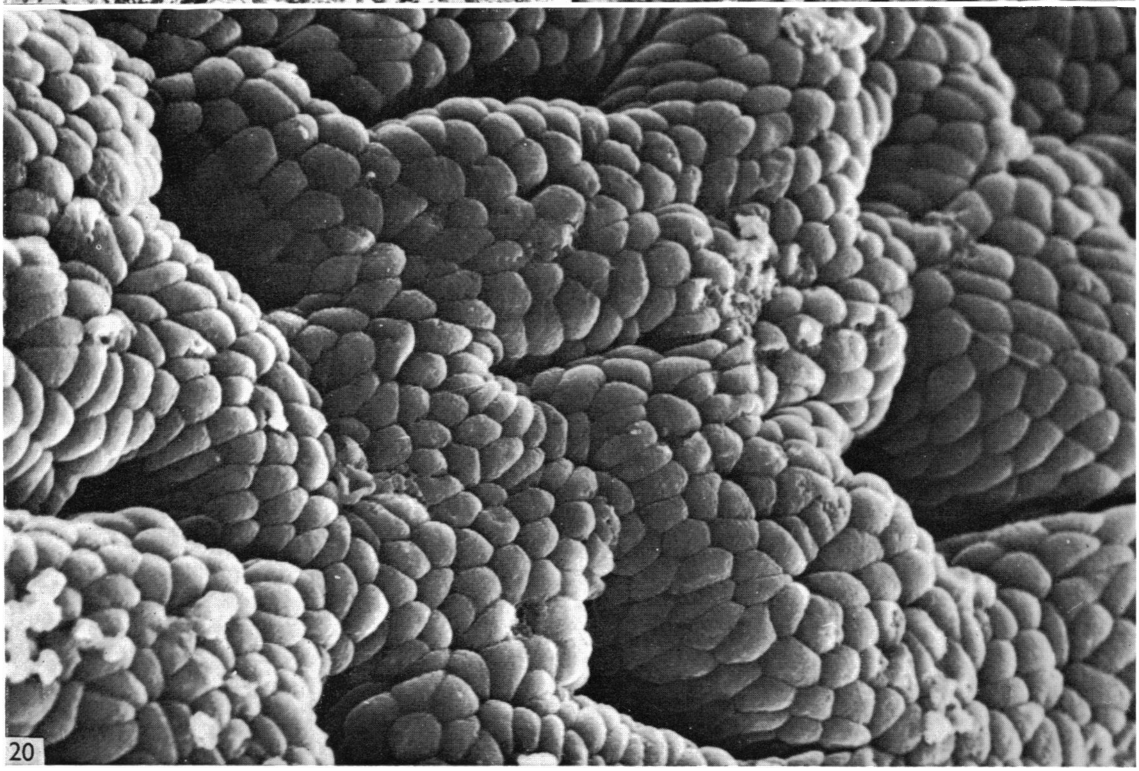
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Electron microscopy

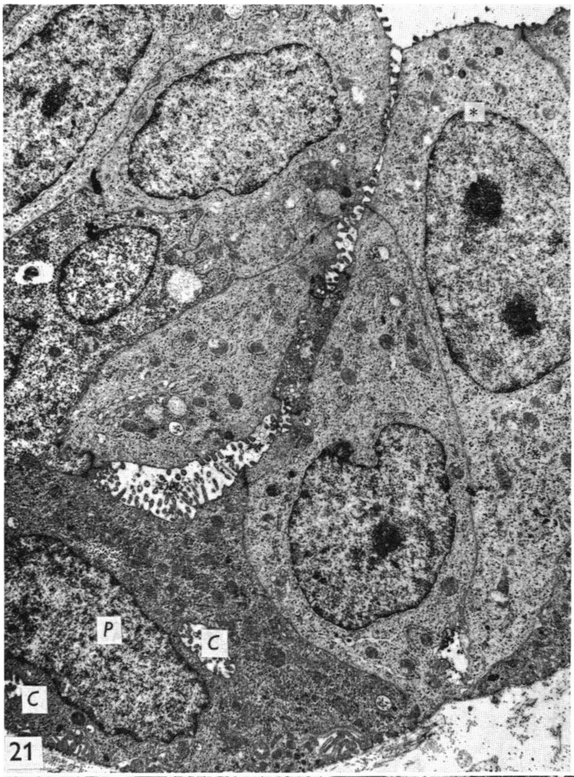
The ultrastructural study confirms and extends the observations made with the light microscope. The gastric lining epithelium of the newborn opossum (1·5 cm) is composed of columnar cells with centrally placed elongated nuclei. Most of the cells appear relatively undifferentiated with only scattered organelles and a few stubby microvilli at the apical surface (Fig. 21). Scattered surface lining cells show small apical collections of mucin granules. Parietal cells also contribute to the gastric lining epithelium, lying immediately adjacent to the gastric lumen (Fig. 22). Parietal cells exposed to the gastric lumen are usually columnar or pyramidal in shape. Parietal cells also are found deep within the epithelium at the bases of surface infoldings, which may correspond to areas of combined gastric pit–gastric gland formation (Figs. 4 and 21). Occasional endocrine cells, which exhibit an electron-lucent cytoplasm with scattered profiles of granular endoplasmic reticulum and occasional granules, also lie within the basal lamina of the surface lining epithelium. Parietal cells of the newborn opossum show numerous mitochondria, scattered elements of granular endoplasmic reticulum and well developed canaliculi (Figs. 21 and 22). Numerous elongated microvilli fill canaliculi and line the apices of parietal cells (Figs. 21 and 22). Parietal cells of the newborn opossum also exhibit considerable electron density in comparison with adjacent cells.

