DEVELOPMENT OF THE INTRASEGMENTAL BRONCHIAL TREE: THE PATTERN OF BRANCHING AND DEVELOPMENT OF CARTILAGE AT VARIOUS STAGES OF INTRA-UTERINE LIFE *

BY

U. BUCHER† AND LYNNE REID

From the Institute of Diseases of the Chest and Brompton Hospital, London

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The purpose of this study is to establish the progress of branching of the intrasegmental part of the bronchial tree at different phases of intra-uterine life and the relation to it of the development of bronchial cartilage.

The importance of the topography of the pulmonary segments to both the surgeon and the clinician has led in recent years to the study of the growth of the segmental structures (Boyden, 1955), and, in order to establish that post-natally new branches of the bronchial tree appear, the most peripheral parts of the respiratory system have previously also been studied (e.g., Willson, 1928). But there is little published work describing the development of the region in between, i.e., the intrasegmental bronchi and bronchioli.

INTRASEGMENTAL BRANCHING

In the five-week foetus (crown-rump length of 8 to 10 mm.) the lobar bronchi are present as small outgrowths from the two primary stem bronchi (Heiss, 1919). Boyden (1955) has described the way in which, in a "burst of activity," these lobar bronchi subdivide so that segmental bronchi are recognizable from the sixth week. During the weeks following, the appearance of the lung is commonly described as "glandular," because its air spaces are enclosed by epithelium surrounded by solid mesenchyme, i.e., the bronchial tree is blind at its distal end and completely lined by epithelium. Increasing vascularization and differentiation into hollow alveoli gives rise to what is known as the "canalicular" phase, which lasts from the sixteenth to the

twenty-fourth week and is marked by the bronchial tree becoming open at its end (Dubreuil, Lacoste, and Raymond, 1936; Palmer, 1936b; Loosli and Potter, 1951); after this the "alveolar" phase is seen, when the patterns resemble that at term (Arey, 1954).

Counts of the number of bronchial branches in single segments have been made by Palmer (1936a), who, in an 18-week foetus (crown-rump length 152 mm.), found 17 divisions between trachea and terminal bronchiolus, counting the left main bronchus as one and, following its pattern of branching, each time selecting the smaller of its divisions and choosing, when two were equal, that which led him in a cranial direction. He does not say what segment he used, but it was possibly the apical segment and the pathway would probably be one of intermediate length. He found (1936b) the same number of divisions in a foetus of 21 weeks (crown-rump length 170 mm.), but also detected capillaries advancing between the epithelial cells of the last three generations included in his count. Broman (1923) found 18-19 generations in the right middle lobe of a newborn baby, counting the trachea as one and including respiratory bronchioles.

In adult lungs the number of branches arising from the axial pathways varies from segment to segment (Hayward and Reid, 1952a), although a characteristic range for each segment can be recognized. Counting the segmental bronchus as one, they found that the number of branches in the lingula, middle lobe, and posterior basal segment was as great as 25; in the apical lower it was about 20. These findings, compared with the embryological results, suggest that new branches appear between birth and adult life, which supports the findings in animal lungs (Willson, 1928; Bremer, 1935).

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†Medizinische Universitätsklinik, Berne, Switzerland. The work reported in this paper was carried out during tenure of a grant from the Janggen-Poehn Foundation, St. Gallen.

CARTILAGE

Tracheal cartilage is first seen at the age of 7 weeks (Arey, 1954). Brenek (1941) has reported a more advanced development of cartilage in the left than the right lung in two foetuses approximately 10½ and 11 weeks (52 and 62 mm. crown-rump measurement respectively), but gives no details of her method of counting.

In adult lungs Hayward and Reid (1952b) counted an average of 10 intrasegmental generations containing cartilage, the minimum, eight, being found in the apical segment of the lower lobes, the maximum, 11, in the lower lingular segments. Von Hayek (1953), following narrow bronchi, counted eight divisions between trachea and the ultimate plate of cartilage, and 13 when counting the larger one at each point of division. The number of branchings distal to the last plate of cartilage varies considerably, for cartilage has been found as close as four divisions from the end of a pathway and in others even seven or eight (Reid, 1958).

MATERIAL AND METHODS

The material examined by us was taken from the lungs of 14 foetuses between the age of 10 weeks and term (Table I). To establish the pattern and progress of branching it was necessary to count the number of branchings (*a*) in different segments in the same foetus and (*b*) in foetuses of various ages.

