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Development of the Esophagus in Human Embryos

Special Reference to Histochemical Study on Carbohydrates

(esophagus/human embryos/histochemistry)

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Using 70 external normal human embryos at developmental stages ranging from 13 to 23 (5.1 to 29.3 mm in crown-rump length), that is, at five to eight weeks postconceptional age, the early development of the esophagus was examined by conventional and histochemical methods. Each material was sectioned serially, 35 embryos were stained with hematoxylin-eosin and in another 35 histochemical methods for determining carbohydrates, PAS, PARS, alcian blue, toluidine blue and colloid iron were used.

In the epithelium, small glycogen particles were seen distributed in the cytoplasm, and were dominant in the base at stage 13. With stage advancement, glycogen particles increased in number and size and were distributed equally in the cytoplasm. Glycoprotein was found on the free surface of the epithelium. In the mesenchyme, glycogen particles were distributed almost equally and showed no remarkable changes during the embryonic period. Acid mucopolysaccharides were equally distributed and were diffuse at stage 13. With stage advancement, these increased in volume, expect for the muscle layer, in which there was no increase.

Vacuoles were found from stages 19 to 22, but there was no occlusion of the lumen in these stages. Histochemically, there were no characteristic findings at these stages. The free glycogen particles found in the vacuoles, were considered to provide evidence of autolysis of the epithelium.

The esophagus is a simple tube-like structure and plays a lesser role than other organs of the digestive tract. Numerous authors have been interested in the development of this organ in human embryos (1—7), particularly the origin of the superficial glands and changes in the esophageal epithelium observed histologically using H. E. staining methods. Their interests have evolved around fetal development.

Schrager (8) found that mucosubstances throughout the gastro-intestinal tract had protective functions. There are also reports of histochemical studies on the development of the gastro-intestinal tract in human embryos and fetuses (9—19).

Recently, Hopwood *et al.* (20) reported the normal distribution of mucosubstances in normal esophageal epithelium of adult humans. McKay *et al.* (21) used a histochemical approach to study human embryos of Streeter's horizon

13 (stage 13, 5mm in crown-rump length) and observed the functional mechanism in operation in embryos at this stage of development. However, there are few reports in the literature of histochemical studies of the esophagus in the human embryos.

We examined the early development of the esophagus, with special reference to the development of carbohydrates and we used conventional and histochemical methods.

MATERIALS AND METHODS

The materials were seventy externally normal human embryos ranging from 5.1 to 29.3 mm in crown-rump length. The developmental stage ranged from 13 to 23 which corresponds to 32 to 52 days of the standardized post-conceptional age (22). Details of the materials are shown in Table I. The

TABLE I. *Embryos*

Stage	No. of embryos		Crown-rump*** length (mm)	Approximate postconceptional age (days)***
	Routine*	Histochem.**	M±S. D.	
13	3	3	5.1 ± 0.15	31.6
14	4	4	6.8 ± 0.08	35.3
15	4	4	8.0 ± 0.07	36.5
16	4	4	9.2 ± 0.08	37.8
17	3	3	11.5 ± 0.13	38.9
18	3	3	13.5 ± 0.36	41.0
19	3	3	15.9 ± 0.30	45.1
20	3	3	19.2 ± 0.26	46.8
21	3	3	21.1 ± 0.26	47.6
22	3	3	22.8 ± 0.53	50.1
23	2	2	28.0 ± 1.02	52.2
Total	35	35		

* Routine histology ; ** Histochemistry

*** These represent standardized figures reported by Nishimura *et al.* (1968)

embryos were obtained through collaborating obstetricians in Japan and were the products of interrupted pregnancies. Immediately upon removal, thirty-five embryos were fixed in Bouin's fluid (A) and placed in 10% formalin. Another thirty-five embryos were fixed in Lillie's fluid (23) (acetic acid alcohol formalin) (B). This same fluid was used for their storage in cold (9°C). Through storage, serial 7μ paraffin horizontal sections were cut and stained with hematoxylin and eosin, and PAS reaction, PARS reaction, alcian blue-PAS double staining, toluidine blue metachromatic reaction and colloid iron-PAS double staining. The purpose of each staining procedure is shown

TABLE II. *Staining Methods for Carbohydrates*

Method	Glycogen	Muco-proteins	Mucopolysaccharides		Glyco-lipids	Glyco-proteins
			Acid	Neutral		
PAS	+	+	−	w +	+	+
PARS	−	+	−	w +	+	+
PAS after diastase	−	+	−	w +	+	+
Alcian blue	−	−	+	−	−	+ +
Toluidine blue	−	−	+ *	−	−	−
Colloid iron	−	−	+	−	−	−

− : negative ; w + : weak positive ; + : positive ; + + : strong positive
* metachromatic This table is a modification of Bancroft's (48).

in Table II. PARS reaction (24) was applied to these sections for determinations of mucoprotein, neutral mucopolysaccharides and glycoprotein.

In A-group (conventional) of 35 specimens, hematoxylin and eosin were used for routine morphology.

In B-group (histochemical), the following stains were applied : PAS reaction, PAS after diastase (25), a combined alcian blue-PAS method (26), toluidine blue method (27), and colloid iron (28).