Definite gastric glands are present in the 2·5 cm opossum (*ca.* 9 days) and are comprised primarily of parietal cells and cells which appear to be undifferentiated (Fig. 23). The latter lie within formed gastric glands immediately adjacent to parietal cells. They are columnar in shape and show only scattered organelles (Fig. 23). In addition to those features which characterized the parietal cell in the newborn, parietal cells of this and all subsequent stages show the presence of a tubulovesicular component within the cytoplasm. The large lipid droplets observed within surface lining cells in the 3·5 cm stage (*ca.* 17 days) do not have a limiting membrane (Fig. 24). Smaller lipid droplets also are observed lying freely in the cytoplasm and are not found within cisternae of the cytoplasmic membranes of the cell or between adjacent cell membranes. Caveolated cells are observed initially at the 3·5 cm stage (Fig. 24) and in later stages. They occur primarily in the surface lining epithelium. This cell type exhibits a narrow apex that protrudes slightly into the lumen, and a wide base which is in contact with the underlying basal lamina. The apex displays short stubby microvilli that are associated with bundles of cytoplasmic filaments extending from the core of each microvillus to the supranuclear region of the cell. Small vesicles and/or tubules extend from the apical surface between microvilli into the cell. Caveolated cells are observed only infrequently.

Fig. 18. A section through the gastric mucosa of a juvenile opossum (28 cm), to show the relative depths of the pits and glands. Epon 812-toluidine blue. × 125.

Fig. 19. Higher magnification of the bases of the gastric glands shows numerous chief cells (arrows) and connective tissue cells. The latter are found adjacent to the bases of the gastric glands both in juvenile and in adult animals. 28 cm opossum. Epon 812-toluidine blue. × 250.

Fig. 20. The gastric surface of a 28 cm opossum. The gastric pits are often irregular or slit-like in appearance. Juvenile. × 750.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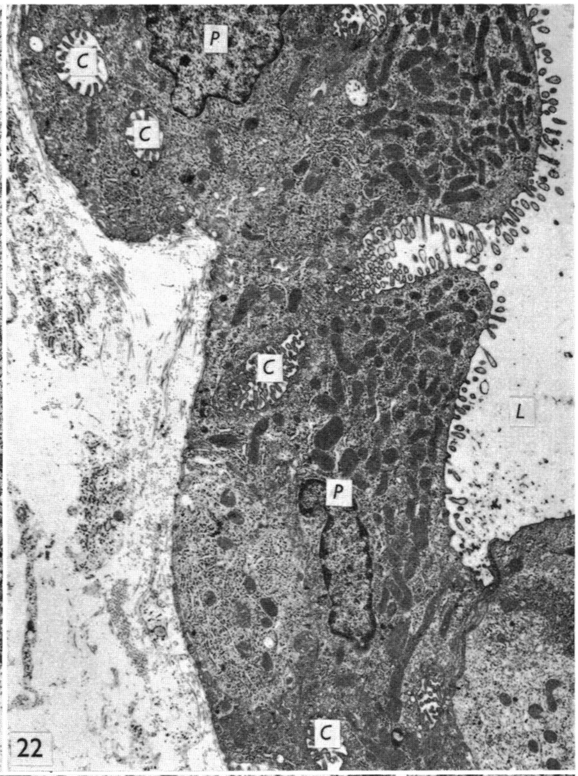
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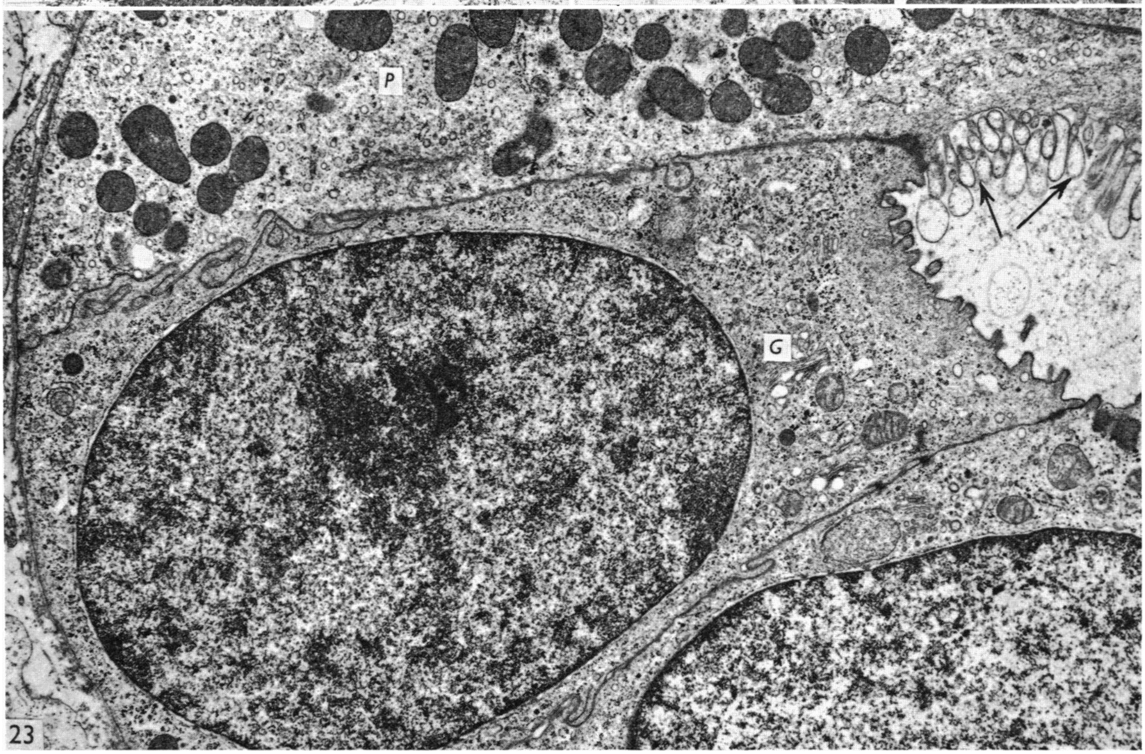
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By 5·5 cm (*ca.* 27 days) the apices of cells in the surface lining epithelium are devoid of lipid droplets but contain secretory granules that have a mottled appearance (Fig. 25). These surface cells appear to be actively engaged in the release of some secretory product (Fig. 25). In the developing gastric glands of the 5·5 cm opossum, cells, which at the light microscopic level seemed undifferentiated, now show scattered electron-dense granules in their apical cytoplasm (Fig. 26). Golgi membranes are intimately associated with forming secretory granules. The cytoplasm shows numerous free ribosomes but a relative paucity of other organelles. The position of such cells in the neck regions of the forming glands, in addition to their ultrastructural characteristics, would indicate that they represent differentiating mucous neck cells.