Although counts were made of pathways in all segments, because of the formidable task of tracing the whole 18 segments at different stages of development certain segments were selected for more intensive study, namely, the apical and anterior segments from the right and left upper lobes, the posterior segment of the right upper lobe, and the segments in the middle lobe and lingula.

Cutting and Staining.—After fixation in formalin, the lung was embedded in paraffin, either whole or in blocks; the trachea was not sectioned. Sections were stained either with haematoxylin-eosin, periodic-acid-Schiff (Hotchkiss-Macmanus), or Verhoeff-van Gieson, occasional sections being selected for additional mucus stains which will be described later.

Serial sections were performed in the planes indicated in Table I, the thickness of the sections being 7 µ; the horizontal plane was generally found the more satisfactory, because more pathways seemed to be cut at right angles than when a vertical plane was used. From the lungs of the younger foetuses all sections were studied, while of the bigger lungs, although not every section was used, never less than every fifth was examined.

Methods of Counting.—Table I gives details of the pathways selected for counting. Our purpose being to compare the development of different segments, we selected the segmental bronchus as the "first generation" and thus avoided reckoning presegmental branching (Fig. 1), which varies considerably from different segments and bears no relation to the length of intrasegmental pathways (Boyden, 1955).

TABLE I
NUMBERS OF SECTIONS, PATHWAYS SELECTED, AND PLANE OF CUTTING

No.	Gestation (Weeks)	Crown-rump Length (mm.)	Length of Life (Days)	Specimen	No. of Blocks	Plane of Cutting	Selected Sections (each 7 µ)	Selected Pathways	Abnormalities
211	10	40	—	Whole foetus	1	Coronal	All	Axial p. of R 1, 3, 4, 5 L 1, 2, 3, 4, 5	Incomplete fissure ML/RLL and RUL/ML
467	12	75	—	Both lungs	1	"	"	R 1, 3, 4, 5 axial L 1, 2, 3, 4, 5 "	
511	13	85	—	Left lung	1	Horizontal	"	L 3, 4 "	Apico-anterior stem
510	14	100	—	" "	1	"	"	L 1, 4, 5 "	Incomplete fissure LUL/LLL
295	16	127 (twin 133)	—	RUL + ML LUL	1 1	Sagittal	" "	R 1, 4, 5 1, 2, 3 non-axial	
36	24	CH 320	3	Both lungs	8	Horizontal	Every eighth	L 1, 4, 5 axial 6 shortest R 1, 3 axial 1, 2, 3 non-axial L 2, 3 R 1, 3, 4 axial 2, 3 non-axial L 1, 4, 5, 6, axial	
235	25	?	—	RUL + ML LUL	3 3	"	" fifth "	R 1, 3, 4 axial 2, 3 non-axial	
613	28	240	?	RUL + ML LUL	3 2	"	All	L 1, 4, 5, 6, axial R 1	Incomplete fissure RUL/ML
234	32	275	5	ML	3	"	"	R 1	
293	35	?	?	LUL	4	"	" fifth " third-fourth	R 1 axial 3 non-axial; shortest	
287	41	?	?	LUL	5	"	" fourth (Block 1: all)	R 1, 3, 5 axial 4 non-axial	
233	About 40	?	3	LUL	3	"	Every tenth	L 3 non-axial	
289	" 40	?	?	LUL	4	"	All	L 1

FIG. 1.—Variation in number (Roman) of presegmental generations. Segmental bronchi, shaded black, standard nomenclature 1–10. (*Thorax*, 1950, 5, 222.)

Where segments are designated by number the nomenclature accepted by the Thoracic Society (1950) has been used. This substantially resembles that of Jackson and Huber (1943), the one adopted by Boyden (1945, 1955). In the Thoracic Society classification the posterior segment of the upper lobe is numbered 2, the anterior segment 3. In the American classification these are reversed.

Not only do the various segmental bronchi represent different orders of generations (Fig. 1) if the trachea is considered the first generation, but they are not all formed at the same time, and to compare bronchial segments which belong to the same period of development it might be thought desirable to relate them to the various phases of bronchial budding. Boyden (1955) points out, however, that even at segmental level the variation in time and site of developing bronchi is such that no sequential pattern can be established.

Axial pathways were chosen for counting the branchings because they yield the maximum number of branches for any one segment and thus enable intersegmental comparison. By "axial pathway" is meant the pathways beginning at the hilum and running to the distal surface of the pleura (Hayward and Reid, 1952a). Sometimes, however, a pathway passes direct to the pleura and then turns at right angles, running parallel to it for some distance (Fig. 2-B). To show any difference between segments a count should be maximal, and therefore should include the whole pathway and not stop short at the point at which it first meets the pleura. Lateral pathways arise from the axial and, running a shorter course to supply the more proximal parts of a segment, have fewer branches.

