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Development of the Digestive System in the North American Opossum (Didelphis virginiana)

With 45 Figures and 68 Tables

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1 Introduction

The North American opossum (*Didelphis virginiana*) generally is regarded as an important animal, phylogenetically. It is considered to represent a prototype marsupial and closely resembles fossil didelphids (Tyndale-Biscoe 1973). Numerous studies concerning the reproductive biology, embryology, and neurobiology of the opossum have been published. More recently, *Didelphis* has become popular as an animal model for gastroenterological studies because of the remarkable anatomical and physiological similarities of the esophagus as compared to that of man.

Most of the studies of early development have concentrated on early cleavage stages and the formation of the three primary germ layers (Hartman 1916, 1919) and fetal membranes (Selenka 1887; McCrady 1938). The ova of *Didelphis* remain in the oviduct only for about 24 h before entering the uterus. A corona radiata is absent and each oocyte is surrounded only by a perivitelline space and a zona pellucida (Talbot and DiCarlantonio 1984). During the short transit period, the egg is fertilized by a single spermatozoon (Rodger and Bedford 1982a,b).

The sperm are paired in the epididymis and female reproductive tract (Biggers and DeLamater 1965; Krause and Cutts 1979), but separate prior to fertilization. After fertilization, a second layer consisting primarily of acid mucopolysaccharide is laid down around the zona pellucida and is thought to be a product secreted by the lining epithelium of the oviduct (McCrady 1938; Rodger and Bedford 1982a). The fertilized ova of *Didelphis* and other metatherians then become surrounded by a third layer, the shell membrane, which is believed to be produced by secretory cells in the distal oviduct (Sharman 1961; Krause and Cutts 1983c). In most marsupials, the shell membrane persists as a physical barrier between the embryo, with its forming fetal membranes, and the endometrium until relatively late in gestation. The shell membrane permits passage of large molecules such as toluidine blue (mol wt. 306), horseradish peroxidase (mol wt. 40 000), and ferritin (mol wt. 460 000) from and to the surrounding uterine fluid. Because of the passage of substances such as peroxidase and ferritin with very large molecular weights, it was concluded that the shell membrane was not significantly involved in regulating the passage of embryonic wastes or nutrients (Hughes and Shorey 1973).

1.1 Endoderm Formation

A morula stage does not form in *Didelphis*, and an inner cell mass also is lacking. A hollow, unilaminar vesicle forms directly and gives rise to all of the embryonic and extraembryonic tissues. Early in the 4th day of gestation, the embryonic sphere consists of a single layer of flattened cells that surrounds a central, fluid-filled cavity (Hartman 1919; McCrady 1938). Late in the 4th day, cells called endodermal mother cells differentiate in the region of the unilaminar sphere that will form the embryo. The endodermal mother cells enlarge, migrate to the interior of the unilaminar sphere, and, by the end of the 5th day of gestation, have formed a definitive endoderm that lines the cavity, thus converting the unilaminar sphere into a bilaminar sphere that consists of ectoderm and endoderm. Mesodermal cells develop in the embryonic region of the bilaminar sphere at about day 6, and by the 8th day extend well beyond the developing embryo to lie between the extraembryonic endoderm and the trophectoderm of the embryonic sphere. The mesoderm consists of a network of large, stellate cells with numerous elongated cytoplasmic processes that extend over considerable distances and are in contact with adjacent mesodermal cells (Krause and Cutts 1985a). The ventral one-third of the embryonic sphere (the side opposite the forming embryo) never is invaded by mesoderm and forms the yolk sac in this species (McCrady 1938; Krause and Cutts 1985b). The endodermal cells of the embryo proper are large, cuboidal cells which at 9 days appear morphologically similar to those that line the interior of the yolk sac (Krause and Cutts 1984, 1985c).

The first 9 days of gestation of *Didelphis* are concerned primarily with the establishment of the three germ layers. During this time, the embryos float freely within the uterine secretions, each prevented from attaching to the uterine mucosa by the surrounding shell membrane. Organogenesis occurs during the last 3½ days of the 12½-day gestation period, and corresponds to the time when the yolk sac placenta is intimately associated with the uterine epithelium (Krause and Cutts 1985b). During the last 3½ days of gestation, development proceeds at an explosive rate to form functional structures that will allow for the survival of the newborn of this species.

1.2 State of Visceral and Other Structures at Birth

Those structures essential for survival at and immediately after birth develop precociously but follow a typically mammalian pattern of development although they usually are modified in some way to allow for the survival of the young following the very abbreviated period of gestation. Structures that are not essential for immediate survival (reproductive organs, lymphoid organs, eyes, ears, and hind limbs) are poorly developed at birth and develop more slowly during the extended postnatal period within the protection of the pouch. Structures that appear most essential for survival at birth include the external nares, which are open and flaring, the olfactory epithelium, lungs, cardiovascular system, mouth (which is large, open, and houses a well-developed

tongue), and the forelimbs, which are well developed with opposable digits and epitrichial claws.

At birth, the young pass from the uterus through a newly formed pathway, the median or pseudovaginal canal, into the urogenital sinus (McCrady 1938). With no aid from the mother, the newborn crawl to the pouch using their well-developed forelimbs and clawed digits, apparently guided by the well-developed sense of smell. Prior to the birth of her litter, the female opossum thoroughly licks and cleans the mammary area and the region between the birth canal and the pouch.

The instinctive licking behavior cleans the pouch and nipple region and also is believed to provide olfactory cues, through the medium of the saliva, that lead the sightless newborn to the pouch and aid them in locating the nipples. Bipolar neurons are present in the olfactory epithelium of the opossum before birth. Axons from these neurons extend into the developing olfactory bulb region of the brain, where they form a network of fibers (Lin et al. 1988). Once within the pouch, the newborn opossum sweeps its head in wide arches and, when the snout touches a nipple, the animal immediately sucks that nipple into the mouth with the aid of the large, well-developed tongue. Having attained a nipple, the young will remain attached to it for the next 60 days of postnatal life.

At birth, the lungs of the opossum are in the initial stages of development only, but are so modified that gaseous exchange can occur as development proceeds, thus allowing for survival of the young (Krause and Leeson 1973, 1975; Krause et al. 1976c). The mesonephros is the functional kidney at birth and remains the primary kidney for at least the 1st week of postnatal life (Krause et al. 1979a). The metanephric kidneys develop primarily during the postnatal period (Krause et al. 1979b; Krause and Cutts 1980b). The periderm of the epidermis also is important to the survival of the newborn and envelops the entire fetus, including the eyes and ears (Krause et al. 1978a). Only the orifices of the oral cavity and nostrils are open to the external environment. The periderm probably helps to prevent dehydration and forms the main protective barrier against bacterial invasion until the immune system becomes fully active.

Recently, a new perception has emerged in the scientific community, recognizing the potential of marsupials as new and unique models for developmental biologists (Tyndale-Biscoe and Janssens 1988). Because of their abbreviated period of intrauterine development, specific questions can be addressed in detail that would be impractical, if not impossible, with common laboratory (eutherian) mammals. *Didelphis*, for example, currently is the model of choice for neonatal nephrologists in the study of renal dysplasia (Steinhardt et al. 1988).

1.3 The Scope of This Review

This volume summarizes and draws together for the first time the morphological, developmental, and quantitative data concerned with the digestive system of the North American opossum, *Didelphis virginiana*. The inter-

relationships between the epithelial lining of the developing digestive system and its associated intrinsic and extrinsic glands are emphasized. As the developmental data published on *Didelphis* are frequently recorded according to snout-rump length measurements, an approximation to age is provided in the Appendix.

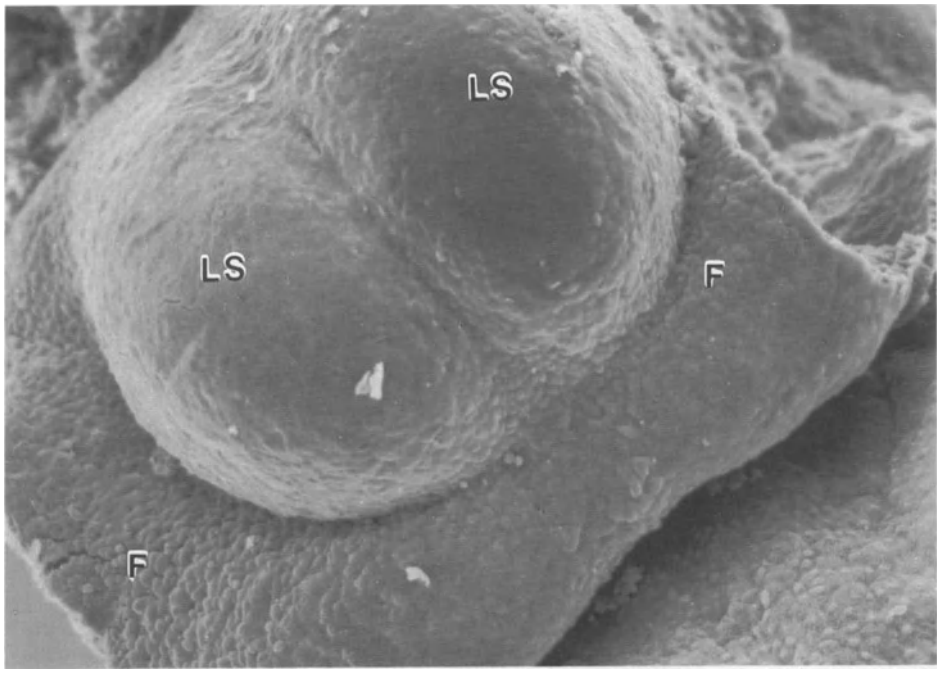
2 Oral Cavity

2.1 Prenatal Development

The first pharyngeal pouches appear during the middle of the 9th day of gestation, slightly preceding development of the pharyngeal floor (McCrady 1938). The pouches form a series of evaginations along the laternal walls of the pharyngeal portion of the developing foregut. Development of the pharyngeal pouches and their derivatives is extremely rapid in *Didelphis* but remains typically mammalian in nature.

By the middle of the 10th day, the developing head is characterized by a frontal eminence, by maxillary and mandibular processes, and by a hyoid arch (Krause and Cutts 1986b). The tongue is prominent at this time, due to rapid proliferation of mesoderm in the center of the mandibular arch. The initial formation of the tongue from two lateral and one medial lingual swellings (Fig. 1) appears to be similar to the development of the tongue in man. The mouth is well formed by day 11 (Fig. 2), as is the tongue, which protrudes from the oral cavity (Krause and Cutts 1986b). Large external nares, eyes, and the orifices of the external auditory meatus also characterize the head of the opossum at this stage of development. The stratified squamous epithelium that lines the oral cavity lies on a delicate connective tissue, separated from it by a thin basement membrane. The epithelium is thickest on the dorsum of the tongue. The external surface of the tongue is relatively smooth and lacks true papillae, although knobs of mucosa are present. The intrinsic musculature of the tongue consists of myotubes with chains of centrally placed nuclei. Faint striations of the peripherally arranged myofibrils can be seen, and the developing skeletal muscle fibers are enveloped in a delicate connective tissue. The majority of the muscle fibers are perpendicular to the dorsum of the tongue at this stage of development (Fig. 3).

Early in day 12, the lateral borders (presumptive lips) of the open mouth begin to grow closed, beginning at the angle of the jaws, and the oral shield begins to form at the apex of the snout. The cells of the epitrichium continue to proliferate and at the time of birth form a covering over the eyes, the orifice of the external auditory meatus, and the remainder of the facial area. Around the oral aperture, this proliferation results in the formation of a cornified epithelial structure, the oral shield, which surrounds the opening to the oral cavity like a flattened collar with prominent points at its edge (Krause and Cutts 1986b). The open mouth and the large, well-developed tongue are essential for nipple attachment and survival after birth (Fig. 4).



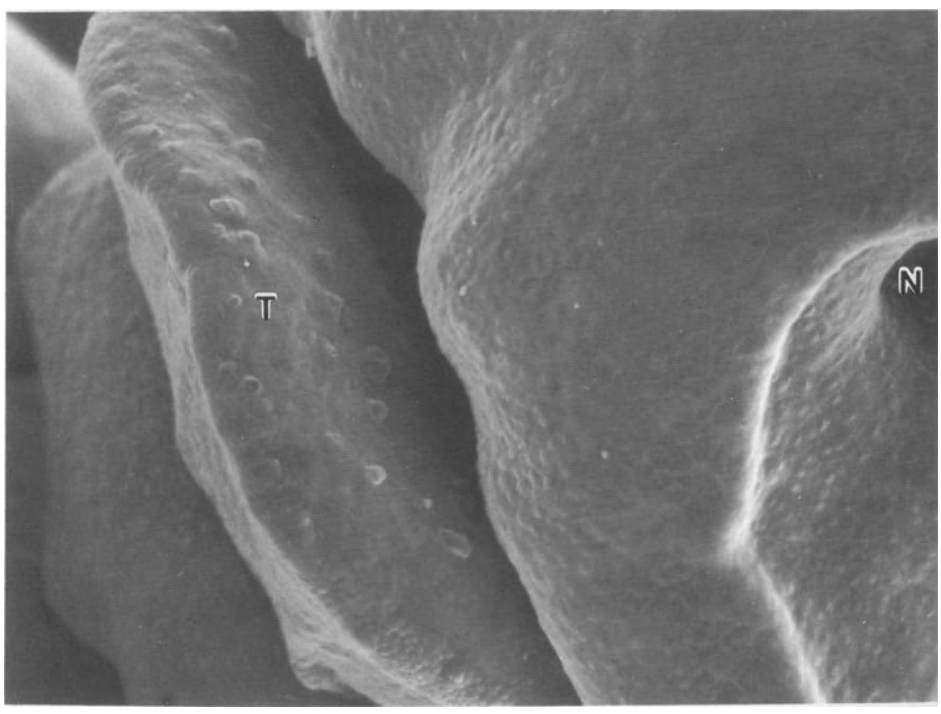
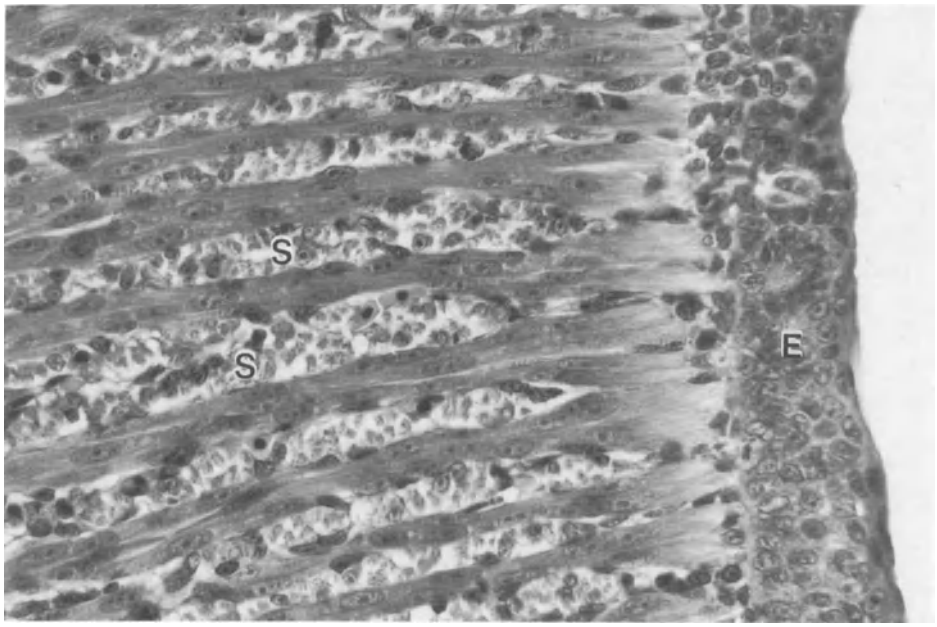


Fig. 1 (*above*). The floor (*F*) of the oral cavity and developing tongue of a 10-day-old opossum embryo following removal of the cranium and maxillary region; the two lateral lingual swellings (LS) remain visible. ×130

Fig. 2 (*below*). A portion of the open mouth and protruding tongue (*T*) of an 11-day-old embryo. One of the external nares (*N*) is shown at the *extreme right*. ×100



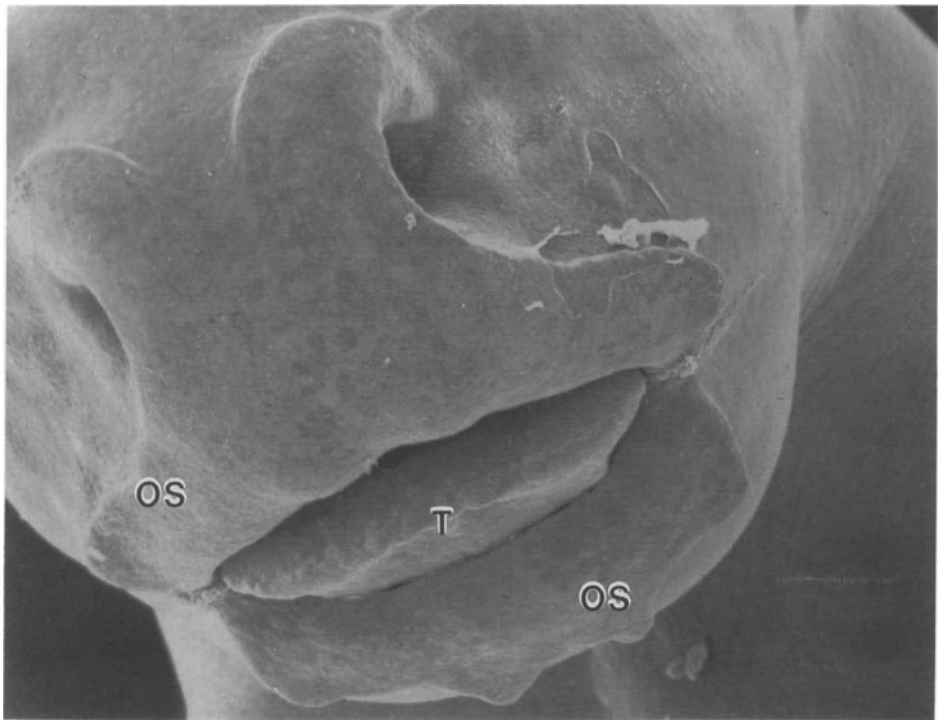


Fig. 3 (*above*). A section through a portion of the tongue of an 11-day-old opossum embryo illustrates the depth of the epithelium (*E*) on the dorsal surface and the perpendicular arrangement of developing skeletal muscle cells. Note the developing skeletal muscle cells (*S*) cut in cross-section. H & E, ×300

Fig. 4 (*below*). The mouth and oral shield (*OS*) of a 12-old-day opossum embryo. The tongue (*T*) is seen protruding from the oral cavity. ×50

2.2 Postnatal Development

Immediately after birth, the opossum young crawl, unaided, to the safety of the pouch, where each locates one of the 13 nipples and sucks it into its oral cavity. Once a nipple has been secured, the young remain attached to that nipple for about the next 60 days of postnatal life (Cutts et al. 1978b): this period within the pouch often is referred to as the fixation period. Immediately after the newborn attaches to a nipple, the lips fuse, and only a small circular orifice remains at the apex of the oral cavity to permit entrance of the nipple into the mouth. Fusion of the lips results from a continuation of the process that began prenatally. Enlargement of that part of the nipple that lies within the oral cavity of the young results in it becoming a bulbous structure that permanently attaches the young to the mother during this period. Although in close apposition, the epithelial tissue of the mother's nipple and that which lines the oral cavity of the young do not fuse during the fixation period of postnatal development.

All marsupial neonates are able to suck (Boyd 1932; Enders 1966). In *Didelphis*, the newborn can imbibe test fluids from nondistortable tubes that have been inserted between the teat and the aperture of the oral cavity (Jurgelski 1971; Griffiths and Slater 1988) but the mechanics of taking milk from the mother's teats differ from those in eutherian species (Cowie 1972). In most eutherians, the nipple within the oral cavity is compressed between the tongue and palate beginning at the base of the teat and extending toward the tip, thereby stripping milk contained within the nipple. As pressure at the base of the teat is released, the nipple again fills with milk due to pressure created by the contraction of myoepithelial cells within the mammary gland, during normal milk letdown. In metatherians, the teat lies within a shallow groove on the dorsal surface of the tongue and the oral cavity is sealed. As the animal sucks, the dorsal surface of the tongue is lowered through contraction of the vertically arranged skeletal muscle fibers that extend from the dorsal to the basal surface of the tongue. As the dorsal surface of the tongue is lowered, a negative pressure is created within the oral cavity (Griffiths and Slater 1988) and milk is drawn from the teat. The ducts within the nipples of marsupials refill by a mechanism of milk letdown similar to that which occurs in eutherian mammals (Griffiths and Slater 1988).

2.2.1 Tongue

At birth, the skeletal muscle fibers in the tongue of the newborn opossum continue to show centrally placed, multiple nuclei, but at about 1 week after birth, typical skeletal muscle fibers with peripheral nuclei characterize the intrinsic musculature of the tongue.

The intrinsic muscles continue to develop, but remain divided into right and left halves by an incomplete median structure, the lingual septum, reflecting their initial development. The muscle is richly innervated, and thick and thin nerve fibers run between muscle cells. Perikarya of the many neurons (ganglia) that occur singly or in small groups also are scattered within the

tongue musculature (Beg and Qayyum 1976). In contrast to the ventral surface, the dorsal surface of the tongue is richly innervated and associated with thick nerve bundles. Only a few nerve fibers are associated with the circumvallate papillae at this stage of development.

By 30 days postnatum, the border of the tongue bears a fringe of simple filiform papillae which are scattered over the dorsum of the tongue also, along with developing fungiform papillae. The latter appear as small, bead-like structures (Beg and Qayyum 1976). Taste buds are present in the dorsum of the tongue at this time, associated with the fungiform papillae. Three circumvallate papillae, arranged in a triangle at the base of the tongue, are present but are not fully developed, and taste buds are lacking in the epithelial walls of these papillae.

The epiglottis of the newborn opossum is intranarial and projects into the posterior nares. It is a tubular structure lined by stratified cuboidal epithelium anchored to a central area of developing cartilage by a delicate connective tissue. Because of the tubular shape and position, fluid can pass around both sides of the epiglottis without interrupting breathing (McCrady 1938). The intranarial epiglottis persists into adulthood.

2.2.2 Salivary Glands

The major salivary glands of the opossum consist of submandibular, greater and lesser sublingual, parotid, and molar glands (Carmalt 1913). Of these, only the development of the submandibular glands has been examined in detail (Leeson et al. 1978). At birth, the submandibular glands of the opossum consist only of a system of primitive ductal elements that terminate in end-pieces composed of proacinar cells (Leeson et al. 1978). The epithelial component is enveloped in, and organized by, a delicate connective tissue that is rich in ground substance. The submandibular glands show progressive growth throughout the postnatal period (Tables 1, 2). It is not until after weaning that the glands show an adult appearance, and even in adult animals there is evidence for continued growth (Leeson et al. 1978). Although the submandibular glands of males have a slightly but consistently greater weight than those of the female opossum, no histological differences have been observed in the submandibular glands with regard to sex either in adult or in postnatal animals. The prolonged period of postnatal development of the submandibular glands can be separated into two basic phases, the first of which extends from birth through the 4th week of postnatal life and is concerned with establishment of the ductal system. The second phase of development lasts for the remainder of the postnatal period and is involved principally with changes in the secretory end-pieces.

The developing ductal elements in the submandibular glands of the newborn opossum are lined by an epithelium that generally is bilayered, but in which the component cells show no apparent specialization. The cells appear flattened and their cytoplasm is electron dense and contains numerous free ribosomes. A distinction cannot be made between intercalated and intralobular ducts. The primitive tubules of the ductal system have wide, irregular lumina

Table 1. Weights of submandibular glands (g: mean ± SD)[a]

Body length (cm)	Male	Female	Combined male and female
1.5[b]	–	–	0.0011 ± 0.0008 (9)
2.0[b]	–	–	0.0018 ± 0.0002 (7)
2.5[b]	–	–	0.0027 ± 0.0008 (12)
3.5	0.0087 ± 0.001 (9)	0.0085 ± 0.0005 (6)	0.0087 ± 0.0001 (15)
4.0	0.0121 ± 0.003 (5)	0.0116 ± 0.003 (5)	0.0119 ± 0.0004 (10)
4.5	0.0182 ± 0.003 (13)	0.0169 ± 0.003 (6)	0.0178 ± 0.003 (19)
5.0	0.0234 ± 0.001 (5)	0.0205 ± 0.0008 (4)	0.0221 ± 0.002 (9)
5.5	0.0237 ± 0.004 (8)	0.0236 ± 0.003 (7)	0.0237 ± 0.003 (15)
6.0	0.0341 ± 0.002 (5)	–	0.0341 ± 0.002 (5)
6.5	0.0408 ± 0.002 (7)	0.0311 ± 0.002 (6)	0.0354 ± 0.006 (13)
7.0	0.0435 ± 0.001 (5)	–	0.0435 ± 0.001 (5)
8.0	0.0557 ± 0.006 (5)	0.0557 ± 0.002 (5)	0.0557 ± 0.004 (11)
9.0	0.0595 ± 0.001 (4)	0.0637 ± 0.004 (3)	0.0616 ± 0.004 (7)
10.0	0.0812 ± 0.005 (8)	0.0802 ± 0.007 (8)	0.0807 ± 0.006 (16)
11.0	0.2216 ± 0.053 (7)	0.2444 ± 0.012 (7)	0.2329 ± 0.039 (14)
12.0	0.2198 ± 0.014 (8)	0.2412 ± 0.009 (7)	0.2305 ± 0.016 (15)
13.0	0.2409 ± 0.0005 (4)	0.2491 ± 0.008 (4)	0.2450 ± 0.007 (8)
15.0	0.2820 ± 0.002 (4)	0.2485 ± 0.005 (4)	0.2652 ± 0.018 (8)
15.5	0.2821 ± 0.017 (3)	0.2579 ± 0.003 (3)	0.2670 ± 0.016 (8)
16.0	0.2896 ± 0.006 (4)	0.2851 ± 0.017 (4)	0.2874 ± 0.013 (8)
17.0	0.3397 ± 0.001 (3)	0.3370 ± 0.015 (3)	0.3384 ± 0.009 (6)
18.0	0.4345 ± 0.01 (4)	0.4685 ± 0.013 (3)	0.4458 ± 0.02 (7)
20.0	0.6124 ± 0.048 (6)	0.5846 ± 0.024 (6)	0.5973 ± 0.035 (12)
22.0	0.6203 ± 0.001 (3)	0.6475 ± 0.013 (3)	0.6384 ± 0.008 (6)
25.0	–	0.9307 ± 0.202 (4)	0.9307 ± 0.202 (4)
28.0	1.5399 ± 0.164 (3)	–	1.5399 ± 0.164 (3)
29.0	–	1.6499 ± 0.076 (3)	1.6499 ± 0.076 (3)
33.0	–	2.5609 ± 0.365 (5)	2.5609 ± 0.365 (5)
37.0	3.4492 ± 0.629 (7)	–	3.4492 ± 0.629 (7)
Adult	5.5504 ± 0.544 (5)	5.3292 ± 0.621 (8)	5.4143 ± 0.58 (13)

[a] The numbers in parentheses, number of animals used.
[b] Sex not determined.

and terminate in end-pieces lined by pyramidal-shaped cells, called proacinar cells (Leeson et al. 1978), which in the newborn are characterized by basally placed, large, vesicular nuclei with distinct nucleoli (Fig. 5). The electron-dense cytoplasm contains numerous free ribosomes and scattered profiles of granular endoplasmic reticulum. The apical cytoplasm of some proacinar cells may contain scattered electron-dense granules. Tight junctions unite the apices of the cells, and desmosomes are scattered between cells in the ductal and proacinar cell regions. Intercellular canaliculi, the lumina of which usually contain an abundance of stubby microvilli, are seen between proacinar cells. Differentiating myoepithelial cells are present (Fig. 5), scattered among the bases of the proacinar cells.

At the end of the 1st week of postnatal life, intralobular and intercalated ducts have differentiated within the ductal system, and by the end of the 3rd week the intralobular ducts are associated with numerous small blood vessels

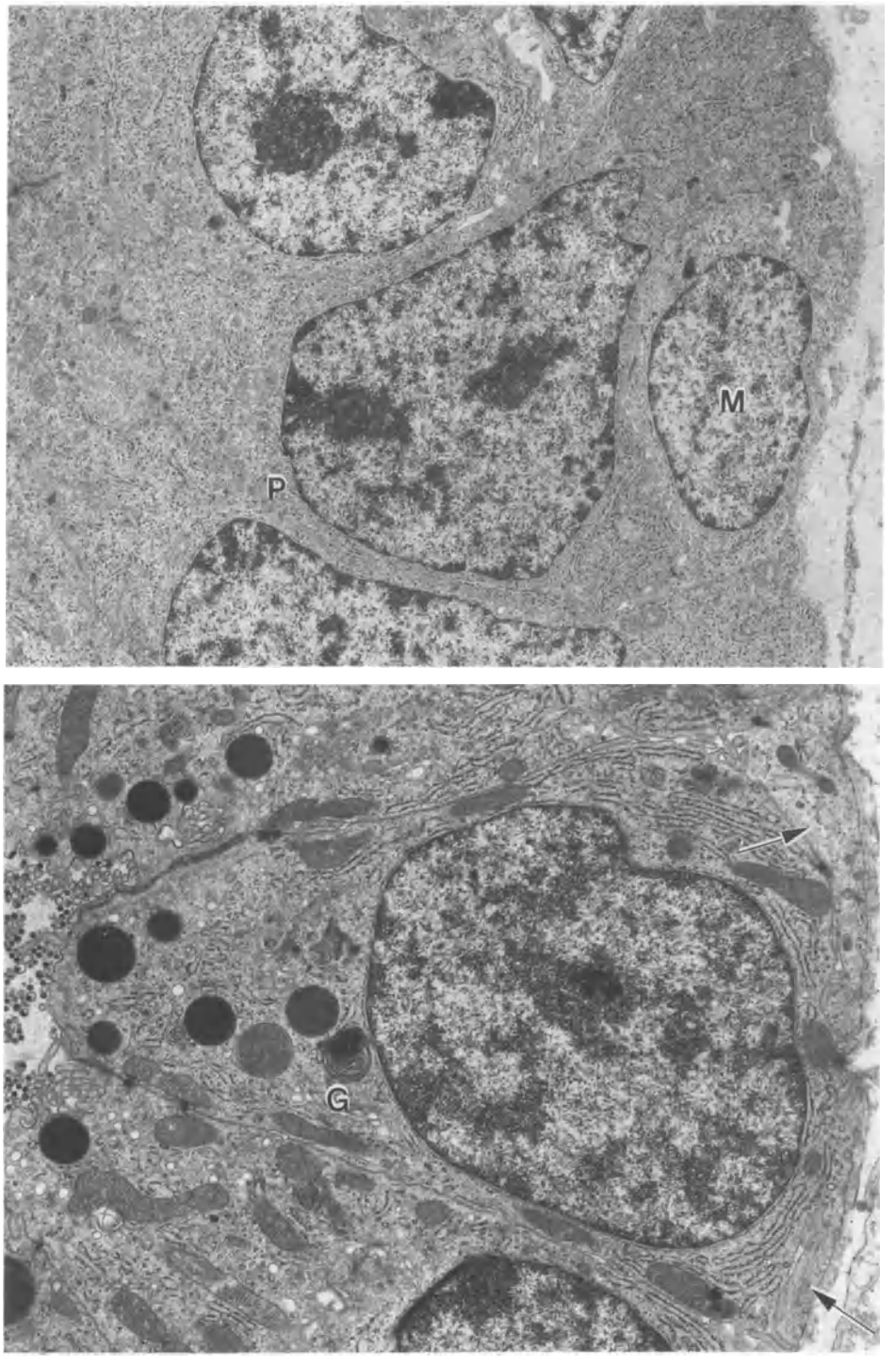
Table 2. Weights of submandibular glands (g) per 100 g body weight (mean ± SD)

Body length (cm)	Male	Female	Combined male and female
1.5[a]	–	–	0.6003 ± 0.007
2.0[a]	–	–	0.5012 ± 0.083
2.5[a]	–	–	0.4237 ± 0.079
3.5	0.5189 ± 0.06	0.4975 ± 0.078	0.5048 ± 0.07
4.0	0.4663 ± 0.015	0.4604 ± 0.028	0.4633 ± 0.018
4.5	0.6060 ± 0.051	0.5201 ± 0.039	0.5789 ± 0.062
5.0	0.5893 ± 0.035	0.5565 ± 0.014	0.5742 ± 0.031
5.5	0.5029 ± 0.028	0.5256 ± 0.01	0.5134 ± 0.024
6.0	0.5289 ± 0.044	–	0.5289 ± 0.044
6.5	0.5798 ± 0.022	0.5058 ± 0.042	0.5334 ± 0.049
7.0	0.3845 ± 0.029	–	0.3845 ± 0.029
8.0	0.3699 ± 0.041	0.3798 ± 0.018	0.3749 ± 0.03
9.0	0.3500 ± 0.031	0.3634 ± 0.008	0.3567 ± 0.021
10.0	0.3101 ± 0.026	0.3144 ± 0.050	0.3123 ± 0.04
11.0	0.4748 ± 0.015	0.5154 ± 0.026	0.4951 ± 0.056
12.0	0.4809 ± 0.035	0.5297 ± 0.02	0.5053 ± 0.037
13.0	0.3992 ± 0.022	0.4424 ± 0.01	0.4208 ± 0.028
15.0	0.2773 ± 0.005	0.3211 ± 0.006	0.2992 ± 0.024
15.5	0.2275 ± 0.005	0.2492 ± 0.014	0.2357 ± 0.014
16.0	0.2232 ± 0.014	0.2373 ± 0.007	0.2302 ± 0.013
17.0	0.2591 ± 0.008	0.2685 ± 0.01	0.2638 ± 0.009
18.0	0.3148 ± 0.003	0.3259 ± 0.008	0.3185 ± 0.007
20.0	0.3088 ± 0.015	0.3318 ± 0.052	0.3192 ± 0.036
22.0	0.2440 ± 0.002	0.2557 ± 0.005	0.2518 ± 0.008
25.0	–	0.3846 ± 0.013	0.3846 ± 0.013
28.0	0.3347 ± 0.019	–	0.3347 ± 0.019
29.0	–	0.3922 ± 0.018	0.3922 ± 0.018
33.0	–	0.3486 ± 0.042	0.3486 ± 0.042
37.0	0.2453 ± 0.025	–	0.2453 ± 0.025
Adult	0.2179 ± 0.024	0.2213 ± 0.062	0.2199 ± 0.049

[a] Sex not determined.

(Leeson et al. 1978). Intralobular ducts lie centrally within each developing lobule, a characteristic of the adult, and are lined by a stratified columnar or pseudostratified epithelium. The cytoplasm of the tall ductal cells is electron lucent and contains numerous mitochondria; basolateral infoldings are absent. Smaller basal cells lie adjacent to the basal lamina, between the bases of the columnar cells. Basal cells are characterized by their central nuclei and an electron-dense cytoplasm. The intercalated ducts are lined by a flattened epithelium and unite the primitive secretory end-pieces consisting of proacinar cells to the remainder of the ductal system. Proacinar cells still are characterized by large, electron-dense granules that now lie adjacent to the apical plasmalemma or to intercellular canaliculi. Short profiles of granular endoplasmic reticulum and mitochondria are found throughout their cytoplasm.

At the end of the 3rd postnatal week, tall light cells are present in the intralobular ducts and show basolateral infoldings. Branching intercalated ducts, lined by a low cuboidal epithelium, now are prominent features of the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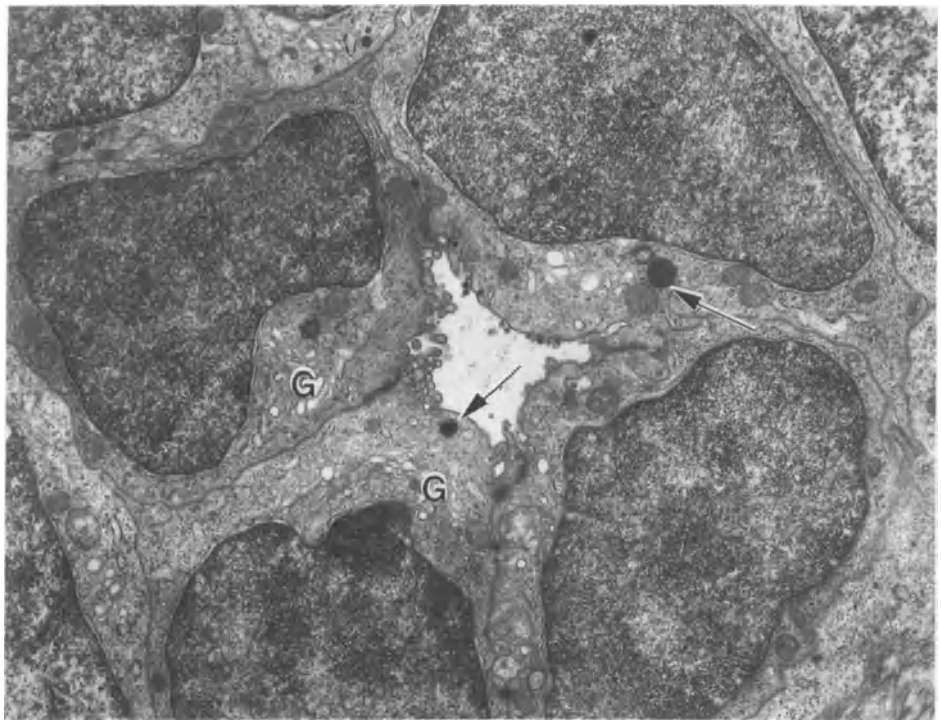
submandibular gland. Numerous small blood vessels have become associated with the intralobular duct system and suggest the beginning of functional activity. The intralobular ducts retain their central position as development continues, and by the 4th postnatal week appear to be structurally mature, and consist of light cells, dark cells, and basal cells. The tall light cells show continued development of basolateral infoldings and an increase in the number of mitochondria. The same three cell types are seen in the interlobular ducts, but the light cells lack the basolateral infoldings (Leeson et al. 1978). Proacinar cells still are characterized by their basal concentrations of granular endoplasmic reticulum and numerous dense granules in the apical cytoplasm (Fig. 6). Intercellular canaliculi continue to be observed.

Expansion of the ductal system continues throughout the second phase of development of the opossum submandibular glands (Figs. 7–9), but the most extensive changes occur within the secretory end-pieces. Prior to 75 days postnatum, the secretory units are acinar in form and the component cells show the cytological features of proacinar cells. After 75 days, the secretory units elongate to form secretory tubules consisting primarily of mucous cells, and this process of tubule elongation continues even after weaning. The cells that form the expanding secretory tubules differ from those that make up the earlier endings, in that the secretory granules now are larger and are more densely packed within the supranuclear cytoplasm. The process of differentiation is subtle, and after weaning the cells appear mature and generally show the characteristics of mucous cells. In addition to the large, closely packed secretory granules, several Golgi complexes occur within the perinuclear cytoplasm and numerous profiles of granular endoplasmic reticulum occupy the basal cytoplasm (Fig. 10). Intercellular canaliculi are absent between the cells of the mucous tubules.

It is not until the animals are free-roving juveniles that the two adult types of secretory cell are encountered in the secretory tubules of the opossum submandibular gland (Wilborn and Shackleford 1969; Leeson et al. 1978). At this time, special serous cells are found at the ends of the mucous tubules in the form of demilunes and are characterized by two types of secretory granule, one of which appears electron dense, while the other is electron lucent. Nuclei often are electron dense and irregular in outline (Fig. 11). Profiles of granular endoplasmic reticulum are scattered throughout the cytoplasm, as are small Golgi complexes, and intercellular canaliculi are present between adjacent

◀ **Fig. 5** (*above*). Proacinar cells (*P*) of the newborn submandibular gland are characterized by large nuclei with distinct nucleoli and an electron-dense cytoplasm. A differentiating myoepithelial cell (*M*) which lies within the basal lamina also is shown. (Leeson et al. 1978) ×8000

Fig. 6 (*below*). Nuclei from proacinar cells of a 32-day-old opossum are basally positioned and surrounded by profiles of granular endoplasmic reticulum. The cytoplasm is further characterized by well-developed Golgi complexes (*G*) and electron-dense secretory granules. Processes of myoepithelial cells (*arrows*) are shown at the *extreme right*. Note the numerous small particles (virus-like particles) in the lumen of the developing tubule shown at the *extreme left*. ×7000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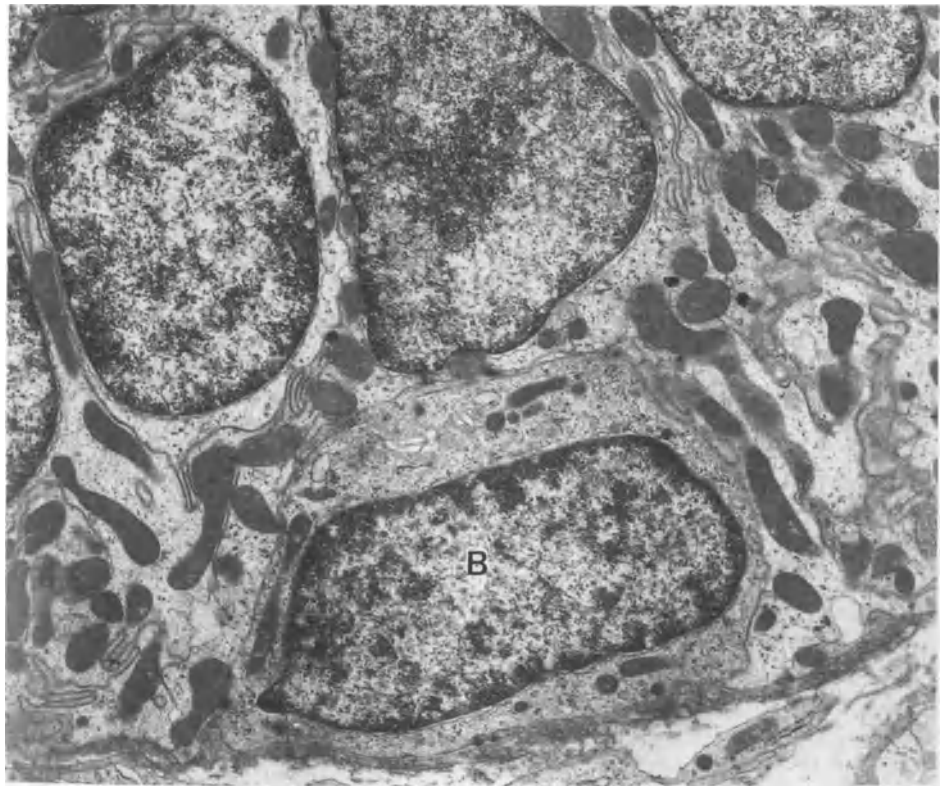
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Table 3. Changes in ductal cells, mucous cells, special serous cells, and mitotic activity during development of the opossum submandibular gland (Lesson et al. 1978)

Body length (cm)	Ductal cells (no. per HPF)	Mucous cells (no. per HPF)	Special serous cells (no. per HPF)	Mitotic activity (no./1000 cells)	
				Ductal	Secreting
1.5	145	12	0	22.1	20.6
2.0	167	19	0	8.3	10.5
2.5	208	30	0	5.2	4.3
3.5	252	43	0	4.0	3.7
4.0	256	44	0	5.2	2.1
4.5	253	44	0	6.1	2.1
5.5	250	70	0	5.1	1.8
6.0	251	62	0	4.3	3.7
7.0	245	63	0	2.0	3.3
8.0	243	58	0	1.2	1.1
10.0	244	63	0	3.8	3.2
11.0	240	60	0	1.1	4.1
13.0	252	92	0	1.0	2.5
16.0	247	103	0	0.85	1.2
22.0	223	54	0	0.6	0.8
26.0	210	51	6	0	1.0
34.0	200	50	38	0	0.3
41.0	181	51	52	0	0

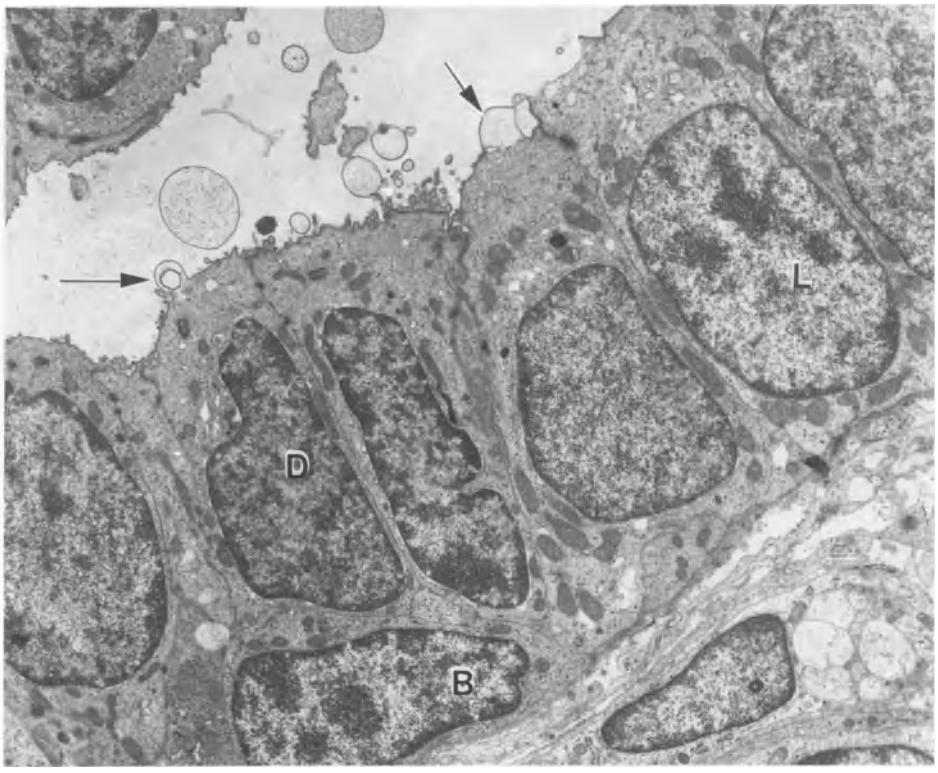
HPF, number of cells counted per high power field.

special serous cells. Proacinar cells, which are present throughout the postnatal period, are no longer observed. Thus, the secretory end-pieces, initially acinar in form, are lined by proacinar cells. As the secretory end-pieces elongate to form a system of branching tubules, mucous cells differentiate in the main body of the tubules. The remaining proacinar cells in the bulbous endings of the tubules eventually are replaced by special serous cells. It is thought that both the mucous and the special serous cells arise from proacinar cells comprising the original secretory units rather than from ductal elements. Mucous cells differentiate prior to the appearance of special serous cells.

