AGE CHANGES IN THE GASTRIC MUCOSA OF THE DOMESTIC PIG

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A Thesis Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

MASTER OF SCIENCE

Major Subject: Veterinary Anatomy

Iowa State University
Of Science and Technology
Ames, Iowa

1962

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INTRODUCTION

Studies made to date on the histological structure of the mucosa of the stomach of the pig have been confined to recording what was observed in animals of a specific age. Sometimes the exact age was given; more often the term young, young adult, adult, or fully grown was the only reference to the age of the animal under consideration. No effort appears to have been made in the past to correlate specifically age with the histological observations made on the mucosa of the stomach of the pig. Does age bring change or changes in the histological structure of the gastric mucosa of the pig? Is there such a thing as a growing mucosa, an average mucosa, or a senile one? If there is, how and at what age does the change take place?

This investigation was undertaken in order to study the possible microscopic changes that occur, during a period of time, from birth to forty-four months and at eight years of age in the gastric mucosa of the pig.

REVIEW OF LITERATURE

Cells of the Gastric Mucosa

Studies made to date on the problem of age changes in the gastric mucosa of the pig had confined themselves to the enzymatic aspect, omitting entirely a histological basis.

Histologists today agree that the stomach mucosa of the pig can be divided into four zones: the esophageal, the cardiac, the fundic, and the pyloric. The esophageal region of the stomach is glandless in all domestic animals (Bensley, 1932). The gastric glands are found in the other three zones.

Histologists also agree on the different types of cells found in the three glandular zones. In the adult pig four types are seen: the chief or peptic cells, the mucous neck cells, the parietal cells and the enterochromaffin cells. In addition, the lining of the glandular stomach is made up of a fifth type, the mucous cells.

The mucous cells

A single layer of tall columnar cells forms the epithelial lining. The distal end of these cells is always filled with an accumulation of mucigenous droplets (Trautman and Fiebiger, 1957) that give a strong positive reaction to the Schiff reagent after oxidation with periodic acid (McManus, 1946). The nucleus in the cell is always basal, oval or rounded in shape, and is usually surrounded by clear cytoplasm. The cells are the highest on the free surface. They become shorter in the

foveolae. The height of the cells is further reduced in the neck and fundus of the glands (Bensley, 1932).

The chief or peptic cells

The chief cells were discovered by Heidenhain in 1870. They are low columnar or pyramidal cells with basal, round nuclei. The basal part of their cytoplasm contains probably ribonucleoprotein material which stains with nuclear dyes. Sometimes it gives the cell a striated appearance (Weber, 1957).

Langley (1879-1882) showed the relationship between the digestive process and the granules of the chief cells. He definitely showed that pepsin pre-existed in the mucous membrane as an antecedent (called now a proenzyme) which he called pepsinogen. Subsequent observers had used the term "zymogen granules." Langley also showed that the granules in question accumulated during period of fasting, and diminished in size during period of active secretion. These secretory granules disintegrate quickly after death due to the acid content of the stomach, unless quickly and properly fixed.

The function of the chief cells is commonly accepted today. The cells produce pepsinogen which is activated into pepsin by the acid content of the stomach. Pepsin acts mainly in the digestion of proteins.

The chief cells are usually found in the lower third or lower half of the gland tubules.

The mucous neck cells

The cells were given the name because of their location in the upper narrower portion of the gland tubules. Bizzozero (1892), as quoted by Bensley (1932), was the first to think that they were different in some respect to the cells in the upper portion of the gland. Bizzozero believed them to be transition form "between the cells of the surface and those constituting the glands." Bensley (1898) proved that these cells were different in structure, content and function from the other cells of the glands. Their function is not known for sure. Their secretion looks like mucus, but their staining reaction is different from the mucus of the lining epithelium. In ordinary preparations, the secretion of the mucous neck cells appear clear or coarsely reticular, and displays the characteristics of mucous cells in general (Bensley, 1932).

The mucous neck cells have a flattened, basal nucleus, presenting sometimes a foamy cytoplasm. They are not always restricted to the neck region of the gland, for they may often be found scattered throughout the body of the gland. In such occasions, they appear as wedge-shaped cells among the parietals. Bensley (1932) described in them small filamentous mitochondria.

The parietal cells

The parietal cells are the oldest known type of cells of the gastric glands of mammals. They are also called oxyntic cells. They are the first to differentiate embryologically in the pig (Kirk, 1910).

They are oblong, triangular or rounded according to their location in the gland. Their name is derived from the somewhat extra-glandular position in which they are most commonly seen. They seem to be squeezed between two adjacent cells and the basement membrane, and present a large rounded clear nucleus. Sometimes two or three nuclei can be found in one cell. Heidenhain (1870)[1] thought they were completely separated from the gland lumen. Stohr (1882)[1] found a process extending from the parietal cell between two adjacent cells, connecting the parietal cell to the lumen. Stohr's finding was later confirmed by Muller (1892),[1] Golgi (1893)[1] and others.[1] Today the existence of such a process is no longer in doubt.

The intracellular canaliculi which are the most distinguishing feature of the parietal cells were observed by Stohr, and later confirmed by Muller and Golgi. The canaliculi appear to be permanent features of the cells. In ordinary preparation under the light microscope, they appear as empty spaces. The canaliculi form an intricate system of secretion channels without a definite pattern, but occupying for the most part the middle of the area between the nucleus and the cellular membrane. The canaliculi connect by one or several intercellular channels with the lumen of the gland. Hally (1959) examined the parietal cell of the mouse under the electron microscope. The most prominent feature in the parietal cell was the intercellular canal which was lined with numerous microvilli. The intercellular channels are

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[1]As quoted by Bensley (1932).

clefts between adjacent mucous cells, and are also lined with microvilli. Similar findings were made in the rat by Kurosomi et al. (1958), in the dog and cat by Sedar (1959), Lawn (1960), and Vial and Orrego (1960). In his study of the parietal cells of the mouse, Hally (1959) found some of what he calls vacuole-containing-bodies (vcb), the number of which seems to fluctuate according to the physiological state of the cell. These vacuole-containing-bodies can be seen in sections 2 micra thick, with the phase contrast microscope.

The cytoplasm of the parietal cells studied under the electron microscope is filled with closely packed mitochondria. The cytoplasm appears clear in between the mitochondria, which are generally spherical in shape or may appear as short thick rods. The findings with the electron microscope corroborate those of Altman (1890),[1] and of Lim (1922) and Ma (1927) with the light microscope.

It is well agreed that the parietal cell secretes hydrochloric acid. However, the manner of formation is still to be clarified. There are several theories, the most commonly accepted one is that neutral salt is excreted by the parietal cell, then the base in combination with bicarbonate is reabsorbed, carbonic anhydrase and urea being involved in the process. "The over-all mechanism is an ion exchange procedure." (Maximow and Bloom, 1957)

Argentaffin or enterochromaffin cells

The argentaffin cells have been more often described in connection with the intestine and referred to "as sometimes found in the stomach,

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[1] As quoted by Bensley (1932).

especially in the pyloric region" (Sharples, 1945). Of late more interest has been given to their consistent presence in the stomach of man and laboratory animals.

The argentaffin cells are polyhedral, containing a round to oval nucleus and numerous spherical cytoplasmic granules. The granules are generally found at the basal end of the cell, but can be found at the apical end, or may overshadow the nucleus. The granules stain black with silver and acquire a yellowish brown color when the tissue is fixed in solutions containing bichromates.

Cordier (1926) gave a complete historical background on the argentaffin cells. Heidenhain (1870)[1] was the first to describe them. Nicholas (1890)[1] found them in the intestine of the lizard. Kultschitzky (1897)[1] discovered them in the dog's intestine and believed them to be "acidophilic leucocytes." Moeller (1899)[1] located them in the human intestine. Schmidt (1905)[1] finding that the granules reduced the chromate salts, called them "yellow bodies." The name of chromaffin was given the argentaffin cells after Schmidt's findings. Bloch (1903)[1] and Aschof (1905)[1] found them in the human at birth. Ciaccio (1907)[1] described them in detail in dogs and guinea-pigs. Ciaccio believed them to produce adrenalin. Ciaccio was the first to call them enterochromaffin cells, Kaufman-Wolf (1911)[1] and Kull (1913)[1] made a short mention of them. Eklof (1914)[1] considered them artifacts. Masson (1914)[1] discovered their affinity for ammoniacal silver, and he called them

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[1]As quoted by Cordier.

argentaffin. Masson attributed to them a nervous function, although he admitted he never established the continuity of the Argentaffins with the submucosal plexus. Tang (1922) studied them in the pig and found them plentiful. Parat (1924) determined that they appear in the human embryo at 4 to 5 months, at about the same time the pancreatic function starts, attributing a secretin function to them.

Macklin and Macklin (1932) listed the names given at one time or another to the argentaffin cells. They are referred to as the cell of Heidenhain, of Nussbaum, of Grutzner and Menzel, of Stohr, of Hamburger, of Nicholas, of Kultschitzky, of Schmidt, of Harvey, and of Ciaccio-Masson. They are also called chromaffins, enterochromaffins argentaffins, yellow cells (gelben Zellen), chromoargentaffins, acidophils, and basal granular cells (basalgekornten Zellen).

Dios-Amado (1925) made an inclusive review of the different hypotheses on the possible significance of the argentaffin cells. He listed them as 1) acidophilic leucocytes (Kultschitzky and Bloch); 2) epithelial cells with an exocrine function (Cordier); 3) epithelial cells with an endocrine function, the production of an adrenalin-like substance (Ciaccio), or the production of secretin (Villemin and Parat), or the production of an unknown substance (Kull); 4) a special cell with a neurocrine secretion (Masson); 5) a simple artifact (Eklof); and 6) simply wandering or migratory cells (Dios-Amado).

Dios-Amado considered the sixth possibility as the real one. He based his opinion on the resemblance of the argentaffins to some cells showing the same staining reaction found in the submucosal tissue.

Wermel and Kacharova (1948) also attributed the production of secretin to the argentaffin cells but went on to explain that

> . . .there is much reason to believe that the endocrine regulation between the pancreas and the intestines involves something greater than a mere stimulation of secretin by the peptic juice. The hormones of the mucosa exert their influence through the medium of the insular apparatus of the pancreas: the mechanism may be of a diverse nature. 1) The hormones may induce the discharge of insulin from the cells (insulinocrinic effect). 2) They may stimulate the accumulation of the secretin (insulinotropic effect). 3) They may activate the genesis of new insular tissue (insulinogenic effect). . . .

The specific product of the enterochromaffin cells was considered by histologists at different times as an o-dihydroxybenzene or m-dehydroxybenzene derivative. Already in 1946 Esparmer had emphasized the probable indolic nature of the substance and thought it was 5-hydroxytriptamine. Esparmer and Asero (1952) considered enteramine as the specific secretion or storage product of the typical enterochromaffin cells of the intestine of vertebrates. They were able to isolate Enteramine as a pure picrate and at the same time were also able to synthesize it; the isolated product and the synthesized one were identical. Esparmer and Asero considered enteramine as a true hormone regulating the flow of circulatory liquids (blood and lymph) through the kidney. In mammals the substance acts primarily and specifically on the contractile structure of the afferent vascular bed of the glomerulus causing the vessel to contract. According to Esparmer and Asero, serotonin is the circulating enteramine. The role of serotonin in the nervous system is currently under intensive investigation (Cantarow and Schepartz, 1958). But it is also the prevalent opinion that the

argentaffin cells are responsible for the intrinsic anti-pernicious anemia factor in men (Dean, 1954; Gillman, 1942).

Tehver (1930) mentioned that the number of enterochromaffin cells depended upon the age of the animal. He found the number of enterochromaffin cells to vary with the age of the animal, being twice as much in young calves and foals as in adult cattle and horses. Tehver also found the distribution of the cells constant for one species. He also mentioned that they developed from endoderm in the first half of gestation in embryos of cattle, sheep, dogs and swine.

Hallier (1937-1938) counted more argentaffin cells in the females than in the males of the same species.

Dawson (1948), on the basis of staining reaction, made a difference between argentophils reacting to the Bodian method and argentaffins reacting to the Masson-Hamperl technique.

Monesi (1960) made the same differentiation on the same basis, but called the cells reacting to the Bodian method argyrophils. In addition Monesi believed that the argentophils-argyrophils and the argentaffins to be the "forerunners of the typical enterochromaffin cells."

The enterochromaffin cells are now considered to be of endodermal origin, that they are part of the cell population of the stomach and intestines, that they produce enteramine (5-hydroxytryptamine) in situ, and have no nervous connections, but their exact function, or their exact contribution to the digestive system, or to the organism as a whole is yet to be settled.

Histogenesis of the Gastric Glands

Lim (1922) reported that the cells of the mucous type found in the neck of the glands preceded the more highly differentiated parietal cells, and the zymogenic cells in histogenesis. Lim's studies were made on adult cats and a few cat fetuses.

Kirk (1910) through serial sections on progressively older pig fetuses formed a different opinion. He found the parietal cells to be the first to differentiate from the adelomorphs. He used the term adelomorphs for embryonic epithelial cells lining the primordium of the stomach. It is essential to keep in mind that Kirk's interpretations were by reference to the adult structure, and were based on granulations found or not found in the cells, and the staining reactions obtained from the three main cell types. Kirk made no mention of the mucous neck cells nor of the argentaffin cells.

The first observations were made by Kirk on 2 centimeter pig embryos and the last ones on a 27-29 cm. fetus. The latter size corresponds roughly to the fetus at term.

At 2 cm. the parts similar to the adult gastric wall were already differentiated except for the muscularis mucosa which appears at 9 or 10 cm. The epithelium was made up of one row of columnar cells of the same height, with nuclei at the same level and with a basement membrane. Stratification was present only in the esophageal region.

At $2\frac{1}{2}$ - 3 cm., the surface line of the epithelium, hitherto level, becomes undulatory in the three parts of the stomach. There was no

corresponding waviness to the basement membrane. The elevations consisted of cells slightly taller and narrower than those of the depressions.

At 3 cm., the glands which were intraepithelial seemed to grow into the lamina propria. The glands appeared earliest in the pyloric region. At this stage, some of the adelomorphs began to differentiate. The basal end of the cell enlarged and became more rounded and broader. The distal end narrowed. The cytoplasmic eosinophilic granulations appeared and canliculi could be demonstrated. The nucleus became spherical. These changed adelomorphs were the earliest parietal cells.

At 6 - $6\frac{1}{2}$ cm., the mucous cells appeared. Their presence was demonstrated by mucicarmine.

The three zones of the stomach, namely pyloric, fundic, and cardiac did not show the same degree of differentiation at the same time. The histogenesis of the three zones, as described by Kirk, can be summarized as follows:

1. Pyloric zone - At 9 cm., the glandular pyloric epithelium consisted of mucous cells. No parietal cells were present except in two or three tubules next to the fundic zone.

At 10 - 11 cm., the cells of the basal part of the glands were so packed with mucus that the nuclei were often flattened. One could say that the cytodifferentiation of the pyloric zone was complete at 9 cm.

2. Fundic zone - At 7 cm., the parietal cells were found everywhere in the glands, and were not confined to the basal region as in the 3 cm. stage. At 8 - 9 cm., every gland of the fundic zone had acquired parietal cells.

At 19 cm., mucous cells appeared in the glands, some adelomorphs were still present. Also the zymogenic cells make their appearance without passing through a mucin stage.

At 25 cm., no adelomorph remained. The cytodifferentiation was complete.

3. Cardiac zone - This zone was the slowest to develop. At 7 - 9 cm., the glands were mostly intraepithelial. At 7 cm., the cells were either adelomorphs or parietals. The parietals were at first confined to the basal region of the glands, but appeared higher up at 10 - 11 cm. At 11 - 12 cm., surface adelomorphs differentiated into mucous cells.

At 13 - 14 cm., the mucous change extended to the bottom of the glands. The mucous differentiation appeared later than in the pyloric zone, but earlier than in the fundic.

At 17 - 18 cm., all the cardiac cells were either mucous or parietal cells.

From 14 cm. on, a marked form divergence was added to the difference in size between cardiac and fundic tubules. The cardiac becoming shallower, but wider in relation to depth, while the fundic tubules preserved their narrow and deep form.

At 15 cm., certain of the groups of parietal cells began to push onward slightly, forming by 18 - 19 cm. secondary tubules made entirely of parietal cells. The secondary tubules were narrower than the primary ones. At 22 cm., several tubules opened into the foveola, some were purely parietal cells, some were purely mucous, while many were made of both cell types. These three types of glands persisted up to the time

of birth (27 - 29 cm.).

At birth, the cytodifferentiation of the cardia was incomplete, or rather "that involution required to bring about the adult cytological status with its single type of cells, was not complete."

4. Esophageal zone - The greater part of this region became stratified during the 6 - 10 cm. stage. The cells remained clear and transparent except those of the deep layer which had acquired a finely granular cytoplasm of the ordinary type. The cells of the superficial stratum had begun to flatten out. At the periphery of the stratified area, the deepest layer of cells was continuous with the simple columnar epithelium of surrounding epithelium.

At 4 - 6 cm., the boundary between the clear cells of the esophageal zone and the granular cells of the cardiac was "sharp to a cell."

Age Changes in Enzyme Activity of Pig Stomach

Lewis et al. (1957) found negligible pepsin activity present in the stomach of young pigs at time of birth, and it remained low until about 3 weeks of age. The pepsin determination was made on dry stomach tissue of baby pigs at various ages. Concurrently the pH of the stomach contents stayed on the acid side from birth to 56 days of age (end of the experiment). There was a marked difference on the acid pH of the stomach contents of pigs fed by the sow and pigs fed on dry ration. The stomach content of the pigs fed on the sow was more acid.

Kvasnitskii and Bakeeva (1940) made gastric fistula under anesthesia in piglets in their first day of life. The gastric secretions

were found to contain pepsin and rennase from the first day, but hydrochloric acid did not appear until 20 - 30 days of age. During the suckling period, the milk clotted rapidly in the stomach of newborn piglets, and the stomach contents were acid. The acidity was probably due, in his opinion, to microbial flora and to the nature of the food rather than to the stomach secretion. Although pepsin was present early in life, it was not utilized until hydrochloric acid appeared. Pepsin was actually put to use only in the last period of suckling (59 days).

Distribution of the Different Secretory Cells of the Fundic Zone in the Stomach of an Adult Pig

Kametaka and Imai (1956) made a study of the distribution of the secretory cells of the fundic zone in the gastric mucosa of an adult pig. They collected 16 samples from the fundic zone. The samples were numbered and the exact relationship with the entire stomach carefully kept. They found that the chief cells increased in number from the neck through the body to the fundus of the glands, but it was the reverse for the parietal cells, while the mucous cells were equally divided in all three parts of the glands. However, in absolute numbers, the chief cells were more numerous in the glands close to the cardiac zone, the mucous cells in the glands close to the pyloric zone, while the parietal cells were equally divided.

