Separation between the digestive and the respiratory lumina during the human embryonic period: morphometric study along the tracheo-oesophageal septum

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ABSTRACT

An isolated tracheo-oesophageal fistula could be caused by close proximity of the epithelia of both organs (O'Rahilly & Müller, 1984; Kluth et al. 1987) at certain embryonic stages, the most frequent location being the tracheal bifurcation. Thus the relative position and degree of separation between the digestive and the respiratory tubes throughout their development may be relevant to the origin of this anomaly. The aim of this study was to analyse along the different segments of the tracheo-oesophageal septum (TES) where the closest relationship between both lumina occurred and what degree of separation was present at each segment. Computer imaging techniques were applied on cross sections of a graded series of normal human embryos (Carnegie stages (CS) 13–23). In addition, the differentiation of the primitive TES was also studied (from CS 12) by light microscopy. Between CS 13 and 16 both tubes tended to separate (phase of separation), principally at the proximal segments of the laryngopharyngeal and the tracheo-oesophageal portions of the TES. During this phase the separation between the trachea and oesophagus was wider than between the larynx and pharynx. From CS 17 to CS 23 the digestive and respiratory lumina reached their widest separation at different levels of the laryngopharyngeal portion. Below these levels they tended to come closer together, principally at the proximal segment of the tracheo-oesophageal portion, but also at the distal part of the laryngopharyngeal portion. During this phase of approximation they reached their closest relationship at the proximal (CS 17) and the distal (from CS 18) segments of the tracheo-oesophageal portion. When finally the distal segment of the trachea (which includes the bifurcation) comes closest to the oesophagus, the coats of both organs have already undergone an appreciable differentiation. According to these observations, the origin of the most frequent isolated tracheo-oesophageal fistula at the bifurcation region could not be explained from the normal development of the TES.

Key words: Development; digestive system; respiratory system.

INTRODUCTION

Oesophageal atresia is often associated with tracheo-oesophageal fistula and in more than half of the cases, both anomalies are part of a wider association of malformations (Fraser et al. 1987; Nebot-Cegarra & Domènech-Mateu, 1989). The disturbances that lead to these anomalies are poorly understood (Merei et al. 1997). The age of recovered human embryos with these anomalies (O'Rahilly & Müller, 1984; Nebot-Cegarra & Domènech-Mateu, 1989), the sequence in their formation in the adriamycin-induced fetal rat model (Merei et al. 1997), the actual knowledge of the mechanism and the genetic control in the differentiation of the trachea and oesophagus (Litingtung et al. 1998; Minoo et al. 1999), as well as of the normal development of the foregut in humans (O'Rahilly & Boyden, 1973; O'Rahilly, 1978; Sutliff & Hutchins, 1994), led us to believe that, probably, the pathogenesis of each of these anomalies is not always the same (Kluth et al. 1987).

Association of oesophageal atresia and tracheo-

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oesophageal fistula could be the result of disorders in foregut development. Several explanations have been proposed to understand its origin: (1) abnormal arrangement of the system of endodermal folds (Kluth et al. 1987); (2) failure of the tracheal bud to develop normally from the primitive foregut (Merei et al. 1997, 1998), with 'trachealisation' of its proximal part and agenesis of the oesophagus (Merei et al. 1997, 1998) or disappearance of the upper posterior part of the oesophagotracheal tube (Possögel et al. 1998); (3) the oesophageal atresia as the initial anomaly preceding the development of the tracheo-oesophageal fistula that arise from the middle branch of a tracheal trifurcation (Crisera et al. 1999); (4) the configurational abnormality in the area of the developing lung bud in Carnegie stage (CS) 12 (Sutliff & Hutchins, 1994). The underlying mechanism may be a disturbance in the epithelial-mesenchymal interaction that leads the patterning of the foregut into trachea and oesophagus (Minoo et al. 1999). The genetic basis could be found in the mutation in sonic hedgehog because this gene is required for the normal development of the trachea, lung and oesophagus (Litingtung et al. 1998).

