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El establecimiento temprano de la nutrición intestinal en el opossum. El sistema digestivo un poco antes e inmediatamente después del nacimiento.

El periodo de gestación en el opossum es muy corto; el intervalo entre la inseminación y el parto dura tan solo trece días, y no mas de diez días pasan entre el principio de la segmentación del óvulo y el nacimiento. El desarrollo del tubo digestivo es muy rápido durante los últimos cinco días de la vida intrauterina. El estómago en el embrión próximo a nacer está comprimido y su cavidad es pequeña; está aún mas reducida en tamaño por pliegues o rugosidades de su mucosa. Pronto des pues del nacimiento las rugosidades desaparecen, a causa de la expansion fisiologica del estómago, producida por la leche. En los estados tempranos, que el embrión pasa en la bolsa de la madre, el estómago es una simple cámara sacular provista de una pared muy delgada y ligeramente diferenciada. El esófago comunica con el estómago en un punto muy próximo al orificio duodenal. En la mucosa, cerca de los orificios de entrada, existen pliegues poco profundos, pero las glándulas no se han desarrollado todavía en ninguna parte del órgano. Las asas primarias del intestino delgado son unas seis en número, y todas ellas aparecen ya en el embrión próximo a nacer. Inmediatamente después del nacimiento, el estómago, distendido por la leche, desplaza al intestino delgado hacia la derecha, pero la identidad de las asas primarias puede reconocerse todavía. En el embrión próximo a nacer se han desarrollado vellosidades en las tres primeras asas. En los jóvenes que habitan la bolsa dichas vellosidades aparecen en todas las regiones del intestino, aunque están mas diferenciadas en el duodeno. Las tunicas externas del intestino son muy delgadas, tanto en el embrión próximo a nacer como en el joven situado en la bolsa. En este ultimo especialmente el intestino delgado es notable por la considerable extensión de la mucosa. En los extremos distales de las células que le revisten existen grandes masas de gránulos que se tiñen especialmente. La capa muscular está indicada solamente por una banda delicada de mioblastos. Los vasos sanguíneos del intestino son especialmente visibles a causa de su gran tamaño y caracter sinusoidal, estando situados en la inmediata proximidad de la mucosa.

THE EARLY ESTABLISHMENT OF THE INTESTINAL NUTRITION IN THE OPOSSUM—THE DIGESTIVE SYSTEM JUST BEFORE AND SOON AFTER BIRTH

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SIX PLATES (TWENTY FIGURES)

As the intra-uterine period in the opossum is very brief, the intestinal nutrition commences in the newborn animals while they are yet relatively very immature. Ten days after the beginning of segmentation, or thirteen days after insemination, the young opossums appear in the pouch; they become at once attached to the teats, and milk soon enters their stomachs. Five days before birth, however, the digestive tract of the opossum is no further advanced than this system in the three-day chick embryo; the foregut extends forward from the broad connection with the yolk-sac, and the first rudiments of the liver, hindgut, and allantois are just appearing. The transformation brought about during these last five days is remarkable in its rapidity.

The stages especially considered in this report are those of the embryo a few hours before birth and the pouch-young about three days old. The external form of the viscera is first examined and the histological structure is then studied. The stomach, small intestine, and large intestine are dealt with in order, special attention being given to the small intestine.

METHODS

This study was commenced and the material used was obtained while I was a member of the staff of The Wistar Institute. The specimens were collected at Austin, Texas, in coöperation with Dr. Carl G. Hartman, of the University of Texas. Doctor

Hartman had collected opossum embryos for several years, and as he had worked out methods for recognizing the various stages of pregnancy, we were able to secure a very complete series.

