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A Human Embryo of Carnegie Stage 12

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= Abstract =**A human embryo obtained from a salpinx removed for the treatment of tubal gestation was serially sectioned and observed. On gross examination, the embryo showed three prominent pharyngeal arches, but not the cervical sinus. The upper arm bud had just begun to appear with a slight elevation in the skin ectoderm. Both the anterior and posterior neuropores had already been closed. The heart was at the cardiac loop stage. The respiratory diverticulum, the dorsal pancreas and the beginning of the omental bursa had appeared. In the pharyngeal region, the adenohypophyseal pocket and the thyroid anlage were observed. The optic evagination showed no regional differentiation yet. From the above findings, we concluded that this embryo belonged to Carnegie stage 12.**

Key Words: *Embryo, Carnegie stage*

INTRODUCTION

For the study of human development, especially during the embryonic period in which the developmental state changes rapidly from time to time, a detailed description of the embryo with time sequence is thought to be elementary and prerequisite. Streeter previously defined twenty-three age groups of human embryos according to the morphological and developmental status of the internal organs, with a time interval of 2-3 days (Streeter 1942, 1945, 1948, 1951). To designate the age groups, he borrowed the term "horizon" used in archaeology and geology, and provided the representative descriptions for horizons XI through XXIII. The concept of Streeter's horizon has been widely accepted by many investigators. In 1987, O'Rahilly and MIler re-examined and modified Streeter's horizons in consideration of Streeter's own and other following investigators' works, and renamed it the "Carnegie staging system" (O'Rahilly and MIler 1987). Thus, it may be preferable to divide the embryonic development of humans into Carnegie stages 1 to 23, instead of Streeter's horizons I to XXIII. Many descriptions and works were done using these classifications of the embryonic period, which enabled comparisons not only of one's work to others, but also between works on humans and those on experimental animals (Butler and Juurlink 1987).

Since the first description of a human embryo using the concept of developmental staging by Chi and Lee(1980) in our country many cases have been reported. However, they are still so few in number that more descriptions of Korean embryos are required.

OM For such reasons, we report here a

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case of an embryo of Carnegie stage 12.

MATERIALS AND METHODS

An embryo was obtained from a resected uterine tube for the treatment of an ectopic pregnancy at a local clinic. The patient was 30 years old. The last menstration began on September 18, 1991, and the operation was done on November 3, 1991. After gross examination, the embryo was fixed in Bouin's solution and embedded in paraplast. A total of 625 horizontal serial sections of 5 μm thickness were made and every fifth section was selected, stained with hematoxylin and eosin, and observed under light microscopy. When necessary, additional sections were also stained and observed.

RESULTS

1. External appearence

The embryo, as a whole, was C-shaped with dorsal convexity(Fig. 1) and the greatest length(GL) was 3.9 mm. The intact umbilical vesicle with a similar volume to the embryo itself was attached to the ventral side of the embryo with a tapering vitelline duct.

The head region showed three prominent pharyngeal arches, the first of which was clearly divided into the maxillary and mandibular parts. The bulky heart was located just caudal to the arches in contact with them. Many somites were observed along the dorsal convexity through the transparent skin ectoderm, but it was difficult to count them accurately without any damage to the embryo because of its small size and fragility. A protruded area presumed to be the primordium of the upper limb bud was distinguishable but not that of the lower limb. The tail, masked by the umbilical vesicle seen from the left side, was short and curled up to the upper and right side of the body.

