A Morphological Study of the Development of the Human Liver

I. DEVELOPMENT OF THE HEPATIC DIVERTICULUM [1]

CHARLES B. SEVERN [2]
Department of Anatomy, University of Michigan, Ann Arbor, Michigan

ABSTRACT The development of the hepatic diverticulum was examined in 38 human embryos representing somite stages 1, 5, 8 and 10 through 29, inclusive. Interpretations were based on light microscopic study of serial sections of these embryos.

The liver primordium was first identified in a five-somite embryo as a flat plate of endodermal cells continuous with, but lying ventral to, the endoderm of the foregut at the anterior intestinal portal. It is positioned caudal and ventral to the developing heart. This plate of endoderm subsequently undergoes a progressive folding due to differential growth of adjacent structures. During the folding process there is a close spatial relationship between the cells of the endodermal plate and the caudal and ventral endothelial lining of the atrium and the sinus venosus. The result of this folding is the establishment of a "T-shaped" diverticulum which projects ventrally and cephalically from the gut tract. The hepatic diverticulum is established by the 20 somite-stage embryo. This mode of development of the hepatic diverticulum is compared to the classical interpretation and to the development of other visceral organs.

The lack of an extensive sequential series of human embryonic material has prevented past investigators from obtaining anything more than general and rather vague concepts as to how the human liver develops. Due to this scarcity of material, most of the descriptions were based on observations of few embryos and represented a limited series of developmental stages. Extrapolations and interpolations of the entire development of the liver were made from the stages available.

The classical description of the development of the liver was written by Lewis ('12), who reviewed the observations made by previous authors. From this review it appears that Thompson ('08), in his description of a 2.5-mm embryo, coined the term "outgrowth" for hepatic diverticulum.

From 1912 to the present several studies have been made concerning the development of the human liver. They include those by Hammar ('26), Bloom ('26), Horstmann ('39), and Elias ('49, '52, '53, '55, '57, '64, '67). These authors have been primarily concerned with the development of the intrahepatic duct system, correlation of the liver's developmental pattern with its definitive architectural pattern, and comparison of the origin and development of the liver in various species of vertebrates. Whereas earlier works on liver development were somewhat imprecise, these later investigations were quite specific in nature and the resulting interpretations yielded conflicting points of view. With the exception of Hammar, who used 88 human embryos ranging from 3.0 mm to 73.5 mm in crown-rump length (CRL) the material used covered a broad spectrum of developmental stages but the number of specimens were few. Bloom described six embryos ranging from 10 mm to 40 mm CRL in size. Horstmann used nine embryos ranging from 16 mm to 330 mm CRL. Elias's study ('55) consisted of seven human embryos.

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[1] This work was supported by the National Institutes of Health, Training grant GM312.

[2] Present address: University of Nebraska College of Medicine, Department of Anatomy, Omaha, Nebraska.

To my knowledge, no attempt has been made to trace the liver back to its primordial stage, nor has anyone traced the morphological development of the human liver from the primordial stage to its adult configuration.

According to current accounts the liver develops as a ventral "outgrowth" of the gut endoderm in the region of the anterior intestinal portal (Arey, '65; Davies, '63; Hamilton et al., '62; Patten, '68; Du Bois, '63). The details of this process have not been described. The availability of an extensive series of human embryos made possible this study of the morphological events involved in formation of the hepatic diverticulum.

MATERIALS AND METHODS

This investigation was based on light microscopic examination of serial sections of 38 human embryos in the Patten Embryology Collection of the Department of Anatomy, The University of Michigan, Ann Arbor, Michigan, and from the collection in the Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland. In table 1 are listed for all embryos the source, collection number, horizon stage, crown-rump length and number of somites, plane of section, section thickness and stain used. Streeter's ('42) criteria based on both external form and internal structure for categorizing embryos into developmental age groups or "horizons" were used when possible to determine the developmental stages of the embryos. Embryos which fell within Horizons IX through XII were classified more specifically according to somite stages. When more than one embryo was available at a developmental stage, the embryos were cross-checked for morphological similarities and/or differences within that stage and also compared to the preceding and following stages. This allowed for a more accurate placement of embryos in a developmental sequence.

The morphological relations of the developing gut tract, heart, regional blood vessels, septum transversum and liver complex were observed. Photomicrographs were taken for clarification and substantiation of some of the observations. Plaster models of the foregut and hepatic diverticulum of embryos of 17 and 23 somites were made by an inverse wax-plate method.

Although the observations to be reported begin with the youngest embryo in the series, the actual studies started with embryos of Horizon XIII, in which the liver and its associated extrahepatic duct system were identifiable. Morphological development was then traced backward, chronologically, to the stage where the liver primordium could not be identified as a specific morphological structure. Then the morphological changes were studied in detail starting with this youngest embryo (one-somite, 1.5 mm, Horizon IX) and progressed throughout the series of specimens.

OBSERVATIONS

Although the hepatic primordium could not be specifically identified in the 1.5 mm embryo, the observations to be presented begin with a description of this embryo as a reference for further description of the hepatic diverticulum in succeeding embryos. It was also necessary to include in the observations of the different staged embryos, descriptions of the configuration of the foregut, heart, and septum transversum as important interrelationships appeared between the development of these structures and of the hepatic diverticulum.

Horizon IX

One-somite embryos (1.5 mm CRL). This embryo consisted of a flattened, elongated disc in which all three germ layers were present (figs. 1–4). The germ layers were distinguishable only by their position and relationship to one another, since the cells of these layers were not significantly different from one another.

The ectoderm of this embryo had the form of a thick middorsal plate of cells that was grooved longitudinally on its dorsal surface. This dorsal plate was identified as the neural plate. The presence of the dorsal groove was characteristic of this horizon. Ventral and contiguous to the neural plate was a layer of endoderm, two to three cells thick, which extended laterally as the single-cell layered yolk sac. Lateral to the midline zone of contiguity between endoderm and ectoderm, these two germ layers were separated from each

TABLE 1

Human embryos studied

Embryo no. [1]	Horizon	CRL (mm)/ No. of somites	Sections: plane [2]/ thickness (μ)	Stain [3]
C-5080	IX	1.5/1	T/10	AlC
C-2795	X	2.0/5	T/6	AlC-OG
C- 391	X	2.0/8	T/10	AlC
C-5074	X	3.3/10	T/10	HE
C-3710	X	3.6/11	T/10	
C-8970	X	/12	T/5	HE
C-6344	XI	2.5/13	T/6	AlC
C-4529	XI	2.4/14	T/10	AlC-OG
C-8962	XI	1.5/15	T/	
C-7611	XI	2.4/16	T/8	HE
C-6784	XI	5.0/17	T/6	H and OG
C-8116	XI	/17	S/8	A
C- 164	XI	3.5/18	T/20	AlC
C-6050	XI	3.0/19	F/10	AlC
C-2053	XI	3.0/20	T/10	AlC
C- 486	XII	/21	T/10	AlC
C-8943	XII	3.9/22	T/8	HE
C-4784	XII	3.0/23	T/10	
C-8505b	XII	3.7/23	S/8	A
C-8505a	XII	3.6/24	T/8	HE
C-7852	XII	3.6/25	T/10	HE
C-8942	XII	3.4/26	F/5	HE
C-4736	XII	3.0/26	T/10	AlC
C-6144	XII	3.3/27	T/10	AlC
C-7999	XII	3.3/28	T/10	HE
C-8941	XII	4.9/28	T–F/6	HE
C-5923	XII	4.0/28	T/10	AlC
C-7724	XII	3.5/29	S/8	HE
C-1062	XII	4.5/29	T/20	AlC
P- 24	X	2.1/12	T/10	HE
P- 71	X	2.5/6	T/10	HE
P- 8	XI	3.0/	T/5	CB
P- 195	XI	3.5/	T/10	HE
P- 44	XII	3.5/	T/10	HE
P- 520	XII	4.0/	T/15	M
P- 36	XII	4.5/28	T/10	HE
P- 555	XII	3.0	T/10	HE
P- 483	XII	4.5	T/10	HE

[1] C, Carnegie collection; P, Patten collection.
[2] T, transverse; F, frontal; S, sagittal.
[3] AlC, Alum cochineal; OG, orange G; HE, hematoxylin and eosin; A, azan; Co, carmine and blue; M, masson.

other by a layer of mesodermal cells. The layer of intervening mesoderm was two to five cells in thickness. In a caudal segment of the embryo (region of the somite) the mesoderm was present in its typical form and consisted of a medial mass, a narrow intermediate portion and a lateral mass which was divided into splanchnic and somatic layers (fig. 4). The space between splanchnic and somatic layers was presumptive coelom. In this region of the embryo the coelomic spaces were at the lateral borders of the embryonic disc; the mesoderm was continuous with the extraembryonic mesoderm of the amnion and yolk sac.