The embryos were removed from the excised uterus in Ringer's solution, as described by Hartman ('19). With scissors a long incision was first made along the uterine horn, cutting rapidly through the muscular layer but without perforating the mucosa, and the latter was then pulled apart with forceps. In order to avoid injury to the embryonic vesicles, which during the last two or three days before birth are closely pressed against each other and intimately applied to the uterine mucosa, the vesicles were separated from one another by cutting off the uterine muscle before opening the lumen; even small pieces of the muscle remaining caused considerable interference by the formation of pockets in the mucosa which surrounded and held portions of the embryonic membranes. This care in separating the vesicles was taken for the reason that I wished to make injections of the blood-vessels in one or more embryos of a litter. Such injections could be made very satisfactorily in these embryos with an intact vascular system and a vigorously beating heart.

The embryos which were to be sectioned were dropped while alive into Bouin's fluid and left for six to twenty-four hours, depending upon the size of the individual. The Bouin's fluid was replaced with 80 per cent alcohol by adding the latter in small quantities to the fixing fluid—taking several hours for the transfer. The alcohol was later changed several times, and finally lithium carbonate—a few drops of a saturated aqueous solution to the ounce of alcohol—was added to remove the picric acid. As the pouch-young were firmly attached to teats in the pouch, they were removed by exerting a slow continuous pull with blunt forceps pressed against the mouth. The specimens were then placed at once into Bouin's fluid, and after the muscular contractions had ceased, incisions were made through the abdominal wall to insure the rapid entrance of the fixing fluid into the body cavity. Embryos and pouch-young were also fixed and stored in 10 per cent formalin.

The whole specimens, which had been fixed in Bouin's fluid, were later run down from 80 per cent alcohol to water—using a fluid-changing device by which one solution was added to the other drop by drop. The apparatus was provided with a glass stirring plunger which was raised and lowered by a cam-shaft, and an overflow-siphon controlled the volume of the solution surrounding the specimen. After staining in alum-cochineal, the specimens were carried back to 80 per cent alcohol, making use of the same device to insure slow interchange of fluids. The abdominal wall was then dissected off so that the external form and position of the viscera could be studied. Next the liver, stomach, and intestines were removed in one piece, and later, in order not to injure the other structures, the liver was dissected off with needles. As a means of recording the appearance of the dissections at the various stages, stereophotographs were made during the progress of the work. The block to be sectioned included the stomach and intestines; it was embedded in paraffin and cut into a series of sections 5μ thick, by Professor Huber's water-on-the-knife method. The sections were stained with Mallory's connective-tissue stain. The use of the cochineal was advantageous in that the embryos were satisfactorily stained for preliminary dissections before imbedding or for subsequent dissections; the stained embryos could also be photographed well; and in the sections the nuclear detail was better shown than with Mallory alone. The latter stain was very useful for showing differentiation among the various tissues. This method of staining the whole embryos in alum-cochineal and the sections with Mallory's connective-tissue stain has been used on specimens of various ages and has proved to be of value. Several series of sections were prepared and used for reference, but the chief observations in this study were made on an embryo of few hours before birth and on a pouch-young about three days old.

DESCRIPTION OF THE GASTRO-INTESTINAL TRACT

Stomach

In the embryo: The stomach in the late embryo is, in general, pyriform in shape, being slightly compressed from above downward; it lies almost in a transverse zone in the left side of the body. As seen in dissections of the viscera in situ, the stomach is found to be entirely masked by the liver, except on the left where a small portion of the greater curvature projects below the hepatic border (fig. 5). Upon removing the liver, the stomach may be seen in its characteristic pyriform shape as shown in figures 7 and 9. The greater curvature is about eight times that of the lesser, as determined by measurements of the curves from the esophageal opening to the pylorus. The esophagus enters the stomach at about the middle of the lesser curvature in rather close proximity to the duodenal opening. The pyloric portion is not drawn out into a tubular form as in human embryos (Lewis, '12), and externally at least there is no indication of a pyloric antrum. The blind pouch above the entrance of the esophagus—the fundus (fig. 9)—is relatively long and tapers rapidly upward to its tip. Except that the pyloric portion is not tubular in form, the stomach as a whole has the appearance of the organ in the late rat and early pig embryos. In naming the various subdivisions of the stomach the terms adopted by Lewis ('12) have been used.