Kametaka and Imai did not find mucous neck cells nor zymogenic cells in the pyloric and cardiac zones, but in some portions of the cardiac zones a few cells similar to parietal cells were observed.

Mitotic Activities of the Cells of the Gastric Mucosa

How the cells of the gastric mucosa, at one time or another, are replaced has not been fully explained. Neither a systematic nor a complete study appears to have been made to date on the mitosis of the cells of the gastric glands of domestic animals under normal conditions. The researches seem to have been confined to laboratory animals.

Bizzozero (1893), as quoted by Bensley (1932), stated that the cells at the bottom of the foveolae are the sources from which the surface cells are replenished. But "the replacement of the cells of the glands proper presents, on the other hand, a different problem for which we do not know yet the solution." (Bensley, 1932). Bensley (1932) reported that Hanna (1910), who had studied the stomach in young mice, observed both chief cells and parietal cells in mitosis. Bensley agreed to the possibility of parietal cell reproducing by direct division and expressed ignorance about another method.

Hunt (1952) found 92% of the mitotic activity in the rat stomach confined to the mucous cells at the bottom of the foveolae, and 5% in the mucous neck cells. Stevens and Leblond (1953) found that in the fundic region only the two mucous containing types of cells showed a significant mitotic activity in the rat. They calculated that it took three days for the total renewal of the cells of the epithelial lining, and seven days for the mucous neck cells. The cells that were being replaced were lost by extrusion. Grant et al. (1953) pointed out that there was evidence that some changes took place during digestion in the

rat. The changes affected the depth of the cell layers, the width of the gastric pits, the mitotic activity, and the staining of the mucus. These effects showed in a wave-like manner, the peak of the wave represented a period of change, while the trough of the wave indicated a period of rest. Grant added that the changes were similar to those observed after the action of a mild irritant, restoration taking place during the interdigestive period.

Hunt (1954) observed the mitotic activity of the rat stomach at intervals after feeding. He found that the activity varied greatly according to the time elapsed after feeding and the amount eaten. The number of mitoses was low after a 48 hour fasting period. The number increased 6 to 8 hours after ingestion of enough food to fill the stomach, and reached its peak 24 hours later. The number and location of the mitoses were much like those found by Hunt in 1952. Hunt believed a transition occurred between the surface mucous cells and mucous neck cells.

Leblond and Walter (1956) pointed out that diurnal activities as opposed to nocturnal, seasonal variations, nutrition, body temperature, state of health, other environmental influences (fear, freedom or captivity) were all possible factors that could influence positively or negatively the proliferation of the cells of the gastric mucosa.

Hunt (1958) studied in the rat the regeneration of the gastric mucosa after extensive cauterization. He concluded that surface cells changed into mucous neck cells, which in turn changed into parietal and

zymogenic cells. He also stated that the rate of regeneration was not significantly different in young and older animals.

MATERIALS AND METHODS

Specimens from 73 normal pigs, 48 females and 25 males (Table 1), were collected. The age of the animals ranged from 1 day to 8 years. Table 1 shows the age spread, with the sex and the number of individuals in each group. The animals marked with footnote [a] were killed in the packing house, the others in the laboratory. The animals that were killed at the packing house were done so as a matter of convenience for the University Swine Farm. The vital statistics on all the animals in this experiment were available for study.

The animals killed in the laboratory were electrocuted and allowed to bleed freely from the axillary area. The abdomen was then quickly incised and the stomach removed. Usually the samples from the animals killed in the laboratory were in the fixative within 5 minutes after death. However, the interval between death and fixation for the animals killed at the packing house was considerably longer.

All the specimens were taken from the same general area of the stomach, namely caudal to the cardiac diverticulum, in the middle of the fundic zone, and at the pyloric antrum. The stomach was routinely inspected for possible gastritis or ulceration. The stomachs of all the animals killed were full at the time of death.

Helly's fluid was used as the fixative of choice for all the specimens (Table 1). The solution is made up of:

Potassium dichromate	2.5 grams
Sodium sulfate	1.0 gram
Mercuric chloride	5.0 grams
Distilled water	100.0 milliliters

To each 20 ml. of the solution, 1 ml. of neutral formalin was added just before use.

Processing of Specimens

All the specimens went through the same processing schedule, namely:

1. fixation for 48 hours, changing the fixative after the first 24 hours;

2. washing for 24 hours in running tap water;

3. dehydrating through 3 successive changes of dioxane, for periods of 4 hours, 4 hours and overnight, respectively;

4. infiltrating for 3 hours, leaving the blocks in successive passages of Altman's mixture or Bioloid of 1 hour each, in the paraffin oven at 58 degrees centigrade; and

5. embedding which followed immediately at the end of the infiltration period.

The first four age-groups were infiltrated and embedded in the Altman mixture, later Bioloid, a commercial preparation replaced the Altman mixture in the department. The melting temperature was the same for both media. All the blocks of stomach tissue were embedded within 5 days after collection.

Staining

In sectioning, 12 slides were routinely made from each zone of each stomach. Nine slides were equally distributed among three stains,

Hematoxylin and Eosin, Crossmon's modification of Mallory triple stain, and a combination Periodic acid-Schiff-Hematoxylin-Aurantia. The other 3 slides were kept in reserve for Getty's combination Wiegert-Heidenhain-Van Gieson, and for trying out other staining methods, and for possible replacement of broken slides.

Hematoxylin and Eosin

This was used as a routine stain. The enterochromaffin cells, stained yellow by the potassium dichromate of the Helly's fluid, contrasted readily with the reddish pink of the surrounding cells. The staining procedure consisted of:

1.	xylol	2 minutes
2.	xylol	2 minutes
3.	absolute alcohol	2 minutes
4.	95% alcohol	2 minutes
5.	80% alcohol	2 minutes
6.	2% Iodine in 70% alcohol	2 minutes
7.	2% sodium thiosulfate, until decolorized	
8.	distilled water	5 minutes
9.	Harris hematoxylin, until nuclei are purple	
10.	tap water	
11.	acid-alcohol, one quick dip, if necessary	
12.	tap water, several changes	
13.	running tap water	20 minutes
14.	80% alcohol	5 minutes
15.	Eosin Y	20 seconds
16.	80% alcohol	3 seconds
17.	80% alcohol	3 seconds
18.	95% alcohol	20 seconds
19.	absolute alcohol	1-1½ minutes
20.	xylol	2 minutes
21.	xylol	2 minutes
22.	xylol	2 minutes

Periodic acid-Schiff-Hematoxylin-Aurantia

The periodic acid-Schiff stained the mucus a deep red. Harris hematoxylin stained the nuclei of the cells blue, and Aurantia stained the parietal cells a golden yellow. The staining procedure consisted of:

1.	the first 8 steps same as in hematoxylin and eosin procedure	
9.	periodic acid	5 minutes
10.	distilled water	10 minutes
11.	Schiff reagent	5-20 minutes
12.	running tap water	15 minutes
13.	Harris hematoxylin, until nuclei are dark purple	
14.	tap water	
15.	acid-alcohol, a quick dip, if necessary	
16.	running tap water	20 minutes
17.	80% alcohol	5 minutes
18.	.5% Aurantia in 70% alcohol	2 minutes
19.	95% alcohol	a quick dip
20.	95% alcohol	a quick dip
21.	absolute alcohol	a quick dip
22.	absolute alcohol	a quick dip
23-25.	3 changes of xylol as in hematoxylin and eosin	

Crossmon's modification of Mallory's triple stain

This stain is primarily for connective tissue, but it also stained orange or bright red the zymogen granules of the chief cells. The staining procedure consisted of:

1.	the first 8 steps same as in hematoxylin and eosin procedure	
9.	Wiegert's hematoxylin	8 minutes
10.	2% sodium bicarbonate	2 minutes
11.	distilled water	1 minute
12.	acid fuchsin-orange G mixture	10 minutes
13.	distilled water, 3 changes	
14.	1½% phosphotungstic acid	3 minutes
15.	distilled water	
16.	anilin blue	1 minute
17.	distilled water, 3 quick changes	
18.	2% acetic acid	5 minutes

19.	95% alcohol	a quick dip
20.	absolute alcohol	a quick dip
21.	absolute alcohol	a quick dip
22.	absolute alcohol	a quick dip
23-25.	3 changes of xylol as in hematoxylin and eosin	

Getty's combination Wiegert-Heidenhain-Van Gieson

This stain is both a connective tissue and elastic fibers stain.

The staining procedure consisted of:

1.	xylol	2 minutes
2.	absolute alcohol	2 minutes
3.	95% alcohol	2 minutes
4.	70% alcohol	2 minutes
5.	running tap water	2 minutes
6.	distilled water	5 minutes
7.	Weigert's elastic tissue stain	45 minutes
8.	95% alcohol, 3 quick changes	
9.	running tap water	2 minutes
10.	distilled water	5 minutes
11.	5% ammonium ferric sulfate in oven at 50 degrees centigrade (or overnight at room temperature)	30 minutes
12.	distilled water, several quick rinses	
13.	Heidenhain solution at 50 degrees centigrade (or overnight at room temperature)	30 minutes
14.	distilled water, until no more color comes out	
15.	differentiating solution, until a grayish hue is obtained	
16.	tap water, until the sections turn blue	
17.	Van Gieson's	15-30 minutes
18.	95% alcohol	a quick dip
19.	absolute alcohol, 2 changes	
20.	xylol	2 minutes
21.	xylol	2 minutes

Coverslipping and mounting followed staining immediately.

Examination of the Sections

In counting cells or measuring the depth of the gastric mucosa, the area of the section seen at high dry magnification (10 x 43) was used as a unit, the field. A Bausch and Lomb filar or screw micrometer was used for measuring. Calibration of the objectives was made with a Bausch and Lomb 0.1 and 0.01 millimeter slide micrometer. The sections were systematically examined, but in counting, the fields were chosen at random. Since there were, in each stain used, three slides for each zone of the stomach, in counting, 4 fields were picked at random on each slide, and the total number divided by 12 for an average.

The parietal cells were counted in the sections stained with the Periodic acid-Schiff-Hematoxylin-Aurantia combination, and the enterochromaffin cells in the slides stained with Hematoxylin and Eosin. The enterochromaffin were also visible in the slides stained with the Crossmon's modification of Mallory's triple stain, and the count on these slides were used as a check.

Table 1. Number and age distribution of pigs studied for age-changes in the gastric mucosa and fixative used

Age	Female	Male	Total number	Fixative
1 day	3	1	4	Helly
3 days	0	1	1	"
7 " (1 week)	3	1	4	"
10 "	1	0	1	"
11 "	1	1	2	"
13 "	1	0	1	"
14 " (2 weeks)	3	1	4	"
17 "	0	2	2	"
20 "	0	1	1	"
21 " (3 ")	0	1	1	"
28 " (4 ")	0	1	1	"
34 "	0	1	1	"
35 " (5 ")	3	1	4	"
42 " (6 ")	3	1	4	"
49 " (7 ")	3	1	4	"
56 " (8 ")	3	1	4	"
63 " (9 ")	3	1	4	"
70 " (10 ")	3	1	4	"
77 " (11 ")	3	1	4	"
84 " (12 ")	3	1	4	"
112 " (16 ")	0	1	1	"
5 months	1	1	2	"
8 "	0	1	1	"
10 " a	0	1	1	"
14 " a	0	1	1	"
19 " a	1	0	1	"
20 " a	2	1	3	"
21 " a	3	0	3	"
26 " a	1	0	1	"
30 " a	1	0	1	"
31 " a	1	0	1	"
44 " a	1	0	1	"
8 years	1	0	1	"
Total	48	25	73	

[a]Animals killed at the packing house.

FINDINGS

The findings are reported according to age, and under each age according to the three glandular zones of the stomach, namely cardiac, fundic and pyloric.

Certain terms that are used are explained below:

Lymphatic elements - Term refers to the lymphocytes seen in the lamina propria. However, since heterophils sometimes, and plasma cells constantly, were observed in association with the lymphocytes, the term "lymphatic elements" was extended to include heterophils and plasma cells. No attempt was made to count or evaluate percentage-wise the heterophils and the plasma cells.

The word "diffuse" qualified the lymphatic elements when they were more or less evenly spread among the fibrocytes. When the same elements were clustered, the word "nodular" was used. There was only one instance in which a bundle of collagenous fibers running for a short arc along the perimeter of a nodule gave the impression of encapsulation.

Blood smears from some of the animals were processed and stained the same way as the stomach specimens for the purpose of identification and comparison.

Branching of the glands - Although the stomach specimens were trimmed at right angle to the mucous membrane, it was not always possible to obtain only longitudinal cuts of the glands throughout the entire section. In estimating the branching, only glands that could be seen from foveola to fundus were traced because enclosed with them, within

the boundary created by the collagenous fibers and muscle strands coming from the muscularis mucosa, were the cross sections of the branches. The amount of branching was estimated in relation to the number of cross sections visible alongside the longitudinal cuts of the glands.

One Day

Cardiac

The glands showed little branching (Figure 1). Some glands were made up entirely of parietal cells, others were a mixture of parietal and mucous cells. The gastric pits extended down to about half the thickness of the mucosa, presenting the shape of a wide V (Figure 2). The parietal cells numbered 22 per field. The depth of the mucosa averaged 99 micra.

Fundic

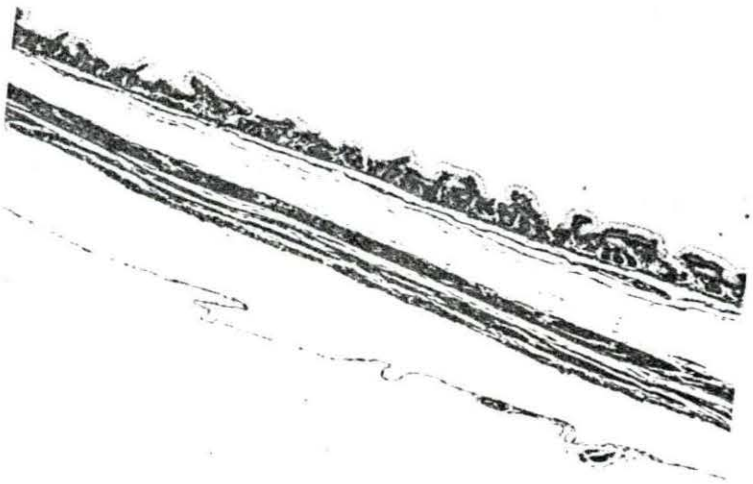
The glands were straight, with very little branching (Figure 3). There were few mucous cells. The chief cells were seen at the fundus and in the body of the glands. The mucous neck cells were not very numerous. The canaliculi of the parietal cells showed a PAS-positive reaction. No cells in mitosis were observed. The depth of the mucosa averaged 240 micra.

Pyloric

The glands showed more branching than in the other two areas. The foveolae were deep and extended down to one third the length of the glands (Figure 4). The glands were made up of largely mucous cells,

Figure 1. One day old. Cardiac zone. Crossmon's modification of Mallory's Triple Stain. 60X. The glands show little branching. The mucosa averages 99 micra.

Figure 2. One day old. Cardiac zone. Crossmon's modification of Mallory's Triple Stain. 400X. The foveolae of the glands appear to be slight depression of the lining epithelium.



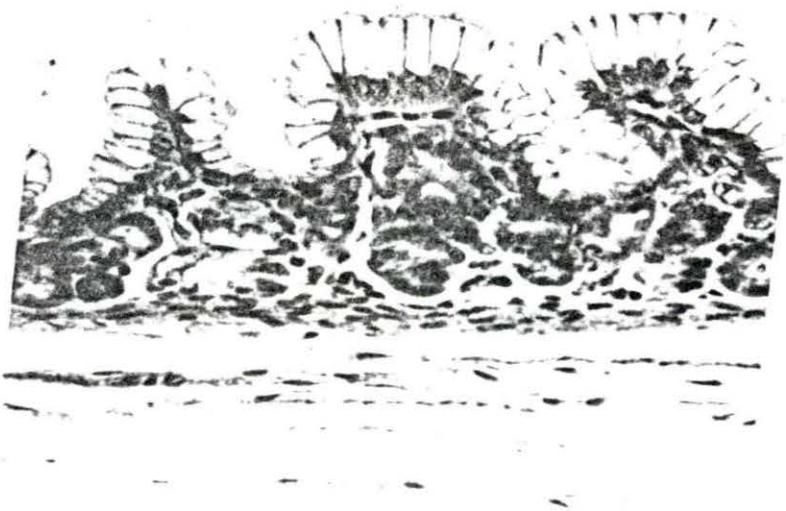
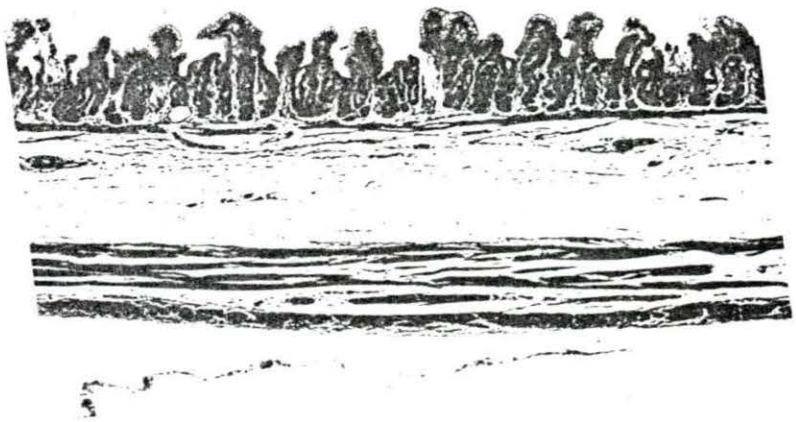
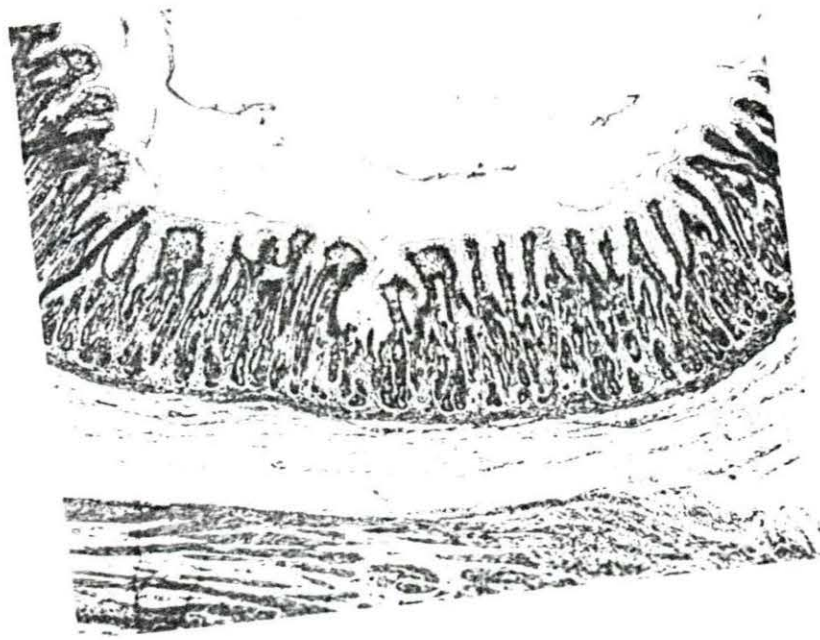


Figure 3. One day old. Fundic zone. Crossmon's modification of Mallory's Triple Stain. 60X. The glands are straight. They already resemble the glands of the adult stomach. The mucosa averages 240 micra.

