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Alveolar Epithelium in Relation to Growth of the Lung

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ALVEOLAR EPITHELIUM IN RELATION TO GROWTH OF THE LUNG

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[Plate 1]

CONTENTS

In the majority of organs which possess a lumen an epithelium is interposed between the parenchyma of the organ and the luminal space. Usually this epithelium lies upon a well-defined layer of fine reticulum fibres which constitutes the basement membrane. The developing lung of the embryo rabbit is no exception to this general rule, at any rate until the 24th day of embryonic life, when the complicated branching lumen is everywhere lined by tall, columnar epithelium supported by a reticular basement membrane. But histological examination of the lung of the adult rabbit shows no sign of epithelium or basement membrane. Indeed, the surface structure of the alveoli of the adult mammalian lung is one of the oldest of the unsolved problems in histology.

A detailed study by several methods of investigation of the stages intervening between the embryo and adult lung shows that the luminal epithelium ceases to be visible after the 26th day of the 32-day gestation period. Before the 26th day, the total volume of epithelium increases but is unaccompanied by any evidence of cell division. Cell rupture is therefore imminent, and its results become apparent between the 24th and 26th days when degenerate nuclei are extruded into the distal part of the respiratory lumen from ruptured cell envelopes. The healthy epithelium of the more

proximal parts of the lumen persists as bronchiolar epithelium, in which quantitative evidence of normal cell division is found.

These facts explain the difficulty in interpreting the picture of cell outlines which is seen when silver nitrate impregnates the cement lines between epithelial cells. In the earlier days of embryonic life the impregnated cell outlines reveal the regular meshwork characteristic of a complete epithelium. In the adult lung no such clear and regular picture is seen, and a close study of the intervening stages discloses that this irregularity of cell outlines starts at the 24th day and progresses with the degenerative changes in the epithelium and extrusion of nuclei. When the lung starts to breathe such traces of impregnated epithelium as were present at term finally disappear. In the adult rabbit, counts of nuclei in the alveolar septa show that there are not enough cells to do more than invest the capillary plexus and to provide nuclei for a few alveolar phagocytes. Moreover, a method of investigation whereby the structure of the alveolar septum may be dissociated fails to reveal any trace of lining epithelium. On histological grounds, therefore, the presence of an alveolar epithelium in the lung of the adult rabbit seems to be ruled out.

Criticism can, however, be levelled against this conclusion on the grounds of lung growth. It has been said that the presence of alveolar epithelium is required to account for further subdivision of the lung lumen during both pre- and post-natal life. New evidence is given in the second part of this investigation which suggests that the complexity of subdivision of the lung lumen is determined by purely physical factors. It is shown that the inequality of growth rates of total lung volume and of volume of the interstitial tissues is the fundamental factor which determines the complexity of lung architecture.

The latter is the result of subdivision of the lumen by a complicated system of septa. The greater the number of septa, the more complex is the subdivision and the higher is the pitch of differentiation. By measuring numbers of septa in terms of the internal surface area of the lumen, a method has been found for quantitative estimation of differentiation. A linear relation is found to exist between this estimate of differentiation on the one hand and the ratio of total lung volume to interstitial volume on the other.

The values of this ratio increase throughout embryonic life. Growth of interstitial tissue does not therefore keep pace with growth of total lung volume. The deficit in terms of volume is made good by the increasing volume of the lumen, but there is another deficit. For if interstitial tissue does not grow as rapidly as lung volume, then elastic fibres may be expected not to grow as rapidly. If this is so (and it is the heart of the problem), they will stretch as the lung expands with growth, and the ratio of total lung volume to interstitial volume will be a measure of the stretch to which elastic fibres are subjected in the growing lung. A linear relation is found between this ratio and numbers of septa, each of which contains a bundle of elastic fibres in its free edge. It is this linear relation which suggests a causal relationship between tension and the structural complexity of lung architecture.

Support for this view in lungs at term and during the early stages of post-natal life is given by the results of artificial distension. The plan of the 5-day lung, the complexity of whose structure is much greater than that at term, can be reproduced by artificial distension of the dead lung at term. Hence there can be no question of any vital processes of growth nor of epithelial activity. Distension alters the lung architecture by altering fibre tension, especially the tension of elastic fibres. In the living lung, it is very probable that active contraction of plain muscle in the mouths of alveolar ducts and their main subdivisions is also involved. The fact that the structural results of respiration and 5 days' growth *in vivo* can be so completely reproduced by artificial distension, as it were *in vitro*, is good reason to believe that subdivision of the respiratory lumen in both cases depends upon the same factor, the tension in elastic fibres.

PART 1. ALVEOLAR EPITHELIUM IN THE RABBIT'S LUNG

Introduction

One of the unsolved problems of lung structure is concerned with the existence of an epithelium covering the alveoli. Epithelium is a continuous sheet of cells separated from the underlying tissues by a basement membrane. This criterion is obeyed up to certain

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stages in the developing lung of the mammalian embryo. In the embryo lung, the epithelium above and the capillary bed below lie in close apposition to the basement membrane. So much is admitted by all observers. In the adult lung, ordinary staining methods fail to reveal an epithelium. The stages intervening between the clearly marked epithelium of the foetus and the obscure surface structure of the adult alveolus has not received thorough investigation.