The mesoderm which at the level of the somite was lateral to the midline juncture of endoderm and ectoderm, continued across the midline and separated the endoderm from the ectoderm at the cephalic end of the embryo (fig. 3). At this level the endodermal layer was about eight cells thick. The cephalic end of the zone of contact of endoderm and ectoderm (immediately caudal to their separation by intervening mesoderm) probably marked the site of the future stomodeum. For descriptive purposes this site of the future stomodeum was referred to as point a. Rostral to this level the dorsal ectoderm consisted of a single layer of cells which was identified as amnion. The endoderm formed a midline plate of two to three cells in thickness (fig. 2). The lateral extent of this endodermal plate paralled the medial borders of the coelomic spaces. The coelomic spaces appeared to approach each other in the midline at a still more rostral level. Just caudal to the level at which these coelomic spaces approximated each other and became a single cavity (pericardial coelom), the endoderm was a single cell layer and was identified as yolk sac. Again, for purposes of description, this anterior point at which the thick two- to three-cell endodermal plate was continuous with the single cell layered yolk sac was designated as point b. The thickened plate of endoderm between points a and b was rostral to the body of the embryo, and represented the entire foregut primordium. The primordial heart was also rostral to the body of the embryo and dorsal to the endoderm between points a and b (fig. 1). In still more rostral sections the coelomic spaces were absent. All that remained was a single cell layer of ectoderm (amnion) and a single cell layer of endoderm (yolk-sac); between the two layers was interposed a two- to three-cell thick layer of mesoderm.

Horizon X

Five-somite embryo (2 mm CRL). In the one-somite embryo the plate of endoderm between points a and b was entirely cephalic to point a; whereas, in the five-somite embryo it lay entirely caudal to point a (fig. 5). In the region of the stomodeum of this older embryo (fig. 6), points a and b appeared at the same transverse level and the intervening endoderm was dorsal, caudal and ventral to the developing heart, pericardium and septum transversum. The endoderm dorsal to the developing heart formed the tubular foregut, which extended from a point a, caudally, to the caudal level of the developing heart. The caudal extent of the endodermal tube was identified as the anterior intestinal portal. The endoderm ventral and cranial to the anterior intestinal portal was in the form of a flat plate. The lateral extent of the plate corresponded to the lateral extent of the pericardial cavity.

The developing pericardium and heart, which were rostral to the stomodeum in the one-somite embryo, were present at the level of, and caudal to, the stomodeum in the five-somite embryo. The lateral body wall had grown laterally and ventrally and enclosed a major portion of the pericardium and heart. The lateral edges of the pericardial mesoderm approximated each other in the midline ventrally and a space (pericardial coelom) was present. Parietal pericardial and epimyocardial layers had formed. An endothelial tube one cell in thickness surrounded by a sheath of cardiac jelly was inside the epimyocardium. Where the parietal pericardia were in contact in the midline there was a thickening of mesodermal cells that separated the endodermal plate from the epimyocardium and its endothelial lining. This mesoderm was continuous with the mesoderm of the parietal pericardium and the mesoderm covering the amnion and yolk sac. Because of its position and its relation to adjacent cellular layers, this mesodermal thickening was interpreted as the developing septum transversum. Caudal to this level, neither the epimyocardia nor the parietal pericardia had fused; they were lateral to the sagittal plane. At this level the endothelium of the cardiac primordia was present as two tubes whose medial walls were in juxtaposition.

At a level caudal to that just described, the two endothelial tubes were not in contact with each other, but projected laterally. In this region the endothelial tubes appeared to straddle the cephalic surface of the endodermal plate. The cells of the endodermal plate appeared to be in direct contact with the cells of the medial walls of the endothelial tubes. Laterally, the endothelial tubes were small in diameter and appeared to be continuous with the developing capillary bed of the yolk sac. The endodermal plate was wider in this area of contact. The area at which the endodermal plate was in direct contact with the endothelial lining of the developing heart (fig. 7) was designated as point c. Just dorsal to point c foregut and endodermal plate were continuous (anterior intestinal portal) (fig. 8).

In the five-somite embryo cells of the tubular foregut and those of the endodermal plate were cytologically similar. The plate was from two- to three-cells thick. These cells possessed little cytoplasm and their tightly packed nuclei were large and spherical to ovoid in shape. The nucleoplasm was granular and contained darkly staining chromatin bodies.

Eight-somite embryo (2 mm CRL). The foregut was more prominent than in the five-somite embryo and was flattened dorsoventrally. Its ventral and lateral walls were thicker than its dorsal wall and were three- to four-cells thick, the nuclei of these cells being compactly arranged and oval in shape. The dorsal wall was a single layer of cells possessing large, spherical nuclei.

The endodermal plate, which was continuous with the foregut, projected ventrally and cephalically from the anterior intestinal portal. It was morphologically and cytologically similar to the ventral and lateral walls of the foregut. The most anterior extent of the endodermal plate (point b) appeared at the level at which the epimyocardium, parietal pericardium and septum transversum were continuous with one another (fig. 11). In the eight-somite embryo point b was no longer at the stomodeal level (fig. 9) as it was in the five-somite embryo, but was at a more caudal level where the ventricular portion of the heart was continuous with the developing atrium.

Just caudal to the level of the stomodeum (fig. 10), the developing heart was a distinct tubular structure situated within a well-defined pericardial cavity. It consisted of two layers, an inner endothelial lining surrounded by an epimyocardial layer. The latter was continuous with the parietal pericardium by a middorsal attachment (dorsal mesocardium). At this level there were also indications of the septum transversum. This septum consisted of loosely arranged mesodermal cells (3- to 4-cells thick) which were located between the parietal pericardium and the yolk sac endoderm. These cells of the septum transversum were continuous laterally and ventrally with the somatic and splanchnic mesoderm covering the amnion and yolk sac.

At a more caudal level (level of the anterior intestinal portal) the parietal pericardia and epimyocardia of opposite sides were no longer in contact with each other in the midline (fig. 12). The thick epimyocardial layer only vaguely outlined a division of the single tube into a bifid structure. The internal endothelial tube branched to the right and to the left and disappeared laterally into the capillary bed of the yolk sac. The fused medial endothelial wall, representing the fusion of paired atrial primordia at this level in the five-somite embryo, was not present in the eight-somite embryo at any level. There was no septum transversum in the midline and the thick endodermal plate was in direct contact with the ventral and caudal surfaces of the branching endothelial tube. At this point of contact (point c), the endodermal plate was slightly wider than at its cephalic end. Although the thickness of the endodermal plate was the same as that of the ventral and lateral walls of the foregut, the cell boundaries were more distinct in the plate than in the foregut. The nuclei were oval but the chromatin was not as compact and the cells contained more cytoplasm than those of the ventral wall of the foregut.

Ten-somite embryo (3.3 mm CRL). In the ten-somite embryo the neural folds had fused in the somite region and formed a neural tube. The neural tube was covered by dorsal ectoderm of the body wall. The cephalic and caudal ends of the neural tube were open (anterior and posterior neuropores). The foregut had elongated and its cephalic end was still compressed dorsoventrally. The dorsal wall of the compressed foregut was one-cell thick. These cells were clearly definable. Their nuclei were large and separated from each other by distinct cytoplasm. The lateral and ventral walls of the foregut were still two- to three-cells thick, but the cells were no longer cuboidal but columnar instead. They contained more cytoplasm and the nuclei were more spherical than those of corresponding cells in the eight-somite embryo.

At the cephalic end of the embryo the endothelial tube of the developing truncus region of the heart divided on either side of the developing foregut (first aortic arch). There was a ventral projection of the ventral wall of the foregut between the divisions of the endothelial tube which was identified as primordial thyroid. The endodermal cells of this projection appeared to be in contact with the endothelial cells of the truncus.

In this embryo, the heart was still a straight double-walled tube. The outer wall consisted of the epimyocardium and the inner wall the endothelial lining. In the region of the anterior intestinal portal the outer epimyocardia and the parietal pericardia were not fused in the ventral midline and the inner endothelial tube projected to the right and left. At the point of this projection the cells of the endodermal plate were still in contact with the ventral and caudal surfaces of the endothelium.

The endodermal plate (between points b and c), which projected ventrally and more cephalically in the five-somite and eight-somite embryos, was positioned more ventrally and was almost perpendicular to the foregut at the anterior intestinal portal in the ten-somite embryo. The endodermal plate was the same thickness as the ventral and lateral walls of the foregut. The greatest thickness and the widest extent of this endodermal plate occurred along the ventral and caudal surfaces of the endothelium at the point of the caudal fusion of the paired primordia of the heart.

The two endothelium-lined vessels caudal to the point of fusion were positioned laterally at more caudal levels. These were the paired primordia of the sinus venosus. In both the five- and eight-somite embryos these two channels were small and appeared to break up close to the point of fusion and became continuous with the capillary beds of the yolk sac. In the ten-somite embryo, however, these vessels, although they were still continuous with the capillary bed of the yolk sac, were large channels which extended caudally the length of the embryo at the edges of the lateral body wall. There also were connections between these large vessels and small blood vessels which appeared *in situ* in the septum transversum.

Eleven-somite embryo. Increased growth along the longitudinal axis of the embryo resulted in the formation of a head and tail fold. In the 11-somite embryo these folds resulted in a C-shaped configuration of the embryo, with its midventral portion being constricted.

In the 11-somite embryo the septum transversum had increased in volume. This produced a greater separation between the parietal pericardium and the endodermal plate. The increase in mass of the septum transversum was ventral and cephalic to the point at which the endoderm was in direct contact with the endothelium of the developing heart (point c). Due to the increased mass of the septum transversum ventral to point c and to the development of the head and tail folds, the developing atrium appeared to be more dorsally positioned. Despite this, the developing heart still remained a straight tubular structure in this embryo.

The endodermal plate was widest in the region of caudal fusion of the heart. The cells of this portion of the endodermal plate were adjacent to the lining cells of the caudal portion of the atrium and the paired primordia of the sinus venosus.