Following the staining preparations, the developing esophagus of the human embryo was observed under a light microscope.

RESULTS

The results are divided into two parts, according to the staining method.

Morphological Results

An outline of the results is summarized in Fig. 1.

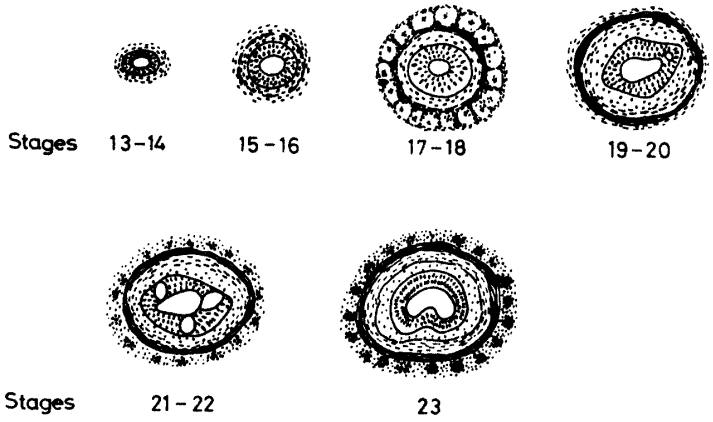

Fig. 1. Schema of early development of the human esophagus.

Stage 13

The epithelium are composed of two cylindrical cell layers with condensed-elongated nuclei. In the mesenchyme, large oval nuclei were surrounded

by immature cells.

Stages 14 and 15

The epithelium consisted of 2 or 3 cylindrical cell layers with elongated nuclei. The mesenchyme was not differentiated and increased in thickness.

Stage 16

The epithelium increased in thickness and there were 2 or 3 cylindrical cell layers. In the mesenchyme, primordium of the circular muscle layer was evident and the mesenchyme increased in thickness, subsequently, the intercellular space broadened.

Stages 17 and 18

The epithelium was composed of 3 or 4 cylindrical cell layers. The circular muscle layer was more evident and there were nerve plexuses outside the muscle layer.

Stage 19

The process of vacuolation was first seen in this stage. The vacuoles produce an irregularity of the lumen of the epithelial lining. The mesenchyme was divided by small vessels into two layers, the inner layer with small oval nuclei and the outer layer with elongated nuclei.

Between stages 20 and 22

The vacuoles are numerous and larger than in the previous stage. The epithelial cell became cuboidal cells with an oval nucleus. The mesenchyme was divided into two layers as in the previous stage. The primordium of the longitudinal muscle layer was evident outside the circular layer.

Stage 23

Vacuoles were seen in the lower area near the cardia of the stomach. The epithelium was composed of two cuboidal cell layers with oval nuclei. Subsequent to the vacuolation, the lumen was not round in this stage. The longitudinal muscle layer was more evident. Spindle shaped cells which were considered to be myoblasts were arranged in such a way so as to divide the mesenchyme.

Histochemical Results

An outline of these results is shown in Table III.

1) Glycogen

In the epithelium, small glycogen particles were distributed over the cytoplasm and were dominant in the base of the epithelial cells at stage 13. With stage advancement, the glycogen particles increased in number and size (Fig. 3), and were distributed equally on the cytoplasm. The activity of glycogen becomes stronger with each stage. Glycogen showed no tendency to decrease in volume through the embryonic period in this study. During the process of vacuolation, the distribution of glycogen showed no characteristic changes in the epithelium, however free glycogen particles were seen in some vacuoles, and such is considered to indicate autolysis of the epithelial cells.

In the mesenchyme, small glycogen particles were observed in immature

TABLE III. *Activity of Some Carbohydrates Demonstrated Histochemically in the Esophagus*

Sites	Carbohydrate	Stage										
		13	14	15	16	17	18	19	20	21	22	23
EPITHELIUM	Glycogen	+	+	++	++	++	++	++	+++	+++	++	+++
	Glycoprotein	−	−	−	−	−	±	+	+	+	+	+
MESENCHYME	Glycogen	+	+	+	+	+	+	+	+	+	+	+
	Acid muco-polysaccarides	±	+	+	+	+	+	++	++	++	++	++

− : negative ; ± : not negative ; + : weak ; ++ : moderate ; +++ : strong activity

cells at stage 13 or 14 (Fig.2). There were small glycogen particles distributed equally and there were no remarkable changes throughout the embryonic period (Figs. 5—7). The activity of glycogen was stronger in the muscle layer, but showed no increase.

2) Glycoprotein and acid mucopolysaccharides

A trace of glycoprotein was found on the free surface of the epithelial cells, and such is considered to reflect the existence of ciliated cells (Figs. 2, 3, 5, 6, 7, and 10). The activity of glycoprotein is at the lumen side of the epithelium.

In the mesenchyme, acid mucopolysaccharides were distributed diffusely at stages 13 or 14 (Fig. 4). With stage advancement, acid mucopolysaccharides increased gradually in volume, probably to keep pace with the degree of mesenchymal differentiation. The activity of acid mucopolysaccharides became moderately strong when the mesenchyme was divided into two layers. In the mesenchyme, acid mucopolysaccharides were distributed equally and showed no particular characteristics.