In this present work a detailed histological description of the life history of epithelium, basement membrane and capillary bed is given, and the limits of the basement membrane and the structure and extent of the capillary bed have been defined as accurately as possible. The resulting picture of lung structure was encouragingly self-consistent and contained significant features which pointed unmistakably in the same direction. Nevertheless, many obscurities remained, due largely to ignorance concerning the facts of lung growth as a whole. This aspect has been studied and has corroborated and amplified understanding of the results from histological investigation. The evidence from both sources favours the view that alveolar epithelium does not exist in at least one adult mammalian species.

Methods

The numbers of rabbit embryos used in the histological section are shown in table 1. A series of human embryos, whose fertilization ages were calculated from menstrual age, is shown in table 2.

Table 1. Series of rabbit embryos

day	numbers of rabbit embryos	fixation *in situ* by perfusion
18	3	—
21	4	—
22	6	—
24	6	—
26	6	—
27	4	—
28	6	3
30	6	2
31 (birth)	6	3
1 hr. after birth	2	2
4 hr. after birth	2	2
6 hr. after birth	4	4
24 hr. after birth	6	3
2 days after birth	6	3
5 days after birth	3	3
10 days after birth	3	3

Table 2. Series of human embryos

fertilization age (days)	fertilization age (days)
50	150
53	157
80	164
91	166
98	168
109	170
112	172
126	180
134	217
137	231
146	266

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Methods of fixation

Depending upon the age of the rabbit embryo, two routine methods have been employed. In the case of embryos younger than the 28-day and some embryos between the 28th and 31st days, after killing by strangling, the lungs were fixed for 48 hr. in 10 % formol saline after opening the thorax. The whole thorax was dehydrated in 70 % alcohol for 48 hr., when the lungs were carefully removed and their edges trimmed before further slow dehydration in 90 % and absolute alcohol. After clearing in chloroform the whole lung was embedded in paraffin at 56° C. Sections were cut at 5, 15, 30μ and very often at 50 or 70μ.

In the case of some foetuses between the 28th and 31st days and all lungs which had breathed, the foetus was killed by intraperitoneal injection of nembutal. The abdomen was opened and the posterior abdominal wall exposed. 3 ml. of 10 % formol saline were injected very slowly into the inferior vena cava and the whole embryo was then immersed in 10 % formol saline. After 48 hr. fixation, the thorax was opened for the first time and the lungs removed. Subsequent treatment was the same as in the first group. In the case of foetuses whose lungs are fluid-filled *in utero*, no significant difference attributable to fixation was found in the histological examination of both groups. In the group of air-filled lungs fixation *in situ* by perfusion is essential.

Staining methods

Ehrlich's acid haematoxylin and eosin. Heidenhain's iron haematoxylin, differentiation to varying depths being carried out on three parallel sections. Van Gieson's stain. Shaw Dunn's modification of Mallory's stain for connective tissue. Weigert's elastic stain. Orcein. Laidlaw's method for reticulum.

Lungs injected to show the capillary bed

The numbers of animals are shown in table 3.

TABLE 3. INJECTED LUNGS IN RABBIT EMBRYOS

day	number injected	number injected and one lung distended
23	2	—
24	2	—
25	2	—
26	3	—
27	2	—
28	3	—
31 (birth)	—	4
1 hr. after birth	—	2
4 hr. after birth	—	2
6 hr. after birth	—	2
1 day after birth	—	4
2 days after birth	—	4

Method of injecting the blood vessels of the lung

This is most easily accomplished in deeply anaesthetized foetuses when respiration is failing but before death. If death has occurred most of the injection passes into the systemic circulation without entering the lungs. If gasping is taking place, the lungs fill with the

injection mass and the foetus may be killed at once by strangling. The injection is best made into the inferior vena cava and without opening the thorax. In foetuses which have breathed or in the adult, the results are incomparably better when injection is made with the thorax unopened. If it should be necessary to distend the lungs, choice between air or formol saline will depend upon whether the lungs are aerated or not. It is a mistake to inject air into lungs which have not breathed or have only gasped, since uniform distension will seldom be obtained owing to the presence of fluid which is normally present in the lumen of embryo lungs.

Choice and preparation of the injection mass

If merely an injection of the capillary bed is required, India ink diluted 8 times with a solution of 0·5 % protein (dried human serum) in normal saline can be used. Weaker dilutions sometimes permit of a nuclear counterstain. When good nuclear counterstaining is essential, the transparent carmine-gelatin mass must be used. It is most easily prepared as follows.

Allow 20 g. of gelatin to swell in 400 ml. of distilled water and warm to 60° C. Dissolve 5 g. carmine in strong ammonia and add to the gelatin. Filter while warm. Place the filtrate in a pH meter and add acetic acid till the voltage is equivalent to pH 7·2. Add a crystal of thymol. It is of assistance to wash out the blood by preliminary perfusion with saline on the acid side of neutrality, to prevent solution of the colloidal carmine. After completing the injection, the embryo should be placed in ice-cold formalin and left there for 48 hr. In the case of an adult rabbit, after completion of the injection, ice-cold formalin can be injected into the trachea in sufficient quantity to distend the lungs to their normal size. Blocks are cut after partial dehydration in 90 % alcohol and are dehydrated and embedded in paraffin wax in the usual way.