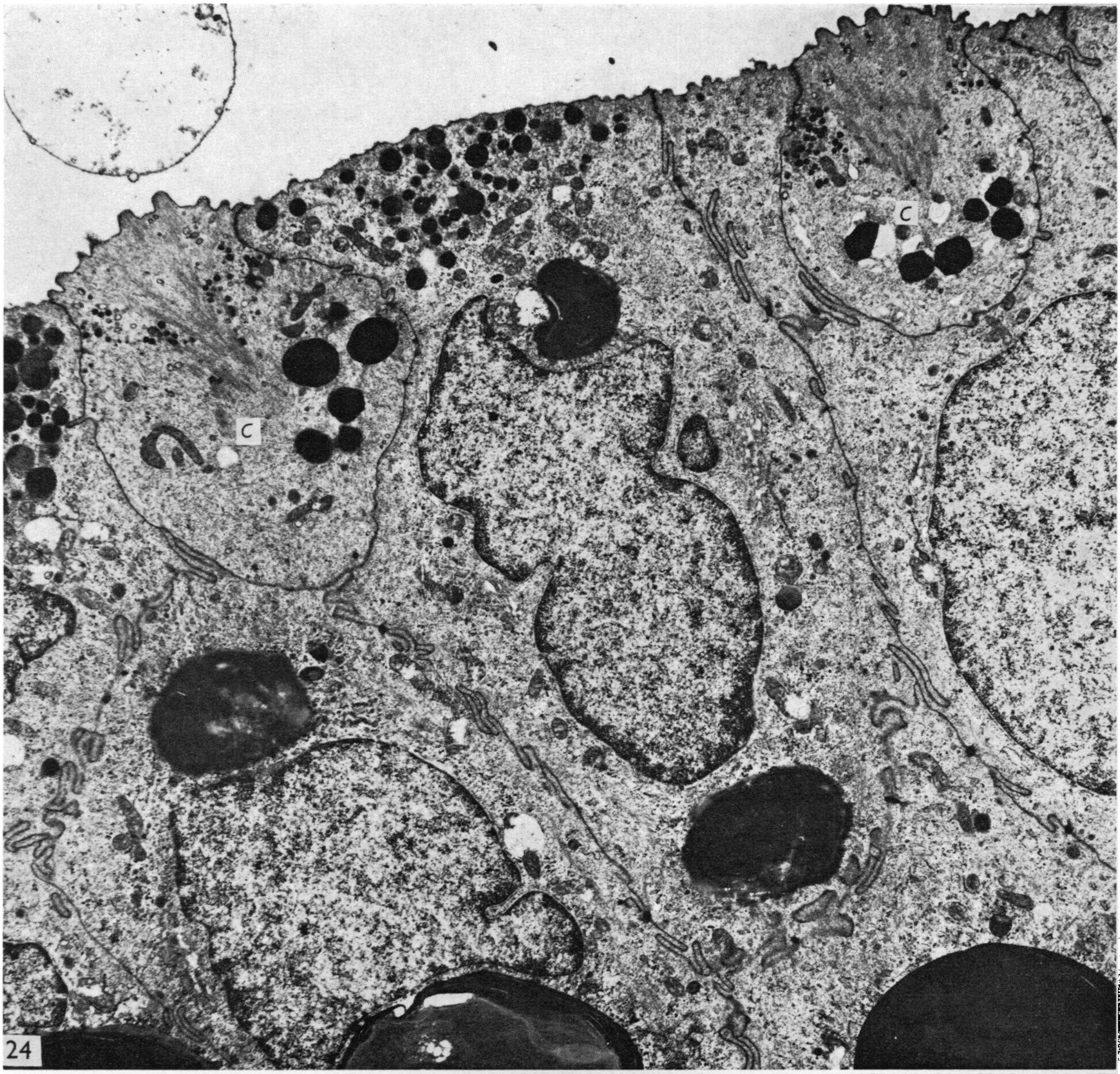
The supranuclear and apical regions of the surface lining cells are filled with secretory granules by the 13 cm stage (*ca.* 75 days) (Fig. 27). The amorphous granules vary considerably in appearance and show regions of increased electron density, giving them a mottled appearance. Although limited by membranes, the granules show a tendency to coalesce into irregularly shaped complexes (Fig. 27). Chief cells are observed for the first time in the 13 cm opossum. These cells show an abundance of granular endoplasmic reticulum in the basal and perinuclear regions, as well as numerous large granules (Fig. 28). The granules are membrane-bound and vary considerably in electron density. Forming granules are associated with numerous Golgi membranes, usually located in the supranuclear region. Parietal cells at this stage differ from those observed in previous stages in that the cytoplasm now contains an increased amount of tubulovesicles (Fig. 28). These occupy a significant volume in parietal cells of all later stages. The morphological features of the surface lining cells, mucous neck cells, and chief cells, in subsequent stages, appear similar to those described previously.