2. Cardiovascular system

The heart was at the cardiac loop stage

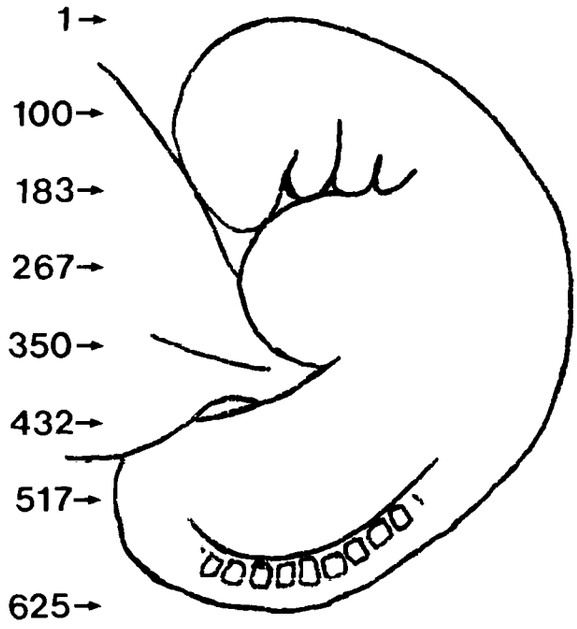

Fig. 1. Overdrawing of the photograph of the embryo. The numbers indicate the section numbers.

without clear-cut demarcations between the four chambers. A marked bulboventricular sulcus was present, and the primitive interventricular foramen was well established (Fig. 2). The wall of the ventricle consisted of a single cell-layered endocardium, three to five cells thick myocardium with abundant cardiac jelly in between. The cardiac jelly, abundant in the wall of the truncus arteriosus, showed scanty cellularity. Some parts of the ventricular walls revealed trabeculated appearence. The left and right atria were roughly demarcated. The atrioventricular canal, the imaginary plane of which was coronally located, could be identified (Fig. 3). The atrial wall retained little cardiac jelly, in contrast to that of the ventricle. The sinus venosus, which drained the common cardinal veins, the umbilical veins, and the hepatocardiac channels, was completely seperated from the left atrium and opened into the dorsal aspect of the right atrium with a still wide sinu-atrial orifice. Rostrally, the boundary between the sinus venosus and the left atrium was indistinct. The truncus arteriosus continued to the aortic sac, which received three pairs of aortic arches from the corresponding pharyngeal arches (Fig. 4). The dorsal aorta ascended