Impregnation by silver nitrate

For impregnation of epithelium or vascular endothelium, I have used 0·2 % silver nitrate in distilled water. I can find no advantage in the several modifications. A preliminary rapid perfusion by 2 % potassium nitrate (suggested by Carleton 1938) removes excess chlorides and reduces interference by precipitates. If the silver solution is injected into the trachea, preliminary perfusion of the pulmonary artery by potassium nitrate can be used with advantage even if the lungs are fully aerated; if an impregnation of vascular endothelium is required, potassium nitrate is followed by silver nitrate in the pulmonary artery. In spite of the intense vaso-constriction produced by silver nitrate, satisfactory impregnation of the capillary bed can generally be found in small areas of the lung.

The lung is removed from the thorax and placed at once in 90 % alcohol in the dark. Blocks are cut after hardening for 48 hr. or longer and left in 90 % alcohol for a further period of 48 hr. Dehydration is then proceeded with and the tissue is embedded in paraffin wax. Sections are cut at 5, 50 and 100 μ, taken to distilled water and placed in a mixture of equal parts of solutions A and B:

solution A		solution B	
metol	30 g.	sodium hydroxide	50 g.
potassium metabisulphite	30 g.	distilled water	1000 ml.
sodium bromide	30 g.		
distilled water	1000 ml.		

Impregnations can sometimes be improved by cautious use of 2 % potassium thiosulphate or by 0·5 % potassium ferricyanide, followed by thorough washing. Its use should, however, be limited to removal of slight precipitate in good preparations intended for subsequent photography. It is dangerous if used to improve bad preparations, and its routine use is likely to lead to results which are deceptive.

A nuclear counterstain is possible where too much silver has not been deposited at the nucleus. It is here that potassium ferricyanide is of great help to remove all impregnation from companion sections and thus to permit the use of any routine staining method.

The routine study of impregnations has been made with a camera lucida and oil-immersion objective. To draw a complicated system of lines is the best way to understand it. Completely erroneous impressions arise unless differences of focal depth are observed before the lines are drawn. Lines appearing to join may be shown to cross when an oil-immersion objective is used.

Histogenesis of the lung of the foetal rabbit

General histological description

It will be convenient to describe the development of the foetal lung before considering certain aspects of this process in detail.

Development of the lung of the foetal rabbit consists in repeated subdivision of the original paired tubes which are surrounded by vascular mesenchyme. During the last week of development the branching lumen, which occupies about 20 % of total lung volume at the 21st day, has expanded to fill about 50 % at term. The remaining portion consists of epithelium, connective tissue and blood vessels.

At the 20th day the epithelium of the respiratory tubes is uniform, closely set, cylindrical and pseudo-stratified. It lies on a well-defined basement membrane of reticulum fibres which contains small quantities of collagen and elastic in the larger tubes. Outside the basement membrane is a cellular mesenchyme, rich in reticulum fibres and containing capillaries which are condensed to form a plexus around the respiratory tubes. By the 22nd day, considerable elongation and subdivision of the tubes has occurred, and the larger tubes are lined by the same tall, columnar epithelium. Distally the epithelium can be separated into two types: the bronchiolar epithelium is cuboidal or low cylindrical, whilst in positions corresponding to the future alveolar ducts the epithelium is flatter and less closely set (figure 1). At the extreme tip of the tubes, the epithelium is cylindrical and pseudo-stratified. Throughout, the basement membrane consists largely of reticulum with a fine deposit of elastic and collagen fibres.

Distribution of reticulum fibres

By the 24th day, three condensations of reticulum are found between adjacent tubes (figure 2), of which the two outer layers form the narrow but densely woven basement membranes of the epithelium. Between them is a third, less dense condensation, composed of thicker fibres of reticulum. Two potential spaces are thus formed in the mesenchyme between adjacent tubes, and injected specimens show that these spaces are filled by the capillary plexus which surrounds each tube (figure 3). These plexuses are very profuse,

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their density being comparable with that of the capillary bed of the adult alveolus. The plexuses have developed as separate vascular condensations around each tube since interconnexions between adjacent plexuses are not numerous. The capillaries are of wide and

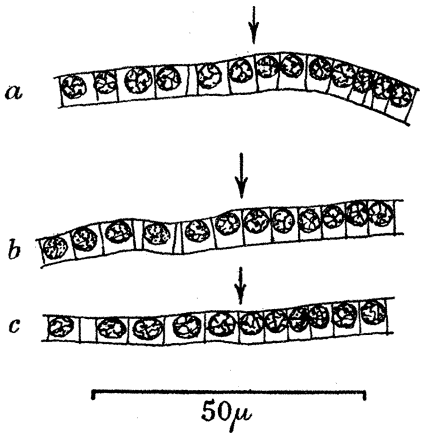

FIGURE 1. The arrow marks the change in character of epithelium at the distal limit of the bronchioles (on the right). *a*, 22nd; *b*, 23rd; *c*, 24th days.