Difficulties.—When following the large bronchi toward the periphery it is often difficult to tell at bifurcations which branch represents an axial pathway. Preliminary examination of various levels with a low-power microscope enabled rough sketches to

be made of the general topography of the segmental bronchi; and it was thus possible to select definitely axial pathways for counting, by choosing a subpleural terminal bronchiolus near the main segmental axis, which was then traced centrally. The final counting of the generations was done by following the selected pathway step by step and noting every branch. Nevertheless, as indicated above, when the direction of the pathway is oblique to the plane of cutting, detection of all the generations is more difficult than when it runs at right angles and each branch can easily be seen.

Our count started with the segmental bronchus and included the branches of the bronchial tree lined with epithelium. Bronchi are taken to be those airways proximal to the most distal piece of cartilage found along any bronchial pathway, tracing it from the hilum; bronchioli are those airways beyond this point, the terminal bronchiolus being the last whose epithelium is complete. More distal are the respiratory bronchioli, those whose epithelium is broken by alveoli opening into their lumen. The distinction between the bronchiolar and the respiratory part of the lung cannot be recognized until after the sixteenth foetal week, as before that age the lungs still have a "glandular" appearance, the whole bronchopulmonary system being covered with a continuous epithelium (Fig. 3). In the early foetuses this included each blind end of the bronchial tree, the so-called "alveolar" bulb, while in the post-sixteenth-week foetuses the last generation counted was the terminal bronchiolus. The effect on the counts of the conversion of the final branches of the bronchial tree to respiratory bronchioli is discussed below.

FIG. 2.—Illustration of intrasegmental branching showing axial pathways, A passing to pleura, B turning at right angles to it; and C the shortest possible course within the segment.

FIG. 3.—Ultimate generations of the bronchial tree at various stages of intra-uterine development; 16 weeks, bronchial tree completely lined with epithelium; 24 weeks, epithelium interrupted by ingrowth of capillaries, i.e., respiratory or alveolar part arises from transformation in the terminal branches; 32 weeks, additional growth respiratory part.

Usually a terminal bronchiolus divides into two branches, but not infrequently these are unequal in their subsequent distribution, one being a respiratory bronchiolus with an incomplete epithelial lining, and the other having an intact epithelial lining which means it is itself terminal or preterminal (Fig. 4). Although the parent bronchiolus before it divides must be considered as the terminal bronchiolus when referring to the first branch, in total counts of genera-

tions of the bronchial tree the most distal terminal bronchiolus was included.

Cartilage.—In every pathway the distance, i.e., the number of generations in which cartilage was present was noted. Precartilage is formed from mesenchyme by proliferation, enlargement, and differentiation of chondroblasts (Arey, 1954). With haematoxylin-eosin stain a homogeneous hyaline matrix is seen between the chondroblasts, which, with special stains, is shown to include collagenous fibres. The matrix, probably a secretion of the cells (Maximow and Bloom, 1952), is composed of a protein of the collagen group together with chondroitin sulphate, the latter being responsible for the basophilia (Pearse, 1960). Cartilage was always easily recognized from the shape and arrangement of the chondroblasts, even where the typical staining properties had not yet developed.

Since the ultimate pieces of cartilage are normally to be found at bifurcations these were examined with special care. The position of the ultimate cartilage was expressed in numbers of preceding generations, e.g., where cartilage or precartilage in a given pathway is said to extend until its twelfth generation, this means that the last bit of cartilage was found within the twelfth generation or at the bifurcation between twelfth and thirteenth.

RESULTS

The following salient features emerge from this study; they concern the way the bronchial tree branches and its cartilage plates develop.

NUMBERS OF BRANCHES.—The maximum number of branches, when development is complete, varies from segment to segment, although for any segment a characteristic range is recognizable. Table II shows the maximum number of generations found along an axial pathway in each of the apical and anterior segments of both upper

FIG. 4.—Reconstruction, from serial sections of a 32-week foetus, of arrangement of terminal bronchioli (the pathways immediately before those into which alveoli open in the periphery of the lung). A to F, respiratory bronchioli; 1, terminal bronchiolus to A; 2 to B; 3 to C and D; 4 to E and F.