The external surface of the stomach is smooth, but faintly delineated folds in the mucosa can be seen through the outer layers. These folds of the mucosa, or rugae, are in general arranged longitudinally and extend from the fundus down into the corpus, being more prominent along the greater curvature. They are filled with loose embryonic mesenchyme and extend far out into the lumen so that irregularly shaped projections and compartments are evident in sections (fig. 11).

In the pouch-young: The stomach in the pouch-young is no longer compressed as in the embryo, but has become tremendously distended by constant filling with milk. The greatly enlarged organ may be made out in the living animal because of

the milk which shimmers through the body-wall as a large white mass. In general, as in the embryo, the stomach lies in the left side of the body with the greater curvature to the left and the lesser curvature toward the right. Measuring the curvatures again from the esophageal opening to the duodenum at its union with the stomach, the ratio is about ten to one—showing an increase in the greater curvature as compared with the ratio of eight to one in the embryo. The esophagus and duodenum still enter the stomach very close together; this striking nearness of these openings into the stomach is a condition similar to that described by Owen ('68) for the *ornithorynchus*. The inferior border of the pyloric portion bulges below the duodenal opening and above blends imperceptibly with the corpus. The organ has expanded in all directions, but the greatest growth has been in the lower part of the cardiac portion. The fundus does not taper to a narrow tip and is much less conspicuous than in the embryo. Especially as regards this part, the stomach is of the simple carnivorous type seen in cat embryos.

By its great expansion the stomach has altered the relations of the other viscera; the small intestine has been crowded to the right side; the lateral margins of the wolffian bodies have been spread far apart; and the liver has been pushed upward and flattened laterally, so that its greatest width is more than twice its vertical thickness. Only a small portion of the stomach now remains capped by the liver, as can be seen by comparison of figures 6, 8, and 10.

The rugae present in the gastric mucosa in the embryo have become obliterated in the pouch-young, and except for a series of slight plaited folds between the esophagus and duodenum, the interior of the stomach, like the outside, has a smooth surface. This smoothing of the mucosa may be accounted for by the physiological distension of the organ with milk. The wall along the greater curvature is much thinner than in the region of the plaited folds referred to. The epithelium of the mucosa is made up of cells of a low columnar type with large vesicular nuclei. The cytoplasm is homogeneous and granular, being slightly more condensed along the cuticular border. Just beneath this border

terminal bars can be seen and the epithelial cells rest upon a delicate but distinct basement membrane. There is some evidence of a differentiation among the epithelial cells in the pyloric region. Here groups of cells appear slightly more darkly stained and more closely packed together. In each section there are found a few cells which in form appear similar to their neighbors, but are more brightly stained with the acid fuchsin; they probably represent a phase in cell activity in which the staining reaction has been modified. In the more attenuated portion of the stomach wall along the greater curvature, the distention has been very pronounced and all layers of the wall have been greatly reduced in thickness. The epithelial cells in some cases have less than half the height of those along the lesser curvature. In the thinnest places, the nuclei have been drawn out parallel with the surface of the epithelium; they are also much farther apart in these regions. The muscular layer is everywhere very thin, being only one or two myoblasts in width and the elongated nuclei are few in number.

Small intestine

A striking feature of the intestine in opossum embryos is the abundance, size, and arrangement of the blood-vessels. In an embryo 8.5 mm. in length—about nine days after the beginning of segmentation and one day before birth—the vessels have a very wide diameter and are also noteworthy for their close approximation to the mucosa, as shown in figure 1 from a transverse section of a loop of intestine. Closely hugging the mucosa, they present the appearance of large sinusoids rather than that of capillaries. The outer borders of the vessels, however, are covered with the mesenchyme, and hence they are not true sinusoids as defined by Professor Minot ('00). In many sections nearly the whole extent of the epithelium of the mucosa is in contact with these large blood-vessels. Four to six folds of the epithelium extend into the lumen, and in each case the crypt of the fold is filled with a large vessel. A reconstruction of a segment of the intestine (fig. 2) indicates a rich plexus of vessels spreading over the outer surface of the mucosa. The crypts of

the folds are filled with vessels which are of great size. This condition does not appear in the drawing of the model, but can be seen in segments of it and in sections of the embryo (fig. 1). In one crypt the mucosa is spread out in a thin layer over a large blood sinus.