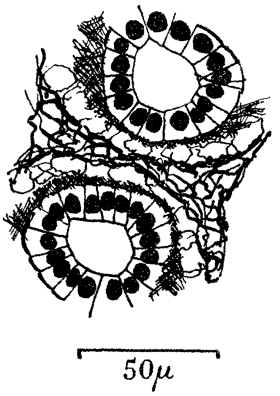

FIGURE 2. Reticulum condensations in intertubular septa. Spaces containing few fibres are seen below the basement membranes for accommodation of the double capillary plexuses. 22 days.

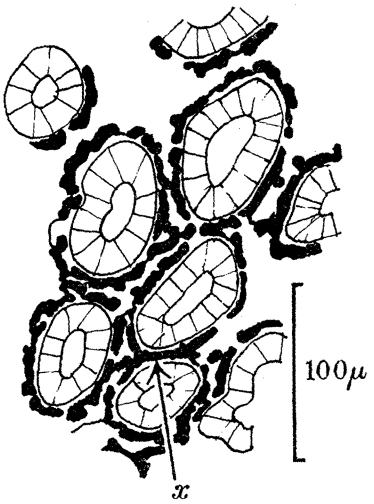

FIGURE 3. Double capillary plexuses in intertubular septa. Early signs of fusion of the plexuses are seen at *x*. 24 days.

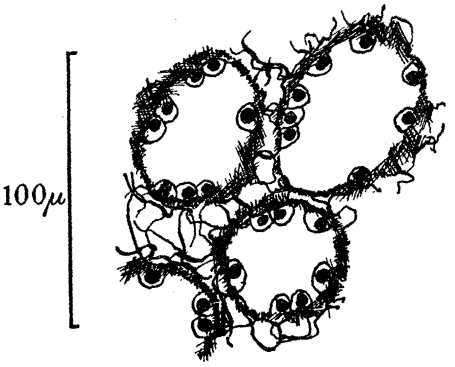

FIGURE 4. Reticulum condensations in intertubular septa at 25 days, for comparison with figure 2. Where fusion of double plexuses has occurred, one space crossed by reticulum fibres is found below the basement membranes for accommodation of the single capillary plexus.

somewhat variable diameter resembling sinusoids, and are closely apposed to the basement membranes.

At the 23rd day, the terminal portions of many tubes have come to lie so closely to each other that the capillary plexuses surrounding them have fused to form a single plexus.

Where such fusion has occurred, the single plexus is now confined by the two basement membranes, and is supported by the looser central condensation of reticulum (figure 4). Nowhere has the capillary plexus penetrated the basement membranes.

Basement membranes

The structure of these basement membranes is best seen in thick sections where the full extent of the septum is lying flat, in the plane of the microscope stage. They are formed by narrow, yet dense felt-works of fine reticulum fibres (figure 5). Thicker fibres join the loose reticulum condensation in the centre of the septum, but these are relatively few and serve to emphasize the individuality of the basement membranes. The central condensation consists in thicker fibres threading their ways through the capillary mesh and thus forming the central support of the capillary wall.

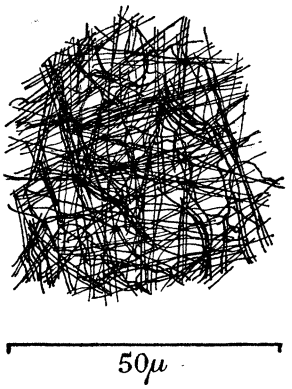

FIGURE 5. Basement membrane at 23 days. Surface view.

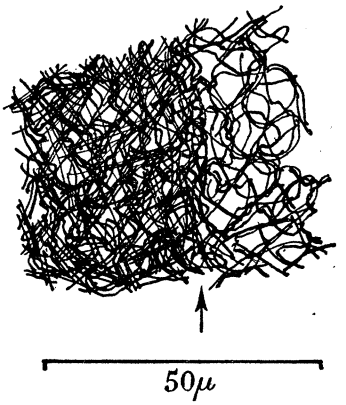

FIGURE 6. The arrow marks the termination of the basement membrane of the bronchiole (to the left). 26 days.

Disappearance of basement membranes

By the 26th day the basement membranes extend no farther than the termination of cuboidal epithelium of the terminal bronchioles (figure 6). Distally, they exist as islands

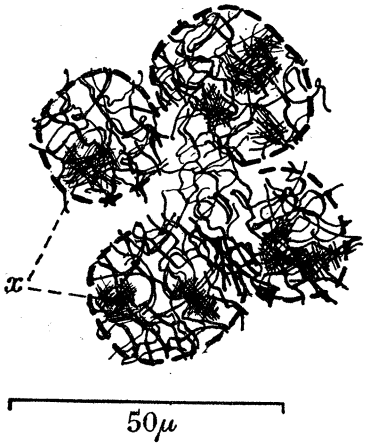

FIGURE 7. Surface view of isolated remains of the basement membrane (*x*). 26 days.