In the process of vacuolation of the esophagus, the obliteration of the lumen as seen in the duodenum was not found in either the morphological or the histochemical specimens.

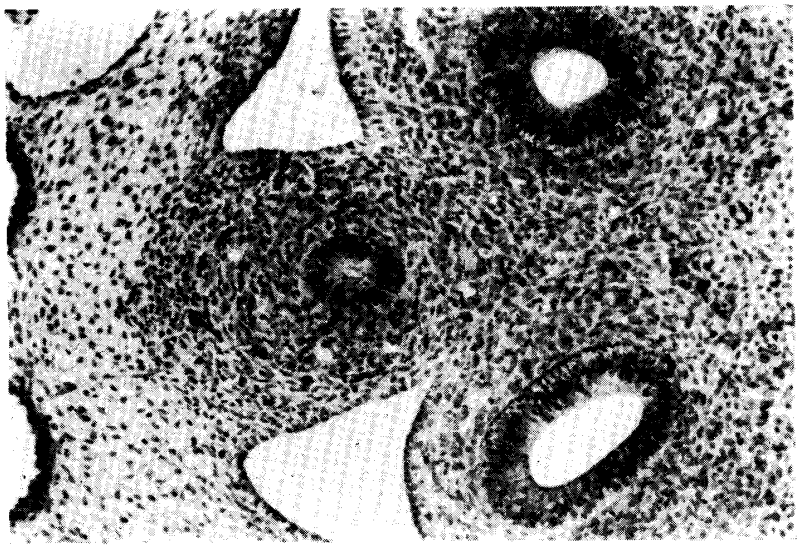

Fig. 2. The PAS-positive granules are distributed in the epithelial cells. The mesenchyme, which is composed of immature cells, is stained diffusely-positive by alcian blue.
(Stage 14, ×100, PAS and Alcian blue)

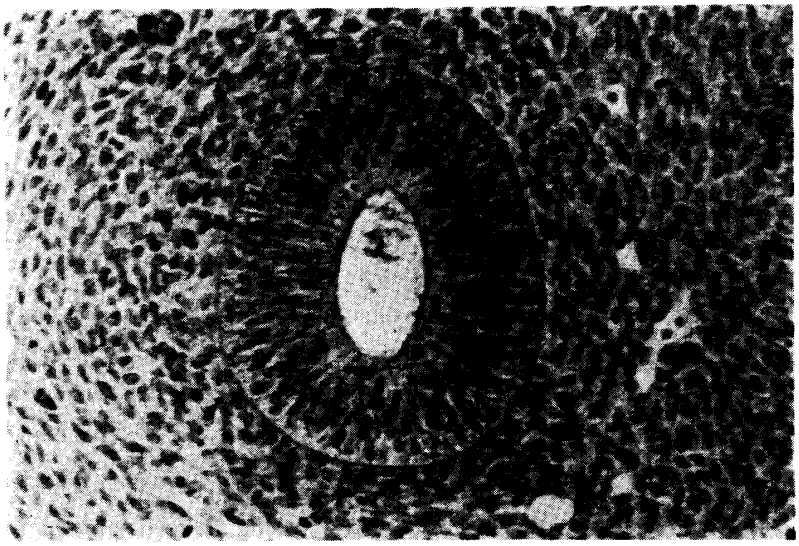

Fig. 3. The PAS-positive granules are distributed in the epithelial cells.
(stage 14, ×400, PAS and Alcian blue)

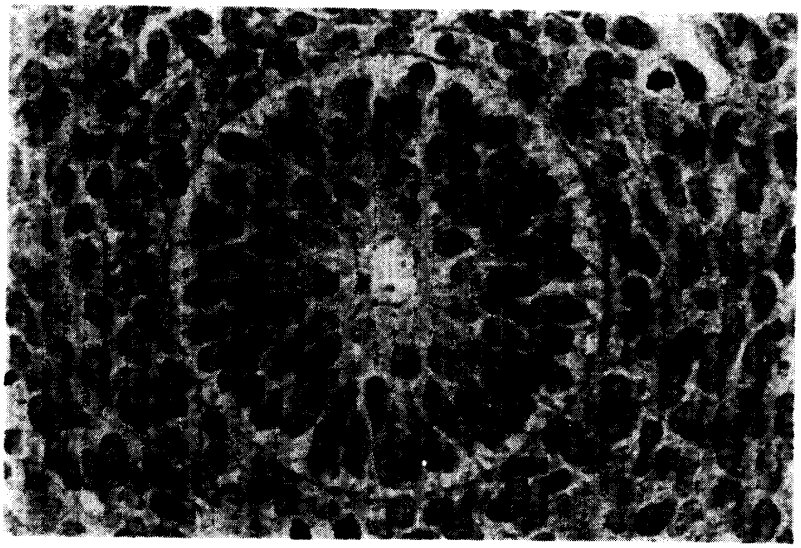

Fig. 4. The epithelial cells did not stain with colloid-iron, but the mesenchyme stained positive weakly and diffusely.
(Stage 14, ×400, Colloid-iron)