TABLE II
MAXIMUM NO. OF BRONCHIAL DIVISIONS ALONG
AXIAL PATHWAYS IN SELECTED SEGMENTS

	Upper Lobe			Middle Lobe and Lingula		Lower Lobe		
	Apical 1*	Posterior 2	Anterior 3	Lateral 4	Medial 5	Anterior 8	Medial 9	Posterior 10
Right	17	20	22	19	26	Right and left 24	18	25
Left	18		24	Inferior 26 Superior 25				

* Segmental numbers as in Thoracic Society Report (*Thorax*, 1950, 5, 222).

lobes, the posterior of the right upper lobe, both segments of the right middle lobe and lingula, and the three basal segments of the lower lobes taken together.

Segments in which the maximum is over 20 are the anterior segment of the upper lobes, both segments of the lingula, the medial segment of the right middle lobe and the anterior and posterior basals.

If the mean of the counts in fetuses over 16 weeks of age is taken, a similar pattern emerges (Table III). The apical segment on both sides

TABLE III
MEAN VALUES OF NO. OF BRONCHIAL DIVISIONS ALONG
AXIAL PATHWAYS IN SELECTED SEGMENTS

	Upper Lobe			Middle Lobe and Lingula		Lower Lobe		
	Apical 1	Posterior 2	Anterior 3	Lateral 4	Medial 5	Anterior 8	Medial 9	Posterior 10
Right	15	16	17		23	Right and left 19		21
Left	15		18	Inferior 20 Superior 22				

gives 15 branches, the anterior 17 on the right and 18 on the left. The posterior on the left gives 16. The lingula gives 22 and 20 and the medial segment of the right middle lobe 23. The anterior basal segment gives 19, the posterior 21 (the former showing on naked-eye examination considerable variation in shape and size).

Rarely do axial pathways in the segments of the upper lobe have as many as 20 generations; the high counts of 20 and 22 were from the same foetus at the sixteenth week. The apical segments of both lower and upper lobes seem to have a similar rate of growth. At the age of 10 and 12 weeks one or two more generations were found in the apical segment of the right upper lobe than in that of the left, and similarly it seemed that

there were rather more in the middle lobe than in the lingula. By the sixteenth week no such difference between the right and left lung could be detected.

COMPLETION OF DEVELOPMENT. — Sufficiently detailed counts were carried out in certain of the upper and middle lobes and lingula to show that in some segments the development is complete by the fourteenth week, while in others it continues until the sixteenth week (Fig. 5). Generally it is in the longer segments with higher maximum counts that growth continues, but by the sixteenth week all the bronchial tree present at birth is formed, and the maximum "burst" of intra-segmental activity would seem therefore to be between the tenth and fourteenth week with a continuation for some segments until the sixteenth week.

LAST PHASE OF DEVELOPMENT. — Soon after the sixteenth week of gestation, in all segments in which counts were made there was a reduction in the number of generations. This is associated with the canalicular phase when, by ingrowth of capillaries between the epithelial cells lining the bronchial tree, an alveolar or respiratory part of the lung appears by conversion of what was the blind end of the bronchial tree (Fig. 3). The maximum number of bronchial branches, i.e., non-respiratory ones, is not regained by birth, which means that after the twenty-fourth week, even if the respiratory part of the lung grows, no type of growth to increase the bronchial divisions completely lined by epithelium seems to occur.

CARTILAGE. — Cartilage continues to grow after bronchial branching is complete, new cartilage appearing until the twenty-fifth week but with no burst of activity such as characterizes branching.

GROWTH RELATED TO BRONCHIAL GENERATIONS. — Cartilage can be identified in the trachea and the main bronchi at the age of 10 weeks, but does not reach the level of the segmental bronchi until the twelfth week, that is, until about six weeks after these are formed. Thereafter, plates of cartilage appear in steady progression further to the periphery (Table IV; Fig. 6, line B) and without increased activity at any special age. Line C illustrates the rate of development of mucous glands, but this is the subject of a separate paper (Bucher and Reid, 1961).