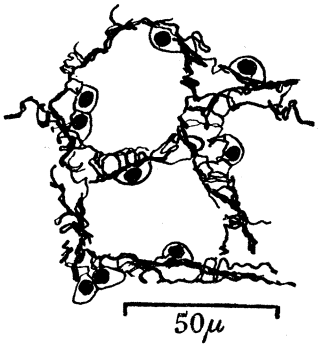

FIGURE 8. Reticulum condensations in intertubular septa. The epithelium and basement membranes have disappeared. 28 days.

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over small areas (figure 7). The continuity of these basement membranes has vanished, and this cannot be the effect of stretching or of separation of their constituent fibres since the quantities of reticulum which are involved are far too large. By the 28th day all trace of the basement membranes has vanished (figure 8).

Epithelium, 23rd to 26th days

Tubes distal to the bronchioles are lined at the 23rd day by a complete cuboidal epithelium. At the 24th day, a slight but definite change is found at the distal limit of the bronchioles which intensifies as the 26th day is reached (see figure 1). Many cells appear to be stretched, and of these several do not contain a nucleus. Striking changes may be found in the remaining nuclei which are often much enlarged, palely staining or fragmented. They may also be observed in all stages of extrusion from the ruptured cell envelope into the lumen of the tube (figure 9). In the remaining epithelial cells of the distal tubes (the future alveolar ducts) mitoses are rarely seen.

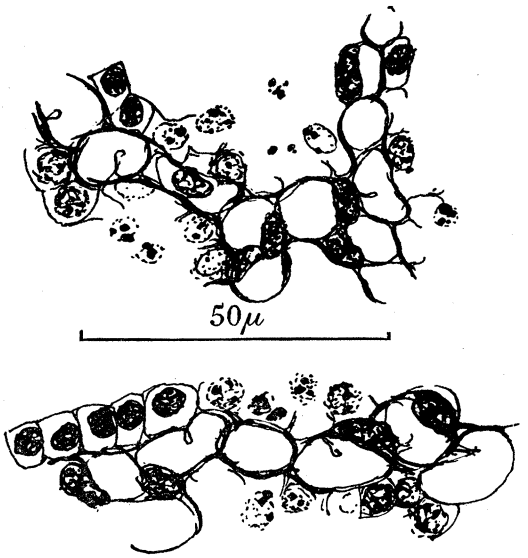

FIGURE 9. Degenerate epithelium of alveolar ducts showing extrusion of nuclei (24 to 26 days).

Desquamation of epithelium

Between the 24th and 26th days larger or smaller numbers of extruded nuclei can be found in the lumen of what have now become the alveolar ducts. The nuclei, fragmented or pycnotic, may be associated with granular debris; sometimes the whole epithelial cell may be found. Here and there the flattened or twisted endothelial nuclei of the capillaries are seen lying in direct contact with the lumen. Earlier, a perfectly definite, complete and nucleated epithelium lying on a well-defined basement membrane has separated the capillary from the lumen. Elsewhere the capillaries are separated only by the non-nucleated remains of the original epithelium, represented by the empty cell envelopes which by slight overlapping at their edges and their well-marked cell membranes somewhat resemble the horny layer of the skin. Desquamation of these cell envelopes may sometimes be observed as minute disorganized laminae fraying off into the lumen, but extrusion of nuclei is more often found than is desquamation of the cell envelope.

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At the 24th to 26th day, in the terminal portions of the respiratory tubes and especially in those which abut against the pleura or against a bronchus or branch of the pulmonary artery, the epithelial island is still present and each cell is nucleated. In the incomplete epithelium of the alveolar ducts, smaller isolated groups of cells may also be found.

An injected specimen of the rabbit's lung at the 25th day does not support the view that there is any capillary proliferation between the epithelial cells. In this species there is no evidence that desquamation of epithelium is either caused or accompanied by capillary proliferation.

Alveolar epithelium in the human lung

The same sequence of events which has been described in the foetal rabbit has been found in man. Though it is impossible to fix the lungs so rapidly (as is possible with the rabbit) as to avoid all possibility of autolysis, enough fresh human embryonic material exists to convince me that autolysis is not a serious matter in the series of embryos which have been studied.

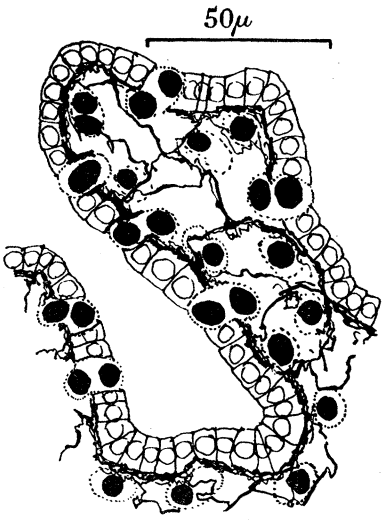

FIGURE 10. The basement membrane is deficient at the points where capillaries protrude between epithelial cells. Black cells are erythrocytes in this and the following figures. Human embryo, 109 days.