The pathogenesis of isolated atresia and fistula could represent faulty development of an already differentiated organ (Kluth et al. 1987). Atresia alone could be produced by compression or/and interruption of the blood supply of the oesophagus, by discordant growth between its mesodermal and endodermal components or because of failure in the recanalisation of the oesophageal epithelium (for review see Merei et al. 1997). Isolated tracheo-oesophageal fistula could be caused by close proximity of the epithelia of both organs (O'Rahilly & Müller, 1984; Kluth et al. 1987).

This last hypothesis suggests that the relative position and degree of separation between the trachea and oesophagus throughout its development might be relevant facts involved in the origin of this anomaly. Despite the fact that the longitudinal growth of both organs (O'Rahilly & Müller, 1984) and the changes of position of the trachea in relation to the vertebral levels have been studied (Müller & O'Rahilly, 1986), and the tracheal bifurcation has been established as the most frequent site of tracheo-oesophageal fistula (Holder & Ashcraft, 1970) and also where the trachea reaches its closest relationship with the oesophagus (O'Rahilly & Müller, 1984; Müller & O'Rahilly, 1986), the sequence of changes in the degree of separation between both tubes during the human embryonic period are still unknown. Where and when are both lumina closest together throughout the embryonic period? What is the relevance of the differences in separation along them? These are questions that our work aims to answer by means of morphometric methods, as well as to assess the possible relationship between the degree of separation of both tubes and the origin of the tracheo-oesophageal fistulae.

MATERIALS AND METHODS

Embryos

The study was carried out on a graded series of normal human embryos belonging to the Bellaterra Collection (Prof. Domènech Mateu). They were classified according to O'Rahilly & Müller (1987). The embryos were fixed in 10% buffered formalin, embedded in paraffin, and serially sectioned in the transverse plane. Developmental data of the specimens as well as information on section thickness and histological staining is provided in Table 1.

Tracheo-oesophageal septum (TES): descriptive conventions

We have considered primitive TES all the tissues interposed between the lumina of the digestive and respiratory tubes. At the same time that the coats of both were differentiating, the interposed tissue be-

Table 1. *Data of the embryos and the sections studied*

Developmental data of the embryos*				Cross-section data		
Embryo designation	CS	POA (d)	CRL (mm)	Thickness (μm)	Stain	Sequential selection to morphometric study
Mar	12	26	4	10	H&E	Discarded†
SS.1	13	28	6	10	H&E	1 in 2
RI-1	14	32	6	10	H&E	1 in 2
Du	15	33	8.4	8	H&E	1 in 3
Du-5	16	37	10.3	8	H&E	1 in 5
Gi.1	17	41	13	10	H&E	1 in 4
Re.1	18	44	15	10	H&E	1 in 4
Du-8	19	48	18	10	H&E	1 in 4
Ri.2	20	50	19	10	H&E	1 in 4
Fu.18	21	52	22	10	H&E	1 in 8
Gi-2	22	54	25	10	H&E	1 in 8
Sam	23	57	30	10	Azan B	1 in 8

* According to the catalogue of the Bellaterra Collection (Prof. Domènech Mateu). † Because the lumen in the respiratory primordium is not present, this embryo has been discarded in the morphometry. For explanation of abbreviations, see note to Table 2.