The extent to which cartilage is found in different axial pathways of a single segment may vary considerably. For example, in a foetus of 24 weeks, along three axial pathways of the anterior segment of the right upper lobe the

TABLE IV
TOTAL NO. OF GENERATIONS AND OF GENERATIONS WITH CARTILAGE (IN BRACKETS) IN VARIOUS SEGMENTS OF FOETAL LUNGS

Age (Wks.)	No.	Right Upper Lobe			Middle Lobe		Left Upper Lobe			Lingula		Right Lower Lobe			Left Lower Lobe		
		1	2	3	4	5	1	2	3	4	5	6	8	9	10	8	10
10	211	8 (—)	8 (—)		7 (—)	8 (—)	6 (—)	5 (—)	7 (—)	6 (—)	6 (—)		5 (—)			4 (—)	
12	467	16 (3)	8 (—)	18 (4)	19 (4)	18 (3)	14 (3)	14 (2)	13 (3)	11 (3)	13 (2)		6 (—)			5 (—)	
13	511								22 (5)	11 (2)	13 (1)		13 (3)		12 (6)	15 (4)	16 (3)
14	510									16 (4)	21 (2)				21 (12)	15 (4)	25 (11)
16	295	16 (6)	20 (7)	22 (8)		26 (9)	21 (5)			15 (3)	25 (8)	16 (5)	24 (7)		24 (6)		
24	294						18 (6)		12 (10)	25 (7)	21 (6)						
36		16 (8)	15 (10)	17 (9)													
25	335	15 (9)	15 (4)	14 (8)						18 (13)	15 (12)	18 (8)					
		17 (8)	17 (12)							14 (7)	15 (9)						
		16 (11)	12 (7)				14 (12)										
28	613	13 (10)	15 (8)				15 (12)								18 (14)		
32	234	14 (9)				22 (16)	14 (10)										
35	293	13 (9)				20 (8)	13 (5)		24 (13)				14 (11)		23 (18)		
							17 (8)		17 (9)								
c. 40	289						15 (12)				23 (16)						
	233						13 (7)		22 (8)		21 (16)						
	287						14 (9)		17 (9)		26 (21)						
							13 (10)				20 (13)

FIG. 5.—Generations of axial pathways at various ages. Upper two lines, the medial segment of the middle lobe and the inferior segment of the lingula show the continued growth to the sixteenth week; shorter segments represented by lower two lines, the apical segments of left and right upper lobes cease to increase at the fourteenth week. Each dot represents the highest count for the particular pathway found in one foetus of a certain age.

FIG. 6.—Summary of intra-uterine development of the intrasegmental bronchial tree. Line A represents the increase in the number of bronchial generations; shaded area between A and A¹ the respiratory part of the bronchial tree (i.e., respiratory bronchioles and alveolar ducts); B the extension of cartilage along the bronchial tree; C the extension of mucous glands. A, B, and C are taken from mean values reached after graphing all counts as in Fig. 5. A derives from Engel's studies and our own counts. The diagram includes adult counts showing the increase in total generations in the post-natal period (data from published figures (ref. see text)).

difference between the number of generations present in each pathway was only three, but in the number of generations with cartilage in their wall it was five. Similarly in a foetus of 25 weeks there was only one generation's difference along three axial pathways of the inferior lingular segment, whereas the extremes of extent of cartilage varied from the seventh to the twelfth generation. At term similar variations in the cartilage were still found (see Cases 289 and 287, Table IV). The figures suggest that the extent of cartilage is related to development of the bronchial generations, in that when the number of generations is higher the further cartilage spreads to the periphery (Table IV). The rate of growth of cartilage, however, does not closely parallel bronchial branching, as it does not show the same burst of activity at any period as does bronchial branching between the tenth and fourteenth weeks.

HISTOGENESIS OF CARTILAGE.—At 10 weeks the plates of cartilage of the main bronchi are developing in zones of precartilage, which consist of concentrations of large cells with round, dark

nuclei (Fig. 7, I), the cytoplasm being palely eosinophilic. The cell limits are distinct and give a slightly positive P.A.S. reaction, but no stronger than many of the undifferentiated mesenchymal cells. The nuclei show very fine, thread-like projections which link one to another. No ground substance and no collagen fibres can be detected. At 12 weeks the central cells of the plates of cartilage in the main bronchi and trachea are bigger and the nuclei appear more widely separated from each other (Fig. 7, II). The cell borders are still slightly P.A.S.-positive, more so in the periphery than in the centre of a plate of cartilage, and less in the distal than in the proximal pieces. These features characterize the precartilage phase.

With development, a ground substance gradually appears between the cells (Fig. 7, III), palely staining and reddish grey with haematoxylin-eosin, though groups of cells (two to four) may remain as a single unit, the outer edge of which is demarcated by a fine P.A.S.-positive line. At 13 and 14 weeks these "capsules" are a very distinct feature of the proximal bit of cartilage (Fig. 7, IV).

FIG. 7.—Stages of cartilage development. I and II (precartilage phase) chondroblasts with their border being slightly P.A.S.-positive; III and IV show increasing development of ground substance between individual cells or groups of cells; clear area of "capsule" remains around the cartilage cell.