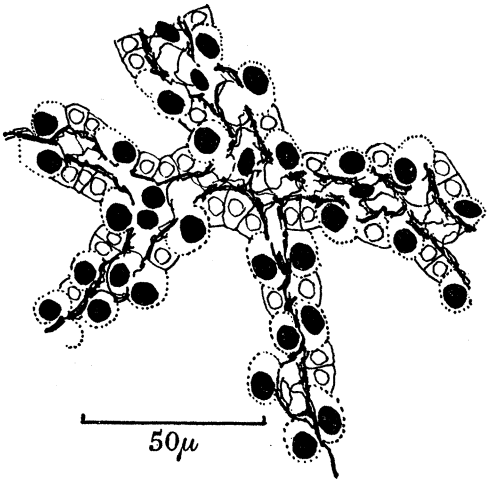

FIGURE 11. More advanced protrusion. Considerable reduction of numbers of epithelial cells. Human embryo, 146 days.

In the rabbit the process of desquamation occurs between the 24th and 26th days and is completed on the 27th day. In the human embryo, the earliest stages of the process can be recognized at the 110th day and are usually complete by the 170th day. It is unlikely, however, that the process of desquamation lasts for so long in the development of one embryo. Four stages of development can be described.

Up to the 110*th day*. The respiratory tubes are lined by a complete, tall-columnar epithelium which gradually becomes less tall. It lies on a well-defined basement membrane of fine, closely woven fibrils of reticulum beneath which lies the capillary plexus and a few loose reticulum fibres in the central part of the septum.

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110*th to* 150*th days*. The earliest signs of incomplete epithelium are seen as a result of the protrusion of capillaries between adjacent epithelial cells. At these points the basement membrane is incomplete, and in surface view the defect appears as a small round hole of the diameter of a capillary. By the 150th day numerous protruding capillaries may be found, and by coalescence the gaps in the basement membrane may become larger. The appearance of the protruding capillaries is exactly that described by Barnard & Day (1937), who attributed it to proliferation of the capillary plexus.

150*th to* 170*th days*. Epithelium has disappeared from large tracts of the surface of the alveolar ducts, though it is still complete in their extreme tips. Occasional epithelial cells, usually single but sometimes in groups, can be seen lying on the capillary plexus or extruding their nucleus into the lumen of the alveolar duct. Retained nuclei may be swollen, pycnotic

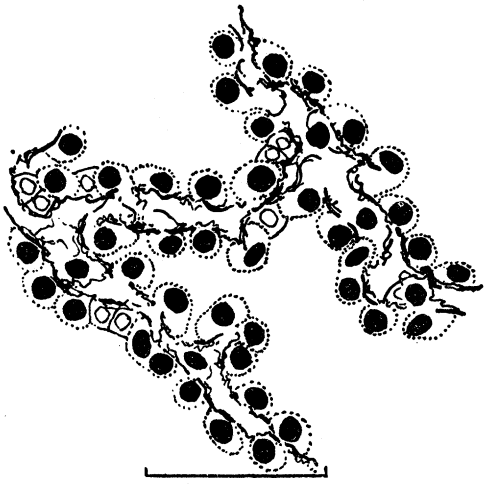

FIGURE 12. Reticulum has formed a new condensation on the deep surface of the capillary bed. Epithelial cells are infrequent since most have desquamated. 166 days.

or fragmented. The basement membrane no longer exists on the luminal surface of the capillary plexus except in the extremities of the respiratory tubes. On the other hand, a narrow but definite condensation of reticulum fibres is now seen on the deep surface of the capillary plexus by which the plexus is separated from the central parts of the septum in which reticulum fibres are sparse. The septum has thus come to consist of a central very loose network of reticulum which lies between two parallel, finely woven condensations of reticulum upon whose luminal surfaces the capillary plexuses are placed. These plexuses appear to lie in direct contact with the respiratory lumen except for an occasional, retained epithelial cell.

170*th day to term*. Except at the extreme tips of the ducts, a continuous epithelium has vanished. Two capillary plexuses lie on the surfaces of all septa which expose two surfaces to a lumen. The plexuses lie on distinct condensations of reticulum between which sparse reticulum fibres run through the central parts of the septum. In the early stages of this period, the central part of the septum is wide and the subcapillary condensations are consequently distinct. As term is reached, more and more narrowing of the septa occurs until the subcapillary condensations have fused in many septa to become the single, central scaffold of reticulum which is characteristic of the post-natal and adult lung.

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The protrusion of capillaries between adjacent epithelial cells is undoubtedly very suggestive of the capillary 'proliferation' described by Barnard & Day (1937). Nothing of the sort is seen in the development of the rabbit. What is known of the development of the pulmonary capillary bed does not support the view that proliferation by active growth takes place, and it may be that the more passive process of protrusion or bulging is associated with increased blood flow.

The capillary plexus in man is separated from the epithelium by a well-marked basement membrane. At the conclusion of the period of desquamation in man, an equally well-marked condensation of reticulum is seen on the deep surface of the capillary bed. It would seem that the structures had changed their relative positions, but it is obvious that two meshworks (capillary plexus and basement membrane) cannot change their relative positions unless 50 % of both continue to lie in their original positions. This, however, is not the case. At least 80 % of the capillary plexus lies on the luminal surface of the reticulum condensation beneath it. Few capillaries are to be found in the central part of the septum. It would seem, therefore, that the original epithelial basement membrane had disappeared with the epithelium and that a new condensation had been formed on the deep surface of the capillary bed.