In addition to this great vascularity of the small intestine of the earlier opossum embryos, there are certain other features of interest in the morphology of the intestinal tract not only in the late embryo, but in the pouch-young.

In the late embryo: The duodenum extends from the pylorus to the right side of the abdominal cavity, bends dorsally in a right angle and proceeds downward and backward to the mid-sagittal region, where it turns sharply back on itself and parallels its course to the pylorus. The duodenum may arbitrarily be said to end at this point. Here, at the upper end of limb 4 in figures 3 and 7, the small intestine makes another complete bend and follows the previous limb, lying mesially to it, for half its length, then turns in a right angle to the left side of the body (fig. 3, 5 to 6) and gradually bends upward to the region beneath the corpus of the stomach. Here for a third time it turns sharply back on itself as far as the midventral plane, where three short loops (fig. 3, 8 to 12) are made before it leads into the large intestine. The small coils are surrounded and partially covered by the larger loops. The position of the union of the small with the large intestine is on the left side of the body just below the middle part of the stomach (behind 12 in fig. 3).

The first double loop of the small intestine—duodenum—has a very wide diameter, being enlarged into an antrum pressing against and encroaching upon the stomach. The widest region of the whole intestinal tract is at the middle of the first limb of this loop; the enlargement is a short distance below the opening of the ductus choledochus. Beyond the duodenum the small intestine—from the beginning of limb 5 in figure 3—gradually diminishes in caliber so that the girth of the smallest coils in the umbilical region is about one-third that of the duodenum in its widest place. The small intestine consists of relatively a few loops, which in most cases have sharp bends. Segments of most

of the coils of the intestine can be seen in a single section, as shown in figure 12, where the great difference in the diameter of the various loops is apparent. Numerous long villi are present in the duodenum and first coils of the small intestine, as shown in figure 12 at 1, 4, 5, 7, 8. In the slender loops 10, 11, and 12 there are no villi, but the mucosa is thrown into four or five folds. The ductus choledochus enters the duodenum in limb 1 eight sections (40μ) below the section figured. Limb 5 of section 254 is shown at a higher magnification in figure 13.

The low columnar cells of the mucosa rest upon a distinct basement membrane. Definite cell-walls are usually made out and the cuticular border ends abruptly in a narrow dense band, bulging out in the middle part of the cell. Intercellular cement lines are prominent in surface views of the tips of the cells, and terminal bars are therefore distinct. The nuclei, which are located in the lower part of the cells near the basement membrane, are usually spherical and contain one or two large nucleoli.

The relations of vessels and the great difference in thickness of the various layers are also indicated in figure 13. Numerous vessels, as also in the earlier embryo (figs. 1 and 2), appear beneath the mucosa, and the core of each villus is practically filled by a large sinus-like blood-vessel. The outer layers of the intestinal wall are very thin; the muscular coat is especially delicate, consisting of a single, or in places, of a double row of myoblasts.

In the pouch-young: After birth, although the intrusive stomach crowds the other viscera out of their former positions, the general arrangement of the coils in the intestines is not fundamentally disturbed. The large loops of the duodenum and the first loops immediately following are similar to their former pattern. This essential similarity in coils of the two stages was strikingly illustrated by a dissection which was made of a formalin-fixed pouch specimen of the same litter as the one shown in figure 6. The coils of the intestine could be spread apart under the binocular microscope so that the various limbs and loops could be compared with those found in the late embryo. As in the latter, the duodenum had by far the largest caliber of any part of the

intestine and again the extreme girth was in the first limb at the region of the first right-angle bend. The last three or four coils of the small intestine were distinctly smaller than the others. This great disparity in the caliber of the different parts of the intestine was found to persist in specimens of at least several weeks' age.