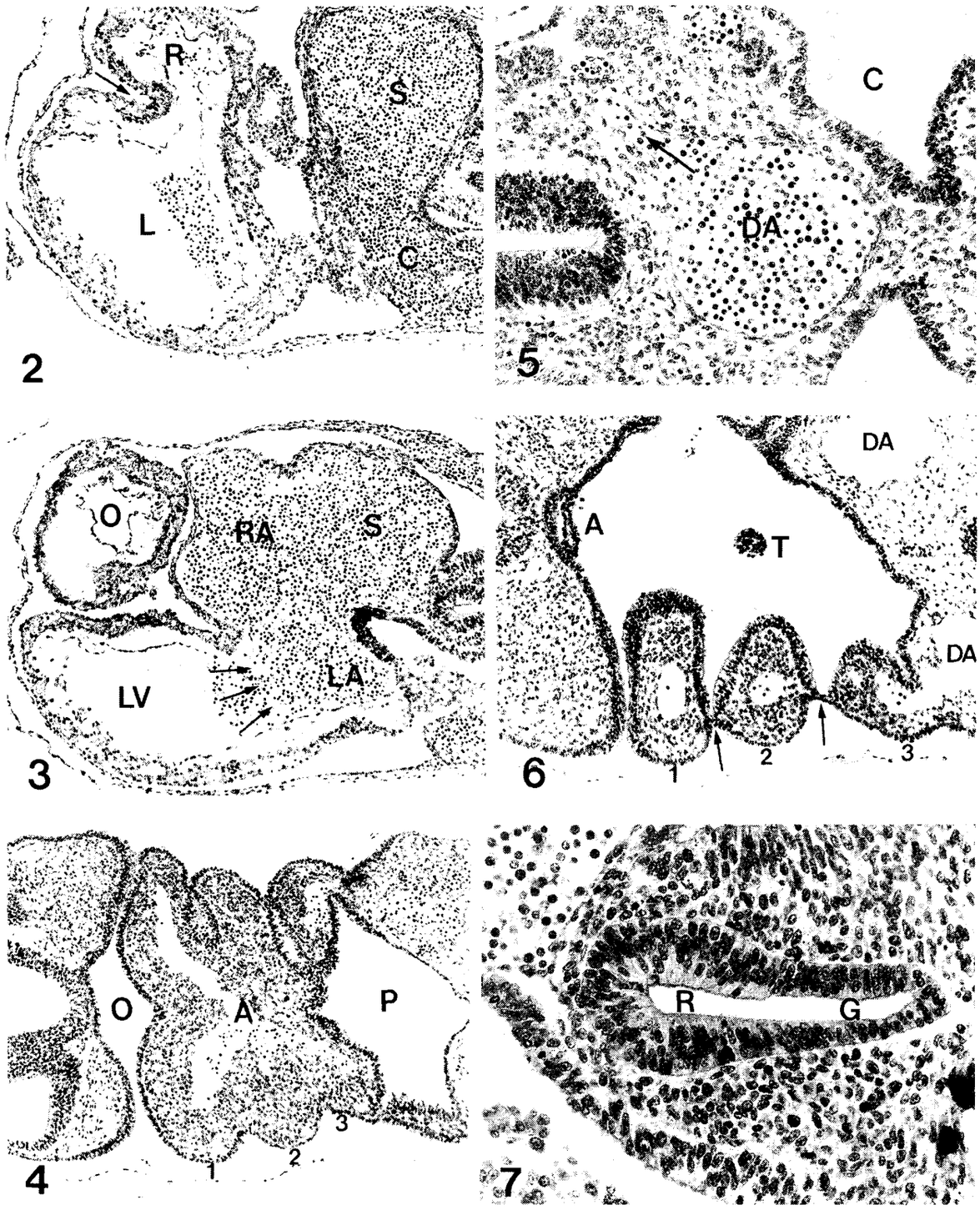

Fig. 2. Section 285. The primitive interventricular foramen. Some trabeculations are seen in the wall of the left ventricle. L: left ventricle, R: right ventricle, S:sinus venosus, C: common cardinal vein(left), Arrow: bulboventricular sulcus. X 80

Fig. 3. Section 342. The atrioventricular canal(arrows). LV: left ventricle, RV: right ventricle, RA: right atrium, S: sinus venosus, O: outflow tract. X 80

Fig. 4. Section 176. Confluence of the aortic arches. The numbers indicate the pharyngeal arches. A: the site of the aortic sac, P: pharynx, O: oral cavity1. X 80

Fig. 5. Section 425. The dorsal aorta(DA) delivering a segmental branch(arrow). C: intraembryonic coelom. X 160

Fig. 6. Section 160. Pharyngeal arches. Apposition of the pharyngeal cleft and pouch is indicated by an arrow. The numbers indicate the pharyngeal arches. T: thyroid primordium, A: adenohypophysial pocket, DA: dorsal aorta. X 100

Fig. 7. Section 271. The respiratory epithelium(R) is thicker than that of the gut(G). X 250

delivering several segmental arteries to the posterolateral directions (Fig. 5), and bifurcated at the level of section 316. In the head region, both dorsal aortas curved over the pharyngeal arches sending each aortic arch, and continued as a terminal branch into the mesenchyme of the maxillary arch. It was also possible to trace the well developed anterior and posterior cardinal veins, up to section 146 rostally and section 416 caudally, respectively. The cut-surface of the right umbilical vein was always larger than that of the left on each horizontal plane.

3. Gastrointestinal tract