Figure 4. One day old. Pyloric zone. Crossmon's modification of Mallory's Triple Stain. 60X. The glands show more branching than in the cardiac and fundic zones. The foveolae extend down close to half the length of the glands. This proportion will remain roughly the same in older animals.





with some parietal cells among them. The mean number of the parietal cells was 15 to 16 per field. Here and there cells in mitosis could be seen. Very little of the lamina propria was left unoccupied by glands. The mucosa averaged 160 micra.

Three Days

Cardiac

The glands were more branched than those of the one day old animals. The gastric pits were of the same depth but narrower. Most of the glands were made up of parietal and mucous cells. The mean number of parietal cells was 18 per field. Here and there a mitotic figure was observed. Lymphatic elements were seen throughout the sections in a diffuse form. Some lymphocytes could be observed in between the cells of the epithelial lining, probably in the process of being extruded. The mucosa averaged 120 micra.

Fundic

The glands showed little branching. The chief cells were confined to the fundus of the glands. The mucous neck cells were few in number. The parietal cells were seen anywhere along the length of the glands. Enterochromaffin cells were present, numbering about 1 per field. Diffuse lymphatic elements were obliterating the remaining lamina propria. Numerous cells were seen in mitosis. The PAS-positive area of reaction in the intracellular canaliculi appeared in some cells as a thin thread, in some others like a smear that surrounded one end of the

nucleus. The depth of the mucosa averaged 250 micra.

Pyloric

The glands showed more branching than in the one day old animal and the gastric pits were not as deep. Mucous and parietal cells were present. The latter were easily recognizable by their large and clear nuclei and the overall size of the cell. The intracellular canaliculi were not as obvious in the pyloric parietal cells as in those of the other zones of the stomach. In the pyloric parietal cells, the cytoplasm appeared more homogeneous. There were approximately 6 to 7 parietal cells and 1 to 2 enterochromaffin cells per field. Cells in mitosis could be observed here and there. Lymphatic elements were in the diffuse form. The depth of the mucosa averaged 250 micra.

One Week

Cardiac

The branching of the glands appeared to have increased. There were about 15 parietal cells per field. The mucous cells in general had increased in number; however, occasionally a whole section of gland was made entirely of parietal cells. There was less than 1 enterochromaffin cell per field. The lymphatic elements took a nodular and diffuse form in three animals, and a diffuse form in the fourth animal of the same age group. The depth of the mucosa averaged 160 micra.

Fundic

The glands showed very little branching. Only a few mucous cells were present, the majority of the cell population being made of parietal and chief cells. There was not a regular pattern of distribution for these two types of cells. The lamina propria showed a diffuse infiltration by lymphatic elements. One of the animals in this age group presented nodules. There was less than 1 enterochromaffin cell per field. The depth of the mucosa averaged 261 micra.

Pyloric

The amount of branching of the glands remained about the same as in the 3 days old group (Figure 5). The mucous cells extended the whole length of the glands. The parietal cells that could be seen, apparently squeezed between mucous cells and the basement membrane, numbered 6 to 7 per field. The depth of the mucous membrane averaged 190 micra.

Ten Days

Cardiac

The amount of branching of the glands appeared to have increased. There were about 18 parietal cells per field. The parietal cells remained in the same number, but were no longer seen in clusters, as in the one week old group. They were distributed more evenly among the mucous cells of the glands. There were only a few cells showing mitosis. The enterochromaffin cells were less than 1 per field. The depth of the mucosa averaged 519 micra.

Fundic

The branching remained the same. The mean number of the parietal cells was 12 per field. The foveolae of the glands reached down to about half the depth of the glands. The mucous cells showed a very strong PAS-positive reaction. Enterochromaffin cells were present, numbering less than 1 per field. Cells in mitosis were rare. The lamina propria showed a diffuse infiltration by the lymphatic elements. The mucosa averaged 420 micra.

Eleven Days

Cardiac

The amount of branching remained approximately the same. There were 13 parietal and less than 1 enterochromaffin cells per field. The lymphatic elements were in the nodular form. The depth of the mucosa averaged 360 micra.

Fundic

The glands were straight. The parietal cells were seen all along the length of the glands. The chief cells, however, seemed to be congregated in the fundus of the glands. Mucous cells were scattered throughout the glands. Only a few cells were in mitosis. Lymphatic elements were in the diffuse form. The depth of the mucosa averaged 437 micra.

Pyloric

The parietal cells numbered about 8 per field. The lymphatic elements were in the diffuse form. The mucous cells showed a strong PAS-positive reaction, not different from that seen in the cardiac mucous cells. One feature, that persisted throughout the subsequent age groups, was that the nuclei of the mucous cells in only the lower third of the pyloric glands had a flattened, somewhat angular appearance. The nuclei of the mucous cells of the other two zones remained rounded, regardless of the location of the cells in the glands. There was less than 1 enterochromaffin cell per field. The depth of the mucosa averaged 450 micra.

Thirteen Days

Cardiac

There were about 13 parietal cells per field. The lymphatic elements were in the diffuse form. The enterochromaffin cells were less than one per field. Only a few cells here and there showed mitotic figures. The depth of the mucosa averaged 390 micra.

Fundic

The chief cells were not very numerous and were concentrated largely in the basal third of the glands. The PAS-positive reaction was still present in the canaliculi of the parietal cells. The mucous neck cells remained confined to the neck of the glands. Few cells showed mitotic figures. The entrochromaffin cells were seen occasionally. The depth

of the mucosa averaged 390 micra.

Pyloric

There were on the average 8 parietal cells per field. The lymphatic elements were diffused throughout the lamina propria. The enterochromaffin cells were rather numerous in spots, with as many as ten seen in one field; however, the mean number was closer to 3. The depth of the mucosa averaged 700 micra.

Two Weeks

Cardiac

There were 10 parietal cells per field. The glands appeared more branched than they were at 10 days. Several nodules of lymphatic elements were observed in the sections. Numerous cells show mitotic figures. The enterochromaffin cells were less than 1 per field. The depth of the mucous membrane averaged 468 micra.

Fundic

The glands were straight with little branching or twisting. At this early age they resembled, only in a diminutive form, the classic picture of the fundic gland of the adult pig. There was no pattern to the location of the parietal and chief cells. In the four individuals comprising this age group, there were numerous cells showing mitosis, but similar to the parietal and chief cells there was no pattern of arrangement to the mitotic figures. The mean number of enterochromaffin cells was less than 1 per field. Lymphatic elements were present in the

nodular form. The depth of the mucosa averaged 631 micra.

Pyloric

The branching of the glands remained the same. The parietal cells numbered 9 per field. Lymphatic elements were in the nodular and diffuse forms. Only a few mitotic figures could be seen. The depth of the mucosa averaged 391 micra.

Seventeen Days

Cardiac

There were 10 parietal cells per field. The enterochromaffin cells were less than 1 per field. The lymphatic elements were in the nodular form, destroying in many areas the architecture of the glands. The branching of the glands remained the same. For the first time, cells in mitosis showed a pattern in their distribution. All the dividing cells appeared to be in a line about midway between the lining epithelium and the muscularis mucosa. A few mitotic figures were seen in the upper half of the glands. The depth of the mucosa averaged 591 micra.

Fundic

The lymphatic elements were in the nodular form, and as in the cardiac zone destroying in places the architecture of the glands. Few cells showed mitotic figures. There were 9 parietal cells and less than 1 enterochromaffin cell per field. The depth of the mucosa averaged 585 micra.

Pyloric

The enterochromaffin numbered 4 per field (Figure 6). Lymphatic elements were in the diffuse form. Only a few cells were showing mitotic figures. There were 9 parietal cells per field. It was not possible to determine the depth of the mucosa in the sections because of the angle of cut.

Twenty Days

Cardiac

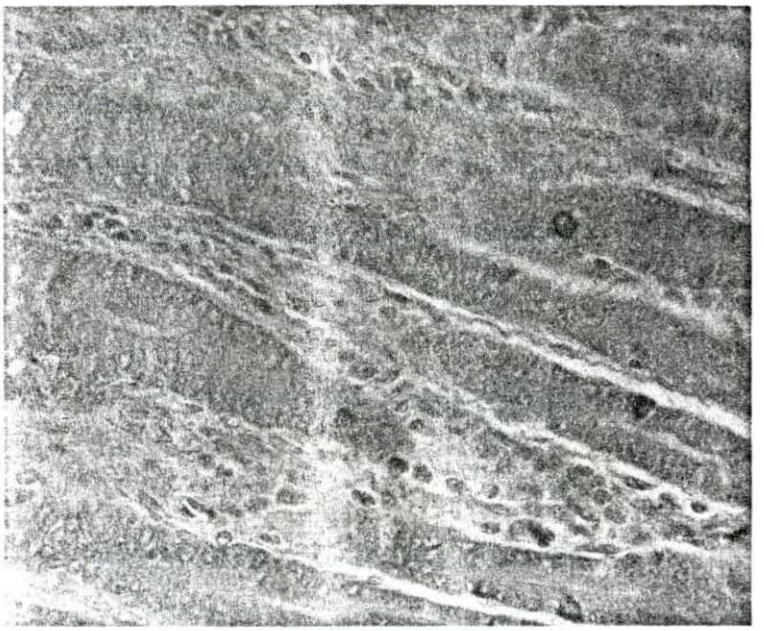
The branching of the glands did not differ from what was observed in the 14 days old. There were 7 parietal cells and between 1 and 2 enterochromaffin cells per field. The lymphatic elements were in the nodular and diffuse forms. Cells in mitosis were seen here and there. The depth of the mucosa averaged 308 micra.

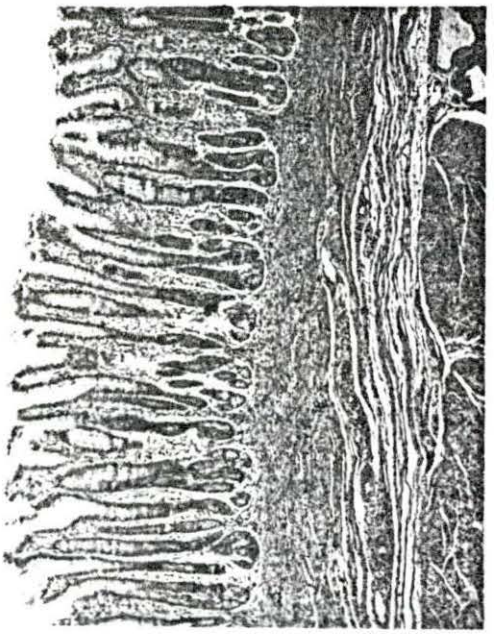
Fundic

There were many cells in mitosis at the various levels in the glands. Except for a PAS-positive reaction in the cytoplasm of some of the mitotic cells, there was no other indication as to what kind of cell was dividing. There was no pattern to the distribution of the chief and parietal cells. The lymphatic elements were in the diffuse form. The enterochromaffin cells were rare. The depth of the mucosa averaged 628 micra.

Figure 5. One week old. Pyloric zone. Crossmon's modification of Mallory's Triple Stain. 60X. The branching of the glands has increased slightly. The lymphatic elements are in the diffuse form.

Figure 6. Seventeen days old. Pyloric zone. Hematoxylin and Eosin stain. 400X. A Wratten filter no. 47 was used to contrast more sharply the entero-chromaffin cells from the mucous cells. The entero-chromaffin cells are the dark cells. The area represents the middle third of the gland. Lymphatic elements are diffusely distributed in the lamina propria.





Pyloric

The parietal cells were observed throughout the sections, but only a few scattered enterochromaffin cells were seen. Because the top of the mucosa had been trimmed off accidentally during processing, the parietal and enterochromaffin cells were not counted and the depth of the mucosa not estimated.

Twenty-one Days (Three Weeks)

Cardiac

The parietal cells were approximately 7 per field. The branching of the glands had remained about the same in the last three age groups (14, 17 and 20 days) in the cardiac and the other two glandular zones of the stomach. The conclusion reached at 21 days, and verified by observations of the following age groups, was that the glands had ceased to branch somewhere between the second and third weeks of life. The chromaffin cells numbered 2 per field. Very few cells were in mitosis. The lymphatic elements were in the nodular and diffuse forms. The depth of the mucosa averaged 395 micra.

Fundic

The greatest number of the chief cells was in the bottom half of the glands, while the parietal cells were observed in every part of the same glands. A few mucous cells were scattered among the parietal and the chief cells. The lymphatic elements were in the nodular and diffuse forms. The enterochromaffin cells numbered less than 1 per field. The

depth of the mucosa averaged 710 micra.

Pyloric

The parietal cells numbered 6 per field. The lympthatic elements were in the nodular and diffuse forms. The PAS-positivity of the mucous cells in the pyloric zone did not appear to be different from that of the mucous cells of the cardiac and fundic zone, as it was in all the other specimens of the present experiment. There were approximately 6 enterochromaffin cells per field. The depth of the mucosa averaged 580 micra.

Twenty-eight Days (Four Weeks)

Cardiac

The parietal cells numbered 7 per field. The lymphatic elements were in the nodular and diffuse forms. There were less than one enterochromaffin cell per field. Very few cells showed mitotic figures. The depth of the mucosa averaged 245 micra.

Fundic

There was no pattern to the location of the chief and parietal cells. Both kinds of cells were observed in all parts of the glands. The chief cells were seen in clusters of 2 or 3, or singly. The mucous cells represented the minority in the cell population. The lymphatic elements were in the nodular and diffuse forms. The enterochromaffin cells and mitotic figures were rare. The depth of the mucosa averaged 675 micra.

Pyloric

The parietal cells numbered 3 and the enterochromaffin cells 4 per field. The lymphatic elements were in the diffuse and nodular forms. Mitotic figures were rare. The depth of the mucosa averaged 621 micra.

Thirty-four Days

Cardiac

The parietal cells numbered 4 and the enterochromaffin cells 2 per field. Cells in mitosis were rare. The lymphatic elements were in the diffuse form. The depth of the mucosa averaged 339 micra.

Fundic

The chief and parietal cells did not show any pattern in their distribution in the glands. The mucous cells were seen in all positions. The enterochromaffin cells numbered 4 per field. The lymphatic elements were in the diffuse form. Cells in mitosis were rare. The depth of the mucosa averaged 837 micra.

Pyloric

The parietal cells were not very conspicuous, numbering only 2 per field. In places they seemed to have retained their general characteristics, in others they looked as if they had shrunk. The nuclei still retained their clear appearance, but the canaliculi had disappeared and the cytoplasm looked more condensed. The enterochromaffin cells numbered 15 per field. The lymphatic elements were in the diffuse form. The pyloric zone of the stomach of the 34 days old was the only one in which

the nuclei of the mucous cells were rounded or oval in all parts of the glands. The depth of the mucosa averaged 630 micra.

Thirty-five Days (Five Weeks)

Cardiac

The parietal cells numbered 3 per field, and the enterochromaffin cells 4 per field. Mitotic figures were numerous and could be observed in almost every stage. The infiltration of the lamina propria by the lymphatic elements was very heavy in all the 4 individuals of this age group, although no nodule was observed. The depth of the mucosa averaged 506 micra.

Fundic

There was a rather heavy concentration of mucous cells in the fundus of the glands, but there was no pattern in the distribution of the chief and parietal cells in the glands. About 1 enterochromaffin cell per field was observed. The lymphatic elements were in the diffuse form and the depth of the mucosa averaged 962 micra.

Pyloric

Both the parietal and the enterochromaffin cells numbered 2 per field. Lymphatic elements were in the diffuse form. In one animal mitotic figures were rare while in the other three composing this age group, numerous cells showed mitotic figures in one stage or another. The mucosa averaged 540 micra.

Forty-two Days (Six Weeks)

Cardiac

The parietal cells numbered 2 or 3 (Figure 7), and the enterochromaffin cells numbered 3 per field. Mitotic figures were observed throughout the sections, but not in heavy concentration. The lymphatic elements were in the diffuse form (Figure 7), with small nodules here and there. The depth of the mucosa averaged 506 micra (Figure 8).

Fundic

The mucous cells were concentrated in the fundus with a scattering in the body of the glands. The enterochromaffin cells numbered less than 1 per field. The depth of the mucosa averaged 1023 micra (Figure 9).

Pyloric

The parietal cells numbered 3 and the enterochromaffin cells 13 per field. The lymphatic elements were in the nodular and diffuse forms. The depth of the mucosa averaged 554 micra (Figure 10).

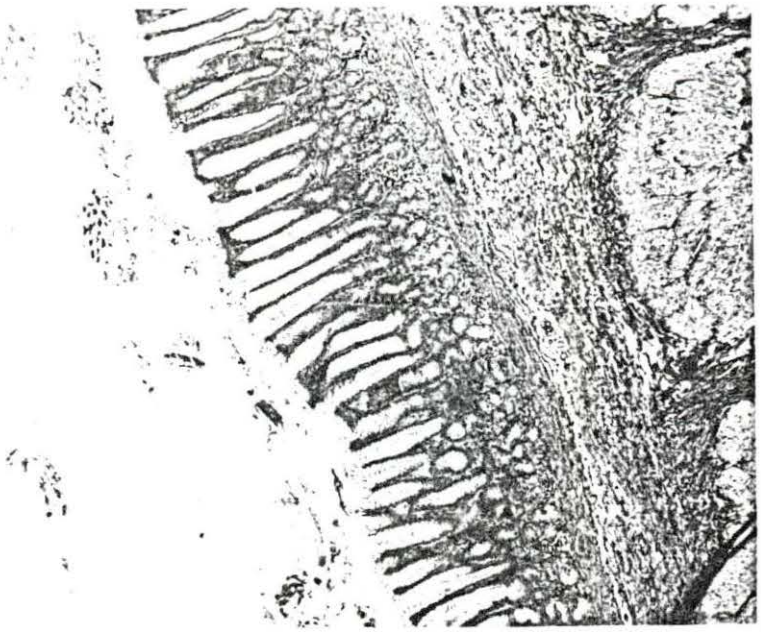
Forty-nine Days (Seven Weeks)

Cardiac

The parietal cells numbered 2 or 3 and the enterochromaffin cells less than 1 per field. The lymphatic elements were in the nodular and diffuse forms, and the diffuse infiltration was heavy. There were only a few cells in mitosis. The depth of the mucosa averaged 479 micra.