Quantitative observations generally support the descriptive, morphological observations made on the opossum submandibular glands. Mitotic activity early in the postnatal period is high, both in the ductal and in the proacinar region (Table 3). The secretory and ductal cells increase in numbers from birth through the end of the 4th postnatal week, with secretory cells increasing more

◀ **Fig. 7** (*above*). An intercalated duct from a submandibular gland of a 32-day-old opossum. Occasional secretory granules (*arrows*) and prominent Golgi complexes (*G*) are seen within the apical cytoplasm. ×6000

Fig. 8 (*below*). The basal region of an intralobular duct from the submandibular gland of a 32-day-old opossum. Note the numerous infoldings of the basolateral plasmalemma and the associated mitochondria. A basal cell (*B*), which is closely opposed to the inner aspect of the basal lamina, is also observed. ×6000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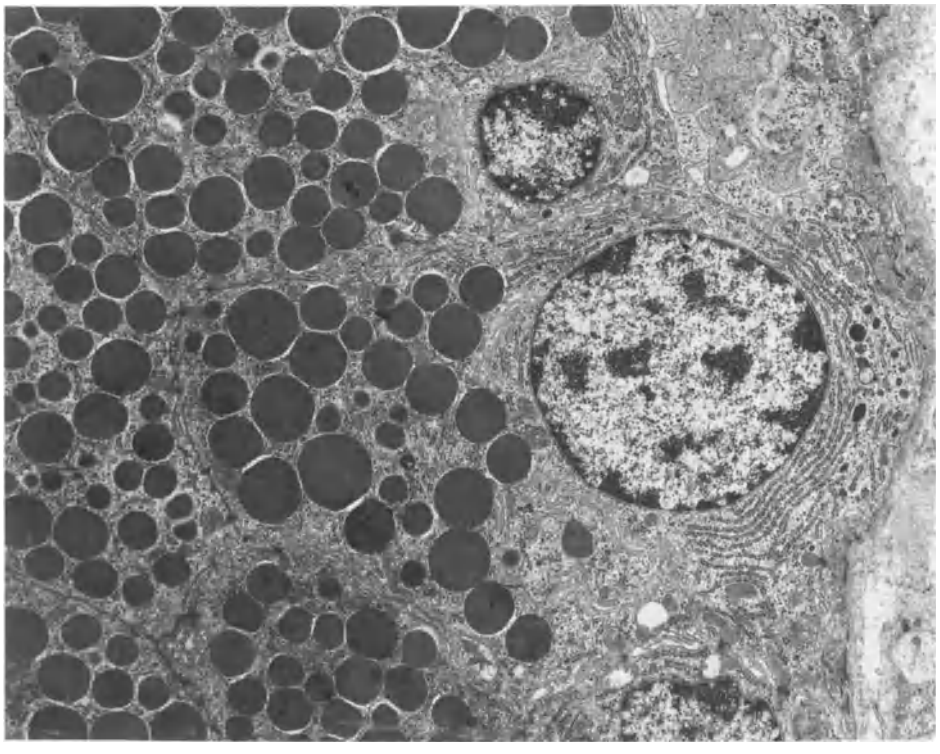


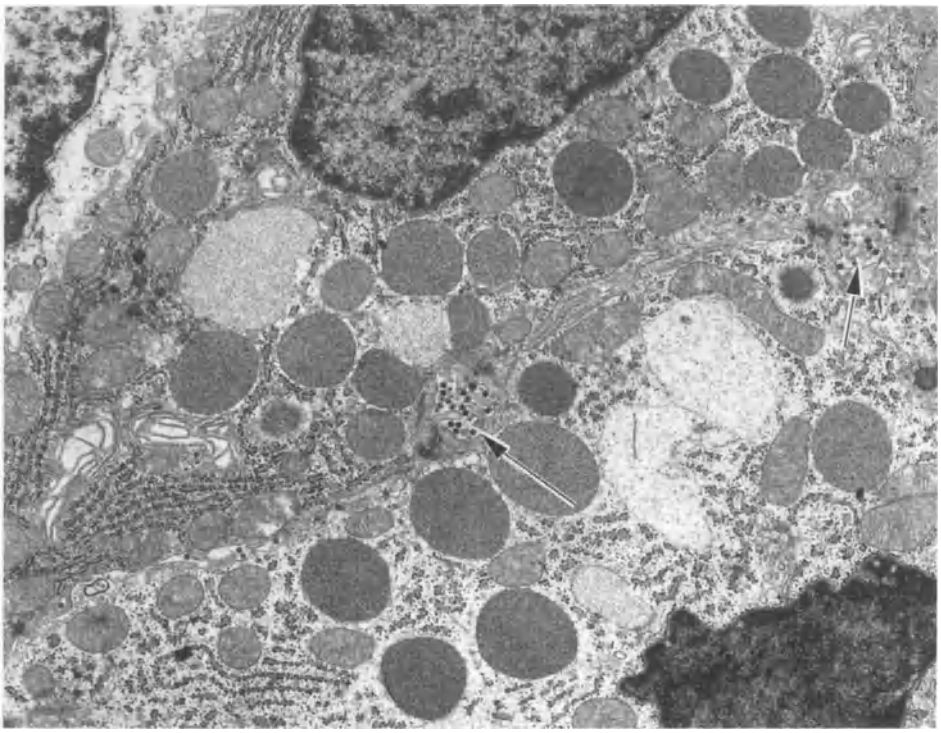


Fig. 11. Portions of two special serous cells taken from the submandibular gland of a juvenile male opossum. Secretory granules appear either electron dense or electron lucent. Numerous profiles of granular endoplasmic reticulum are seen within the surrounding cytoplasm. Note the virus-like particles within the intercellular canaliculi (*arrows*). (Leeson et al. 1978) ×7000

rapidly than ductal cells. Although mitotic activity declines, it remains fairly high in the ductal cells between the 18th and 32nd days postnatum, and it is during this period of development that the intralobular ducts show extensive cytodifferentiation. The numbers of ductal and secretory cells are maintained at reasonably constant levels for the succeeding 40 days, after which the ductal cells continue to increase in number until after weaning: they then progressively decline to stable values (Leeson et al. 1978). The increase in the number of ductal cells correlates with a second peak of mitotic activity in the intralobular ducts at 68 days postnatum. Mitotic activity associated with proacinar cells remains relatively high until 60 days postnatum, corresponding

◀ **Fig. 9** (*above*). A segment of an interlobular duct taken from the submandibular gland of a 32-day-old opossum. Tall light (*L*) and dark (*D*) cells as well as a basal cell (*B*) are shown. Note the apical blebbing (*arrows*) seen on some cells. ×6000

Fig. 10 (*below*). Cells comprising a portion of a secretory tubule from a submandibular gland of a 105-day-old opossum are filled with electron-dense secretory granules. Numerous profiles of granular endoplasmic reticulum and small Golgi complexes occupy the perinuclear cytoplasm. ×6000

to the time immediately prior to elongation of end-pieces and formation of secretory tubules.

The secretory units continue to elongate and branch until after weaning and consist primarily of mucous cells. Only after weaning has taken place are the special serous cells seen, forming caps at the ends of the mucous tubules (Leeson et al. 1978). Special serous cells are characterized ultrastructurally by low concentrations of granular endoplasmic reticulum and by secretory granules that, although similar in appearance to mucous granules, do not stain like mucous granules following histochemical staining. Special serous cells contain only small amounts of mucosubstance which may be neutral but never acidic (Pinkstaff 1980). Proacinar cells that contain heterogeneous secretory granules are present at the 105th day and may represent an intermediate form in the process of differentiation into the special serous cell type. Special serous cells develop very late in the submandibular glands of the opossum and apparently arise from proacinar cells. Once special serous cells have differentiated, proacinar cells are no longer seen. The function of the special serous cell type is unknown.

The sequence of changes that occurs during the postnatal development of the opossum submandibular gland is not unique and is similar to that of the porcine parotid gland (Koppang and Getty 1970). In this species, maturation of the ducts also precedes differentiation of acinar cells. In contrast, maturation of acini preceding that of the ducts has been reported in the submandibular glands of the rat and mouse (Gabe 1950; Jacoby 1959; Leeson and Jacoby 1959).

Except in newborn animals, numerous inclusions are consistently observed in the lumina of the mucous tubules and intralobular ducts of the submandibular glands of all postnatal stages examined (Krause et al. 1978c). These particles are abundant and often appear to form into irregular aggregates (Figs. 6, 11). Similar particles are not seen in the cytoplasm or in the nuclei of the component cells of the ducts and tubules. The spherical particles are fairly uniform in size, show a central electron-dense core, and are limited by a distinct membrane. The particles range from 100 to 150 nm in diameter, while the central cores show diameters ranging from 50 to 70 nm. The size, location, and morphology of these particles are quite distinct from adjacent secretory granules found within the cells that make up the mucous tubules. The morphology of the particles is similar to that reported for a number of viruses (Dalton and Haguenou 1973). Unlike most viruses described to date, however, the particles seen in the submandibular gland of *Didelphis* are not present in the cytoplasm or nucleus of the component cells (Krause et al. 1978c). It also is possible that these small inclusions represent an additional type of secretion by the secretory cells composing the opossum submandibular gland. Growth factors such as neural growth factors and epidermal growth factor, found in high concentrations in the submandibular gland of other species (Liebow 1989), have not been demonstrated to date in *Didelphis*.

2.2.3 Development of Dentition

Development of teeth in *Didelphis* occurs primarily during the postnatal period and, while generally similar to that of other mammalian species, it does differ in several respects. As in other mammals, the teeth take origin from a dental lamina, a band-like epithelial structure that lies over the opposing surfaces of the upper and lower jaws. This epithelial band gives rise to outgrowths that penetrate the underlying mesenchyme and eventually give rise to the ectodermal component of teeth. The under-surface of this epithelial downgrowth invaginates to form the cap stage of tooth development. Deepening of the invagination results in the formation of the bell stage, and it is at this time that cells in the mesenchyme adjacent to the inner dental layer differentiate into odontoblasts and begin producing dentin. Simultaneously, the epithelial cells of the outer dental layer differentiate into ameloblasts and lay down enamel in the form of elongated prisms. The enamel of most marsupials, including *Didelphis*, is unique in that it contains enamel tubules that course between and around enamel prisms and interprismatic regions (Lester 1970; Boyde and Lester 1967; Osborn 1974). The enamel tubules of *Didelphis* are cytoplasmic processes within the enamel and are thought to represent spindle-shaped cytoplasmic extensions of the odontoblasts into the enamel (Lester 1970; Osborn 1974; Risnes and Fosse 1974). The enamel tubules are continuous at the dentinoenamel junction with odontoblast tubules. Thus, developing marsupial enamel is analogous to bone, dentin, and cementum in that it contains the processes of cells (Lester 1970).

Although studies on early tooth development are few, several interesting observations have been made. The most detailed studies have been carried out in pouch-young animals that measured from 11 to 49 mm in crown-rump length (Berkovitz 1967, 1978). Unlike most eutherians, tooth replacement in *Didelphis* involves only one tooth, a deciduous molar which is replaced by the last tooth of the premolar series (Berkovitz 1978). All teeth of *Didelphis* develop initially from the free edge of the dental lamina, and the invaginations of the dental lamina are so slight that the early tooth buds give the impression of arising directly from the oral epithelium. As development continues, an undifferentiated epithelial downgrowth occurs on the lingual side of each enamel organ, but these downgrowths do not differentiate into tooth germs and therefore do not result in the formation of permanent teeth as occurs in eutherian species (Berkovitz 1978). The lone exception is the third premolar (Berkovitz 1967).

At 25 days postnatum, all five incisor tooth germs are present in the maxilla. The second, third, and fourth incisors all develop at about the same time, whereas the first and fifth incisors appear slightly later (Berkovitz 1978) and are in the bud stage of development at 25 days (at which time the second, third, and fourth incisors are in the bell stage) (Berkovitz 1967). This order of development differs from that reported for the majority of eutherians, in which the order of incisor development is from anterior to posterior. The mandibular incisors of *Didelphis* do develop in order from anterior to posterior as for most eutherian species, and by 25 days postnatum all are in the cap stage of development (Berkovitz 1967). The canines are in the bell stage of develop-

ment at this time, with enamel and dentin both formed at the apex. The second premolar of *Didelphis* is the first premolar to appear; the replacing third premolar develops from the dental lamina that lies between the second premolar and the deciduous molar (Berkovitz 1978). At 25 days postnatum, the first premolar is in the cap stage of development and the second premolar is in the bell stage, with enamel and dentin being present. Premolar teeth of eutherian mammals develop in sequence from posterior to anterior. The maxillary canine and second premolar teeth of *Didelphis* are slightly more advanced in their development than are the corresponding mandibular teeth.

The order of development of the first four cusps in the lower first permanent molar is similar to that seen in other marsupials (Berkovitz 1978) and eutherian species (Butler 1956). In *Didelphis* the metacone is the largest and first molar cusp to calcify in the upper deciduous and first permanent molar, in contrast to the order of development and calcification of other marsupial (Berkovitz 1967, 1978) and eutherian forms (Butler 1956). The first cusp to calcify in the mandibular molar teeth is the protoconid.

Although the general conformation of the underlying teeth can be seen under a rather transparent gingival covering for some time prior to weaning, tooth eruption through the epithelial surface does not begin until about postnatal day 75 (Krause and Cutts 1980a). Weaning begins around the 85th postnatal day (Cutts et al. 1978b) but is not completed for several days. Tooth eruption begins when a small circular crater forms in the gingival epithelium that covers the crown of the tooth prior to its actual emergence. As the tooth emerges into the oral cavity, the crater expands in diameter to accommodate the tooth. Canines, some premolars, and molars appear to erupt at about the same time (~75 days), and the overlying and surrounding gingival surfaces show similar morphological features without regard to the type of underlying tooth. Observations by scanning electron microscopy suggest that the crown of the tooth does not actively push through and rupture an intact gingival surface, but rather the overlying gingival surface degenerates first and the tip of the crown emerges into the formed defect (Krause and Cutts 1980a). Crater formation appears to be a controlled phenomenon and follows a definite pattern, producing an opening that tightly surrounds the emerging tooth. This cratering of the gingiva may be the result of enzymes released by the dental and/or oral epithelium (Weinmann 1966) or may represent small focal areas of pressure necrosis.

2.3 Adult Teeth

The dental formula for *Didelphis* is incisors 5/4, canines 1/1, premolars 3/3, molars 4/4 × 2 and totals 50 teeth, all of which are rooted. The maxillary incisors are conical, small, and unequal in size; the first incisor is larger than the others and is separated from them. The mandible contains one less incisor than does the maxilla. The canines are large and conical, and the molars are tricuspidate. The last premolar is preceded by a multicuspidate, molariform deciduous tooth. The arrangement of three premolars and four molars is the reverse of that in typical eutherian mammals, which generally have four premolars and three molars.

2.4 Adult Tongue

The tongue of the adult opossum is elongate and ovoid in shape, tapering to a rounded tip. The thin free portion of the tongue thickens posteriorly and the mucosa of the lateral posterior border forms narrow, elongated ridges that have a serrated appearance. The dorsum of the tongue shows five types of papillae: filiform, fungiform, conical, compound filiform, and vallate (Krause and Cutts 1982). The simple filiform and fungiform papillae are typically mammalian in structure, and the latter may or may not be associated with taste buds. The fungiform papillae vary in number and usually are of small size, as in most polyprotodont marsupials (Sonntag 1924). *Didelphis*, like all diprotodont and most polyprotodont marsupials, shows a triangular arrangement of three vallate papillae on the dorsal surface of the posterior aspect of the tongue (Sonntag 1924; Krause and Cutts 1982). The apex of the triangle is pointed posteriorly and the posterior-most papilla is the largest. The mucosal surface around the vallate papillae is smooth and consists of a thick, keratinized, stratified squamous epithelium. The interior of each vallate papilla is filled by a large core of lamina propria that lacks glands. The epithelium covering the vallate papillae thins near the base and contains the majority of the taste buds associated with the opossum tongue. Ducts from adjacent serous glands open into the cleft that surrounds each papilla. The serous glands are extensive in their development and lie between fascicles of the striated muscle that makes up the body of the tongue. Numerous ganglia also are present in the surrounding connective tissue.

Compound filiform papillae are abundant immediately anterior to the region of the vallate papillae. They resemble coronets and show a fringe of processes that extend from the circumference of their apical surfaces (Fig. 12). The epithelium that covers these papillae shows extensive keratinization, especially of the projections at the margins of the papillae (Krause and Cutts 1982). The compound type of filiform papilla is characteristic of the tongues of most marsupials, including *Didelphis*. Its function is unknown.

The conical papillae are large with blunt free ends and are found only in an oval patch on the anterior portion of the tongue. In *Didelphis* these types of papilla are heavily keratinized and are separated from one another by simple, and occasionally compound, filiform papillae. The only other marsupials reported to date that show a similar patch of large conical papillae are two other *didelphids*: *D. marsupialis* and *D. azarea*. Their function in the oral cavity also is unknown.

Typical foliate papillae are absent in *Didelphis* and the posterolateral borders where they normally occur in other species show, instead, an elongated ridge with numerous, irregular finger-like projections. The covering stratified squamous epithelium is less keratinized than elsewhere on the dorsum of the tongue and is devoid of taste buds. The lamina propria which forms the cores of these structures contains numerous capillaries (Krause and Cutts 1982). Foliate papillae also are absent in most polyprotodont marsupials but have been reported in some diprotodont marsupials (Sonntag 1924).

Behaviorly, opossums appear to show little discrimination in taste unless the food has a particularly strong flavor; the animals do respond to very bitter

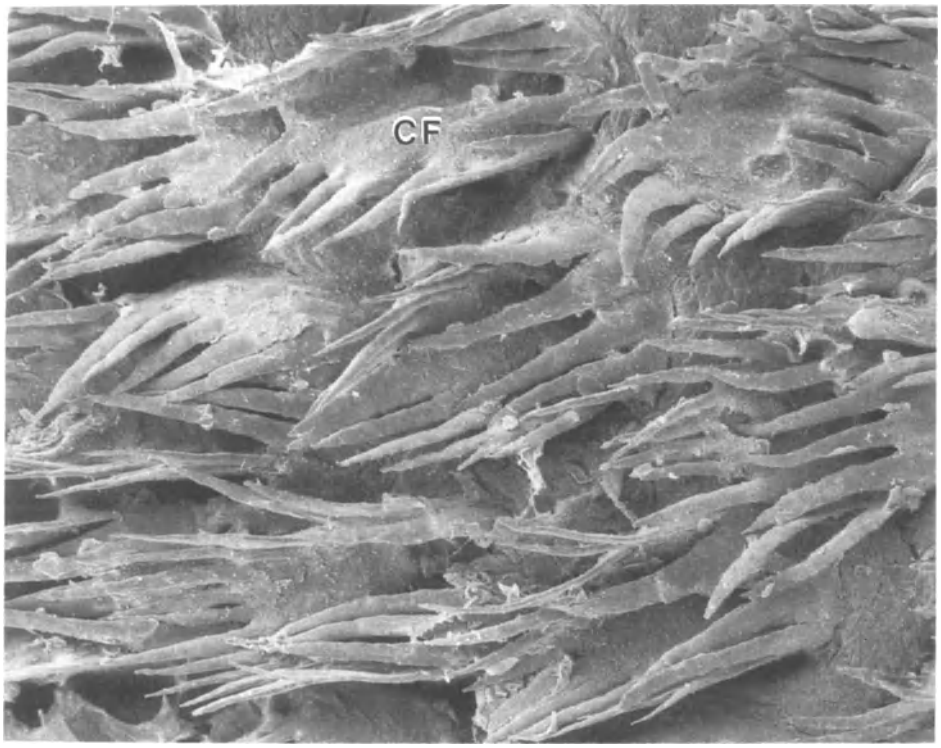


Fig. 12. Compound filiform papillae (*CF*) from an adult female opossum. Note the fringe of keratinized projections around the apex of each papilla. ×100

or highly sweetened foods. The posterior of the tongue is most sensitive to bitter taste, and the anterior half of the tongue to sour stimuli. The taste responses of the opossum are reported to be more similar to those of carnivores than to those of rodents (Pressman and Doolittle 1966; Tamar 1961). *Didelphis* is considered to be one of the most omnivorous mammals known, and while it will eat almost anything, the animal does show a preference for insects and meat when these are available. The North American opossum is an opportunistic eater and its diet varies considerably, consisting of whatever food is available in the area and during the season of the year.

2.5 Jaws and Associated Musculature

The opossum for the most part is omnivorous and often feeds on carrion, particularly small mammals (rabbits and rodents). *Didelphis* is capable of exerting tremendous pressure with its jaws and has little difficulty in crushing bones, fruit pits, and other hard materials. The ability to crush bone and consume this material is important to the homeostasis of this species because of its exceptional need for calcium. Blood calcium levels of the opossum are high (15 mg/dl) as compared to most common laboratory animals (6.5 mg/dl).

The primary muscles of mastication are the masseter and temporalis muscles, which contain a predominance (98%) of type II skeletal muscle fibers (Hansen et al. 1987). Type II fibers correspond to fast contracting fibers while type I muscle fibers correspond to slow contracting fibers (Dubowitz and Brooke 1973; Burke et al. 1971, 1973). The snapping of the opossum jaw is characterized by an initial high rate of jaw closure (Thexton and Hiiemae 1975, 1977), and type II fibers are adapted for strong, fast contractions. *Didelphis* has a four-stage chewing cycle which in general is similar to that seen in other mammals (Crompton and Hiiemae 1970; Crompton et al. 1975, 1977). Movements of the chewing cycle are produced by cranial and lower jaw movements and can be subdivided into: (1) fast closing, (2) powerstroke, (3) slow opening, and (4) fast opening movements (Hiiemae and Jenkins 1969; Hiiemae and Crompton 1971; Hiiemae and Thexton 1974). Tongue movement serves to move food within the oral cavity and to direct it toward the teeth during chewing (Hiiemae and Crompton 1971).

2.6 Adult Salivary Glands

The parotid glands are of moderate size and appear as thin, lobulated structures that curve around the inferior margins of the external auditory meatus. A prominent excretory duct drains each gland and crosses the masseter to open into the superior vestibular region of the mouth. The parotid of *Didelphis* presents the histological profile of a purely serous gland (Shackleford and Wilborn 1968). The secretory units are very closely packed and the ductal system consists of intralobular and interlobular ducts (Quintarelli and Dellovo 1969). The cells that make up the secretory units stain with the periodic acid-Schiff method but fail to stain by alcian blue staining methods, suggesting that neutral mucins are present (Quintarelli and Dellovo 1969). However, sialic acids have been localized in the opossum parotid in biochemical assays (Junqueira and Fava-de-Moraes 1965).

The greater sublingual gland of the opossum is a mixed, compound, tubuloacinar gland in which the majority of the secretory units are mucous tubules (Junqueira and Fava-de-Moraes 1965; Pinkstaff 1975). Demilunes or cap cells often are associated with the ends of the mucous tubules and are serous in nature (Shackleford and Wilborn 1968); the serous cells on occasion also form acini. The serous demilune cells and the serous cells that form acini contain sulfomucins (Pinkstaff 1975, 1980). The cells of the mucous tubules contain either sulfomucins or nonsulfated acidic mucosubstances, and cells containing either may exist side by side in the same mucous tubule (Pinkstaff 1975). The ductal system is simple and consists primarily of interlobular ducts that have the morphological appearance of typical excretory ducts. Intralobular ducts are infrequent and lack basal striations. Intercalated ducts are not observed (Pinkstaff 1975).

The submandibular gland of *Didelphis* has been studied more extensively than have the other salivary glands of this species (Junqueira and Fava-de-Moraes 1965; Quintarelli and Dellovo 1969; Shackleford and Wilborn 1968; Wilborn and Shackleford 1969). It is a mixed, compound, branched tubular

gland surrounded by a capsule of rather dense connective tissue. The secretory units consist of elongated, branched tubules formed by mucous secretory cells, with demilunes of special serous cells at the ends of the tubules. The cells that make up the mucous tubules are characterized by a high concentration of neutral mucosubstances (Pinkstaff 1975). Ultrastructurally, special serous cells lack well-developed granular endoplasmic reticulum, suggesting that the cells have only a minor role in the production of protein substances, and the function of the special serous cell is unknown. Myoepithelial cells of the submandibular gland are associated with intercalated ducts and secretory units. Their concentration is greater in the opossum than in rat or human submandibular glands (Wilborn and Shackleford 1969).

One of the most distinctive features of the opossum submandibular gland is the concentration of intralobular ducts in the center of each lobule. This arrangement is present in the early postnatal stages, and by the end of the 4th postnatal week most ducts appear structurally mature. The intercalated ducts are short segments in the opossum submandibular gland that connect the secretory units to the distinctive intralobular duct system. Component cells of the intercalated ducts show morphological features that indicate little secretory activity (Leeson et al. 1978). The striated intralobular ducts constitute nearly 30% of the opossum submandibular gland and in this regard are similar to those described in two Australian marsupials, *Trichosurus vulpecula* and *Perameles nasuta*. This percentage is nearly four times that reported for some species of edentates, lagomorphs, carnivores, artiodactyls, and man (Young and van Lennep 1978).

A tubular segment consisting of granule-filled secretory epithelial cells lies between the short intercalated duct proper and the cells of the secretory unit. Although the term "granular intercalated duct" has been proposed for this segment (Shackleford and Wilborn 1968), the cells that line it (as pointed out by Young and van Lennep 1978) in no way differ from typical protein-secreting cells and should be considered as part of the secretory end-piece. Furthermore, the classical granular convoluted (striated) ducts of rodents develop first as typical striated ducts before becoming protein-secreting cells (Young and van Lennep 1978). Like the intralobular ducts, interlobular ducts differentiate early in the opossum submandibular gland and appear structurally mature by the end of the 4th postnatal week. They never assume the prominence of the intralobular ducts.

Opossum saliva collected after administration of pilocarpine is hypotonic to serum and contains 9.3 mEq potassium/liter and 7.3 mEq sodium/liter, with a potassium/sodium ratio of 8:7. In addition to moistening and initiating the digestion of food, saliva is spread over the feet, tail, and legs to help regulate body temperature through evaporation of saliva from body surfaces (Higginbotham and Koon 1955).

Secretions of the opossum submandibular glands may be involved in other important functions also. In several eutherian species the submandibular glands secrete a polypeptide called epidermal growth factor (EGF), which is a powerful mitogen and has the capacity to protect the gastric mucosa from damage by a variety of irritants (Konturek et al. 1988). Removal of the submandibular glands delays healing of induced gastric ulceration and has a

hypoplastic effect on the gastric mucosa. EGF, released into the gut lumen by the salivary glands, is thought to serve as a natural growth-promoting factor for the gastrointestinal mucosa (Dembiński et al. 1982).

To date, the literature contains no information concerning the localization and/or synthesis of factors such as neural growth factors (NGF), EGF, renin, or other products that are thought to be secreted by the salivary glands and to be involved in regulating the development and function of the digestive system. EGF and NGF are found in high concentrations in the submandibular glands of other species (Murphy et al. 1979, 1980, 1981; Steidler and Houghton 1980). EGF is thought to stimulate the gastrointestinal mucosa and tissues within the oral cavity (Liebow 1989). NGF from the submandibular glands also is thought to have a role in stimulating and directing development of the innervation of the gastrointestinal tract (Levi-Montalcini 1987; Liebow 1989). The precocious development and large size of the submandibular glands in *Didelphis* strongly suggest that these glands may play an important role in influencing not only structures developing within the oral cavity but also those developing in the remainder of the digestive system.

3 Esophagus

3.1 Prenatal Development

The endoderm consists of a single layer of cuboidal cells that lines the flattened interior surface of the developing opossum embryo until about the middle of the 9th day of gestation (Krause and Cutts 1985b). Just after the formation of the branchial pouches and pharyngeal membranes at the end of the 9th day of gestation, the first trace of the foregut appears, beginning as a minute diverticulum in the floor of the pharynx. The lungs also develop as endodermal outgrowths from this newly formed, endodermal-lined tube, and early in day 10, the esophagus is established as an independent entity (McCrady 1938).

3.2 Postnatal Development

In the newborn opossum, the esophagus is characterized by a lumen of considerable diameter with a lining epithelium 2–3 cells thick that bears numerous invaginations extending from its surface. A muscularis mucosae is absent and the muscularis externa consists only of a few, loosely knit myoblasts (Krause et al. 1976a). A delicate, scant connective tissue separates these two layers.

Ultrastructurally, the lining epithelium generally is stratified in type and consists of two layers, but regions that have a pseudostratified appearance also are present (Fig. 13). Stubby microvilli covered by a thin glycocalyx characterize the plasmalemma of the surface cells, which have a cuboidal to columnar shape. The apices of the adjacent surface cells are united by tight (occluding) junctions; scattered desmosomes are present between the lateral surfaces of adjacent cells, the membranes of which show considerable implication. Cells in the basal stratum rest on a delicate basal lamina, and the basal plasmalemma shows scattered hemidesmosomes. The cytoplasm of the cells in both strata contains numerous free ribosomes, scattered profiles of granular endoplasmic reticulum, collections of intermediate filaments, scattered mitochondria, and occasional Golgi complexes. Numerous small vesicles are present in the apical cytoplasm of cells that border the lumen of the esophagus. Nuclei show an abundance of euchromatin and scattered clumps of heterochromatin that occur most frequently near the nuclear envelope. One or more nucleoli are present.

At the end of the 1st postnatal week, the lumen of the esophagus is less patent than in the newborn, and the lining epithelium is organized into two distinct strata (Fig. 14). The cells of the surface layer are columnar in shape

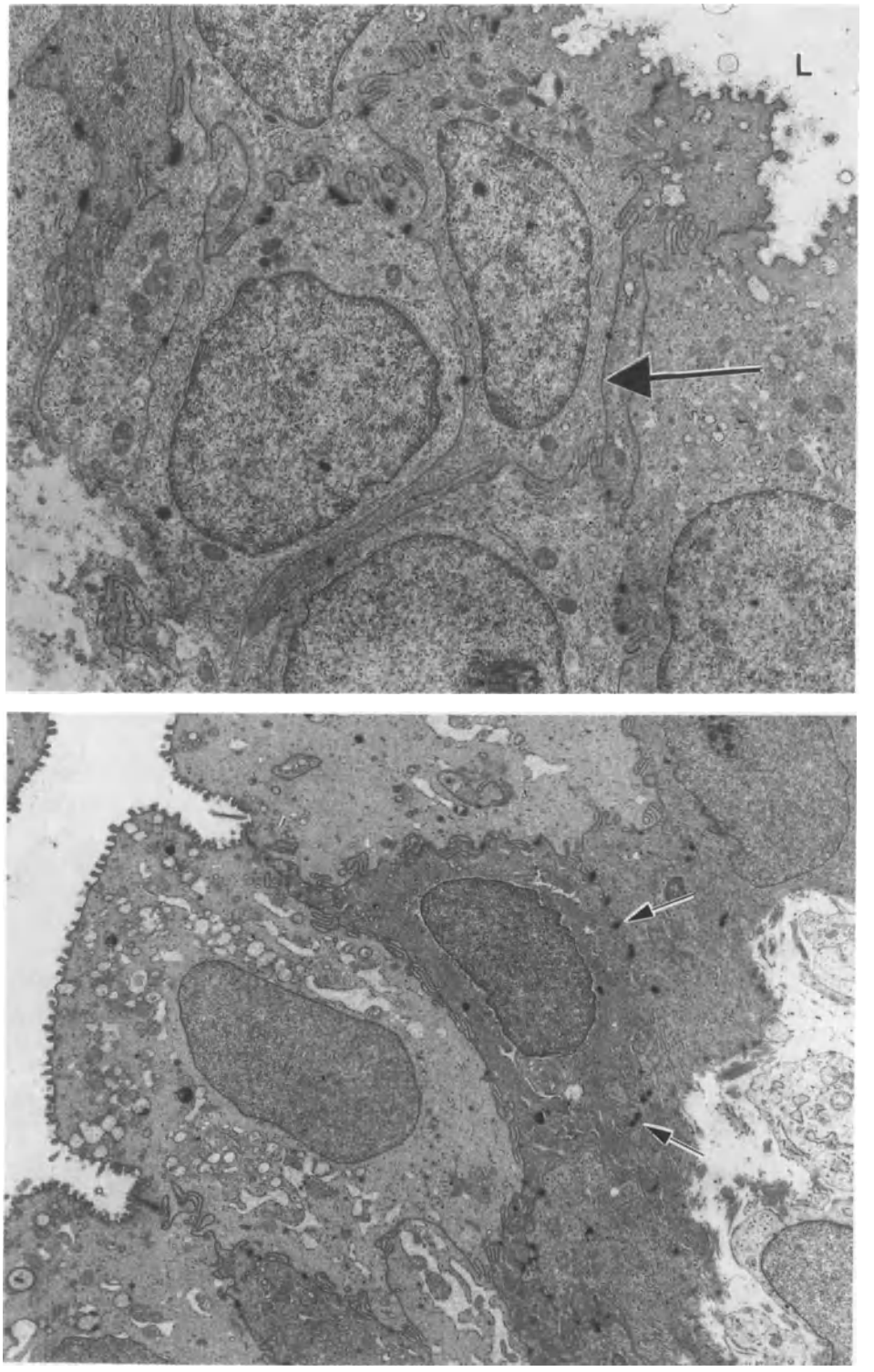
with spherical or oval nuclei; the cell apices continue to be united by occluding junctions. The hyaloplasm is more electron lucent and homogeneous and contains fewer organelles when compared to that of the newborn. Numerous small vesicles continue to characterize the apical cytoplasm of the surface cells, which remain in close association and show scattered desmosomes and interdigitations of adjacent cell membranes. Surface cells of similar appearance have been described lining the esophagus of the 6-mm pig (Flint 1907).

The basal stratum consists of a single layer of smaller, darker-staining, irregularly shaped cells with central nuclei. Nucleoli are common. The basal cell membrane is relatively smooth, lies on a delicate basal lamina, and shows scattered hemidesmosomes. Scattered desmosomes are found between the cell membranes of adjacent cells. The cytoplasm contains numerous free ribosomes and scattered profiles of most other organelles.

Ciliated cells and occasional goblet cells, typical of those seen in the respiratory tract, are scattered within the esophageal epithelium (Krause et al. 1976a) and perhaps reflect a common origin from endoderm at the same site in both systems. Ciliated cells also are present in the developing esophagus of several avian species (Edgar 1947; Ivey and Edgar 1952; Hinsch 1967) and some mammals (Sevcenko et al. 1972; Sevcenko and Vacek 1973), including man (Johnson 1910; Johns 1952). The esophageal wall shows an increased cellularity of the connective tissue as compared to the newborn, and the layers of the muscularis externa are more clearly defined by the end of the 1st postnatal week. The establishment of the muscularis externa and the associated increase in muscle tone is thought to be responsible for the decrease in luminal diameter of the esophagus that is seen at this time.

As development continues, the esophageal epithelium progressively increases in depth but continues to maintain the two strata established during the 1st postnatal week. The basal stratum remains a single layer of smaller cells that rest on the underlying basal lamina, but the surface stratum now consists of a layer, two or three cells deep, of large, light-staining cells. These cells have become more fusiform in shape and their cytoplasm shows an increase in the number of intermediate (keratin) filaments. The surface cells retain their stubby microvilli, and the adjacent cell membranes show an increase in the degree of lateral infolding and implication. Desmosomes also increase in number. Despite an increase in thickness of the esophageal epithelium, goblet and ciliated cells still are present. Mesenchymal cells within the underlying connective tissue appear to be organized into a discrete layer by the end of the 2nd postnatal week. These cells differentiate into the smooth muscle cells of the muscularis mucosae by the end of the 3rd postnatal week (Krause et al. 1976a).

Cells in the surface strata of the esophageal epithelium have undergone marked changes by the end of the 3rd week of postnatal life. The surface cells now are flattened and electron dense, and their nuclei are oriented parallel to the luminal surface (Fig. 15). The nuclei of cells that underlie the surface layer tend to show a similar orientation. At this stage of development and in older animals, the esophageal epithelium shows three basic strata: a germinal layer consisting of a single layer of cells, a spinosal layer, and a layer of flattened cells that retain their nuclei. Cells that line the esophageal lumen continue to


L

show short, stubby microvilli covered by a thin glycocalyx. Ciliated cells and goblet cells still are seen between the surface squamous cells of the esophageal epithelium in 21-day-old animals (Fig. 16), but usually are not present in the esophagus after that time. However, unlike in other mammalian species, ciliated cells may persist in the esophageal epithelium of much older animals, though they are restricted to the epithelium that lies between the large, permanent transverse folds that characterize the distal 1–5 cm of the esophagus in *Didelphis* (Krause et al. 1976a; Marklin et al. 1979). The epithelium between these permanent folds consists of two thin strata and retains the immature characteristics of much younger animals. Even in the adult, the epithelium of this region appears morphologically very similar to that seen in 14-day-old animals (Krause et al. 1976a).

In *Didelphis*, the esophageal epithelium of suckling animals and the adult does not undergo complete keratinization, and cells that line the lumen retain their nuclei. The degree of cornification is thought to be related primarily to the diet of a species (Goetsch 1910), and animals such as rodents and ruminants that swallow coarse foods show extensive cornification. In species such as carnivores and man that generally consume soft foods, a thick cornified layer is lacking and the surface cells retain their nuclei and appear morphologically similar to those seen in the opossum.

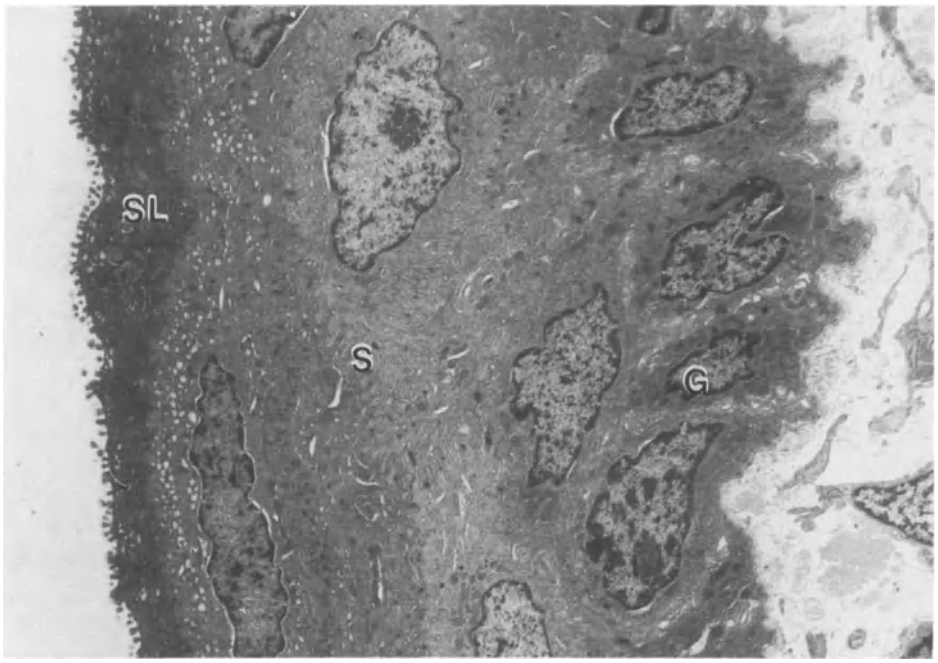
3.2.1 Esophageal Glands

Coincident with the morphological changes of the esophageal epithelium, the esophageal glands arise as solid outgrowths from the basal surface of the epithelium at the end of the 3rd week of postnatal life (Krause et al. 1976a). The glands continue to expand throughout the postnatal period and follow a pattern of development similar to that of other species, including man (Flint 1907; Johnson 1910; Mottet 1970). In mammalian species other than *Didelphis*, the glands begin their development prenatally, rather than after 3 weeks into the postnatal period, as in the opossum.

During the first 60 days of postnatal life, the esophageal glands of the opossum consist primarily of ducts and typical mucous cells. Serous (light) cells begin to differentiate at the ends of the secretory units just prior to weaning (about the 75th postnatal day). The esophageal glands continue to develop and expand after weaning, and in the cervical and thoracic segments they are confined primarily to the submucosa. In contrast, glands that develop in the distal esophagus are confined primarily to the lamina propria. The majority of secretory components consist of mucous cells, whereas serous cells usually

◀ **Fig. 13** (*above*). Scattered regions of esophageal epithelium from the newborn opossum are pseudostratified in appearance, with cells that extend from the esophageal lumen (*L*) to the basal lamina (*arrow*). ×8000

Fig. 14 (*below*). The esophageal epithelium of a 7-day-old opossum consists of two distinct strata: a surface layer of columnar cells and a basal layer of small, irregular, electron-dense cells. Numerous desmosomes (*arrows*) are seen between the basal cells. ×5000



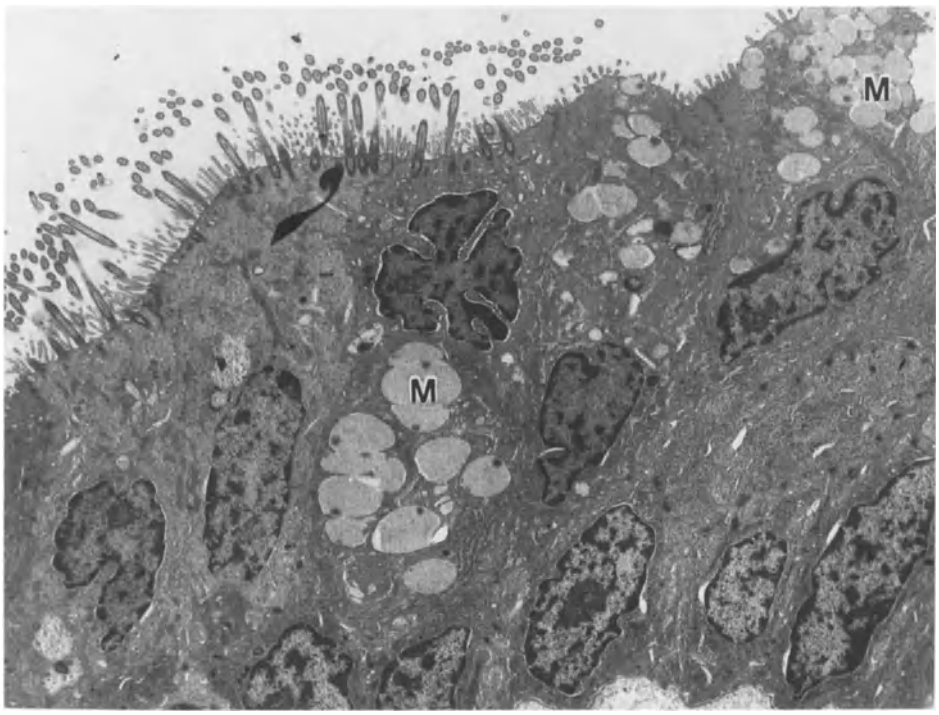


Fig. 15 (*above*). The esophageal epithelium of a 21-day-old opossum consists of three strata: a surface layer (*SL*) of flattened cells oriented parallel to the surface, a spinosal layer (*S*), and a germinal layer (*G*) consisting of a single layer of cells. ×4000

Fig. 16 (*below*). A region of esophageal epithelium from a 21-day-old opossum containing ciliated cells and cells filled with mucin granules (*M*). ×4000

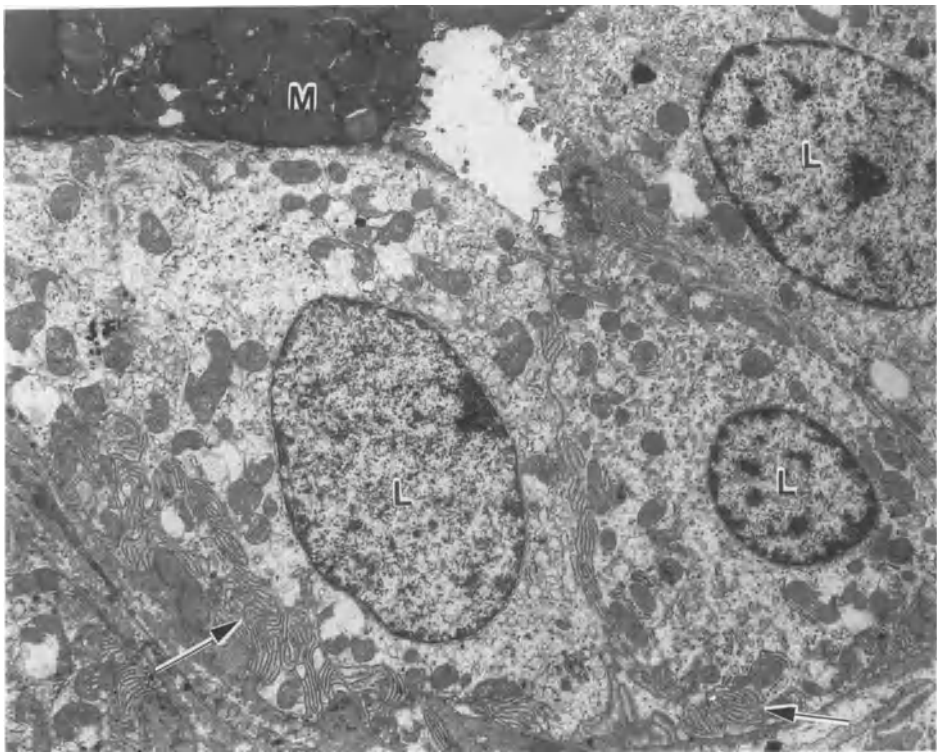


Fig. 17. A region of an esophageal gland showing three light cells (*L*). Note the extensive infoldings (*arrows*) of basolateral cell membranes. A portion of a mucous cell (*M*) is shown at the *extreme upper left*. ×4000

occupy only the terminal portions of the secretory units in a manner similar to the serous demilune cells of the submandibular or sublingual glands (Krause et al. 1976a). Mucous and serous cell types have been observed in the esophageal glands of other mammalian species (Goetsch 1910), and Johns (1952) has described both cell types in man.