Calibrated pH papers were used to determine the pH of stomach contents at the stages examined. Stomach contents of the majority of opossums prior to weaning showed a pH ranging from 6·0 to 6·5. It was not until solid food was observed in the stomach that a low pH (1·5–2·0) was recorded with consistency.

Fig. 21. An invagination from the gastric lining epithelium of a newborn opossum. The lining epithelium (*) is comprised of columnar shaped cells with centrally placed oval nuclei. A parietal cell (*P*) lies on a delicate basal lamina at the base of the invagination. It shows numerous mitochondria, scattered amounts of glycogen, and well developed canaliculi (*C*). Parietal cells of the newborn also exhibit greater electron density than surrounding cells. × 4500.

Fig. 22. Areas of the gastric lumen (*L*) are often lined by parietal cells (*P*), which also show well developed canaliculi (*C*) and numerous mitochondria. The parietal cells exhibit a well developed microvillus border on their surface. Newborn opossum. × 4000.

Fig. 23. An undifferentiated cell near the base of a developing gastric gland. The cell is columnar in type and shows only scattered elements of granular endoplasmic reticulum and mitochondria. Golgi membranes (*G*) are found near the apex. A parietal cell (*P*), with numerous mitochondria, scattered vesicles, and large 'empty' microvilli (arrows), also is observed. 2·5 cm opossum (9 days). × 7200.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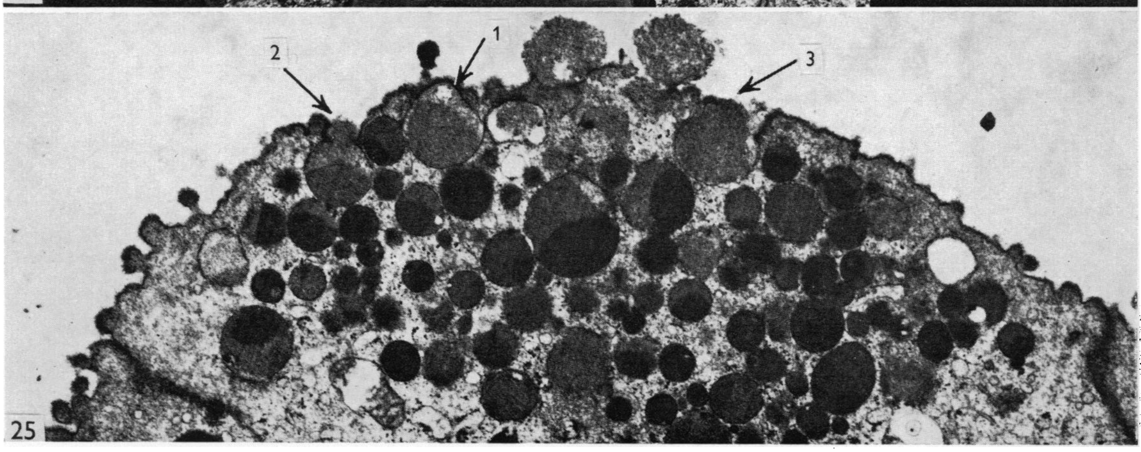
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Quantitative data

Figure 29 shows the stages of development of the gastric mucosa. The depth of the surface epithelium is established at birth and remains unchanged throughout the succeeding stages of postnatal development. The thin, incomplete muscularis mucosae present at birth increases in thickness to reach a constant depth by the 5·5 cm stage (27 days). The greatest change occurs in the thickness of the lamina propria as a result of the increasing length of the gastric glands. These elements show a gradual and rather uniform increase in depth up to the 10·0 cm stage (60 days). The greatest development of the glands occurs in the 15 days between the 10·0 and 13·0 cm stages, during which time the glands double in length. There is a continued lengthening of the glands between the 13·0 and 22·0 cm stages, but at a reduced rate.

The upper part of Figure 30 shows the number of parietal and chief cells at the different stages of development. No chief cells are seen until the 13·0 cm stage (75 days), after which their numbers increase rapidly. Parietal cells, present at birth, increase progressively in number to reach a stable population by the 13·0 cm stage. At birth, and up to the 3·5 cm stage, a small proportion of the parietal cells is found intermingled with the surface epithelial cells. In later stages, parietal cells are restricted to the glands.