Figure 7. Six weeks old. Cardiac zone. Crossmon's modification of Mallory's Triple Stain. 400X. Two parietal cells can be observed in the section (indicated by arrow). The mucus stained a bright red by the PAS-Hematoxylin-Aurantia combination appears black in the picture. There is a diffuse infiltration of lymphatic elements.

Figure 8. Six weeks old. Cardiac zone. Crossmon's modification of Mallory's Triple Stain. 60X. The glands show extensive branching. There is a heavy infiltration by lymphatic elements. The mucosa averaged 506 micra.



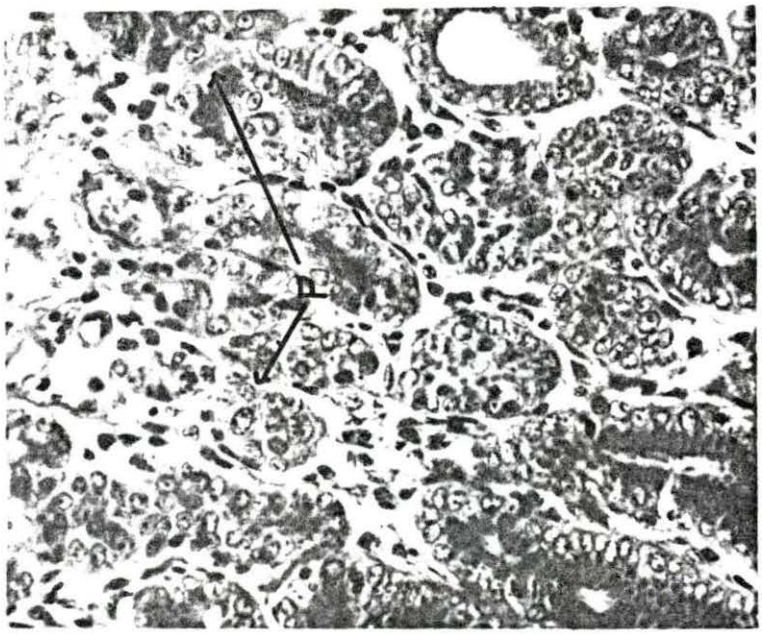

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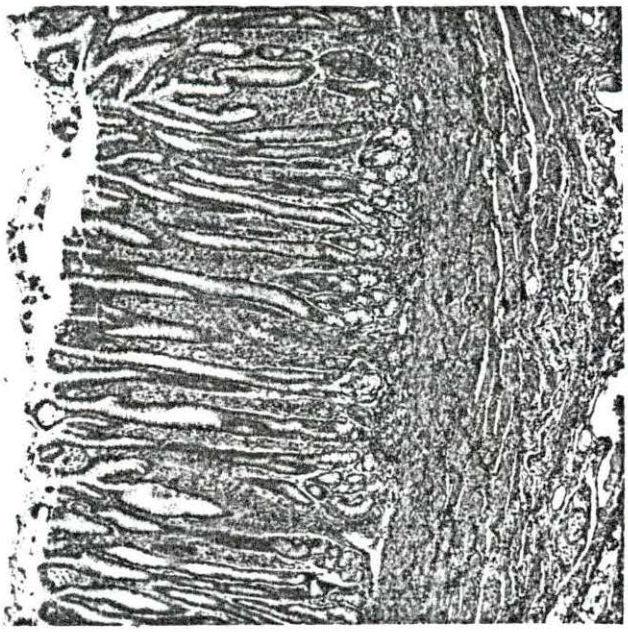
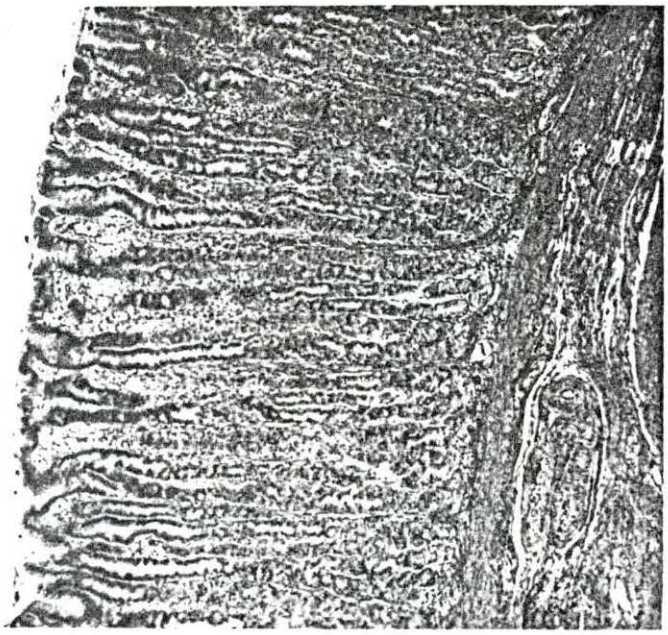
Figure 9. Six weeks old. Fundic zone. Crossmon's modification of Mallory's Triple Stain. 60X. The foveolae are shallow. The branching of the glands has not increased. The mucosa averaged 1023 micra.

Figure 10. Six weeks old. Pyloric zone. Crossmon's modification of Mallory's Triple Stain. 60X. The foveolae of the glands are deeper extending at times over half the length of the glands. The mucosa averages 554 micra.





Fundic

The mucous cells were more numerous at the fundus of the glands. The parietal cells outnumbered the chief cells by two to one, and there was no pattern to their distribution in the glands. Lymphatic elements were in the diffuse form. Only a few cells showed mitotic figures. The enterochromaffin cells numbered less than 1 per field. The depth of the mucosa averaged 869 micra.

Pyloric

The parietal cells numbered 2 to 3, and the enterochromaffin cells about 1 per field. Numerous cells were in mitosis. The lymphatic elements were in the diffuse and nodular forms. The depth of the mucosa averaged 753 micra.

Fifty-six Days (Eight Weeks)

Cardiac

The parietal cells numbered 1 and the enterochromaffin cells less than 1 per field. Lymphatic elements were in the diffuse and nodular forms. Numerous cells showed mitotic figures. The depth of the mucosa averaged 595 micra.

Fundic

The arrangement of the cells was not different from what was observed in the fundic region of the 7 weeks old. There were few cells in mitosis. Lymphatic elements were in the nodular and diffuse forms. The depth of the mucosa averaged 1171 micra.

Pyloric

The enterochromaffin cells numbered 4 and the parietal cells 2 per field. There were numerous cells in division. The lymphatic elements were in the diffuse form. The depth of the mucosa averaged 843 micra.

Sixty-three Days (Nine Weeks)

Cardiac

The enterochromaffin cells numbered less than 1 and the parietal cells 1 per field. Few cells showed mitotic figures. The lymphatic elements were in the diffuse form and the depth of the mucosa averaged 456 micra.

Fundic

There was an abundance of mucous cells outnumbering the parietal cells, which up to this present age group were in predominance. There was less than 1 enterochromaffin per field. The lymphatic elements were in the diffuse form. The depth of the mucosa averaged 1360 micra.

Pyloric

The parietal cells numbered 1 and the enterochromaffin less than 1 per field. Cells in mitosis were rare. The lymphatic elements were in the diffuse form. The depth of the mucosa averaged 734 micra.

Seventy Days (Ten Weeks)

Cardiac

The parietal cells numbered 2 and the enterochromaffin cells less

than 1 per field. The lymphatic elements were in the diffuse and nodular forms. A few cells in mitosis were observed here and there. The depth of the mucosa averaged 523 micra.

Fundic

The mucous cells appeared to outnumber the combined population of the parietal and chief cells. The enterochromaffin cells were less than 1 per field. Mitotic figures were rare. Lymphatic elements were of the diffuse form. The depth of the mucosa averaged 1263 micra.

Pyloric

The parietal cells numbered 1 and the enterochromaffin less than 1 per field. Lymphatic elements were of the diffuse form. Cells in mitosis were rare. The depth of the mucosa averaged 781 micra.

Seventy-seven Days (Eleven Weeks)

Cardiac

The parietal cells numbered 1 and the enterochromaffin cells less than 1 per field. Lymphatic elements were of the diffuse form. The depth of the mucosa averaged 583 micra.

Fundic

The mucous cells appeared to have outnumbered the parietal and the chief cells combined. The lymphatic elements were in the diffuse form. The enterochromaffin cells numbered less than 1. Mitotic figures were rare. The depth of the mucosa averaged 1305 micra.

Pyloric

The parietal cells numbered less than 1 and the enterochromaffin cells 3 to 4 per field. Mitotic figures were rare. The lymphatic elements were in the diffuse form. The depth of the mucosa averaged 922 micra.

Eighty-four Days (Twelve Weeks)

Cardiac

Both the parietal and the enterochromaffin cells numbered less than 1 per field. The lymphatic elements were in the diffuse and nodular forms. The depth of the mucosa averaged 677 micra.

Fundic

The arrangement of the cells remained the same. The enterochromaffin cells were less than 1 per field. Mitotic figures were rare. The lymphatic elements were in the diffuse form. The depth of the mucosa averaged 1360 micra.

Pyloric

Both the parietal and the enterochromaffin cells numbered 1 per field. Mitotic figures were rare. The lymphatic elements were in the diffuse and nodular forms. The depth of the mucosa averaged 796 micra.

One Hundred and Twelve Days (Sixteen Weeks)

The three zones of the stomach, in their gland arrangement and cellular content, were almost the exact replicas of photographs of the

pig stomach usually seen in textbooks.

Cardiac

The parietal cells numbered 1 and the enterochromaffin cells less than 1 per field. Numerous cells were in mitosis, displaying all four stages of division. Lymphatic elements were in the nodular and diffuse forms. The mucosa averaged 1260 micra (Figure 11).

Fundic

Mitotic figures were rare. The lymphatic elements were in the diffuse form. The enterochromaffin cells numbered less than 1 per field. The mucosa averaged 1500 micra (Figure 12).

Pyloric

The parietal cells numbered 1 per field and the enterochromaffin less than 1 per field. Cells in mitosis were rare. The mucosa averaged 1260 micra (Figure 13).

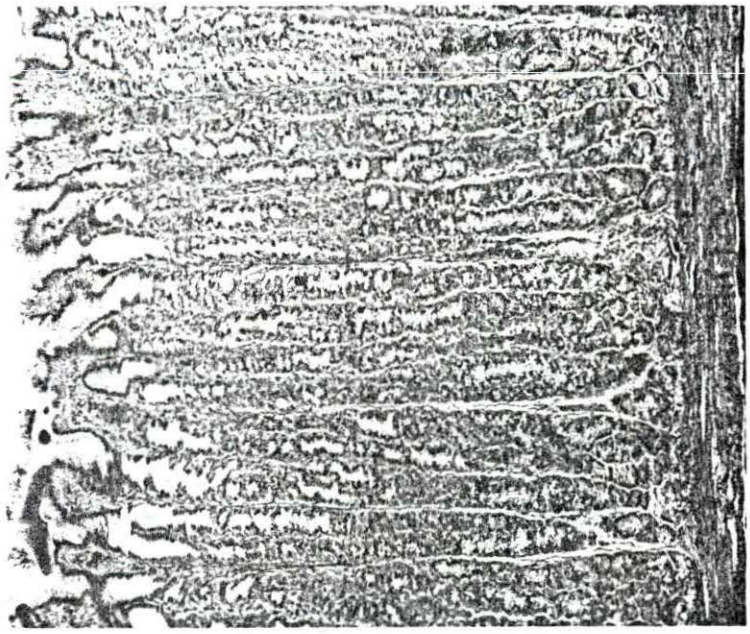
Five Months

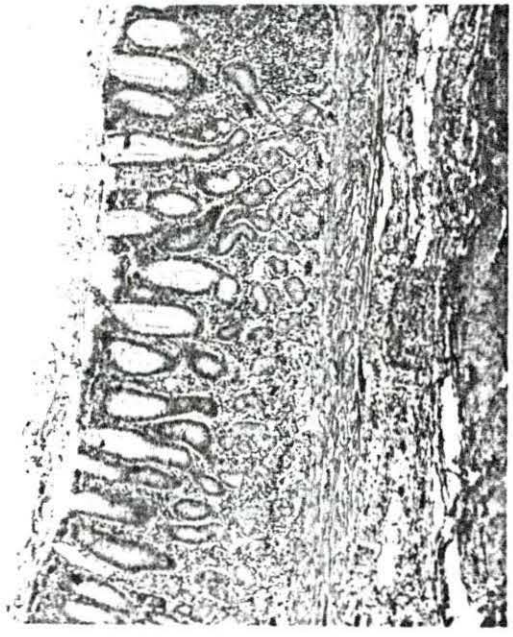
Cardiac

The parietal cells numbered less than 1 and the enterochromaffin 1 per field. The lymphatic infiltration was very heavy in both individuals of this age group, and one of the nodules extended into the submucosa. Strands of collagenous fibers extending along the perimeter of the nodule gave the impression of encapsulation. Mitotic figures were rare. The depth of the mucosa averaged 720 micra.

Figure 11. Sixteen weeks. Cardiac zone. Crossmon's modification of Mallory's Triple Stain. 60X. The mucosa averages 540 micra.

Figure 12. Sixteen weeks. Fundic zone. Crossmon's modification of Mallory's Triple Stain. 60X. The mucosa averages 1500 micra.





Fundic

The mucous cells appeared to be the predominant member of the cell population. The lymphatic elements were in the diffuse form. The enterochromaffin cells were rare and the depth of the mucosa averaged 1800 micra.

Pyloric

The parietal cells numbered 1 and the enterochromaffin less than 1 per field. The lymphatic elements were in the diffuse form. Mitotic figures were rare. The depth of the mucosa averaged 1530 micra.

Eight Months

Cardiac

Both parietal and enterochromaffin cells numbered less than 1 per field. Mitotic figures were rare and the lymphatic elements were in the diffuse form, and the depth of the mucosa averaged 540 micra.

Fundic

The mucous cells still seemed to represent the greatest percentage of the cell population. The chief and parietal cells showed no definite pattern in their distribution in the glands. The lymphatic elements were in the diffuse form, with an occasional submucosal nodule. The enterochromaffin cells numbered less than 1 per field. The depth of the mucosa averaged 2000 micra.

Pyloric

Both the parietal and the enterochromaffin cells numbered less than 1 per field. The lymphatic elements were in the diffuse form, and mitotic figures were rare. The depth of the mucosa averaged 1270 micra.

Ten Months

Cardiac

The parietal and the enterochromaffin cells numbered less than 1 per field. The lymphatic elements were in the diffuse and nodular forms. Mitotic figures were absent. The depth of the mucosa averaged 360 micra.

Fundic

The mucous cells still appeared to be the most predominant cellular element (Figure 14). The parietal and the chief cells did not show any pattern in their distribution in the glands. The enterochromaffin cells and mitotic figures were rare. The depth of the mucosa averaged 2000 micra.

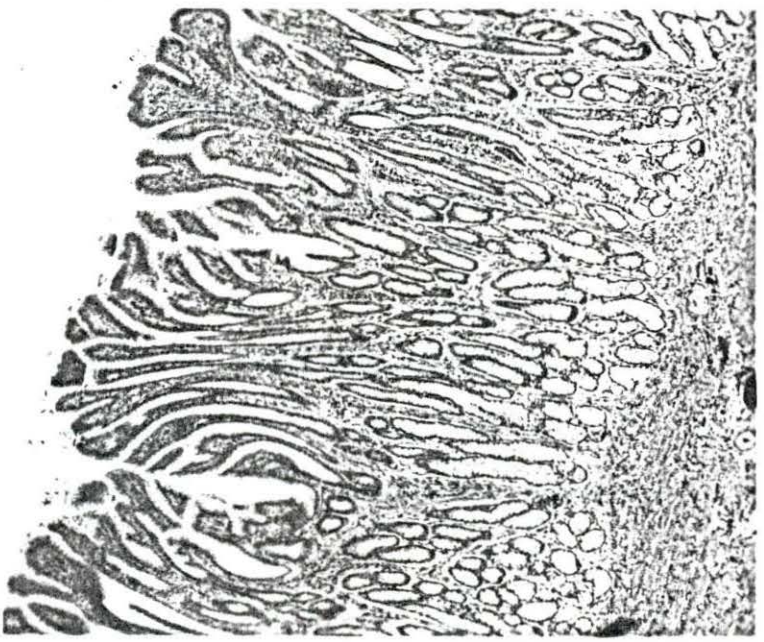
Pyloric

Both the parietal and the enterochromaffin cells were less than 1 per field. The lymphatic elements were in the diffuse form. The depth of the mucosa averaged 1620 micra.

Figure 13. Sixteen weeks. Pyloric zone. Crossmon's modification of Mallory's Triple Stain. 60X. The mucosa averages 1260 micra. The glands show well their branching. The nuclei of the mucous cells are flattened.

Figure 14. Ten months. Fundic zone. Crossmon's modification of Mallory's Triple Stain. 400X. There is a predominance of mucous cells (m). The parietal cells (p) show no definite pattern as to their arrangement. Likewise, there was no specific pattern in the chief cells (c).


E
P
C



Fourteen Months

Cardiac

Both the parietal and the enterochromaffin cells numbered less than 1 per field. The lymphatic elements were in the diffuse form. The depth of the mucosa averaged 620 micra.

Fundic

In this animal, the majority of the chief cells were observed to be in the upper two thirds of the glands. The parietal cells showed no preference as to location. The mucous cells outnumbered the last named two types of cells. The lymphatic elements were in the diffuse form. The depth of the mucosa averaged over 2000 micra.

Pyloric

Both the parietal and the enterochromaffin cells numbered less than 1 per field. The lymphatic elements were in the diffuse form and mitotic figures were rare. The depth of the mucosa averaged 1420 micra.

Nineteen Months

Cardiac

Both the parietal and the enterochromaffin cells numbered less than 1 per field. Mitotic figures were rare. The lymphatic elements were in the diffuse and nodular forms. The depth of the mucosa averaged 765 micra.

Fundic

The chief cells were observed mostly in the upper two thirds of the glands. The parietal cells were seen in every location in the glands. The lymphatic elements were in the diffuse and nodular forms. The depth of the mucosa averaged 1382 micra.

Pyloric

The parietal and the enterochromaffin cells both numbered less than 1 per field. The lymphatic elements were in the nodular and diffuse forms. The depth of the mucosa averaged 900 micra.

Twenty Months

Cardiac

The parietal and the enterochromaffin cells both numbered less than 1 per field. The lymphatic elements were in the nodular and diffuse forms (Figure 15). Mitotic figures were rare. The depth of the mucosa averaged 720 micra (Figure 15).

Fundic

The arrangement of the cells continued to be the same, with the mucous cells predominating. The enterochromaffin cells numbered less than 1 per field. Mitotic figures were rare. The lymphatic elements were in the diffuse and nodular forms. The depth of the mucosa averaged 2000 micra (Figure 16).

Figure 15. Twenty months. Cardiac zone. Crossmon's modification of Mallory's Triple Stain. 60X. A lymphatic nodule extending the whole depth of the mucosa. The lymphatic elements are also in the diffuse form. The mucosa averages 720 micra.