In the pharyngeal region, the first three pharyngeal pouches extended laterally far enough so that the lining epithelium came to meet the skin ectoderm resulting in a membrane-like appearence (Fig. 6). The upper and median part of the oral epithelium was in close contact with the neural tube. The respiratory diverticulum was observed in the ventral side of the esophagus, but the lung buds and the esophagotracheal septum were not. The epithelium of the respiratory tract, which shared a common lumen with the esophagus, was thicker than that of the latter (Fig. 7). The site of the future stomach could be identified by the presence of the beginning omental bursa, which appeared as a slight indentation of the coelomic surface on the right side in this embryo (Fig. 8). The gastric region revealed a more active proliferation of coelomic epithelial cells than any other part of the alimentary canal. The dorsal pancreas with the epithelium thickened was observed in the dorsal half of the duodenum (Fig. 9). Just caudal to the dorsal pancreas and in the ventral side of the canal, the hepatic diverticulum bulged extending ventrally and slightly rostrally. From the diverticulum, proliferating hepatic parenchymal cells with eosinophilic cytoplasm spread out radially into the stroma to become trabeculated cell cords intermingled with the hepatic venous plexus (Fig. 10). One third to one half of the stroma was infiltrated by parenchymal cells. At section 430, the gut communicated with the umbilical vesicle(yolk sac) via a narrow duct (Fig. 11). The lining epithelium changed transitionally from stratified in the gut to simple cuboidal in the lumen of the duct. The umbilical vesicle was also lined by a single layer of large, cuboidal cells with eosinophilic cytoplasm, and was covered with a single layer of flattened cells. In addition, plenty of vascular plexus was interposed between the two layers (Fig. 12). The end of the hindgut opened to the cloaca, an expansive lumen located in the ventrally flexed caudal part of the body of the embryo. From the cloaca, the allantois bulged out with a tapering lumen and was dorsally directed. The tip of the allantois lodged between both umbilical arteries (Fig. 13).