Summary of observations for Horizon X. By the end of Horizon X, the following morphological structures were established: the foregut; the hepatic primordium (a thick endodermal plate which extended ventrally and cephalically from the anterior intestinal portal, and caudal and ventral to the developing heart); a straight tubular heart consisting of truncus, ventricle and atrium; the first aortic arch and the paired primordia of the sinus venosus; septum transversum; thyroid primordium; and a neural tube, open at cephalic and caudal ends.

Horizon XI

Thirteen-somite embryo (2.5 mm CRL). In the 13-somite embryo, in addition to the greater length of the foregut, its cephalic end had flattened out considerably, being four to five times greater in width than in height (developing pharynx). There was little change in the cytology of its cells, the only noticeable one being an increase in the quantity of cytoplasm in the ventral and lateral walls. This cytoplasm was displaced to the luminal surface of the foregut lining. The middorsal wall of the foregut was flat, as was the ventral surface of the neural tube. These two surfaces were separated by a rod-like structure of columnar cells (notochord) which appeared to be continuous with the endodermal cells of the dorsal wall of the foregut. As in the 10- and 11-somite embryos, the midventral wall of the foregut (thyroid primordium) projected ventrally into the region in which the truncus appeared to divide around the developing pharynx. The endodermal cells appeared to be in contact with the endothelial cells of this bifurcation.

The tubular heart had increased in length and volume and filled the pericardial cavity. The cardiac loop was apparent in this embryo. The ventricle bulged ventrally and caudally filling the right ventral portion of the pericardial sac, then curved to the left and filled the left ventral portion of the pericardial sac. It then turned dorsally and cephalically and was continuous with the atrium which was positioned mid-dorsally in the pericardial sac. The atrium was at the same level as the lowest point of the bulging ventricle. At the level at which the ventricle was continuous with the atrium the septum transversum was thicker. The mass of the septum transversum had increased both ventrally and laterally to point c. The increase appeared to be in a dorsal direction, extending just ventral to the developing atrium, which in this embryo, was more dorsally positioned. This dorsal extent coincided with the point at which the pericardial mesoderm divided to the right and to the left. In the area in which the pericardial mesoderm had not fused in the ventral midline the endothelial lining of the atrium divided into vessels which branched laterally, right and left, representing the area of caudal fusion of the heart. On the caudal and ventral surfaces of this division the endodermal plate was in direct contact with the endothelial lining and there was no intervening mesoderm of the septum transversum. The lateral endodermal cells of the medial walls of the vessels which branched right and left. Dorsal to this zone of contact the endodermal plate was continuous with the ventral and lateral walls of the foregut. In this embryo the endodermal plate was almost perpendicular to the anterior intestinal portal.

Sixteen-somite embryo (2.4 mm CRL). There was an increase in the overall size of the heart, septum transversum, lateral body wall and foregut in the 16-somite embryo. With the increased size of the heart the cardiac loop was more pronounced. The ventricle bulged ventrally and caudally to the atrium. The size of the atrium itself was increased. There was now a definite single sinus venosus which was continuous caudally with paired omphalo-umbilical trunks. These trunks represented the confluence of the primordia of the omphalomesenteric and umbilical vessels. While in the 13-somite embryo the cells of the thick endodermal plate were in direct contact with the endothelial cells of the ventral and caudal walls of the atrium, in the 16-somite embryo the cells of the endodermal plate were in direct contact with the endothelial cells of the ventral and caudal walls of the sinus venosus. No midline septum was present in the sinus venosus.

As previously stated, the size of the lateral body walls increased, thus enclosing more of the lateral and ventral portions of the embryo. The increased mass of the septum transversum was lateral rather than in the midline. The endodermal plate ventral to point c was no longer flat, but rather dome-shaped (fig. 15). At point b it still remained a flat plate (fig. 14). The overall configuration of the thickened endodermal plate was that of a gradual slope (fig. 13). Its ventral and cephalic tip (point b) was cephalic to point c, which was cephalic to the anterior intestinal portal. The caudal position of the anterior intestinal portal relative to point c was suggestive of an increased growth in the caudal end of the foregut and a correlative fixed position of the endoderm at point c.

Seventeen-somite embryo (5 mm CRL). The 17-somite embryo exhibited the first signs of the endodermal plate developing into the hepatic diverticulum. Whereas the endodermal plate between points b and c was dome-shaped in the 16-somite embryo, there were definite bilateral folds of the lateral edges of this endodermal plate in the 17-somite embryo(fig. 17). These folds occurred in the region where the omphalo-umbilical trunks were confluent with the capillary bed of the yolk sac and at which there was an increased amount of mesoderm at the lateral edges of the septum transversum. The ventral and caudal bulge of the ventricle had increased and there had also been an increase in the cellular mass of the septum transversum laterally and in the ventral midline. The midline increase in mass further separated the ventral endodermal plate from the layer of pericardial mesoderm. The endodermal cells were still in contact with the endothelial cells of the sinus venosus and of the omphalo-umbilical trunks. The anterior intestinal portal was caudal to the level of this zone of contact.

Sagittal sections of another 17-somite embryo verified the observations given above of a 17-somite embryo which was sectioned transversely. Furthermore, they brought out other facts for consideration. In the midline, the anterior intestinal portal was caudal to the level of contact between endoderm and endothelium (point c). This caudal shift in the anterior intestinal portal created a fold in the endoderm. The dorsal portion of this fold formed the ventral wall of the caudal portion of the foregut. The ventral portion, between the anterior intestinal portal and point c, formed the dorsal wall of the hepatic diverticulum. The growth of the endoderm appeared to have been between points a and c. Point c appeared to be relatively fixed as the site at which the endoderm was in contact with the caudal endothelial lining of the sinus venosus (fig. 18). In this 17-somite embryo there was also a connection between the endodermal plate and the mesodermal cells of the caudal portion of the pericardium just ventral and dorsal to the sinus venosus. The endoderm appeared continuous with the pericardial mesoderm in these two areas. The rest of the endodermal plate, which projected ventrally from the zone of contact, was separated from the layer of parietal pericardium by cells of the septum transversum. There was a transverse groove in the endodermal plate located at its point of contact with the endothelium of the sinus venosus and the omphalo-umbilical vessels. Laterally the omphalo-umbilical vessels were reduced in size and continuous with the small blood channels of the yolk sac and of the septum transversum. The lateral extremes of the endodermal plate were folded caudally.

In a three-dimensional view (figs. 25–32), the flat endodermal plate seen in younger stages of embryos had begun to take the shape of a hollow "T-shaped" structure. The walls of the right and left extensions of the "T" were somewhat thicker than the remaining walls. There appeared to be more nuclei in these areas; they were eccentrically displaced and grouped peripherally with a relatively large amount of cytoplasm near the lumen.

Nineteen-somite embryo (3.0 mm CRL). The hollow "T-shaped" diverticulum was positively identified in the 19-somite embryo (fig. 19). The thickness of the walls of this diverticulum was similar to that of the walls of the caudal end of the foregut, with which it was continuous. The lateral walls of the cephalic end of the diverticulum were somewhat thicker and appeared to project laterally into the septum transversum.

The only other morphological change to be noted was the development of the omphalomesenteric veins. The medially positioned omphalomesenteric veins and the laterally positioned umbilical veins were confluent with the right and left branches of the sinus venosus. These veins were identified in previous embryos as the omphalo-umbilical trunks. There was still a close relationship between the cephalic end of the diverticulum and the sinus venosus.

Twenty-somite embryo (3.0 mm CRL). A cross sectional view of the ventral diverticulum showed an increased thickness of the septum transversum. The cells of the septum transversum were adjacent to the lateral and ventral walls of the diverticulum. The right and left growth of the cephalic end of the diverticulum and the approximation of these sites of growth to the developing veins were apparent. One could see the continuity between the foregut and the diverticulum (fig. 20).

Summary of observations for Horizon XI. In Horizon XI (embryos having from 13–20 somites) there was further overall growth and development of the embryos as compared to those of the previous horizon. The heart had begun to rotate and had increased in size: the ventricle bulged ventrally and caudally, the atrium was positioned more dorsally and cephalically to the ventricle and the single sinus venosus was established. The omphalomesenteric veins and the umbilical veins were identified as separate vessels. There was an increased development of the septum transversum and of the lateral body wall. The foregut had elongated and was separable into pharyngeal and esophageal regions. In the floor of the pharynx were signs of the developing thyroid. At the caudal end of the foregut the hepatic diverticulum was established.

DISCUSSION

In the present study it would appear that the hepatic diverticulum was formed concurrently with the establishment of the gut tract and that it was established in part by differential growth of the endoderm, in part by differential growth of surrounding structures and in part because of a relatively fixed contact point between a segment of the endoderm and the endothelial lining of the heart.