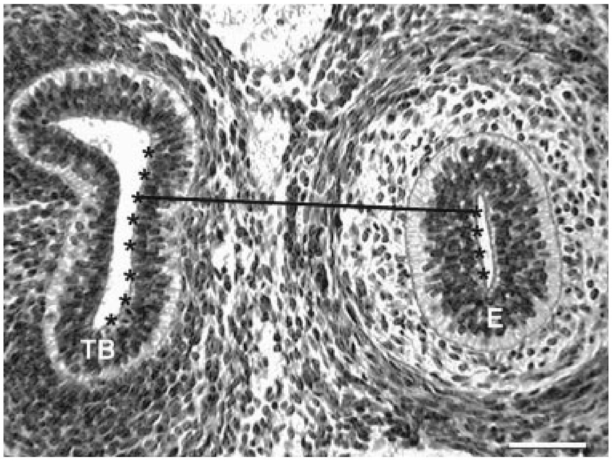

Fig. 1. Cross-section of the embryo Re-1 (CS 18). On each selected section the minimum distance (line) between the respiratory and the digestive lumina was obtained among all the distances measured from the coordinates of opposite points (asterisks) on the apical surface of their epithelial layers. From this stage (CS 18) the distal part of the trachea come closest to the oesophagus. Note the considerable degree of differentiation reached in the walls of the oesophagus (E) and the tracheal bifurcation (TB). Bar, 25 μm.

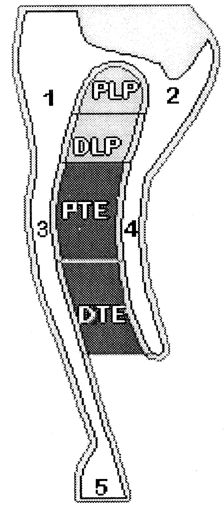

Fig. 2. Diagrammatic representation of the segments of the tracheo-oesophageal septum. Two portions were defined at the septum: the laryngopharyngeal portion (light areas) between the pharyngeal (1) and the laryngeal (2) lumina, and the tracheo-oesophageal portion (dark areas) between the oesophageal (3) and tracheal (4) lumina. Each portion was divided in 2 equivalent segments: the proximal (PLP) and the distal (DLP) laryngopharyngeal segments and the proximal (PTE) and the distal (DTE) tracheo-oesophageal segments. 5, gastric cavity.

tween them was becoming the septum. While these tubes did not reach a sufficient degree of differentiation to allow a clear distinction between the larynx and the trachea, and between the pharynx and the oesophagus, we delimited them in accordance with the descriptions made by Zaw-Tun (1982), Zaw-Tun & Burdi (1985) and Sañudo & Domènech-Mateu (1990). Thus, we have divided the TES in laryngopharyngeal and tracheo-oesophageal portions.

Morphometric methods

In order to evaluate at every stage the level where the lumina of the respiratory and digestive tubes were closest, distances between both were calculated on sequentially selected cross-sections. The sequence was determined in relation to the crown-rump length (CRL) of the embryo and the thickness of the sections (Table 1). The first section was randomly chosen from the separation point between the digestive and respiratory lumina. Sections below the tracheal bifurcation as well as the bronchial tree were not considered. These measurements could not be performed in the embryo Mar (CS 12) because it had a solid respiratory primordium.

The distances between both lumina were calculated from the coordinates of the opposite points on the apical surface of each epithelial (endodermal) layer. To obtain the coordinates an image-analyser consisting of a microscope (Nikon, Optiphot-2), video-camera (Sony, CCD Iris), workstation with monitor (Silicongraphics) and suitable software (Visilog 5) were used. These distances were calculated by means of the following equation,

$$DDR = \sqrt{((X_d - X_r)^2 + (Y_d - Y_r)^2)}$$

where DDR is the distance (μm) between 2 points on the internal wall of the digestive (coordinates $= X_d$ and Y_d) and respiratory (coordinates $= X_r$ and Y_r) tubes, respectively. From each section, the minimum distance between the lumina of both tubes (MDDR) was selected (Fig. 1). DDR and statistic calculations were done by SPSS 9.0.

Statistics

Moving average of the MDsDR. In order to smooth the extreme values, the moving average of the MDsDR was calculated at 3 adjacent sections: the proper section, the previous one and the next. The moving averages of each portion of the TES were grouped as the values of 2 equivalent segments: the proximal (PLP) and the distal (DLP) segments of the laryngopharyngeal portion and the proximal (PTE) and the distal (DTE) segments of the tracheo-oesophageal portion (Fig. 2).