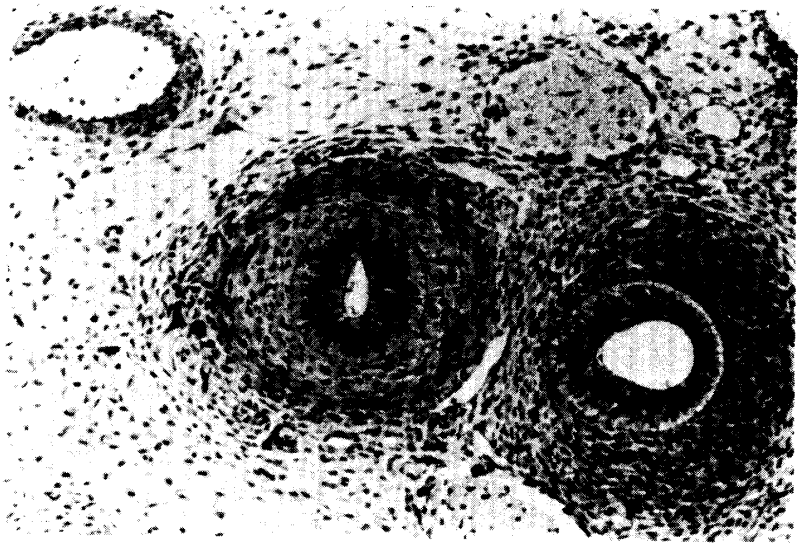

Fig. 5. The upper portion of the esophagus is shown. The epithelium has two or three layers of cells which have PAS-positive particles. The mesenchyme stained diffusely-positive with alcian blue. The muscle layer is already evident.
(Stage 17, ×100, PAS and Alcian blue)

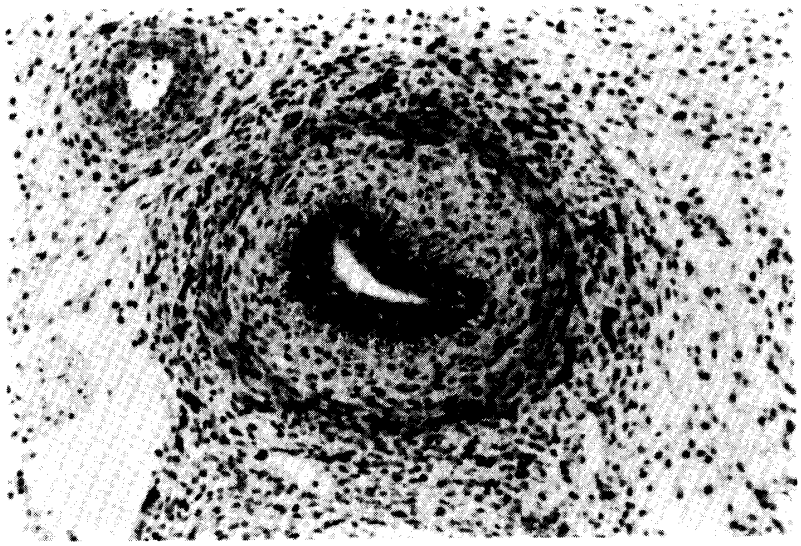

Fig. 6. The epithelium is composed of 3–4 layers of cells with PAS-positive particles. The mesenchyme stained diffusely-positive with alcian blue.
(Stage 18, ×100, PAS and Alcian blue)

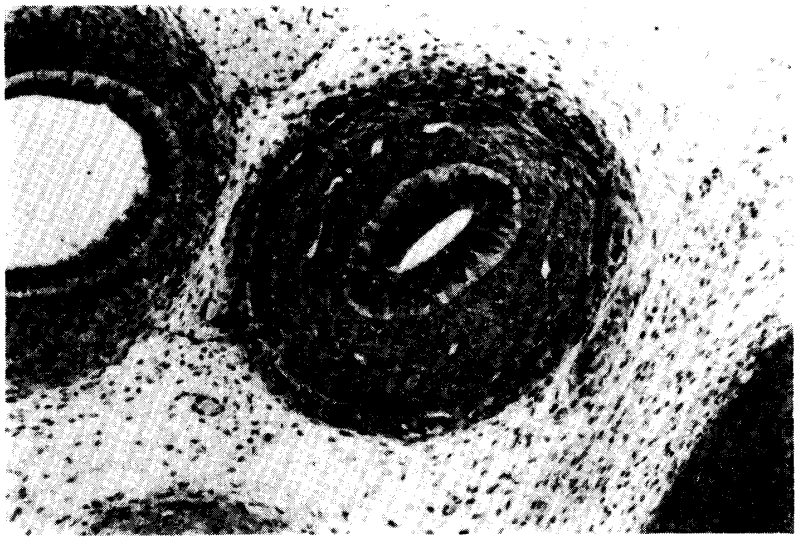

Fig. 7. The epithelial cells have PAS-positive particles, which have increased in size. The mesenchyme stained with alcian blue more intensely-positive at this stage.
(Stage 19, ×100, PAS and Alcian blue)