It was noted in the observations that the endodermal primordium of the foregut and hepatic diverticulum was differentiated from the yolk sac by the thickness of its cell layers. It was distinctly thicker than the single-cell layer of the yolk sac. In tracing this thicker endodermal layer throughout the separate somite-staged embryos it was noted that there were no areas of "isolated" cell proliferation and that this endoderm, although it increased in length, remained rather uniform in thickness until after the establishment of the hepatic diverticulum. In following the morphological configuration of this thicker endodermal segment it was noted that in the one-somite embryo this endodermal segment consisted of a flat plate of cells which projected rostral to the rostral end of the developing embryo. In the five-somite embryo it had a "U-shaped" configuration with the dorsal limb of the "U" forming the ventral and lateral walls of the foregut (fig. 25). For purposes of description points a, b, and c were utilized as reference marks for the hypothetical segmentation of the endoderm. Point a represented the endoderm at the stomodeal plate; b, the point at which the thick endoderm abruptly became the single-cell layer of the amnion; and c the point at which there was contiguity between the endoderm and the endothelium of the developing caudal region of the heart. In older embryos (8-somites) the configuration of the endoderm showed a change in its morphological configuration. Instead of a "U-shaped" configuration the endoderm had a "J-shaped" configuration (fig. 26) which suggested an increase in the length of the thick segment of endoderm. This increase appeared to occur between points a and c, with a developmental lag in the growth of the endoderm between points b and c. The perpendicular position of segment b-c to segment a-c appeared to be due to an increased size and length of the cephalic end of the embryo which resulted in a ventral flexure of the head and an increase in the size and length of the developing heart. In the 16-somite embryo the endodermal plate was no longer flat but rather was related to an increased lateral mass of the septum transversum and to increased foldings of the lateral body wall (fig. 28). There was a continued folding of the endoderm primarily ventral and lateral to point c in the 17-somite embryo, but the most striking change in the configuration of the endoderm was the caudal shift of the anterior intestinal portal relative to point c. Whereas, the anterior intestinal portal was at the same level as point c in the younger embryo; it was caudal to this point in the 17-somite and older embryos. Again this suggested an increase in length of the endoderm between points a and c. This time, however, due to the caudal position of the anterior intestinal portal relative to point c, definite point of fixation was indicated in the area of contiguity between the endoderm and endothelium of the sinus venosus. The consequence of this fixation and the increased length of the endoderm between points a and c resulted in formation of the dorsal wall of the hepatic diverticulum. As indicated in the 19-, 20-, and 23-somite embryos, the endodermal segment between points c and b had folded caudally, ventrally, and laterally to form the ventral and lateral walls of the hepatic diverticulum (figs. 29–32). The folding of this segment of endoderm appeared to be directly related to an increase in mass of the cells in the septum transversum and to an apparent area of fixation point c. The diverticulum was "T-shaped" and suggested a bilobed structure. The bilobed configuration of the hepatic diverticulum was attributed also to the area of contiguity of endoderm to endothelium and not only to the sinus venosus, but also in part to the paired omphalo-umbilical trunks.

The endothelial lining in contact with the endoderm at point c was the caudal and ventral lining of the atrium in embryos up to 13-somite stage. In the 16-somite embryo the endothelial lining in contact with the endoderm was that of the caudal and ventral surfaces of the sinus venosus. No further fusion of the endothelial lining of the developing heart was observed in any of the embryos older than five somites. This perhaps implies that the basic tubular structure of the heart, formed from a midventral fusion of paired primordia, occurs prior to the eight-somite stage and that further growth of the tubular heart and the development of the sinus venosus from a bifid to a single structure occurs cephalad to the zone of contact between endothelium and endoderm.

The area of endothelial-endodermal contiguity (point c) might have the following significance. If this is a point of true fixation one might expect to find some type of malformation that would indicate this. In subsequent studies two such abnormalities were noted. The first of these was an endodermal cyst located on the wall of the sinus venosus of a 15 mm pig embryo (fig. 22). One can assume that an area of contiguity must have existed between the endoderm and the endothelial lining of the developing heart at some stage of development and that, instead of the endoderm detaching as it normally does after establishment of the hepatic diverticulum, this area of contact persisted and a segment of the hepatic diverticulum was broken off and carried along with the dorsally and cephalically migrating sinus venosus.

If one speculates as to what might happen if the paired cardiac primordia failed to fuse in the ventral midline thus allowing no point of contiguity between the endoderm and endothelium either at its cephalic or caudal end, one would expect that neither a thyroid nor a liver would develop. One would also suspect that the foregut would probably not develop. In two cases of acardia amorphus recently examined this was found to be the case. In neither instance was there a fused tubular heart nor any evidence of a liver, diaphragm, thyroid, lung, or esophagus (figs. 23, 24).

A further observation concerning the relationship of the development of the hepatic diverticulum and the development of the septum transversum and the pericardium merits attention. In the five-somite embryo the ventral segment of the thick endoderm (point b) was found at the same level as the point of fusion of the pericardium in the ventral midline. In embryos of 8, 10, 13, and 16 somites there was a shift in the point of caudal closure of the pericardial sac relative to point b. It appeared to move dorsally in relation to the ventral end of the endodermal plate, toward the zone of contact between endoderm and the endothelial lining of the atrium (point c). If one considers the relationships between the endodermal plate, the fusion of the pericardium and the extent of the septum transversum at point b in the five-somite embryo and these same relationships in the 16-somite embryo, it would appear that, at least in part, the development of the septum transversum was dependent on the development of the pericardium. In the same manner, growth of the endodermal plate between points b and c may be dependent to some extent on closure of the pericardium and/or the development of the septum transversum.

The observations and discussion presented in this study are contrary to the theory (which originated from Thompson's interpretation in 1908) that the hepatic diverticulum is formed by an "outgrowth" of the gut tract. It has already been noted that there were no notable localizations of marked (cellular) proliferations until after the establishment of the hepatic diverticulum. In the 25-somite embryo a localized proliferation of cells occurred at the most caudal end of the ventral wall of the diverticulum (point b). This localized proliferation created a bulge in the ventral wall of the diverticulum and was found in older embryos to be the developing cystic duct-gallbladder. In contrast to the mode of formation of the hepatic diverticulum, the formation of the cystic duct-gallbladder *was* an "outgrowth" typical of the primordia of the pancreas, parotid, submandibular gland and lung. It was not until after the establishment of the bulge of the cystic duct-gallbladder that the thickened endoderm continued caudal development to the ventral walls of the ductus choledochus and midgut. This would suggest that although points a and b were hypothetical points of reference it is quite possible that the endoderm which formed the foregut and the hepatic diverticulum was established as early as the one-somite embryo or perhaps even earlier.

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PLATE 1

EXPLANATION OF FIGURES

Schematic diagram and photomicrographs of a one-somite human embryo. Embryo 5080, Horizon IX. Photomicrographs are by courtesy of the Carnegie Institution of Washington.

1 Diagram of a sagittal section showing the respective levels at which figures 2, 3 and 4 were taken. The endoderm is represented by the solid black area, neural ectoderm by the stippled area and mesoderm by the white area. Point a represents the area of the presumptive stomodeum. Point b represents the point at which the thickened endoderm is continuous with the single-cell layered yolk sac. (H) indicates presumptive heart.

2 Photomicrograph of a transverse section immediately caudal to the level at which the thickened endoderm (E) is continuous with the yolk sac. $\times$ 75.

3 Photomicrograph of a transverse section through the presumptive stomodeum. Note the mesoderm (M) extending across the midline and separating endoderm from ectoderm. $\times$ 75.

4 Photomicrograph of a transverse section through the somite region. Note the contiguity of cells of the ventral neural plate with the dorsal archenteron (arrow), and also the position of the somite mesoderm. $\times$ 75.