Figure 16. Twenty months. Fundic zone. Crossmon's modification of Mallory's Triple Stain. 60X. The mucosa averages 2000 micra; extending beyond the frame of the picture, there is a nodular (arrow) and diffuse infiltration of lymphatic elements.

n

Pyloric

Both the parietal and the enterochromaffin cells numbered less than 1 per field. The lymphatic elements were in the diffuse and nodular forms. There were very few mitotic figures. The depth of the mucosa averaged 1310 micra (Figure 17).

Twenty-one Months

Cardiac

Both the parietal and the enterochromaffin cells numbered less than 1 per field. There were many mitotic figures, located anywhere in the glands, throughout the sections. The lymphatic elements were in the nodular and diffuse forms. The depth of the mucosa averaged 675 micra.

Fundic

The arrangement of the cells was similar to that previously observed. The enterochromaffin cells were rare. Only a few mitotic figures were observed in the whole of the sections. The lymphatic elements were in the diffuse form. The mucosa averaged over 2000 micra.

Pyloric

Both the parietal and the enterochromaffin cells numbered less than 1 per field. There were numerous mitotic figures. The lymphatic elements were in the nodular and diffuse forms. The depth of the mucosa averaged 1360 micra.

Twenty-six Months

Cardiac

The parietal and the enterochromaffin cells were rare. There were very few mitotic figures in all the sections. The lymphatic elements were in the diffuse form. The depth of the mucosa averaged 675 micra.

Fundic

The arrangement of the cells was similar to that previously observed in the 21 months old animal. There were very few mitotic figures to be seen. The lymphatic elements were in the diffuse form. The depth of the mucosa averaged 2000 micra.

Pyloric

Both the parietal and the enterochromaffin cells were rare. The mitotic figures were rare. The lymphatic elements were in the diffuse form. The depth of the mucosa averaged 1500 micra.

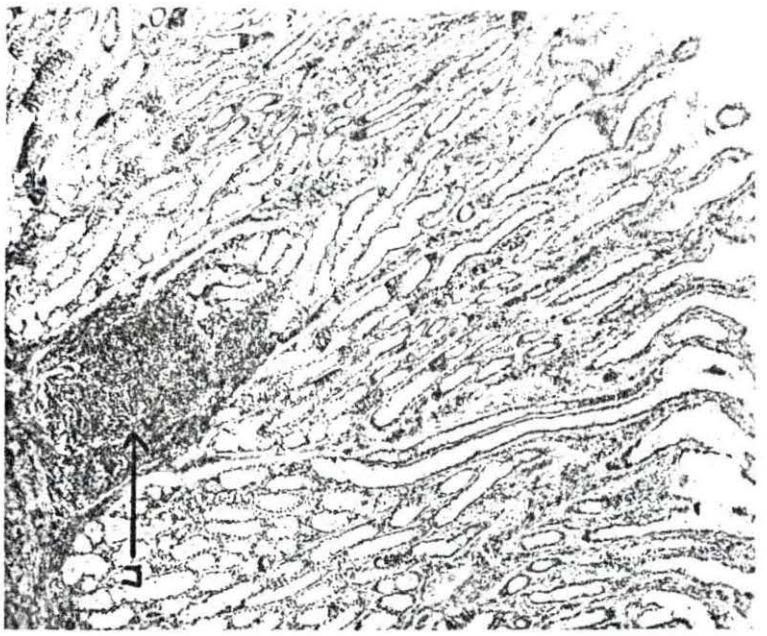
Thirty Months

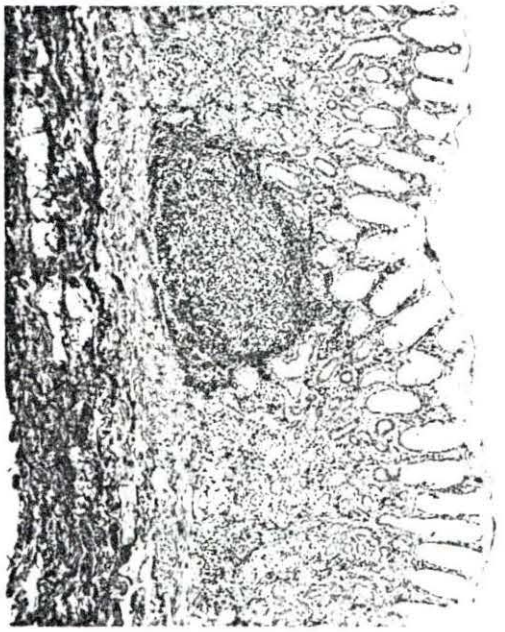
Cardiac

Both the enterochromaffin and the parietal cells were rare. Only a few mitotic figures were observed. The lymphatic elements were in the diffuse and nodular forms (Figure 18). The depth of the mucosa averaged 483 micra.

Figure 17. Twenty months. Pyloric zone. Crossmon's modification of Mallory's Triple Stain. 60X. The mucosa averages 1310 micra. The lymphatic elements are in the nodular (arrow) and diffuse forms.

Figure 18. Thirty months. Cardiac zone. Crossmon's modification of Mallory's Triple Stain. 60X. The mucosa averages 483 micra. A lymphatic nodule with what appears to be a germinal center.





Fundic

The arrangement of the cells in the glands was not different from what was previously observed. The mitotic figures were rare. The depth of the mucosa averaged over 2000 micra (Figure 19).

Pyloric

The parietal and the enterochromaffin cells were rare. Very few cells were showing mitotic figures. The branching of the glands was typical of the description found in current literature, and very similar to the branching of glands seen in textbooks. The depth of the mucosa averaged 1600 micra (Figure 20).

Thirty-one Months

Cardiac

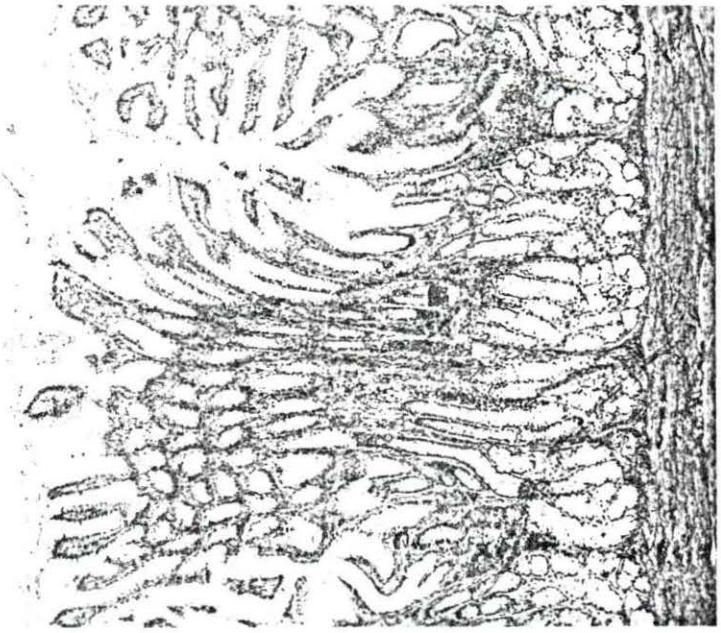
The parietal and the enterochromaffin cells were rare. A few cells were observed showing mitotic figures. The lymphatic elements were in the diffuse and nodular forms. The depth of the mucosa averaged 630 micra.

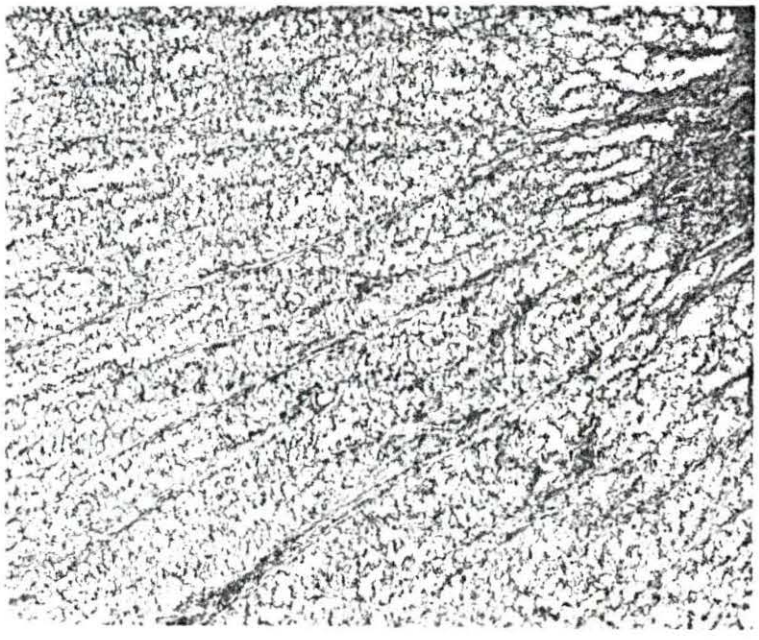
Fundic

The general aspect of the glands was the same, with predominance of the mucous cells (Figure 21). The parietal and the chief cells did not show any definite pattern as to their location in the glands (Figure 21). The lymphatic elements were in the diffuse and nodular forms. Cells with mitotic figures were extremely rare. The depth of the mucosa averaged 1500 micra.

Figure 19. Thirty months. Fundic zone. Crossmon's modification of Mallory's Triple Stain. 60X. The mucosa averages 2000 micra extending diagonally, beyond the frame of the picture.

Figure 20. Thirty months. Pyloric zone. Crossmon's modification of Mallory's Triple Stain. 60X. The mucosa averages 600 micra. The branching of the glands has not changed.





Pyloric

The parietal and the enterochromaffin cells were rare. There were very few cells in mitosis. The lymphatic elements were in the diffuse and nodular forms and the depth of the mucosa averaged 1100 micra.

Forty-four Months

Cardiac

The parietal and the enterochromaffin cells were rare and only a few cells here and there were in mitosis. The lymphatic elements were in the diffuse and nodular forms. The depth of the mucosa averaged 405 micra (Figure 22).

Fundic

The arrangement of the glands and the distribution of the cells in the glands were the same as in the 31 months old. Mitotic figures were very rare. The lymphatic elements were in the diffuse form. The mucosa averaged 1805 micra (Figure 23).

Pyloric

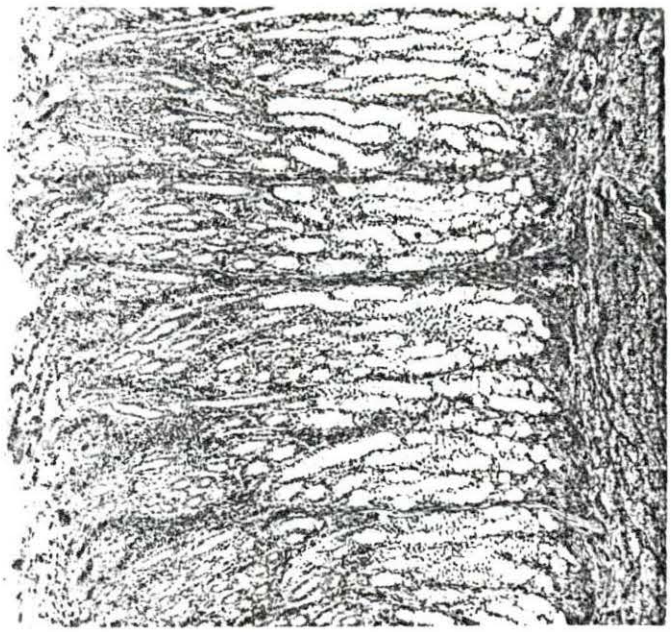
The parietal and the enterochromaffin cells were rare. Only a few cells showed mitosis. The lymphatic elements were in the diffuse and the nodular forms. The depth of the mucosa averaged 1000 micra (Figure 24).

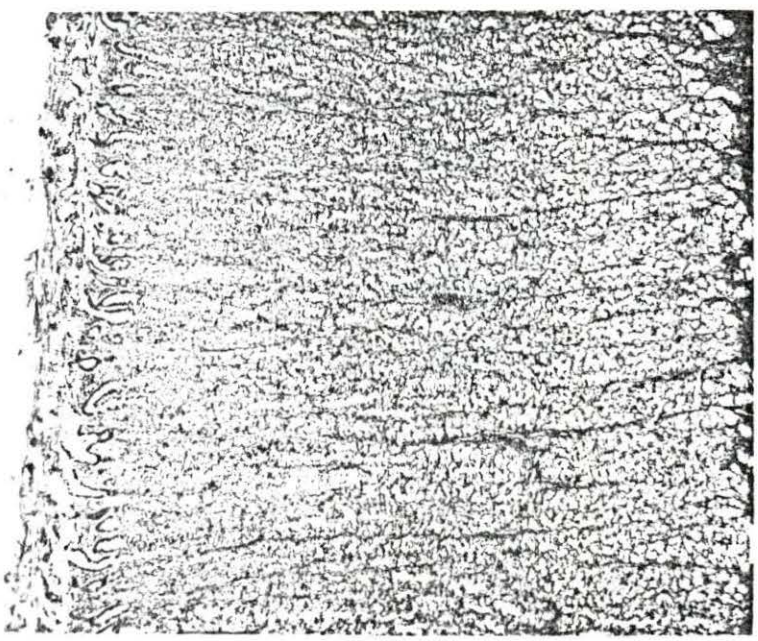
Figure 21. Thirty-one months. Fundic zone. PAS-Hematoxylin-Aurantia staining combination. 400X. Fundus of glands showing the predominance of mucous cells (m) in the area, and the lack of definite pattern in the arrangement of the parietal (p) and chief (c) cells.

Figure 22. Forty-four months. Cardiac zone. Crossmon's modification of Mallory's Triple Stain. 60X. The mucosa averages 405 micra. The lymphatic elements are diffusely distributed.

Figure 23. Forty-four months. Fundic zone. Crossmon's modification of Mallory's Triple Stain. 30X. The magnification was reduced in order to fit the mucosa within the frame of the picture. The mucosa averages 1805 micra.

Figure 24. Forty-four months. Pyloric zone. Crossmon's modification of Mallory's Triple Stain. 60X. The mucosa averages 1000 micra. The lymphatic elements are in the diffuse form. The section was slightly oblique.





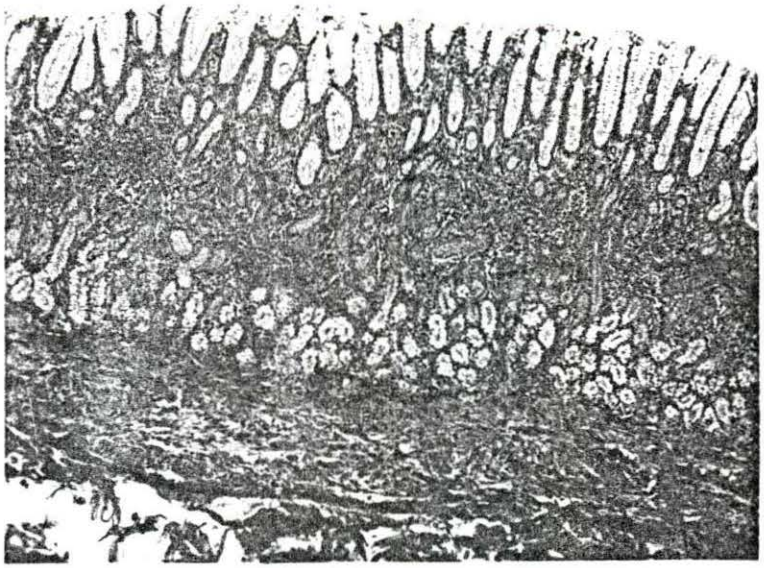
Eight Years

The cardiac (Figures 25 and 26), fundic (Figure 27) and pyloric (Figure 28) zones did not show any difference from those of the 30 or 44 month animals in the arrangement of the cells in the glands, in the branching of the glands, and the presence of lymphatic elements (Figure 26). However, there was a very definite increase in the amount and size of the collagenous fibers in the lamina propria and the trabeculae originating from the muscularis mucosa. The depth of the mucosa in the cardiac, fundic and pyloric zones was respectively 510, 1580, and 815 micra.

A series of pictures (Figures 29 through 38) showed the gradual increase in the size and the amount of the collagenous fibers. The fibers seemed to be no larger than those seen in the 20 months old, but the quantity seemed to be increased. The elastic fibers appeared to have remained the same in size and in amount throughout the different ages. The fundic zone was chosen but the same observations were made on that part of the lamina propria of the cardiac and pyloric zones. All of the pictures were taken at 625X. The Crossmon's modification of the Mallory's Triple Stain was used.

Figure 25. Eight years old. Cardiac zone. Crossmon's modification of Mallory's Triple Stain. 60X. The mucosa averages 510 micra.

Figure 26. Eight years old. Cardiac zone. Crossmon's modification of Mallory's Triple Stain. 160X. The same section as in Figure 25 but taken at higher magnification to show the diffuse but rather heavy infiltration by the lymphatic elements.



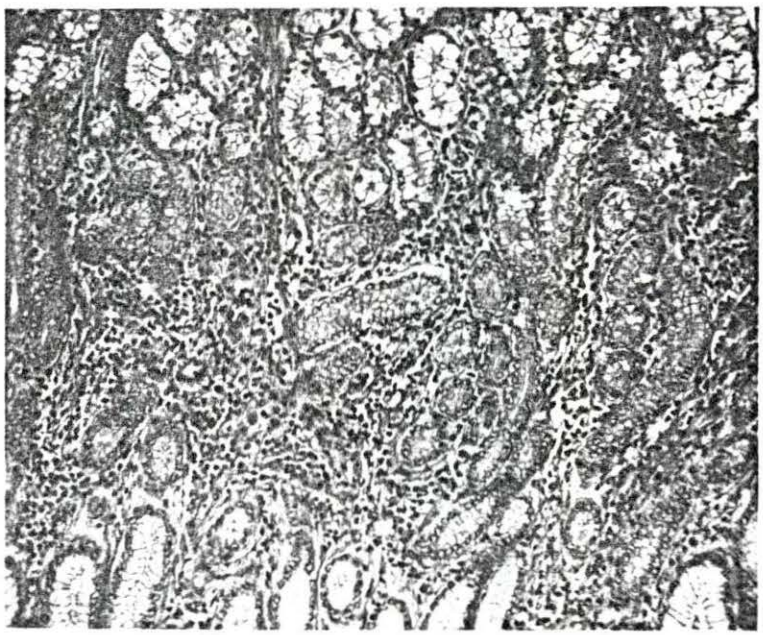
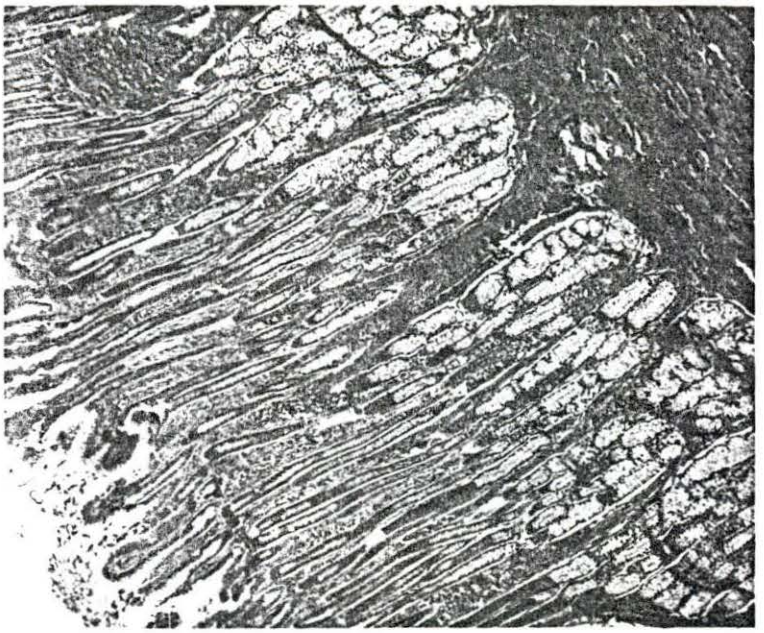


Figure 27. Eight years old. Fundic zone. Crossmon's modification of Mallory's Triple Stain. 60X. The mucosa averages 1580 micra.