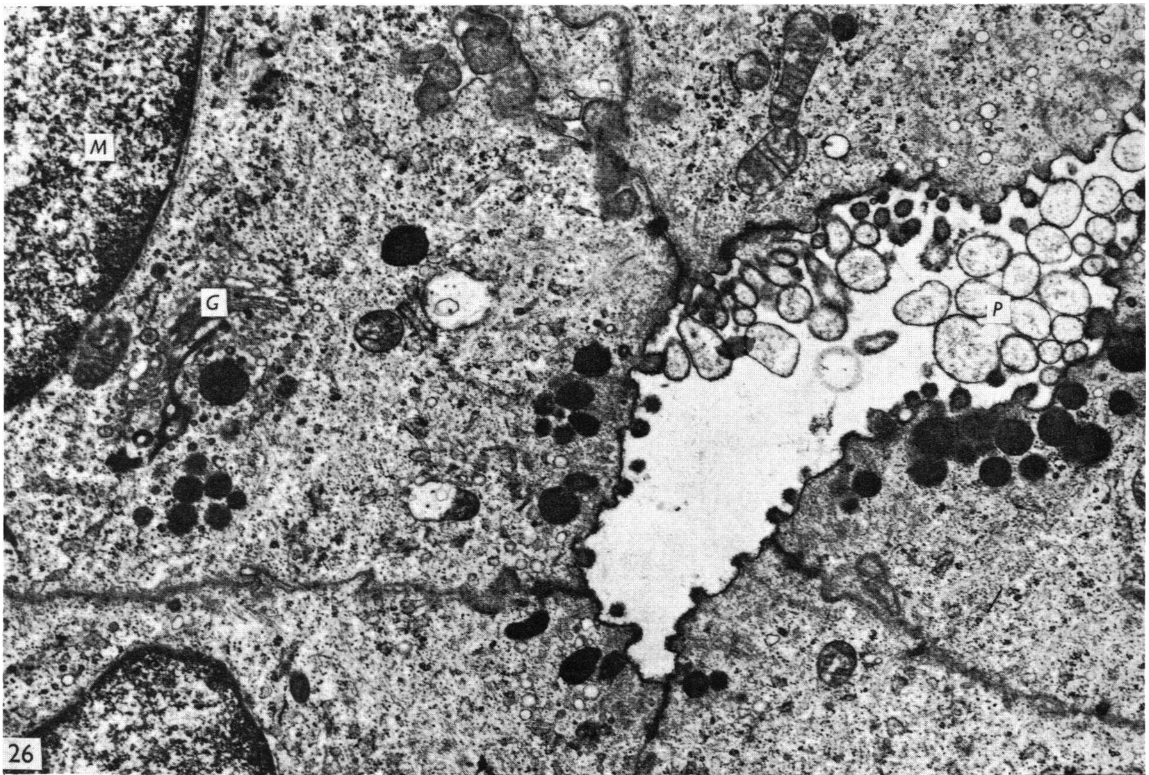
The mitotic activity of the surface epithelium and of the glands is shown in the lower portion of Figure 30. Mitotic activity in the lining epithelium reaches a maximum at the 3·5 cm stage, after which it gradually declines. The glandular epithelium shows a rather sustained period of mitotic activity up to the 10·0 cm stage, with a secondary peak between the 5·5 cm and 10·0 cm stages. The peak precedes the appearance of chief cells in the glands which coincides with the marked increase in length of the gastric glands at 13·0 cm.

DISCUSSION

The gastric mucosa of the newborn opossum is composed only of a simple columnar epithelium and is similar to the gastric mucosa of the newborn of two Australian marsupials, the native cat (Hill & Hill, 1954) and the red kangaroo (Griffiths & Barton, 1966). A similar gastric mucosa is found in pouch-young of one of the monotremes, the echidna (Krause, 1972). Unlike the former species, the gastric mucosa of the echidna is surfaced by stratified squamous epithelium in the adult and is devoid of gastric glands (Krause & Leeson, 1974). In the three marsupial species

Fig. 24. A section through the gastric lining epithelium of the 3·5 cm opossum (*ca.* 17 days). The cytoplasm of component cells shows numerous lipid droplets which do not appear to be limited by a definite membrane. The apices of two caveolated cells (*C*) are shown at the top of the micrograph. ×7500.

Fig. 25. The apex of a surface lining cell filled with secretory granules. The granules vary in size and individual granules exhibit differences in electron density, giving them a mottled appearance. Generally, they are comprised of a flocculent material limited by a membrane. The granules appear to fuse with the apical cell membrane (arrow 1), which later ruptures to discharge the secretory material into the lumen (arrows 2, 3). Aggregates of secretory material are observed on the external surface of the apical cell membrane. 5·5 cm opossum (*ca.* 27 days). ×6000.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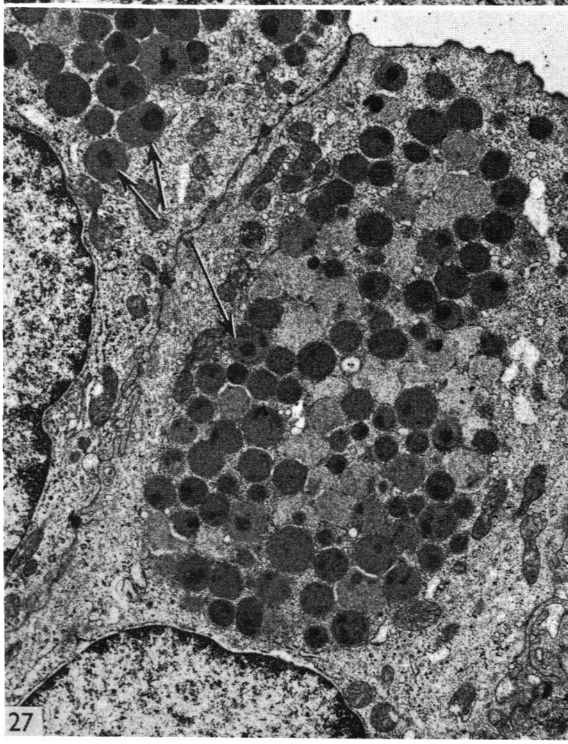
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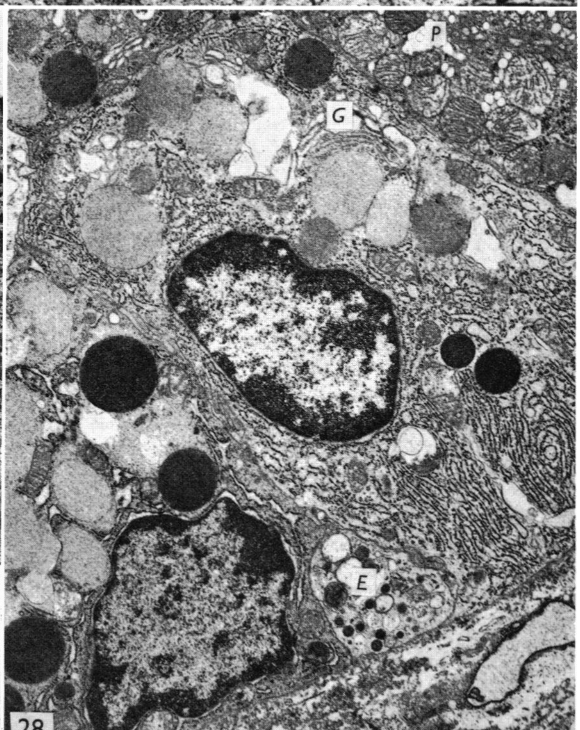
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examined to date, fovea and gastric glands are not present at birth and develop during the postnatal period. However, many eutherian species, including pig (Kirk, 1910), rat (Kammeraad, 1942), rabbit (Menzies, 1958), man (Johnson, 1919; Salenius, 1962; Nomura, 1966), show development of fovea and outgrowth of gastric glands before birth. Fovea and gastric glands of the opossum appear to develop simultaneously, and are initimately associated throughout development. The surface lining epithelium shows an initial invagination into the underlying connective tissue during the first day of postnatal life (1·5 cm). Parietal cells, which stain intensely with toluidine blue, are found at the bases of such invaginations and these also are found within the surface lining epithelium in the newborn. The invaginations continue to develop, and the glands, which expand further into the underlying connective tissue, consist primarily of parietal cells and 'undifferentiated' cells. Further expansion of glands occurs as development progresses and, by 4·5 cm (*ca.* 20 days), fovea are well established. The quantitative studies indicate that the greatest development of the glands occurs between the 10·0 and 13·0 cm stages. By 13·0 cm (*ca.* 75 days), small clusters of chief or zymogen cells are present at the bases of the glands, and by 28·0 cm (juvenile) near adult proportions are found. Chief cells continue to make up only a small population of cells and are restricted to the most basal regions of the glands. The order in which parietal and chief cells make their appearance is similar to that observed in other species; however, the long interval after birth before chief cells begin to appear seems to be peculiar to the opossum.