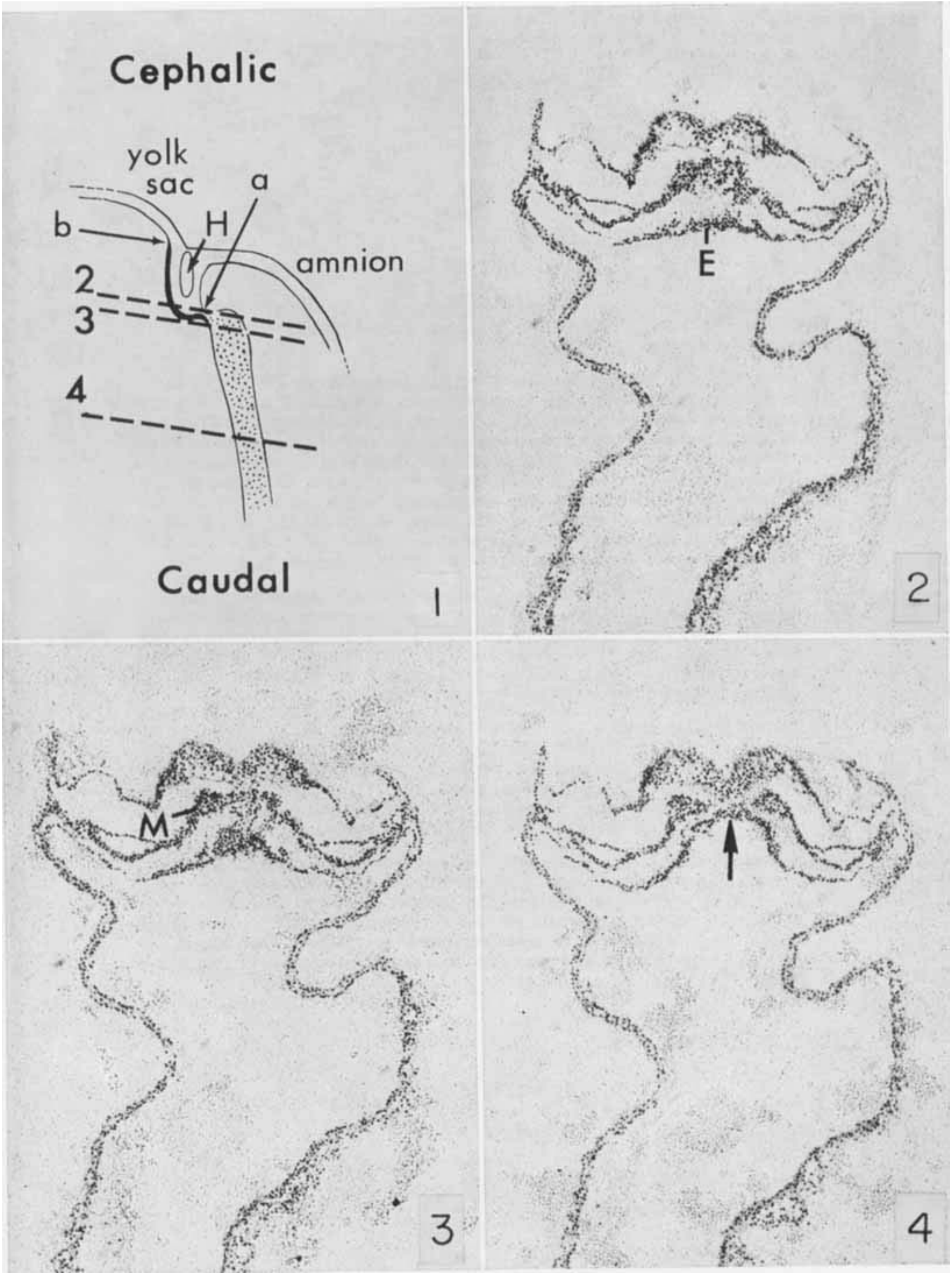

Cephalic
yolk
sac
a
b
H
amnion
2
3
4
Caudal
1
E
2
M
3
4

PLATE 2

EXPLANATION OF FIGURES

Schematic drawing and photomicrographs of a five somite human embryo. Embryo 2795, Horizon X. Photomicrographs are by courtesy of the Carnegie Institution of Washington.

5 Diagram of a sagittal section showing the configuration of thickened endoderm (dark area) in relationship to the neural plate (stippled area) and developing heart (H). Observe the "U-shaped" configuration of the endoderm. Point a represents the presumptive stomodeum. Point b represents the site at which the thick endoderm is continuous with the single-cell layered yolk sac. Point c represents the area of contiguity of endoderm and the endothelial lining of the heart. AIP (anterior intestinal portal) is the point at which the flat plate of endoderm is continuous with the ventral and lateral walls of the foregut. Transverse dashed lines represent levels of figures 6, 7, and 8.

6 Photomicrograph of a transverse section through the stomodeum. Observe the endothelial lining of the truncus arteriosus adjacent to the foregut (F), the fusion of the parietal pericardia in the ventral midline (arrow), and the mesodermal cells continuous with the somatic and splanchnic layers of mesoderm separating the thickened endoderm (E) from the parietal pericardium. $\times$ 75.

7 Photomicrograph of a transverse section through the caudal level of the developing heart. Observe the fusion of the medial walls of the endothelial lining of the paired atria (A) and the lateral displacement of the epimyocardia and parietal pericardia (P). Also observe the contiguity of the thickened flat plate of endoderm with the caudal and ventral endothelial lining of the fused atria (arrow). $\times$ 75.

8 Photomicrograph of a transverse section through the anterior intestinal portal. Observe the continuity of the thickened endodermal plate with the ventral and lateral walls of the foregut (arrow). Also observe the paired endothelial primordia of the sinus venosus (SV) immediately lateral and ventral to the anterior intestinal portal. The paired endothelial primordia of the sinus venosus are continuous with the blood island of the yolk sac. $\times$ 75.

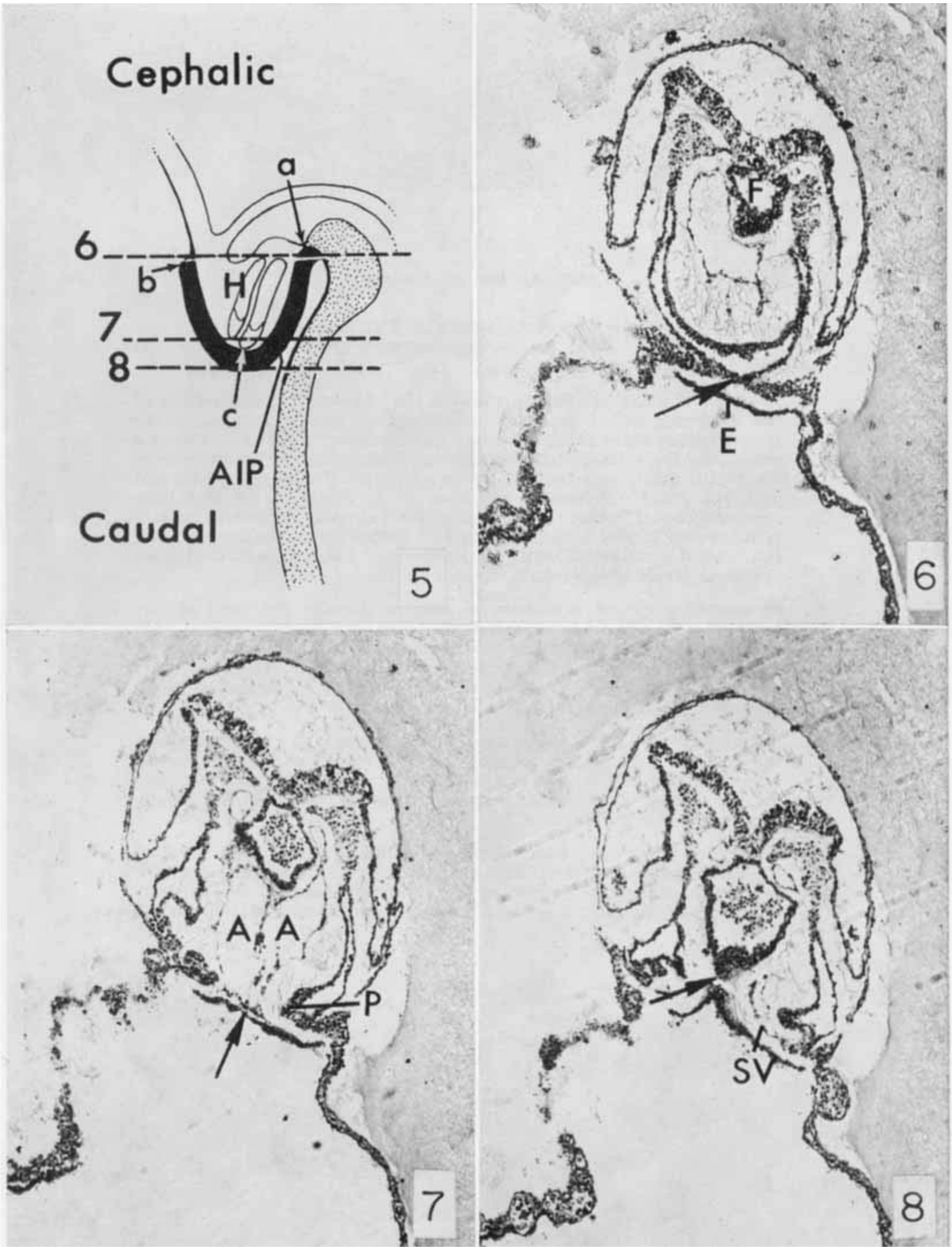

Cephalic
a
6
b
H
7
8
c
AIP
Caudal
5
F
E
6
A
A
P
7
SV
8

PLATE 3

EXPLANATION OF FIGURES

Schematic diagram and photomicrographs of an eight-somite human embyro. Embryo 391, Horizon X. Photomicrographs are by courtesy of the Carnegie Institution of Washington.

9 Diagram of a sagittal section showing the "J-shaped" configuration of the thickened endoderm (dark area) and its relationship to the adjacent neural plate (stippled area) and the developing heart. Point a represents the presumptive stomodeum. Point b represents the site at which the thick endoderm is continuous with the single-cell layered yolk sac. Point c represents the area of contiguity of the endoderm and endothelial lining of the heart. AIP (anterior intestinal portal) is the point at which the flat plate of endoderm is continuous with the ventral and lateral walls of the foregut. Transverse dashed lines represent levels of figures 10, 11 and 12.

10 Photomicrograph of a transverse section through the level of the ventricle (V). Observe the well-defined pericardial sac (parietal pericardium, epimyocardium and dorsal mesocardium). Also observe the mesodermal cells of the septum transversum (ST) which separate parietal pericardium from the yolk sac. There is no thickened endoderm ventral to the pericardium at this level.

11 Photomicrograph of a transverse section through the level at which the ventricle and atrium are continuous. Observe the continuity between epimyocardium, parietal pericardium and the mesodermal cells of the septum transversum (arrow). Observe the thickened endodermal plate at this level. $\times$ 75.

12 Photomicrograph of a transverse section through the caudal level of the undivided atrium (A). The epimyocardia and parietal pericardia are positioned laterally. Observe the contiguity between the endothelial lining of the atrium and the thickened endodermal plate (arrow). $\times$ 75.