The villi of the small intestine of the pouch-young are covered by tall columnar cells with large centrally placed nuclei. A delicately striated cuticle rests upon a distinct cement line and terminal bars are very prominent. In the distal ends of the cells are large granular strands and masses which vary considerably in shape and size. The masses are located in a zone which occupies about the middle third of the space between the nucleus and the cuticular border of the cell. The distribution of these granules may be more extensive, but distally there is always a narrow strip of cytoplasm devoid of granules. These cytoplasmic inclusions stain brilliantly with eosin, and with the acid fuchsin of the Mallory stain. Iron hematoxylin brings them out distinctly. After staining with alum-cochineal in toto, the granular masses are faintly visible; following this stain with gentian violet applied to the tissue after sectioning, the masses become intensified but remain much paler than the nucleoli. These granular aggregations, varying in shape and size, appear in nearly all the cells of the villi and in some of the cells of the intervillous spaces. The strands are made up of fine granules; the larger masses are likewise composed of granules or consist of a limiting border of granules enclosing a less densely stained homogeneous interior. The ultimate round granules also vary considerably in size; minute isolated ones may be seen between the larger groups. Some of the cells are filled with a non-staining substance so that they appear practically empty; a small amount of protoplasm clings to the nucleus and a few strands may stretch across to the cell-membrane. Many other cells are partially vacuolated; these occur more abundantly in the first large coils of the intestine. The cells on the left side of the villus shown in figure 18 are of this type.

There are no glands present in any part of the intestine, but between the villi the epithelial cells are in many places more closely packed, more darkly stained, and of smaller size than those of the villi. A group of these cells is shown in figure 18. In corresponding regions of the two stages, the villi are taller and more highly specialized in the pouch-young (cf. figs. 13 and 17). Of the loops in the older specimen the one at 7-8 in figure 16 has the largest lumen. Here the villi are much lower than in the coils nearer the stomach, but the details of the mucosa and of the other layers are very similar to those found in the duodenum. The smaller coils have increased in size only slightly and the villi are imperfectly formed in some of the limbs and absent in others. The striking thinness of the external layers of the intestine is more marked in the pouch-young than in the embryo. This feature of the pouch-young is well illustrated by the photographs (figs. 17 and 18). The relation of the mucosa and the blood-vessels is again very intimate; the entire core of each villus is occupied by a large sinus-like vessel.

Large intestine

In the embryo: The large intestine begins at its junction with the small intestine, the point of origin being just below the corpus of the stomach on the left side of the abdominal cavity. From this point it swings gradually toward the midplane of the body and leads a nearly straight course downward to the cloaca. The diameter of the large intestine is nearly the same throughout its extent, being about equal to that of the most slender of the coils of the small intestine. The caecum is a long slender blind outgrowth from the large intestine immediately below the beginning of this part of the gut. The caecum grows out at a sharp angle and extends ventrally just beneath the stomach, so that the blind end is seen in ventral views of the viscera (fig. 7).

The epithelial tube of the colon is small and laterally constricted with the dorsal edge bent toward the left and the ventral edge toward the right so that in cross-section it appears as a widely opened letter S. Since the epithelial tube is small, the

combined width of the external layers is relatively greater than it is in the preceding portions of the digestive tract. The epithelial lining of the colon consists of undifferentiated low columnar cells with large elongated nuclei and homogeneous granular cytoplasm. There are many thin-walled blood-vessels in the loose mesenchyme of the submucosa. The muscular coat is a thin layer made up of about three rows of myoblasts without differentiation into longitudinal and circular coats. The layer of mesenchyme surrounding the muscularis is slightly denser than that constituting the submucosa.