Ultrastructurally, the mucous cells of *Didelphis* contain numerous mucin granules that show a peculiar pattern of variable electron densities (Krause et al. 1976a). These membrane-bound secretory granules are intimately associated with adjacent Golgi membranes and fill the apical and supranuclear cytoplasm. The mucous cells show a definite polarity, with their nuclei confined to the basal cytoplasm, where they are surrounded by profiles of granular endoplasmic reticulum. In contrast, the cytoplasm of the serous (light) cells is electron lucent and shows numerous mitochondria, scattered Golgi profiles, and occasionally structures that are believed to be secondary lysosomes. The secretory granules and numerous profiles of granular endoplasmic reticulum that normally are associated with serous cells in the salivary glands are absent in this cell type in the esophagus, and the cells are characterized instead by extensive infoldings of the basolateral cell membranes and the presence of numerous, secretory intercellular canaliculi (Fig. 17). The fine structure of

Table 4. Thickness and mitotic activity of esophageal epithelium during development of the opossum (Krause et al. 1976a)

Body length (cm)	Thickness of epithelium (μm)	Mitotic activity (no. mitotic cells/mm^2)
1.5	16.0	6.4
2.5	19.8	22.4
3.5	24.8	48.0
4.5	30.5	60.4
6.5	64.7	48.8
10.0	70.6	34.3
13.0	96.3	12.8
20.0	124.8	9.6

the light cells of the esophageal glands appears similar to that of the hydrotic cell, described in the opossum bronchial glands (Sorokin 1965; Krause and Leeson 1975), and again may reflect a common origin from a similar region of endoderm. Myoepithelial cells invest both cell types in the secretory units of the opossum esophageal glands (Krause et al. 1976a) and also have been reported in the esophageal glands of cattle (Wakuri and Muto 1972).

3.2.2 Quantitative Data on the Development of Esophageal Epithelium

The depth of the esophageal epithelium progressively increases throughout the postnatal period (Krause et al. 1976a), with the most dramatic increase occurring during the first 75 days of postnatal life (Table 4). The number of mitotic figures in the lining epithelium of the esophagus increases rapidly during the first 3 postnatal weeks to reach a peak at about 21 days. Mitotic activity then declines to reach stable values by the 75th postnatal day (Table 4). The number of mitotic figures within the epithelium is greater than in any other layer of the esophagus, in keeping with a tissue in which there is both an increase in thickness and a need to replace cells exfoliated from its surface (Krause et al. 1976a). The peak of mitotic activity within the esophageal epithelium coincides with the initial appearance of the esophageal glands, which form as basal outgrowths from the epithelium at this time.

The factors or mechanisms involved in the transformation of a stratified columnar epithelium consisting of two layers to the multilayered stratified squamous epithelium seen in older animals and the adult are unknown. The change seems to be unrelated to the consistency of food in the diet, since pouch-young opossums are permanently attached to the same nipple for the first 60 days of postnatal life and the diet is liquid (milk). The dramatic change that occurs in the epithelium takes place midway during this period. The ciliated and goblet cells perhaps represent an expression of stem cells in the endoderm which also give rise to the epithelial lining of the respiratory system. At the time when the morphology of the epithelial lining changes, the esophageal glands begin to develop as outgrowths from this epithelium, an

Table 5. Mitotic activity (mean ±SD) of the subepithelial layers of the esophagus (Krause et al. 1976a)

Body length (cm)	Number of mitoses (per mm^2)			
	Lamina propria	Muscularis mucosae	Submucosa	Muscularis externa
1.5		8.22 ± 0.61[a]		4.68
2.5		13.36 ± 1.04[a]		26.72 ± 3.9
3.5		9.49 ± 0.63[a]		16.78 ± 1.7
4.5	1.37 ± 0.07	9.92 ± 0.58	2.76 ± 0.09	11.75 ± 2.5
6.5	6.58 ± 0.16	5.17 ± 0.31	4.41 ± 0.29	7.22 ± 1.8
10.0	7.21 ± 0.33	4.33 ± 0.44	9.37 ± 0.38	6.06 ± 1.4
13.0	5.79 ± 0.24	2.38 ± 0.39	4.12 ± 1.06	3.65 ± 2.3
20.0	0.59 ± 0.02	1.22 ± 0.11	0.78 ± 0.05	1.07 ± 0.7

[a] Stages at which there is no well-defined muscularis mucosae. The figures given are for the total mitoses seen within the layer bounded by the epithelium and the muscularis externa.

event that also appears to be unrelated to the consistency of diet. It may be that epithelial cells which form the germinal layer of the esophageal lining epithelium are so programmed that at a specific time (age) the contained genome expresses a different phenotype in response to as yet unknown factors. The underlying connective tissue also shows dramatic changes at this time, becoming more cellular and giving rise to the muscularis mucosae. The changes seen in the connective layers may be related directly to the changes that occur in the overlying epithelium, and signals that originate in the underlying connective tissue also may be involved in the transformation of the lining epithelium. This is supported by the need for intimate interaction between these two components for normal glandular development to occur in most organs (Krause and Cutts 1986a). Changes in the esophageal lining epithelium occur simultaneously with the initial development of glands, but retention of a primitive stratified cuboidal epithelium with ciliated cells in the distal esophagus of the adult suggests that not all epithelial cells in the germinal layer are programmed in a similar fashion.

3.2.3 Quantitative Data on Mitotic Activity in the Subepithelial Layers

Table 5 shows the mitotic activities in the subepithelial strata of the developing esophagus. The absence of a well-defined muscularis mucosae up to 14 days precludes subdivision of the subepithelium into its constituent layers prior to 21 postnatal days. Mitoses recorded for the earlier stages represent the combined mitotic activity of the lamina propria, the muscularis mucosae, and the submucosa. Several peaks of mitotic activity occur, with an initial peak at 7 days in the combined subepithelial layers and in the muscularis externa. Following establishment of a definite, but delicate, muscularis mucosae at 14 days, the decline in mitotic activity within the muscularis mucosae is relatively slow; even in juvenile animals the rate of cell division exceeds that of the lamina propria and submucosa. This sustained pattern of mitotic activity is thought to relate to

Table 6. Thickness of the esophageal epithelium in the adult opossum (Marklin et al.. 1979)

Distance from pharynx (cm)	Thickness of epithelium (μm)
0.6	214.52
2.0	71
3.8	37
5.5	35
7.3	83.85
8.8	49.98

the presence of a thick, well-formed muscularis mucosae in the adult (Marklin et al. 1979). Similarly, formation of a well-defined muscularis externa at the end of the 1st postnatal week is accompanied by a peak of mitotic activity in this layer at this time (Krause et al. 1976a). As in the muscularis mucosae, a relatively high rate of mitosis is maintained throughout its subsequent development (Table 5). The peak of mitotic activity within the submucosa between 21 and 75 postnatal days is related to the growth of the esophageal glands which extend into this layer.

3.3 Structure of Adult Esophagus

The esophageal epithelium attains its maximal thickness at the proximal end and thins progressively toward the esophageal-gastric junction (Table 6). The epithelium associated with the transverse folds varies from nonkeratinized stratified squamous to an epithelium that consists of two to three layers of cuboidal or polygonal cells. The latter epithelium, together with ciliated cells, lies deep within the transverse folds (Krause et al. 1976a).

The connective tissue of the underlying lamina propria is fairly compact and shows no unusual properties. Along most of its length, it is crossed by ducts of the esophageal glands that are housed within the submucosa. Esophageal glands within the lamina propria may be present in the proximal end of the esophagus, but are particularly prominent distally within the transverse folds (Marklin et al. 1979). Diffuse accumulations of lymphocytes also are present within the lamina propria, particularly in the distal region.

The muscularis mucosae first appears as slips of smooth muscle about 5 mm distal to the pharyngoesophageal junction and then thickens progressively to form a single, continuous layer of loosely spiralling smooth muscle (Marklin et al. 1979). This compact band splays out in the region of the transverse folds to form a much thickened layer, then forms a much thinner layer as it continues into the stomach (Table 7).

Connective tissue elements of the submucosa are typical of those observed in other species. Autonomic ganglia are present within the submucosa and are scattered throughout the length of the esophagus; perikarya of neurons in the submucosal plexus increase in density distally (Table 8). In general, the ganglia

Table 7. Thickness of the muscularis mucosae in the esophagus of the adult opossum (Marklin et al. 1979)

Distance from pharynx (cm)	Depth of muscularis mucosae (μm)
0.6	0.833
2.0	1.095
3.8	1.614
5.5	1.809
7.3	2.429
8.9	3.952
9.3	6.191
9.8	2.462

Table 8. Number of ganglia per cm^2 and the number of perikarya per ganglion in the submucous plexus of the opossum esophagus (Christensen and Rick 1985)

Region	Ganglia per cm^2	Perikarya per ganglion
Proximal esophagus	4.5 ± 1.3	8.1 ± 1.6
Middle esophagus	5.5 ± 1.8	9.4 ± 0.9
Distal esophagus	9.7 ± 1.9	8.7 ± 1.0

Values are means ± SD.

are small, sparse, and irregularly distributed (Christensen and Rick 1985). Neurons in this layer stain positively for vasoactive intestinal polypeptide (VIP), and VIP-immunoreactive fibers invest the opossum esophageal glands (Christensen et al. 1987b). It is not known whether or not the VIP released in this area promotes secretion by the opossum esophageal glands, as is the case in Brunner's glands of the rat (Kirkegaard et al. 1981).

3.3.1 Histochemistry of Esophageal Glands

Esophageal glands within the submucosa show the same morphological features as those described previously in the lamina propria. Mucin granules (Table 9) in the mucous cells of opossum esophageal glands stain intensely with the periodic acid-Schiff method before and after digestion with saliva, suggesting that the reactive material is not glycogen. The granules stain with Alcian blue (at both pH 2.5 and 1.0) and with aldehyde fuchsin, and they are metachromatic when stained with toluidine blue. Positive staining with alcian blue at pH 1.0, positive staining with aldehyde fuchsin, and metachromasia with toluidine blue indicate that sulfated mucins are present (Krause et al. 1976a). Methylation at 60°C, followed by saponification, removes formed sulfate esters from the mucins, as indicated by the loss of alcinophilia at pH 1.0. Thus, mucins secreted by the opossum esophageal glands appear complex

Table 9. Histochemistry. Results of the histochemical study showing the staining of the mucin granules of the esophageal glands (Krause et al. 1976a)

PAS (before and after treatment with saliva)	Toluidine blue (0.1% and 1.0%) pH = 1.0	Aldehyde fuchsin	Aldehyde fuchsin and Alcian blue (0.25%) pH = 2.5	Alcian blue (0.1% and 1.0%) pH = 2.5	Alcian blue–PAS (0.1% and 1.0%) pH = 2.5	Alcian blue (0.1% and 1.0%) pH = 1.0	Alcian blue–PAS (0.1% and 1.0%) pH = 1.0
+++	+++ (metachromatic)	+++ (purple)	+++ (blue)	+++ (blue)	+++ (blue, red, blue-red)	+++ (blue)	+++ (blue, red, blue-red)
				Methylation in acid methanol at 37°C for 4 h			
				++ (blue)	+++ (red)[a]	++ (blue)	++ (red)[a]
					+++ (blue)		+++ (blue)
					+++ (blue-red)		+++ (blue-red)
				Saponification in 1.0% KOH in 70% ethanol for 20 min after prior methylation at 37°C			
				++ (blue)	+++ (red)[a]	++ (blue)	+++ (red)[a]
					+++ (blue)		+++ (blue)
					+++ (blue-red)		+++ (blue-red)
				Methylation in acid methanol at 60°C for 4 h			
				–	+ (red)	–	+ (red)
				Saponification in 1.0% KOH in 70% ethanol for 20 min after prior methylation at 60°C			
				+ (blue)	++ (red)	–	++ (red)

[a] Majority of cells.

The intensity of the staining reaction is designated as follows: +++, intense staining; ++, moderate staining; +, light staining; –, no staining.

Table 10. Thickness of the muscularis externa in the adult opossum (Marklin et al. 1979)

Distance from pharynx (cm)	Depth of muscularis externa (μm)	
	Inner muscle layer	Outer muscle layer
0.6	356.3	354.6
2.0	238.2	115.6
3.8	478.1	203.1
5.5	556.3	253.2
7.3	698.4	306.3
8.8	543.8	335.3

and are thought to contain neutral and acidic glycoproteins, and some of the acidic forms may be sulfated. Individual mucous cells may contain either or both neutral and acidic glycoproteins, as indicated by their staining reactions as has been reported in human esophageal glands (Lambert et al. 1973). The light (serous) cells that occur at the ends of the secretory units of opossum esophageal glands do not stain with the histochemical procedures listed above. As noted earlier, these cells lack abundant profiles of granular endoplasmic reticulum and secretory granules and therefore may be involved in the production of a secretion that is rich in ions rather than in the secretion of mucin. A similar function has been proposed for hydrotic cells of similar appearance in the bronchial glands of the opossum (Sorokin 1965).

3.3.2 Muscularis Externa

The muscularis externa arises at the base of the inferior constrictor muscle of the pharynx, where it forms as a wide band of striated muscle. The outer longitudinal and inner circular layers are of equal thickness at the origin of the esophagus, but over the succeeding 2 cm the muscle layers thin out, with a greater reduction occurring in the outer layer (Marklin et al. 1979). Distal to this point, the muscularis externa progressively increases in thickness. The greatest increase occurs in the circular layer, which becomes the predominant component of the muscularis externa (Table 10). Near the esophageal-gastric junction, the inner layer thins out, whereas the outer layer retains its dimensions or even increases slightly in thickness.

The distribution of striated and smooth muscle differs in the two layers of the muscularis externa. Proximally, the outer and inner layers consist entirely of striated muscle. The striated muscle of the outer layer extends further distally than that of the inner layer, and thus the extent of mixed muscle is correspondingly less (Table 11). Purely smooth muscle is present in both layers in the distal half of the esophagus. The transition from striated to smooth muscle occurs as a random intermingling of the two muscle types (Marklin et al. 1979).

Histochemical typing of the striated muscle in the opossum esophagus indicates that the muscularis externa consists primarily of a single fiber type

Table 11. Striated, mixed, and smooth muscle in the muscularis externa of the adult opossum (Marklin et al. 1979)

Inner layer			Outer layer		
Esophageal segment (cm)	Type of muscle	% of total muscle	Esophageal segment (cm)	Type of muscle	% of total muscle
0–1.78	Striated	18.8	0–2.81	Striated	30.3
1.78–4.50	Mixed	28.5	2.81–4.27	Mixed	14.7
4.50–9.56	Smooth	52.7	4.27–9.56	Smooth	55.0

Table 12. Histochemical classification of fiber types in the striated muscle of the opossum and human esophagus (Shedlofsky-Deschamps et al. 1982)

	Opossum	Human	Dubowitz-Brooke classification (1973)		
			Type I	Type IIA	Type IIB
SDH	Intermediate	Intermediate to weak	Strong	Intermediate	Weak
NADH	Intermediate	Intermediate to weak	Strong	Intermediate	Weak
ATPase (pH)					
10.4	Strong	Strong	Weak	Strong	Strong
4.6	No reaction	Strong	Strong	No reaction	Strong
4.3	No reaction	No reaction	Strong	No reaction	No reaction
PAS	Strong	Intermediate	Intermediate	Strong	Intermediate

SDH, succinic acid dehydrogenase; NADH, nicotinamide adenine dinucleotide dehydrogenase; ATPase, adenosine triphosphatase; PAS, periodic acid-Schiff reagent.

that can be classified as a type II fiber (Shedlofsky-Deschamps et al. 1982). The histochemical reactions used for this determination are shown in Table 12, which also summarizes the staining reactions of different fiber types in human skeletal muscle as presented by Dubowitz and Brooke (1973). The histochemical identification of the striated muscle fibers of the esophagus as type II fibers suggests that the muscularis externa of the opossum consists of fast-acting, fatigue-resistant fibers (Burke et al. 1971). Type I fibers have also been observed at the proximal end of the esophagus and are thought to represent a contribution of fibers from the inferior pharyngeal constrictor (Marklin et al. 1979; Shedlofsky-Deschamps et al. 1982). These observations are in agreement with manometric and electromyographic studies of the upper esophageal sphincter which have shown that the inferior pharyngeal constrictor makes a significant contribution to this region in the opossum, with only a small contribution from the esophageal musculature (Asoh and Goyal 1978b). Type I fibers are seen in this region of the human esophagus and also extend from the pharyngeal musculature, suggesting that in man and the opossum, pharyngeal muscle contributes to the upper esophageal sphincter (Shedlofsky-Deschamps et al. 1982).

Table 13. Distribution of motor end plates in the muscularis externa in the opossum and human esophagus (Shedlofsky-Deschamps et al. 1982)

Distance from pharynx (cm)	No. end plates/cm^2	
	Human	Opossum
1	138.31	102.7
2.5	–	45.3
3.5	77.8	–
5	–	19.02
6	58.47	–
7.5	–	7.11
8.5	47.79	–
11.0	38.13	–

A lower esophageal sphincter has been demonstrated manometrically (Tuch and Cohen 1973) and pharmacologically, (Christensen 1970) and studies using the opossum esophagus have provided abundant data about this region (Asoh and Goyal 1978a; Christensen 1982; Christensen et al. 1973a,b, 1979, 1987a, 1989; Christensen and Percy 1984; Christensen and Roberts 1983; Cohen and DiMarino 1976; Cohen and Green 1973; Daniel et al. 1979; De Carle and Christensen 1976; De Carle et al. 1976; Domoto et al. 1983; Goyal and Rattan 1973, 1976, 1978; Gutierrez et al. 1977; Lipshutz and Cohen 1971; Lipshutz et al. 1971; Mukhopadhyay 1978; Orsi and Ferreira 1978; Rattan and Goyal 1975, 1978, 1979, 1980, 1983; Rattan et al. 1972, 1988; Robison et al. 1984; Schlippert et al. 1979; Schulze and Christensen 1977; Schulze et al. 1977a,b, 1978; Schulze-Delrieu and Crane 1982; Seelig and Goyal 1978; Sengupta et al. 1987; Weisbrodt and Christensen 1972). Histological studies indicate sphincter-like expansions of the muscularis mucosae and muscularis externa in the distal esophagus of the opossum (Marklin et al. 1979). The expanded regions of these muscle layers do not coincide, and expansion of the muscularis mucosae occurs distal to that of the muscularis externa. Because the layers of the muscularis externa diminish in thickness as the muscularis mucosae expands, the structure of the lower esophageal sphincter in the opossum may be more complex than previously thought and probably does not reside solely in the muscularis externa. The muscularis mucosae, bacause it expands considerably in this region to form a loose spiral arrangement of smooth muscle fibers, also may contribute to the sphincteric action in the lower esophagus. Similar expansions of the muscularis mucosae have been reported in the lower esophageal region of the rabbit (Cecio et al. 1976), cat (Clerc 1983), and man (Giordano-Lanza and Manieri 1961). The presence of numerous vagal mechanoreceptors in the mucosa of this region in the cat (Clerc and Mei 1983) further supports the concept that the muscularis mucosae plays a role in the closure mechanism of the lower esophageal sphincter.

As demonstrated by acetylcholinesterase staining, motor end plates within the muscularis externa of the opossum esophagus are most numerous at the proximal end and progressively decrease in number distally (Table 13). The

Table 14. Number of ganglia and perikarya per cm^2 and nerve bundle diameter in the myenteric plexus of the opossum esophagus (Christensen et al. 1983)

Region	Ganglia/cm^2	Perikarya/cm^2	Nerve bundle diameter (μm)
Striated-muscled esophagus	2.9 ± 0.9	43.5 ± 21.9	11.7 ± 0.8
Striated- and smooth-muscled esophagus	17.1 ± 2.3	254.8 ± 38.7	17.0 ± 1.2
Smooth-muscled esophagus	33.8 ± 2.3	563.6 ± 69.9	19.3 ± 1.4

Values are means ± SD.

decrease corresponds to the decrease in striated muscle fibers. Although more numerous in man due to the greater thickness of the muscularis externa and length of the esophagus, the pattern of distribution of motor end plates is similar to that in the opossum (Shedlofsky-Deschamps et al. 1982). The appearance of the myenteric plexus in the opossum esophagus differs considerably depending on whether the region consists wholly of striated muscle, a mixture of striated and smooth muscle, or smooth muscle only (Christensen and Robison 1982; Christensen et al. 1983). In the region of striated muscle, the myenteric plexus is sparse and irregular and consists of thin branching fascicles; the constituent ganglia are small. In the region of mixed muscle, the plexus is more abundant and the nerve fascicles become thickened and branch more frequently; ganglia are more abundant and larger (Christensen et al. 1983). The plexus becomes even more prominent and the ganglia larger and more numerous in the proximal smooth muscle segment of the esophagus (Table 14). Thus, as would be anticipated, the densities of ganglia and perikarya progressively increase from the region of purely striated muscle to the region of purely smooth muscle (Marklin et al. 1979; Christensen et al. 1983). Interestingly, the number of ganglia and perikarya decrease in the region of the lower esophageal sphincter (Tables 15, 16). When this region of the opossum esophagus is stained for choline acetyltransferase to demonstrate cholingergic neurons, select populations of neurons (38%) within the myenteric plexus are reactive. Significant differences were not observed between the number of neurons positive for choline acetyltransferase in the distal esophagus and those in the region of the lower esophageal sphincter (Seelig et al. 1984). Vagal stimulation of the lower esophagus causes opposite responses in these two regions: contractions of the esophageal body and relaxation of the lower esophageal sphincter (Goyal and Cobb 1981). Pharmacological studies have demonstrated the presence of nonadrenergic neurons in this region (Goyal and Rattan 1978; Goyal and Cobb 1981), and peptidergic neurons that contain VIP also have been observed (Christensen et al. 1987b). VIP-positive perikarya are present in the myenteric and submucosal plexuses of the opossum esophagus, and VIP-immunoreactive fibers are reported to occur in the muscularis mucoae throughout the esophageal smooth muscle and in the striated muscle regions as well (Christensen et al. 1987b). Galanin, a peptide known to inhibit smooth muscle contraction in the esophagus, has been found in the opossum esophagus (Sengupta and Goyal 1988). Generally, the distribution of galanin parallels that of VIP but was not observed in the striated musculature or associated with

Table 15. Density of ganglia along the esophageal body and sphincter (Christensen and Robison 1982)

Segment position[a]	Animal number					Mean ± 1 SE (ganglia/cm^2)
	1	2	3	4	5	
3 cm above LES	75	65	54	61	51	61 ± 4[b]
2 cm above LES	47	47	43	57	46	48 ± 2[c]
LES	48	33	25	23	21	30 ± 5

[a] LES, lower esophageal sphincter.
[b] Greater than LES and 2 cm above LES ($P < 0.05$).
[c] Greater than LES ($P < 0.05$).

Table 16. Density of perikarya along the esophageal body and sphincter (Christensen and Robison 1982)

Segment position[a]	Animal number					Mean ± 1 SE (cells/cm^2)
	1	2	3	4	5	
3 cm above LES	906	1176	600	772	636	818 ± 105[b]
2 cm above LES	679	660	321	570	414	529 ± 70[c]
LES	333	172	79	234	141	192 ± 43

[a] LES, lower esophageal sphincter.
[b] Greater than LES and 2 cm above LES ($P < 0.05$).
[c] Greater than LES ($P < 0.05$).

blood vessels. Fibers reactive to substance P also have been demonstrated in the smooth muscle of the muscularis externa and in the muscularis mucosae of the opossum esophagus (Krause et al. 1985). These observations, together with the decreased thickness of the muscularis externa and the expansion of the muscularis mucosae, again suggest that the physiological character of this region is more complex than originally thought. The sphincter activity of this region may depend as much on the special properties of its smooth muscle as on special innervation (Christensen and Robison 1982; Christensen et al. 1987b).

The primary function of the esophagus is to transport ingested food to the stomach. The lower esophageal sphincter (LES) prevents reflux of materials from the stomach into the esophagus. The LES region in man and other species normally is tonically contracted, resulting in a high intraluminal pressure which prevents retrograde reflux of materials (Biancani et al. 1973, 1982; Christensen 1983; Hillemeier 1985). Activation of peristalsis in the smooth muscle that composes the body of the esophagus combined with relaxation of smooth muscle in the LES region is thought to occur as a result of neurotransmitter activity. VIP is thought to be the neurotransmitter responsible for stimulating peristalsis in the esophageal body and may be responsible for the relaxation of the LES region in man and other species as well (Biancani et al. 1984; Christensen et al. 1987b).

4 Stomach

4.1 Prenatal Development

The initial formation of the stomach occurs during the early part of the 10th prenatal day, when it appears as a distal expansion of the esophagus (McCrady 1938). The stomach rudiment continues to expand and, by the 12th prenatal day, is lined by a simple columnar epithelium surrounded by a delicate mesenchymal tissue without apparent subdivision. Enteroendocrine cells of undetermined nature are scattered within the gastric epithelium prior to birth.

4.2 Postnatal Development

Growth of the stomach, as estimated from total wet weight, is summarized in Table 17. There is progressive growth of the stomach prior to and after weaning, with no obvious differences with regard to the sex of the animals. The stomach shows a marked increase in weight during the postweaning period. Relative to body weight, the stomach increases greatly in weight during the 1st postnatal week; it then shows a progressive decline until about the 40th postnatal day, thereafter progressively increasing through weaning (Table 18).

The stomach of the opossum is somewhat unusual in that the small intestine leaves and the esophagus enters from the same region, separated by only a few millimeters of tissue. When the stomach of older pouch-young animals is opened along the lesser and greater curvatures and the mucosa examined, two distinct regions can be identified. The mucosa that lines most of the stomach (body and fundus) is darker and contains numerous rugae as compared to the lining of the distal (pyloric) portion, which shows fewer rugae and is much lighter in color. The boundary between these regions is sharp.

4.2.1 Fundic Mucosa

The gastric mucosa of the newborn opossum consists of a simple columnar epithelium that lies on a distinct basal lamina. The epithelial cells are united at their apices by typical tight junctions, and desmosomes are scattered between adjacent cell membranes. The cytoplasm often contains scattered lipid droplets of variable size (Krause et al. 1976b). The fundic region of the gastric mucosa is the first to differentiate, and because of this earlier development oxyntic

Table 17. Weights of stomach (g: mean ± SD)[a]

Body length (cm)	Male	Female	Combined male and female
1.5[b]	–	–	0.0012 ± 0.0001 (5)
2.0[b]	–	–	0.0013 ± 0.0002 (9)
2.5[b]	–	–	0.0059 ± 0.001 (7)
3.5	0.0114 ± 0.0008 (9)	0.0204 ± 0.018 (6)	0.0168 ± 0.095 (15)
4.0	0.0157 ± 0.0003 (5)	0.0154 ± 0.0004 (5)	0.0155 ± 0.0004 (10)
4.5	0.0179 ± 0.004 (13)	0.0196 ± 0.005 (6)	0.0184 ± 0.004 (19)
5.0	0.0195 ± 0.002 (5)	0.0192 ± 0.001 (4)	0.0194 ± 0.002 (9)
5.5	0.0273 ± 0.003 (7)	0.0215 ± 0.003 (6)	0.0246 ± 0.004 (13)
6.0	0.0348 ± 0.003 (5)	–	0.0348 ± 0.003 (5)
6.5	0.0344 ± 0.002 (7)	0.0304 ± 0.014 (6)	0.0341 ± 0.002 (13)
7.0	0.0555 ± 0.005 (5)	–	0.0555 ± 0.005 (5)
8.0	0.0916 ± 0.007 (5)	0.0887 ± 0.001 (5)	0.0902 ± 0.006 (10)
9.0	0.1102 ± 0.008 (4)	0.1244 ± 0.005 (3)	0.1173 ± 0.0.10 (7)
10.0	0.1725 ± 0.011 (7)	0.1687 ± 0.033 (7)	0.1706 ± 0.023 (14)
11.0	0.2742 ± 0.076 (7)	0.2918 ± 0.036 (7)	0.2830 ± 0.058 (14)
12.0	0.3563 ± 0.007 (8)	0.3521 ± 0.011 (7)	0.3542 ± 0.009 (15)
13.0	0.3874 ± 0.028 (4)	0.3643 ± 0.018 (4)	0.3759 ± 0.025 (8)
13.5	0.4270 ± 0.005 (3)	0.3922 ± 0.008 (3)	0.4096 ± 0.019 (6)
15.0	0.8197 ± 0.01 (4)	0.8054 ± 0.066 (4)	0.8125 ± 0.011 (8)
15.5	0.9104 ± 0.004 (5)	0.9879 ± 0.052 (3)	0.9395 ± 0.035 (8)
16.0	1.032 ± 0.036 (4)	1.0599 ± 0.033 (4)	1.0459 ± 0.035 (8)
17.0	1.359 ± 0.087 (4)	–	1.359 ± 0.087 (4)
18.0	1.5912 ± 0.039 (4)	1.7086 ± 0.022 (3)	1.6303 ± 0.068 (7)
20.0	2.3617 ± 0.026 (6)	2.3943 ± 0.387 (5)	2.3764 ± 0.246 (11)
22.0	2.7578 ± 0.314 (3)	2.6818 ± 0.167 (3)	2.7071 ± 0.257 (6)
25.0	–	4.5989 ± 0.9 (3)	4.5989 ± 0.9 (3)
27.5	6.8744 ± 0.404 (3)	–	6.8744 ± 0.404 (3)
29.0	–	6.9005 ± 0.415 (3)	6.9005 ± 0.415 (3)
33.0	–	9.9789 ± 0.474 (5)	9.9789 ± 0.474 (5)
37.0	18.2599 ± 2.46 (7)	–	18.2599 ± 2.46 (7)
Adult	31.8514 ± 3.34 (5)	35.6211 ± 4.65 (8)	34.1712 ± 4.47 (13)

[a] The numbers in parentheses, number of animals used.
[b] Sex not determined.

glands are the first to show subdivision into glandular and foveolar components. Parietal cells, as well as enteroendocrine cells, are present within the gastric epithelium in the fundic region of the newborn. The parietal cells lie adjacent to the underlying basal lamina and, on occasion, may border the lumen of the stomach (Fig. 18). Clefts within the gastric mucosa of the newborn opossum usually contain parietal cells at their bases. These clefts are thought to represent the initial formation of the oxyntic glands and their associated foveolae (gastric pits), which develop simultaneously in this species.

Ultrastructurally, the columnar cells of the gastric epithelium appear relatively undifferentiated and show only scattered organelles and a few short microvilli at the apical surface (Krause et al. 1976b). Some cells may show small apical collections of mucin granules near the apical plasmalemma. Parietal cells of the newborn opossum are characterized by numerous mito-

Table 18. Weights of stomach (g) per 100 g body weight (mean ± SD)

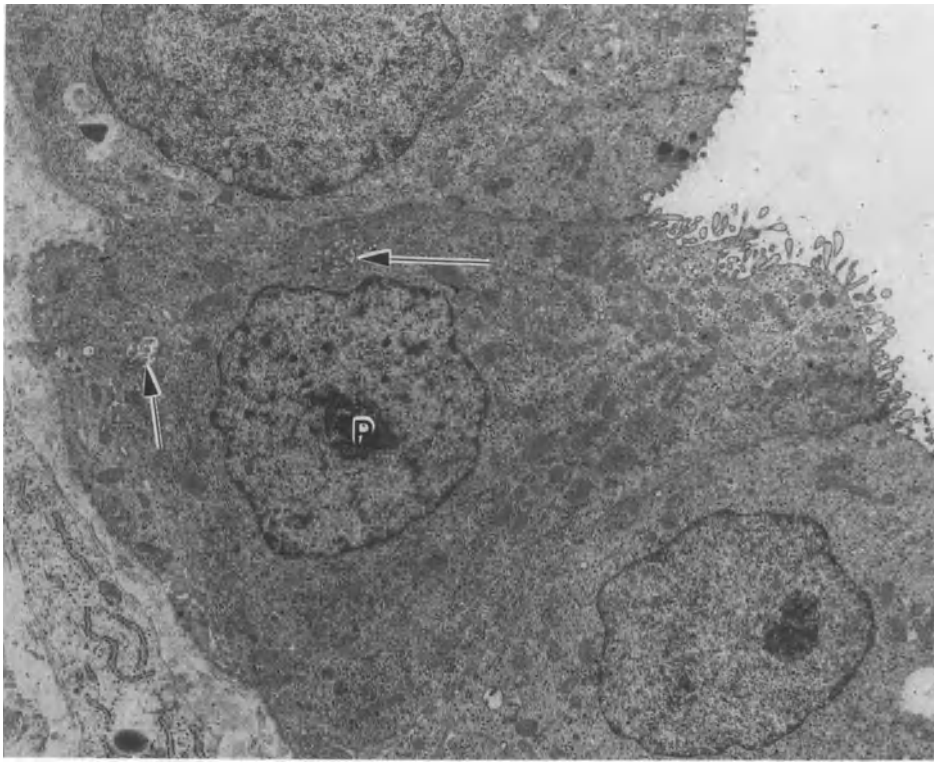
Body length (cm)	Male	Female	Combined male and female
1.5[a]	–	–	0.7635 ± 0.082
2.0[a]	–	–	1.6104 ± 0.112
2.5[a]	–	–	1.5940 ± 0.62
3.5	0.6965 ± 0.088	1.3576 ± 0.56	0.9750 ± 0.26
4.0	0.6022 ± 0.015	0.6073 ± 0.03	0.6048 ± 0.02
4.5	0.5971 ± 0.074	0.5965 ± 0.081	0.5969 ± 0.074
5.0	0.4943 ± 0.051	0.5193 ± 0.032	0.5054 ± 0.043
5.5	0.5817 ± 0.037	0.4787 ± 0.021	0.5342 ± 0.081
6.0	0.5499 ± 0.04	–	0.5499 ± 0.04
6.5	0.4761 ± 0.026	0.5529 ± 0.031	0.5222 ± 0.048
7.0	0.4915 ± 0.066	–	0.4915 ± 0.066
8.0	0.5937 ± 0.015	0.6071 ± 0.032	0.6004 ± 0.024
9.0	0.6467 ± 0.041	0.7103 ± 0.002	0.6785 ± 0.045
10.0	0.6591 ± 0.059	0.6481 ± 0.056	0.6536 ± 0.055
11.0	0.6667 ± 0.079	0.6180 ± 0.062	0.6424 ± 0.073
12.0	0.7792 ± 0.03	0.7780 ± 0.019	0.7749 ± 0.024
13.0	0.6410 ± 0.011	0.6566 ± 0.049	0.6488 ± 0.033
13.5	0.6879 ± 0.005	0.6448 ± 0.037	0.6843 ± 0.035
15.0	0.9148 ± 0.007	0.9172 ± 0.017	0.9160 ± 0.012
15.5	0.8030 ± 0.01	0.8731 ± 0.049	0.8293 ± 0.045
16.0	0.8077 ± 0.019	0.8673 ± 0.017	0.8375 ± 0.036
17.0	0.9214 ± 0.047	–	0.9214 ± 0.047
18.0	1.1689 ± 0.035	1.2064 ± 0.004	1.1814 ± 0.033
20.0	1.2416 ± 0.066	1.2766 ± 0.086	1.2575 ± 0.074
22.0	1.0872 ± 0.037	1.0591 ± 0.066	1.0685 ± 0.049
25.0	–	1.7252 ± 0.036	1.7252 ± 0.036
27.5	1.4966 ± 0.022	–	1.4966 ± 0.022
29.0	–	1.6403 ± 0.098	1.6403 ± 0.098
33.0	–	1.3616 ± 0.051	1.3616 ± 0.051
37.0	1.2821 ± 0.062	–	1.2821 ± 0.062
Adult	1.2537 ± 0.179	1.5742 ± 1.41	1.4509 ± 0.221

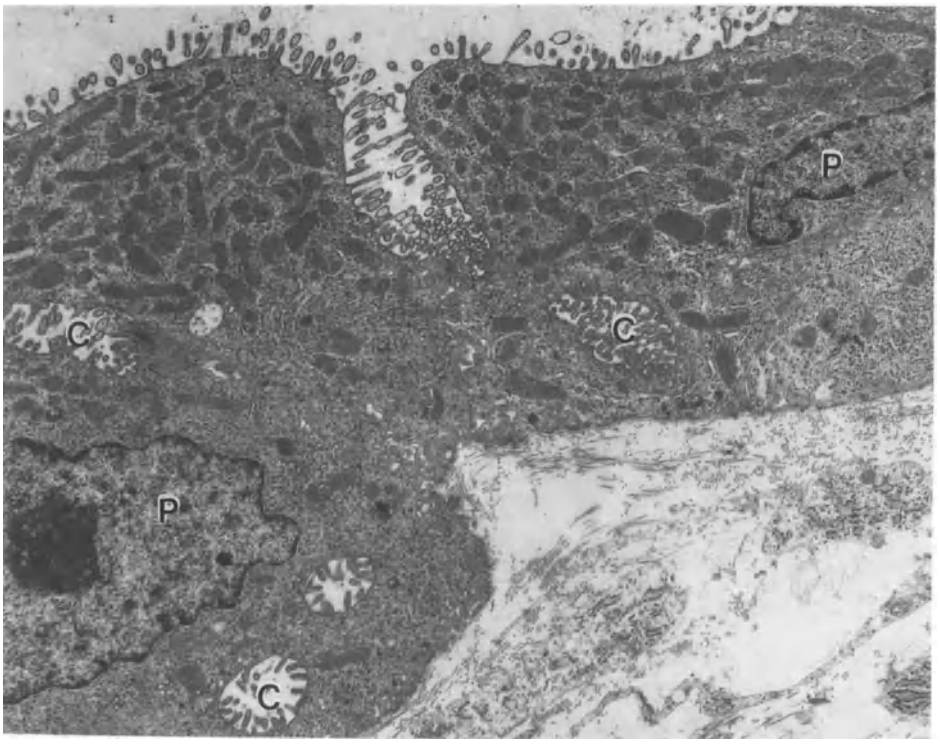
[a] Sex not determined.

chondria, well-developed canaliculi, and scattered elements of granular endoplasmic reticulum. The cytoplasmic matrix often shows considerable electron density in comparison to adjacent cells. Mitochondria of parietal cells stain intensely for succinate dehydrogenase at this time. A tubulovesicular component is present in the cytoplasm of the parietal cells adjacent to canaliculi, which often are filled with numerous elongated microvilli. Parietal cells in the stomach of the newborn respond to exogenous administration of

Fig. 18 (*above*). A recently differentiated parietal cell (*P*) in the gastric lining epithelium of a newborn opossum. Note the initial formation of canaliculi (*arrows*). ×6000 ▶

Fig. 19 (*below*). Adjacent, stimulated parietal cells (*P*) lining the gastric lumen of a newborn opossum stomach show an increase in length and width of canaliculi (*C*). (Krause et al. 1976b) ×6000


P


P
C
C
P
C

pentagastrin by the production of hydrochloric acid with reduction of the luminal pH (Cutts and Krause 1980a). After such stimulation, the parietal cells show a loss of the tubulovesicular component and a corresponding increase in the number and length of microvilli within canaliculi, and the canaliculi themselves increase in width and length (Fig. 19). Such ultrastructural changes following stimulation typify parietal cells of adults of other species (Helander and Hirschowitz 1974; Ito and Schofield 1974), including man (Tarnawski et al. 1980).

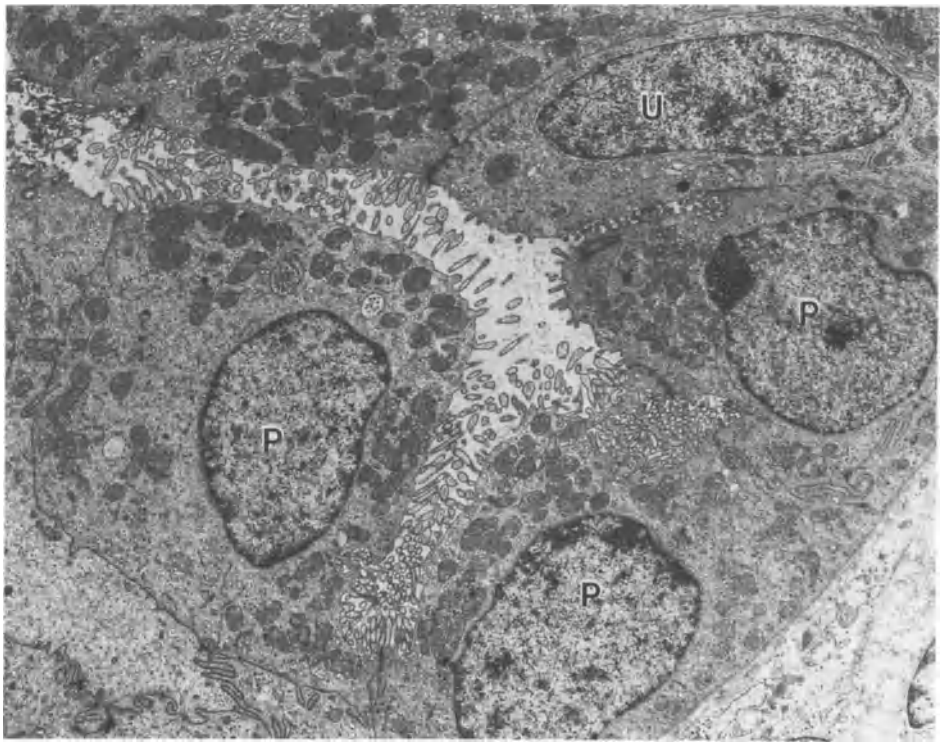
The occasional enteroendocrine cells observed in the fundic region of the newborn stomach show an electron-lucent cytoplasm that contains scattered profiles of granular endoplasmic reticulum, bundles of intermediate filaments, and occasional small, electron-dense granules of variable size and morphology. The enteroendocrine cells also demonstrated by silver staining and immunocytochemistry are found at the base of the gastric lining epithelium on the luminal side of the basal lamina.

The simple columnar epithelium that makes up the gastric mucosa of *Didelphis* appears to be similar in morphology to the gastric mucosa of newborns of two Australian marsupials, the red kangaroo (Griffiths and Barton 1966) and the native cat (Hill and Hill 1955). In *Didelphis* a large population of cells is seen within the gastric mucosa that are immunoreactive for both pepsinogen and prochymosin (prorennin) at the time of birth (Krause et al. 1987). In all three species the gastric glands are not present at birth, and glands and foveolae both develop during the prolonged postnatal period. Although the surrounding mesenchyme shows no subdivision at this stage of development, scattered myoblasts of a presumptive muscularis externa are present near the external surface of the stomach wall. This primitive muscular layer consists of a single layer of myoblasts, one to three cells deep.