Figure 28. Eight years old. Pyloric zone. Crossmon's modification of Mallory's Triple Stain. 60X. The mucosa averages 815 micra.



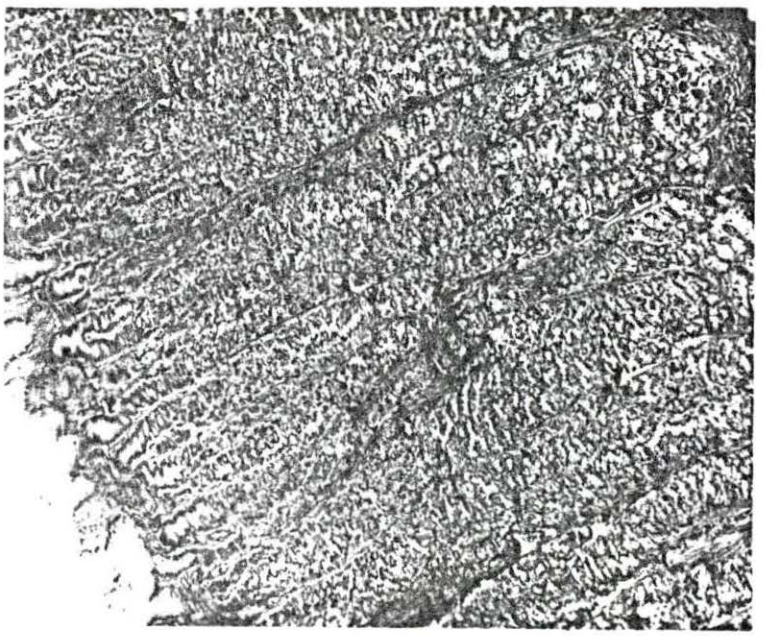
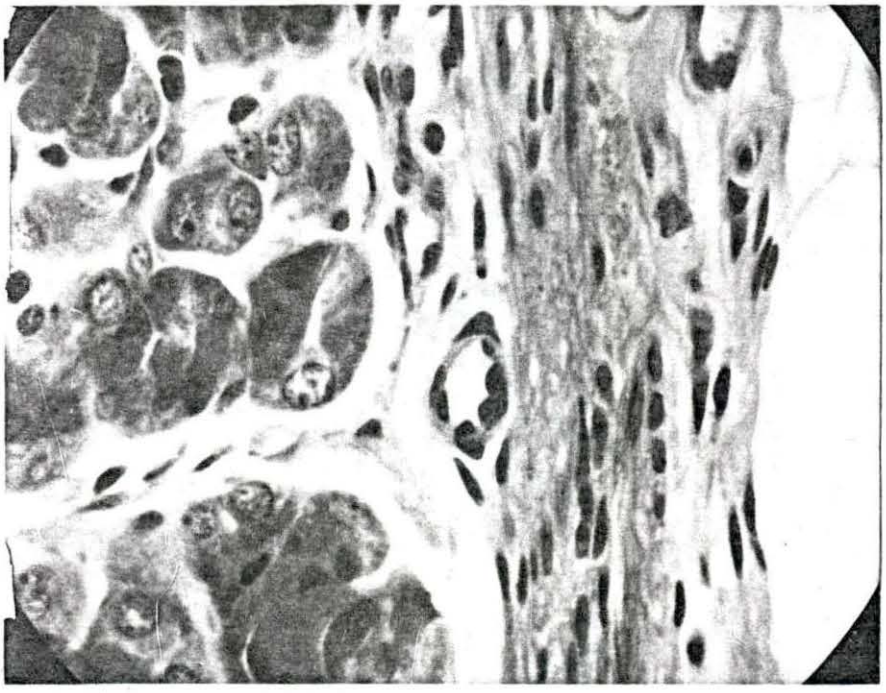


Figure 29. One day old. Fundic zone. Crossmon's modification of Mallory's triple stain. 625 X. Lamina propria, below the fundus of the glands visible at the top, the muscularis mucosae (muscle fibers stained red) and the submucosa. The collagenous fibers (stained blue) are very fine and are more visible in the submucosa than in the lamina propria. The collagenous fibers are faintly seen among the fibers of the muscularis mucosae. There was some shrinkage in the section.

Figure 30. One week old. Fundic zone. Crossmon's modification of Mallory's triple stain. 625 X. Lamina propria, below the fundus of the glands visible at the top, the muscularis mucosae (fibers stained red), and the submucosa. The collagenous fibers (stained blue) are visible in the muscularis mucosae, but are scarcely visible in the lamina propria. There was some shrinkage in the section.



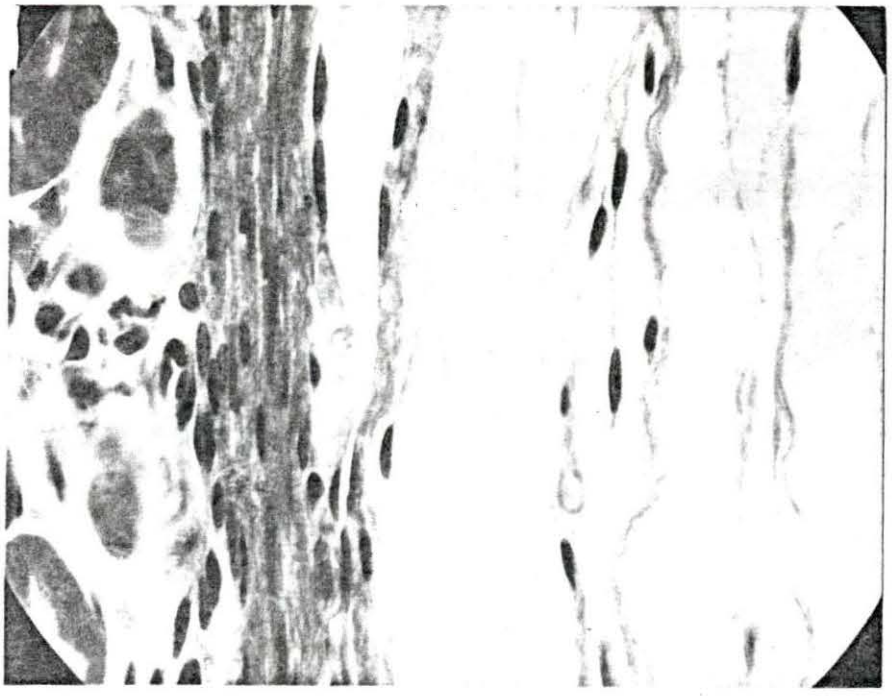
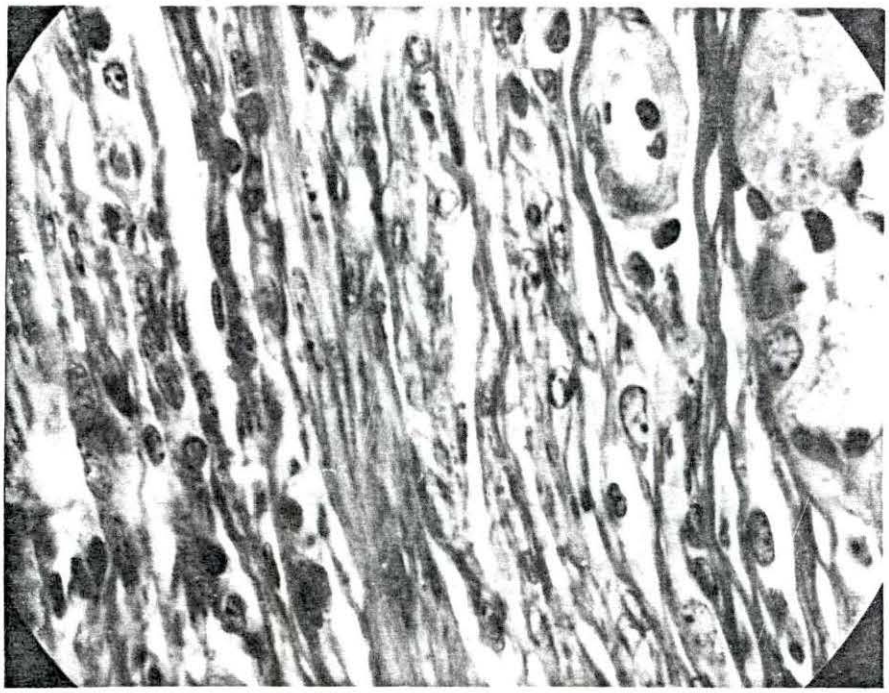


Figure 31. Four weeks old. Fundic zone. Crossmon's modification of Mallory's triple stain. 625 X. Lamina propria, below the fundus of the glands visible at the top, the muscularis mucosae (muscle fibers stained red), and the submucosa partly visible. The collagenous fibers (stained blue) have increased in number and in size, in the lamina propria and among the muscles fibers of the muscularis mucosae. There is some shrinkage in the section.

Figure 32. Two months old. Fundic zone. Crossmon's modification of Mallory's triple stain. 625 X. Lamina propria, below the fundus of the glands visible at top, and the muscularis mucosae (muscle fibers stained red). The collagenous fibers (stained blue) are larger and more numerous than those of the four weeks old.



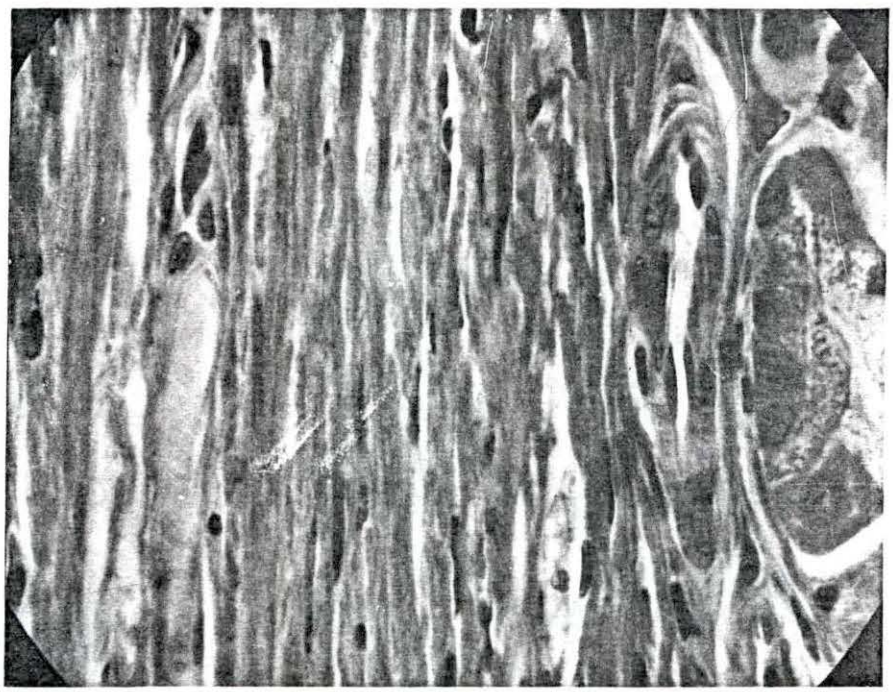
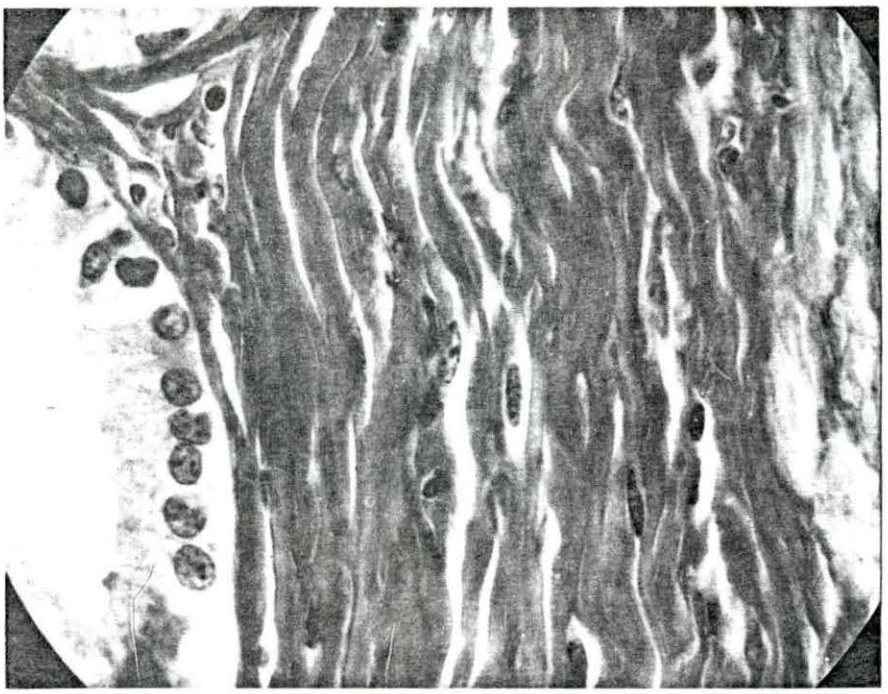


Figure 33. Five months old. Fundic zone. Crossmon's modification of Mallory's triple stain. 625 X. Lamina propria, below the fundus of the glands visible at the top. Only the upper part of the muscularis mucosae (muscle fibers stained red) is visible. The collagenous fibers (stained blue) appear to be regularly arranged. They are thicker and more numerous than those of the two months old. There does not seem to be an increase in the number of the fibers.

Figure 34. Ten months old. Fundic zone. Crossmon's modification of Mallory's triple stain. 625 X. Lamina propria, below the fundus of the glands visible at top. The collagenous fibers (stained blue) appear larger and more numerous than in the five months old. The arrangement of the fibers appears also regular in this animal. A few muscle fibers (stained red) can be seen. The difference in color with the preceding pictures is due to the photographic processing.



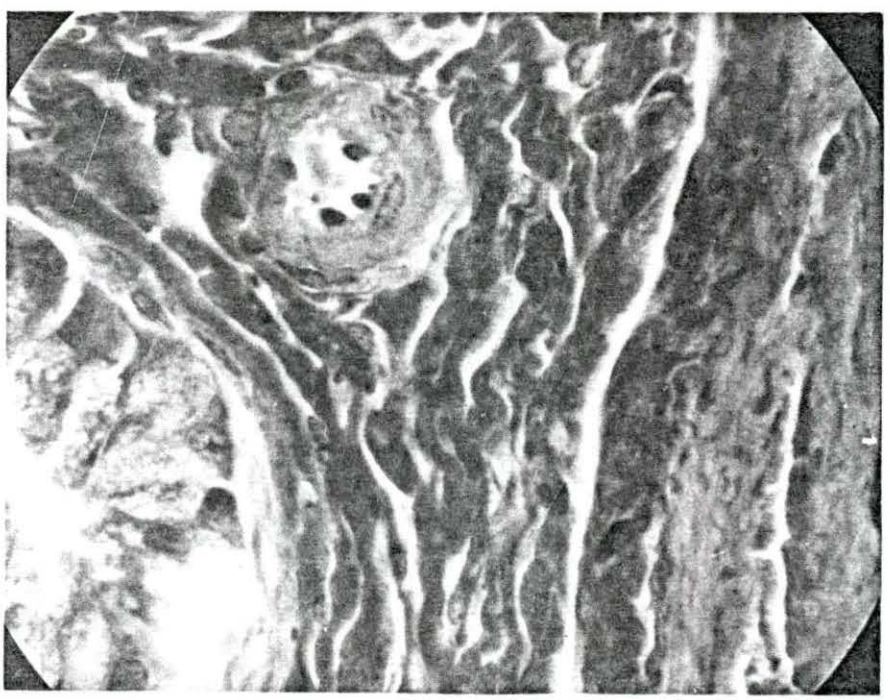
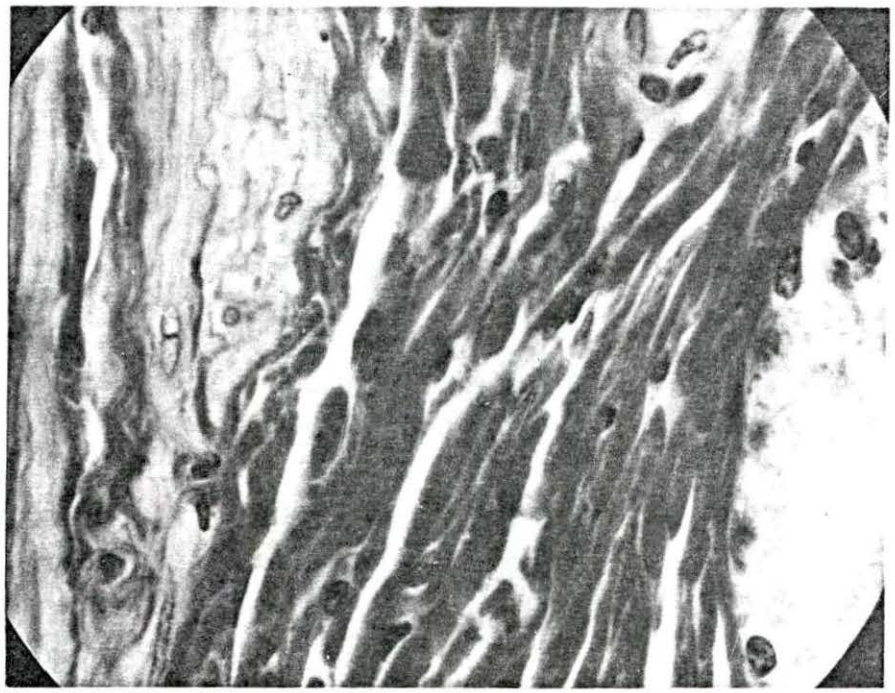


Figure 35. Fourteen months old. Fundic zone. Crossmon's modification of Mallory's triple stain. Lamina propria, below the fundus of the glands visible at the top. The collagenous fibers (stained blue) do not appear different in size and in number from those of the ten months old. A few muscle fibers (stained red) of the muscularis mucosae can be seen.