Between the capsules lies a homogeneous mass, which stains red with haematoxylin-eosin, deep blue with alcian blue, violet with toluidine blue, but hardly pink with P.A.S. (whereas mucus in the glands stains bright pink), although the edges of the capsules give slightly stronger reaction. At all stages the centrally placed cells are polygonal in outline, but those at the periphery are spindle-shaped and stain more darkly with haematoxylin-eosin than the central ones (Fig. 8). More distally, cartilage has the appearance of the younger cartilage or precartilage described above.

At 16 weeks the ground substance, especially near the edge of the proximal plates of cartilage, stains slightly positively with P.A.S., but staining with alcian blue, mucicarmine, or toluidine blue is still much more marked. In some instances eosinophilia has given way to slight basophilia.

This staining behaviour does not alter markedly until near term, when the matrix is more strongly P.A.S. positive and basophilia may be more obvious in the proximal cartilage. Neither in the proximal nor in the distal plates can collagenous fibres be distinguished with the van Gieson staining, although at 12 weeks silver impregnation reveals an irregular net of intercellular reticulin, which is specially concentrated in the perichondrial region. Later, these fibres are less obvious

within the cartilage, often seeming very short. Even at 24 weeks some of the most distal plates of cartilage are still in the form of precartilage and at term even the distal plates show the more immature staining properties described above.

DISCUSSION

The counts reported here are much higher than those obtained in earlier studies elsewhere. Both Palmer (1936) and Broman (1923) started their counting at a more proximal level than the segmental bronchus. Adjusting for the fact that we counted the segmental bronchus as 1, Palmer found 14 generations in the right middle lobe in an 18-week foetus and Broman found 15-16, a count which must be further reduced for comparison with ours, as it went beyond the terminal bronchiole to include the respiratory bronchioles. High maximal counts such as we established are obtained only by choosing axial pathways, i.e., those having the longest pathway within a segment. The number of segments we have examined, and even of the pathways within a single segment, are much higher than those made in previous studies.

Like Boyden (1955), we found that at 10 and 12 weeks a few (one to five) more intrasegmental generations were present in the right lung than in

FIG. 8.—Plate of cartilage from 13-week foetus. Drawing at the right, magnification of area within square in drawing on the left. From the earliest time that precartilage can be recognized the peripheral cells are flattened while the central cells are polygonal in shape.

the left (Fig. 1, Table IV), but that if presegmental generations were included in the counting the two sides showed much the same activity. While presegmental branches remain the same, at later stages the number of intrasegmental branches in the left lung overtakes that in the right, for we detected no difference between the two.

Our most outstanding finding is that the period in which the bulk of bronchial formations occurs is relatively short, occurring between the tenth and fourteenth weeks (Fig. 5); in this period 65–75% of all branches are formed and later formation of new epithelialized bronchioli is evidently minimal. Boyden (1955) speaks of a burst of activity in bronchial branching between the second and sixth weeks, when the segmental and subsegmental bronchi are formed. There is an even greater burst of activity in the intrasegmental bronchial tree between the tenth and fourteenth weeks, but it seems to slow down in some segments earlier than in others. While in the segments of the upper lobes formation ceases at the fourteenth week, in the lingula and the middle lobe branching goes on until the sixteenth week. This prolonged activity probably also includes the anterior and posterior basal segments and accounts for the final difference in the number of generations as between the segments. This continued growth in the longer pathways may be bound up with the caudad growth of the lung. Wells and Boyden (1954) have emphasized that at an early stage of development the lungs do not fill the pleural space and that growth occurs particularly in a cephalo-caudad direction.

After the twentieth week in all pathways we counted between two and six generations less than in earlier counts in the same pathway. This is due to the fact that we counted (by definition) fully epithelialized generations only, while between the sixteenth and twenty-fourth weeks, i.e., during the canalicular phase, the last few generations of the bronchial tree partly lose their cuboidal epithelium by ingrowth of capillaries and form the primitive respiratory bronchioli. This method of counting could be repeated with more certainty than one based on change in the nature of the epithelium.