By the end of the 1st week of postnatal life, short but definite oxyntic glands are present in *Didelphis* and contain parietal, enteroendocrine, and undifferentiated cells. The undifferentiated cells generally are columnar in shape and show only scattered organelles, although in some cells free ribosomes may be numerous (Fig. 20). The mucosal surface now shows a considerable amount of infolding due to the formation and growth of foveolae and glands (Table 21). The epithelial cells of the gastric lining contain an increase in lipid droplets; these continue to increase in size and number through the 2nd postnatal week. Some of the droplets ultimately measure 15 μm in diameter. The lipid droplets lack a limiting membrane and lie free within the cytoplasm. They are not seen within the cisternae of the endoplasmic reticulum or between adjacent cell membranes. Undifferentiated cells

Fig. 20 (*above*). A region of a forming oxyntic gland from a 7-day-old opossum containing several parietal cells (*P*) and an undifferentiated cell (*U*). ×5000 ▶

Fig. 21 (*below*). Three undifferentiated cells (*U*) lining an oxyntic gland of an 14-day-old opossum. A large, stimulated parietal cell (*P*) is shown at the base. Note the difference in concentration of mitochondria between the parietal cell shown in this figure and the parietal cells shown in Fig. 20. ×5000


U
P
P
P

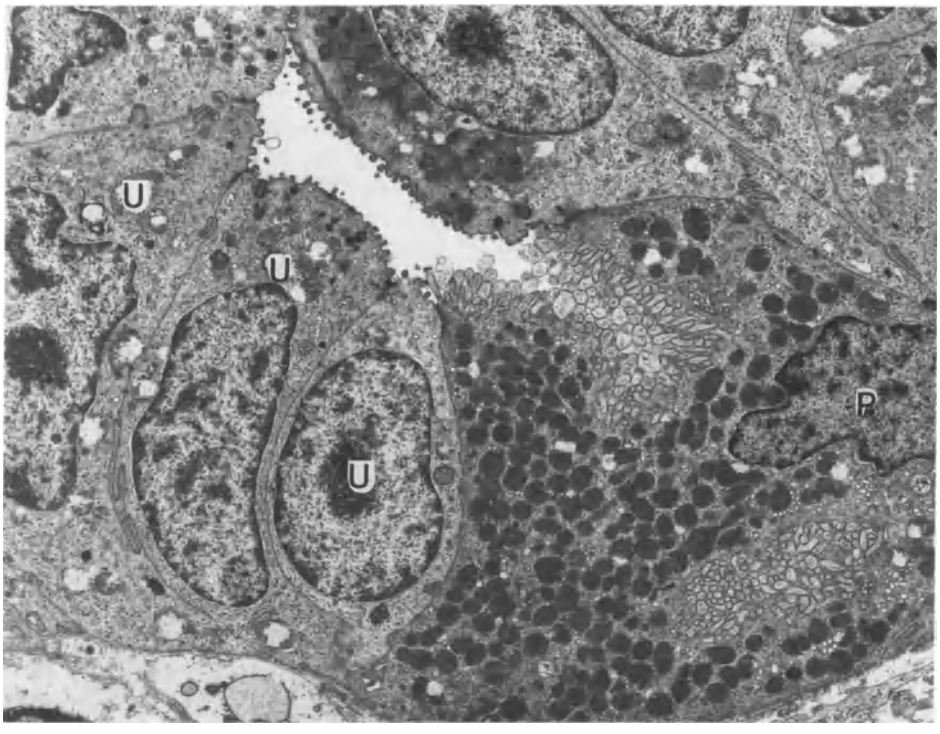

U
U
U
P

Table 19. Development of the gastric mucosa in the opossum (Krause et al. 1976b)

Body length (cm)	Depth of surface epithelium (μm)	Depth of lamina propria (μm)	Depth of glands (μm)	Depth of muscularis mucosae (μm)
1.5	25.00	36.54	–	3.85
3.5	25.00	51.92	48.08	8.65
5.5	24.04	67.31	60.58	14.42
8.0	25.96	84.62	70.84	14.42
10.0	26.92	119.23	113.46	13.46
13.0	27.88	226.92	219.23	13.38
22.0	25.68	334.62	321.15	14.58

continue to represent a significant proportion of the cells in the expanding oxyntic glands (Fig. 21).

Occasionally, caveolated cells also are observed, both within the forming foveolae and in the oxyntic glands at this and later stages of development. They are seen most often within the surface lining epithelium. Caveolated cells are characterized by a narrow apex that protrudes slightly into the lumen, and a wide base that is in contact with the basal lamina. The cell apex shows short, broad microvilli filled with bundles of cytoplasmic filaments that may extend into the supranuclear region of the cell. Caveolae and/or tubules extend from the apical cell membrane between microvilli, for a considerable distance into the cell cytoplasm. Their function is unknown.

Lipid droplets are not as frequent in the gastric epithelium by the end of the 3rd postnatal week (Krause et al. 1976b), and small clusters of mucin granules are apparent in the apices of most cells of the surface lining epithelium. Cells of the gastric epithelium are devoid of lipid droplets by the end of the 4th postnatal week, but the secretory granules continue to increase in number. Ultrastructurally, secretory granules have a mottled appearance, are limited by a membrane, and fill the apical and supranuclear regions of the surface lining cells by the 75th postnatal day. The appearances of these amorphous secretory granules vary considerably. Regions of increased electron density give the granules a mottled appearance. The mucin granules tend to coalesce into irregularly shaped complexes despite being limited by membranes. The surface cells contain an abundance of granular endoplasmic reticulum in the basal and perinuclear cytoplasm. Numerous Golgi complexes, associated with developing granules, are located in the supranuclear cytoplasm. The oval nuclei show definite polarization toward the base of the cells and usually contain at least one well-developed nucleolus.

Quantitative studies indicate that the greatest development of the oxyntic glands occurs between the 60th and the 75th postnatal day (Table 19). Prior to and during this time, the oxyntic glands continue to consist primarily of three cell types: parietal, immunoreactive enteroendocrine, and undifferentiated cells. The increase in depth of the oxyntic glands prior to the 75th day (before the onset of weaning) is due largely to an increase in the number of parietal cells (Table 20). The parietal cells are thought to arise from undifferentiated columnar cells within the developing oxyntic glands (Krause et al. 1976b). This

Table 20. Parietal and chief cells in the gastric mucosa of the opossum (Krause et al. 1976b)

Body length (cm)	No. of cells/mm^2	
	Parietal cells	Chief cells
1.5	32.05	0
3.5	133.33	0
5.5	178.21	0
8.0	246.16	0
10.0	258.97	0
13.0	310.26	105.13
22.0	320.51	212.80

Table 21. Mitotic activity of gastric epithelium in the developing opossum (Krause et al. 1976b)

Body length (cm)	Mitotic cells (no./mm^2)	
	Surface epithelium	Glandular epithelium
1.5	9.33	5.00
3.5	43.00	20.28
5.5	31.53	23.07
8.0	23.33	32.67
10.0	20.67	18.86
13.0	10.87	7.53
22.0	2.00	3.49

undifferentiated cell type continues to show numerous free ribosomes and occasional small Golgi complexes; it may also contain small electron-dense granules. These ultrastructural features suggest that these cells may represent presumptive mucous neck cells, which have been reported to give rise to both zymogen and parietal cells in other species (Stevens and Leblond 1953; Messier and Leblond 1960) and in regenerating fundic mucosa (Lawson 1970).

Chief cells are not observed in the oxyntic glands of the opossum until about the 75th postnatal day (Table 20) and first occur at the bases of the glands (Krause et al. 1976b). They are thought to arise from undifferentiated cells that still are present within the developing oxyntic glands. A peak in the mitotic activity of the glandular epithelium occurs prior to the appearance of chief cells (Table 21), further supporting this concept. After definitive chief cells have been established, an increase in their numbers may be due to continued development of undifferentiated cells and/or perhaps the division of other chief cells (Willems et al. 1972a). Chief cells in the oxyntic glands of *Didelphis* remain restricted to the bases of the glands and form only a relatively small population, even in adult animals. Following the appearance of chief cells, the oxyntic glands continue to increase in depth throughout adulthood and now increase their length by normal proliferative activity in the neck region of the glands (Krause et al. 1976b).

Table 22. Development of gastric mucosa in the opossum (Acuff et al. 1989)

Area	Age (days)	Length (μm)	
		Pit	Gland
Cardia	29	60	44
	40	50	39
	73	150	112
	95	130	233
	Juv.	77	510
	Adult	229	669
Fundus	29	30	23
	40	40	51
	73	50	111
	95	60	130
	Juv.	122	382
	Adult	220	526
Pylorus	29	68	39
	40	70	48
	73	88	112
	95	130	150
	Juv.	200	450
	Adult	154	518

Ultrastructurally, the cytoplasm of the chief cells contains an abundance of granular endoplasmic reticulum that fills the basal, perinuclear, and supranuclear regions. Well-developed Golgi complexes and numerous, large, membrane-bound granules that vary considerably in electron density further characterize the chief cell population. Although chief cells are late to develop, once formed they have structural features that are typical of those found in eutherian mammals (Ito 1967), including man (Stachura et al. 1981).

4.2.2 Glands of the Pylorus and Cardia

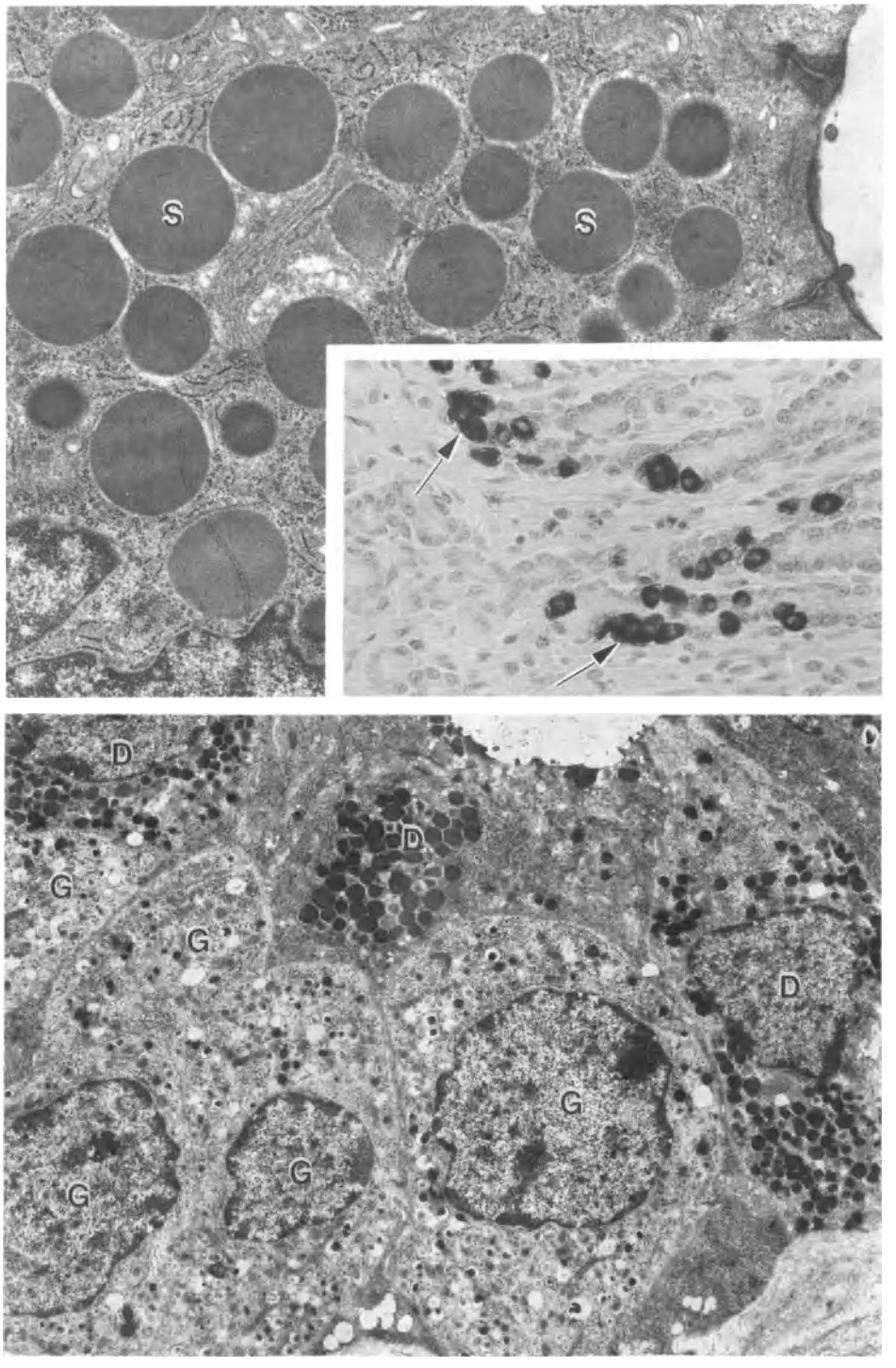
The pyloric glands begin to differentiate at about the 4th postnatal week but are not obvious structures until about 50 days postnatum (Acuff et al. 1989). They begin as outgrowths from the surface lining epithelium and form simultaneously with their associated foveolae. Like the oxyntic glands, they develop throughout the postnatal period and into adulthood. Glands and foveolae gradually increase in length until 95 days of age (weaning), by which time they have tripled their length (Table 22). During this period of development, the secretory tubules of the pyloric glands form, branch, coil, and extend through the developing lamina propria. The pyloric glands are simple, branched tubular glands, two or more of which may drain into a single foveola. The pyloric glands of the opossum tend to be organized into discrete secretory lobules separated by connective elements of the surrounding lamina propria. The ratio of the length of the foveolae to the depth of the pyloric glands is about 1.0:3.4.

Pyloric glands consist primarily of light-staining, pyramidal-shaped secretory cells with basally positioned nuclei. These mucin secretory cells are characterized ultrastructurally by an abundance of granular endoplasmic reticulum in the basal and perinuclear cytoplasm and by large, well-developed Golgi complexes that occupy the supranuclear region. The apical half of the cell is filled with secretory granules that compress the nucleus toward its base. The secretory granules are membrane bound, amorphous in nature, and show considerable electron density (Fig. 22). Enteroendocrine cells also are numerous in pyloric glands, as can be seen after staining by the Churunkian-Shenk silver method (Acuff et al. 1989).

The enteroendocrine cells within the pyloric region of postweaned opossums tend to form a narrow band of cells, closely crowded together at the base of the foveolae and in the neck segments of the glands (Fig. 23). The ultrastructural morphology of the enteroendocrine cells varies according to the specific type, but all generally show an electron-lucent cytoplasm, occasional profiles of granular endoplasmic reticulum, and scattered secretory granules whose morphology depends on the cell type observed. Several enteroendocrine cells often lie adjacent to one another around the circumference of the neck of individual pyloric glands, where they form clusters (Fig. 24).

It is not until the 4th postnatal week that the cardiac glands and their associated foveolae appear. These arise together as invaginations of the gastric epithelium (Table 22). Both components differentiate and develop simultaneously, and by the time of weaning the foveolae are fully developed and the glands are well established (Acuff et al. 1989). The depth of the cardiac glands increases more rapidly than that of the foveolae so that, just prior to the onset of weaning, glands are almost twice as long as are the foveolae. At weaning, the cardiac glands have attained their adult appearance and frequently are organized into small lobule-like complexes. Human cardiac glands show a similar organization (Krause et al. 1978b). Opossum cardiac glands begin at the esophageal-gastric junction and extend for only a few millimeters distally (Fig. 25). They are simple, branched, tubular glands, several of which may drain into a single foveola. The ratio of length of the foveolae to the depth of the gland is about 1:2.8 (Acuff et al. 1989). The secretory tubules of the cardiac glands consist primarily of mucous cells with a light-staining cytoplasm and basally positioned nuclei. Ultrastructurally, the secretory cells of the cardiac glands appear to be very similar to the cells that compose the opossum pyloric glands. Like the cells of the pyloric glands, the secretory cells of the cardiac glands contain membrane-bound, amorphous, secretory granules of considerable electron density that fill the apices of the cell. Well-developed Golgi complexes occupy the supranuclear cytoplasm, and profiles of granular endoplasmic reticulum fill the perinuclear and basal regions.

In all three regions of the opossum stomach, the foveolae and their associated glands grow simultaneously and increase their lengths into adulthood. The fundic region is the first portion to differentiate and, because of this early development, is the first region to show subdivision into glandular and foveolar components. At weaning the ratio of foveolae to gland length is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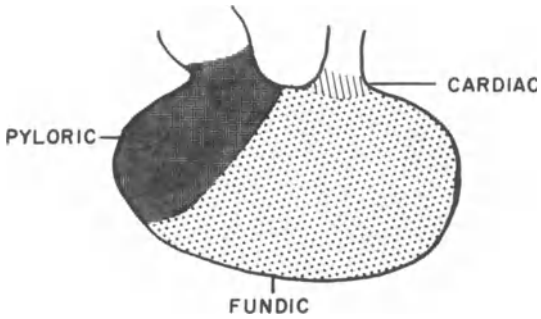


Fig. 25. A line diagram of the opossum stomach shows the distribution of the cardiac, fundic (oxyntic), and pyloric glands

equivalent to that in the adult. Formation of the pyloric glands is delayed and the foveolae remain the longest element, even at 75 days postnatum. It is not until after weaning that the length of the cardiac glands surpasses that of the foveolae (Acuff et al. 1989).

If the development of the gastric mucosa, other than its glands, is considered, then the depth of the surface epithelium is established at birth and remains unchanged throughout development (Krause et al. 1976b). The muscularis mucosae is thin and incomplete at birth but rapidly increases in thickness to reach a constant depth by the end of the 4th postnatal week (Table 19). The most dramatic change occurs in the depth of the lamina propria, which increases as a result of the increasing length of the gastric glands. The connective tissue cells and connective tissue fibers show a corresponding increase in number. The lamina propria is fairly scant around oxyntic glands, of moderate amount about cardiac glands, and abundant around the pyloric glands. In all three regions of the stomach, a considerable amount of lamina propria is present between the bottoms of the glands and the muscularis mucosae. By the time of weaning, smooth muscle cells and numerous connective tissue cells, primarily mast cells, eosinophils, macrophages, and lymphocytes, are found within the delicate connective tissue of the lamina propria. The number of these cells increases after weaning and continues to increase throughout adulthood. The connective tissue cells are thought to present an immunological barrier to the passage of foreign antigens from the gastric lumen. Similar accumulations of these connective tissue cell types are seen in the lamina propria of the stomach, small intestine, and colon of the mouse (Deane 1964). The close relationship of macrophages, plasma cells, lymphocytes, and eosinophils in the gastrointestinal mucosa of the mouse, opossum, and other species is important in the defense against foreign antigens

◀ **Fig. 22** (*above*). The apical region of a mucous cell taken from a pyloric gland illustrates the nature of the electron-dense secretory granules (*S*). ×10000
Fig. 23 (*inset*). Gastrin-immunoreactive cells (*arrows*) within the pyloric glands of the opossum. ×250
Fig. 24 (*below*). An electron micrograph taken from the neck segment of a pyloric gland illustrates portions of five G cells (*G*) and three D cells (*D*) clustered together. ×6000

(Butzner and Befus 1989; Freier 1989). The dramatic increase in number of eosinophils that occurs in the lamina propria of the young-adult opossum may be a response to the infestation of gastric round worms (*Physaloptera turgida*) and other parasites which are common in the opossum (Potkay 1977).

Computer-assisted, three-dimensional reconstructions of smooth muscle projections from the muscularis mucosae into the lamina propria of the adult fundic stomach have shown these elements to form a unique net-like arrangement around the oxyntic glands in *Didelphis* (Seelig et al. 1985). It has been proposed that nerve-stimulated contractions of this smooth muscle network compress the oxyntic glands and thus aid in secretion by emptying the glandular contents into the gastric lumen.

4.2.3 Mucin Histochemistry

The secretory granules within the cells of the gastric lining epithelium stain with Schiff's reagent before and after digestion with saliva, and fail to stain with Alcian blue (at either pH 1.0 and 2.5) or aldehyde fuchsin (Krause et al. 1976b), suggesting that the secretory product is a neutral mucin (glycoprotein). Similar observations have been reported for mucins of the surface lining epithelium of the dog (Spicer et al. 1967; Spicer and Sun 1967) and man (Lev 1966; Cathcart et al. 1974). Chief cells of *Didelphis* fail to stain by these methods, indicating the absence of sulfated mucins in this cell type. Secretory granules within mucous cells of the pyloric and cardiac glands are PAS positive before and after digestion with saliva (Acuff et al. 1989). Failure of these secretory granules to stain with Alcian blue (pH 1.0 or 2.5) suggests that, like those within the surface lining epithelium, they contain neutral glycoproteins and perhaps lack strong carboxyl and/or sulfate groups (Mowry 1956, 1960; Spicer 1960; Spicer and Sun 1967).

4.2.4 Immunohistochemistry of Gastric Proteinases

Immunoreactivity to bovine pepsinogen and prochymosin (prorennin) is seen in cells of adult and developing oxyntic glands but not in the pyloric or cardiac glands of *Didelphis* (Krause et al. 1987). Immunoreactivities to pepsinogen and prochymosin occur primarily, but not exclusively, within the same cell type. Prochymosin-immunoreactive cells show intense immunoreactivity, whereas those stained for pepsinogen are only weakly reactive. Reactive cells of the newborn opossum are scattered within the simple sheet of gastric epithelium that lines the lumen of the stomach, but thereafter are found within the developing oxyntic glands. Two periods of cellular differentiation and development are seen in the postnatal opossum stomach. The first is concerned with the establishment of the parietal cell population and an undifferentiated cell type. The second period begins at weaning and extends into the postweaning period; it is concerned with the establishment of the chief cell population. The depth of the gastric mucosa increases by about 70% from the weaning opossum to the juvenile animal and continues to increase in depth into adulthood. Prior

Table 23. Gastric proteinase-containing cells in the developing opossum stomach (Krause et al. 1987)

	Pepsinogen (cells/mm^2)	Prochymosin (prorennin) (cells/mm^2)
Newborn	1141.2 ± 61.3	1552.5 ± 63.6
7 days postnatum	761.7 ± 133.3	983.5 ± 138.0
70 days postnatum	127.5 ± 41.7	117.7 ± 17.6
93 days postnatum	830.46 ± 23.3	774.8 ± 96.3
Weaned juvenile	1086.6 ± 102.0	1178.6 ± 120.3
Adult	759.7 ± 38.3	767.1 ± 37.6

Values are means ± SD.

to weaning, immunoreactivities to prochymosin and pepsinogen occur primarily in an undifferentiated cell type however, in very young animals; some immunoreactivity is present in parietal cells as well. Following the appearance of chief cells, immunoreactivities to both gastric proteinases occur primarily in the chief cell population (Krause et al. 1987). Thus, pepsinogen and prochymosin are produced by the same cell type in the oxyntic glands of *Didelphis*, but the cell type differs prior to and after weaning. A similar shift from one cell type to another during development has been reported for gastric proteinases in the stomach of the calf (Andrén et al. 1982; Andrén and Björck 1986; Miura et al. 1988). Co-localization of prochymosin and pepsinogen immunoreactivities has been demonstrated in the same secretory granules of chief, mucous neck, and transitional cells of the calf stomach at the ultrastructural level (Yamada et al. 1988). In *Didelphis*, the number of cells that contain gastric proteinases decreases from the newborn to just prior to weaning, but then increases after weaning (Table 23) only to decrease once again in adult animals. It is not clear whether the decrease in the number of immunoreactive cells in pouch-young and in adult animals is an actual decrease or is merely an apparent decrease resulting from the progressive growth of the stomach. Thus, the gastric mucosa of the suckling opossum appears capable of producing both chymosin (rennin) and pepsin to aid in the digestion of milk prior to the appearance of chief cells. In addition to cells within the oxyntic glands of the calf and opossum, pepsinogen has been demonstrated in mucous neck cells of the pig (Yasuda et al. 1966; Liebman and Samloff 1979) and man (Samloff 1971; Samloff and Liebman 1973; Hirsch-Marie et al. 1976).

A third cell type, apparently a transition between the mucous neck and chief cells of the human oxyntic glands, also has been shown to contain pepsinogen immunoreactive granules (Cornaggia et al. 1986). This observation, together with observations on the developing oxyntic gland of *Didelphis*, suggests that during development chief cells originate either from an undifferentiated cell type within the oxyntic glands or by progressive transformation of mucous neck cells. Once established, chief cells of the mouse have the ability to divide and contribute, at least in part, to their own population, as shown by autoradiography (Willems et al. 1972a).

Table 24. Argyrophil cells in the opossum stomach (no./mm^2) (Acuff et al. 1989)

Age (days)	Cardia	Fundus	Pylorus
29	569 ± 77	600	270 ± 30
40	400 ± 44	544 ± 7	280 ± 7
73	222 ± 98	411 ± 18	331 ± 44
95	78 ± 9	237 ± 59	211 ± 16
Juv.	109 ± 2	160 ± 14	273 ± 49
Adult	23 ± 3	74 ± 16	130 ± 26

Values are means ± SD.

Table 25. Percent distribution of argyrophil cells in the opossum stomach (Acuff et al. 1989)

Age (days)	Cardia	Fundus	Pylorus
29	39.6	41.7	18.7
40	32.7	44.5	22.8
73	12.0	42.6	34.4
95	14.8	45.1	40.1
Juv.	20.2	29.4	50.4
Adult	8.9	27.9	63.2

4.2.5 Enteroendocrine Cells

One day prior to birth (11½ days of gestation) scattered enteroendocrine cells can be identified in the simple columnar gastric lining epithelium of the opossum embryo, and they continue to be scattered throughout the gastric lining epithelium in the newborn. These can be demonstrated by the Churunkian-Shenk silver method of staining. The silver-stained enteroendocrine cells usually are concentrated in the basal regions of the epithelial invaginations and remain closely associated with the developing glands in all three regions of the opossum stomach (Acuff et al. 1989). In the oxyntic glands, the argyophil cells are scattered randomly until about the 40th day, after which they are concentrated in the distal half of the gland. As development continues, the cells become scattered through the distal three-fourths of the oxyntic glands and the concentration of argyrophil cells in these glands gradually decreases (Tables 24, 25). Argyrophil cells also are associated with the expanding regions of the cardiac and pyloric glands (Acuff et al. 1989). Although the total concentration of positive cells gradually decreases during development of the stomach, those present in the pyloric glands form an increasing proportion of such cells in the stomach (Table 25). During development, argyrophil cells in the cardiac and oxyntic glands remain fairly scattered, whereas in the pylorus of the weaned juvenile, a shift in their location is seen, from the basal region of the pyloric glands to the region of the neck and isthmus. In all three glands and throughout development of the gastric mucosa, there is a relationship between argyrophil cells and the region of the glands, where the mitotic activity is highest. This observation suggests

that the enteroendocrine cells within the gastric mucosa either have a relatively short life span or are strategically positioned to influence the differentiation of other cell types at this location via a paracrine mechanism.

In the oxyntic glands, argyrophil cells show a variable morphology: some are stellate with long, branching, dendritic-like processes, while others are elongate or spindle shaped. Argyrophil cells generally are round or oval in the pyloric glands, whereas in glands of the cardia both oval and spindle-shaped cells are found (Acuff et al. 1989).

If the peroxidase-antiperoxidase method (Sternberger 1979) is used to examine that enteroendocrine cell population demonstrated by silver staining methods, then five types of enteroendocrine cells can be identified in the gastric mucosa. Cells immunoreactive for gastrin, bovine pancreatic polypeptide (BPP), glucagon, somatostatin, and the amine, serotonin (5-HT) have been identified (Krause et al. 1985, 1986) and are present in the newborn, scattered within the gastric lining epithelium (Tables 26, 27).

Glucagon-immunoreactive cells are seen almost exclusively in the region of the fundus, and gastrin-immunoreactive cells are restricted to the epithelial lining of the pylorus (Table 27). 5-HT- and somatostatin-immunoreactive cells are present in the gastric lining epithelium of the pyloric and fundic regions. Cells immunoreactive to BPP are the most numerous enteroendocrine cell type seen in the newborn and initially appear to be confined to the epithelial lining at the confluence of the cardia, fundus, and pylorus (Krause et al. 1986). As soon as the oxyntic glands begin to differentiate near this junction, glucagon- and BPP-immunoreactive cells become restricted to these glands. By the end of the 1st postnatal week, BPP-immunoreactive cells are confined primarily to the fundic region, as occurs in all older postnatal stages examined (Table 27). Similarly, cells positive for pancreatic polypeptide first appear in the antrum of the pig at the end of the 6th week of gestation and by 2 weeks after birth are present in the oxyntic glands, where they remain throughout development into adulthood (Alumets et al. 1983). Glucagon-immunoreactive cells are first detected in the fundus of man at 10 weeks and remain confined to the oxyntic glands (Stein et al. 1983). Both BPP- and glucagon-immunoreactive cells are confined to the oxyntic glands of the opossum throughout development, and their numbers show the same pattern of decline with age (Tables 26, 27).

A marked increase in the number of all five immunoreactive cell types is seen by the end of the 1st postnatal week in the opossum, with the most prominent increase occurring in the cells immunoreactive for gastrin. These cells remain restricted to the pyloric portion of the stomach, confined to a narrow band that includes the bottoms of the foveolae and the ducts of the pyloric glands. Gastrin-immunoreactive cells are restricted to the pylorus in man (Dubois et al. 1976; Larsson et al. 1977; Stein et al. 1983), the pig (Alumets et al. 1983), and the mouse (Kataoka et al. 1985). The populations of 5-HT- and somatostatin-immunoreactive cells shift from the cardiac and fundic regions to the pyloric region of the opossum stomach, attaining a near adult-type distribution soon after weaning (Table 27). Gastrin-immunoreactive cells and somatostatin- and 5-HT-positive cells are confined in a narrow band at the junction between the foveolae and the isthmus of the opossum pyloric glands early in postnatal life. Somatostatin- and 5-HT-immunoreactive cells have been

Table 26. Endocrine cell types in the stomach of developing opossums (Krause et al. 1986)

Body length (cm)	Somatostatin		Gastrin		5-HT		BPP		Glucagon	
	Cells per mm^2	Relative increase[a]	Cells per mm^2	Relative increase[a]	Cells per mm^2	Relative increase[a]	Cells per mm^2	Relative increase[a]	Cells per mm^2	Relative increase[a]
1.5	17.12	1.0	6.85	1.0	26.54	1.0	31.67	1.0	3.42	1.0
2.5	71.20	4.2	180.08	26.3	54.4	2.1	83.76	2.7	29.32	8.6
12.5	124.39	7.3	86.79	12.7	57.86	2.2	14.47	0.5	5.79	1.7
20.5	140.88	8.2	95.10	13.9	102.14	3.9	10.57	0.3	3.52	1.0
30.0	145.80	8.5	96.02	14.0	72.89	2.8	9.94	0.3	6.63	1.9
Adult	112.43	6.6	137.91	20.1	41.97	1.6	4.50	0.15	2.99	0.9

[a] Relative increases are calculated using the 1.5 cm value as the baseline.

Table 27. Distribution of endocrine cells in the stomach during development (Krause et al. 1986)

Hormone	Body length (cm)	Total endocrine cells/mm^2 (mean ± SD)	Distribution (%)		
			Cardia	Fundus	Pylorus
Gastrin	Newborn	7.14 ± 2.6	0	0	100
	2.5	179.9 ± 2.6	0	0	100
	12.5	87.4 ± 4.1	0	0	100
	20.5	96.6 ± 9.4	0	0	100
	30.0	98.4 ± 8.2	0	0	100
BPP	Newborn	32.4 ± 5.6	0	55	45
	2.5	82.7 ± 9.7	0	100[a]	0
	12.5	13.3 ± 5.8	7	93	0
	20.5	11.2 ± 2.8	4	96	0
	30.0	9.0 ± 1.5	0	100[a]	0
5-HT	Newborn	26.9 ± 4.4	0	92	8
	2.5	55.6 ± 8.0	0	71	29
	12.5	57.1 ± 12.0	11	29	60
	20.5	102.9 ± 6.9	2	17	81
	30.0	68.4 ± 5.6	4	21	75
Glucagon	Newborn	3.6 ± 1.2	0	100	0
	2.5	29.7 ± 2.1	8	90	2
	12.5	5.6 ± 1.4	0	100	0
	20.5	4.2 ± 1.5	0	100	0
	30.0	7.2 ± 0.9	0	100	0
Somatostatin	Newborn	15.6 ± 3.9	0	84	16
	2.5	70.9 ± 5.4	0	52	48
	12.5	125.9 ± 17.2	5	14	81
	20.5	137.3 ± 19.8	4	24	72
	30.0	148.3 ± 16.5	11	7	82

[a] BPP: at 2.5 cm, 35% of positive cells at cardiopyloric junction; at 30.0 cm, 25% at this junction.

reported to decrease in the fundus and increase in the pylorus of the prenatal human stomach, their numbers in the pylorus eventually exceeding those in the fundus (Creutzfeldt and Arnold 1978; Stein et al. 1983). Although this close relationship between these three enteroendocrine cells begins early in the opossum, it is not fully established until weaning and thereafter is characteristic of the pylorus of this species. Ultrastructurally, it is not uncommon to find eight to ten enteroendocrine cells immediately adjacent to one another (Fig. 24). A similar close association of somatostatin- and glucagon-immunoreactive cells is reported to occur in the developing fundic stomach of the human fetus (Stein et al. 1983).

In *Didelphis*, gastrin-immunoreactive cells are at their greatest density at the end of the 1st postnatal week (Krause et al. 1986), which coincides with the rapid increase in number of parietal cells and the development of oxyntic glands (Krause et al. 1976b). Mitotic activity within the oxyntic mucosa is at its greatest during the first weeks of postnatal life. Cells immunoreactive for pancreatic polypeptide and glucagon also are at their highest concentration during the 1st week of life, but then decrease markedly in number as devel-

Table 28. Distribution of endocrine cells in the stomach (Krause et al. 1985)

Organ	Area	Cell/mm^2	%
Stomach	Cardia	5.59	1.86
	Fundus	22.07	7.36
	Pylorus	271.87	90.67

Table 29. Absolute number of immunoreactive cells per mm^2 of mucosa of the stomach (Krause et al. 1985)

	Stomach area		
	Cardia	Fundus	Pylorus
CCK	–	–	–
Glucagon	–	2.69	–
Gastrin	–	–	138.4
BPP	–	4.26	–
Somatostatin	4.59	7.25	101.9
Secretin	–	–	–
Motilin	–	–	–
Neurotensin	–	–	–
5-HT	1.00	7.87	31.57
GIP	–	–	–

opment progresses. In contrast, somatostatin- and 5-HT-immunoreactive cells do not occur in adult numbers until weaning as new cells are established.

In the stomach of the adult opossum, 90.7% of the enteroendocrine cells occur in the pyloric region (Table 28); of these, the majority are gastrin- and somatostatin-immunoreactive cells, and there are a lesser number of cells immunoreactive for 5-HT (Table 29). The oxyntic glands contain cells that are immunoreactive for glucagon, BPP, somatostatin, and 5-HT. The same four enteroendocrine cell types are present in the oxyntic glands of the koala (Yamada et al. 1987), but in the honey possum only cells that are immunoreactive for 5-HT and somatostatin were observed (Yamada et al. 1989).

4.2.6 Submucosa

At birth the submucosa of the stomach of the opossum is poorly defined. It is separated from the lamina propria only by a thin, incomplete muscularis mucosae that consists of a layer of scattered myoblasts. Throughout development and in the adult, the submucosa consists of collagen, reticular, and elastic fibers embedded in an amorphous ground substance. It contains those connective tissue cells that normally are associated with loose connective tissue, and houses blood vessels of larger caliber that provide smaller tributaries to supply the physiological needs of the gastric mucosa. Neurons of

Table 30. Thickness and mitotic activity of the muscularis externa of the stomach in the opossum (Cutts et al. 1978a)

Body length (cm)	Thickness of muscularis externa (μm)	No. mitotic cells per 1000 cells
1.5	9.44	13.88
2.5	29.58	39.22
3.5	47.19	17.16
5.5	56.63	5.72
8.0	144.75	3.27
10.0	180.62	2.12
13.0	226.56	0.49
20.0	339.84	0
Adult	662.24	0

the submucosal plexus are present. These are poorly developed in the newborn but increase in size and number through the first 4 weeks of postnatal life, after which time they appear to form a fairly stable population. In the adult, the submucosal plexus is much less dense than that observed in the small instestine (Christensen and Rick 1985).

4.2.7 Muscularis Externa

The muscularis externa of the newborn opossum stomach consists of a thin layer of smooth muscle, two to three cells thick, incompletely separated into outer and inner layers (Cutts et al. 1978a). The inner layer predominates and forms a complete investment for the stomach. In contrast, the outer layer is formed by a single layer of scattered myoblasts and appears to be discontinuous. The muscularis externa is well developed and clearly organized into its two component strata by the end of the 1st postnatal week. The outer layer, although thinner than the inner, now is continuous and several cells thick. Throughout development and into adulthood, the muscularis externa increases in thickness, with the inner layer continuing to remain the thickest and most prominent (Table 30). An additional third layer, the innermost oblique layer, is present in *Didelphis*, but only in the proximalmost portion of the stomach.

Mitotic activity is highest during the first 2 postnatal weeks, with a peak occurring at the end of the 1st week (Table 30). Although the number of dividing cells declines rapidly, a considerable amount of mitotic activity continues well into the postnatal period. A low rate of mitotic activity can be seen in the muscularis externa of juvenile opossums, and occasional mitotic figures can be found even in the adult. Most of the dividing cells occur at the junction of the two muscle strata and at the junction of the inner muscle layer with the submucosa. Only occasionally are mitotic figures seen in the central regions of the two muscle layers (Cutts et al. 1978a).

Development of the opossum muscularis externa is thought to involve both the proliferation of smooth muscle cells and the hypertrophy of the established

Table 31. Overall rate of development of the muscularis externa in the stomach from birth to juvenility (20-cm stage) (Cutts et al. 1978a)

Days of development	89
Depth of muscularis externa (μm)	
Initial	11.37 ± 1.54
At 89 days	343.91 ± 9.37
Gain in depth	332.54
Overall rate of gain (μm/day)	3.74

muscle. In the opossum stomach, the primary period of smooth muscle cell proliferation extends through the first 4 weeks of postnatal life, during which time there is little increase in the total thickness of the muscularis externa (Cutts et al. 1978a). Thereafter, mitotic activity continues, even in the adult, but at a much reduced rate. Continued proliferative activity in smooth muscle of the gastrointestinal tract in adult rats and mice also has been demonstrated (Messier and Leblond 1960), and the adult muscularis externa is considered to be a slowly renewing tissue (Kaye et al. 1971). Hypertrophy appears to be a major factor in establishing the total thickness of the muscle wall in the opossum stomach and begins immediately following the main period of cell proliferation (the 4th postnatal week). Hypertrophy of the muscle cells continues for about 3 weeks, during which time the rate of expansion of the muscularis externa is about three times as great as that of the preceding 4 weeks. A second increase in thickness of the muscularis externa occurs during the period from weaning to adulthood. The overall rate of development of the muscularis externa is shown in Table 31.

Neurons (neuroblasts) of the myenteric plexus are present between the forming layers of the muscularis externa in the newborn opossum stomach. The component elements of the plexus are poorly developed initially, but increase in number and size through the first 4 weeks of postnatal life, thereafter forming a relatively stable population (Cutts et al. 1978a). Thus, elements of the myenteric plexus appear to develop in concert with the differentiation and proliferation of the smooth muscle cells that form the muscularis externa. By the time that most of the smooth muscle cells have been established, the elements of the myenteric plexus have been defined.

The myenteric plexus of the proximal opossum stomach consists of thin, intersecting nerve bundles with a uniform distribution of large ganglia interposed at the intersections (Christensen et al. 1983). Neurons within the ganglia are compactly arranged. Most ganglia in the proximal stomach are intrafascicular, except for a few parafascicular ganglia located just distal to the cardia. Superimposed on this basic pattern of nerve elements are several thick nerve fascicles. The myenteric plexus of the proximal region also contains thick nerve fascicles that radiate from the lesser to the greater curvature, bypassing the ganglia, and these nerve bundles have been termed "shunt fascicles." The shunt fasicles that bypass ganglia in the proximal stomach are believed to be projections of vagal branches and may shunt vagal impulses from ganglia in the lesser curvature to those in the greater curvature. Because this region of

Table 32. Ganglion density, perikaryon density, and nerve bundle diameter in the myenteric plexus of the opossum stomach (Christensen et al. 1983)

Region	Ganglia/cm^2	Perikarya/cm^2	Nerve bundle diameter (μm)
Proximal stomach	52.0 ± 1.0	2166.8 ± 251.8	59.7 ± 3.1
Distal stomach	54.6 ± 1.3	2550.2 ± 125.3	54.9 ± 4.0

Values are means ± SD.

the stomach is involved in volume adaptation, the shunt fascicles are thought to be involved in this function (Christensen et al. 1983). The myenteric plexus of the distal stomach (pylorus) consists of thicker fascicles with large intrafascicular ganglia uniformly distributed in areas where the fascicles interesect. Shunt fascicles are not seen in the distal stomach (Christensen et al. 1983). A quantitative comparison of the myenteric plexus in these two regions of the opossum stomach is shown in Table 32.

In the distal pylorus, the inner circular layer of the muscularis externa expands into a thick ring of smooth muscle often referred to as the pyloric sphincter. In this region one to three thick nerve fascicles encircle the pylorus.

Scattered nerve fibers that show immunoreactivity for substance P, vasoactive intestinal polypeptide, gastrin-releasing polypeptide, and leu-enkephalin have been observed in the stomach wall of *Didelphis* (Krause et al. 1985). Substance P is the most uniformly and widely distributed peptide of this group and is prominent in the nerve fibers associated with the muscularis mucosae. They are particularly abundant near the esophageal-gastric junction. Nerve fibers reactive to all four substances are present within the lamina propria. Although they often pass close to the glands of a specific region, the fibers appear to be more closely associated with the smooth muscle cells that course between the glandular units within the gastric mucosa. Occasional immunoreactive fibers are seen in the submucosa, in the inner and outer layers of the muscularis externa, and around perikarya of the myenteric and submucosal ganglia (Krause et al. 1985). Cholinergic excitatory innervation usually is dominant in the outer longitudinal muscle layer. Noradrenergic inhibitory innervation is more complete in the proximal innermost oblique muscle layer and the pyloric sphincter than in the other two strata that form the muscularis externa (Anuras et al. 1974; Christensen and Torres 1975). Both cholinergic and noradrenergic innervation are distributed through the vagi.

4.2.8 Interaction Between Exocrine and Endocrine Components of the Stomach

Secretions from certain enteroendocrine cells are believed to exert inductive or inhibitory effects on the gastrointestinal tract, or to initiate secretion and/or motility during development (Johnson 1987). Secretory granules within somatostatin-containing enteroendocrine cells of the gastric mucosa are

transported in a proximodistal direction in the cell processes, away from the cell body, and are released via paracrine secretion into the intercellular space (Larsson 1984). Thus, enteroendocrine cells may control the function of cells they contact by way of a directed, process-mediated delivery of a secretory product, which is known as paracrine secretion.

Hyperplasia of the oxyntic mucosa with an increase in the number of parietal cells results from chronic administration of pentagastrin (Crean et al. 1969; Stanley et al. 1972). Gastrin also appears to be selectively targeted to specific epithelial cell types in the gastric mucosa, as well as having more general trophic actions. Gastrin stimulates DNA synthesis and the mitotic activity of glandular stem cells, and it increases the number of parietal cells; it does not, however, increase the number of chief cells (Willems et al. 1972a,b; Willems and Lehy 1975; Caes and Willems 1984; Majumdar 1984) or the growth of the pyloric mucosa. Exogenous somatostatin counteracts the trophic effect of exogenous and endogenous gastrin on the oxyntic mucosa and causes hypoplasia of parietal cells (Lehy 1984; Senegas-Balas et al. 1985). Chronic administration of somatostatin also increases the number of chief cells in mice (Senegas-Balas et al. 1985). In *Didelphis* the highest concentration of gastrin-immunoreactive cells occurs prior to weaning, corresponding to the time when the greatest number of parietal cells appear and when the oxyntic glands attain their greatest length. The largest number of mitotic figures observed within the oxyntic mucosa also occurs during this period (Krause et al. 1976b). Only after the oxyntic glands have been established do the numbers of somatostatin cells equal the numbers of gastrin cells in the pylorus of *Didelphis*. Thus, somatostatin-containing cells may modulate the differentiation of parietal cells through their action on gastrin-containing cells. Chief cells also make their initial appearance at this time.