Determination of phases during the embryonic period. The possible tendency for both tubes to move closer together or to separate along their lengths has been determined by means of Pearson's correlation coefficient between the moving averages of the MDsDR and the position of the sections ordered in accordance with the sequence used in their selection (Table 1). We defined phase of separation or approximation when there was significant positive or negative correlation, respectively, during consecutive stages.

Proper separation between digestive and respiratory lumina along the TES

Proper separation obtained at the segments of the TES. At each level (section) the separation between the digestive and respiratory lumina was a consequence of the separation reached in the more proximal sections as well as the proper separation of the level. In order to determine the proper separation at a level, the difference between the moving averages of the MDsDR obtained from the level and the previous one was determined. The previous value of the most proximal section was considered 0 because it corresponded to the bottom of the common lumen of the digestive and the respiratory pathways (separation point). When there was an increment of distance (separation) the value of the proper separation was positive, whereas when there was a decrement (approximation) it was negative. We defined the proper separation of a segment as the sum of the proper separations obtained along all its levels.

Degree of proper separation at each embryonic stage. In order to compare the proper separation of the segments of the TES of an embryo (stage), we defined in each segment the degree of proper separation as the percentage of the total separation of the stage. This last parameter was calculated by adding together the absolute values of the proper separation of the segments.

Phasic degree of proper separation. In order to compare the proper separation of the different segments of the TES throughout the separation or approximation phases, we have determined the degree of proper separation obtained at a segment during a phase (phasic degree) by the same methods used to calculate the degree at each embryonic stage but adding together all the values obtained during the phase.

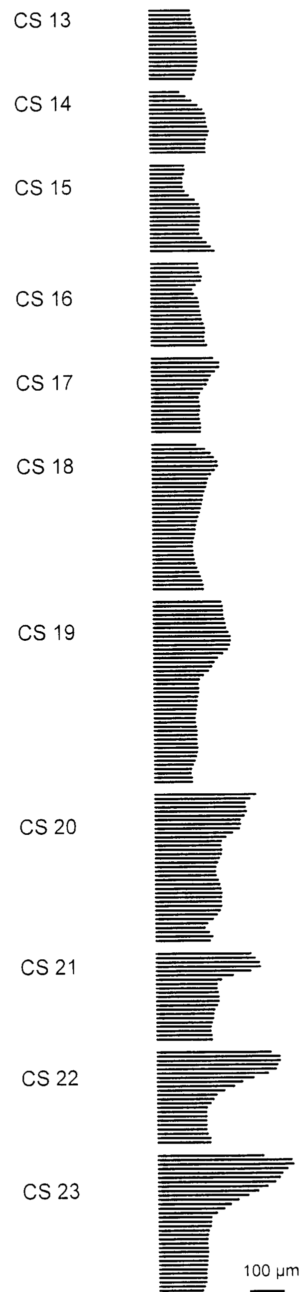

Fig. 3. Minimum width along the tracheo-oesophageal septum. The length of each craniocaudally ordered line corresponds to the moving average of the minimum distances between the digestive and respiratory lumina that has been obtained on each section of a graded series of human embryos. Bar, 100 µm. For explanations of the abbreviations, see footnote to Table 2.

RESULTS

Differentiation in the TES

At CS 12 the respiratory primordium was a solid epithelial bud as well as the tissue that joined it to the foregut wall. We have thus considered this bridge as the initial TES. From CS 13 it was possible to delimit the laryngeal and the tracheal lumina, and the mesenchyme progressively lodged between the respiratory and digestive epithelia. From CS 14 it was condensed at both epithelial tubes. Among the mesenchymal derivatives the following were noted: from CS 17 the muscle coats of the oesophagus appeared (Fig. 1); from late CS 20 the lamina of the

Table 2. *Morphometric data of the segments of the tracheo-oesophageal septum (TES) of a graded series of human embryos*