The pulmonary lobule

Following desquamation of the alveolar epithelium of the embryo, the point of termination of bronchiolar epithelium can be supposed to represent the apex of the cone formed by the pulmonary lobule. There is no other structure than this termination of bronchiolar epithelium which lies even roughly in the same relative position. In lungs of a given age, the length of the alveolar ducts measured from the termination of bronchiolar epithelium to the lobular periphery is fairly uniform, but there is reason to believe that the distal termination of epithelium may not occupy the same relative position in lobules from lungs of different age groups. Broman's (1923) observations in the cow and in man and Willson's (1928) observations in the mouse show that the number of bronchial divisions separating

TABLE 4. RELATION OF LUNG VOLUME TO LENGTH OF ALVEOLAR DUCTS

	length of alveolar ducts (μ)				lung volume (ml.)	
age	measured	corrected for shrinkage (see table 11, part 2)	increase	(increase)^3	measured	calculated
			Rabbit			
28 days (embryo)	210	225			1·1	
adult	800	800	3·54	44	45·75 [=]*	48·5
			Man			
term	400	400			50	
adult	1500	1500	3·75	52·5	6000 [>]*	2625
			Mouse			
term	190	190			0·03	
adult	400	400	2·1	9·3	0·7 [>]*	0·28

* The mouse and man possess respiratory bronchioles. The rabbit does not, and in this species the cube of the increase by growth in alveolar duct length is a fair measure of increase in lung volume. In the mouse and in man measured lung volume is almost three times as large as the calculated figure.

the trachea from the alveolar ducts increases between birth and maturity. Inexplicable on any other hypothesis, Bremer (1935) suggested that this increase in number of bronchial divisions is the result of a peripheral advance of bronchiolar epithelium, so that the alveolar ducts of the young are converted into the bronchioles of the adult.

Data show that this manner of growth does not take place in the rabbit. For the cube of the increase in length of alveolar ducts between two age groups multiplied by lung volume of the younger age group is found to be a fair measure of total lung volume (see table 4). In man and the mouse, on the other hand, such calculation is far below measured lung volume. It is, indeed, probable that the manner of bronchiolization of alveolar ducts postulated by Bremer (1935) is limited to those species which possess respiratory bronchioles, and that it is absent in those species which do not possess them, as, for instance, the rabbit.

Development of the capillary bed

Foetal stage

The changes in arrangement of the reticulum condensations in the intertubular septa have been related to desquamation of epithelium and its basement membranes on the one hand and, on the other, to fusion of originally independent capillary plexuses.

Throughout the foetal life of the rabbit, most of the intertubular septa (exposing two surfaces to adjacent lumina) contain two plexuses (Schultze 1871). These are developed as separate condensations around tubes. In two characteristic situations the septa present only one surface to a lumen: first in the subpleural extremities of tubes, and secondly in tubes whose extremities abut against an interstitial structure. In these situations, the capillary bed is single, and this affords evidence that the primordial capillaries have condensed to form a plexus which envelops the developing lumen. The capillary plexuses in these situations differ also from those throughout the largest part of the lung, in that their mesh is very much less complex. Such is the case in the later stages of foetal life (figure 13) and also in the adult (figure 14), where the contrast in man has been illustrated by Miller (1937). Schultze (1871) appreciated that the larger mesh of subpleural alveoli was the result of persistence of a single plexus, the complexity elsewhere being the result of fusion of two originally separate plexuses. Analogous conditions are found in the human foetus and in amphibian or reptilian lungs. In the latter species, thick septa draw up two sheets of the capillary net, one on each side of the thick central stroma (Williams 1859), a condition which persists into their adult life.

It is not difficult to imagine the stages leading to development of the relatively loose plexus found in subpleural alveoli. It is a very different matter to imagine the development of so profuse a capillary net as that which invests the rabbit's lung at term. Recognition of its development from fusion of two plexuses resolves the difficulty at least temporarily.

Post-natal stage

The capillary bed would appear to be unfavourably placed for enlargement by the recognized method of formation of capillary loops. The plexus is confined by adjacent alveolar surfaces and its mesh is narrower than the average diameter of its constituent capillaries. It would appear, therefore, to be rigidly confined to the septum and to be of

such an order of complexity that capillary proliferation is out of the question. Yet it is clear that the onset of respiration must increase the respiratory surface and that the first month of life must involve a still greater increase. How, therefore, does the capillary plexus accommodate itself to further growth of the lung?

At term, and indeed at all stages of the rabbit's life, the mesh size of the capillary bed is of two types. The larger mesh is about half the complexity of the other, and is found in those alveolar septa which present only one surface to the respiratory lumen. In these

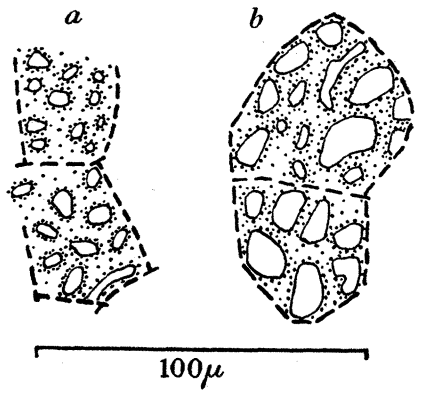

FIGURE 13. Comparison of mesh of capillary bed from normal alveolus (*a*) and subpleural alveolus (*b*). 1-day old rabbit.