The muscularis externa of the newborn opossum is extremely thin and is composed of a single layer of myoblasts, one to three cells in depth. By the ninth day it is of considerable thickness and exhibits a well defined inner circular layer. At 3·5 cm both the inner circular and the outer longitudinal layers are established. Throughout the postnatal period the coats of the muscularis externa continue their development and increase in thickness.

Ultrastructurally, parietal cells of the newborn opossum show numerous mitochondria, canaliculi, and a well developed border of long microvilli lining both the canaliculi and the apical cell surface. The cytoplasm contains numerous free ribosomes and appears more electron-dense than that of surrounding epithelial cells. Parietal cells of the newborn opossum, unlike those of a number of species reported, actually line the gastric lumen and are in direct contact with luminal contents. The remainder of the lining surface is comprised of a tall columnar undifferentiated

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Fig. 26. What is thought to be a developing mucous neck (*M*) cell within a gastric gland shows scattered electron-dense granules, Golgi complexes (*G*) and occasional mitochondria. The apex of a parietal (*P*) also is shown. 5·5 cm opossum (*ca.* 27 days). × 10000.

Fig. 27. The apices of surface lining cells exhibit a heterogeneous population of secretory granules. The granules vary somewhat in electron density and are comprised of a flocculent material limited by a membrane. The granules often show areas of increased electron density, giving them a mottled appearance (arrows). 13 cm opossum (*ca.* 75 days). × 5500.

Fig. 28. A chief cell from the 13 cm opossum. The basal portion of the cell is filled with granular endoplasmic reticulum. The apex of the cell contains membrane-bound secretory granules, which vary in size and in electron density. Scattered Golgi complexes (*G*) are noted in the apical cytoplasm in relation to the granules. A portion of the parietal (*P*) and a endocrine cell (*E*) also are shown. × 6000.

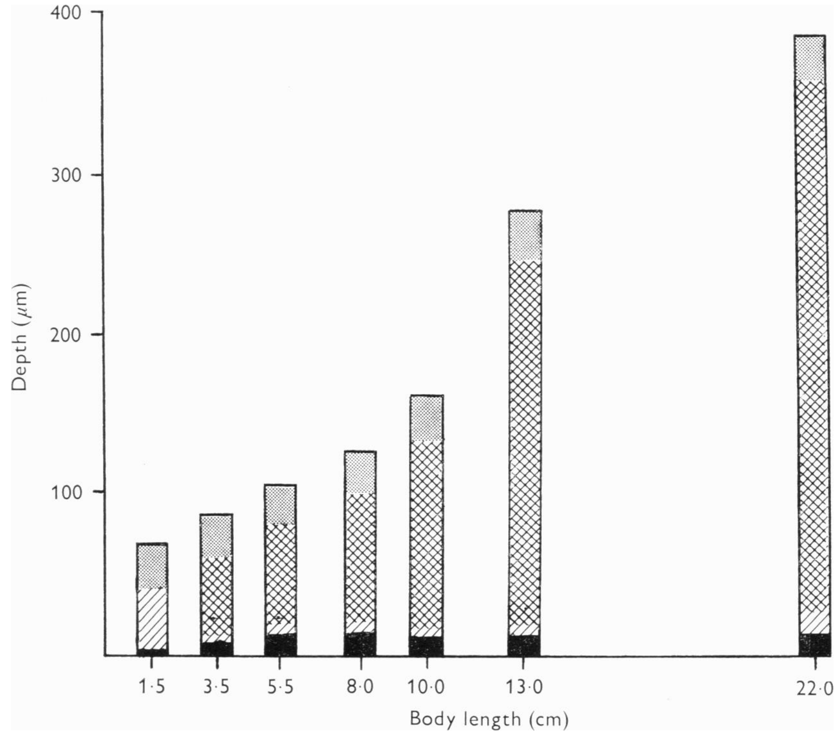

Fig. 29. Development of the mucosa and its constituent layers. The total height of each bar represents the total depth of the mucosa at each stage. The upper stippled area represents the depth of the surface epithelium. The depth of the lamina propria is indicated by the diagonal lines, while the depth of the glands within the lamina propria is indicated by the area of cross-hatching. The muscularis mucosae is indicated by the solid black portions.