	Moving averages of the minimum distances between the lumina of the digestive and the respiratory tubes (μm)								Proper separation (μm)				Degree of proper separation (%)			
	PLP		DLP		PTE		DTE									
CS	Mx	Mn	Mx	Mn	Mx	Mn	Mx	Mn	PLP	DLP	PTE	DTE	PLP	DLP	PTE	DTE
13	166.3	163.3†	166.6	166.6	190.1	172	192.7*	172.3	166.3	0.3	23.5	−17.9	80.0	0.1	11.3	−8.6
14	142.6	118.9†	191	167.1	228.9	209.8	235.4*	220.7	142.6	48.4	37.9	−7.0	60.4	20.5	16.1	−3.0
15	139.2	129.3†	137.1	137.1	197.6	178.3	254.4*	191.9	129.3	7.8	59.2	58.1	50.8	3.1	23.3	22.8
16	203	190.3	199.7	163.9†	201.6	190.8	222.2*	211	203.0	−32.1	30.7	20.6	70.9	−11.2	10.7	7.2
17	269.3*	245	235.3	219.3	205.1	187.7†	195.6	188.9	252.3	−33.1	−26.4	2.2	80.4	−10.5	−8.4	0.7
18	258.2	176.1	260.1*	221.9	217.4	169.2	201.5	159.3†	258.2	−36.3	−52.7	32.3	68.0	−9.6	−13.9	8.5
19	285.5	269.4	305.7*	288.5	275.4	167.2	174.4	146.6†	285.5	8.6	−126.9	−15.3	65.4	2.0	−29.1	−3.5
20	400.3*	356.8	353.6	307.9	281.3	239.5	263.9	196.9†	362.5	−54.6	−47.9	−43.3	71.3	−10.8	−9.4	−8.5
21	409.7	375.3	413.6*	374.3	305.6	241.6	232.4	216.9†	409.7	−35.4	−131.1	−21.7	68.5	−5.9	−21.9	−3.6
22	487*	451.3	470.5	382.1	339.1	198.9	210.9	195.3†	476.4	−94.3	−183.2	12.1	62.2	−12.3	−23.9	1.6
23	534.1*	417.7	490.7	462.9	431.8	195	196.4	170.5†	510.4	−47.5	−266.2	−26.2	60.0	−5.6	−31.3	−3.1

* The maximum value obtained at a stage. † The minimum value obtained at a stage.
Abbreviations (and for Table 1 and Figs 1–3): B, Bielschowsky; CRL, crown-rump length; CS, Carnegie stage; DLP, distal segment of the laryngopharyngeal portion of the TES; DTE, distal segment of the tracheo-oesophageal portion of the TES; H&E, Groat's haematoxylin and eosin; Mn, minimum value; Mx, maximum value; POA, postovulation age; PLP, proximal segment of the laryngopharyngeal portion of the TES; PTE, proximal segment of the tracheo-oesophageal portion of the TES.