Since we found the number of epithelialized generations in foetuses of the age of 24 and 40 weeks to be slightly lower than the figures previously reported for the total number of bronchi and bronchioli in corresponding segments of the adult lung (Hayward and Reid, 1952a), it certainly seems that at some time after the twenty-fourth week of foetal life a transformation must take place of respiratory bronchioli to those com-

pletely lined with epithelium. Animal studies in particular have been directed to determining whether such increase takes place before or after birth and the nature of any growth that occurs, but, in respect of pulmonary anatomy and structure especially, the reservation always necessary when arguing from one species to another is of particular significance. In human lung, Broman (1923) found between birth and adult life an increased number of generations before respiratory bronchioli in the right middle lobe. Engel (1947) also has confirmed an increase in the adult as compared with a child's lung. In animal lungs a similar increase has been shown, for example, in newborn and adult white mice by Willson (1928). These comparisons are between the lung at birth and in adult life. From the appearance in animal lungs suggestions have been offered for the way in which this conversion is brought about. Bremer (1935) observed buds from the last bronchioli in the lung of opossum embryos, a species born early in its development; in newborn rabbits he found sprouts emerging at the periphery of alveoli, but in cats he also observed regression of alveoli and spreading of the cuboidal epithelium from more proximal parts, respiratory bronchioli and alveolar ducts seeming to undergo transformation into terminal bronchioli with thickening of their wall by ingrowth of muscle fibres. Policard and Galy (1945) also suggest that after birth respiratory bronchioles (*bronchioles de transition*) are transformed again into fully epithelialized bronchioli (*bronchioles vraies*) by "*desalveolisation*." They even state that respiratory bronchioli are no longer present in lungs of old persons.

On the other hand, Boyden (1961) suggests that in the dog such transformation does not occur, rather the reverse. He has selected the caudal pathway in the infracardiac lobe, as this can be recognized with certainty in the animal. By recognizing the pattern of branching rather than depending on total counts, he showed a metameric interruption of the peripheral epithelium rather than downgrowth. Unfortunately none of the earlier studies has been carried out on the dog, so that while casting some doubt on previous work it cannot be taken to refute it.

As mentioned above, our counts at about the twentieth week of pregnancy are generally lower than those for the adult (Hayward and Reid, 1952a). The difference suggests that there must be an increase between the two, but our results do not establish whether these respiratory bronchioli are converted to terminal bronchioli before birth, i.e., whether there is a further increase before

birth in the number of airways completely lined with epithelium. In the two segments in which counts would have seemed sufficiently large to justify deduction, the results are somewhat conflicting.

In comparing different segments, either the average of counts made or the single highest count may give an incomplete picture because either may cloak the other. Taking first the apical segment of the upper lobe, the maximum number of pathways after the sixteenth week never exceeds 17, although the highest count before this is 21, while the average of the seven counts between the twelfth and twenty-fifth weeks is 16.5, and of the 13 counts at the twenty-fifth week and later 14. This would seem to indicate that there is no conversion of respiratory to completely epithelialized bronchioli before birth.

The lingula, on the other hand, shows a reverse trend; in one of the lungs at term a count of 26 is included although the maximum before 24 weeks is 25; the average of three early counts is 23 and of the five later ones 22.5. This may reflect a greater range in the lingula, as this segment varies considerably in its shape, particularly its length, and it may also be that our counts are too few for any satisfactory deduction to be made. On the other hand, the variation between the apical segment and the lingula may reflect an actual difference between the two, as we have already seen that the duration and degree of growth is different in the two segments.

ACCESSORY ALVEOLAR COMMUNICATION.—In human lungs "of all ages" as well as those of cats and rabbits, Lambert (1955) found and described accessory communications between distal bronchioli and adjacent alveoli, which consisted of short tubules lined with epithelium and sometimes containing macrophages. We have seen these communications in adult lung, but have not detected them in this foetal material; although serial sections were used and the lungs were mostly well expanded, they were not specially preserved in a fully inflated state. Lambert (1960) has identified these tubules in many children from a few weeks old, but, unlike the adult ones, these specimens were not reconstructed to ascertain over how many generations the tubules were present. In the adult, Lambert (1955) found them over the ultimate three or four generations of a pathway, and it may be that they arise mainly in post-natal development during transformation of alveolar ducts into epithelialized bronchioli, a process which, it has been suggested, occurs as a feature of post-natal growth. The spaces would then

represent points of incomplete spread of epithelium.

APPEARANCE OF CARTILAGE.—Comparison of the development of the intrabronchial cartilage and the branching of the bronchi (Fig. 6) shows that at 16 weeks, when the ante-natal formation of new bronchioli is nearly completed, further cartilage had still to appear and does so until the twenty-fourth week, when it remains at the same level, leaving about five (range three to nine) generations of bronchioli free.