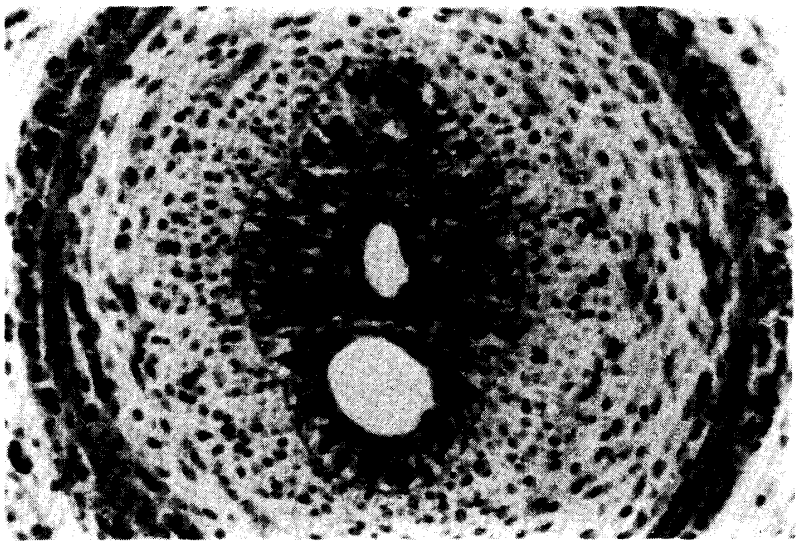

Fig. 8. A large vacuole is seen in the epithelium, thereby two lumens. The epithelial cells appear to contain PARS-positive particles.
(Stage 20, ×100, PARS)

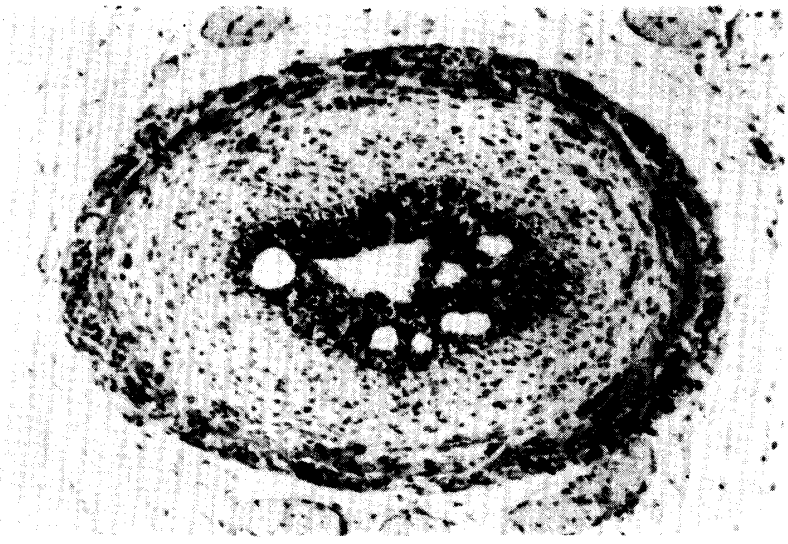

Fig. 9. There are some various-sized vacuoles in the epithelium. The PARS-positive particles are distributed in the epithelial cells, but the mesenchyme was not stained with PARS.
(Stage 21, ×100, PARS)

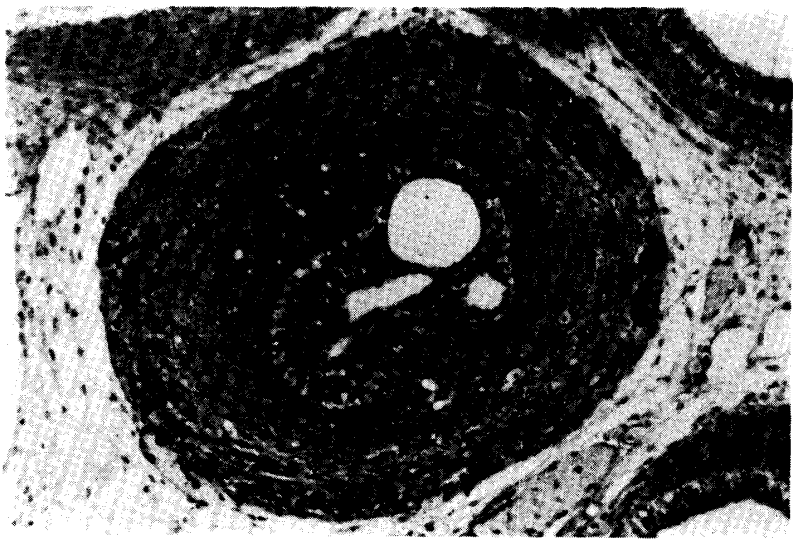

Fig. 10. In comparsion with Fig. 7, the mesenchyme stained intensely-positive with alcian blue but not so the epithelium. The process of vacuolation is evident. (Stage 21, ×100, Alcian blue)