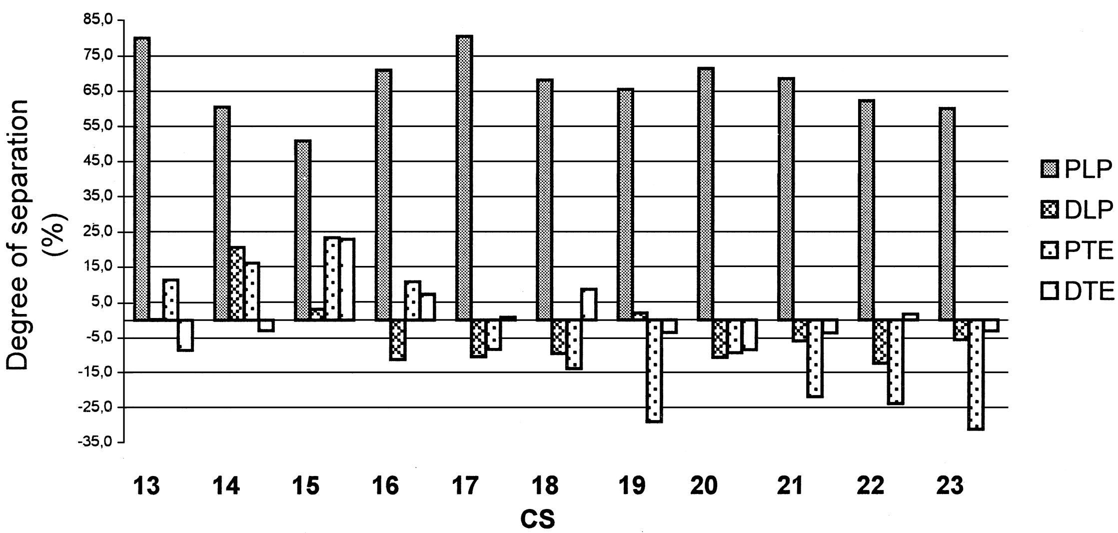

Fig. 4. Comparison of the degrees of proper separation between the digestive and respiratory lumina obtained at the segments of the tracheo-oesophageal septum of each embryo. For explanations of abbreviations, see footnote to Table 2.

cricoid cartilage formed and at CS 23 some tracheal cartilage was partially located between the tracheal and oesophageal lumina.

Maximum and minimum separation between the lumina of the digestive and respiratory tubes

The moving averages of the MDsDR of each embryo are shown in Figure 3.

The value 0 of the point of separation between the larynx and the pharynx was discarded. The minimum values of the moving averages of the MDsDR were obtained at segments of the TES located more and more caudally (Table 2). The maximum values of the moving averages of the MDsDR are listed in Table 2.

Phases determined by the tendencies of the moving averages of the MDsDR

Two phases were determined: phase of separation (CS 13–16) and phase of approximation (CS 17–23).

Proper separation and degrees of proper separation obtained in the laryngopharyngeal and tracheo-oesophageal segments of the TES

The values of the proper separations and the degrees of proper separation between the digestive and respiratory lumina obtained at each segment of the TES and at each stage are shown in Table 2 (see also Fig. 4). The values of the phasic degrees of proper separation between both lumina obtained at each

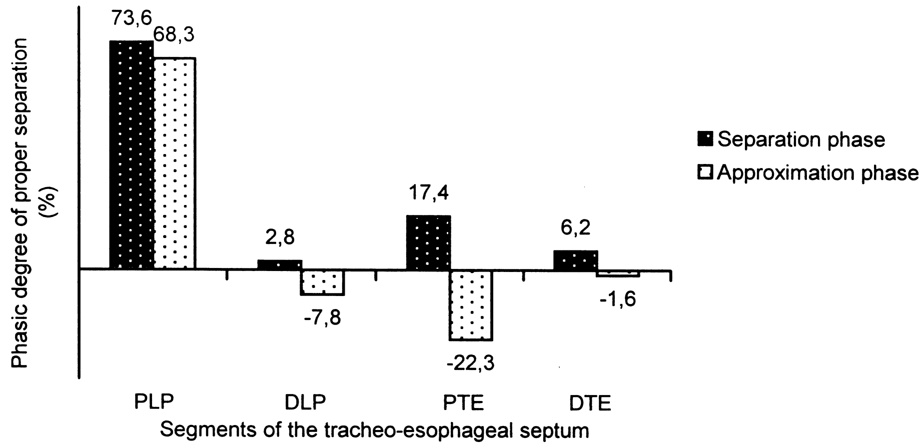

Fig. 5. Phasic degree of proper separation obtained at the segments of the tracheo-oesophageal septum during the phase of separation and approximation. The values of each segment correspond to the percentage that the proper separations obtained from it represent in relation to the total separation (absolute values) of the phase. For explanations of abbreviations, see footnote to Table 2.

septal segment during the phases of separation and approximation are shown in Figure 5.