Figure 36. Twenty months old. Fundic zone. Crossmon's modification of Mallory's triple stain. 625 X. Lamina propria, below the fundus of the glands visible at the upper left hand corner. The size of the collagenous fibers (stained blue) has remained the same, but their number has increased. The muscularis mucosae could not be included in the photograph at this magnification.



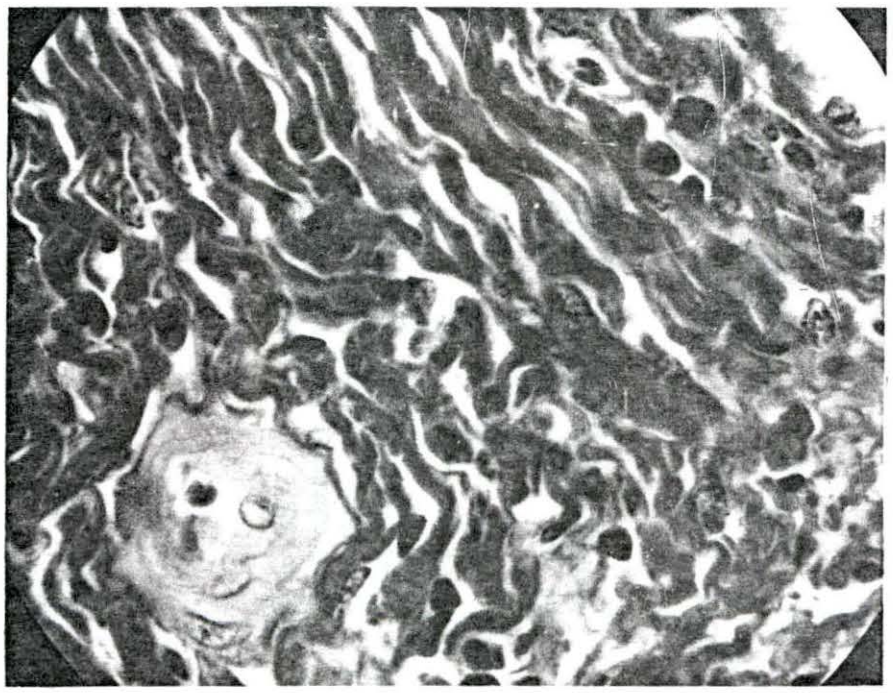
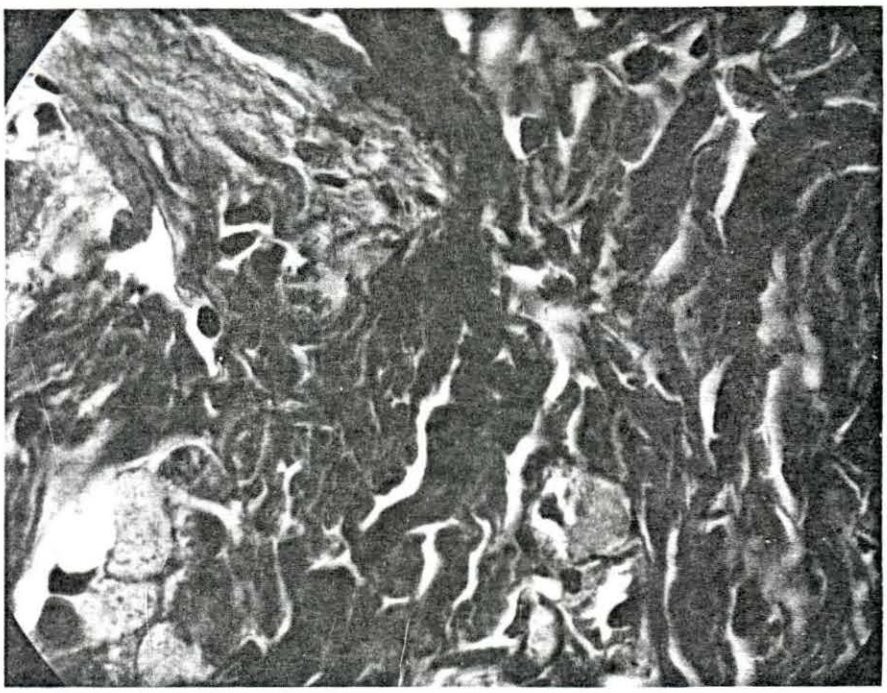


Figure 37. Forty four months old. Fundic zone. Crossmon's modification of Mallory's triple stain. Lamina propria, below the fundus of the glands visible at the top. There does not seem to be much change. The size and the number of the collagenous fibers (stained blue) appear to have remained the same as in the 20 months old.

Figure 38. Eight years old. Fundic zone. Crossmon's modification of Mallory's triple statin. 625 X. Lamina propria, below the fundus of the glands, visible at the top. There does not seem to be much difference in the size and number of the collagenous fibers observed in the lamina propria of this animal and the fibers of the twenty month old.



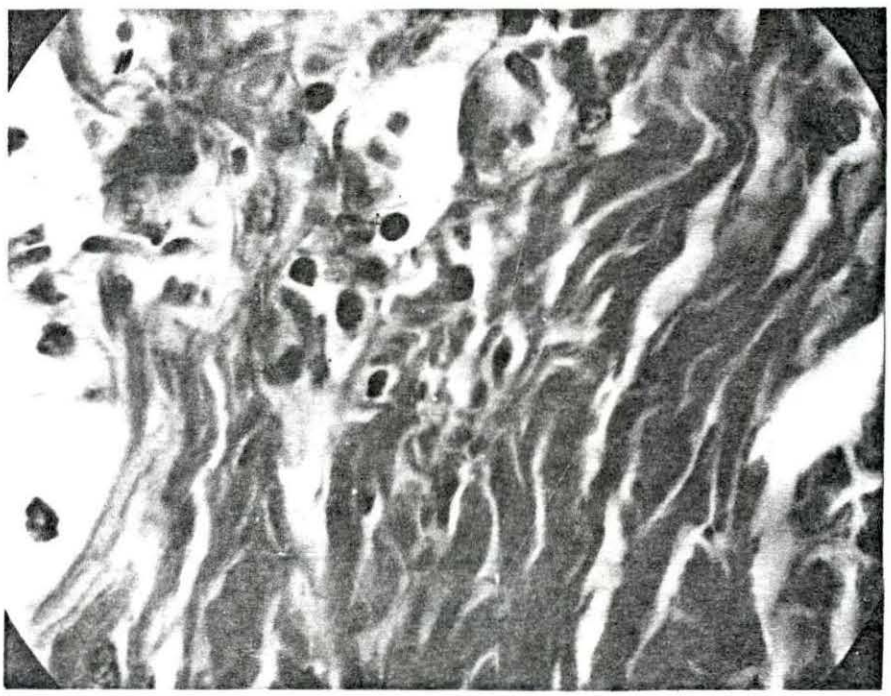


Table 2. Distribution per field of the parietal cells in the cardiac and pyloric zones of the stomach of the pig

Age	Cardiac	Pyloric
1 day	22	15-16
3 days	18	6-7
7 " (1 week)	15	6-7
10 "	18	12
13 "	10	8
14 " (2 weeks)	10	9
17 "	10	9
20 "	7	6
21 " (3 ")	7	6
28 " (4 ")	7	3
34 "	4	2
35 " (5 ")	3	2
42 " (6 ")	2-3	3
49 " (7 ")	2-3	2-3
56 " (8 ")	1	2
63 " (9 ")	1	1
70 " (10 ")	2	3
77 " (11 ")	1	-1[a]
84 " (12 ")	1	1
112 " (16 ")	1	1
5 months	-1	1
8 "	-1	-1
10 "	-1	-1
14 "	-1	-1
19 "	-1	-1
20 "	-1	-1
21 "	rare	rare
26 "	rare	rare
30 "	rare	rare
31 "	rare	rare
44 "	rare	rare
8 years	very rare	very rare

[a] -1 stands for less than one.

Table 3. Average depth of the gastric mucosa of the pig, according to the different zones; the depth of the mucosa is expressed in micra

Age	Cardiac	Fundic	Pyloric
1 day	99	240	160
3 days	120	250	200
7 " (1 week)	160	261	248
10 "	262	519	420
11 "	360	457	405
13 "	390	720	627
14 " (2 weeks)	468	631	591
17 "	338	585	-
20 "	308	628	-
21 " (3 ")	395	710	580
28 " (4 ")	245	675	621
34 "	339	837	630
35 " (5 ")	506	962	540
42 " (6 ")	435	1023	554
49 " (7 ")	479	869	753
56 " (8 ")	595	1171	843
63 " (9 ")	456	1360	734
70 " (10 ")	523	1265	781
77 " (11 ")	583	1305	922
84 " (12 ")	677	1360	796
112 " (16 ")	540	1500	1260
5 months	720	1800	1530
8 "	540	2000	1200
10 "	360	2000	1620
14 "	620	2000	1460
19 "	765	1360	900
20 "	720	2000	1310
21 "	675	2000	1360
26 "	675	2000	1500
30 "	483	2000	1500
31 "	630	1500	1000
44 "	405	1805	900
8 years	510	1580	815

DISCUSSION

The observations made on the histology of the gastric mucosa of the day-old pig agreed for the most part with those made by Kirk (1910) on the fetus just before term.

The cytodifferentiation of the fundic zone at one day of age was found to be complete. The four types, mucous or surface, mucous neck, parietal and chief cells were already present. The shape of the glands hinted of what they would look like when the animal has reached the adult age.

The pyloric zone had two types of cells, mucous and parietal, instead of only the mucous cells described by Kirk. The parietal cells broke the continuity of the mucous cells which seemed to represent the majority of the cells of the pyloric glands. The specimens were taken far enough from the fundic zone to eliminate the possibility that the glands could be in a transition area.

In the cardiac zone, the same two types of cells were found, but the arrangement was very different. Whereas in the pyloric zone the parietal cells appeared out of place, in the cardiac zone they seemed to represent an integral part of the cell population. They formed in place glands of their own, without the participation of one single mucous cell, and the parietal-only glands existed side by side with glands made up of both the parietal and mucous cells. An actual count per field showed that the number of parietal cells in the cardiac zone was only slightly higher than in the pyloric zone (Table 2).

In the three gastric zones of the one-week and two-weeks old animal there was an increase in the depth of the mucosa. The glands showed little difference in the arrangement of their cells from those of the one-day old, except that the parietal cells in the cardiac and pyloric zones had decreased in number.

In the cardiac zone of the three-week old, the glands made up exclusively of parietal cells that were observed in the one-day, one-week and two-week old had disappeared, or they had acquired some mucous cells because all the glands seen in the sections had by then both parietal and mucous cells. A migration of the parietal cells from the cardiac or pyloric zones toward the fundic zone was hard to conceive and no evidence to support such a view could be discerned. However, the parietal cells were observed free in the lumen of the glands without showing any sign of degeneration, and they were also observed bulging into the lumen, as if the pressure from the surrounding cells was slowly pushing them out. It could be said that the parietal cells of the cardiac and pyloric zones were lost by extrusion. Both the cardiac and the pyloric zones acquired the adult appearance by shedding the parietal cells, and by an increase in the population of the mucous cells.

In the four-week old, the glands of all three gastric zones seemed to have reached the maximum development as to the amount of branching. The word "seemed" is used because sections from only one area of one zone could not begin to show exactly how the branching proceeded. Blocks from several areas in one zone, if cut serially and reconstructed, could give a more exact idea on how the branching occurred.

From the fourth week on, the glands increased in length bringing about an increase in the depth of the mucous membrane. The measurements of the depth of the gastric mucosa of the pig from one day to forty-four months of age indicated that the thirty-two age groups could be placed into three different periods of development.

In the first period, that extends from one day to fifteen months of age, the depth of the mucosa increased more or less steadily. The glands had already reached their maximum branching in all three zones by the third week of age. The increase in length was possible only by a multiplication of the cells above and beyond a mere replacement of the worn out or dying cells.

The increase in the depth of the mucosa of the three gastric zones during the first period could be accounted for only by a steady multiplication of the individual cells. Mitosis was observed in many cells in all three zones, in all the individuals, but never in number sufficient to account for the steady growth. It was quite possible that cell renewal in the gastric mucosa varied with the time elapsed after feeding as Hunt (1954) had indicated, or was under the influence of several factors (Leblond and Walter, 1956), such as diurnal or nocturnal activities, seasonal variations, nutrition and other environmental conditions. In this experiment, no attempt was made to eliminate or take into consideration any of those factors that might or could influence cell renewal.

In the cardiac and pyloric zones, there was mitosis and there was growth, but mitosis did not seem to affect the parietal cells. There was

a steady decrease in the number of the latter in those two zones (Table 2). By the fourth week the parietal cells were down to one third of their original number. By the twelfth week, they were occasionally seen. So, any cell in mitosis seen in the cardiac and pyloric zones of the animals could be assumed to be a mucous cell.

The fundic zone, with its four types of cells, presented a different situation. Since a cell in mitosis gave no indication as to what the daughter-cells would be, and since in the fundus and body of the glands, parietal cells were seen side by side with chief and mucous cells, there was practically no way of deciding which cell is dividing. In the neck of the glands, a cell in mitosis can be assumed to be a mucous neck cell. In none of the seventy-three specimens studied was mitosis observed in the lining or surface epithelium, while division was sometimes seen in the neck of the glands. Hence the possibility that the lining epithelium was built up from, or replaced by the mucous neck cells.

Hunt's experiments (1958) tended to prove that the cells of the lining epithelium or surface cells change into mucous neck cells, and the latter into parietal cells. Later in the regeneration process the mucous neck cells would change into zymogenic or chief cells. Hunt reached these conclusions after cautery of the mucous membrane and observations of the healing process on the following sixty-two days. It was difficult to consider the processes following cautery as normal physiological conditions. The events following extensive damage to the mucosa could not and should not be paralleled with events that take place in a normal mucosa under day to day living conditions. Even in accepting

Hunt's conclusions as being what normally could take place in the rat stomach, it did not necessarily imply that it would be the same pattern for the pig. In the histogenesis of the gastric mucosa of the pig, Kirk (1910) found that the parietal cells came first, then the chief cells; the mucous cells came last without passing through a mucin stage. Only one worker quoted by Bensley (1932) appears to be on record for having seen the parietal cells in division. The lack of evidence points to direct division as the method of multiplication in the case of the chief and parietal cells. Biopsy of the stomach of less than three-week old pigs at regular intervals day or night, before or after feeding, coupled with the use of labelled compounds, could help elucidate the question of mitosis in the cells of the gastric mucosa.

Fewer cells in mitosis were observed in the second period which extended arbitrarily from fifteen to twenty months of age. The depth of the mucosa, in the animals between fifteen and twenty months of age, remained more or less level. The cell divisions were apparently for the purpose of maintenance, because the glands seemed to have stopped growing.

In the third period, from twenty months to ninety-six months of age, the depth of the mucosa started to decrease. Cells in mitosis were observed, but the divisions were not able apparently to keep up with the losses due to death or wear. Could the decrease in the depth of the mucosa past the twentieth month be considered as the beginning of senescence for the stomach? Yes, if in senescence were considered only the changes that occur in the structure and necessarily in the function

of an organ between the time of peak maturity and the time of death of the individual. Changes did take place in the stomach. There was a shortening of the glands, and an increase both in size and amount of the collagenous elements in the lamina propria. It was difficult to dis-associate senescence from the overall aging process, and in the case of the stomach, the changes that occur after the twentieth month were simply a continuation and a magnification of changes that started in the first day of extra-uterine life. The space between the fundus of the glands and the muscularis mucosae which in the one-week old was occupied by "a delicate, sparse connective tissue framework" (Trautman and Fiebiger, 1957) became filled in the twenty month old with almost regularly arranged, coarse collagenous fibers. No measurements were made on the collagenous fibers, but a series of colored pictures taken at the same magnification showed the changes that took place in the connective tissue elements of the lamina propria. The increase in the amount and the size of the collagenous fibers of the lamina propria will slow down the motility, and decrease the capacity for expansion of the stomach. Motility of the stomach is essential for penetration of the gastric enzymes into the ingested foodstuffs. Digestion begins with the action of the enzymes. Lack of motility will affect digestion which in turn will influence absorption in the intestines.

Considering the enzymes involved in the digestive process, there was partial agreement between the work of Kvasnitskii and Bakeeva (1940) and that of Lewis et al. (1957). Lewis et al. found very little pepsin activity until the sixth week, while Kvasnitskii and Bakeeva believed

that the enzymes are not utilized before the eighth week. The last two workers found hydrochloric acid present only on the twenty-fifth day, attributing the acidity present before the twenty-fifth day to the microbial flora of the ingested food. Lewis et al. neglected to do any hydrochloric acid determination, recording only the acidity of the stomach contents. As indicated by Lewis et al., the pepsin activity of the dry stomach tissue at six weeks was eight times higher than it was at two weeks. The glands of the animals in this experiment did not show a similar increase in the number of the zymogenic cells. The stomach, already at three weeks, had the structure in miniature of that of the adult, yet it was around the sixth or eighth week that it began to function like an adult's. It was very likely that the body requirement, at six or eight weeks, necessitated the full use of the stomach. As the animal becomes older, is there in the production of digestive enzymes, a pattern comparable to the one seen in the growth of the mucosa? An increase in the length of the glands may mean more cells that can produce enzymes or hydrochloric acid. A shortening of the glands may conversely mean a decrease in the number of the cells capable of producing enzymes or hydrochloric acid.

Of all the different types of cells making up the cell population of the gastric mucosa, the enterochromaffin or argentaffin have been probably the least understood when it comes to function. A good deal of research had been done on them in the past fifteen to thirty years in an effort to elucidate the role played by them in the mammalian physiology. The substance they elaborate had been isolated and was called

synonymously enteramine, 5-hydroxytriptamine, and serotonine. They were not attributed any specific or direct role in the digestive process, like the parietal or zymogenic cells for instance. It was thought at one time they were responsible for the intrinsic anti-anemia factor (Johnson, as quoted by Gilman, 1942). Wermel and Kacharava (1948) concluded they produced secretin. Debray and Besancon (1960) believed that in the human, their secretion might influence the motility of the small intestine. Apparently, no definite conclusion concerning the physiological role of the substance secreted by the enterochromaffin cells has been reached to this date.