An increase in the number of gastrin-immunoreactive cells as well as gastrin follows administration of bombesin in the rat (Lehy 1984), and cerulein has been shown to stimulate the proliferation of the pyloric epithelium (Caes and Willems 1984). These results suggest that the enteroendocrine cells of the pylorus form a dynamic population of cells that is capable of responding to various hormonal stimuli. In *Didelphis*, the vast majority of gastric enteroendocrine cells of adult animals are located in the pylorus (Tables 28, 29), where they are confined to a band that includes the bottoms of foveolae and the ducts of the pyloric glands (Krause et al. 1985). This particular region is a site of high mitotic activity that provides replacement cells for the surface lining epithelium and the underlying glands in adult animals (Stevens and Leblond 1953; Eastwood 1977; Lee and Leblond 1985a,b; Lee 1985). That the majority of enteroendocrine cells in the opossum stomach are found in this area of high mitotic activity suggests that the endocrine cells may have a relatively short life span and/or influence the differentiation of other cell types which originate from this region.

5 Small Intestine and Colon

5.1 Prenatal Development of Small Intestine and Colon

The endoderm of the 9-day opossum forms the innermost layer of the embryo and also lines the interior of the chorion (Krause and Cutts 1985b). At this stage, the embryo appears as a flattened disk and consists of ectoderm, mesoderm, and endoderm, Cell boundaries between individual endodermal cells are distinct, and microvilli on the apical surfaces of the cells are short and scattered (Fig. 26). No obvious differences are seen between the endodermal cells that line the interior of the yolk sac chorion and those that form the third layer of the opossum embryo (Krause and Cutts 1985b,c). The endoderm consists of a single layer of cuboidal cells which are joined by junctional complexes and often bear elongated processes that unite with similar processes of adjacent endodermal cells. The intercellular space formed between the processes usually appears empty. The cytoplasm of the endodermal cells is characterized by numerous free ribosomes, occasional profiles of granular endoplasmic reticulum, and scattered mitochondria. There is little morphological evidence to suggest absorptive activity by the endoderm at this time in *Didelphis*. Similar observations have been reported in the *Philander opossum* (Enders and Enders 1969).

The nuclei of opossum endodermal cells contain one or more prominent nucleoli and show an abundance of euchromatin (Krause and Cutts 1985b). Mitotic figures are common in the endodermal layer.

During the next few hours, development of the gut in *Didelphis* proceeds at a rapid rate. As a result of cephalocaudal folding of the embryo, the cephalic and caudal ends of the underlying yolk sac become incorporated into the embryo proper and form the foregut and hindgut, respectively, as in other mammalian forms (McCrady 1938). Simultaneously, the embryonic disk undergoes lateral folding to form blindly ending tubes that are lined by endoderm. The wide midgut spans the interval between the forming foregut and hindgut.

As in other species, most of the small intestine distal to the entrance of the bile duct forms from the midgut region. A narrow gut tube is established by the 10th prenatal day. Midway through the 10th day, a diverticulum from the hindgut establishes contact with the proctodeum and forms the cloacal membrane (McCrady 1938). The tail gut is at the height of its development early on day 11; the cecal diverticulum appears early in day 12. As in other mammalian species, the distal colon, rectum, and anal canal of *Didelphis* form from the hindgut region.

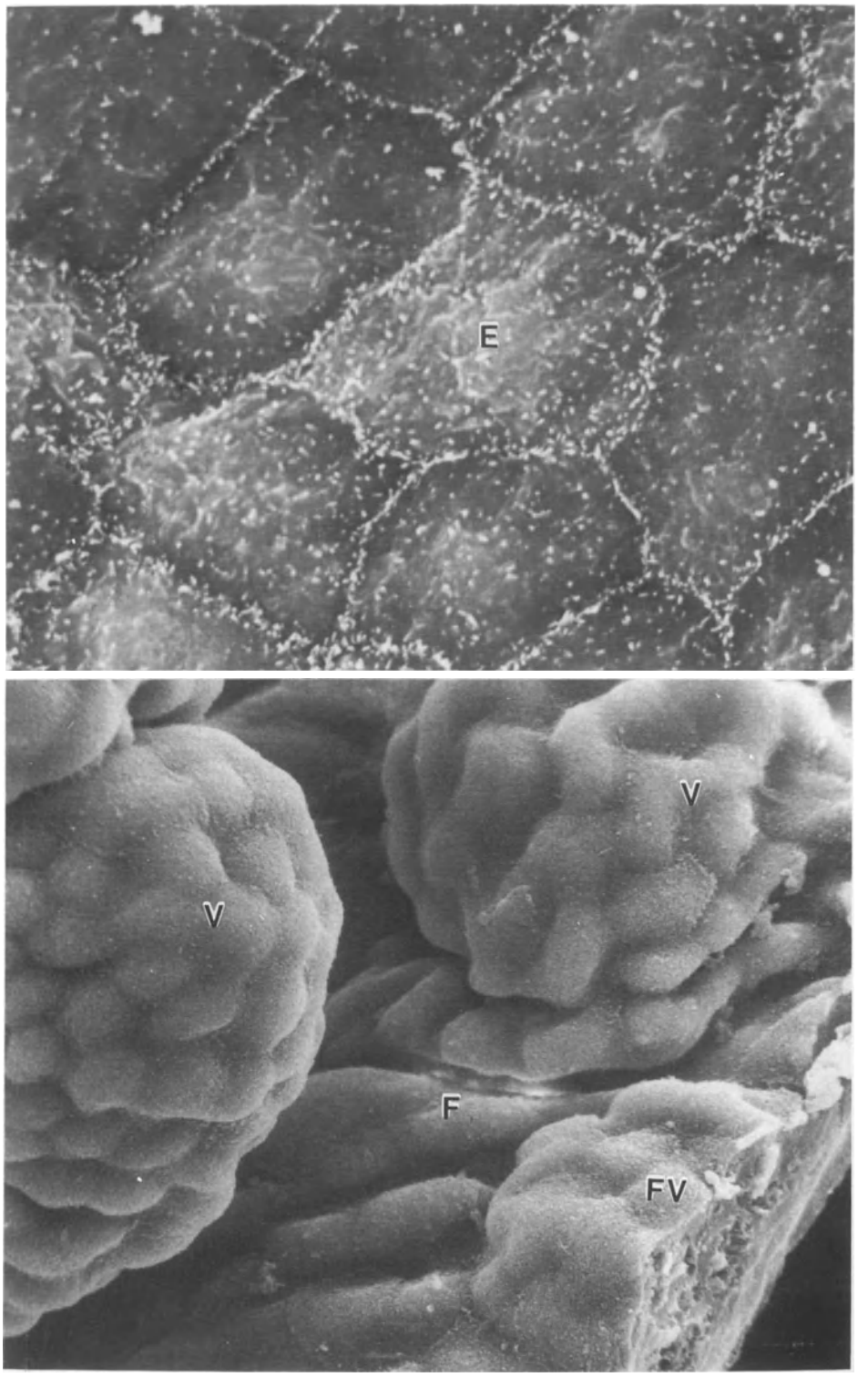

E
V
V
F
FV

Table 33. Weights of gut tube (g: mean ± SD)[a]

Body length (cm)	Male	Female	Combined male and female
1.5[b]	–	–	0.0044 ± 0.0003 (9)
2.0[b]	–	–	0.0117 ± 0.0009 (7)
2.5[b]	–	–	0.0198 ± 0.005 (12)
3.5	0.0436 ± 0.005 (9)	0.0510 ± 0.004 (6)	0.046 ± 0.009 (15)
4.0	0.0482 ± 0.005 (5)	0.0477 ± 0.005 (5)	0.0479 ± 0.005 (10)
4.5	0.0495 ± 0.006 (13)	0.0533 ± 0.011 (6)	0.0507 ± 0.008 (19)
5.0	0.063 ± 0.006 (5)	0.0575 ± 0.009 (4)	0.0605 ± 0.066 (9)
5.5	0.0708 ± 0.008 (7)	0.0725 ± 0.009 (6)	0.0716 ± 0.008 (13)
6.0	0.0921 ± 0.001 (4)	–	0.0921 ± 0.001 (4)
6.5	0.1126 ± 0.006 (5)	0.1002 ± 0.007 (6)	0.1052 ± 0.009 (11)
7.0	0.2008 ± 0.017 (5)	–	0.2008 ± 0.017 (5)
8.0	0.3014 ± 0.007 (4)	0.3062 ± 0.016 (4)	0.3038 ± 0.012 (8)
9.0	0.3957 ± 0.016 (3)	0.3977 ± 0.009 (3)	0.3967 ± 0.012 (8)
10.0	0.4841 ± 0.028 (7)	0.4953 ± 0.081 (7)	0.4896 ± 0.058 (14)
11.0	2.0727 ± 0.555 (7)	1.9942 ± 0.267 (7)	2.0335 ± 0.528 (14)
12.0	2.0374 ± 0.101 (6)	2.1554 ± 0.142 (6)	2.0964 ± 0.132 (12)
13.0	2.7193 ± 0.001 (3)	2.2654 ± 0.079 (3)	2.4919 ± 0.253 (6)
15.0	4.4123 ± 0.107 (4)	4.2394 ± 0.126 (4)	4.3254 ± 0.143 (8)
15.5	4.7689 ± 0.123 (5)	4.6131 ± 0.008 (3)	4.7104 ± 0.132 (8)
16.0	6.5053 ± 0.011 (4)	6.5359 ± 0.37 (4)	6.5211 ± 0.243 (8)
17.0	7.8225 ± 0.372 (4)	–	7.8225 ± 0.372 (4)
18.0	8.7904 ± 0.031 (4)	8.9515 ± 0.063 (2)	8.8441 ± 0.091 (6)
20.0	10.8200 ± 0.827 (6)	10.4941 ± 0.926 (5)	10.6718 ± 0.836 (11)
22.0	15.0538 ± (1)	15.0586 ± 0.064 (3)	15.057 ± 0.045 (4)
25.0	–	17.5536 ± 0.277 (3)	17.5536 ± 0.277 (3)
27.5	22.5232 ± 0.541 (3)	–	22.5232 ± 0.541 (3)
29.0	–	23.4914 ± 1.28 (3)	23.4914 ± 1.28 (3)
33.0	–	30.5645 ± 1.39 (3)	30.5645 ± 1.38 (3)
37.0	78.0532 ± 19.55 (7)	–	78.0532 ± 19.55 (7)
Adult	161.967 ± 16.2 (5)	143.057 ± 10.23 (8)	150.329 ± 19.87 (13)

[a] The numbers in parentheses, number of animals used.
[b] Sex not determined.

5.2 Postnatal Development of Small Intestine

Growth of the small intestine and colon as indicated by the total wet weight is shown in Table 33. A progressive growth of the intestinal tract occurs prior to and after weaning with no obvious difference between male and female animals. The gut shows a marked increase in weight after weaning. Relative to body weight, the weight of the intestinal tract remains fairly constant during

◀ **Fig. 26** (*above*). The luminal surface of endodermal cells (*E*) forming the innermost layer of the 9-day-old opossum embryo. ×3000
Fig. 27 (*below*). The intestinal floor (*F*), two villi (*V*), and what is thought to be a forming villus (*FV*) from the duodenum of a newborn opossum. (Krause et al. 1977a) ×800

Table 34. Weights of gut tube (g) per 100 g body weight (mean ± SD)

Body length (cm)	Male	Female	Combined male and female
1.5[a]	–	–	2.6933 ± 0.092
2.0[a]	–	–	3.2287 ± 0.213
2.5[a]	–	–	2.9963 ± 0.622
3.5	2.6322 ± 0.171	2.9003 ± 0.27	2.7930 ± 0.264
4.0	1.8454 ± 0.11	1.8799 ± 0.165	1.8627 ± 0.134
4.5	1.6566 ± 0.089	1.6388 ± 0.126	1.6509 ± 0.099
5.0	1.5813 ± 0.127	1.5601 ± 0.129	1.5718 ± 0.121
5.5	1.5109 ± 0.104	1.6188 ± 0.122	1.5599 ± 0.121
6.0	1.4293 ± 0.036	–	1.4293 ± 0.036
6.5	1.5608 ± 0.082	1.6290 ± 0.095	1.6017 ± 0.092
7.0	1.7654 ± 0.036	–	1.7654 ± 0.036
8.0	2.0215 ± 0.079	2.0929 ± 0.192	2.0571 ± 0.142
9.0	2.3120 ± 0.297	2.2682 ± 0.096	2.2898 ± 0.200
10.0	1.8559 ± 0.153	1.9131 ± 0.168	1.8845 ± 0.157
11.0	4.5386 ± 0.598	4.3657 ± 0.396	4.3379 ± 0.55
12.0	4.4595 ± 0.186	4.7191 ± 0.299	4.5889 ± 0.274
13.0	4.5097 ± 0.248	4.0776 ± 0.044	4.2936 ± 0.285
15.0	4.9241 ± 0.097	4.8259 ± 0.105	4.8751 ± 0.107
15.5	4.2059 ± 0.099	4.0762 ± 0.059	4.1572 ± 0.105
16.0	5.0934 ± 0.101	5.3509 ± 0.338	5.222 ± 0.268
17.0	5.9713 ± 0.149	–	5.9713 ± 0.149
18.0	6.4566 ± 0.037	6.3204 ± 0.059	6.4112 ± 0.081
20.0	5.6734 ± 0.267	5.5469 ± 0.263	5.6159 ± 0.26
22.0	5.9346	5.9469 ± 0.022	5.9429 ± 0.018
25.0	–	6.7168 ± 0.271	6.7168 ± 0.271
27.5	4.9153 ± 0.247	–	4.9153 ± 0.247
29.0	–	5.5646 ± 0.184	5.5646 ± 0.184
33.0	–	4.1722 ± 0.186	4.1722 ± 0.186
37.0	5.4531 ± 0.158	–	5.4531 ± 0.158
Adult	6.3322 ± 0.219	6.3195 ± 0.379	6.3153 ± 0.298

[a] Sex not determined.

the first 2 postnatal weeks, then declines slightly, only to increase markedly at 60 days (Table 34). Thereafter the weight of the gut remains fairly constant.

5.2.1 Intestinal Mucosa

The diameter of the small intestine of the newborn opossum is not uniform along its length. The proximal region shows a well-developed lumen with scattered villi, whereas the distal segments have a diameter only about one-third that of the duodenum; small, immature villi fill the lumen of the distal small intestine (Krause et al. 1977a). A well-developed vascular bed is interposed between the intestinal lining epithelium and the surrounding intestinal wall. Small elevations often extend from the intestinal floor between villi and are thought to represent the initial evaginations of new villi that form with continued growth of the gut (Fig. 27). Additional villi apparently develop as a

Table 35. Quantitative data (means ± SD) for developing small intestine (Krause et al. 1977a)

	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 ep. cells	–	–	3.0 ± 0.2	5.9 ± 0.27	17.6 ± 0.14	39.7 ± 1.1	53.7 ± 2.3
Goblet cells/1000 ep. cells	–	–	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	NM	124.7 ± 7.6	NM	168.5 ± 15.3	179.6 ± 21.4

NM, not measured.

Table 36. Distribution of Paneth cells (Krause et al. 1977a)

	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

result of evaginations of the intestinal epithelium, together with the underlying mesenchyme and vasculature, into the intestinal lumen. Early in the postnatal period, the villi show marked differences with regard to the height and state of differentiation (Table 35). Some villi are tall and club-like, whereas others are short and finger-like; these morphological differences are taken to represent different stages in the development of the villi. Villi in various states of maturation continue to be seen until just prior to weaning, when all appear to be uniform in general conformation and height. A similar pattern of late villus formation and growth has been reported in other vertebrates (Hilton 1902; Kammeraad 1942), including man (Johnson 1910).

Intestinal glands are not present in the newborn opossum and do not appear until relatively late in the postnatal period, making their initial appearance at about the end of the 7th postnatal week. The intestinal glands develop as outgrowths from the intestinal floor between adjacent villi as in other species (Hilton 1902; Kammeraad 1942), but are short, even in the adult (Table 35).

The intestinal lining epithelium consists mainly of principal intestinal epithelial cells, but does contain scattered enteroendocrine cells of various types. Mitotic figures are prominent. The depth of the intestinal lining epithelium remains fairly constant throughout development, and mitotic figures within the intestinal epithelium gradually decline until about 75 days postnatal, at which time there is a slight peak in activity (Table 35) coincident with the appearance of the intestinal glands, which become well defined at this time. Goblet and Paneth cells first appear at the end of the 2nd postnatal week and progressively increase in number as development continues. Initially, Paneth cells are scattered randomly among the principal intestinal epithelial cells that cover the villi, and only a few Paneth cells are present at the bases of the villi (Tables 35, 36). As development continues, Paneth cells become more concentrated at the bases of villi and, with the formation of intestinal glands, become even more concentrated in these structures (Table 36). A number of Paneth cells, however, remain localized along the sides and apices of villi, even in adult animals. The location of Paneth cells within the intestinal epithelium that covers villi is quite unlike the localized distribution in other vertebrate species, where they are confined largely to the bottoms of intestinal glands (Oppel 1897; Klein 1906; Castro et al. 1959; Wheeler and Wheeler 1964; Krause 1971b). Ultrastructurally, the Paneth cells contain an abundance of

granular endoplasmic reticulum that is scattered throughout the cytoplasm. The secretory granules are large, homogeneous, and exhibit considerable electron density (Krause et al. 1977a). Developing granules are intimately associated with the Golgi complexes that occupy the supranuclear region and contain a light, amorphous material that varies considerably in electron density. The granules are limited by a membrane. Numerous transport vesicles are present between nearby Golgi membranes and elements of the granular endoplasmic reticulum. These observations suggest that the synthesis of Paneth cell granules in the opossum is similar to that in the mouse (Trier et al. 1967), in which protein synthesis occurs in the granular endoplasmic reticulum; the protein then is transported to the Golgi complex, via transport vesicles, to be packaged as secretory granules.

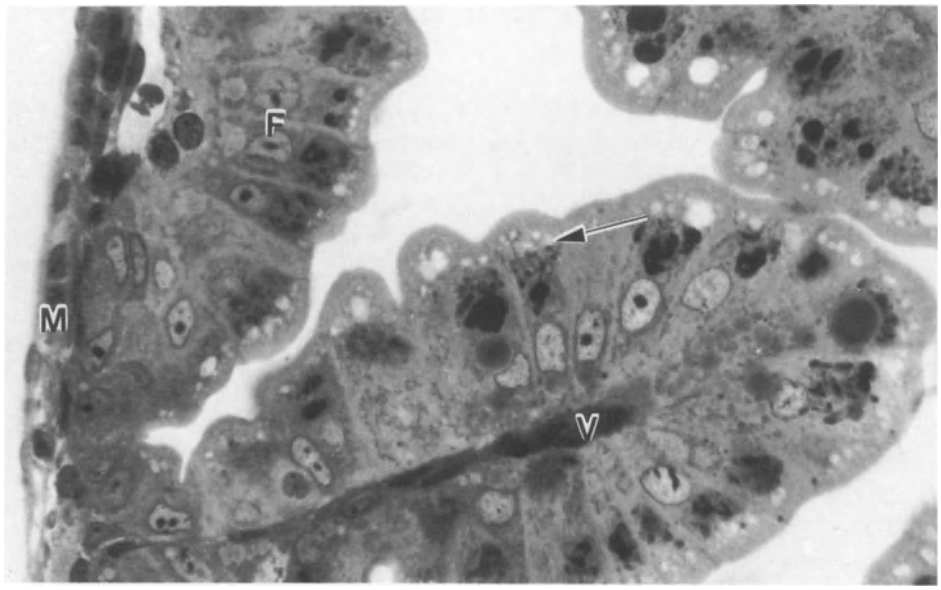
Goblet cells occur only in limited numbers early in postnatal life and do not constitute a significant population until just prior to weaning (Table 35). They stain with periodic acid-Schiff, Alcian blue, and aldehyde fuchsin methods, and are distinguished easily from the Paneth cells. Goblet cells show ultrastructural features typical of those reported in other vertebrate species.

Intestinal absorptive (principal) cells cover the villi and intestinal floor. They show an extensive apical endocytic complex, large lipid droplets, and dense irregular aggregates of material within the supranuclear region (Fig. 28). The absorptive cells lie on a large but delicate vascular bed that not only underlies the absorbing epithelium on the intestinal floor, but also extends into and forms the cores of the villi. The vascular bed is thought to aid in increasing the absorptive capacity of the small intestine.

The apical endocytic complex of the absorptive intestinal cells consists of numerous vesicles and/or anastomosing tubules that take origin from invaginations of the plasmalemma between microvilli (Fig. 29). Once formed, the tubulovesicular component expands into large, membrane-bound vacuoles that may appear empty or contain light, flocculent material. Large, irregular complexes of electron-dense material, limited by a membrane, are directly associated with, and lie immediately subjacent to, these empty-appearing vacuoles. The surrounding cytoplasm contains numerous mitochondria, scattered profiles of granular endoplasmic reticulum, and other organelles characteristic of normal intestinal epithelial cells. Small vesicles, possibly a form of transport vesicle, often are seen in the supranuclear cytoplasm and contain a light amorphous material. Lipid droplets of varying size are scattered throughout the cytoplasm.

By the end of the 1st postnatal week, the intestinal epithelial cells show an increased accumulation of lipid, most of which forms large droplets that may measure 18 μm or more in diameter. By the end of the 2nd postnatal week, the amount of lipid appears to diminish and now is present as small droplets. Later in the postnatal period, but prior to weaning, the intestinal epithelial cells show a reduction in the number of inclusions present, and the endocytic activity appears more pronounced in those cells that cover the tips of villi than in the cells at the bases of villi.

By the 75th postnatal day, fewer inclusions are seen in the intestinal epithelial cells, but in some animals a well-developed endocytic complex



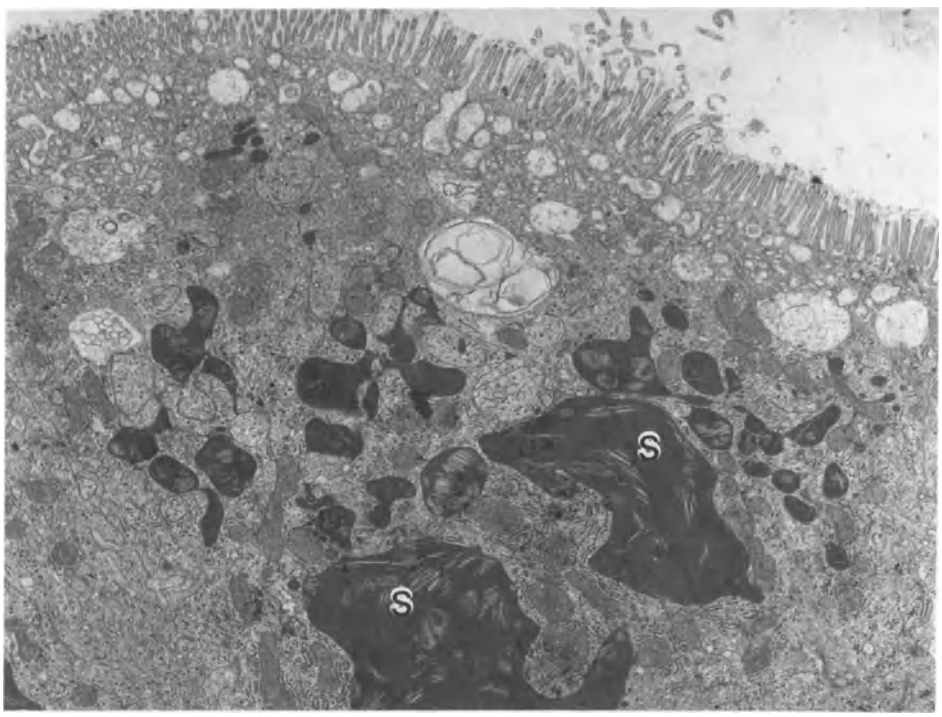


Fig. 28 (*above*). A segment of the duodenal wall from a newborn opossum illustrating a villus (*V*), a region of intestinal floor (*F*), and the very thin muscularis externa (*M*). Note the apical endocytic complex (*arrow*) and large vacuoles within the intestinal absorptive cells. ×400

Fig. 29 (*below*). The apical endocytic complex from an intestinal absorptive cell of a newborn opossum. The large supranuclear vacuoles (*S*) contain electron-dense material. In contrast, the smaller vacuoles more closely associated with the invaginations of the apical cell membrane contain a light-staining amorphous material. ×800

together with its associated inclusions may persist until weaning. At weaning (95 days postnatum) only scattered, minute vacuoles are seen in the apical cytoplasm and the apical endocytic complex is absent. Lymphoid wandering cells now appear within the intestinal epithelium with increasing frequency. The intestinal epithelium of the weaned opossum appears morphologically similar to that reported in the majority of other mammals. The principal cells are tall columnar cells with a prominent microvillus border, and the apices of adjacent cells are united by junctional complexes. The cells show a definite polarity, have basally placed nuclei, and lie on a distinct basal lamina.

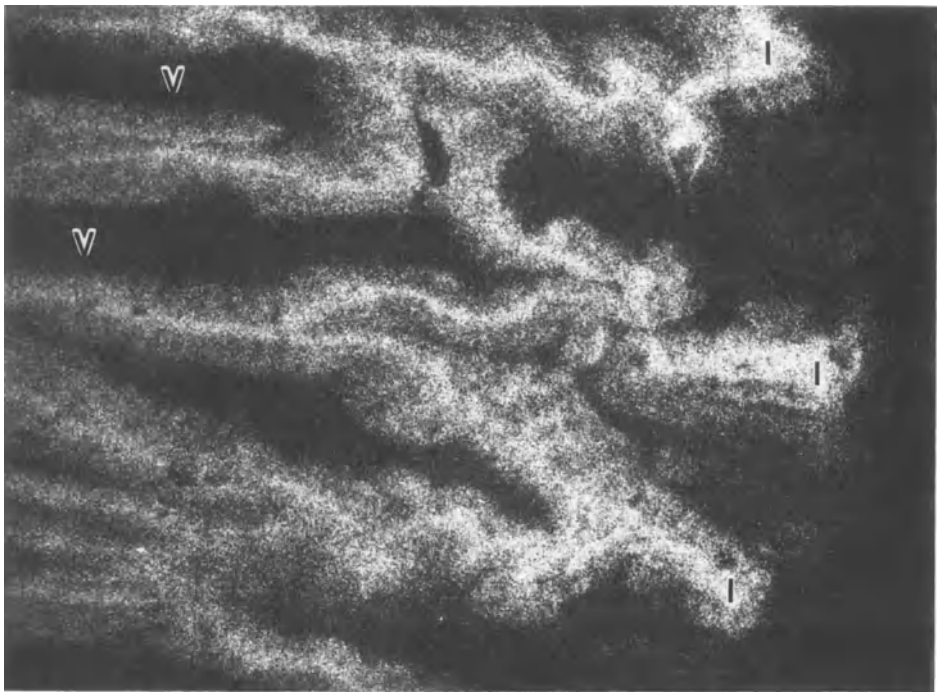
Thus, during the suckling period, the intestinal epithelium that lines the entire small intestine appears to be modified primarily for absorption. During the early periods of development this modification apparently results in the delayed appearance of intestinal glands and populations of other cell types, including the principal intestinal epithelial cells. The large vascular bed that lies immediately beneath the intestinal epithelium probably enhances the absorptive capacity of the small intestine. Endocytic complexes and vacuoles within intestinal epithelial cells have been reported in a number of fetal and suckling eutherian mammals (Clark 1959; Clarke and Hardy 1969, 1971; Cornell and Padykula 1969; Graney 1968; Hugon 1970; Kraehenbuhl and Campiche 1969; Lev and Orlic 1973; Orlic and Lev 1973; Staley et al. 1972; Williams and Beck 1969) as well as one prototherian, the echidna (Krause 1972a). The endocytic complex is essential in sequestering macromolecular protein from the intestinal lumen and is of considerable importance since it is one of the mechanisms by which passive immunity is passed from mother to offspring.

In most species, nonspecific absorptive activity is largely restricted to those epithelial cells that lie on villi within the distal small intestine. The selective transfer of immunoglobulins across the intestinal epithelium involves binding of the immunoglobulins to Fc receptors located in the microvillus cell membrane of intestinal absorptive cells that line the proximal small intestine (Rodewald 1973; Weaver and Walker 1989). Invaginations develop at the base of microvilli and form clathrin-coated vesicles which avoid lysosomal digestion. The coated vesicles move to the lateral cell membranes of the intestinal absorptive cells and discharge their contents into the intercellular space, thereby allowing immunoglobulins to cross the epithelial barrier intact (Weaver and Walker 1989). In the opossum, unlike most species, the lining cells of the entire small intestine are modified for absorption. Absorptive activity also occurs in cells that line the intestinal floor and is not restricted to cells that cover villi as in other species. However, the pathway for lipid absorption in the intestinal tract of the suckling opossum is thought to be similar to that of other species. Lipid enters the cisternae of endoplasmic reticulum and/or Golgi complex, and chylomicra are formed that ultimately are released between adjacent lateral cell membranes (Friedman and Cardell 1972a,b; Sage and Jersild 1971). The opossum does differ from many other suckling species with respect to the dimensions of the lipid droplets that accumulate in the supranuclear region, particularly during the 2nd postnatal week. The nature of the electron-dense material within the large supranuclear vacuoles is unknown, but it may represent an accumulation of sequestered protein, as reported in other species.

The large supranuclear vacuoles divide the intestinal absorptive cells into apical and basal regions and are rich in acid and alkaline phosphatase, suggesting a highly developed lysosomal system, as in other species.

In some species the system of vacuoles has considerable importance in the breakdown of absorbed materials (Shervey 1966; Kraehenbuhl and Campiche 1969; Cornell and Padykula 1969). Transport of absorbed macromolecules by intestinal epithelial cells has not only nutritional but also immunological importance. In species such as the pig and horse, passive immunity is transferred from mother to offspring largely by intestinal absorption of intact antibodies during the suckling period. In other species, such as the guinea pig and rabbit, intestinal absorption and transport of intact antibodies does not occur (Kraehenbuhl and Campiche 1969), and in these species the absorbed materials are broken down by enzymatic activity within the supranuclear vacuoles. The mouse and rat represent intermediate species where antibodies are transmitted to the young by the placenta and by intestinal absorption during suckling. The mechanism by which absorbed material is directed to the lysosomal complex of the supranuclear vacuoles to be broken down, or by which it bypasses this complex, is unknown but appears to vary between species. In the neonatal rat, the lysosomal complex can select not only among gamma globulins but also among homologous serum protein fractions (Bamford 1966). Whether or not the intestinal epithelial cells of the suckling opossum are involved in the transport of intact macromolecules through the epithelial barrier for passive immunity has not been determined. However, the transient, poorly developed placental attachment in the opossum would favor such a hypothesis (Krause and Cutts 1985b). In addition, it is known that three Australian marsupials, the tammer, possum, and quokka, derive all maternal immunoglobulins from colostrum and milk via the intestinal route (Yadav 1971).

The apical endocytic complex persists in the absorptive intestinal epithelial cells until just prior to weaning, at which time it is lost, first from the cells that cover the bases of the villi and then progressively, from cells toward the tips of the villi. Similar events occur in the rat at weaning. Simultaneously, there is a progressive loss of the endocytic complex from cells in a proximal-distal sequence down the intestinal tract (Krause et al. 1977a). The loss of the apical endocytic complex is thought to be due not to decreased activity by individual intestinal absorptive cells but to replacement by new generations of cells that are incapable of absorbing intact macromolecules, as has been reported in the rat (Clarke and Hardy 1969). There is evidence that cessation of macromolecular absorption by the intestinal epithelial cells occurs in neonates of the hamster, rabbit, guinea pig, and mouse before replacement of the initial intestinal absorptive cell population (Rundell and Lecce 1972). Such observations suggest the loss of macromolecular absorption (closure) may not be a direct consequence of the turnover of the cell population and that the process may be much more complex. Factors such as diet, growth factors, and hormones may direct the genome of the intestinal stem cells to differentiate and provide the cell type that normally is associated with the adult intestinal epithelium.



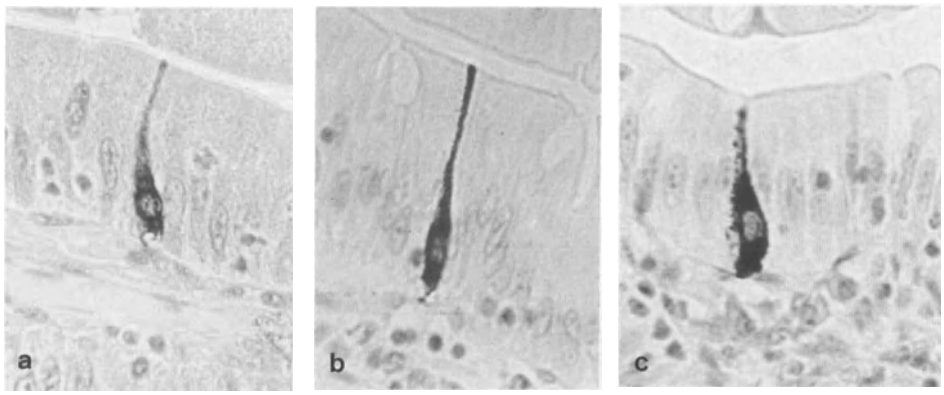


Fig. 30 (*above*). A radioautograph of a section from the proximal small intestine labeled with ^{125}I-enterotoxin demonstrates receptor localization for heat-stable enterotoxin in intestinal epithelial cells on villi (*V*) and lining intestinal glands (*I*). Dark field. ×150
Fig. 31 (*below*). **a** A gastrin-immunoreactive cell present within the intestinal epithelium. ×400. **b** A 5-HT-immunoreactive cell located within intestinal epithelium. ×400. **c** A secretin-immunoreactive cell within intestinal epithelium. ×400

Intestinal epithelial cells of all development stages examined (including adult opossums) have high receptor density for a heat-stable enterotoxin produced by *Escherichia coli* (Forte et al. 1988, 1989; Krause et al. 1990; White et al. 1989). Heat-stable enterotoxin binds specifically to receptors in the plasmalemma that form the microvillus (striated) border of *Didelphis* (Fig. 30) and, in this species, activates guanylate cyclase. Heat-stable enterotoxin is a major cause of diarrhea in children (Sack et al. 1975; Donta et al. 1977; Black

Table 37. Glucagon-immunoreactive cells in the intestinal tract of the developing opossum (no./mm^2: mean ± SD) (Krause et al. 1989b)

Organ	Body length (cm)				
	Newborn	2.5	12.5	20.5	30.0
Duodenum					
Proximal	4.77 ± 0.23	0.00	0.00	0.00	0.00
Distal	8.42 ± 4.07	0.00	0.00	0.00	0.00
Small intestine					
Seg 1	18.38 ± 3.42	0.00	0.00	0.00	0.00
Seg 2	10.39 ± 2.46	0.00	0.11 ± 0.15	0.00	0.00
Seg 3	11.34 ± 6.40	0.00	0.00	0.00	0.00
Seg 4	12.22 ± 8.75	0.00	0.00	0.00	0.00

Table 38. BPP-immunoreactive cells in the intestinal tract of the developing opossum (no./mm^2: mean ± SD) (Krause et al. 1989b)

Organ	Body length (cm)				
	Newborn	2.5	12.5	20.5	30.0
Duodenum					
Proximal	13.75 ± 13.47	1.80 ± 2.54	0.00	0.00	0.00
Distal	12.70 ± 7.98	2.66 ± 3.77	0.00	0.00	0.00
Small intestine					
Seg 1	9.33 ± 0.64	0.00	0.00	0.00	0.00
Seg 2	8.41 ± 3.39	0.85 ± 1.21	0.00	0.00	0.00
Seg 3	9.13 ± 1.52	0.00	0.49 ± 0.34	0.00	2.34 ± 0.83
Seg 4	17.80 ± 1.66	0.00	0.15 ± 0.21	0.00	3.24 ± 0.99

et al. 1982a,b) and in laboratory and domestic animals (Burgess et al. 1978). In *Didelphis* it causes a marked increase in the volume of intestinal secretion (secretory diarrhea). Heat-stable enterotoxin inhibits sodium chloride absorption and stimulates chloride secretion by the intestinal epithelial cells. The occurrence of membrane receptors for heat-stable enterotoxin in epithelial cells other than those that line the intestinal tract (gallbladder, cystic duct, common bile duct, trachea, epithelial cells of the duodenal glands, and proximal tubules of the kidneys) suggests the presence of a naturally occurring endogenous ligand that may be linked to the regulation of sodium chloride transport in various epithelia of the opossum (Krause et al. 1990).

5.2.2 Enteroendocrine Cells

Enteroendocrine cells that are immunoreactive for glucagon (glicentin), bovine pancreatic polypeptide (BPP), serotonin (5-HT), gastrin, somatostatin, motilin, gastric inhibitory peptide (GIP), secretin, and cholecystokinin (CCK)

Table 39. 5-HT-immunoreactive cells in the intestinal tract of the developing opossum (no./mm^2: mean ± SD) (Krause et al. 1989b)

Organ	Body length (cm)				
	Newborn	2.5	12.5	20.5	30.0
Duodenum					
Proximal	26.48 ± 8.68	37.17 ± 13.92	47.46 ± 2.15	27.56 ± 3.23	119.22 ± 10.42
Distal	26.71 ± 6.78	16.37 ± 5.19	51.21 ± 17.49	21.50 ± 4.16	129.81 ± 15.90
Small intestine					
Seg 1	19.15 ± 12.96	28.20 ± 9.85	27.05 ± 6.40	15.53 ± 6.54	91.14 ± 6.28
Seg 2	17.07 ± 7.58	27.81 ± 5.26	20.53 ± 0.87	17.08 ± 3.50	108.57 ± 13.25
Seg 3	22.33 ± 7.03	9.94 ± 7.79	46.65 ± 4.87	16.77 ± 1.15	53.15 ± 29.70
Seg 4	21.91 ± 8.41	21.08 ± 6.86	35.53 ± 2.08	42.24 ± 7.03	87.09 ± 13.40

Table 40. Gastrin-immunoreactive cells in the intestinal tract of the developing opossum (no./mm^2: mean ± SD) (Krause et al. 1989b)

Organ	Body length (cm)				
	Newborn	2.5	12.5	20.5	30.0
Duodenum					
Proximal	12.22 ± 0.56	11.43 ± 3.20	10.50 ± 1.34	35.79 ± 4.41	16.76 ± 4.40
Distal	21.96 ± 2.40	15.76 ± 5.20	10.78 ± 3.39	41.07 ± 10.17	27.62 ± 8.15
Small intestine					
Seg 1	7.86 ± 9.90	6.25 ± 1.50	2.33 ± 0.39	24.96 ± 7.54	21.79 ± 8.39
Seg 2	4.66 ± 5.23	12.22 ± 8.74	2.15 ± 0.77	21.66 ± 0.73	20.72 ± 7.80
Seg 3	2.38 ± 1.68	9.72 ± 4.90	0.18 ± 0.25	20.71 ± 5.75	15.67 ± 7.19
Seg 4	7.37 ± 5.40	3.33 ± 4.70	0.00	10.25 ± 1.38	12.88 ± 9.20

are scattered within the intestinal epithelium of the newborn opossum (Krause et al. 1989b). Of these peptide-containing cells, somatostatin-, gastrin-, and 5-HT-immunoreactive cells show an adult distribution (Krause et al. 1985). The number and distribution of individual types of immunoreactive enteroendocrine cells are shown in Tables 37–46. Enteroendocrine cells immunoreactive for glucagon and BPP are present throughout the small intestine of the newborn, but rapidly decrease in number so that by the end of the 1st postnatal week they are seen only as occasional, scattered cells (Tables 37, 38). The 5-HT-immunoreactive cells are present throughout the small intestine, but they are concentrated proximally. Their numbers increase continuously up to weaning, then decrease, only to increase once more during the postweaning period (Table 39). Cells immunoreactive for gastrin progressively increase in number from birth until weaning, then decline (Table 40). Somatostatin-immunoreactive cells occur throughout the length of the small intestine of the newborn and gradually decrease in number until weaning, at which time their numbers increase dramatically to equal those of weaned animals (Table 41). Cells that are immunoreactive for motilin, GIP, secretin, and CCK generally

Table 41. Somatostatin-immunoreactive cells in the intestinal tract of the developing opossum (no./mm^2: mean ± SD) (Krause et al. 1989b)

Organ	Body length (cm)				
	Newborn	2.5	12.5	20.5	30.0
Duodenum					
Proximal	5.23 ± 7.41	14.43 ± 2.84	6.55 ± 0.52	32.17 ± 3.33	35.40 ± 3.47
Distal	6.66 ± 9.42	7.96 ± 6.55	5.79 ± 1.26	29.39 ± 5.80	28.96 ± 4.22
Small intestine					
Seg 1	10.64 ± 2.11	4.73 ± 3.38	1.91 ± 0.42	20.26 ± 2.95	28.11 ± 2.61
Seg 2	11.78 ± 2.26	4.67 ± 3.82	2.12 ± 0.59	17.87 ± 2.73	22.12 ± 3.39
Seg 3	12.70 ± 8.98	2.77 ± 3.93	2.78 ± 0.69	26.51 ± 5.66	19.75 ± 1.88
Seg 4	5.74 ± 1.83	7.22 ± 6.14	1.55 ± 1.40	21.79 ± 6.23	20.51 ± 2.57

Table 42. Motilin-immunoreactive cells in the intestinal tract of the developing opossum (no./mm^2: mean ± SD) (Krause et al. 1989b)

Organ	Body length (cm)				
	Newborn	2.5	12.5	20.5	30.0
Duodenum					
Proximal	1.99 ± 2.81	2.95 ± 0.12	13.23 ± 1.53	21.43 ± 4.91	17.48 ± 3.87
Distal	1.96 ± 2.77	3.16 ± 2.84	11.94 ± 4.23	21.50 ± 4.08	24.49 ± 7.46
Small intestine					
Seg 1	0.00	1.23 ± 1.74	4.19 ± 2.10	8.40 ± 2.96	15.45 ± 8.58
Seg 2	0.00	0.00	0.25 ± 0.18	2.31 ± 0.32	2.09 ± 1.47
Seg 3	0.00	0.00	0.75 ± 0.73	0.33 ± 0.24	0.17 ± 0.13
Seg 4	0.00	0.00	0.16 ± 0.22	0.00	0.19 ± 0.13

are concentrated in the duodenum early in development, then increase in number and are found more distally with age (Tables 42–45). Cells immunoreactive for motilin and secretin remain concentrated primarily in the proximal small intestine, whereas CCK- and GIP-immunoreactive cells become more evenly distributed throughout the small intestine with age. A similar proximal to distal progression of enteroendocrine cells has been reported in the developing gut of the rat (Larsson et al. 1974), pig (Alumets et at. 1983), and man (Larsson et al. 1975; Chayvialle et al. 1980).

Enteroendocrine cells immunoreactive for neurotensin are unique in that they do not appear until about the 75th postnatal day and are observed in the distal segments of the small intestine (Table 46). They increase in number with age and follow a distal to proximal progression in their distribution in the intestinal epithelium of the small intestine.