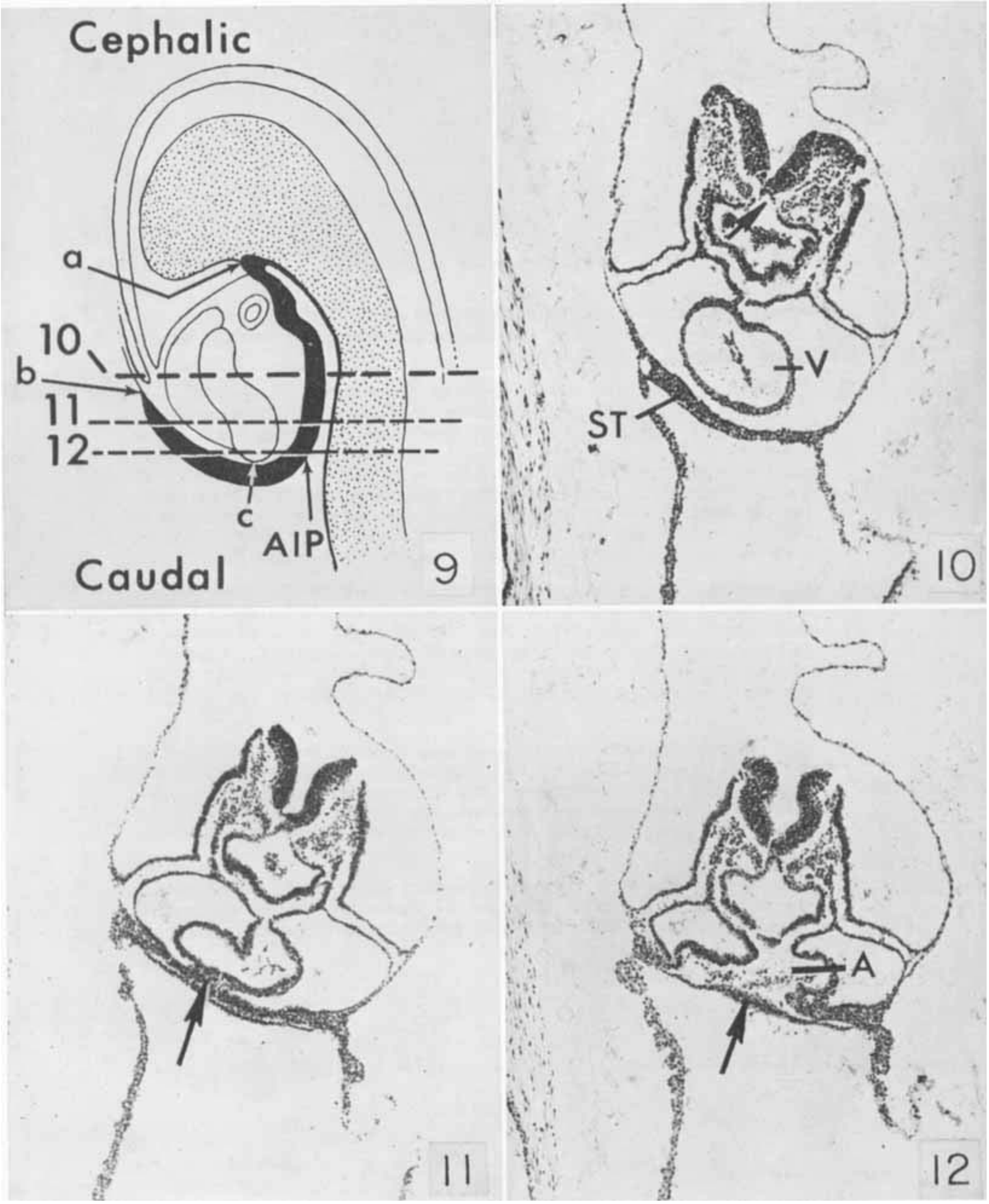

Cephalic
a
10
b
11
12
c
AIP
Caudal
9
V
ST
10
11
A
12

PLATE 4

EXPLANATION OF FIGURES

Schematic diagram and photomicrographs of a 16-somite human embyro. Embryo 7611, Horizon XI. Photomicrographs are by courtesy of the Carnegie Institution of Washington.

13 Diagram of a sagittal section showing the reversed "L-shaped" configuration of the thickened endoderm (dark area) in relationship to the adjacent neural plate (stippled area) and developing heart. Point b represents the site at which the thick endoderm is continuous with the single-cell layered yolk sac. Point c represents the area of contiguity of endoderm and endothelial lining of the heart. AIP (anterior intestinal portal) is the point at which the flat plate of endoderm is continuous with the ventral and lateral walls of the foregut. Transverse dashed lines represent levels of figures 14, 15, and 16.

14 Photomicrograph of a transverse section through the level of the ventricle (V) and atrium (A). At this level the pericardium is well established and is separated from the thickened plate of endoderm (E) by mesodermal cells of the septum transversum (ST). The thickness of the endodermal plate ventral to the heart is approximately the same as that of the ventral and lateral walls of the pharynx. × 75.

15 Photomicrograph of a transverse section through the caudal level of the sinus venosus. Observe the contiguity between the endothelial lining of the sinus venosus and the endodermal plate (arrow). Also observe the mesoderm (M) between the lateral pericardia and the endodermal plate and the dome-shape of the thickened endoderm. × 75.

16 Photomicrograph of a transverse section through the level of the anterior intestinal portal. Observe the approximation of the paired omphalo-umbilical (OU) trunks to the endoderm of the anterior intestinal portal. × 75.

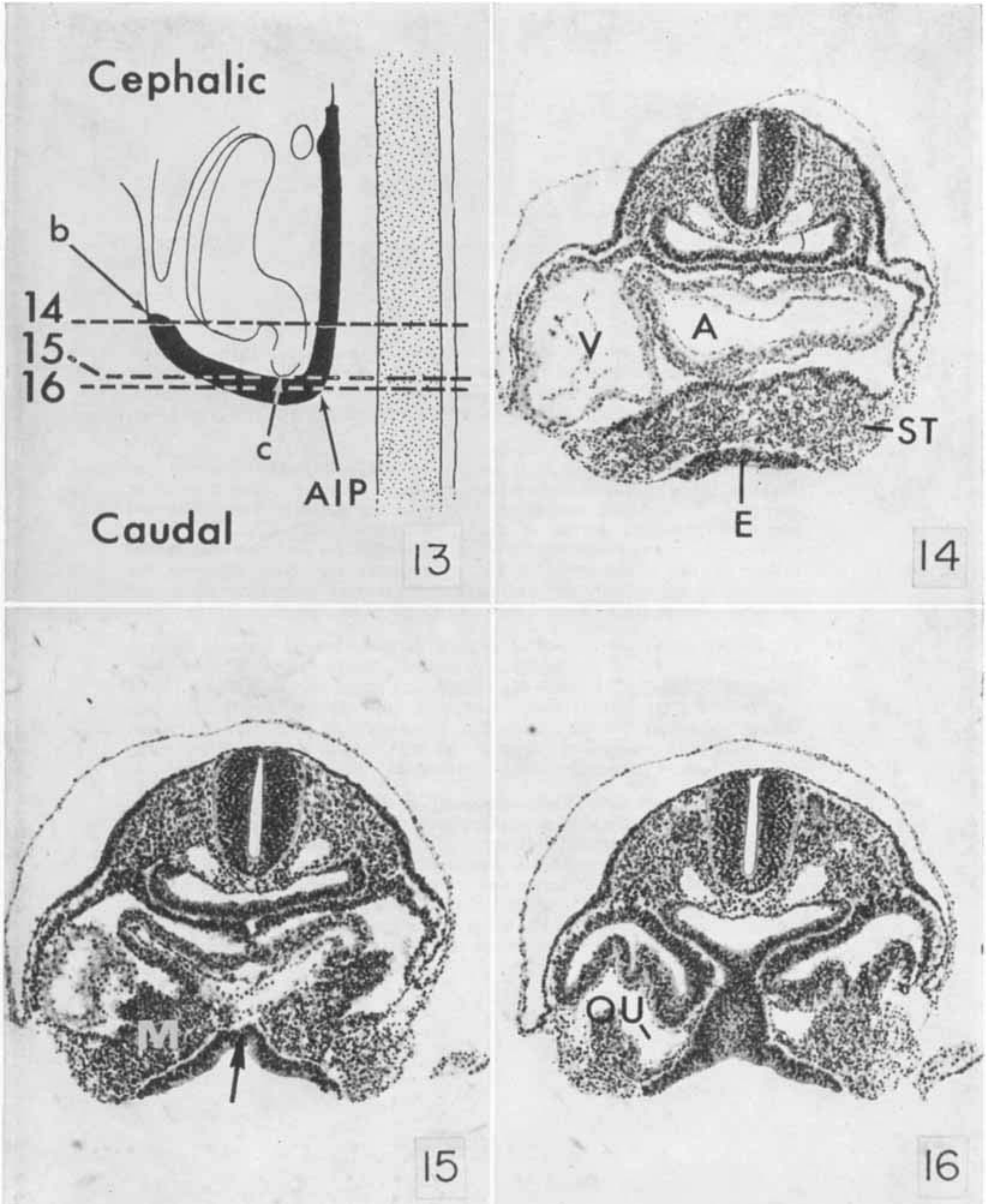

Cephalic
b
14
15
16
c
AIP
Caudal
13
V
A
ST
E
14
M
15
OU
16

PLATE 5

EXPLANATION OF FIGURES

Photomicrographs are by courtesy of the Carnegie Institution of Washington.

17 Photomicrograph of a transverse section of a 17-somite human embryo through the level of the sinus venosus (SV). Observe the mesoderm lateral and dorsal to the thickened endoderm and the ventral folding (arrow) of the lateral edges of this endoderm. Embryo 6784, Horizon XI. × 75.

18 Photomicrograph of a sagittal section of a 17-somite human embryo. Observe the general configuration of the thickened endoderm. That segment of thickened endoderm (between the arrows) ventral to the anterior intestinal portal is the presumptive hepatic diverticulum. Observe the area of contact between the endoderm and the endothelial lining of the sinus venosus (c, as appears in text). Observe the amount of mesoderm separating the thickened endoderm from the parietal pericardium ventral to c. Embryo 8116, Horizon XI. × 75.