In the pouch-young: The large intestine grows a little more rapidly than the other portions of the intestine, but in the pouch-young studied it is still the least differentiated region of the intestinal tube. The girth is only a little greater than that of the smallest of the coils of the ileum. The caecum remains a long slender lateral outgrowth as in the younger stage. There is in addition a short epithelial diverticulum which has grown out from the large intestine near its union with the small intestine. The significance of this diverticulum is not known.

The mucosa has grown so that it is about twice its former width. The tall columnar cells extend from the cuticular border to a distinct basement membrane. The cytoplasm is evenly stained and undifferentiated. The nuclei are confined largely to the lower portion of the cells, but they are not arranged in a single row; with the folding of the epithelial tube the regular distribution of the nuclei is apparently disturbed; they are found in groups opposite the folds and arranged in a triple layer. The lumen is pentagonal in transverse section. The increased caliber of the large intestine may be accounted for by the increase in size of the lumen and to the growth in thickness of the mucosa; the external layers, however, are relatively thinner than in the embryo.

DISCUSSION AND SUMMARY

The period of gestation in the opossum is very short; the interval from copulation to parturition is only of thirteen days' duration, and of this period three days have passed before the

beginning of segmentation of the ovum. Selenka ('87) calculated that segmentation did not begin until the end of the fifth day, but from much more extensive material Hartman ('19) estimates that the first division of the fertilized ovum occurs two days earlier. During the next three days the embryo develops to a stage with an undifferentiated fore- and hindgut. From this primitive condition the digestive tract, during the last five days before birth, is rapidly transformed to a functioning state. This rapid change from the primitive gut into an alimentary system capable of digesting milk, even at a stage of relatively incomplete differentiation, presents many problems of great interest.

The stomach of the late embryo may be described as consisting of a cardiac and a pyloric portion, more or less arbitrarily marked off on the lesser curvature at the angular incisure. The cardiac portion, using the terminology adopted by F. T. Lewis ('12), may be said to include a prominent blind pouch or fundus tapering rapidly to a tip and a large lower part or corpus. The different parts blend imperceptibly with each other along the greater curvature. In the opossum embryo the pars pylorica is not tubular in form as described by Lewis for the human embryo, and as Lewis and I reported ('14 and '15) for the cat, rat, pig, and sheep. In a younger opossum embryo—one measuring 7 mm. in length—the whole digestive tract by reconstruction (Heuser, '18) showed a more elongated portion leading into the duodenum, and the stomach resembled the organ in the 10-mm. human embryo (Lewis, '12, fig. 5). The gastric canal, which Lewis demonstrated to be an epithelial differentiation in human embryos, and as we likewise found to be the case in the other mammals named, has not been definitely made out in the opossum embryos examined; however, it is believed that it would be revealed by wax reconstructions of the gastric epithelium in a proper series of stages.

The stomach is small in the late embryo and the lumen is further reduced in size by the presence of folds or rugae in the mucosa. The rugae vary considerably in number and prominence in embryos of this stage, being less conspicuous in two other

individuals of the same age. Near the pylorus there are a few shallow folds in the mucosa, indicating a very early stage in the development of pyloric glands, and at the opening of the esophagus, less definitely marked folds are found where the cardiac glands will later develop. The epithelial lining of the stomach, however, is made up of low columnar cells, which are at this stage undifferentiated in all parts of the organ.

After birth the distention of the stomach with milk results in the disappearance of the rugae which were present in the embryo. In the living animal the position and large size of the stomach are very evident on account of the milk which can be seen through the transparent body-wall. The greatest enlargement has occurred in the portion of the corpus along the greater curvature and in the upper part of the pyloric region. The fundus ends bluntly and is not prominent as in the embryo.