4. Central nervous system

The anterior and posterior neuropores had already been closed. The neural tube revealed two prominent flexures, the cephalic and the cervical. The pontine flexure was not recognized. The optic vesicle, the outpouching of the forebrain, was apparent. However, the ectoderm overlying the optic vesicle was not thickened yet, in other words, the optic disc was not yet delineated. Two cranial ganglia, presumed to be those of the trigeminal and vestibulofacial nerves, were apparently formed (Fig. 14). They were not seperated completely from the neural tube, evidence that they were formed by the migrated neural crest cells from the tube (Fig. 15). The ganglia of the glossopharyngeal and vagus nerves were not observed as a seperated entity from the neural tube. However, some neural crest cells were migrating from the tube just posterior to the otocyst to become ganglia of the glossopharyngeal and/or vagus nerve. The roof of the rhombencephalon was thinned out to a one- to two-cell layer. Throughout the neural tube, abundant mitotic feature was seen in the neuroepithelial layer. The marginal layer began to appear, especially caudal to the midbrain. In the vicinity of the neural tube, a lot of vascular plexuses had been formed (Fig. 16).

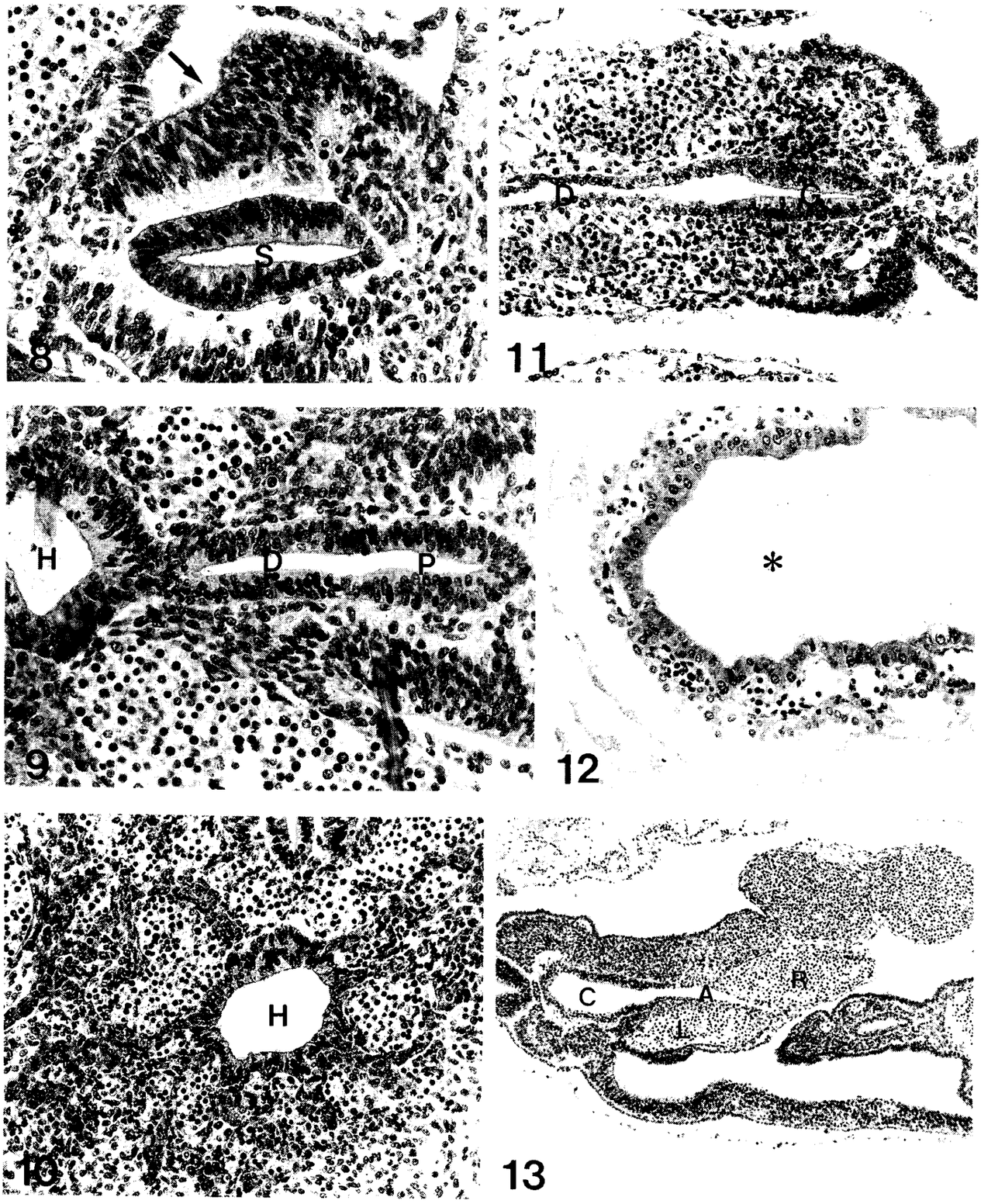

Fig. 8. Section 311. Stomach(S). The right coelomic epithelium is indented to indicate the beginning of the omental bursa(arrow). X 250

Fig. 9. Section 357. The dorsal pancreas(P) is being formed from the dorsal side of the duodenum(D). H: hepatic diverticulum. X 250

Fig. 10. Section 344. The liver. Parenchymal cell strands are intermingled with hepatic venous plexus. H: hepatic diverticulum. X 160

Fig. 11. Section 430. The gut is connected to the vitelline duct. G: gut, D: vitelline duct. X 160

Fig. 12. Section 407. The wall of the umbilical vesicle. The asterisk is in the lumen. X 160

Fig. 13. Section 539. The tip of the allantois(A) is located between umbilical arteries. C: cloaca, R right umbilical artery, L: left umbilical artery, V: umbilical vein. X 60

5. Ear

At both sides, the otocyst was well formed with a 3-4 cell layer of lining epithelium resembling that of the neural tube rather than the skin ectoderm. Though the pit was obliterated, a connecting stalk still remained.

6. Urinary system

The mesonephric tissue began to occur at section 420 on the left and at section 405 on the right. They extended caudally as far as to the last somite level. Almost the entire length of the mesonephric cord was segmented into numerous successive nephric vesicles each with a pin-hole lumen. Such successiveness was more characteristic for the caudally located vesicles (Fig. 17). A few mesonephric vesicles in the most rostral region showed two solid cell strands extruding from the dorsal and ventral walls (Fig. 18). The ventral strand was connected to the coelomic epithelium, presumed to be the vestigial peritoneal funnel. The dorsal strand was connected to the mesonephric duct, suggesting it was the excretory tubule. The mesonephric duct, located dorsal and slightly lateral to the mesonephros, showed varying appearences from section to section. The lumen of the duct was vivid in several sections, or obliterated in others. Whether the caudal end of the duct was connected to the cloaca was hard to verify, since they became more and more tapered as they descended, and the composing cells had no identifiable characteristics from the surrounding mesenchymal cells. However, they were traced caudally to the somite fourth to the last.