19 Photomicrograph of a frontal section of a 19-somite human embryo through the level of the heart and hepatic diverticulum. The diverticulum is a "T-shaped," hollow structure. There is an epithelial projection from its lateral walls. Separate omphalomesenteric and umbilical veins can be identified. The diverticulum is in direct contact with the caudalmost extension of the endothelial lining of the sinus venosus (arrow). Embryo 6050, Horizon XI. × 75.

20 Photomicrograph of a transverse section of a 20-somite human embryo immediately caudal to the level of the anterior intestinal portal. The lumina of the hepatic diverticulum (D) and the foregut (F) are continuous. The diverticulum at this level exhibits a dilation to the right and to the left. Observe the contiguity between the lateral walls of the diverticulum and the medial endothelial linings of the omphalo-umbilical vessels (OU). Also observe the amount of mesoderm of the septum transversum (ST) between the ventral wall of the diverticulum and yolk sac endoderm. Embryo 2053, Horizon XI. × 75.

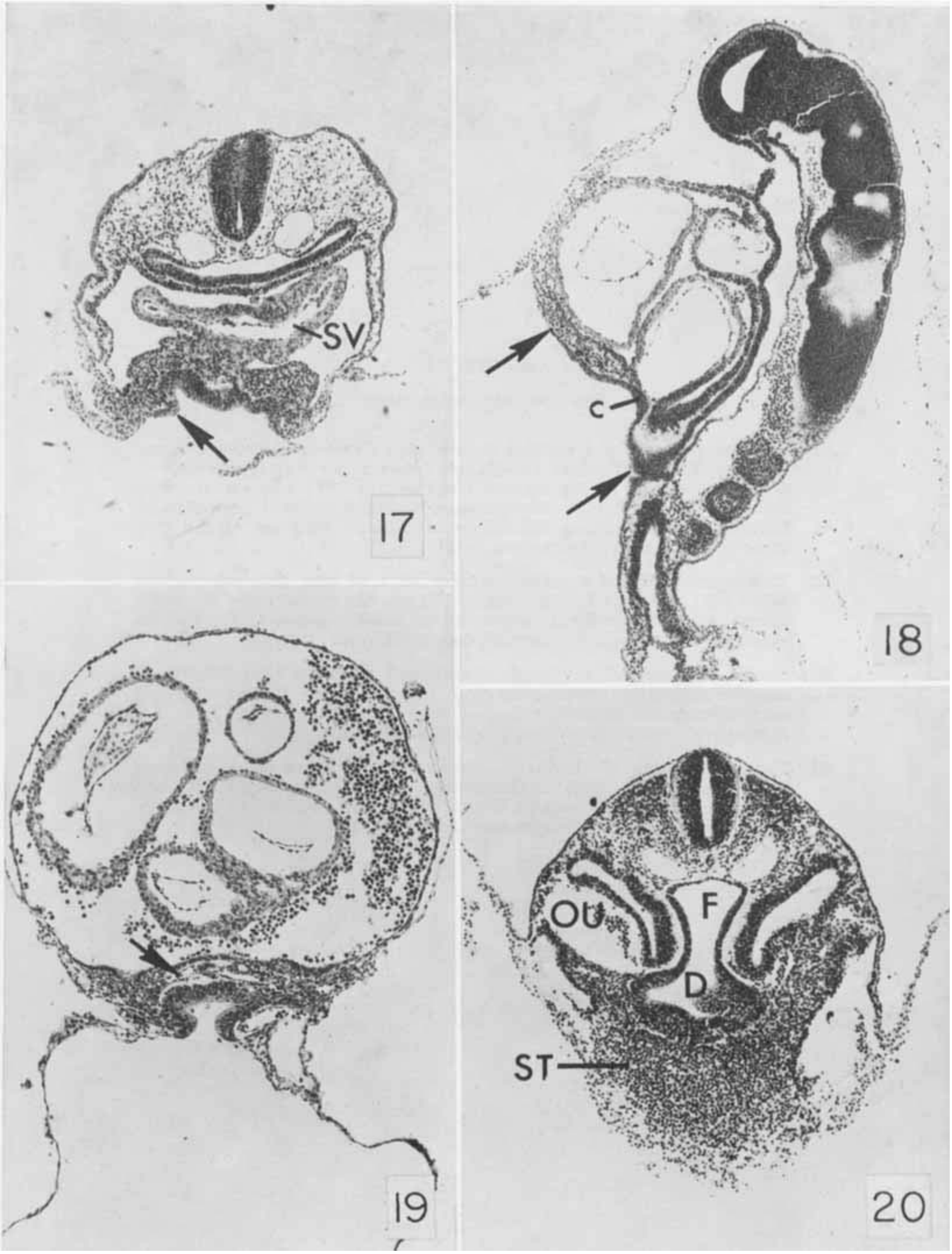

SV
17
c
18
19
OU
F
D
ST
20

PLATE 6

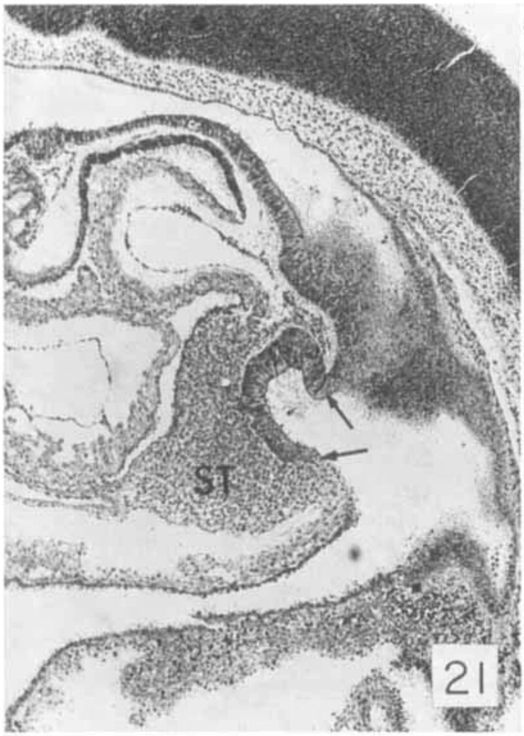
EXPLANATION OF FIGURES

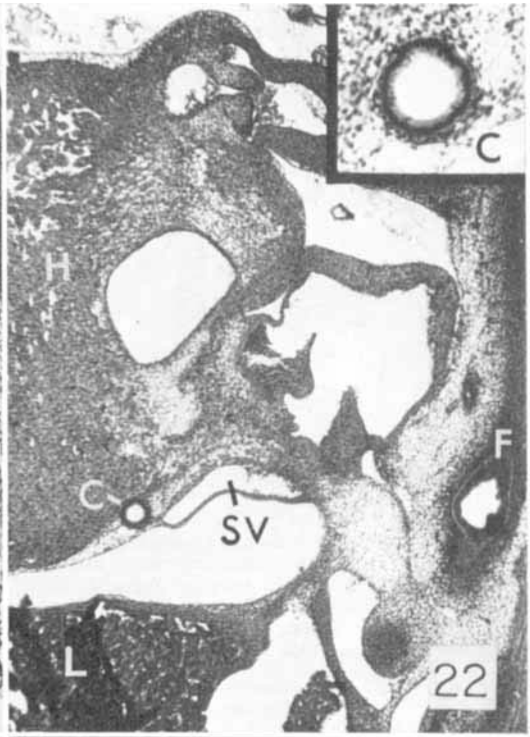
21 Photomicrograph of a sagittal section of a 23-somite human embryo. The hepatic diverticulum (endoderm between arrows) is established. Observe the mass of the septum transversum (ST) between the ventral wall of the hepatic diverticulum and the parietal pericardium. Embryo 8505B, Horizon XII. Photomicrograph courtesy of the Carnegie Institution of Washington. $\times$ 75.

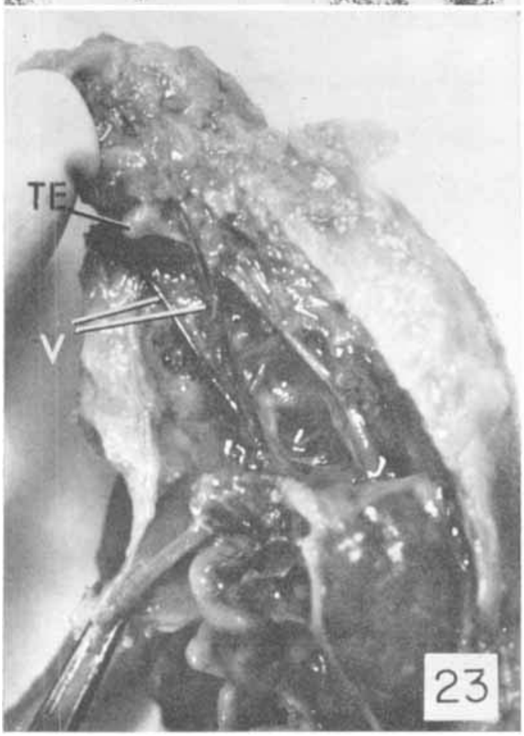
22 Photomicrograph of a sagittal section of a 15 mm pig embryo. The heart (H), Liver (L), and foregut (F) are shown. Observe the endodermal cyst (C) in juxtaposition to the sinus venosus (SV). The insert is a higher magnification of the endodermal cyst.

23 Photomicrograph of the head, thorax and abdomen of a five-month acardia amorphus. Observe the paired vessels in the thorax (V) and also absence of heart, esophagus, lungs, diaphragm and liver. TE represents a rudimentary tracheoesophageal bud.