DISCUSSION

Although there are a few reports concerning development of the human esophagus during prenatal periods, few included a discussion of the organogenesis of the esophagus at the early embryonic stage, rather they dealt with the fetal stage. Johns (6) examined the developmental changes that occur in the esophageal epithelium, with particular reference to the development of the superficial glands. He found that the embryonic esophagus in man was lined by stratified columnar epithelium which in general was three cells deep, from the 3 mm to the 16 mm in crown-rump length (CRL), it was two layered and subsequently proliferated to become many layers between 23 and 34 mm in CRL. In the former, the findings in the present study were in good agreement. In the latter, we found two layers at stage 16, this being earlier than his finding. Maruyama (7) studied, morphologically and histologically, the development of the esophagus in Japanese embryos and mentioned that the inner surface of the esophagus was partly covered with columnar epithelium at the 6.3 mm in CRL, which increased remarkably at 9.38 mm in CRL and larger specimens. In our work, we found that the epithelia were already composed of two cylindrical cell layers at stage 13 (5.2 mm in CRL) and remarkably increased in thickness, but were 2 or 3 cylindrical cell layers, at stage 16 (9.5 mm in CRL).

The situation of the nuclei and the state of the cytoplasm of submucous tissue were quite similar to the results of the present study. According to Hopkins (29), the esophageal epithelium proliferates until the lumen is almost filled with cells during the seventh and eighth weeks. At stages 17—18 (11.5—13.5 mm in CRL), we also found the lumen nearly filled with epithelial cells composed of 3 or 4 cylindrical cell layers.

With regards to epithelial occlusion of esophageal development, the views are conflicting in some textbooks of embryology (30—33). Kreuter (34) suggested that a physiological occlusion of the lumen of the esophagus ("solid" stage), found in the 19 to 20 mm stage, might lead to atresia if recanalization did not occur. Subsequent work has led to some doubt as to whether or not a complete occlusion of the lumen can occur. Maruyama (7) found esophageal occlusion in two specimens of 6.3 mm and 33.0 mm in CRL. Johns (6) noted vacuolization in the esophageal epithelium of embryos from 13 to 16 mm in CRL (stages 17 to 18), but did not find epithelial occlusion. Smith (38) investigated the early development of the trachea and esophagus in human embryos, and discussed the relation to atresia of the esophagus and tracheoesophageal fistula. His investigation revealed little evidence of epithelial occlusion in stages 10 to 15, although narrowing of the esophagus in the region just caudal to the bifurcation of the trachea was noted and there was a reduction in the caliber of the esophageal lumen.

The process of vacuolation was observed by us from stages 19 to 22, in agreement with findings of other authors (4, 6, and 35). Kreuter (34) reported that the esophagus become occluded by epithelial proliferation, yet this was denied by other authors (4, 6, 35, and 36). We also did not find any obliteration of the lumen. As Hopkins (29) pointed out,however, it is so rare to find a mucosal diaphragm across the lumen of the esophagus, that occlusion rarely takes place.

Botha (37) reported that there is no complete occlusion of the esophageal lumen and no evidence to suggest that the lumen is re-established by vacuolization as seen in the esophagi of certain lower vertebrates observed by Reese (38).

Maruyama (7) reported that the inner circular layer of lamina muscularis was evident for the first time in the 16 mm embryo, and the primordium of muscularis mucosae appeared in the 28 mm embryo in the state of a few bundle of embryonic muscular fibers. The external longitudinal muscular layer was composed of smooth muscle in the embryos smaller than 100.5 mm. Smith (35) observed the submucosal and muscular layers of the esophagus in stage 18 (14—16 mm in CRL) embryos. According to Botha (37), the circular layer is formed first, at the end of the 6th week (12 mm in CRL). Hopkins (32) stated that the circular muscle coat appears early in the sixth week (9 mm), and the longitudinal musculature is indicated by the ninth week (30 mm). In the present study, primordium of the circular muscle layer was first recognized at stage 16 and that of the longitudinal muscle layer at stage 21. Thus, we found that the stage of appearance of the each musclar layer was earlier than the data in previous studies. According to Smith (35), the mesenchyme throughout plays only a passive role, and angiogenesis occurs in response to epithelial growth. Maruyama (7) first observed esophageal blood vessels in the 16 mm embryo. We observed the small vessels in mesenchymal layers at stage 19 (15.6 mm in CRL), at much the same time as Maruyama's.

Fetal tissues are abundant in glycogen, but the role of glycogen stored in

the immature tissues is unkown. The fetal epithelia may serve as glycogen stores for the metabolic needs of the developing organism (42). A possible precursor of glycoprotein, epithelial glycogen has been related to other metabolic processes which may have relevance in the fetal period (16). Glycogen is an energy source, but in the esophagus, glycogen does not decrease after the process of the vacuolation. Therefore, histoenzymoligical or histoimmunological studies are required.

As Needham (39) has pointed out, the regularities discovered by morphological investigations will always have their validity, and will, in a sense, be unaffected by anything that biochemistry may discover. McKay *et al.* (21) reported a limited histochemical survey of a 5 mm embryo (stage 13), which represents the first of a series of such embryos, and the following year, a similar study of 6 and 7 mm (stage 14) embryos (40). The epithelia of the pharynx, the lung and the gut present the same histochemical pattern and these cells all contain abundant glycogen (21). The basement membrane of the gut epithelium contains glycoprotein at 5 mm in CRL. In another study of 6 mm and 7 mm embryos, both epithelium and mesemchyme of the esophagus were shown to contain glycogen, glycoprotein and ribonucleoprotein (40). At stages 13 and 14, we confirmed these findings by McKay *et al.* (21, 40).