epithelium although occasional scattered cells do show small accumulations of secretory granules. Immature mucous cells, which give rise to the parietal cell type, have been reported during normal development in rabbit (Menzies, 1958), man (Nomura, 1966) and mouse (Matsuyama & Suzuki, 1970). In the opossum also, additional parietal cells appear to arise from undifferentiated columnar cells within the developing gastric glands. By 8·0 cm (*ca.* 50 days) many of the undifferentiated cells contain small electron-dense granules and numerous ribosomes. The forming granules are directly associated with Golgi complexes. These cells may be differentiating mucous neck cells, which have been reported to give rise to both parietal and chief cells in adult animals of other species (Stevens & Leblond, 1953; Messier & Leblond, 1960) and in regenerating gastric mucosa (Lawson, 1970). Chief cells are first observed in the 13·0 cm opossum (*ca.* 75 days) and are a considerable distance from the neck region of the developing gland. It is possible that they arise from undifferentiated cells still present within the developing gastric glands. It is perhaps significant that there is a peak of mitotic activity in the glandular epithelium prior to the appearance of chief cells. Normally the parietal cell is not considered to give rise to

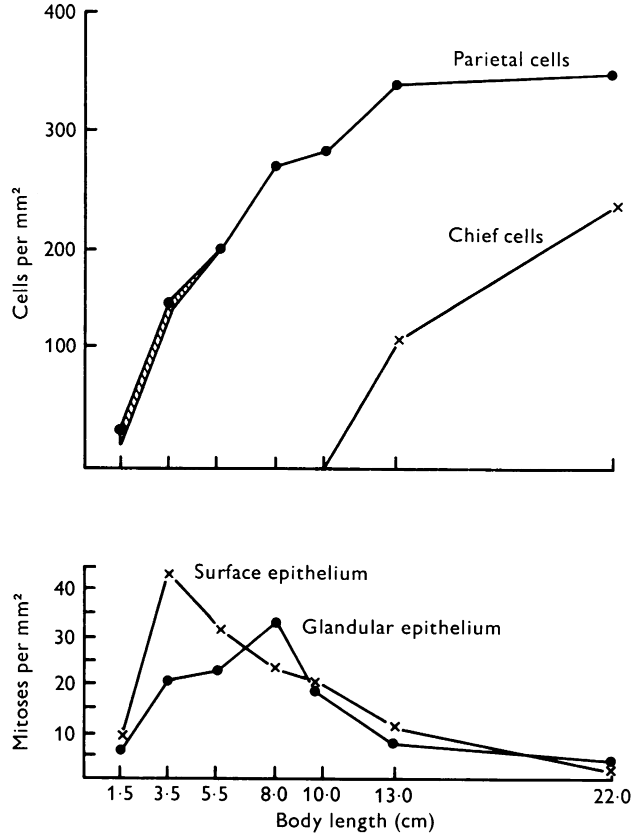

Fig. 30. The upper part of the figure shows the number of parietal and chief cells calculated per mm^2. Initially some parietal cells are found scattered among the surface epithelial cells and not contained within glandular elements. These are shown by the area encompassed by the diagonal lines. The lower part of the figure shows the mitotic activity of the surface and glandular epithelium.

any other cell type or to undergo mitotic activity for purposes of self replication (Ragins, Winckze, Liu & Ditbrenner, 1968). In contrast to the parietal cell, chief cells have been reported to undergo mitosis and to contribute to their own population of cells (Willems, Galand, Vansteenkiste & Zeitoun, 1972). This may explain why the chief cell in this particular species is restricted to the bases of the glands in relatively small numbers, even in the adult animal. Until adult proportions are attained, the glands may increase their length further by normal proliferative activity in the neck region.

Although tubulovesicles are not observed in parietal cells of the newborn opossum, the cells show well developed canaliculi with long microvilli. Scattered cytoplasmic tubulovesicles are observed in parietal cells by the 9th postnatal day and at all subsequent stages. Microvilli of the canaliculi and apical cell surface also increase in size and appear empty. Changes in the tubulovesicular compartment, and the depletion and accumulation of microvilli in the canaliculi, are thought to be associated with acid production by the parietal cell (Sedar & Friedman, 1961; Leeson, 1974; Ito & Schofield, 1974). The volume of the tubulovesicular compartment in parietal cells of the opossum increases at a time when solid food is first observed in the gastrointestinal tract. It is at this time also that the pH of stomach contents drops to 2·0–2·5, indicating increased functional activity of the parietal cells. A similar sequence of events is present in the rat also, although the timing is different. The gastric pH of fetal rats (18 days gestation) is reported to be near 6·5 and at birth near 5·8 (Manville & Lloyd, 1932). The gastric pH decreases to about 2·7 in 10 day old rats (Helander, 1969*a*, *b*). Parietal cells of the 10 day old rat also show an increase in the tubulovesicular compartment, as well as a marked development of the canaliculi (Helander, 1969*a*, *b*).