DISCUSSION

The respiratory primordium appears as a widened terminal portion of the laryngotracheal sulcus at CS 10. By the end of the CS 11, the respiratory primordium is identified as a knoblike thickening of the epithelial wall. From CS 12 the respiratory primordium (lung primordium) grows caudally on a lengthening stalk that develops in proximodistal sequence (O'Rahilly & Müller, 1984; Sutliff & Hutchins, 1994). The cephalic end of the stalk develops into the larynx, while the rest becomes the trachea (Zaw-Tun, 1982; Zaw-Tun & Burdi, 1985; Sañudo & Domènech-Mateu, 1990). At CS 13 the buds of the main bronchi are present (O'Rahilly & Boyden, 1973; O'Rahilly & Müller, 1984).

As a consequence of the rapid growth of the heart and liver, the portion of foregut between the respiratory and hepatic primordia undergoes an early growth in length and as a result, is stretched (Zaw-Tun, 1982). The laryngeal part of the pharynx, the oesophagus, the stomach and the proximal part of duodenum are derived from this portion.

The respiratory primordium grows caudally into the mesenchyme ventral to the foregut (O'Rahilly & Müller, 1984). This splachnic mesenchyme, through epithelial-mesenchymal interactions, has been proposed as crucial in the morphogenesis of the trachea and the lungs (Shannon, 1994; Minoo et al. 1999). The part of this mesenchyme that comes to lie between the respiratory and digestive tubes, together with their epithelia, constitute the TES (O'Rahilly & Müller, 1984). The coats of the opposite walls of the respiratory and digestive tubes arise from the differentiation of this primitive septal tissue, as well as the connective tissue that ultimately represents the definitive TES. Nowadays it is accepted that in the human embryo the respiratory primordium, and subsequently the trachea, grows caudally independently of the digestive tube (Zaw-Tun, 1982). Thus the TES do not arise from the growing together of epithelial ridges into a common chamber, according to the classic explanation (Smith, 1957).

Since in the origin of the isolated tracheo-oesophageal fistula there should be some alteration in the normal embryogenesis that results in the fusion between the digestive and respiratory lumina (see Introduction), the relative changes of position and the degree of separation that occur between them during the human embryonic period are relevant facts but not yet understood. Our study has been an attempt in this direction, applying morphometric methods on serial cross sections of a graded series of human embryos.

In the planning of our study, we considered that because the respiratory system has a caudal hanging growth, the distance that at every level separated it from the adjacent digestive tube was a consequence both of the separation obtained in the proper level and of the other one passively acquired by the separation obtained in the more proximal levels. According to whether the distance at a given level was greater or smaller than that measured at the previous level, its proper separation was positive (separation) or negative (approximation), respectively.

The PTE was the only segment where the proper separations always had signs in concordance with the phase (Fig. 4, Table 2): positive at separation phase and negative at approximation phase, and where important degrees of separation were obtained.

During the separation phase (CS 13–16), the spacing out between the digestive and respiratory lumina occurred with greater intensity in the PTE than in the DTE, while it was insignificant in the DLP (Fig. 5). However, throughout all embryonic stages studied the separation obtained in the PLP was much longer than in the other segments (Figs 4, 5, Table 2), because of the increased growth of the larynx and the particular characteristics in the formation of its cavities (Zaw-Tun & Burdi, 1985; Sañudo & Domènech-Mateu, 1990). We think that at the beginning of this phase, when both the larynx and the trachea start to elongate (O'Rahilly & Müller, 1984), the mechanical influence of the initial gastric dilatation (Macarulla et al. 1996) displacing ventrally the overlying respiratory primordium could explain why the larynx and the trachea diverged from the supragastric parts of the foregut. Because the oesophagus elongates more rapidly than the trachea (O'Rahilly & Müller, 1984) the stomach is progressively displaced to a more caudal position than the respiratory primordium and its bronchial derivatives. According to O'Rahilly & Müller (1984), at CS 15 the lobar buds appear growing dorsally and embracing the oesophagus, but we have not observed that the tracheal bifurcation subsequently became the part of the trachea closest to the oesophagus as these authors postulated. On the contrary, throughout the separation phase the digestive and respiratory lumina were always closer at the laryngopharyngeal segments of the TES than at the tracheo-oesophageal segments, and they reached their maximum separation at the DTE (Table 2), which ended in the tracheal bifurcation. As the oesophagus is not totally fixed to the oesophageal hiatus until CS 17 (Nebot-Cegarra & Domènech-Mateu, 1980), the changes of shape, volume (Macarulla et al. 1996) and position (Nebot-Cegarra et al. 1999) that the stomach undergoes during previous stages, could have some influence on the position of the oesophagus.