In the small intestine of human embryos, Jirásek and Koldovský (15) found that the endodermal epithelium of the primitive intestine (stages 10−14) contains glycogen and a small amount of ribonucleoproteins. According to Lev (41) and Garbarsch (17), the villus surfaces were lined by the multilayered, vacuolated, glycogen-rich epithelium with centrally located nuclei in the previllus stage in the 7 week old embryo. These findings were much the same as ours in the present study with respect to the onset of glycogen and existence of glycogen in some vacuoles at stage 22.

The abundance of fetal epithelial glycogen is in accord with the observation that glycogen-synthesizing enzymes are more prominent in fetal than in adult epithelia in many species, including man (43, 44).

In the histochemical observations, the ciliated epithelium, which has acid mucopolysaccharides was first detected at stages 22 or 23 in the present study, and such parallels findings of others (1, 2, 45). In adults, Hopwood *et al.* (46) reported histochemical findings of the normal human esophageal epithelium and showed that each of the three layers of cells contained neutral mucosubstances in their cell coats and that the superficial cells were also covered with an acid mucosubstance.

The epithelium changed from cylindrical cells to cuboidal ones between stages 20 and 22, and we found remarkable changes. Electron microscopic examinations and other histochemical methods should be done for elucidation.

The differentiation of the mesenchyme occurs gradually, and in the process, small vessels play an important role as they divide the mesenchyme into layers at stage 19. These observations are in agreement with findings of Nagy (47), who mentioned that blood vessels were important in the development of the intestinal tract.

Thus, the early development of the esophagus is much the same as the development of other areas of the alimentary tract.

REFERENCES

1) Neumann, E. (1876) Flimmerepithel in Oesophagus menschlicher Embryonen. *Arch. Mikr. Anat.* **12**, 570–574
2) Schaffer, J. (1904) Die oberen cardialen Oesophagusdrüsen und ihre Entstehung. *Virchows Arch. Pathol. Anat.* **177**, 181–205
3) Schridde, H. (1904) Uber Magenschleimhautinseln vom Bau der Cardial drüsenzone und Fundusdrüsesregion und den unteren oesophagealen Cardialdrüsen gleichende Drüsen im oberten Oesophagusabschnitte. *Virchow Arch. Pathol. Anat.* **175**, 1–16
4) Johnson, F. P. (1910) The development of the mucous membrane of the esophagus, stomach and small intestine in the human embryo. *Am. J. Anat.* **10**. 521–559
5) Lewis, F. T. (1910) The development of the esophagus. In : Keibel and Mall's Manual of Human Embryology. **2**, pp. 355–368, Lippincott, Philadelphia
6) Johns, B. A. E. (1952) Developmental changes in the oesophageal epithelium in man. *J. Anat.* **86**, 431–441
7) Maruyama, H. (1956) Studies on the development of the oesophagus in Japanese embryo. *Acta Anat. Niigata'ensia* **43**, 13–58 (Abs. in English)
8) Schrager, J. (1970) The chemical composition and function of gastro-intestinal mucus. *Gut* **11**, 450–456
9) Nagakawa, T. (1959) Histogenetic and cytological studies on the duodenal mucous membrane in human foetuses. *Arch. Histol. Jpn.* **16**, 495–522 (Abs. in English)
10) Furui, T. (1959) Cytological studies on the intestine of human foetus. *J. Jpn. Obstet. Gynecol. Soc.* **11**, 1742–1750 (Abs. in English)
11) Sano, H. (1960) Histochemical studies on phosphatases in the intestinal canal and kidney of human fetuses. *J. Tokyo Med. Coll.* **18**, 1947–1959 (Abs. in English)
12) Nakayama, A. (1961) Histochemical study on intestinal epithelium of human embryo, with special reference to basal granulated cells, goblet cells and distribution of glycogen. *J. Tokyo Med. Coll.* **19**, 1511–1525 (Abs. in English)
13) Andersen, H., Bierring, F., and Matthissen, M. (1964) On the nature of the meconium corpuscles in human foetal intestinal epithelium. *Acta Pathol. Microbiol. Scand.* **61**, 377–393
14) Graumann, W. (1964) Polysaccharide. Ergebniss der Polysaccharidehistochemie : Mensch und Säugetiere. In : Handbuch der Histochemie. Bd. 2, pp. 132–134, Fischer, Stuttgart
15) Jirásek, J. E., Uher, J., and Koldovský, O. (1965) A histochemical analysis of the development of the small intestine of human fetuses. *Acta Histochem.* **22**, 33–39
16) Lev, R. and Weisberg, H. (1969) Human foetal epithelial glycogen : a histochemical and electronmicroscopic study. *J. Anat.* **105**, 337–349
17) Garbarsch, C. H. (1969) Histochemical studies on the early development of the human small intestine. *Acta Anat.* **72**, 357–375
18) Lev, R., Siegel, H. I., and Bartman, J. (1972) Histochemical studies of developing human fetal small intestine. *Histochemie* **29**, 103–119
19) McFall, W. T. and Kraus, B. S. (1963) Observations on the glycogen content of foetal oral mucous membranes. *J. Periodontol.* **34**, 416–421
20) Hopwood, D., Logan, K. R., and Coghill, G. (1977) Histochemical studies of mucosubstances and lipids in normal human esophageal epithelium. *Histochem. J.* **9**, 153–161
21) McKay, D. G., Adams, E. C., Hertig, A. T., and Danziger, S. (1955) Histochemical horizons in human embryos. *Anat. Rec.* **122**. 125–151
22) Nishimura, H., Takano, K., Tanimura, T., and Yasuda, M. (1968) Normal and abnormal development of human embryos : First report of the analysis of 1,213 intact embryos. *Teratology* **1**, 281–290
23) Lillie, R. D. (1965) Carbohydrates. In : Histopathologic Technic and Practical Histochemistry. pp. 228–280, McGraw-Hill, Chichester