Immunoreactive enteroendocrine cells within the intestinal epithelium of the developing and adult opossum, although randomly scattered, tend to occur as small clusters of cells expressing similar immunoreactivity (Krause et al. 1985, 1989b). Such grouping results in considerable variation in the distribution of a specific enteroendocrine cell type within a given intestinal segment and

Table 43. GIP-immunoreactive cells in the intestinal tract of the developing opossum (no./mm^2: mean ± SD) (Krause et al. 1989b)

Organ	Body length (cm)				
	Newborn	2.5	12.5	20.5	30.0
Duodenum					
Proximal	4.44 ± 6.28	0.46 ± 0.64	2.22 ± 0.76	12.09 ± 6.33	1.20 ± 0.45
Distal	0.00	0.00	2.10 ± 0.73	10.54 ± 2.94	1.52 ± 0.44
Small intestine					
Seg 1	3.03 ± 4.28	1.38 ± 0.99	1.59 ± 0.19	8.65 ± 0.87	3.27 ± 1.44
Seg 2	7.97 ± 6.35	0.00	1.56 ± 0.14	13.56 ± 2.17	1.95 ± 1.42
Seg 3	0.00	0.00	1.74 ± 1.13	1.19 ± 0.09	2.42 ± 1.81
Seg 4	0.00	0.00	0.00	0.00	0.36 ± 0.51

Table 44. Secretin-immunoreactive cells in the intestinal tract of the developing opossum (no./mm^2: mean ± SD) (Krause et al. 1989b)

Organ	Body length (cm)				
	Newborn	2.5	12.5	20.5	30.0
Duodenum					
Proximal	0.98 ± 1.38	4.04 ± 0.92	1.78 ± 0.97	14.81 ± 1.66	14.83 ± 2.24
Distal	1.77 ± 2.51	3.63 ± 2.82	1.02 ± 0.32	11.31 ± 12.49	11.03 ± 4.03
Small intestine					
Seg 1	0.00	0.00	0.54 ± 0.57	0.00	9.07 ± 6.20
Seg 2	0.00	0.00	0.00	0.00	0.69 ± 0.21
Seg 3	0.00	0.00	0.00	0.00	1.38 ± 0.22
Seg 4	0.00	0.00	0.00	0.00	1.39 ± 0.35

Table 45. CCK-immunoreactive cells in the intestinal tract of the developing opossum (no./mm^2: mean ± SD) (Krause et al. 1989b)

Organ	Body length (cm)				
	Newborn	2.5	12.5	20.5	30.0
Duodenum					
Proximal	0.00	9.19 ± 1.29	7.21 ± 2.41	16.40 ± 8.17	13.40 ± 1.28
Distal	1.45 ± 2.05	9.22 ± 3.22	6.13 ± 1.65	31.07 ± 13.98	14.88 ± 2.92
Small intestine					
Seg 1	3.92 ± 5.54	1.28 ± 1.81	3.00 ± 1.04	25.49 ± 7.42	14.23 ± 4.22
Seg 2	0.00	0.00	2.15 ± 0.73	16.98 ± 3.02	15.55 ± 9.54
Seg 3	3.70 ± 5.23	0.00	1.42 ± 0.69	11.06 ± 5.28	13.27 ± 9.27
Seg 4	1.11 ± 1.57	0.00	0.00	0.00	9.04 ± 7.99

Table 46. Neurotensin-immunoreactive cells in the intestinal tract of the developing opossum (no./mm^2: mean ± SD) (Krause et al. 1989b)

Organ	Body length (cm)				
	Newborn	2.5	12.5	20.5	30.0
Duodenum					
Proximal	0.00	0.00	0.00	0.00	0.00
Distal	0.00	0.00	0.00	0.00	0.40 ± 0.56
Small intestine					
Seg 1	0.00	0.00	0.00	0.00	1.34 ± 1.89
Seg 2	0.00	0.00	0.00	6.82 ± 2.41	16.54 ± 4.37
Seg 3	0.00	0.00	5.89 ± 1.45	37.32 ± 6.53	33.81 ± 11.76
Seg 4	0.00	0.00	22.08 ± 10.14	147.87 ± 35.50	52.27 ± 33.60

may account for the large standard deviation seen in the numbers of some immunoreactive cells (Krause et al. 1989b). The clustering together of specific immunoreactive enteroendocrine cell types at random foci suggests that enteroendocrine cells differentiate in small clones rather than as scattered, individual cells. Undifferentiated cells (possibly "stem" cells) within the intestinal glands are linked by functional gap junctions (Bjerknes et al. 1985), and these cells are thought to respond to some form of feedback from cells on villi via a chalone-like mechanism (Cairnie 1976; Rijke et al. 1976; Al-Nafussi and Wright 1982). Differentiation and proliferation of intestinal epithelial cells apparently are under some form of regulation. The life span of the enteroendocrine cell in the opossum is thought to be relatively short (7–10 days), as in other species. These cells are exfoliated from the tips of the villi, along with other cells that make up the intestinal epithelium, during the normal, continuous replacement and renewal of this tissue. What factors control their differentiation in the opossum is unknown.

Enteroendocrine cells on villi usually are spindle shaped and each possesses an elongated, apical cytoplasmic process (Fig. 31) that makes contact with the intestinal lumen. Some enteroendocrine cells within the intestinal glands likewise show this morphology. A number of round and oval immunoreactive cells that are confined to the basal region of the intestinal epithelium also are observed. This latter type of enteroendocrine cell lacks the apical cytoplasmic process but often shows a process that runs along the basal lamina and extends beneath adjacent cells. Such features are observed especially in somatostatin-immunoreactive cells, although other immunoreactive enteroendocrine cells on occasion may show a similar morphology. A few of the spindle-shaped enteroendocrine cells may show both apical and basal cytoplasmic processes. No apparent correlation can be made between the open and closed morphological forms of enteroendocrine cells and a specific immunoreactivity in *Didelphis*.

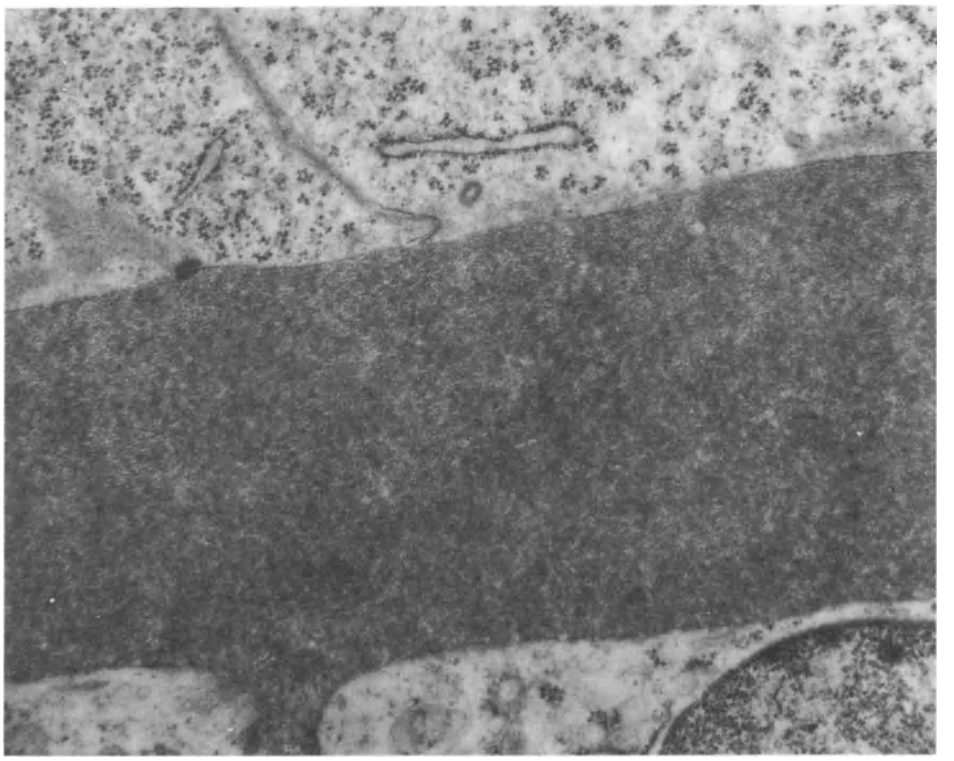
The enteroendocrine cells show several common ultrastructural features. Most show an electron-lucent cytoplasm that contains scattered profiles of granular endoplasmic reticulum, small Golgi complexes, scattered filaments, secretory granules, and occasional mitochondria. The secretory granules vary

in size and morphology depending on the specific enteroendocrine cell type, but they have not been characterized ultrastructurally in the opossum.

5.2.3 Lamina Propria

A delicate, vascular connective tissue lies between the intestinal epithelium and the developing muscularis externa early in the postnatal period. Not until about the end of the 3rd postnatal week do smooth muscle cells appear, having differentiated from the adjacent mesenchymal cells in the proximal small intestine. A definite muscularis mucosae is present in the small intestine by the end of the 4th week, and smooth muscle cells can be observed extending into the connective tissue core of the villi. As development progresses, the connective tissue elements make up a greater proportion of the lamina propria. The extensive capillary bed seen in the early stages of development remains intimately associated with the intestinal epithelium, separated from it only by a thin basement membrane. This capillary network remains in close association with the intestinal epithelium over the villi and with the developing intestinal glands throughout all older developmental stages, including the adult. Larger blood vessels, lacteals, coarse connective tissue fibers, and connective tissue cells progressively come to form a major portion of the cores of villi in the expanding lamina propria. At weaning, the lamina propria is well developed and contains numerous connective tissue cells, primarily lymphocytes, macrophages, and plasma cells.

In weaning animals, two membranes appear beneath the intestinal glands and limit the lamina propria (Krause and Leeson 1969a). After weaning, these are well established; one membrane forms a series of cup-like structures that embrace the bottoms of the intestinal glands, while the other forms a layer on the luminal side of the muscularis mucosae. Both laminae show continued development throughout life and may attain a depth of 25 μm or more. The external membrane forms a continuous sheet pierced only by blood vessels and lymphatics that enter the lamina propria from the submucosa. Silver, orcein, and Alcian blue staining methods show a concentration of dense material within the interior membrane that is surrounded by a thick matrix of homogeneous material. Some regions of the membranes appear to have fibrillar connections with surrounding connective tissue fibers. Ultrastructurally, the internal membrane consists primarily of a finely granular or fibrillar material (Fig. 32). The internal membrane is thought to replace or blend with the basement membrane that underlies the epithelium of the intestinal glands. The basal cytoplasm of adjacent intestinal epithelial cells frequently shows concentrations of electron-dense material and is thought to contribute, at least in part, to the internal membrane. The external membrane is less regular in outline than the internal membrane due to the extension of fibrillar material at its interface with the muscularis mucosae. The surface of the external membrane that faces the lamina propria also is irregular in outline. Unlike the internal membrane, the external membrane contains numerous formed fibrils, some of which show an indefinite periodicity. They form a close meshwork but do not display a regular arrangement.



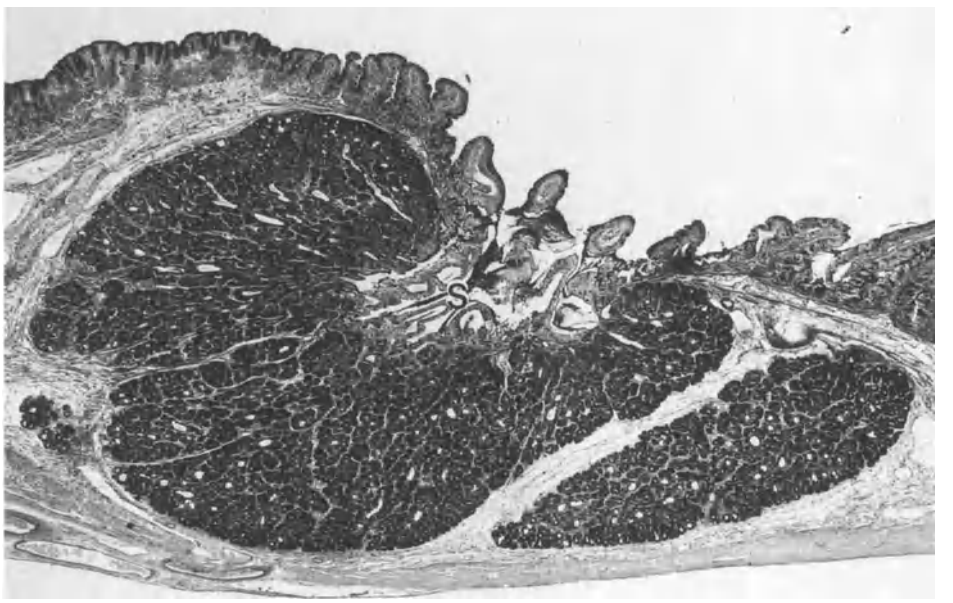


Fig. 32 (*above*). An electron micrograph of the internal limiting membrane. The cytoplasm of two intestinal epithelial cells is shown near the *top* of the electron micrograph. ×10 000
Fig. 33 (*below*). Brunner's glands from an adult male opossum. The duodenal sinus (*S*) is shown near the center of the glandular mass. (Krause and Leeson 1969b) Periodic acid-Schiff, ×12

Both membranes stain with the PAS method after digestion with diastase and/or saliva and fail to stain with Alcian blue (at either pH 2.5 or 1.0) or with toluidine blue. Prior treatment with collagenase reduces the membranes to about one-half their original thickness (Krause and Leeson 1969a), but the reduction is not uniform, some areas being more susceptible to collagenase digestion than others. The electron microscopic and histochemical observations suggest that the membranes are made of a collagen-like protein enveloped in a complex, neutral glycoprotein of unknown composition (Krause and Leeson 1969a). Since both membranes appear just after weaning, their appearance is most likely related to the ingection of materials other than mother's milk. Although an analysis of opossum milk has yet to be done, several antiparasitic, antibacterial, antiviral, and antifungal factors have been reported in eutherian milk (Hanson et al. 1988; Hernell and Bläckberg 1988). It is quite possible that similar factors exist in opossum milk and that with loss of these agents at weaning, the lamina propria responds to a variety of factors present in the opossum's new diet. As the membranes increase in width, numerous connective tissue cells, primarily macrophages, lymphocytes, plasma cells, and eosinophils, fill the interstices between membranes, resulting in a thick, complete sleeve of connective tissue cells that surrounds the intestinal tract throughout its length. It is of interest that only a few Peyer's patches are found along the intestinal tract of juvenile and adult opossums. The large population of connective tissue cells sandwiched between the limiting membranes of the lamina propria may act as a defensive barrier against the invasion of foreign organisms from the intestinal lumen. Similar appearing membranes within the intestinal lamina propria occur in a number of Australian marsupials (Krause 1972b) as well as the platypus (Krause 1975a).

5.2.4 Submucosa

The submucosa remains undefined until about 21 days into the postnatal period and becomes apparent with the appearance of the muscularis mucosae. Initially, it is composed of a scant, delicate, connective tissue, but it becomes more compact and consists of larger connective tissue fibers as development progresses. It is organized into plicae proximally and, as in other species, houses larger blood vessels that course around the circumference of the small intestine, providing smaller tributaries to the mucosa.

5.2.5 Submucosal (Meissner's) Plexus

Little if any information is available concerning the submucosal nerve plexus in the developing opossum, but it has been studied in detail in the adult animal (Christensen and Rick 1985). Ganglia within the submucosa generally are interconnected by nerve bundles of variable sizes and have an irregular distribution. Most ganglia consist of three or more neurons (Table 47); single or two nerve cell bodies do occur but are very rare (Christensen and Rick 1985).

Table 47. Number of ganglia per cm^2 and number of perikarya per ganglion in the submucosal plexus of the opossum small intestine (Christensen and Rick 1985)

Organ	Ganglia per cm^2	Perikarya per ganglion
Duodenum	161.8 ± 21.2	11.2 ± 0.6
Jejunum	214.8 ± 15.1	10.4 ± 0.6
Ileum	148.8 ± 9.0	10.0 ± 0.6

Values are means ± SD.

5.2.6 Duodenal (Brunner's) Glands

The duodenal glands of the opossum begin to differentiate from the intestinal lining epithelium just distal to the gastrointestinal junction soon after birth. Each invagination results in the initial formation of the duct system of a single duodenal gland (Krause and Leeson 1969c). As development continues, the duct system branches repeatedly, follows a tortuous course, and gives rise to a complex structure. Portions of the initial duct system radiate laterally in a stellate fashion, then subdivide into several intralobular ducts. By the 3rd week of postnatal life, most of the developing ducts are intralobular ducts. Secretory units (tubules and acini) first appear at about the 5th week of postnatal life. The duodenal glands develop independently but in very close approximation to one another. The evagination and development of several closely associated duodenal glands result in formation of funnel-shaped mucosal depressions between glands, and the depression may be lined by gastric and/or intestinal epithelium (Krause and Leeson 1969b). As a result, the compound tubuloacinar glands of the adult drain via a single excretory duct either into sinuses or independently into the intestinal lumen. Human duodenal glands, as well as those of most other species investigated to date, develop prior to birth from the bottoms of intestinal glands (Johnson 1910; Krause 1987). In species such as the rat, a few proximal glands may form directly from the intestinal epithelium early in development, but glands that form later and more distally differentiate from intestinal glands (Krause and Leeson 1967).

In the adult opossum, the duodenal glands form a large, lobed, glandular collar immediately distal to the pyloric sphincter (Fig. 33). The limited distribution and complex nature of the duodenal glands is a feature shared by most marsupial species (Krause 1972b, 1973). The two monotremes (the platypus and the echidna) also have complex duodenal glands, but in these species the glands drain into the distal stomach (Krause 1970, 1971a). The large, complex nature of the duodenal glands in these more primitive mammals makes them quite unlike those seen in most eutherian species, in which small individual glands are scattered for variable distances along the proximal small intestine (Krause 1975b, 1980, 1981; Landböe-Christensen 1944).

The secretory units consist of tall, pyramidal-shaped cells that contain numerous secretory granules which stain intensely with the Schiff reagent. The intensity of the reaction is unaffected by prior treatment with saliva or diastase,

and the granules fail to stain with toluidine blue or Alcian blue, suggesting the presence of a neutral glycoprotein. The ductal epithelium is Alcian blue positive at pH's 1.0 and 2.5, indicating the presence of acidic glycoproteins.

Scattered enteroendocrine cells which are immunoreactive for somatostatin, gastrin, motilin, BPP, and 5-HT also are present in the duodenal glands of the opossum (Krause 1987). Motilin-immunoreactive cells often are seen concentrated within the intestinal epithelial lining of the funnel-shaped depressions that receive the ducts of the duodenal glands (Krause et al. 1985).

Although the duodenal glands generally are considered to consist of mucin-producing cells, the ultrastructural features of the cells are more characteristic of serous cells. The round or oval nuclei are located near the bases of the cells, and secretory granules fill the apical cytoplasm. Profiles of granular endoplasmic reticulum fill the basal and perinuclear regions and often extend into the apical region of the cells as well. Golgi elements are well developed, extensive, and occupy the supranuclear region, where they are associated with numerous secretory granules at their maturing face and with an abundance of transport vesicles that originate from granular endoplasmic reticulum at the forming face.

Synthesis of glycoproteins by the cells that form the opossum duodenal glands is thought to follow a pathway similar to that described in the duodenal glands of the mouse. The protein moiety is synthesized by the granular endoplasmic reticulum and is carried via transport vesicles to the Golgi complex, where it is complexed to the carbohydrate moiety (Rohr et al. 1967; Schmalbeck and Rohr 1967). A similar pathway exists in a variety of cells involved in the synthesis of glycoprotein (Alberts et al. 1989). The amorphous secretory granules from cells of the opossum duodenal glands are limited by a membrane and have a mottled appearance due to scattered regions of increased electron density. Granules with a similar mottled appearance occur in the secretory cells of the human cardiac, pyloric, and duodenal glands (Krause et al. 1977b, 1978b; Krause 1987). It is thought that the regions of variable electron density indicate different substances within a given secretory granule. Ultrastructural immunocytochemical studies have shown that such a segregation of materials does occur within the secretory granules of human pancreatic A cells (Ravazzola and Orci 1980), serous cells of human salivary glands (Machino et al. 1986), and mucous neck cells of the calf stomach (Yamada et al. 1988).

The secretions of the duodenal glands form a thin coat of glycoprotein over the intestinal epithelium following a meal. This layer is thought to provide a zone of stability for the epithelial surface and may delay the diffusion of hydrogen ions and other substances toward the mucosa, allowing sufficient time for them to be neutralized by the bicarbonate that originates from the surface epithelium.

Epidermal growth factor (EGF) has been identified immunohistochemically in the duodenal glands of the mouse (van Noorden et al. 1977), rat (Kirkegaard et al. 1983), and man (Heitz et al. 1978). Although EGF is known to stimulate cell growth and differentiation and to have cytoprotective effects on the intestinal mucosa (Dembiński et al. 1982; Hollenberg 1979; Konturek et al. 1988; Ménard 1989), its precise role in the secretion of the duodenal glands is unknown.

Table 48. Thickness and mitotic activity of muscularis externa of the small intestine (Cutts et al. 1978a)

Body length (cm)	Thickness of the muscularis externa (μm)	No. mitotic cells per 1000 cells
1.5	18.75	15.0
2.5	25.00	12.5
3.5	28.13	3.8
5.5	37.50	5.0
8.0	59.38	2.5
10.0	70.62	1.3
13.0	114.38	0
20.0	350.00	0
Adult	456.25	0

5.2.7 Muscularis Externa

The external muscle wall of the small intestine is thin and poorly developed in the newborn opossum. Although inner and outer strata can be defined, only the inner layer is completely formed and this consists of but a single layer of myoblasts (Cutts et al. 1978a). The outer stratum also consists of myoblasts, but these are scattered and the layer appears to be discontinuous. Myoblasts from the inner layer of the muscularis externa contain bundles of myofilaments, numerous dense bodies, and attachment plaques (Cutts et al. 1978a). These cells show no ultrastructural features that are not found in similar developing smooth muscle cells of later stages, including the adult. Both inner and outer layers appear to develop equally with time, and mitotic activity is highest during the 1st postnatal week (Table 48). Mitotic figures, although rare, can be found in juvenile and adult animals. As in the stomach, the increase in the number of smooth muscle cells in the muscularis externa of the small intestine is due to apposition of newly differentiated cells at the surfaces of the inner and outer layers. Increase in the thickness of the muscularis externa initially involves proliferation of smooth muscle cells which then is followed by hypertrophy of the established smooth muscle. Proliferative activity occurs primarily during the first 4 postnatal weeks with only a slight increase in thickness of the muscularis externa (Table 48) occurring during this time. A period of hypertrophy begins at the end of the proliferative period and continues for 3 weeks, during which time the rate of expansion is about three times that of the preceding 4 weeks. A second period of hypertrophy occurs in the period of development between juvenile and adult animals and begins when solid food is first encountered in the intestinal tract (Cutts et al. 1978a). The overall rate of development for the muscularis externa of the small intestine from birth to juvenile animals is shown in Table 49.

The longitudinal stratum of the muscularis externa is supplied primarily by excitatory cholinergic innervation whereas the circular layer is supplied primarily by nonadrenergic, noncholinergic inhibitory innervation (Anuras et al. 1977). Similar nonadrenergic inhibitory nerves are found in the esophago-

Table 49. Overall rate of development of the muscularis externa in the small intestine from birth to juvenility (20-cm stage) (Cutts et al. 1978a)

Days of development	89
Depth of muscularis externa (μm)	
Initial	13.6 ± 1.49
At 89 days	350.22 ± 5.6
Gain in depth	336.63
Overall rate of gain (μm/day)	3.78

gastric junction, in the oblique layer of stomach muscle, and in muscle of the gastroduodenal and ileocecal junction (Anuras et al. 1977; Conklin and Christensen 1975). The vagi convey both excitatory and inhibitory influences to the small intestine, and the inhibitory influences have been found to be predominant (Gidda and Goyal 1980). The longitudinal and circular layers of the duodenal muscularis externa respond differently to various gastrointestinal hormones (Anuras and Cooke 1978). VIP raises tension in the longitudinal strata but reduces tension in the circular layer. Cerulein and CCK stimulate smooth muscle in the circular layer but have little effect on the longitudinal layer. Glucagon reduces tension in both layers of smooth muscle, and pentagastrin and secretin apparently have little effect on either.

5.2.8 Myenteric (Auerbach's) Plexus

As in the stomach, the myenteric plexus of the small intestine is present at birth, but is poorly developed. The component elements increase in size and number during the first 4 postnatal weeks, then appear to remain stable (Cutts et al. 1978a). This segment of time corresponds to the period of most active proliferation of smooth muscle cells in the muscularis externa. In the rabbit and rat, innervation of the muscularis externa of the gut tube occurs while smooth muscle still is differentiating (Gershon and Thompson 1973), and similar events appear to occur in the opossum. Such a hypothesis is supported by the observation that in *Didelphis* the first period of hypertrophy in the muscularis externa occurs after the myenteric plexus appears to have been established (Cutts et al. 1978a).

The myenteric plexus of the adult consists of a regular network of intersecting nerve fascicles with intrafascicular ganglia located at the intersections (Christensen et al. 1983). Although ganglia increase in number distally along the small intestine, the number of neurons associated with each ganglion does not (Table 50).

5.3 Postnatal Development of Colon

The colon of the newborn opossum has a small lumen, and most of it is lined by a pseudostratified columnar epithelium, although some proximal regions are lined by simple columner epithelium. Intestinal absorptive cells in the colonic

Table 50. Ganglia and perikarya per cm^2 and nerve bundle diameter in the myenteric plexus of the opossum small intestine (Christensen et al. 1983)

Organ	Ganglia/cm^2	Perikarya/cm^2	Nerve bundle diameter (μm)
Duodenum	24.3 ± 1.5	1062.6 ± 18.2	66.2 ± 3.7
Midjejunum	37.2 ± 2.6	1386.0 ± 178.0	76.6 ± 2.8
Ileum	52.5 ± 1.8	1201.6 ± 102.3	82.4 ± 4.0

Values are means ± SD.

epithelium show a well-developed apical endocytic complex and contain numerous large lipid droplets, as do the cells of the small intestine (Krause et al. 1977a). Goblet cells are present within the epithelial lining of the newborn colon and account for nearly 12% of the surface lining cells (Table 51). Villi are not apparent in the newborn or in subsequent postnatal stages, and the colonic lining epithelium lies on a distinct basement membrane. Unlike in the opossum, numerous villi of various shapes and sizes have been described in the rat caecum during the 1st week of postnatal life (Ono 1980). Villi also have been described in human fetal colon (Bell and Williams 1982). The depth of the epithelium does not change significantly during development, and goblet cells increase progressively in number so that during weaning they constitute 60% of the epithelium. Mitotic activity within the lining epithelium is prominent during the 1st postnatal week, then decreases rapidly with development (Table 51). Intestinal glands, although absent in the newborn, are well established by the end of the 1st postnatal week and show a progressive increase in depth thereafter.

By the end of the 2nd postnatal week only scattered inclusions are seen in the colonic absorptive cells, and the apical endocytic complex is no longer observed by the end of the 3rd week.

5.3.1 Enteroendocrine Cells

In addition to goblet and intestinal absorptive cells, the colonic epithelium contains three known types of enteroendocrine cells immunoreactive for either somatostatin, 5-HT, or neurotensin (Krause et al. 1989b). All are present at birth. Cells immunoreactive for 5-HT are scattered throughout the colon at birth, increase in number with development, and become more concentrated distally (Table 52). Somatostatin-immunoreactive cells initially are more concentrated proximally, then become more uniformly distributed along the length of the colon (Table 53). Cells immunoreactive for neurotensin, although seen initially in the proximal colon of the newborn opossum, do not form a significant population until 75 days and then are found throughout the colon during development. Their numbers increase with development and show a greater concentration distally (Table 54).

The enteroendocrine cells of the colon, like those of the small intestine, consist of open and closed types. Both forms may show basal cytoplasmic

Table 51. Quantitative data (means ± SD) for developing colon (Krause et al. 1977a)

	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 ep. cells	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 ep. cells	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

Table 52. 5-HT-immunoreactive cells in the intestinal tract of the developing opossum (no./mm^2: mean ± SD) (Krause et al. 1989b)

Organ	Body length (cm)				
	Newborn	2.5	12.5	20.5	30.0
Caecum	0.00	42.59 ± 52.57	7.04 ± 0.69	15.03 ± 1.91	60.69 ± 27.90
Colon					
Proximal	57.78 ± 57.23	73.28 ± 19.20	17.70 ± 1.79	17.47 ± 0.45	85.73 ± 29.35
Middle	70.09 ± 49.60	51.11 ± 10.15	33.30 ± 2.09	37.42 ± 2.06	96.21 ± 27.90
Distal	4.76 ± 6.74	27.77 ± 39.30	36.97 ± 3.62	46.61 ± 0.67	114.37 ± 6.89

Table 53. Somatostatin-immunoreactive cells in the intestinal tract of the developing opossum (no./mm^2: mean ± SD) (Krause et al. 1989b)

Organ	Body length (cm)				
	Newborn	2.5	12.5	20.5	30.0
Caecum	0.00	0.00	1.64 ± 1.18	3.27 ± 1.65	12.28 ± 3.45
Colon					
Proximal	31.52 ± 23.06	18.14 ± 8.3	4.76 ± 2.83	10.61 ± 2.61	11.42 ± 4.07
Middle	9.52 ± 13.46	6.03 ± 1.17	2.49 ± 0.65	26.03 ± 5.43	11.66 ± 3.26
Distal	0.00	2.81 ± 0.85	4.36 ± 10.72	21.45 ± 3.49	9.01 ± 2.65

Table 54. Neurotensin-immunoreactive cells in the intestinal tract of the developing opossum (no./mm^2: mean ± SD) (Krause et al. 1989b)

Organ	Body length (cm)				
	Newborn	2.5	12.5	20.5	30.0
Caecum	0.00	0.00	3.04 ± 0.79	3.70 ± 2.95	4.61 ± 2.11
Colon					
Proximal	3.03 ± 4.29	0.00	2.62 ± 0.27	8.35 ± 1.50	10.15 ± 9.39
Middle	0.00	0.00	7.67 ± 7.11	32.81 ± 7.34	40.78 ± 12.19
Distal	0.00	0.00	39.73 ± 5.77	68.90 ± 15.15	60.04 ± 7.23

processes, and there is no obvious correlation between their morphology and a specific type of immunoreactivity. Although randomly scattered, the immunoreactive cell types tend to occur in small clusters, as in the small intestine.

The total number of immunoreactive cells in the entire intestinal tract gradually decreases from birth to about 75 days postnatum, then increases dramatically at weaning and continues to increase in postweaned animals (Fig. 34).

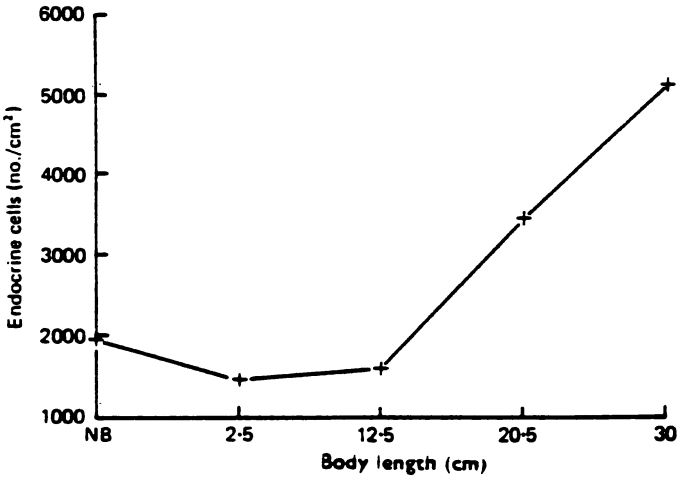


Fig. 34. Total number of enteroendocrine cells in the intestinal mucosa of the developing opossum. (Krause et al. 1989b)

5.3.2 Lamina Propria

During development, the morphology of the lamina propria of the colon is very similar to that of the small intestine and, as in the small intestine, two membranes develop after weaning and limit the lamina propria (Krause et al. 1977a). The membrane on the luminal surface of the muscularis mucosae is the more prominent, while that which envelops the bottoms of intestinal glands is poorly developed and never attains the width of its counterpart in the small intestine (Krause and Leeson 1969a). Numerous macrophages, lymphoid cells, and eosinophils fill the lamina propria after the appearance of the internal and external membranes. A definite muscularis mucosae is present by the end of the 2nd postnatal week and consists of a thin layer of differentiating myoblasts. It continues to increase in thickness throughout the postnatal period by addition of newly differentiated myoblasts to its inner and outer surfaces. The colonic muscularis mucosae lies fairly close to the colonic lumen, which is highly anaerobic, suggesting that in order to maintain normal function the muscularis mucosae must rely on its intrinsic blood supply for oxygen (Percy et al. 1986). If denied oxygen, the muscularis mucosae of the opossum colon fails to maintain normal tone and spontaneous phasic contractions (Percy et al. 1984; Percy and Christensen 1986). This sensitivity to oxygen depletion is somewhat curious in view of the close association with the anaerobic environment of the lumen.

5.3.3 Submucosa

Initially, the submucosa consists of a scant, delicate connective tissue that becomes defined after the 2nd postnatal week with the appearance of the

Table 55. Number of ganglia per cm^2 and number of perikarya per ganglia in the submucosal plexus of the opossum colon (Christensen and Rick 1985)

Organ	Ganglia per cm^2	Perikarya per ganglion
Proximal colon	13.3 ± 2.7	15.5 ± 1.2
Midcolon	11.4 ± 1.3	17.3 ± 1.7
Distal colon	8.3 ± 1.1	14.6 ± 1.7
Rectum	5.4 ± 1.9	11.3 ± 1.4

Values are means ± SD.

Table 56. Thickness and mitotic activity of the muscularis externa of the colon (Cutts et al. 1978a)

Body length (cm)	Thickness of muscularis (μm)	Mitotic cells (per 1000)
1.5	43.75	8.75
2.5	46.87	5.60
3.5	59.38	3.13
5.5	68.75	1.87
8.0	115.63	0
10.0	125.00	0
13.0	200.00	0
20.0	398.72	0
Adult	475.06	0

muscularis mucosae. With subsequent development, the connective tissue becomes more compact and consists of larger connective tissue fibers and houses the larger blood vessels of the gut wall, which provide smaller vessels for the mucosa.

5.3.4 Submucosal (Meissner's) Plexus

As in the small intestine, the submucosal plexus of the colon consists of ganglia interconnected by a network of nerve bundles of varying size (Christensen and Rick 1985). However, unlike the submucosal plexus of the small intestine, a decreasing gradient of nerve cell density occurs distally along the colon (Table 55). Data are not available concerning the development of the submucosal plexus in the opossum.

5.3.5 Muscularis Externa

The muscularis externa of the colon is well developed at birth (Table 56) and is three times as thick as it is in the stomach and small intestine. It also differs in that the inner and outer components are clearly defined at birth (Cutts et al.

Table 57. Overall rate of development of the muscularis externa in the colon from birth to juvenile (20-cm stage) (Cutts et al. 1978a)

Days of development	89
Depth of muscularis externa (μm)	
Initial	42.81 ± 2.18
At 89 days	396.34 ± 3.74
Gain in depth	353.53
Overall rate of gain (μm/day)	3.97

Table 58. Ganglia and perikarya per cm^2 and nerve bundle diameter in the myenteric plexus of the opossum colon (Christensen et al. 1983)

Organ	Ganglia/cm^2	Perikarya/cm^2	Nerve bundle diameter (μm)
Caecum	15.4 ± 1.7	490.8 ± 94.3	43.5 ± 2.4
Proximal colon	29.2 ± 2.5	1119.3 ± 244.1	57.5 ± 4.8
Midcolon	28.2 ± 1.7	1088.8 ± 106.8	42.2 ± 2.0
Distal colon	18.4 ± 2.7	993.2 ± 108.3	36.9 ± 2.7
Rectum	13.6 ± 2.5	188.5 ± 28.4	33.9 ± 4.2

Values are means ± SD.

1978a). Development of the inner layer becomes more pronounced with time and results in the prominence of this layer in the adult. The number of mitotic cells is lower in the colon than in the small intestine and stomach. From a peak at birth, the mitotic activity in the muscularis externa of the colon quickly diminishes (Table 56). As in the other regions of the gastrointestinal tract, mitotic figures are present primarily on the surfaces of the two strata that form the muscularis externa. Occasional dividing cells continue to be encountered in weaned animals and even are present in reproductively active adults. Although the thickness of the muscularis externa differs from organ to organ early in development, after weaning the muscle wall is similar in the stomach, small intestine, and colon (Cutts et al. 1978a). During the period from birth to weaning, the overall rate of development of the muscularis externa is roughly the same for all three organs (Table 57). The muscularis externa of the colon, stomach, and small intestine is characterized by an early period of proliferation (the first 4 postnatal weeks) followed by two periods of hypertrophy.

5.3.6 Myenteric (Auerbach's) Plexus

Elements of the myenteric plexus are poorly developed at birth, but become prominent structures of the muscularis externa by the end of the 4th postnatal week (Cutts et al. 1978a).

The myenteric plexus of the adult opossum colon is formed by a network of intersecting nerve bundles with ganglia at the intersections (Christensen et al. 1983). The ganglia are large and intrafascicular. This pattern is found

throughout most of the colon, but there is a progressive decrease in density of ganglia and nerve cell bodies distally (Table 58). The myenteric plexus of the rectum, defined by Christensen et al. (1983) as that segment of the colon below the pelvic brim, is sparse and irregular, and intersecting nerve bundles vary considerably in diameter. Intersections often lack associated ganglia, which, when present, generally are small and may be parafascicular. Ganglia within the rectum are reported to be 36% parafascicular, 38% intermediate, and 26% intrafascicular in type (Christensen et al. 1983). Several aggregates of nerve bundles that often contain small ganglia also are present in the rectal segment and have been called labyrinthine nodes. Ganglia continue to be present to the level of the internal anal sphincter.

In the more distal segments of the rectum, nerve elements of the myenteric plexus form a stellate plexus in which large ganglia are more evenly distributed (Christensen et al. 1984). Superimposed upon this plexus is an array of thick nerve bundles that course along the long axis of the colon and may extend proximally for about 20% of its total length. These have been termed shunt fascicles by Christensen et al. (1984) and have been described in man (Stach 1971). The shunt fascicles contain myelinated nerve fibers and bypass many of the more distal ganglia, but along their course they do give branches to more proximal ganglia. The shunt fascicles are thought to represent specialized communication pathways of sacral visceral nerves between the distal rectum and more proximal regions of the colon by means of which centers in the central nervous system can selectively influence function in different regions of the colon (Christensen et al. 1984). The presence of myelinated fibers, which are infrequent in the myenteric plexus, would support such a hypothesis. In addition, it has been shown that sacral nerves (primarily S_3 and S_4) are responsible for relaxation of the internal anal sphincter.

During the normal defecation reflex, pressure on the internal anal sphincter decreases in response to fecal distension of the distal colon, and shunt fascicles may be the pathway by which the peristaltic contractions of the distal colon are coordinated during defecation. Pressure in the resting anal canal in *Didelphis* is not affected by skeletal muscle relaxants, suggesting that the resting pressures of the anal canal in this species are due primarily to activity of the smooth muscle of the internal anal sphincter and not to the skeletal muscle of the external anal sphincter (Culver and Rattan 1986). Similarly, reflexive relaxation of the internal anal sphincter can be prevented by inhibiting release of neurotransmitters from preganglionic sacral nerves and through activation of opioid receptors (Rattan and Culver 1987). Vasoactive intestinal polypeptide (VIP) is a potent relaxant of the smooth muscle of the internal anal sphincter (Rattan and Shah 1987; Nurko and Rattan 1988). The rectoanal reflex may be mediated, at least in part, through release of VIP from intramural myenteric inhibitory neurons (Nurko and Rattan 1988; Nurko et al. 1989). It has been suggested that such neurons lie in the sacral inhibitory pathway, which may be involved in the release of acetylcholine from preganglionic fibers that in turn, activate muscarinic and nicotinic receptors on postganglionic, noncholinergic, nonadrenergic inhibitory neurons. The latter myenteric neurons release VIP, which relaxes the smooth muscle of the internal anal sphincter (Rattan and Shah 1987).

The myenteric plexus of the rectum resembles that present in the smooth muscle region of the esophagus, and both rely perhaps more on extrinsic than on intrinsic innervation. The sphincteric character of both regions suggests that the activity seen in these areas also may depend heavily on special properties of their smooth muscle (Christensen et al. 1983).

The measured transit time of materials moving through the gastrointestinal tract of the opossum is relatively rapid in comparison to several other species examined (Clemens and Stevens 1980).

5.4 Caecum

In general, the development and the adult structure of the caecum resemble those of the remainder of the colon. As in the colon, enteroendocrine cells are observed that are immunoreactive for 5-HT, somatostatin, or neurotensin (Krause et al. 1989b). Enteroendocrine cells immunoreactive for 5-HT are present in relatively high numbers by the beginning of the 1st postnatal week, decrease in number prior to and during weaning, then increase after weaning (Table 52). Although this cell type is present in the proximal and middle regions of the newborn colon, both regions also tend to show a decrease in the number of 5-HT cells prior to and during weaning, but an increase after weaning. In contrast, 5-HT-immunoreactive cells in the distal colon show a progressive increase in number throughout development. Somatostatin- and neurotensin-immunoreactive cells are not seen until about the 75th day of postnatal life. Somatostatin-immunoreactive cells progressively increase in number during development (Table 53), whereas neurotensin-immunoreactive cells form a relatively stable population after their appearance in the caecum (Table 54).

The myenteric plexus of the caecum differs somewhat in that it consists of a sparse network of thin, intersecting nerve bundles with small intrafascicular ganglia at the intersections (Christensen et al. 1983). The myenteric plexus at this region contains fewer ganglia and nerve cell bodies per area than other regions of the colon (Table 58).

5.5 Comparison of Enteroendocrine Cells in the Gastrointestinal Tract of the Adult

If the gastrointestinal tract is subdivided into four regions (stomach, duodenum, small intestine, and colon) and the enteroendocrine cell population counted without regard to specific types, then nearly half of these cells are found in the duodenum and the remainder of the small intestine (Table 59). Thirty-three percent are confined to the mucosa of the stomach, with the remainder being in the colon (Krause et al. 1985). If each major region is further subdivided into specific segments and the total population of enteroendocrine cells considered, then the majority (90%–91%) of these cells in the gastric mucosa are found in the pylorus (Table 60). In the duodenum and the remainder of the small intestine, the enteroendocrine cells are evenly distri-

Table 59. Distribution of total endocrine cells by organ (Krause et al. 1985)

Organ	Cells/mm^2	% of total
Stomach	299.8	33.1
Duodenum	136.8	15.1
Small intestine	307.8	34.0
Colon	160.4	17.7
Total	904.5	100.0%

Table 60. Distribution of endocrine cells in each organ (Krause et al. 1985)

Organ	Area	Cells/mm^2	%
Stomach	Cardia	5.59	1.86
	Fundus	22.07	7.36
	Pylorus	271.87	90.67
Duodenum	Proximal	71.53	52.28
	Distal	65.27	47.71
Small intestine	10 cm	61.02	19.82
	20 cm	57.91	18.82
	30 cm	62.25	20.22
	40 cm	58.18	18.90
	Distal ileum	68.43	22.23
Colon	Caecum	17.49	10.90
	Proximal	33.58	20.94
	Mid	51.48	32.09
	Distal	57.85	36.07

buted, while those in the colon increase in number distally (Table 60). Nearly 50% of the total number of enteroendocrine cells occur in the pylorus and duodenum, and cells of this region contain the greatest diversity of peptides (Krause et al. 1985, 1989b). Developmentally, it is this region of the foregut that gives rise to precursors of the endocrine and exocrine portions of the pancreas and the liver.

5.6 Interaction Between Enteroendocrine and Exocrine Components of the Gut

Some gastrointestinal peptides are thought to exert inductive or inhibitory effects on the intestinal tract during development and to initiate motility and/or secretion (Johnson 1987). In the opossum, there appears to be little correlation between the loss of the apical endocytic complex in absorptive intestinal epithelial cells and the appearance of a specific type of enteroendocrine cell (Krause et al. 1989b). However, there is an apparent correlation between the loss of the endocytic complex and the dramatic increase in the total number of

enteroendocrine cells in the gut at weaning. Whether this is a direct correlation of the enteroendocrine cell population influencing the absorptive intestinal epithelial cell population or whether it represents the influence of other factors on the stem cell population within the intestinal glands of the opossum is unknown. Many of the physiological changes that occur within the gastrointestinal mucosa, such as closure and the appearance of enzymes and receptors, are thought to be mediated, at least in part, by adrenal corticosteroids just prior to and immediately after weaning (Klein 1989; Morisset 1989). It may be that the various enteroendocrine cells are receptive to certain stimuli from the gut lumen (amino acids, acidity, glucose) and that secretion of peptides and amines at weaning is also important for the functional maturation of the intestinal mucosa. In other species, secretion from Paneth cells can be elicited by cholecystokinin, catecholamines, and 5-HT (Ahonen 1973; Ahonen and Penttilä 1975a,b; Balas et al. 1974) and inhibited by atropine administration. Cholinergic stimuli can induce mucus secretion by goblet cells (Specian and Neutra 1982; Phillips et al. 1984). Such observations suggest that certain gastrointestinal peptides, amines, and factors from the enteric nervous system act as potential regulators of postnatal development and gut function. The presence on some enteroendocrine cells with long basal cytoplasmic processes that extend between adjacent cells in the intestinal epithelium, and a paracrine mode of secretion, also support such an hypothesis.

6 Pancreas

6.1 Prenatal Development

The dorsal anlage of the pancreas evaginates from the presumptive duodenal region of the foregut late in the 10th day of gestation, slightly dorsal and caudal to the hepatic diverticulum. The connection between the dorsal anlage and the foregut disappears late in the 11th day, and an accessory pancreatic duct does not develop. Simultaneously, the ventral pancreatic anlage forms during day 11, originating as a branch of the hepatic diverticulum. Just prior to birth (12th day of gestation), the ventral and dorsal anlagen unite and are connected to the proximal duodenum by a single excretory duct derived from the ventral portion (McCrady 1938).

6.2 Postnatal Development

Growth of the pancreas, as indicated by total wet weight, is shown in Table 61. Prior to the 25th postnatal day, the pancreas is difficult to visualize and impossible to dissect intact from the adjacent vicera. In those animals in which the intact pancreas can be removed with confidence, a very slow, progressive increase in pancreatic weight occurs prior to weaning, with a marked increase in weight occurring during and after weaning (Table 61). No obvious differences in pancreatic weights were noted between male and female animals. Relative to body weight, pancreatic weight remains fairly constant prior to weaning, increases during the weaning period, and then declines to adult levels (Table 62).

The pancreas of the newborn opossum is small and quite immature in microscopic appearance. It consists of primitive exocrine tubules that surround a central region of endocrine cells (King et al. 1978). Although endocrine cells are present, islets are poorly defined and blend imperceptibly with the expanding exocrine component. The exocrine tubules end as solid clusters of undifferentiated cells among which large, pyramidal proacinar cells and scattered endocrine cells are observed.