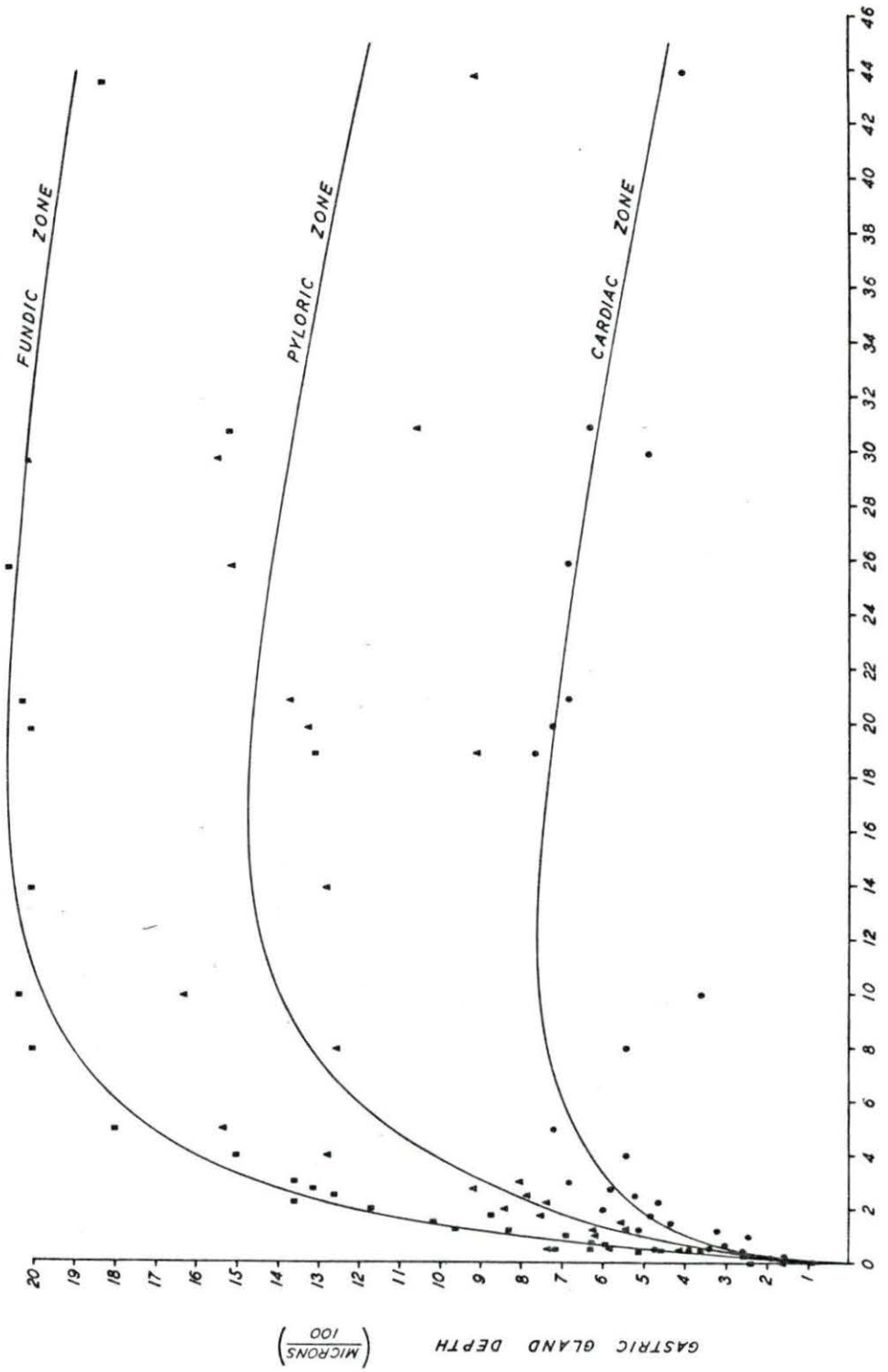
The enterochromaffin cells were found with almost constant regularity in all three zones of the stomach, in all the animals of this present study. Between the twentieth and the forty-second day of age, there was a sharp rise in the number of enterochromaffin cells in the pyloric zone. The number tapered down after the forty-second day, and by the fifty-sixth day was down to the same number it was before the twentieth day. There was no explanation that can be offered as to the cause and significance of the increase in number of the enterochromaffin cells in these age groups. All the animals were in excellent physical condition.

Because some attention has been given lately to finding of stomach ulcers in otherwise normal pigs, all the stomachs were carefully examined as soon as the specimens were removed. No ulcer was found in any of the animals of this experiment. The stomach ulcers are apparently becoming more prevalent among swine, but no specific etiological agent has been

incriminated as yet.

The lymphatic elements represented the most consistent finding through the different age groups, being present in the nodular or the diffuse forms from three days to forty-four months, and in the eight years old animal. Weidenreich et al. (1933) had noted lymphatic nodules and aggregates of nodules in the cardiac region of the pig, and had found them more numerous in the young than in the older animals. His oldest animals were five months old. Weidenreigh et al. mentioned the possibility of the existence of a pathological condition to explain their presence. The observations made in this study do not agree with those of Weidenreich et al.; the presence of the lymphatic elements either in diffuse or nodular forms was constant in all age groups, in all the three zones of the stomach. All the animals could have passed for food for human consumption, except the very young for which immaturity would have been a cause for condemnation. The animals came from different environments. They did not show any gastritis; it could well be that the consistent presence of the lymphatic elements in the three zones of the stomach could simply be a normal defense mechanism that started early and persisted throughout the life of the animal.

Figure 39. Growth of the three different zones of the gastric mucosa, in relation to each other


FUNDIC ZONE
PYLORIC ZONE
CARDIAC ZONE
GASTRIC GLAND DEPTH
(MICRONS/100)
AGE (MONTHS)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

SUMMARY AND CONCLUSIONS

1. Two thousand and twenty-four sections made from two hundred and sixteen specimens taken from the three glandular zones of the stomach of seventy-two pigs were examined microscopically.

2. The age of the animals ranged from one day to eight years.

3. The specimens were taken from the same general area of the stomach, namely caudal to the cardiac diverticulum, in the middle of the fundic region, and at the pyloric antrum. They were taken far enough away from the adjacent zones to be out of a transition area.

4. Four different staining methods were used: a) Hematoxylin and Eosin, as a routine stain; the enterochromaffin cells with their yellow color contrasted very well. b) Periodic acid-Schiff-Hematoxylin-Aurantia combination for the mucous and parietal cells. c) Crossmon's modification of Mallory's Triple Stain for the collagenous fibers and the zymogen granules of the chief cells. d) The Weigert's-Heidenhain-Van Gieson combination for collagenous and elastic fibers.

5. The observations were on the type, the distribution and the morphology of cells, the branching of the glands, and the depth of the mucosa.

6. All the five types of cells, namely mucous, mucous neck, parietal, zymogenic and argentaffin cells, seen in the three glandular zones of the adult stomach were present at birth, but their arrangement was different from that of the adult. The glands made up of these cells were then mere twisted tubules, with little branching.

7. The cardiac zone reached the adult stage between the third and fourth week by rearrangement of the mucous cells and the gradual loss of the parietal cells. The parietal cells were lost by extrusion into the gland lumen. The parietal cells never completely disappeared from the cardiac zone.

8. The fundic zone at birth had the five types of cells observed in the fundic zone of the adult animal, namely the parietal, argentaffin, zymogenic mucous and mucous neck cells. However, the fundic zone did not display a specific or consistent pattern in the arrangement of the cells which composed it. The mucous cells from the ninth week on seemed to have become the predominant element of the cell population.

9. The pyloric zone at birth showed less parietal cells than the cardiac. While in the cardiac zone the parietal cells form glands of their own, in the pyloric zone, the parietal cells were part of glands made up mostly of mucous cells. The parietal cells decreased in number as the animal became older, without, however, disappearing completely.

10. By the fourth week of age, the glands of all zones appeared to have reached their maximum branching, without reaching their maximum growth.

11. The growth of the mucosa in all three glandular zones was achieved by an increase in the number of the individual cells. Mitoses were observed in every age group, but never in sufficient number to account for the steady increase of the depth of the mucosa. Even after the mucosa had stopped growing, mitoses in much lesser number could still be observed in the three zones of the stomach.

12. Evidence found by other investigators pointed to a certain rhythm in the cell division in the glandular stomach of laboratory animals. The rhythm was not observed in the specimens studied in this experiment. However, the reason may be due to the fact that no effort was made to regulate feeding before death, nor to regulate the time of day at which the animals were to be killed.

13. The lamina propria displayed definite changes between the first day of life and eight years of age. There is a steady increase in the size and amount of collagenous fibers; the elastic fibers remained about the same.

14. Lymphatic elements were consistently present in all three zones of the stomach, either in nodular or diffuse forms, from three days to eight years of age. The mucosa of the stomach was examined in each of the seventy-two animals for possible gastritis but in all cases the observations were negative.

15. There were no ulcers present in any of the stomachs of the animals killed for this experiment.

16. The order of depth for the three zones was the same for all the individuals in this study; the fundic being the deepest, next the pyloric followed by the cardiac zone.

17. Although not consistent with every age group, the growth of the mucosa in the three gastric zones showed a definite pattern, an increase from one day to fourteen months, a plateau between fourteen and twenty months, and a decrease from twenty months on. The possibility that the

arrest of the growth of the mucosa might be an indication of senescence was discussed.

BIBLIOGRAPHY

Barter, R. and A. G. E. Pearse (1953). Detection of 5-hydroxytriptamine in mammalian enterochromaffin cells. Nature 172: 810.

Bensley, R. R. (1902-1903). The differentiation of the specific elements of the gastric glands of the pig. Am. J. Anat. 2: 105-106.

_____ (1910). The cardiac glands of the mammalian stomach. Anat. Rec. 4: 375-390.

_____ (1932). The gastric glands. In Cowdry, E. V., ed. Special cytology. pp. 199-230. 2nd ed. New York, Paul B. Hoeber Inc.

Cantarow, A. and B. Schepartz (1958). Biochemistry. 2nd ed. Philadelphia, W. Saunders Co.

Cordier, R. (1926). Recherches morphologiques et expérimentales sur la cellule chromoargentaffine de l'épithelium intestinal des vertébres. Arch. Biol. 36: 427-463.

_____ and L. Lison (1930). Études histologiques de la substance chromoargentaffine de la cellule de Kultchitzky. Bull. d'Hist. Appliq. à la Physiol. 1: 140-149.

Crossmon, G. (1937). A modification of Mallory's connective tissue stain with a discussion of the principles involved. Anat. Rec. 69: 33-38.

Dawson, A. B. (1927). The various elements in the epithelium of the fundic mucosa with special reference to those cells whose endodermal origin is doubtful. Anat. Rec. 35: 99-107.

_____ (1948). Argentophil and argentaffin cells in the gastric mucosa of the rat. Anat. Rec. 100: 319-329.

Dean, H. W. (1954). Alimentary tract. In Greep, R. O., ed. Histology. pp. 540-579. New York, The Blackiston Company, Inc.

Debray, C. and F. Besancon (1960). Le rôle physiologique, chez l'homme, de la sérotonine (5-hydroxytryptamine) dans la motricité de l'intestin grêle. Recherches électromanometriques. Comp. Rend. Soc. de Biol. 154: 1747-1757.

Dios-Amado, L. (1925). Sur l'existence des cellules argentaffines dans le tissu conjonctif des villosités intestinales. Compt. Rend. Soc. de Biol. 93: 1548-1549.

_____ (1925). Sur la significance des cellules de Nicolas. Compt. Rend. Soc. de Biol. 93: 1550-1551.

Esparmer, V. and B. Asero (1952). Identification of enteramine, the specific hormone of the enterochromaffin cell system as 5-hydroxytriptamine. Nature 169: 800.

Getty, R. (1945). The histopathology of a focal hepatitis and of its termination ("Sawdust" and "Telang" liver) in cattle. Unpublished M.S. Thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.

Gillman, J. (1942). The structure of the basal granular cells (argentaffin) in the human (Bantu) alimentary canal with special reference to the anti-anemic factor. S. Afric. J. Med. Sci. 7: 144-159.

Grant, R., H. I. Grossman and A. C. Ivy (1953). Histological changes in the gastric mucosa during digestion and their relationship with mucosal growth. Gastroenterology 25: 218-231.

Hale, A. J. (1958). Observations on the nature of the chromaffin reaction. J. of Physiol. 141: 193-197.

Hallier, S. (1937-1938). Über die Wirkung wiederholter Kleiner Gaben von Insulin, Klityran und Tonephin aud die basalgekornten Zellen in Darm der weissen Ratte und des Meerschwein-chen. Ztschr. f. Zellforsch. 27: 52-64.

Hally, A. D. (1959). The fine structure of the gastric parietal cell in the mouse. J. of Anat. 93: 217-224.

_____ (1959). Functional changes in the vacuole containing bodies of the gastric parietal cells. Nature 183: 408.

Hunt, T. H. (1952). Mitotic activity in the gastric glands of the rat. Anat. Rec. 112: 346.

_____ (1954). Variations in mitotic activity on the rat stomach at intervals after feeding. Anat. Rec. 118: 392.

_____ (1958). Regeneration of the gastric mucosa in the rat. Anat. Rec. 131: 193-211.

Kametaka, M. and H. Imai (1956). Studies of the artificial digestion assay. III. Distribution of the three types of secretory cells in the gastric mucosa of the swine (In Japanese, English summary). Jap. J. of Zootech. Sci. 27: 43-48.

Kirk, E. G. (1910). On the histogenesis of gastric glands. Am. J. of Anat. 10: 472-520.

Kowalczyk, T., W. G. Hoeskstra, K. L. Puestow, I. D. Smith and R. H. Grummer (1960). Stomach ulcers in swine. J. Am. Vet. Med. Assoc. 137: 339-344.

Kurosomi, F., S. Shibasaki, G. Uchida and Y. Tanaka (1958). Electron microscope studies of the gastric mucosa of normal rats (In Japanese, English summary). Arch. Histol. Jap. 15: 587-592.

Kvasnitskii, A. V. and E. N. Bakeeva (1940). Gastric secretion and digestion of unweaned pigs. Trud. Inst. Svinovod, Kiev. 15: 3-42. (Original not available for examination; abstracted in Vet. Bull. 13: 222, 1943.)

Langley, J. N. (1880-1882). On the histology of the mammalian gastric glands and the relation of pepsin to the granules of the chief cells. J. of Physiol. 3: 269-290.

Lawn, A. M. (1960). Observations of the fine structures of the parietal cells of the rat. J. of Biophys. and Biochem. Cytol. 7: 161-166.

Leblond, C. F. and B. E. Walter (1956). Renewal of cell population. Physiol. Rev. 36: 255-276.

Lewis, C. J., R. A. Hartman, C. H. Liv, R. O. Baker and D. V. Catron (1957). Digestive enzymes of the baby-pig - pepsin and trypsin. J. of Agric. and Food Chem. 5: 687-689.

Lim, R. K. S. (1922). The gastric mucosa. Quart. J. Microsc. Sci. 66: 187-212.

Ma, W. C. (1927). A method for the demonstration of the intracellular secretion canaliculi of the parietal cells of the mammals. Anat. Rec. 35: 337-339.

Macklin, C. C. and H. T. Macklin (1932). The intestinal epithelium. In Cowdry, E. V., ed. Special cytology. pp. 1771-1822. 2nd ed. New York, Paul B. Hoeber Inc.

Maximow, A. A. and W. Bloom (1957). A textbook of histology. 7th ed. Philadelphia, W. B. Saunders Co.

McManus, J. F. A. (1946). Histological demonstration of mucin after periodic acid. Nature 158: 202.

Monesi, V. (1960). Differentiation of argyrophil and argentaffin cells in organotypic cultures of embryonic chick intestine. J. Embryol. Exp. Morph. 8: 302-313.

Parat, M. (1924). Contribution à l'histophysiologie des organes digestifs de l'embryon. L'apparition corrélative de la cellule de Kultschitzsky et de la sécrétine chez l'embryon. Compt. Rend. Soc. de Biol. 90: 1023-1024.

Read, A. M. and F. R. C. Johnstone (1961). The distribution of parietal cells in the gastric mucosa of the cat. Ant. Rec. 139: 525-530.

Scott, H. R. and B. P. Clayton (1953). A comparison of the staining affinity of aldehyde fuchsin and the Schiff reagent. J. of Histochem. and Cytochem. 1: 336-352.

Sedar, A. W. (1959). An attempt to correlate the fine structure of the parietal cell with the functional state of the gastric mucosa. Anat. Rec. 133: 337.

Sharples, W. (1945). A note on the relatively high number of argentaffin cells of the mucosa of the human stomach. Anat. Rec. 91: 237-241.

Sloss, M. W. (1954). The microscopic anatomy of the digestive tract of the Sus scrofa domestica. Am. J. Vet. Res. 15: 578-593.

Stevens, C. E. and C. P. Leblond (1953). Renewal of the mucous cells of the gastric mucosa of the rat. Anat. Rec. 115: 231-241.

Tang, E. H. (1922). Über die Panethsche Zellen Sowie die gelben Zellen des Duodenums beim Schwein und den anderen Wirbeltieren. Arch. f. Mikrosk. Anat. 96: 436-472.

Tehver, J. E. (1930). Über die enterochromaffinen Zellen der Haussäugetiere. Ztschr. f. Mikrosk. Anat. Forsch. 21: 462-496.

Trautman, A. and J. Fiebiger (1957). Fundamental of the histology of domestic animals. (Translated by R. E. Habel and E. L. Biberstein.) Ithaca, N. Y., Comstock Publishing Associates.

Vial, J. D. and H. Orrego (1960). Electron microscope observations of the fine structure of the parietal cells. J. Biophysic. and Biochem. Cytol. 7: 367-372.

Weber, A. and A. W. Stinson (1957). Lectures and laboratory outlines. Microscopic anatomy of domestic animals. (Mimeographed) Minneapolis, Minnesota, University of Minnesota, Division of Veterinary Medicine.

Weidenreich, F., M. Baum and A. Trautman (1933). Lymphgefäßsystem. In Bolk, L., E. Göppert, E. Kallius and W. Lubosch, eds. Handbuch der vergleichenden Anatomie der Wirbeltiere. VI Band, pp. 745-854. Berlin, Germany, Urban und Schwarzenberg.

Wermel, E. M. and E. A. Kacharova (1948). A study of the basal granular cells of the mucosa of the small intestine in the production of secretin. Anat. Rec. 101: 595-604.

ACKNOWLEDGMENTS

The author wishes to express his deep appreciation to Dr. Robert Getty for guidance, encouragement, constructive criticism and time given so willingly during the working out of the problem.

The author is very grateful to Dr. Martin J. Ulmer of the Department of Zoology for the use of the then only available Aurantia dye, and later for his information on source of supply.

The author wishes to thank Dr. Hideo Tamate, now back at Tohoku University, Sendai, Japan, for his help and advice in overcoming some staining difficulties.

The author wishes to thank Miss Rose Aspengren for her technical assistance in the preparation of some of the slides.

The author takes the opportunity to acknowledge with appreciation the support, in part, from the Department of Health, Education and Welfare, U. S. Public Health Service, under grant #H-4487.