24) Hotchkiss, R. D. A. (1948) Microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. *Arch. Biochem.* **16**, 131–139
25) Lillie, R. D. (1949) Studies on histochemistry of normal and pathological mucins in man. *Anat. Rec.* **103**, 611–624
26) McManus, J. F. A. and Mowry, R. W. (1964) Staining methods. In : Histologic and Histochemical. pp. 63–64, Harper and Row, New York
27) Kramu, H. and Windrum, G. M. (1955) Sulphation techniques in histochemistry with special reference to metachromasia. *J. Histochem. Cytochem.* **2**, 196–201
28) Müller, G. (1955) Über eine Verein fachung der Reaktion nach Hale (1946). *Acta Histochem.* **2**, 68
29) Hopkins, W. A. (1972) The esophagus. In : Embryology for Surgeons (Gray, S. W. and Skandalakis. J. E., eds.) pp. 63–67, Saunders, Philadelphia
30) Patten, B. M. (1968) Histogenesis of the walls of the digestive tube. In : Human Embryology. pp. 379–385, McGraw-Hill, New York
31) Hamilton, W. J. and Mossman, H. W. (1972) The esophagus. In : Human Embryology. Prenatal development of form and function. pp. 334–337, W. Heffer and Sons, Cambridge
32) Arey, L. B. (1974) The digestive tube. In : Developmental Anatomy. pp. 245–255, W. B. Saunders, Philadelphia
33) Moore, K. L. (1977) The digestive system. In : The Developing Human. pp. 197–198, W. B. Saunders, Philadelphia
34) Kreuter, E. (1905) Die angeborenen Verschliessungen und Verengerungen des Darmkanals im Lichte der Entwicklungsgeschichte. *Dtsch. Z. Chir.* **79**, 1–89
35) Smith, E. I. (1957) The early development of the trachea and esophagus in relation to atresia of the esophagus and tracheoesophageal fistula. *Contrib. to Embryol.* **36**, 41–62
36) Schridde, H. (1908) Uber die Epithelproliferationen in der embryonalen menschlichen Speiseröhre. *Virchows Arch. Pathol. Anat.* **191**, 178–192
37) Botha, G. S. (1959) Organogenesis and growth of the gastroesophageal region in man. *Anat. Rec.* **133**, 219–239
38) Reese, A. M. (1926) The occlusion of the esophagus and trachea in crocodillia and snakes. *Am. J. Anat.* **37**, 195–212
39) Needham, J. (1950) Biochemistry and Morphogenesis. Cambridge Univ. Press, Cambridge
40) McKay, D. G., Adams, E. C., Hertig, A. T., and Danziger, S. (1956) Histochemical horizons in human embryos. *Anat. Rec.* **126**, 433–464
41) Lev, R. (1968) A histochemical study of glycogen and mucin in developing human foetal epithelia. *Histochem. J.* **1**, 152–165
42) Moog, F. and Ortiz, E. (1960) The functional differentiation of the small intestine. VII. The duodenum of the foetal guinea-pig, with a note on the growth of the adrenals. *J. Embryol. Exp. Morphol.* **8**, 182–194
43) Takeuchi, T., Higashi, K., and Watanuki, S. (1955) Distribution of amylophosphorylase in various tissues of human and mammalian organs. *J. Histochem. Cytochem.* **3**, 485–491
44) Kobayakawa, S. (1957) Histochemical and biochemical study on the distribution of amylophosphorylase in human embryos. *Acta Med. Fukuoka* **27**, 1659–1688
45) Jahrmaeker, F. (1906) Uber die Entwicklung des Speiseröhrenepithels beim Menshen. Inaug. Diss., Marburg. (Cited by Johnson, 1910)
46) Hopwood, D., Kathleen, R. L., and Milne, G. (1977) Mucosubstances in the normal human oesophageal epithelium. *Histochemistry* **54**, 67–74
47) Nagy, V. (1912) Uber die Histogenese des Darmkanals bei menschlichen Embryonen. *Anat. Anz.* **40**, 147–156
48) Bancroft, J. D. (1967) Carbohydrates and amyloid. In : An Introduction to Histochemical Technique. pp. 62–94, Butterworths, London