Although the degree to which cartilage extends in pathways of corresponding segments varies considerably, in general terms it seems that cartilage spreads further to the periphery in the longer pathways. Thus we found eight to 10 generations with cartilage in the relatively short axial pathways of the upper lobes and in the lingula 13 to 21. On the other hand, the number of cartilage-free bronchioli proved to be independent of the total length of the pathway, i.e., even in shorter segments a high number might be free.

The number of bronchi with cartilage found in the foetuses after the twenty-fifth week corresponds exactly to the average figures given for the adult lung by Hayward and Reid (1952b) and von Hayek (1953). This means that at about the twenty-fifth foetal week the number of bronchi, defined as those parts of the pathways which contain cartilage, is finally established. This is important, because any increase in the total number of generations of a pathway occurring in late foetal or in post-natal life is an increase in the number of bronchioli.

The ground substance of the cartilage is slow to mature, particularly the mucoprotein. Whereas on its first appearance it already stains typically with alcian blue, toluidine blue, and mucicarmine, it hardly gives a positive reaction with P.A.S. and still has a distinct eosinophilia. Only in the second half of foetal life, basophilia of the matrix and a positive stain with the periodic-acid-Schiff method are evident, and this only in proximal cartilage. The staining reactions which develop earlier may depend on the presence of chondroitin sulphuric acid, while the aggregation of polysaccharide to mucoprotein, being responsible for the positive reaction with periodic-acid-Schiff and for basophilia, seems to develop later (Pearse, 1960).

CLINICAL APPLICATION.—Chronological studies of bronchial development make it possible to give a more precise description and interpretation of congenital anomalies of the lung. From the number of bronchial generations present or involved in a deformity it may be possible to

deduce, as is possible with congenital cardiac anomalies, when foetal arrest occurred. This is especially urgent if environment is to be related to pulmonary defect—a field of investigation not yet opened up. A quantitative expression of bronchial development by reference to generations is particularly useful in the study of hypoplasia, in which it has recently been applied (Reid, 1961; Reid and Areechon, in press).

SUMMARY

The main growth of the intrasegmental bronchial tree occurs between the tenth and fourteenth weeks, during which period 70% of the number of generations present at birth are formed. The longer segments of the lung continue growing until the sixteenth week, and, as a result, the axial pathways of the lingula and middle lobe and anterior and posterior basal segments at term include more generations than those of the apical segments of the upper lobes. In the latter, 14 to 18 generations, on an average, are finally reached by 14 weeks whereas in the former another three to 10 generations are added.

After the twenty-fourth week the total number of fully epithelialized generations (including the terminal bronchiolus) is slightly reduced—by two to four generations—as the result of the transformation of the last few generations into respiratory bronchioli.

Comparison with the available data concerning adult lungs, however, shows that the number of divisions of the bronchial tree lined with epithelium is less at 24 weeks than that described in the adult, and suggests that the transformation of respiratory bronchioli into fully epithelialized branches occurs between the twenty-fourth week of intra-uterine development and adult life. Our material does not establish whether such transformation occurs during foetal life, as only two segments in which counts are frequent, the lingula and the apical segment of the upper lobe, give rather conflicting data.

The general pattern of growth applies to all the segments investigated. After 14 weeks there is no significant difference between the intrasegmental pathways on the right and the left. Although up to this time intrasegmental bronchial pathways on the left have one to five generations fewer than corresponding pathways on the right, the number of presegmental generations is higher on the left than on the right side and the development of the intrasegmental bronchial tree starts earlier on the right side.

The spreading of intrasegmental cartilage occurs steadily between the tenth and twenty-fourth

weeks. By 25 weeks the total number of bronchi containing cartilage is the same as for the adult.

In spite of the great variations in spreading of cartilage, which in general is related to the length of a pathway, it seems that the number of generations free of cartilage is independent of the total length of the pathway.

The cellular formation of cartilage is achieved in two weeks on the average, but the development of the final histochemical properties of the intracellular matrix takes much longer, about 15 to 20 weeks, so that even at term the distal plates of cartilage do not have a mature intracellular matrix.

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*Since this paper went to press, Boyden has lent me his report (in press, *Journal of Thoracic and Cardiovascular Surgery*) on the medial segment of the right middle lobe, for infants of 2 days, 37 days, 88 days, and 6 years 8 months of post-natal age. The counts along an axial pathway—including terminal, but not respiratory, bronchioli—vary from 20 to 26 generations. These are within the adult range, which suggests that new bronchi do not form in this period. Counts in the two-day lung were as high as those in the child of 6 years 8 months.