Cells that make up the remainder of the exocrine tubules are smaller than proacinar cells and the tubular epithelium ranges from simple squamous to simple columnar. Although most tubules consist of a simple epithelium, some areas of stratification occur in which two or three layers of cells are present. The regions of stratification show intense cellular proliferation and are sites of the paratubular buds which are characteristic of the pancreas in the new-

Table 61. Weights of pancreas (g: mean ± SD)[a]

Body length (cm)	Male	Female	Combined male and female
5.0	0.0092 (1)	0.0119 ± 0.0005 (5)	0.0114 ± 0.0011 (6)
5.5	0.0098 ± 0.0002 (3)	0.0110 ± 0.0006 (3)	0.0104 ± 0.0008 (6)
6.5	0.0261 ± 0.0005 (2)	0.0215 ± 0.0018 (5)	0.0228 ± 0.0026 (7)
10.0	0.1180 ± 0.003 (3)	0.1278 ± 0.0177 (3)	0.1229 ± 0.0137 (6)
13.0	0.1010 ± 0.006 (3)	0.1256 (1)	0.1072 ± 0.0118 (4)
15.0	0.2334 ± 0.022 (6)	–	0.2334 ± 0.022 (6)
17.0	0.6917 ± 0.1039 (2)	0.7039 (1)	0.6958 ± 0.085 (3)
18.0	0.7046 ± 0.0127 (2)	0.982 (1)	0.7971 ± 0.131 (3)
20.0	1.4984 (1)	1.4428 (1)	1.4706 ± 0.027 (2)
Adult	11.190 ± 0.44 (3)	12.8953 ± 0.42 (3)	12.0426 ± 0.43 (6)

[a] The numbers in parentheses, number of animals used.

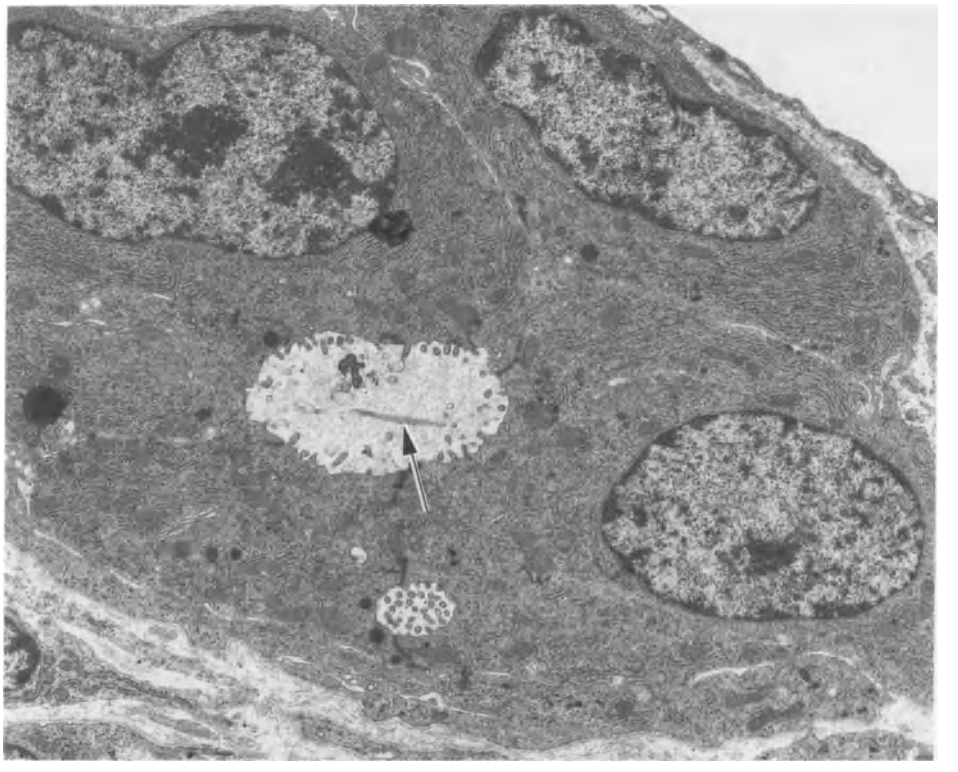
Table 62. Weights of pancreas (g) per 100 g body weights (±SD)[a]

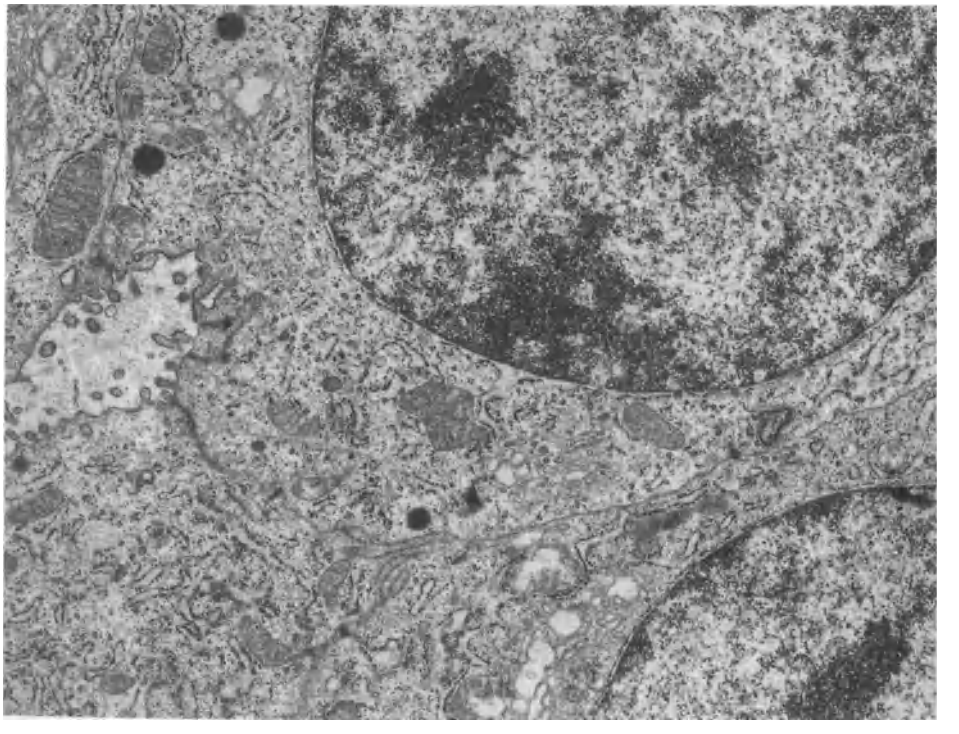
Body length (cm)	Male	Female	Combined male and female
5.0	0.2788 (1)	0.3108 ± 0.017 (5)	0.3055 ± 0.0195 (6)
5.5	0.2239 ± 0.0109 (3)	0.2315 ± 0.013 (3)	0.2277 ± 0.0125 (6)
6.5	0.3449 ± 0.0146 (2)	0.2997 ± 0.051 (5)	0.3189 ± 0.189 (7)
10.0	0.5187 ± 0.0189 (3)	0.5658 ± 0.064 (3)	0.5423 ± 0.053 (6)
13.0	0.3056 ± 0.018 (3)	0.3806 (1)	0.3243 ± 0.036 (4)
15.0	0.3783 ± 0.038 (6)	–	0.3783 ± 0.038 (6)
17.0	0.5940 ± 0.069 (2)	0.5915 (1)	0.5931 ± 0.056 (3)
18.0	0.7046 ± 0.013 (2)	0.8466 (1)	0.7519 ± 0.067 (3)
20.0	0.7568 (1)	0.7716 (1)	0.7642 ± 0.007 (2)
Adult	0.5455 ± 0.0517 (3)	0.5314 ± 0.153 (3)	0.5384 – 0.115 (6)

[a] The numbers in parentheses, number of animals used.

born and week-old opossum. The paratubular buds are outgrowths from the exocrine tubules and eventually differentiate into the exocrine and endocrine components of the pancreas. Cells that are immunoreactive either for insulin, glucagon, somatostatin, BPP, or 5-HT are present, either scattered in small groups or as isolated cells within the exocrine tubules. All five types of immunoreactive cells are present in the group of endocrine cells that occupies the central region of the pancreas (Krause et al. 1989a).

Ultrastructurally, proacinar cells contain abundant free ribosomes, scattered profiles of granular endoplasmic reticulum, and a few secretory granules which, when present, often are large (Figs. 35, 36). Some cells may contain scattered lipid droplets. The apices of the cells are united by tight junctions. The lumina of the exocrine tubules often are irregular, and projections of the lumen may extend a considerable distance between adjacent cells. These luminal extensions often contain numerous microvilli from adjacent cell surfaces.





Developing acini, centroacinar cells, and small intralobular ducts are present by the end of the 1st postnatal week. The ductal cells are characterized by an electron-lucent cytoplasm and contain scattered organelles that are much less abundant than in cells of the developing acinar units. The ductal cells also possess occasional, scattered, elongate cilia that extend from cell apices and course for considerable distances within the lumina of the ductal system where they often lie parallel to the lumen (Figs. 37, 38). Cilia are present in all stages of development, including weaned, juvenile animals. The developing acinar cells show increasing amounts of granular endoplasmic reticulum, and an occasional cell contains scattered zymogen granules which are round in outline, limited by a membrane, and electron dense. The Golgi complex is not a prominent organelle of the supranuclear cytoplasm of most acinar cells during the first 2 postnatal weeks. The expanding epithelial units are grouped together by a loose, delicate connective tissue resulting in early lobulation of the pancreas.

The same types of immunoreactive cells that are observed in the newborn are present in the pancreas of the week-old opossum. Single cells or small clusters of endocrine cells remain closely associated with the expanding ductal and acinar epithelium, and a considerable number of endocrine cells still remain confined to the central region of the pancreas.

With continued growth, the intralobular ducts become lined by an epithelium that ranges from simple squamous to simple columnar. The smaller ducts, lined by simple squamous epithelium, usually terminate in two or more acini. The centroacinar cells present are identical in morphology to adjacent ductal cells outside the acinar units. During the first few weeks of postnatal life, the pancreatic lobules are characterized by numerous small ducts that radiate spoke-like from a common, central intralobular duct toward clusters of differentiating acini.

The pancreatic lobules progressively enlarge by continued growth of the ductal system, concomitant with an increase in the number of acini. The number of acinar cells filled with zymogen granules gradually increases, and by the end of the 5th week many acinar cells are almost as large as those of the adult. As the exocrine pancreas continues to expand and mature, other newly formed regions remain that are immature in appearance (Fig. 39). By the 60th postnatal day, nearly half of the acinar cells of the opossum pancreas are filled by zymogen granules.

The mitotic rates in acinar cells, endocrine cells, and cells in the small ducts are highest during the 1st postnatal week (Table 63). The exocrine component of the pancreas undergoes an extremely rapid rate of differentation and growth, and during the 1st postnatal week about 3.5% of the epithelial cells in this segment are in cell division. Mitotic activity within the acini then

◀ **Fig. 35** (*above*). Proacinar cells from the developing pancreas of a newborn opossum. A portion of a cilium lies within the lumen of the expanding tubule (*arrow*). ×5000
Fig. 36 (*below*). Increased magnification of the apical and supranuclear regions of proacinar cells illustrates details of the cytoplasm. Newborn opossum. ×10000

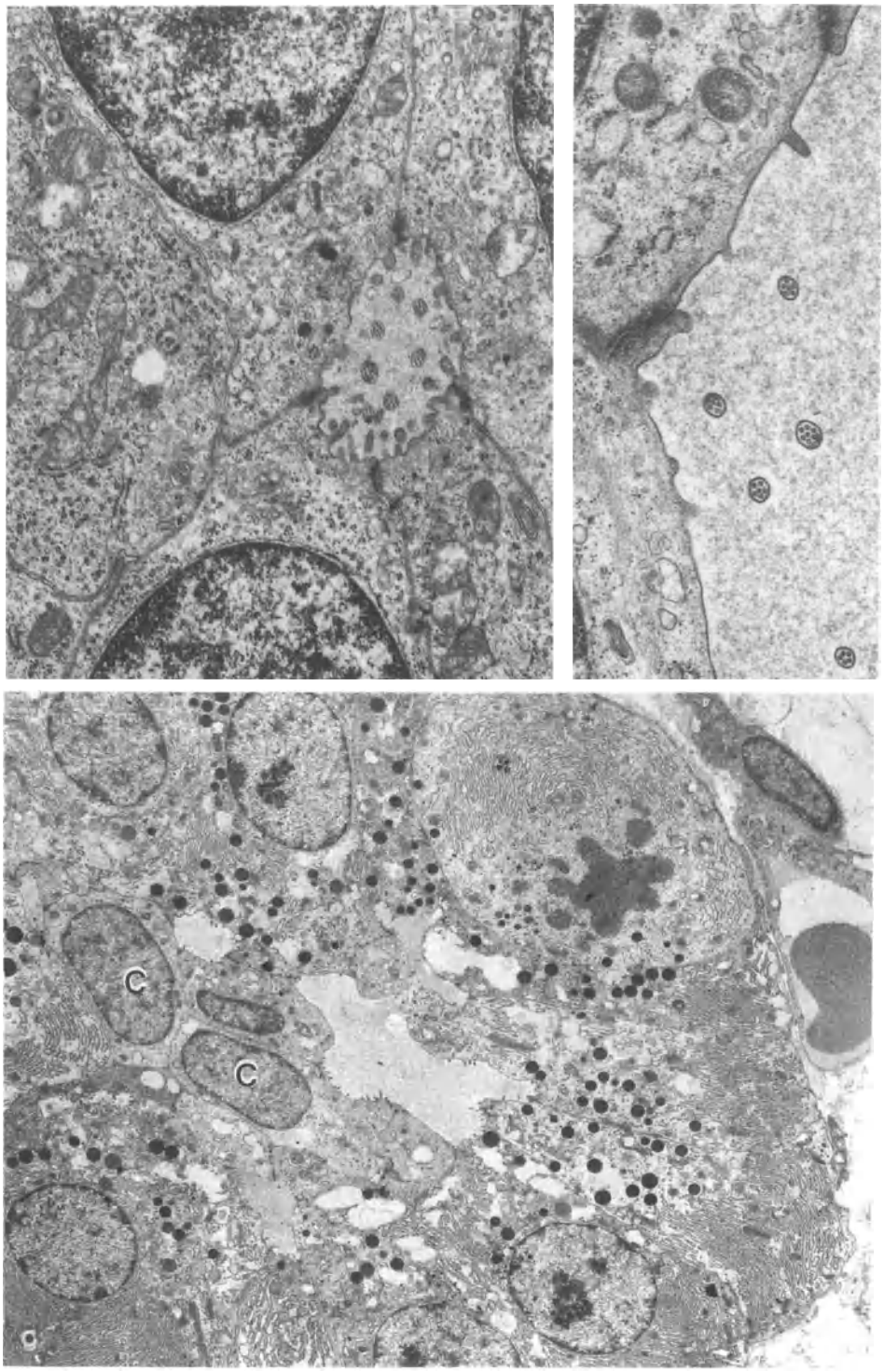

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Table 63. Mitotic activity in the pancreas of the developing opossum (no./1000 cells)

Body length (cm)	Large ducts	Small ducts	Acinar cells	Islet cells
1.5	0	22.11	22.11	11.33
2.0	0	24.66	29.22	10.78
2.5	0	10.72	5.78	6.99
3.5	0	6.67	4.00	4.56
4.0	6.56	4.61	0.89	2.28
4.5	5.27	3.06	4.44	4.66
5.5	3.06	5.33	2.66	1.00
6.5	5.00	7.94	4.61	6.11
7.0	5.67	3.78	13.55	5.99
8.0	6.99	6.99	16.56	3.44
10.0	3.89	6.77	7.83	1.88
11.0	8.67	6.22	14.99	7.11
13.0	2.67	6.78	16.11	1.77
15.0	1.06	0.33	1.06	1.22
16.0	0.99	4.00	6.99	9.44
19.0	0.33	4.72	8.33	6.88
28.0	0.33	0.67	0.72	3.33

decreases until the end of the 2nd postnatal week, after which it increases dramatically, maintaining relatively stable levels until well after weaning, when the activity gradually decreases (Table 63). Similarly, the rate of cell division within the intralobular ducts decreases until the end of the 2nd postnatal week, then maintains a relatively stable rate until after weaning, when it gradually decreases (Table 63). The relatively high rate of mitotic activity in the cells of the intralobular duct system is perhaps related not only to expansion of the exocrine pancreas, but also to differentiation and development of intralobular pancreatic islets which take their origin from this site (Krause et al. 1989a). Mitotic activity among the pancreatic endocrine cells also is highest in the newborn opossum. The rate of mitosis then decreases markedly until the 3rd postnatal week, after which it increases steadily until well after weaning; even then a relatively high rate of mitosis is maintained within the ductal system. The low mitotic activity during the first 4 postnatal weeks is perhaps due to the ill-defined nature and paucity of intralobular islets at this period of development. The marked mitotic activity in the cells of the acini, intralobular ducts, and islets emphasizes that postnatal development of the exocrine and endocrine components of the opossum pancreas is prolonged and that growth of

◀ **Fig. 37** (*upper left*). Cilia often are encountered within the lumina of developing pancreatic acini. 7-day-old opossum. ×10 000
Fig. 38 (*upper right*). Increased magnification of cilia from an intralobular duct illustrates the atypical, random arrangement of contained microtubules. 7-day-old opossum. ×15 000
Fig. 39 (*below*). An electron micrograph of a region of exocrine pancreas taken from a 60-day-old opossum illustrates the morphology of centroacinar cells (*C*) and surrounding acinar cells, one of which is in division. Note the increase in amount and organization of granular endoplasmic reticulum. Zymogen granules are more abundant also. ×4000

Table 64. Number of immunoreactive endocrine cells (mean ± SD) in the developing pancreas (Krause et al. 1989a)

Hormone	Body length (cm)	No. cells/mm² pancreas	No. islets/mm² pancreas	No. cells per islet	Mean area/ islet (mm²)	Region of pancreas
Somatostatin	NB	71.33 ± 10.1	–	–	–	Tail
	NB	78.9 ± 13.4	–	–	–	Head
	2.5	80.77	–	–	–	Tail
	2.5	66.67	–	–	–	Head
	12.5	30.65 ± 10.1	7.59 ± 3.8	4.08 ± 0.57	0.0027 ± 0.0009	Tail
	12.5	31.58 ± 4.03	7.98 ± 2.24	4.11 ± 0.52	0.0021 ± 0.0007	Head
	30.0	6.49 ± 2.93	1.13 ± 0.52	5.69 ± 0.91	0.014 ± 0.008	Tail
	30.0	5.98 ± 2.17	0.88 ± 0.45	4.59 ± 0.59	0.0057 ± 0.009	Head
5-HT	NB	91.26 ± 34.3	–	–	–	Tail
	NB	51.5 ± 45.3	–	–	–	Head
	2.5	46.91 ± 11.9	–	–	–	Tail
	2.5	76.79 ± 1.79	–	–	–	Head
	12.5	11.72 ± 4.2	4.38 ± 1.03	2.76 ± 0.94	0.0022 ± 0.0011	Tail
	12.5	15.76 ± 7.6	6.14 ± 1.0	2.81 ± 1.7	0.0023 ± 0.0005	Head
	30.0	2.64 ± 1.39	0.449 ± 0.045	5.14 ± 2.5	0.0053 ± 0.0011	Tail
	30.0	2.42 ± 2.0	0.51 ± 0.17	3.33 ± 2.4	0.0054 ± 0.001	Head
BPP	NB	220.86 ± 20.8	–	–	–	Tail
	NB	232.87 ± 56.9	–	–	–	Head
	2.5	243.67 ± 7.95	–	–	–	Tail
	2.5	355.44 ± 7.06	–	–	–	Head
	12.5	97.1 ± 67.3	10.0 ± 4.25	8.23 ± 2.55	0.0029 ± 0.0009	Tail
	12.5	75.2 ± 20.04	9.3 ± 1.1	7.95 ± 1.48	0.0025 ± 0.0003	Head
	30.0	8.91 ± 5.12	1.02 ± 0.33	9.1 ± 3.4	0.0086 ± 0.0007	Tail
	30.0	7.39 ± 1.3	0.78 ± 0.15	7.5 ± 1.4	0.0087 ± 0.002	Head

Insulin	NB	44.17 ± 0.27	–	–	–	Tail
	NB	36.76 ± 7.54	–	–	–	Head
	2.5	43.06 ± 13.38	–	–	–	Tail
	2.5	41.2 ± 18.75	–	–	–	Head
	12.5	42.79 ± 6.88	5.94 ± 1.0	8.78 ± 0.85	0.0024 ± 0.0004	Tail
	12.5	54.44 ± 19.2	7.20 ± 2.3	7.81 ± 1.72	0.0021 ± 0.0008	Head
	30.0	19.25 ± 0.42	0.51 ± 0.07	38.81 ± 5.9	0.0078 ± 0.002	Tail
	30.0	21.84 ± 8.26	0.55 ± 0.14	40.0 ± 15.29	0.0098 ± 0.005	Head
Glucagon	NB	314.5 ± 106.2	–	–	–	Tail
	NB	292.4 ± 111.0	–	–	–	Head
	2.5	173.62 ± 51.04	–	–	–	Tail
	2.5	225.04 ± 3.99	–	–	–	Head
	12.5	39.99 ± 14.7	5.84 ± 0.37	7.92 ± 2.9	0.0027 ± 0.0009	Tail
	12.5	48.8 ± 6.18	6.64 ± 1.38	8.07 ± 3.0	0.0029 ± 0.009	Head
	30.0	22.8 ± 7.7	1.19 ± 0.29	16.78 ± 4.63	0.0083 ± 0.003	Tail
	30.0	23.09 ± 3.03	1.31 ± 0.34	20.98 ± 3.84	0.0081 ± 0.003	Head

NB, newborn.

both these components continues well into adulthood. The period of postnatal development prior to the onset of weaning is mainly concerned with the *formation* of ducts and acini of the exocrine pancreas, whereas the development that occurs after weaning appears to be limited to the *expansion* of established ducts, acini, and islets (King et al. 1978).

Although immunoreactive endocrine cells can be identified easily in the pancreata of newborn and week-old opossums, the boundaries of individual islets cannot be ascertained with certainty. Therefore, only the number and type of immunoreactive cells present have been evaluated and in Table 64 are expressed as the number of immunoreactive cell per mm^2 of total pancreatic tissue. The number of each type of immunoreactive cell per mm^2 of pancreatic tissue remains relatively high in the week-old opossum (Krause et al. 1989a). Early in development, endocrine cells that are immunoreactive for BPP and glucagon are the most numerous of the endocrine cell types present (Table 64). Glucagon-immunoreactive cells are localized at the periphery of some developing intralobular pancreatic islets. Other types of immunoreactive endocrine cells (somatostatin, 5-HT, BPP, insulin) are scattered within the pancreatic islets in the newborn and week-old opossums. During the 1st week of postnatal life, the number of somatostatin- and insulin-immunoreactive cells remains relatively constant, whereas cells immunoreactive for glucagon, BPP, and 5-HT decrease in number (Table 64).

Endocrine cells appear to proliferate from the terminal regions of the intralobular ducts where the ducts abut differentiating acinar units. Such groups of endocrine cells remain closely associated with the exocrine epithelium of origin, even in adulthood. The endocrine cells often appear to form a collar of tissue that envelops the distalmost extent of the intralobular duct, and the cells usually are separated from the exocrine lumen by intervening ductal epithelium. The intralobular ducts, lined by a simple cuboidal epithelium, usually lie near the center of expanding pancreatic lobules and may give rise to several intercalated ducts (King et al. 1978). The interlobular and main pancreatic ducts are lined by tall columnar epithelium and lie in the interlobular connective tissue.

Pancreatic lobules progressively enlarge by continued branching and elongation of the ductal system together with an increase in the number and growth of acini. By the end of the 4th postnatal week, many of the developing islets arising from ducts are surrounded by acinar epithelium, and small- and medium-sized islets often appear to be enveloped by a single layer of acinar cells. By the end of the 5th week, this single layer apparently expands to form acini that now are grouped at the periphery of most intralobular islets. Islets which are in direct continuity with intralobular ducts still are present and rarely may be isolated within the interlobular connective tissue.

As the developing islets increase in size and number, undifferentiated cells within the exocrine epithelium associated with them also proliferate to envelop the islets. Similar observations have been reported in eutherian species (Githens 1986). The simultaneous expansion of the exocrine and endocrine components results in most of the islets assuming a central location within the expanding lobules. Changes in the islets after the 9th week of development are primarily the result of an increase in the number of cells and size of the islets,

and to the degree of vascularity. Most of the islets are fairly well defined and have an adult configuration by weaning, but many intralobular islets retain a close association with the duct of origin and remain localized centrally within expanding pancreatic lobules. Islets continue to be present in the scant connective tissue between lobules, but these are rare.

The central area of developing islets seen in the newborn opossum is thought to give rise to primary (interlobular) islets (King et al. 1978). A second generation of islets then arises from endocrine cells contained within the exocrine component of the pancreas, resulting in the appearance of intralobular islets which differentiate and proliferate first within the primitive exocrine tubules, then within the intralobular duct system. As the endocrine and exocrine components expand, the forming intralobular islets remain near their point of origin, become surrounded by the exocrine pancreas, and come to lie near the center of the lobules (King et al. 1978).

The interlobular islets are believed to arise from the central mass of endocrine cells in very young animals, and rarely from intralobular ducts. In the latter case, an association with the exocrine component is never established and the fully developed islets come the reside not within a lobule, but in the interlobular connective tissue. Formation of interlobular and intralobular islets has been reported in several other mammals including: sheep (Laguesse 1896), mouse (Mori and Haga 1960), rat (Hard 1944), guinea pig (Bensley 1911), and man (Pearse 1903; Ferner and Stoeckenius 1950; Hultquist and Thorell 1953; Robb 1961; Conklin 1962; Liu and Potter 1962; Like and Orci 1972; Laitio et al. 1974). Interlobular islets in the developing pancreas of the sheep are said to degenerate (Laguesse 1896), whereas in the opossum some interlobular islets persist in the adult as scattered entities in the interlobular connective tissue.

Independent (isolated) immunoreactive endocrine cells continue to be found scattered within the intralobular duct system, in the acinar units, in significant numbers even in older animals, in which they are found between acinar cells (Table 65). These are thought to represent sites of future islet formation as the pancreas continues to expand and grow throughout the life of the opossum (Krause et al. 1989a). In addition, independent cells may contribute small amounts of peptide to the exocrine secretion of the pancreas. Beta cells in the exocrine pancreas of suckling young of a South American opossum (*Didelphis albiventris*) contribute insulin to the exocrine secretion of the pancreas (Coutinho et al. 1984), but whether or not the small amounts of these peptides in the exocrine secretion influence the physiological activity of ductal cells, and whether similar events occur in *Didelphis virginiana*, is unknown. Isolated cells immunoreactive for glucagon, insulin, pancreatic polypeptide, and somatostatin also are present in the exocrine pancreas of an adult Australian possum, *Trichosurus vulpecula* (Reddy et al. 1986).

The number of endocrine cells in the islets that react for glucagon, BPP, somatostatin, and 5-HT decreases considerably in 74-day-old opossums, but endocrine cells immunoreactive for insulin form a relatively stable population from birth to weaning. All five types of immunoreactive endocrine cells present in the intralobular islets also occur in interlobular islets. In the latter, however, the cells remain scattered within the islets and do not show the organized distribution that is seen in the intralobular form.

Table 65. Number of independent immunoreactive endocrine cells (mean ± SD) scattered within the exocrine pancreas and percent of interlobular islets (mean ± SD) in the developing opossum pancreas (Krause et al. 1989a)

	Body length (cm)	Head		Tail	
		Independent cells/mm^2	Interlobular islets (%)	Independent cells/mm^2	Interlobular islets (%)
Somatostatin	NB	–	–	–	–
	2.5	–	–	–	–
	12.5	6.19 ± 0.84	3.79 ± 0.07	6.64 ± 0.62	1.92 ± 0.06
	30.0	0.98 ± 0.50	1.29 ± 0.47	1.01 ± 0.18	1.45 ± 1.02
5-HT	NB	–	–	–	
	2.5	–	–	–	
	12.5	6.89 ± 0.27	2.94 ± 0.06	5.97 ± 0.16	2.66 ± 0.03
	30.0	1.01 ± 0.24	1.36 ± 0.72	0.94 ± 0.32	1.08 ± 0.8
BPP	NB	–	–	–	–
	2.5	–	–	–	–
	12.5	5.22 ± 7.39	1.83 ± 0.79	5.68 ± 8.04	2.02 ± 0.79
	30.0	0.70 ± 0.1	4.38 ± 0.21	0.82 ± 0.4	3.73 ± 1.2
Insulin	NB	–	–	–	–
	2.5	–	–	–	–
	12.5	5.76 ± 0.21	4.92 ± 4.91	4.38 ± 0.25	4.07 ± 2.94
	30.0	0.46 ± 0.11	1.93 ± 0.48	0.53 ± 0.12	1.46 ± 0.55
Glucagon	NB	–	–	–	–
	2.5	–	–	–	–
	12.5	7.17 ± 1.77	1.61 ± 0.31	7.14 ± 1.51	2.20 ± 0.03
	30.0	0.63 ± 0.14	2.08 ± 0.67	0.58 ± 0.27	3.06 ± 2.24

NB, newborn.

The majority of endocrine cells immunoreactive for somatostatin, glucagon, BPP, and 5-HT occur at the periphery of the intralobular islets, but some are scattered within the islets as well. BPP- and glucagon-immunoreactive cells often form an inconsistent peripheral layer, two to three cells deep. They also occur in clusters that are scattered along the periphery but most often are present at the ends of elongate islets. Cells immunoreactive for somatostatin also are present along the edge of islets but usually form a layer of two to three cells, deep to the margin of the islets (Fig. 40). A small population of cells showing 5-HT immunoreactivity also are found at the periphery of the islets, but these cells are the least numerous of the endocrine cells in the pancreas and 5-HT activity often is co-localized in cells that are immunoreactive for BPP (Krause et al. 1989a). Not all cells immunoreactive for 5-HT show immunoreactivity for BPP, and these may represent an independent endocrine cell type in the opossum pancreas. Endocrine cells immunoreactive for insulin are found primarily in the central region of the islets (Fig. 40).

Co-localization of peptides and monoamines in different pancreatic endocrine cells varies considerably among species. Monoamines have been described in alpha and beta cells of the pig (Cegrell 1970; Lebovitz and Feldman 1973; Owman et al. 1973), sand rat (Hahn von Dorshe and Titlbach 1977), and several avian species (Watanabe et al. 1988). 5-HT immunoreactivity has

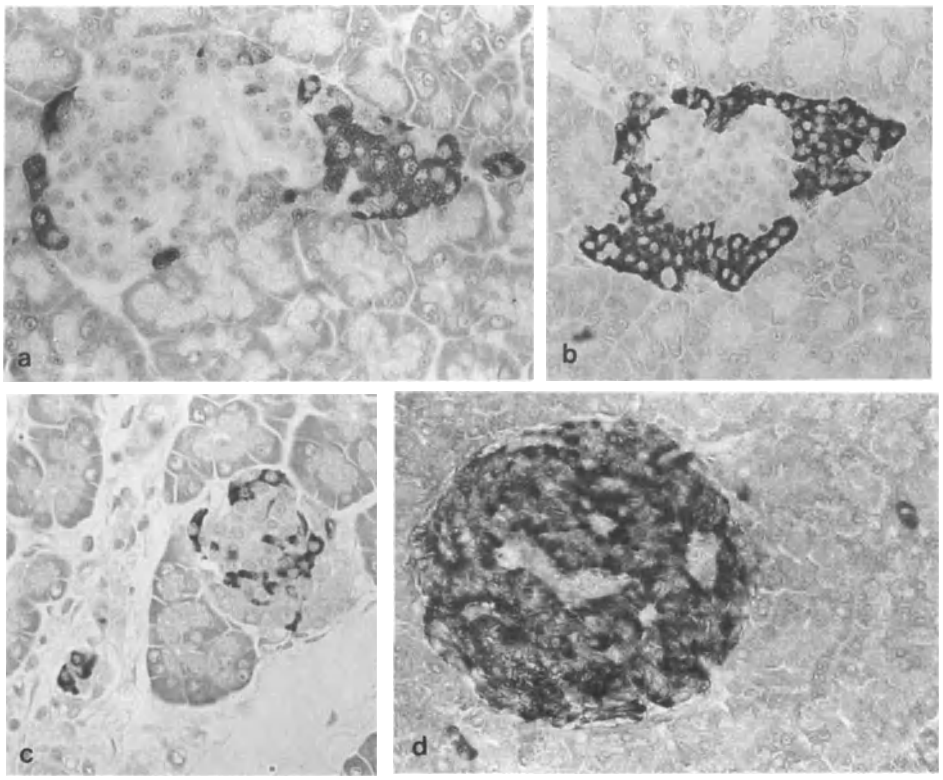


Fig. 40 a–d. Pancreatic islets from a recently weaned opossum illustrate the distribution of: **a** BPP-immunoreactive cells, ×250; **b** glucagon-immunoreactive cells, ×250; **c** somatostatin-immunoreactive cells, ×250; **d** insulin-immunoreactive cells, ×250

been reported in beta cells of the pig and guinea pig, in alpha cells of the dog (Cegrell 1968; Jaim-Etcheverry and Zieher 1968; Owman et al. 1973; Lundquist et al. 1975), caiman, and bovine (Yamada et al. 1986; Nakajima et al. 1988), and in pancreatic polypeptide cells of cattle (Nakajima et al. 1988). Dopamine also has been co-localized in pancreatic polypeptide-immunoreactive cells of *Didelphis* (Larsson et al. 1976).

Considering total pancreatic tissue prior to weaning, cells that are immunoreactive for glucagon are the most numerous endocrine cells present, followed in decreasing order by cells immunoreactive for BBP, somatostatin, insulin, and 5-HT (Table 64). However, if the number of endocrine cells per mm^2 of *intralobular islet* is considered, then insulin-immunoreactive cells are the most numerous, followed by cells immunoreactive for glucagon, BPP, somatostatin, and 5-HT (Table 64). The total number of pancreatic islets, independent immunoreactive cells in the exocrine pancreas, and the total number of immunoreactive cells in islets per mm^2 of pancreatic tissue decrease after weaning (Tables 64, 65). Generally, as the exocrine pancreas continues to expand, the mean area of the pancreatic islets also increases (Table 64). If the number of each type of endocrine cell per mm^2 of pancreatic islet is

considered, then cells immunoreactive for insulin show the greatest increase in number, followed by cells immunoreactive for glucagon and 5-HT (Table 64). The number of cells immunoreactive for somatostatin and BPP per intralobular islet remain relatively stable prior to and after weaning.

The high concentration of endocrine cells in the pancreas during its early development and the subsequent decrease in cell concentration are thought to be the result of the rapid expansion of the exocrine pancreas. Both the exocrine and endocrine components of the opossum pancreas continue to grow and expand until well after weaning, but the continued growth of the endocrine component is overshadowed by the exocrine portion as development continues (Krause et al. 1989a).

The five types of immunoreactive cells present in the intralobular islets also are found in the interlobular islets (Table 65). No difference in the percentage of each immunoreactive cell type either in the intralobular or interlobular islets or in the independent endocrine cells is observed between the head and tail regions of the opossum pancreas (Table 64). The Australian marsupial *Trichosurus vulpecula* also lacks a topographical distribution of the four major endocrine cells between head and tail regions (Reddy et al. 1986); this finding contrasts with reports for several eutherian species, including man (Orci et al. 1976; Orci and Unger 1975; Baetens et al. 1979; Gersell et al. 1979; Reddy et al. 1985). *Trichosurus* and *Didelphis* both show a greater proportion of cells immunoreactive for insulin than do other marsupials (red and gray kangaroo, euros), in which glucagon cells predominate (White and Harrop 1975).

In *Didelphis* the pancreatic endocrine cells that are immunoreactive for somatostatin, BPP, and 5-HT often are irregular in shape and may have cytoplasmic processes that extend between adjacent cells to meander through the periphery of the islets. Similar processes have been described for cells immunoreactive for somatostatin in the pancreas of *Trichosurus* (Reddy et al. 1986), suggesting that paracrine regulation of islet function (Orci and Unger 1975; Watkins et al. 1980) may occur in marsupials. Islet hormones (insulin, glucagon, somatostatin, pancreatic polypeptide) are known to influence the function of the exocrine pancreas (Henderson et al. 1981), and a similar situation may exist within the islets themselves as the various peptides and amines have direct access to other islet cells due to the arrangement of the microvasculature within the islets (Bonner-Weir and Orci 1982). Cells immunoreactive for insulin and glucagon generally are round or oval and lack long processes.

The amino acid sequences of opossum insulin, glucagon, and pancreatic polypeptide have been determined, and insulin and pancreatic polypeptide show a high degree of conservation in their primary structure as compared to other mammals (Yu et al. 1989). It is of interest that chicken glucagon is identical to opossum glucagon, with both differing from the typical mammalian form by replacement of asparagine by serine at its penultimate C-terminal position (Yu et al. 1989).

The intralobular pancreatic islets in *Didelphis* continue to increase in size and number throughout development, as reported for the rat (Hellman 1959), in which beta and alpha cells increase rapidly late in fetal life (McEvoy and Madson 1980) and beta cells continue to increase after birth (McEvoy 1981).

Similar events occur in the opossum but much later in development, near and after weaning. Beta cells also increase throughout development and well after weaning in the human pancreas (Rahier et al. 1981). As in the opossum, many endocrine cell types in pancreata of human neonates occur in small clusters or as isolated cells, and nearly 15% occur in the ducts and acini of neonates, with small clusters persisting in infants, but with less frequency (Rahier et al. 1981).

Development of the opossum pancreas occurs almost entirely during the long period of postnatal development that typifies this species. Although prolonged, the general pattern of development for exocrine and endocrine components is typical of that in other mammals (Falkmer and Patent 1972; Pictet and Rutter 1972).

7 Liver

7.1 Prenatal Development

During the middle of the 10th fetal day, the hepatic diverticulum arises from the foregut in the midventral line, at the level where omphalomesenteric veins empty into the sinus venosus. Early in the 11th day, that portion of the diverticulum that runs most craniad becomes the hepatic duct. Several solid cords of cells expand from this area, extend into the mesenchyme of the ventral mesentry, and grow in the direction of the sinus venosus. The liver enlarges rapidly during the 11th and 12th days and is actively hemopoietic prior to birth. A well-defined ventral segment of the original liver diverticulum is the anlage of the gallbladder (McCrady 1938).

7.2 Postnatal Development

Growth of the liver, as estimated from its total weight, is shown in Table 66. The most notable feature is the progressive growth of the organ throughout the postnatal period prior to weaning, with no differences with regard to sex of the animals. The liver shows a marked increase in weight during the postweaning period. Relative to body weight, however, the liver progressively decreases in weight following the 3rd postnatal day (Table 67), and it is not until weaning that weights comparable to those in adults are obtained. Thereafter steady growth occurs.

The liver of the newborn opossum is markedly immature in its microscopic appearance. Hepatocytes show no organization into plates and are concentrated around central veins, while at the periphery of the developing lobules the cells cluster into groups separated by large islands of hemopoietic cells (Fig. 41). Cells of the granulocyte and erythrocyte series, as well as scattered megakaryocytes, form the majority of the hemopoietic elements. Numerous mitoses are seen within the groups of hemopoietic cells, and occasionally mitotic figures can be found among hepatocytes. Hepatocytes are small and contain pale, vesicular nuclei with distinct nucleoli (Cutts et al. 1973).

Ultrastructurally, the hepatocytes contain lipid droplets of varying sizes, abundant profiles of granular endoplasmic reticulum, and mitochondria with sparse, transversely arranged cristae (Fig. 42). Glycogen is present but is not a prominent feature of the cytoplasm in hepatocytes of the newborn. Occasional bile canaliculi are found between some hepatocytes, and these frequently contain a few, short projections of microvilli from the surrounding hepatocytes.

Table 66. Weights of liver (g: mean ± SD)[a]

Body length (cm)	Male	Female	Combined male and female
1.5[b]	–	–	0.0049 ± 0.0006 (6)
2.0[b]	–	–	0.0191 ± 0.003 (7)
2.5[b]	–	–	0.0252 ± 0.055 (12)
3.5	0.0517 ± 0.005 (9)	0.0550 ± 0.004 (6)	0.0537 ± 0.004 (15)
4.0	0.0651 ± 0.005 (5)	0.0768 ± 0.002 (5)	0.0709 ± 0.007 (10)
4.5	0.0816 ± 0.017 (13)	0.0807 ± 0.022 (6)	0.0813 ± 0.018 (19)
5.0	0.090 ± 0.002 (5)	0.0985 ± 0.003 (4)	0.0988 ± 0.003 (9)
5.5	0.123 ± 0.013 (7)	0.1172 ± 0.016 (6)	0.1203 ± 0.014 (13)
6.0	0.1367 ± 0.006 (4)	–	0.1367 ± 0.006 (4)
6.5	0.1581 ± 0.003 (4)	0.134 ± 0.012 (6)	0.1436 ± 0.015 (10)
7.0	0.187 ± 0.016 (5)	–	0.187 ± 0.016 (5)
8.0	0.3988 ± 0.007 (4)	0.3945 ± 0.058 (4)	0.3966 ± 0.038 (8)
9.0	0.4578 ± 0.059 (3)	0.4493 ± 0.055 (3)	0.4536 ± 0.051 (6)
10.0	0.796 ± 0.062 (7)	0.7175 ± 0.161 (7)	0.7567 ± 0.124 (14)
11.0	1.5905 ± 0.519 (7)	1.8014 ± 0.336 (7)	1.6960 ± 0.434 (14)
12.0	1.9863 ± 0.211 (6)	1.9111 ± 0.163 (6)	1.9487 ± 0.183 (12)
13.0	2.2851 ± 0.381 (3)	2.3576 ± 0.098 (3)	2.3213 ± 0.253 (6)
15.0	3.8103 ± 0.141 (4)	3.9398 ± 0.054 (4)	3.8751 ± 0.121 (8)
15.5	4.6535 ± 0.161 (5)	4.6929 ± 0.118 (3)	4.6683 ± 0.139 (8)
16.0	5.7284 ± 0.372 (4)	5.6822 ± 0.288 (4)	5.7053 ± 0.309 (8)
17.0	6.9743 ± 0.471 (3)	6.6830 ± 0.155 (3)	6.6828 ± 0.332 (6)
18.0	7.6913 ± 0.18 (4)	7.3659 ± 0.161 (2)	7.5828 ± 0.230 (6)
20.0	10.111 ± 0.344 (6)	10.0922 ± 1.62 (5)	10.0985 ± 1.04 (11)
22.0	12.8413 ± (1)	12.9193 ± 0.058 (3)	12.8933 ± 0.061 (4)
25.0	–	15.6687 ± 4.47 (3)	15.6687 ± 4.47 (3)
27.5	24.1194 ± 1.113 (3)	–	24.1194 ± 1.113 (3)
29.0	–	26.3517 ± 0.256 (4)	26.3517 ± 0.256 (4)
33.0	–	38.9981 ± 1.83 (5)	38.9981 ± 1.83 (5)
37.0	58.1936 ± 4.15 (7)	–	58.1936 ± 4.15 (7)
Adult	151.532 ± 20.29 (5)	132.9394 ± 19.43 (8)	140.09 ± 20.05 (13)

[a] The numbers in parentheses, number of animals used.
[b] Sex not determined.

Small, membrane-bound vesicles occur within the cytoplasm of hepatocytes immediately adjacent to canaliculi.

Extensive areas of the developing sinusoids appear to lack an endothelium, and rarely lining cells are seen in relation to bordering hepatocytes (Krause et al. 1975). In the regions where sinusoids lack lining cells, hemopoietic cells and hepatocytes are in close contact. When sinusoidal cells are observed, they appear elongated and their cytoplasm contains scattered elements of granular endoplasmic reticulum, cytoplasmic vacuoles, and mitochondria (Fig. 42). Thin cytoplasmic processes often extend from the perimeter of sinusoidal cells to lie in relation to neighboring hepatic cells, forming a perisinusoidal space that is narrow and which often contains scattered collagen fibers. Short microvilli from neighboring hepatocytes may extend into the perisinusoidal space. In those regions of sinusoids that lack a lining endothelium, microvillus projections from the surface of adjacent hepatocytes are not apparent and collagen fibers are not seen.