Bensley ('02), in his comprehensive paper, described the stomach of the adult opossum as a simple structure, resembling closely in shape and arrangement of parts those of insectivores and carnivores. He showed in a diagram (fig. 10 A) that the fundic gland zone occupied the greater portion of the mucous surface, with the remainder covered by pyloric glands, and stated also that the cardiac gland zone occupied an extremely narrow zone at the termination of the esophagus. Except that the glands have not yet developed, the arrangement of parts in the early pouch-young is similar to that described for the adult. However, the epithelial cells are yet of a simple type, and although certain cells in the pyloric region are slightly more darkly stained and more closely packed together, there is no differentiation among them. A few slight plaited folds occur near the pylorus and also around the entrance of the esophagus. Glands, however, are nowhere present nor can any of the elements be regarded as cells associated with the formation of acid as found in the functioning stomach of higher mammals.

The coils of small intestine in the late embryo are placed medially in the abdominal cavity, lying between the liver and the wolffian bodies. The first loops extend toward the right side, partially surrounding the remaining smaller coils. Mall

('98) showed that the primary coils of the intestine in the human embryo could be recognized very early and that even the various loops of the adult intestine, as well as their position, could be made out in embryos of five weeks. The first three of the primary loops, I believe, can be identified in the 7-mm. opossum embryo; the smaller distal coils, however, are not as yet indicated.

Villi have developed in the first three loops of the small intestine of the late opossum embryo. It was not determined whether the villi originate singly as thickenings of the epithelium, as Johnson ('10) has described for the human embryo, or whether they are preceded by longitudinal folds. Four to six low folds are present in the mucosa of the last slender coils and there are a few diverticula of the mucosa similar to those found by Lewis and Thyng ('08) in various mammals. One diverticulum of this nature is shown in limb 12 of figure 12. The epithelial lining is made up of undifferentiated cells of a low columnar type, filled with homogeneous cytoplasm and with nuclei proximally placed near the basement membrane. The entire width of the intestinal wall, exclusive of the mucosa, is remarkably thin; in some places the whole wall beneath the basement membrane is equal to about one-third the height of the epithelial cells above it. The muscular coat particularly is very delicate; it consists of a fine strand of myoblasts only one or two in thickness.

In the pouch-young the small intestine has been crowded to the right by the enlarged stomach. The primary coils, however, can still be recognized; the numerals on figures 3 and 4 have been placed on comparable limbs of the coils. Villi are present in all parts of the small intestine, although they are older and more advanced in the duodenum. The external coats are yet very thin and the intestine is noteworthy for the very large surface of the mucosa. The muscular layer is only faintly indicated by a few circular myoblasts, so that peristaltic contractions must be very feeble. The intestinal blood-vessels are especially noticeable on account of their large size and sinusoidal character, being closely applied to the mucosa.

The cytoplasm of the cells covering the villi is coarsely granular and the large vesicular nuclei are placed centrally in the cell.

In many places between the villi there are patches of epithelial cells which are smaller and more darkly stained than those covering the villi. In the distal ends of the cells covering the villi one sees very prominent masses which can usually be resolved into granules with the high power. These granules stain brilliantly with eosin and with acid fuchsin. The chief interest in them lies in speculation as to whether they are products of secretion or of absorption; no data, other than that afforded by histological examination, have been obtained.

The large intestine had changed to a much smaller degree relatively in the transformation from the late embryo to the pouch-young. Although the external caliber of the colon has increased considerably in the interval of growth, there is but little advance in the epithelial or mesoblastic differentiation. In the pouch-young the epithelial lining-cells have doubled in height; the lumen is considerably increased in size and the mesenchyme is markedly condensed. These morphological changes are, however, much less pronounced than are the differentiations which have occurred in the upper portions of the small intestine. The process of differentiation apparently proceeds from above downward, in the direction of the physiological passage of food-stuffs. The relatively slight dilation of the colon and the histological findings indicate that the residue of the food-stuffs reaching the colon in the opossum pouch-young is relatively small; practically all of the milk must be disposed of in the upper portions of the alimentary canal.