The pH optimum for lipolytic activity in the rat stomach is reported to be 6·5, but significant hydrolysis does occur at 3·5 (Clark, Brause & Holt, 1969). The enzyme involved evidently is quite stable, even at low pH. Studies on the gastric absorption of lipids in suckling rats have shown that medium-chain fatty acids are readily absorbed through the gastric mucosa of suckling rats and are utilized by the body (Egelrud, Olivecrona & Helander, 1971). The opossum, like the rat (Helander & Olivecrona, 1970), shows morphological evidence that lipids are absorbed by the surface lining cells of the gastric mucosa during the first 3 weeks after birth. Like the rat, the lipid droplets in the surface lining cells of the opossum lack bounding membranes. Lipid droplets are not observed within Golgi cisternae or within cisternae of the endoplasmic reticulum. Further, chylomicra are not observed in the intercellular space or near the basal lamina of the surface epithelium. The mechanism of gastric absorption of lipid in both the opossum and rat appears to be significantly different from the mode of lipid absorption in the small intestine. In the latter, lipid is broken down to free fatty acids and monoglycerides which are absorbed by the intestinal epithelial cells (Johnson & Young, 1968). Lipid droplets within the intestinal cells are usually surrounded by a membrane, either of the Golgi complex or of the endoplasmic reticulum (Strauss, 1968). In contrast the large lipid droplets of the gastric lining epithelium appear free within the cytoplasmic matrix and have no limiting membrane. In addition, the intestinal epithelial cells have the ability to produce chylomicra, which pass to intercellular spaces before release into nearby lacteals (Strauss, 1968). Such a mechanism does not appear to exist in the gastric lining epithelium of either the suckling opossum or the rat (Helander & Olivecrona, 1970). Although some fatty acids may diffuse through the gastric mucosa, lipid also may be taken up indiscriminately and appear as droplets in the surface epithelial cells (Egelrud *et al.* 1971). The mechanism of uptake and the fate of such lipid droplets in the rat and opossum is unknown at present.

By the 5·5 cm stage (*ca.* 27 days), cells which are thought to be differentiating mucous neck cells make their appearance in the developing gastric glands. The cells contain numerous free ribosomes and scattered apical granules. The forming granules are directly associated with Golgi membranes and are small and electron-dense. Mucous neck cells of similar appearance are found in the 20 day rat embryo (Helander, 1969*a*, *b*).

It is interesting that chief cells do not appear within the gastric mucosa of the opossum until just prior to weaning (13 cm = 75 days). The cytoplasm of such cells is characterized by the presence of an extensive granular endoplasmic reticulum and contains large membrane-bound granules which vary considerably in electron density. By way of comparison, similar appearing chief cells are found in the gastric mucosa of the developing rat by the 21st day of intrauterine development. It is difficult to explain why chief cells are so delayed in their development in the opossum, since an acid medium necessary for their enzymatic activity is apparently established 9 days after birth when tubulovesicles first appear within parietal cells. However, extensive development of tubulovesicles does not occur until just prior to weaning. It is possible that the delay in differentiation of chief cells may be related in some way to the mechanism of transfer of immunoglobulins from the mother to the offspring. Early differentiation of chief cells undoubtedly would have an adverse effect upon the transfer if it occurs via the milk, a route which is known to be present in many species, including the rat (Rodewald, 1970).

The surface lining epithelium stains with PAS but fails to stain with alcian blue or aldehyde fuchsin. These results indicate that the secretory product is a neutral mucin. Similar results have been reported for the surface lining epithelium of the dog (Spicer, Leppi & Henson, 1967; Spicer & Sun, 1967) and man (Lev, 1966; Cathcart, Fitts, McAlhany & Spicer, 1974). Chief cells of the opossum fail to stain with PAS, aldehyde fuchsin or alcian blue, whereas chief cells of the dog stain both with aldehyde fuchsin and with alcian blue, indicating the presence of sulfated monosubstances in this particular species.

Endocrine cells are present in the gastric mucosa of the newborn opossum and are observed in the forming gastric glands at all developmental stages examined, as well as in the adult. They exhibit morphological features similar to those of endocrine cells described in the gastrointestinal tract of several species, including cat (Vassallo, Solcia & Capella, 1969), rabbit (Capella, Solcia & Vassallo, 1969), pig (Capella & Solcia, 1972), and man (Vassallo, Capella & Solcia, 1971; Rubin, 1972).

Caveolated cells also are present within the gastric mucosa of the suckling opossum. They were first noted in the 3·5 cm opossum (*ca.* 17 days) and were found infrequently in subsequent stages. Nabeyama & Leblond (1974) have reviewed the literature concerning this cell type, which has been described by several different investigators under a variety of names. The functional significance of these cells has yet to be established.

At the time of weaning numerous connective tissue cells, primarily macrophages, lymphocytes and esosinophils, are found in the lamina propria surrounding the gastric glands. The number of such cells increases after weaning and continues to increase into adulthood. These cells may represent a barrier against invasion by foreign organisms from the gastric lumen. Similar observations have been reported in the mouse (Deane, 1964).

SUMMARY

The postnatal development of the gastric mucosa in the opossum has been traced with the light, transmission and scanning electron microscopes. The formation of fovea and gastric glands occurs simultaneously during the postnatal period. During the first 60 postnatal days the developing gastric glands are composed of

undifferentiated cells, parietal cells and scattered endocrine cells. Chief cells are not present until just before weaning (13 cm, i.e. *ca.* 75 days). Juvenile and adult animals show only a small population of chief cells, and these are confined to the bases of the gastric glands. The pH of stomach contents ranges from 6·0 to 6·5 until the time of appearance of solid food within the stomach, when it drops to 2·0–2·5.

The surface cells lining the gastric lumen contain a considerable amount of what appears to be lipid during the first 3 weeks after birth, and this may indicate that the gastric mucosa is involved in the absorption of lipid during this period. The mode of lipid absorption appears to be different from that described for the intestinal tract of several other species.

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