24 Photomicrograph of the head, thorax and abdomen of a full-term acardia amorphus. Observe the paired vessels in the thorax (V) and absence of heart, esophagus, lungs, diaphragm and liver. TE represents a rudimentary tracheoesophageal bud.


ST
21


C
H
C
SV
F
L
22


TE
V
23

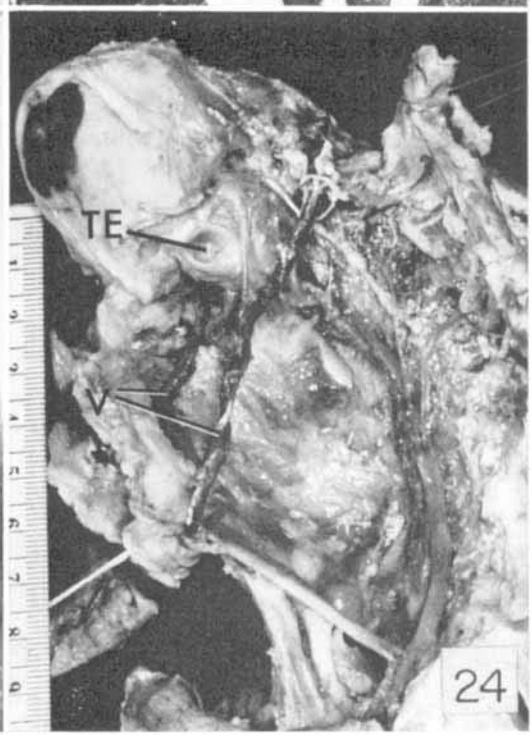

TE
V
24

PLATE 7

EXPLANATION OF FIGURES

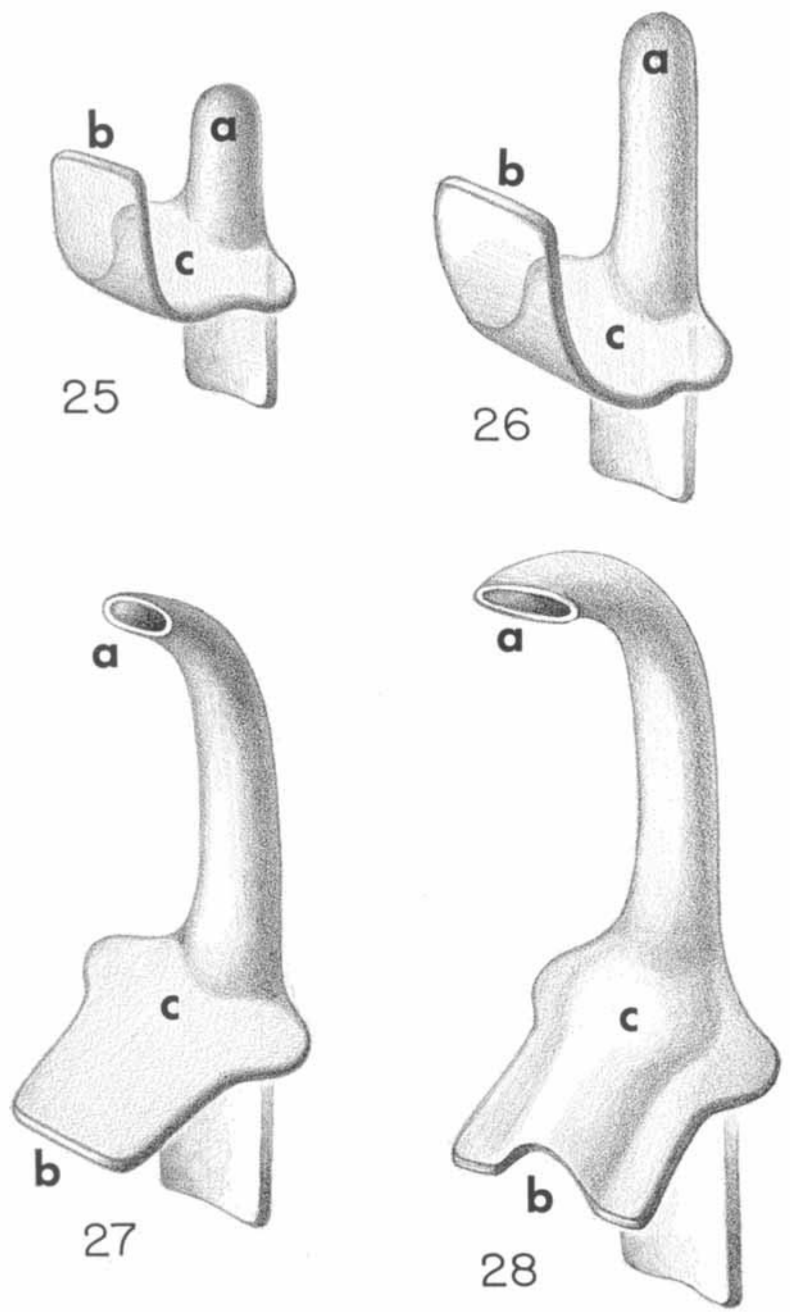
Figures 25 through 28 are three-dimensional drawings of a ventrolateral view of different stages of the developing foregut and hepatic diverticulum made from reconstructions and models. Letter (a) indicates the stomodeal area of the tubular foregut. Letter (b) identifies the leading edge of thickened endoderm where it is continuous with the one-cell layered yolk sac. Letter (c) indicates the zone of contact between the endoderm and the caudal endothelial lining of the developing heart.

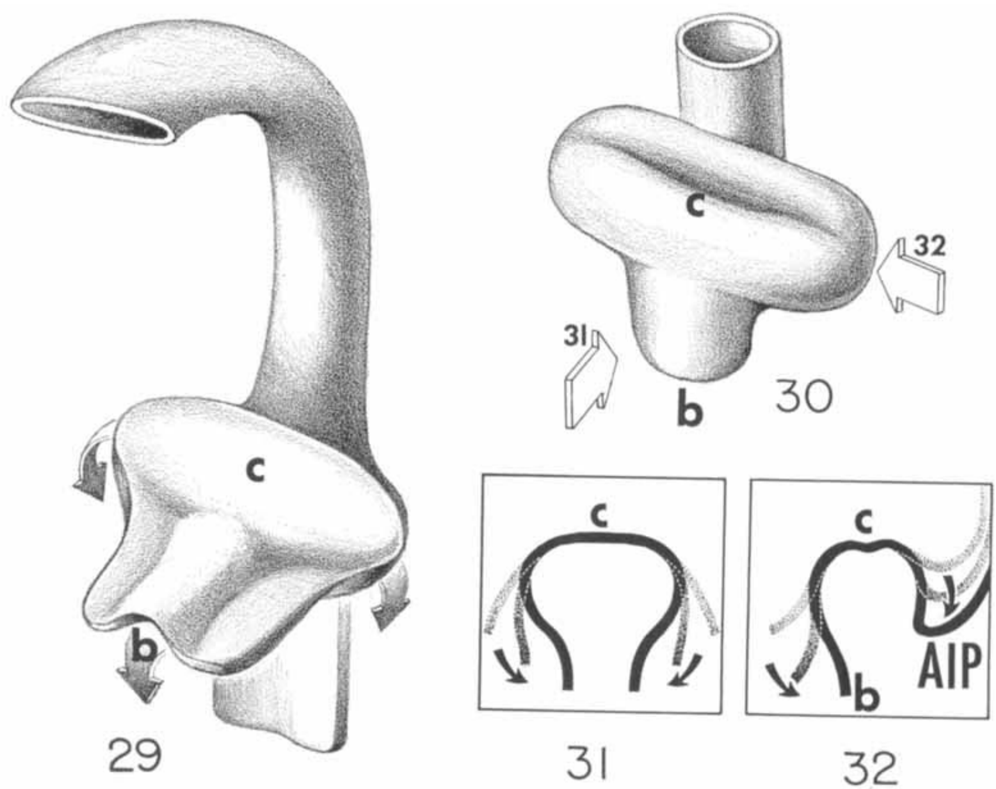
25 Drawing shows the general configuration of the endoderm as it exists in a five-somite human embryo.

26 Drawing shows the general configuration of the endoderm as it exists in an eight-somite human embryo.

27 Drawing shows the general configuration of the endoderm as it exists in a 13-somite human embryo.

28 Drawing shows the general configuration of the endoderm as it exists in a 16-somite human embryo.


b
a
c
25
a
b
c
26
a
c
b
27
a
c
b
28



EXPLANATION OF FIGURES

29 Drawing shows the general configuration of the endoderm as it exists in a 19-somite human embryo. Letter (b) represents the leading edge of thickened endoderm where it is continuous with the one-cell layered yolk sac. Letter (c) represents the zone of contact between the endoderm and the caudal endothelial lining of the developing heart.

30 Drawing shows the general configuration of the endoderm as it exists in a 23-somite human embryo. Letters (b) and (c) have the same representation as in figure 29. Arrows show the directions of the views for figures 31 and 32.

31 Drawing shows a section of figure 30 as viewed from the ventral side. Stippled lines and arrows show the direction of folding of the endoderm. Letter (c) represents the zone of contact between the endoderm and the caudal endothelial lining of the developing heart.

32 Drawing shows a section of figure 30 as viewed from the lateral side. Stippled lines and arrows represent the direction of folding of the endoderm. Letter (c) indicates the zone of contact between the endoderm and the caudal endothelial lining of the developing heart.