In summary it may be said that the changes in the gastrointestinal canal from the late embryo to the early pouch-young, in which the digestion of milk must occur, are marked. Certain of the changes, as the enormous dilatation of the stomach, must be largely due to mechanical causes; other alterations, as in the formation of villi and the differentiation of the epithelial cells in the upper parts of the small intestine, must be accounted for by the natural processes of development of the embryo, aided or hastened possibly by the early functional requirements of the organs.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Transverse section of a loop of the small intestine in an 8.5-mm. opossum embryo. Wistar series 16177, section 555. The section shows the relation of the large sinus-like blood-vessels to the intestinal mucosa. A diverticulum of the epithelium is seen in the lower right portion of the section. $\times 125$.

2 Wax-plate reconstruction of a segment of the small intestine in an 8.5-mm. opossum embryo. Wistar series 16177, sections 553-576. The model shows a freely anastomosing plexus of blood-vessels closely pressed upon the mucosa. $\times 125$.

3 Outline drawing of the viscera in an opossum embryo a few hours before birth to show the positions of the sections shown in figures 11, 12, and 13. From embryo no. 154. $\times 18$.

4 Outline drawing of the viscera in a young opossum about three days old to show the positions of the sections shown in figures 14, 15, and 16. From pouch-young no. 156. $\times 18$.

PLATE 2

EXPLANATION OF FIGURES

5 Dissection of an 11.75-mm. opossum embryo to show the positions and relations of the viscera in the body. The stomach and intestines of this embryo—one a few hours before birth—are shown in figures 3, 7, and 9. These organs were then embedded and sectioned. Embryo no. 154. $\times 12$.

5

PLATE 3

EXPLANATION OF FIGURE

6 Dissection of an 11.75-mm. pouch-young of the opossum. This specimen is about three days old. The greatly distended stomach has crowded the intestines toward the right. The stomach and intestines are shown again in figures 4, 8, and 10. Photographs of sections of the same organs are shown in figures 14 to 20. Pouch-young no. 156. $\times 12$.

PLATE 4

EXPLANATION OF FIGURES

7 Dissection of the stomach and intestines from an 11.75-mm. opossum embryo. Ventral view of the organs from the specimen shown in figure 5. From embryo no. 154. $\times 18$.

8 Dissection of the stomach and intestines from an 11.75-mm. pouch-young of the opossum. Ventral view of the organs from the specimen shown in figure 6. From pouch-young no. 156. $\times 18$.

9 Same organs as shown in figure 7 oriented to show the form of the stomach. From embryo no. 154. $\times 18$.

10 Same organs as shown in figure 8 oriented to show the form of the stomach. From pouch-young no. 156. $\times 18$.

PLATE 5

EXPLANATION OF FIGURES

11 Photograph of a section of the stomach of the late opossum embryo. The position of this section and those shown in figures 12 and 13 are indicated in figure 3. From section 89 of embryo no. 154. $\times 25$.

12 Photograph of section 203. From embryo no. 154. $\times 25$.

13 Photograph of limb 5 in section 254 of the small intestine. From embryo no. 154. $\times 100$.

14 Photograph of section 128 of the early pouch-young. The positions of the sections in the series are indicated in figure 4. From pouch-young no. 156. $\times 20$.

15 Photograph of section 195. From pouch-young no. 156. $\times 20$.

PLATE 6

EXPLANATION OF FIGURES

Photographs of sections passing through the stomach and intestines of a pouch-young of the opossum. From pouch-young no. 156.

16 Section 295. $\times 20$.

17 Limb 5 of the small intestine in section 195 (fig. 15). $\times 100$.

18 From limb 4 of the small intestine in section 295 (fig. 16). Some of the cells of the villi are largely filled with a non-staining substance. A group of darkly stained and smaller cells is also shown between the villi. $\times 400$.

19 Limb 7 of the small intestine in section 295 (fig. 16). The prominent granular masses in the epithelial cells are here shown in a tangential section of the villi. $\times 400$.

20 Villus from limb 1 of the small intestine in section 128 (fig. 14). $\times 400$.