Table 67. Weights of livers (g) per 100 g body weight (mean ± SD)

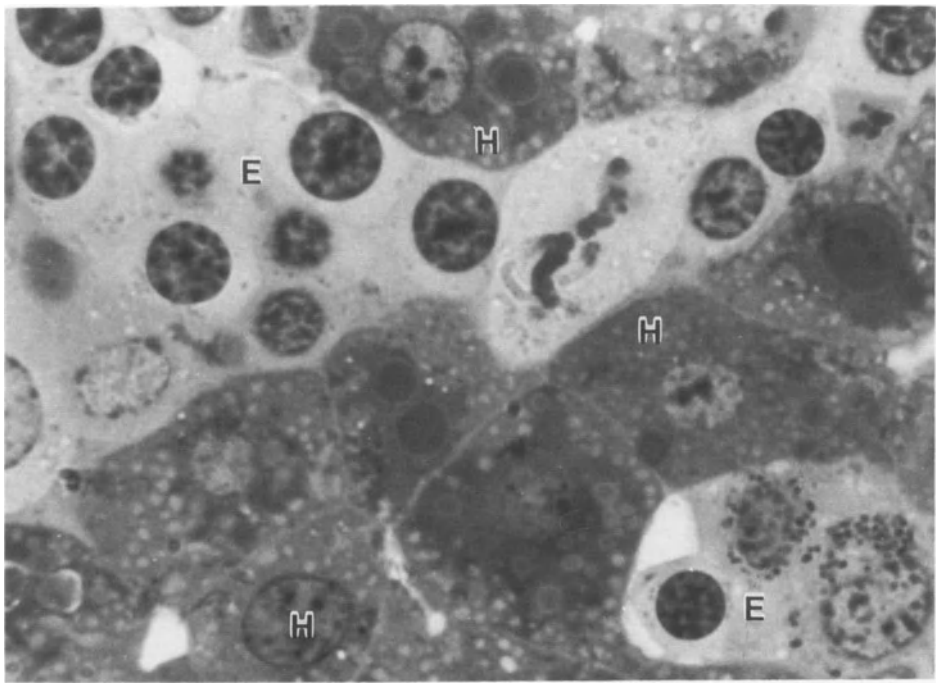
Body weight (cm)	Male	Female	Combined male and female
1.5[a]	–	–	2.0186 ± 0.024
2.0[a]	–	–	5.2242 ± 0.51
2.5[a]	–	–	3.7953 ± 0.468
3.5	3.142 ± 0.393	3.1328 ± 0.255	3.1365 ± 0.304
4.0	2.4945 ± 0.134	3.0328 ± 0.207	2.7636 ± 0.295
4.5	2.715 ± 0.354	2.463 ± 0.332	2.6354 ± 0.359
5.0	2.4863 ± 0.079	2.6724 ± 0.098	2.5647 ± 0.163
5.5	2.5949 ± 0.129	2.6115 ± 0.113	2.5996 ± 0.113
6.0	2.1199 ± 0.051	–	2.1199 ± 0.051
6.5	2.2078 ± 0.065	2.1766 ± 0.141	2.1828 ± 0.109
7.0	1.6484 ± 0.157	–	1.6484 ± 0.157
8.0	2.5992 ± 0.199	2.7004 ± 0.426	2.6498 ± 0.313
9.0	2.6307 ± 0.223	2.5580 ± 0.181	2.5943 ± 0.185
10.0	3.0542 ± 0.311	2.7681 ± 0.528	2.9112 ± 0.442
11.0	3.8276 ± 0.465	4.0744 ± 0.276	3.951 ± 0.389
12.0	4.3399 ± 0.381	4.1838 ± 0.389	4.262 ± 0.377
13.0	3.7711 ± 0.476	4.2486 ± 0.277	4.0099 ± 0.436
15.0	4.252 ± 0.136	4.4875 ± 0.119	4.3697 ± 0.172
15.5	4.1038 ± 0.118	4.1466 ± 0.098	4.1198 ± 0.106
16.0	4.4837 ± 0.279	5.6486 ± 0.197	4.5661 ± 0.241
17.0	5.3161 ± 0.237	5.3243 ± 0.163	5.3199 ± 0.167
18.0	5.6486 ± 0.092	5.2003 ± 0.028	5.4992 ± 0.242
20.0	5.3101 ± 0.196	5.3724 ± 0.249	5.3384 ± 0.212
22.0	5.0624	5.258 ± 0.192	5.0889 ± 0.029
25.0	–	5.7947 ± 0.607	5.7947 ± 0.607
27.5	5.2479 ± 0.232	–	5.2479 ± 0.232
29.0	–	5.4823 ± 0.097	5.4823 ± 0.097
33.0	5.3217 ± 0.275	–	5.3217 ± 0.275
37.0	4.0741 ± 0.328	–	4.0741 ± 0.328
Adult	5.9212 ± 0.583	5.7234 ± 0.512	5.7994 ± 0.525

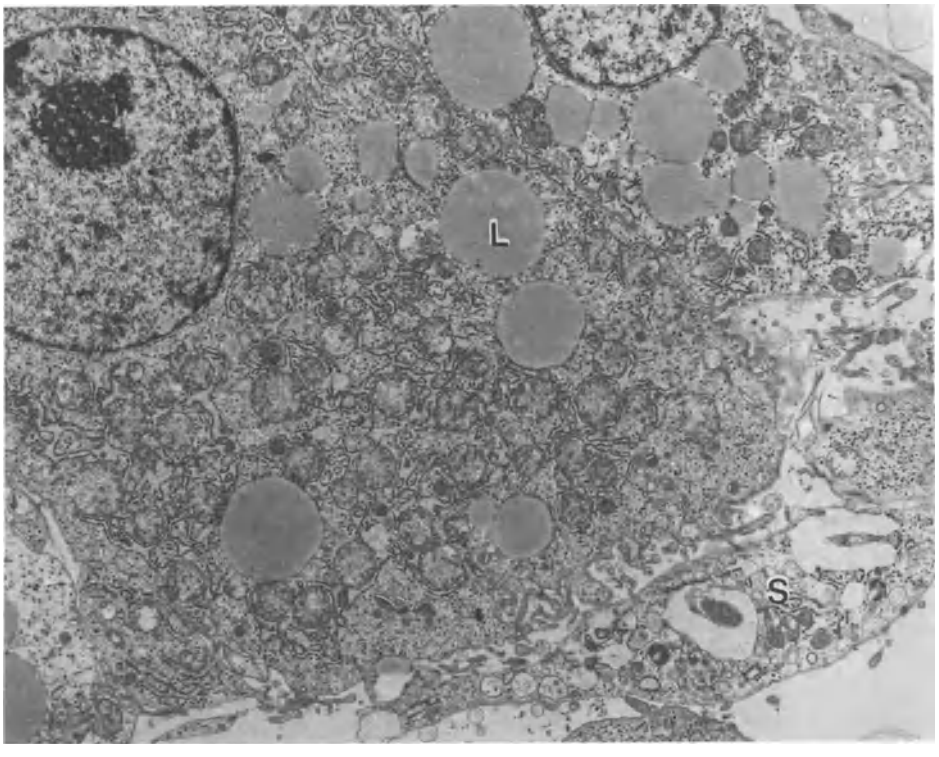
[a] Sex not determined.

During the first 21 days after birth, hepatocytes become organized into irregular plates (cords), and a lobular arrangement of the liver is established. At the end of the 1st postnatal week, the hepatocytes show a more uniform arrangement into plates that radiate from the central vein. Bile canaliculi are a consistent feature observed at this time (Fig. 43). However, hepatocytes still lack organization at the periphery of the forming lobules and appear as groups

Fig. 41 (*above*). The liver of the nowborn opossum is characterized by clusters of hepatocytes (*H*) separated by hemopoietic cells (*E*). Note the lipid droplets of varying sizes within the hepatocytes. Epon 812–toluidine blue, ×1000 ▶

Fig. 42. (*below*). The cytoplasm of a hepatocyte from the liver of a newborn opossum contains profiles of granular endoplasmic reticulum, scattered mitochondria, and lipid droplets (*L*). A portion of a sinusoidal endothelial cell (*S*) is shown at the *lower right*. (Krause et al. 1975) ×6000


E
H
H
H
E


L
S

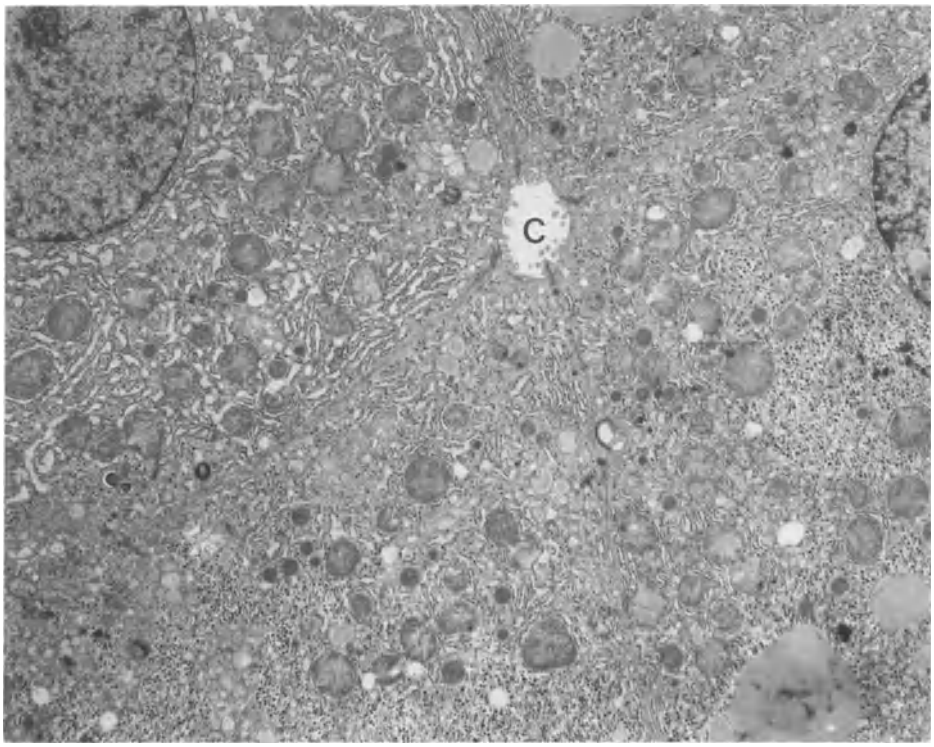


Fig. 43. Portions of four hepatocytes taken from the liver of a week-old opossum that show more mature ultrastructural features and border a bile canaliculus (*C*). (Krause et al. 1975) ×5000

separated by scattered, irregular islands of hemopoietic cells. Hepatocytes show an increase in the amount of lipid deposition, and although the lipid droplets vary considerably in size, they generally appear larger than in the liver of the newborn opossum. Small hepatocytes, relatively free of lipid droplets, are present near the periphery of the lobules, and these cells are thought to be organizing into the limiting plate of the lobule. A marked increase in mitotic activity and an increase in the number of hepatocytes can be observed during this period of development. Hepatocytes in the developing limiting plates continue to show an abundance of granular endoplasmic reticulum, and scattered Golgi complexes are seen in relation to bile canaliculi. Glycogen, mainly in the form of alpha particles or rosettes, is abundant and, in most of the liver cells, forms large accumulations in the cytoplasm. Although lining cells are seen with greater frequency in the sinusoids at this stage, large areas remain that lack a sinusoidal lining, and in such areas hemopoietic cells continue to maintain their intimate relationship with adjacent hepatocytes. Collagen fibrils occur with greater frequency and abundance in the forming perisinusoidal space. The cytoplasm of sinusoidal cells continues to be characterized by vacuoles.

Irregular plates of hepatocytes extend throughout each lobule by the end of the 2nd postnatał week and are separated by wide spaces. Numerous lipid

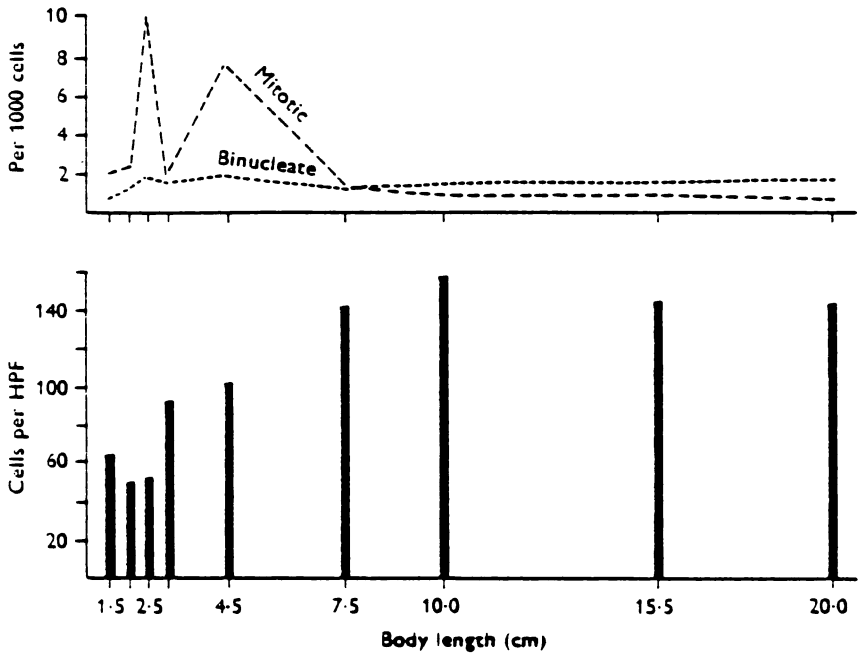


Fig. 44. Quantitative data for hepatic cells. The *lower figure* shows the number of hepatic cells per high power field (*HPF*) at different stages of development. In the *upper figure* the corresponding number of mitotic and binucleate hepatic cells are shown per 1000 hepatic cells. (Cutts et al. 1973)

droplets of variable size, glycogen deposits, and an increased amount of granular endoplasmic reticulum constitute the prominent features of the hepatocyte cytoplasm. Small groups of hemopoietic cells persist in most of the sinusoids but contain mainly cells of the erythrocyte series and megakaryocytes.

A lobular arrangement is well established by the end of the 3rd postnatal week, with irregular plates of hepatocytes extending from a wide central vein to the edge of the lobule. An increased mitotic activity occurs in these cells at this time and precedes an increase in the number of hepatocytes. Sinusoids now are defined clearly, but many continue to contain small groups of hemopoietic cells consisting mainly of cells of the erythrocyte series. Numerous megakaryocytes are present.

Small bile ductules are seen for the first time at the end of the 3rd postnatal week. The ductules are lined by low cuboidal cells and lie within a small amount of connective tissue at the periphery of the lobules. Scattered microvilli project from the apical surface of these cells into the lumen of the ductule. Clearly defined portal areas that contain small bile ducts also are seen for the first time, near the periphery of established lobules.

Lipid droplets and glycogen remain abundant in the cytoplasm of hepatocytes through the 7th postnatal week. Lipid droplets are often large and may attain a diameter in excess of 8 μm.

Peaks of mitotic activity seen in hepatocytes occur at the end of the 1st and 3rd postnatal weeks (Fig. 44). Each peak precedes a subsequent increase in the

total number of hepatocytes and is accompanied by a modest increase in the number of binucleate cells. Aside from these two periods, the numbers of binucleate hepatocytes and of those in mitosis remain fairly constant (Fig. 44). Hepatocytes reach maximum numbers by the 6th postnatal week and thereafter remain relatively constant to maturity. The apparent decrease in number of hepatocytes during the 1st week coincides with a period of marked hemopoietic activity. Primitive blood cell precursors (stem cells) are present in the opossum liver during the first few days of the 1st postnatal week. They occur in small numbers and soon disappear. Following the 1st postnatal week, with continued differentiation of blood cells, decline and extinction of hemopoietic elements soon results. Hemopoietic stem cells may produce a factor that retards division in hepatocytes until much of the hepatic hemopoiesis is complete. Once the primitive blood cell precursors are depleted, mitotic activity increases among hepatocytes.

Individual lobules and periportal areas are clearly defined by the end of the 6th postnatal week. Hepatocytes crowded at the periphery of lobules still contain large quantities of lipid droplets. Blood-forming elements occur only as small, scattered foci at the periphery of the lobules. These consist of megakaryocytes and a few cells of the erythrocyte series. Mitotic activity among hemopoietic elements no longer occurs, but an occasional hepatocyte can be seen in mitosis.

It is not until just after weaning that the liver architecture becomes adult in appearance (Cutts et al. 1973). Prior to that time the liver resembles that at the 6th postnatal week. Lipid droplets and glycogen remain abundant through the 7th week. By postnatal week 10, there are fewer, smaller lipid droplets. The remaining hepatocyte cytoplasm is characterized by the presence of granular endoplasmic reticulum, glycogen, and numerous, small, spherical mitochondria. Golgi complexes are seen adjacent to bile canaliculi. Scattered among these hepatocytes are hepatocytes that show greater electron density.

The architecture of the liver in the adult opossum is typically mammalian. Lobules arranged around central veins are bounded incompletely by connective tissue elements, and blood-forming elements are absent except for the occasional megakaryocyte. Periportal areas generally are small and contain a sparse connective tissue that envelops blood vessels and tributaries of the bile duct. Hepatocytes are uniform in size and contain pale, vesicular nuclei. A few binucleate hepatic cells are present, as in earlier stages.

Hemopoiesis is a prominent feature of the opossum liver, especially during the first 2 weeks of postnatal life (Fig. 45). Seventy-nine percent of the cells of the newborn liver are hemopoietic in origin and have an intense mitotic rate of 40/1000 cells. Hemopoietic cells account for 85% of the liver cells by the end of the 1st postnatal week, but this number rapidly declines so that by the 6th week they constitute only about 7% of the cells present in a liver section.

The decrease in hemopoietic cells is concomitant with the increase in hepatic cells in the developing opossum liver, and similar events have been described in the mouse (Nichols and Simmons 1970). From birth to about the end of the 9th postnatal week there is a progressive increase in the number of hepatocytes in *Didelphis*; during this same period the number of blood-forming cells decreases. The liver is the only source of new erythrocytes in peripheral

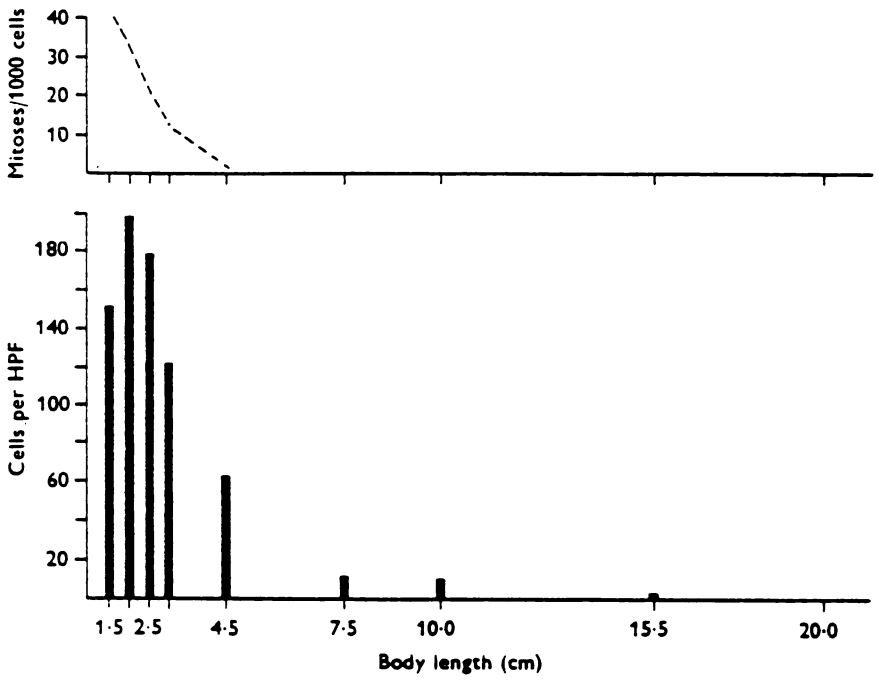


Fig. 45. Quantitative data for hemopoietic cells in the liver of the postnatal opossum. The *lower figure* shows the total number of hemopoietic cells (erythrocytic, granulocytic, and megakaryocytic) per high power field (*HPF*), while the *upper figure* shows the number of mitotic cells per 1000 hemopoietic cells. (Cutts et al. 1973)

blood during the first postnatal days (Cutts et al. 1980), and there is little or no contribution being made by the spleen at this time (Cutts and Krause 1982). Very few circulating leukocytes are present at birth, and these consist mainly of neutrophils (Cutts and Krause 1980b). During the first 2 weeks of postnatal life, leukocytes rapidly increase in number in the blood, primarily as a result of hepatic hemopoiesis. Megakaryocytes are prominent in the liver up to the end of the 3rd postnatal week, and although their subsequent decline is rapid, they can be observed occasionally even in adult animals (Cutts et al. 1973; Paone et al. 1975).

Early in the postnatal period in *Didelphis*, liver growth occurs principally as a result of hyperplasia. Numerous mitotic figures are seen until the 6th week, with peaks of mitotic activity occurring at the end of the 1st and 3rd postnatal weeks. Each peak precedes a subsequent increase in the total number of hepatocytes. The peaks of mitotic activity coincide with a modest increase in the number of binucleate cells. Hepatocytes reach their maximum number by the end of the 6th week and thereafter maintain a constant level. This observation, plus the uniformity of cell size throughout most of the postnatal period, suggests a lack of hypertrophy in the opossum liver. This situation is quite unlike that in the rat, rabbit, and pig, in which liver growth is a result of both hyperplasia and hypertrophy (Bischoff et al. 1969; Greengard et al. 1972; Leeson and Cutts 1972).

The presence of numerous lipid droplets is characteristic of hepatic cells during the postnatal period of *Didelphis*. The accumulation reaches a peak at the end of the 1st week, then gradually declines so that only a few droplets remain in juvenile animals. In the cat, guinea pig, mouse, and rabbit, lipid stores are high at birth and are depleted gradually to reach adult levels during the following 1–2 weeks (Chaves 1923; Imrie and Graham 1920; Deane 1944; Leeson and Cutts 1972). Agranular endoplasmic reticulum is scarce in the hepatocytes of opossums younger than 85 postnatal days. At this stage and in older animals, an increased but variable amount of agranular endoplasmic reticulum is seen. This increase occurs during and after weaning and may result from a change in diet (Krause et al. 1975). Golgi elements usually are small and consist of two to five short cisternae. The complexes tend to be situated between the nuclei and bile canaliculi of hepatocytes and are observed throughout the postnatal period.

7.3 Gallbladder and Common Bile Duct of the Adult Opossum

The gallbladder of the adult opossum is a spherical structure that passes abruptly into a straight, tenuous cystic duct (Dubois and Hunt 1932a). It has a typical mammalian structure and consists of a mucosa, a muscularis, and a surrounding serosa or adventitia.

The common bile duct has been subdivided into five regions according to the morphology of a given segment (Dubois and Hunt 1932b). Segment 1 is the upper one-third of the common bile duct, which lacks smooth muscle in the surrounding wall; segment 2 consists primarily of the middle portion and is characterized by scattered peripheral bundles of longitudinally arranged smooth muscle, while segment 3 is a short portion that lies parallel to the pancreatic duct. In segment 3 both the common bile duct and the pancreatic duct are encircled by longitudinally and circularly arranged bundles of smooth muscle. Segment 4 is an elongated region of the ampulla of Vater where the pancreatic and common bile ducts share a common lumen. The surrounding wall shows a thickening of the muscle coats, and this region lies completely outside the duodenal wall unlike in most eutherian mammals, including man. The fifth segment consists of an intramural duct that extends from the ampulla. The surrounding musculature is distinct from that of the duodenal wall. There are no published reports concerned with the development of this portion of the bilary system in *Didelphis*.

Table 68. Mean content of cholesterol, bile acids, and phospholipid in opossum bile (Nakayama and Johnston 1957)

	Cholesterol (mg %)	Cholic acid (mg/ml)	Deoxycholic acid (mg/ml)	Chenodeoxy-cholic acid (mg/ml)	Phospholipid (mg/ml)
Hepatic bile	23.1	1.6	0.50	2.4	9.1
Gallbladder bile	137.9	25.5	5.1	13.2	27.5

7.4 Opossum Bile

Bile constituents from the gallbladder and hepatic bile have been analyzed in the adult opossum (Haselwood and Wootton 1950; Wilber 1952; Nakayama and Johnston 1957). Cholesterol levels are comparable to those in common laboratory animals, and cholic acid and chenodeoxycholic acid have been identified as the major bile acids. The mean amounts of these substances in hepatic and gallbladder bile are summarized in Table 68.

8 Factors That Influence the Differentiation and Growth of Glands Associated with the Gastrointestinal Mucosa

Various factors influence the differentiation, growth, and function of the glands associated with the gastrointestinal mucosa and also play an important role in regulating and maintaining the balance between epithelial cell proliferation and loss of cells from the gastrointestinal mucosa. Although the nature of the activities of such substances in *Didelphis* is unknown, there appears to be no reason to believe that they function in any way different to those in other mammals. Differentiation and growth, as well as epithelial cell proliferation and loss, are influenced by luminal, dietary, neural, and hormonal factors. Preprogrammed intrinsic factors also may play an important role in the regulation of the processes. The effect of such factors on gastrointestinal differentiation and growth have been summarized in elegant reviews by Klein and McKenzie (1983a,b), and by Johnson (1987) and Henning (1987). These factors include hypophysial hormones or tissues under hypophysial control, endogenous gastrointestinal hormones from enteroendocrine cells, neurotransmitters from the enteric nervous system, factors present in luminal content (amniotic fluid, milk, or dietary factors), and intrinsic mechanisms such as prostaglandins and chalones. Gastrin-, CCK-, somatostatin-, BPP-, glucagon-, secretin, motilin-, GIP-, neurotensin-, and 5-HT-immunoreactive cells are present in the gastrointestinal mucosa of the newborn opossum (Krause et al. 1986, 1989b). Cells with neurotensin immunoreactivity are unique in that, although present in the proximal colon of the newborn, the cells disappear and a significant population is not seen again in the intestinal mucosa until the 74th postnatal day, just prior to weaning.

Certain gastrointestinal peptides may exert inductive or inhibitory effects on the gastrointestinal mucosa and glands during development and may initiate secretion in *Didelphis* as in other mammalian species (Simopoulos et al. 1989; Walsh 1987). For example, the period in which gastrin-immunoreactive cells are most abundant in the gastrointestinal mucosa is also that period when the oxyntic glands show the greatest increase in length due to increase in the number of parietal cells (Krause et al. 1976b, 1986, 1989b). It has been shown that exogenous administration of pentagastrin to other species causes an increase in depth of the oxyntic mucosa (Crean et al. 1969; Stanley et al. 1972). Gastrin, which is thought to be selectively targeted to specific cell types in the oxyntic mucosa in promoting growth, is known to stimulate mitotic activity and DNA synthesis of glandular stem cells and to increase the number of parietal cells (Willems et al. 1972b; Willems and Lehy 1975; Caes and Willems 1984; Majumdar 1984). However, exogenous gastrin does not stimulate the growth of the pyloric mucosa or increase the number of chief cells. Cells immunoreactive

for somatostatin also increase in the gastrointestinal mucosa during development. After the oxyntic glands have been established, the total number of somatostatin-immunoreactive cells exceeds the number of gastrin-immunoreactive cells (Krause et al. 1986, 1989b). It is at this time that chief cells are seen in the oxyntic glands of *Didelphis*. Administration of somatostatin to other species decreases the proliferative activity of progenitor cells stimulated by gastrin, resulting in a hypoplasia of parietal cells, thus counteracting the stimulatory effects of exogenous pentagastrin on the fundic mucosa (Lehy 1984; Senegas-Balas et al. 1985). These results strongly suggest that somatostatin modulates the differentiation of parietal cells through action on gastrin-containing cells.

The pancreas and liver both develop from the region of the foregut that will become the duodenum and whose mucosa shows the greatest diversity and number of enteroendocrine cell types. Hence, it is not surprising that several gastrointestinal peptides from enteroendocrine cells of this region of the gut dramatically influence the activities of the liver and pancreas. Secretin, gastrin, neurotensin, and CCK affect secretion by the pancreas. Secretin promotes a bicarbonate-rich secretion by the pancreatic ductal system whereas CCK promotes synthesis and release of enzymes by pancreatic acinar cells (Petersen et al. 1978; Solomon 1987). Some gastrointestinal peptides modulate pancreatic endocrine function also (Yamada et al. 1980, 1983). In addition to peptides that originate from the gastrointestinal mucosa, peptides from the endocrine portion of the pancreas may influence the function of its exocrine and endocrine components as well (Grube 1986; Henderson et al. 1981). Pancreatic polypeptide has an inhibitory effect on pancreatic enzyme secretion and plays an important role in the regulation of pancreatic secretion (Langlois et al. 1989). Somatostatin inhibits pancreatic secretion also; insulin promotes synthesis by pancreatic acinar cells, whereas glucagon inhibits the synthesis and release of enzymes but stimulates the release of bicarbonate (Henderson et al. 1981).

Many gastrointestinal peptides from the proximal intestinal mucosa also influence the liver and biliary system. Bile formation occurs primarily at two anatomical sites: (a) bile canaliculi and (b) bile ductules and ducts. In the bile canaliculi, bile flow is the result of bile acid-dependent and bile acid-independent mechanisms (Javitt 1976a,b). Secretin increases ductular-ductal bile secretion, which is rich in bicarbonate (Jones et al. 1971; Boyer and Bloomer 1974), whereas glucagon increases canalicular bile secretion by the bile acid-independent mechanism (Kaminski et al. 1975; Meyers and Jones 1979). CCK stimulates contraction of the gallbladder and relaxation of the hepatopancreatic sphincter and increases bile flow, but the site of action of CCK in the promotion of bile secretion has yet to be determined. Somatostatin inhibits canalicular bile formation in man and rats, and may decrease ductular-ductal bile secretion as well (Ricci and Fevery 1981; Kaminski and Deshpande 1983; Magnusson et al. 1989). Insulin and glucagon both influence carbohydrate metabolism in hepatocytes.

The precise role played by these gastrointestinal peptides in the normal development of the liver and pancreas is uncertain, but it is known that CCK influences or promotes pancreatic growth (Brants and Morisset 1976). Although

CCK exists in several molecular forms, little is known about their role in development. Exogenous CCK markedly affects pancreatic growth in adult animals, and combinations of secretin and CCK increase the growth of the rat pancreas to a greater degree than when separate doses of the same peptides are administered (Dembiński and Johnson 1980; Johnson 1987; Solomon et al. 1978, 1983). Effects of exogenous CCK on pancreatic growth of neonates are less well understood, but CCK-8 in young rats causes hyperplasia and hypertrophy (Werlin and Stefaniak 1982a,b, 1983). Secretin injected into neonatal rats also results in hypertrophy and hyperplasia of the pancreas (Morisset 1980), whereas somatostatin inhibits growth of the pancreas (Morisset et al. 1982; Morisset 1984).

At birth the pancreas of the opossum appears to be non-functional and consists essentially of exocrine epithelial tubules developing around a central region of endocrine cells (King et al. 1978; Krause et al. 1989a). Acini, centroacinar cells, and intralobular ducts are present by the end of the 1st postnatal week, but more mature pancreatic lobules are not a prominent feature of the opossum pancreas until near weaning, when the ductal system and acini are well established. The postweaning period is concerned primarily with expansion of established ducts and acini (King et al. 1978). The number of cells immunoreactive for CCK and gastrin in the gastrointestinal mucosa is relatively high during this period in *Didelphis* as compared to levels in the adult (Krause et al. 1985, 1989b). In contrast, the number of cells immunoreactive for secretin does not reach adult levels until the postweaning period.

Like CCK and secretin, neurotensin is reported to have a stimulatory effect on secretion by the exocrine pancreas (Baca et al. 1982; Khalil et al. 1986), and administration of neurotensin to young rats results in hyperplasia of the pancreas (Feurle et al. 1987). Cells immunoreactive for neurotensin appear in the intestinal mucosa of *Didelphis* at a time when the pancreas shows the greatest degree of growth (Krause et al. 1989b), whereas cells that are immunoreactive for somatostatin show a relative decrease in number in the opossum pancreas during the period of rapid growth (Krause et al. 1989a). The concentration of somatostatin-immunoreactive cells also is lowest in the gut when the pancreas shows the greatest amount of growth (Krause et al. 1989b). Cells reactive for pancreatic polypeptide within islets are present in relatively high numbers in the pancreas of *Didelphis* prior to weaning. Exocrine secretion is inhibited by pancreatic polypeptide administration in other species (Adrian et al. 1978; Beglinger et al. 1984; Langlois et al. 1989), but the effect of pancreatic polypeptide on pancreatic growth in the opossum is unknown.

Cells that are immunoreactive for pancreatic polypeptide are the most numerous cell type in the endocrine pancreas of the opossum prior to weaning, when the exocrine component is being established. Pancreatic polypeptide inhibits exocrine secretion in most mammals and may have a similar function in the opossum during the early postnatal period, thus allowing for continued differentiation and growth of the exocrine pancreas. Just prior to weaning (~74 days postnatum), chief cells appear in the oxyntic mucosa, and this correlates with the time at which cells reactive for pancreatic polypeptide decrease in the pancreas and cells immunoreactive to secretin increase in the gut. What influence these gastrointestinal peptides have on the development of the biliary

system and liver of *Didelphis* or of other species is unknown, but since several of the gastrointestinal peptides do influence the activity of the biliary system and hepatocytes, it would seem logical that they also may influence development. This apparently is the case for the pancreas.

Regulation of hepatic and pancreatic growth is influenced by the contents of the gut lumen and may involve factors such as epidermal growth factor, prostaglandins, and chalones, in addition to gastrointestinal peptides (Lebenthal 1989; Ménard 1989). The submandibular and duodenal glands both develop precociously in the opossum as compared to the gastrointestinal mucosa and, in other species, these glands produce large amounts of epidermal growth factor (Heitz et al. 1978; Kirkegaard 1983). How this latter factor, if present in the opossum, might influence gastrointestinal structures during development is unknown.

Secretions from classical endocrine organs such as the pituitary, thyroid, and adrenal glands are known to influence development of the gastrointestinal tract and its associated glands (Morisset and Jolicoeur 1980; Morisset et al. 1981). Some evidence suggests that classical endocrine hormones may interact with cells within the gastrointestinal mucosa which then serve as intermediaries in regulating the growth of associated digestive glands (Enochs and Johnson 1977; Werlin and Stefaniak 1983; Johnson 1981, 1987). Palmiter-Thomas (1987) demonstrated four molecular forms of intestinal CCK in neonatal rats; each form showed a distinct change in amount with postnatal age. Intestinal extracts from neonatal rats contained significantly more CCK after administration of hydrocortisone (Palmiter-Thomas 1987), suggesting that hydrocortisone may act on CCK-containing cells to regulate both the amount and the type of CCK produced in the gut. Both may be very important in regulating pancreatic growth during development.

In *Didelphis* the pituitary, thyroid, parathyroid, and adrenal are immature in appearance at birth and during the 1st postnatal week. It is not until much later in the prolonged postnatal period that they begin to show mature features (Carmichael et al. 1987; Krause and Cutts 1983a,b; Sherman and Krause 1990). These observations suggest that classical endocrine organs may have little influence on early development of the gastrointestinal mucosa and its associated glands, but may play a major role near or at weaning, when these structures mature physiologically and show adult morphology. It may be significant that it is at this stage of development that the enteroendocrine cells are at their greatest number.

Didelphis would appear to be an ideal animal model with which to study development of the digestive tract and its interaction with endocrine organs that influence its differentiation and development. Classical endocrine organs essentially develop in parallel with the digestive system during the lengthy postnatal period.

9 Concluding Remarks

The opossum is an ideal animal model in which to study the formation and establishment of endoderm and its subsequent differentiation into fore-, mid- and hindgut structures. The marsupial model is not complicated by the formation of a morula or an inner cell mass as in eutherian species. Only a unilaminar sphere is formed, followed by development of bilaminar and trilaminar spheres due to differentiation of the endoderm and mesoderm, respectively. One region of the trilaminar sphere becomes the embryo while the remainder forms the fetal membranes. Culture methods for studying cleavage and early blastocyst formation in metatherians are available and have been used to study the early developmental biology of Australian marsupials (Selwood and Young 1983; Selwood 1986, 1987, 1989) as well as later developmental stages of *Didelphis* (New and Mizell 1972).

Because of the short gestation period (12½ days) the opossum model can be used to study most of the developmental events of the digestive system since these occur primarily when the young are within the pouch and available for a variety of manipulative procedures. *Didelphis* would appear to be ideal for an examination of the effects of diet on normal differentiation, development, and function of the digestive system. For example, parathyroid hormone-related peptide occurs in opossum milk (Thurston et al. 1990) and is thought to influence calcium metabolism prior to functioning of the parathyroid gland. Since in the opossum most endocrine organs are established during the postnatal period, signaling mechanisms and the early interactions between each endocrine organ and the various components of the digestive system could be examined in this species. Thus, *Didelphis*, because of its very immature state at birth, can be used to address many basic developmental questions without the complications of intrauterine surgery, to an extent that would be difficult with eutherian mammals.

The general development of the digestive system of *Didelphis* is the same as that in other mammals, although some subtle modifications occur which presumably compensate for the abbreviated gestation period and allow the organism to survive in the external environment of the pouch. Peculiarities such as the very late appearance of chief cells in the oxyntic glands and the delayed maturation of salivary glands, pancreas, and liver can be used to advantage by the investigator.

The distribution of the various types of endocrine cells in the opossum gastrointestinal mucosa is established early and is similar to that reported for man and other species (Sjölund et al. 1983; Solcia et al. 1987; Takagi et al. 1990). The opossum pancreas is a large, compact, glandular structure, similar

in morphology to that of the dog and man. Thus, the opossum has an advantage over rats, mice, and several other common laboratory animals in which the pancreas is a small, thin mesenteric structure.

Like other visceral structures, the organs of the digestive system of *Didelphis* are very immature (embryonic) at birth, making the pouch-young of this species ideal for studying this system under a variety of experimental conditions. Because the young are accessible within the pouch, where they remain attached to the same nipple for the first 60 days of postnatal life, each neonate can be subjected to different chemical, physical, operative, or dietary manipulations under a known identical environment. Experimental materials can be administered intragastrically by simply passing a small catheter along the nipple and through the oral cavity and esophagus to the stomach, without removing the young from the nipple or pouch (Jurgelski 1971, 1974). Because of the relatively large litter size, littermates at the same stage of development can be used as control and experimental animals. Attention by biomedical investigators needs to be directed to the quantitative and qualitative analysis of opossum milk. The opossum and other marsupials rely on the mother's milk for a long period (approximately 90 days in the opossum), at a time when most of the differentiation and development of organs take place. The milk of eutherians is relatively uniform in composition throughout lactation in a given species, once full lactation is achieved. In contrast, the milk composition (sugars, proteins, lipids) of marsupials changes throughout lactation (Green and Merchant 1988). The milk in these species shows a severalfold increase in content of solids between early and late lactation. The carbohydrate content is high in early milk and low in late milk, and the lipid content increases in late lactation. In addition to nutritional substances, several hormones and hormone-related substances (prostaglandins, nucleotides, NGF, EGF) are present in the milk of a variety of eutherian species, including man (Koldovsky et al. 1988; Ménard and Arsenault 1988). NGF and EGF are present in eutherian milk and are thought to have profound effects on the development of the structures that compose the digestive system (Lebenthal 1989; Ménard 1989). To date an analysis for hormones or growth factors in marsupial milk, including that of *Didelphis*, has not been done.

In addition to EGF and NGF possibly occurring in opossum milk, two glands (the submandibular and duodenal glands) also are known to produce large amounts of NGF or EGF in eutherian species. Both glands are well developed in the opossum and develop precociously with respect to other structures associated with the digestive tract. These observations suggest that both glands may play an important role in opossum development. Although neither NGF nor EGF has been looked for in the opossum, it is quite likely that they are present and function in a way similar to that observed in other species.

Organs such as the esophagus, stomach, and pancreas of *Didelphis* anatomically are very similar to those of man and thus are unlike those in most laboratory animals. As pointed out by Henning (1987), future investigations on gastrointestinal development and the mechanisms concerned with its regulation must be done on species other than rats and mice since in many aspects of gastrointestinal development, rodents are quite different from man. The developing opossum would appear to fulfill this need.

Didelphis also appears to be an ideal mammalian model in which to study changes in the digestive system due to aging. For their size, opossums generally are regarded as among the shortest-lived mammals, with a life expectancy in the wild of about 2 years. By the end of their second winter, the emaciated appearance, the worn teeth, and the state of reproductive decline give a general impression of the animal being just "worn out" (Hunsaker 1977; Seidensticker and Lumpkin 1989).

Didelphis has become the animal model of choice for the study of the esophagus, because the morphology and physiology of this organ are similar to those in man. The opossum esophagus has been of special value in investigations concerning properties of the upper and lower esophageal sphincters and factors that control their activity. Such studies have been extended to the pyloric, ileocecal, and internal anal sphincteric regions, as well as other aspects of normal gastrointestinal function.

This manuscript presents a summary of the published data concerning the development of the digestive system of the North American opossum, *Didelphis virginiana*. It is hoped that this review will stimulate research interest in an experimental animal model that is underused for developmental studies not only of the digestive system, but of other organ systems as well. The opossum, due to its very short gestation period of only 12½ days, should prove to be an excellent experimental model with which to address specific developmental questions that would be difficult if not impossible to tackle utilizing most eutherian species.

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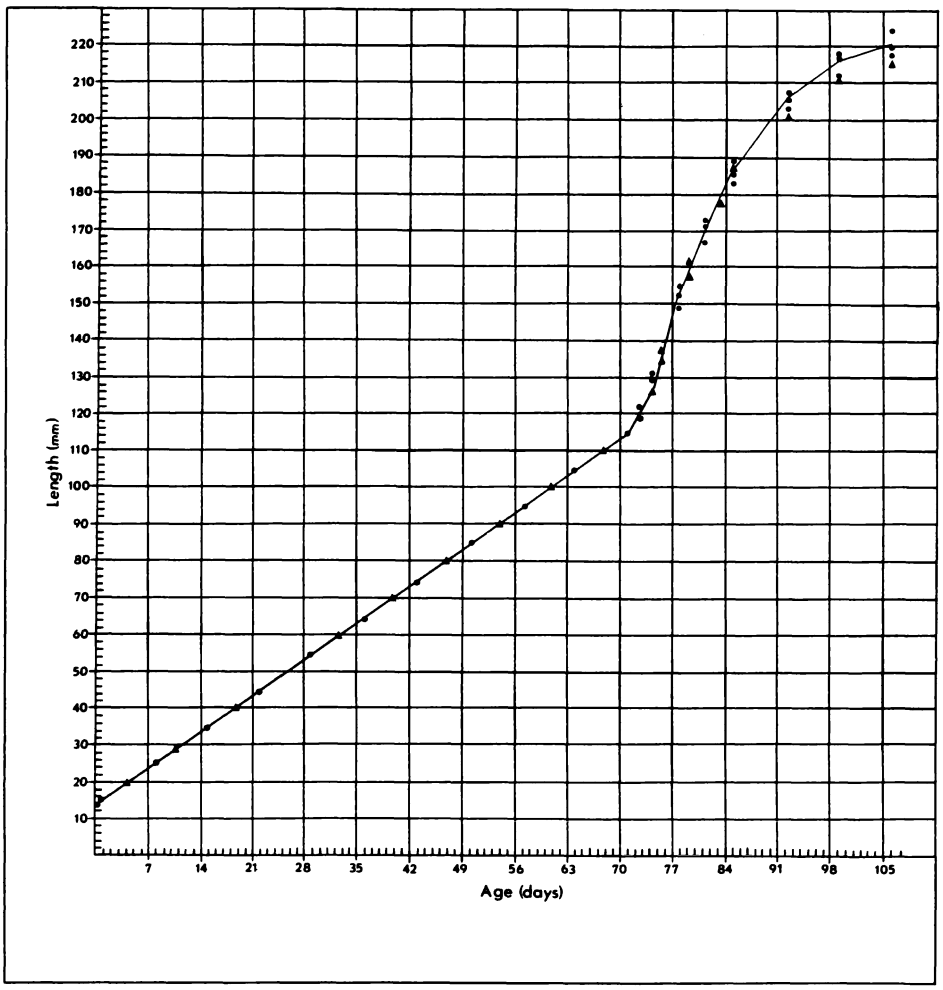
Appendix

The figure (●, data obtained from litters born in captivity; ▲, data from litters born in the wild. Each point on the curve is the mean for 3–9 animals.) shown below (from Cutts et al. 1978b) relates body length (snout-pump length) to age for the first 106 days of postnatal life. During the first 10 weeks, growth is linear and the increase in length is remarkably constant regardless of litter size or sex.



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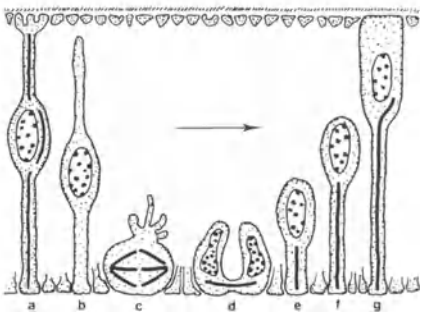

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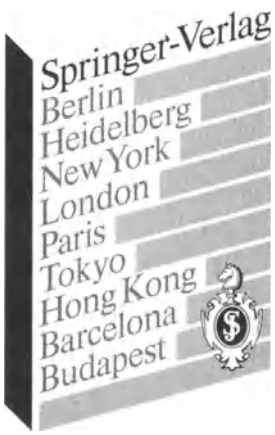

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In this investigation, the development of different larval pigment patterns in two species of tailed amphibians is used as a model system for a comparative morphogenetic analysis. The study presents new data on the interactive behaviour of melanophores and xanthophores during their arrangement into horizontal or vertical cellular arrays.

Volume 119

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A Critique of Hypotheses

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Volume 121

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