7. Miscellaneous

The upper arm buds appeared as an elevated skin ectoderm with a condensation of mesoblastic cells beneath it. The skin ectoderm of the arm bud was thick compared to that of its surroundings - the cells were cuboidal instead of flat. In the pharyngeal region, the beginning of the primordium of the future adenohypophysis, the adenohypophysial pocket, formed as an indentation of the upper oral epithelium. It was observed from section 132 to 136 in a cephalocaudal direction. The thyroid primordium was observed to project into the pharyngeal cavity as a tuberculum thyroideum(thyroid tubercle). On histological sections, it appeared as an island of cell nests in the pharynx (Fig. 6), because it was sectioned perpendicularly to its longitudinal axis. The island was observed from section 133 to 136, locating on the midline and between the first and second pharyngeal arches.

The somites showed somewhat different appearences depending on their locations. Namely, the occipital somites were broken up in their ventromedial sides with some preservation of their architecture only in the dorsolateral side, the dermatome. The dermatome was in close contact with the skin ectoderm. In the middle part of the body, the myotome was clearly recognized in each somite, while the sclerotome was broken up(Fig. 19). The most caudal somites still retained a cyst-like structure each with a prominent somitocoele, because the sclerotomal cells had not migrated so extensively (Fig. 20). The number of clearly identified somites was 26 with one or two additional somites.

DISCUSSION

The exact count of somites was very difficult especially in the occipital region, where the boundary between two adjacent somites was not so clear. However, the number of counted somites suggested that this embryo is at least older than stage 11, belonging to stage 12 or 13 (O'Rahilly and Miller 1987). The external features such as the presence of arm buds, the absence of leg buds, three prominent pharyngeal arches, and the abscence of the distinct cervical sinus favor the view that this embryo belongs to stage 12. Other findings that appear to put this embryo into stage 12 are the narrowing down of the opening between the gut and the umbilical vesicle to make he vitelline duct, closure of the caudal neuropore which is known to be seen in advanced