During the phase of approximation, the digestive and respiratory lumina (CS 17–23) reached their greatest separation at the laryngopharyngeal segments of the TES (Table 2). Below this level, both lumina were closest at the tracheo-oesophageal segments (Table 2), as a consequence of the approximation which occurs in the DLP, and principally in PTE (Fig. 5). From CS 18, it was at the DTE where both tubes were closest (Table 2), more as a consequence of the approximation carried out at proximal segments than of the insignificant proper changes which occur in this one (Figs 4, 5). These chronological data are in disagreement with O'Rahilly & Müller (1984), since they observed from CS 15 the region of the tracheal bifurcation as the closest to the oesophagus. These authors did not precisely state what the distance considered was; perhaps they alluded to the separation between the external margins of both organs, while we measured distances between luminal surfaces (Fig. 1). In this phase the oesophagus continues elongating more quickly than the trachea (O'Rahilly & Müller, 1984), and its thoracic and abdominal parts become delimited by the oesophageal hiatus. The trachea stabilises its descent from CS 18 (Müller & O'Rahilly, 1986) and the respiratory tree grows and spreads into both pericardioperitoneal canals (from CS 21 pleural cavities) so that at CS 20 the lungs half fill them (O'Rahilly & Boyden, 1973). These data show a scenario different from the separation phase, because the expansion of the respiratory tree adapting into these coelomic spaces could cause tensions on the trachea and the larynx and, as a consequence, modifications in their orientation.

The growth and migration of other organs, and the changes in the body size and flexures could also be involved in modifications in the separation between the digestive and respiratory tubes. It is possible that the oesophagus straightens subsequently in response to the stretching that it undergoes when the liver and heart grow rapidly (Zaw-Tun, 1982).

Close proximity of the epithelia of the trachea and the oesophagus (O'Rahilly & Müller, 1984; Kluth et al. 1987) and a favourable structural arrangement (O'Rahilly & Müller, 1984) have been proposed as the main conditions to produce isolated tracheo-oesophageal fistula. The most frequent site of the fistula is in the vicinity of the tracheal bifurcation (Holder & Ashcraft, 1970). In consequence, when the region of the tracheal bifurcation came closest to the oesophagus (CS 18) we should expect at this level a poorly differentiated mesenchymal tissue, but on the contrary in both organs it has acquired a considerable degree of differentiation from CS 17 (Fig. 1). This fact could lend indirect support to Kluth et al. (1987), who suggested that the tracheo-oesophageal fistula is induced by an abnormal localisation of the organs, resulting in too close an apposition of the epithelia of the oesophagus and the trachea. Nevertheless, it has not been possible to corroborate any of these hyphotheses in man, since we are not aware of any published case of a human embryo with an isolated tracheo-oesophageal fistula (Nebot-Cegarra & Domènech-Mateu, 1989).

In conclusion, during the human embryonic period the digestive and respiratory tubes undergo separation of their lumina in accordance with 2 opposite phases. From CS 13 to CS 16, both tubes have a tendency to separate principally at the PLP but also at the PTE, and reach the widest separation of their lumina at the tracheo-oesophageal segments of the TES. Between CS 17 and 23, below the point at which they reach their widest separation (laryngopharyngeal septal segments), the tubes tend to come closer together principally at the PTE but also at the DLP. When from CS 18 the trachea and oesophagus reach their closest relationship at the DTE (including the tracheal bifurcation), the coats of both organs have already undergone an appreciable differentiation. Our data suggest that there is no relation between the distal approximation that normally involves both organs and the most frequent appearance of the isolated tracheo-oesophageal fistula at the region of bifurcation.

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