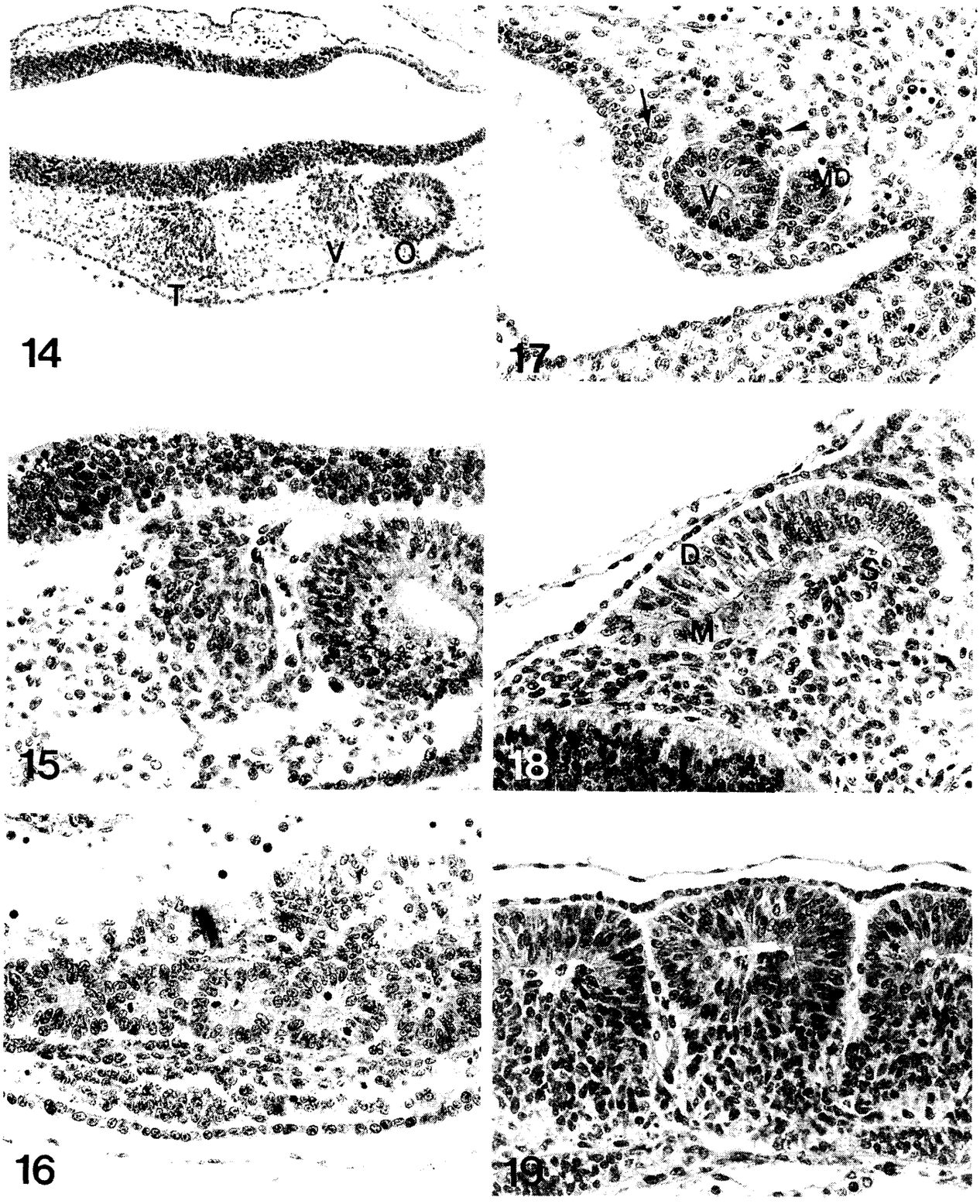

Fig. 14. Section 65. Two cranial ganglia in front of the otocyst(O). T: trigeminal, V: vestibulofacial. X 100

Fig. 15. Section 65. Higher magnification of the vestibulofacial ganglion seen in Fig. 14. Cells emerging from the neural tube are seen. X 250

Fig. 16. Section 584. A series of mesonephric vesicles is seen. X 250

Fig. 17. Rostrally located mesonephric vesicle(V). MD: mesonephric duct, arrow: peritoneal funnel, arrowhead: excretory tubule. X 250

Fig. 18. Section 450. A somite in the middle of the body. Myotome(M) is identifiable. D: dermatome, S:sclerotome. X 250

Fig. 19. Section 616. Caudally located somites. X 250

members of this stage, and three to five cells in the thickness of the myocardium with moderate trabeculation. However, the otocyst is attached to the skin ectoderm by a stalk, which is a characteristic finding in the less developed embryos of stage 13. In stage 13, the respiratory system shows recognizable trachea and the right and left lung buds, which were abscent in our results. This embryo showed only a respiratory diverticulum. Morever, it is possible for one "to outline the parts of the optic evagination, namely, retina, pigment layer of retina, and optic stalk region" in horizon XIII (Streeter 1945), which was impossible in this embryo, because the optic vesicle was of a simple, somewhat rounded shape. In addition, the skin ectoderm in the area of the future optic disc showed no evidence of differentiation, which favors the view that this embryo belongs to stage 12. This embryo lacks the septum primum in the heart, one possible structure in stage 12. However, the septum primum has been reported to be present only in 7 out of 25 embryos of stage 12, although present in all stage 13 embryos (McBride *et al.* 1981).

The early form of Rathke's pouch, the adenohypophysial pocket, was observed. It has been reported to begin to appear in an embryo of horizon XIII instead of XII by Chi and Lee (1988). However, they observed only one embryo of horizon XII. O'Rahilly *et al.* (1984) inspected 21 embryos of stage 12 and found the adenohypophysial pocket in 18 embryos. Thus, the pocket may be another characteristic of a member of stage 12. We were unable to trace the two observed distinct ganglia in front of the otocyst to confirm which ganglia they were. However, some reports certify that they are the trigeminal and vestibulofacial ganglia (Streeter 1945; O'Rahilly *et al.* 1984; O'Rahilly and Miller 1987). In this embryo, the thyroid primordium took the form of a cellular hump projecting into the pharynx, a feature not mentioned in many well known embryology textbooks. However, that the thyroid anlage might project into the pharynx as a tubercle was reviewed by O'Rahilly(1983), and this embryo may be such a case. Sgalitzer(1941) differentiated normal thyroid tubercle and abnormality in thyroid anlage. He demonstrated two embryos of stage XII with abnormal thyroid anlage. It was not only invaginated into the pharynx, but also contained mesenchymal core and blood vessels. Our embryo showed no such findings, which indicated that its tubercle must have been normal.

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