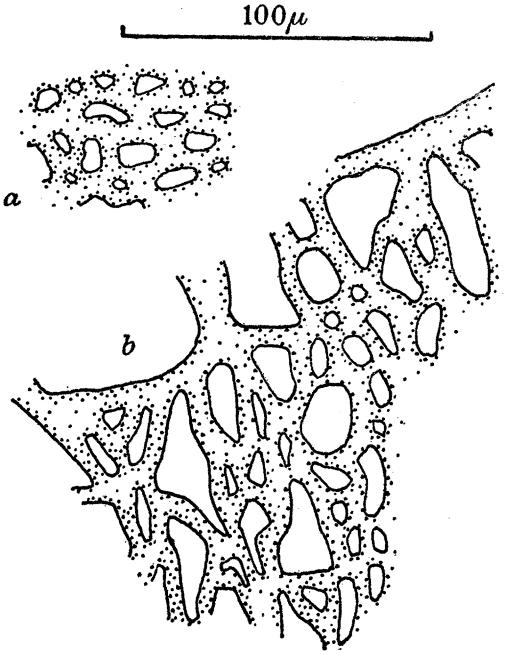

FIGURE 14. Comparison of mesh of capillary bed from normal alveolus (*a*) and subpleural alveolus (*b*). Adult rabbit.

situations, below the pleura and abutting against interstitial structures, the capillary bed has been formed from one plexus. In all other situations the capillary plexus is developed from two plexuses placed back to back.

Post-natal growth of the lung will therefore have different effects on these plexuses. In the plexuses of subpleural type, growth of the respiratory tree must enlarge the mesh of the plexus in all dimensions unless it is modified by capillary proliferation within the plexus itself. In plexuses of normal type (exposing two surfaces to the lumina), growth and distension of the lumen must distend and enlarge each plexus, but it could also promote further and more complete fusion of the plexuses, so that by degrees their original double nature would become obliterated.

Figure 15 shows that such local fusion actually takes place and that between birth and the 10th day of life the double character slowly disappears. Even by 48 hr. of life, the double character in many places could hardly be guessed without knowledge of the earlier state. It can, however, be recognized in all stages of development of septa including the earliest, knob-like stage (figure 16).

Such fusion, amplifying that already noticed to occur in the embryo, provides for no more than a temporary increase in complexity. It cannot provide for growth of the plexus when continual increase of surface area imposes still further demands on the capillary bed. Unless the plexus stretches, some other method of reproducing itself must be found, and it would seem that only two ways exist by which this can be accomplished.

By endothelial loops

This is the usual method by which a capillary bed enlarges. Evidence can be found that it occurs in the lung where it appears to be limited to the largest intercapillary spaces of plexuses of the subpleural type. Even here it seems to be relatively uncommon.

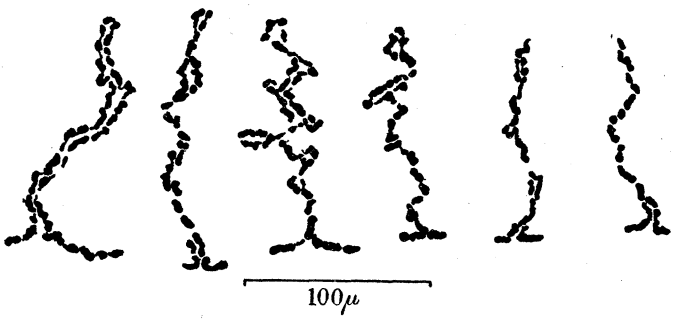

FIGURE 15. Stages in fusion of double capillary plexuses in intertubular septa. 48 hr. of life.

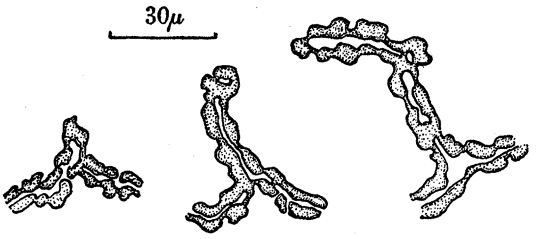

FIGURE 16. Double plexus in early stages of formation of interalveolar septa. 48 hr. of life.

By collapse of capillaries and adherence of endothelium

Such a method of growth has not, so far as I am aware, been described. By increasing the numbers of intercapillary spaces as a result of local adherence of their walls it would permit of enlargement of the capillary bed without loss of its complexity. Such a method would permit of increase in numbers of capillaries within the existing dimensions of the plexus.

Evidence for this view is derived from observations on injected lungs fixed *in situ*.

In all injected lungs during the period of growth, minute circular spaces can be found measuring 2 to 3μ in diameter (figure 17). They are found in what would otherwise be very wide capillaries. Their edges are clear and well defined and they contain no trace of the injection mass. They appear, therefore, to be minute intercapillary spaces. They are only very rarely observed in the adult rabbit's lung, as would be expected if the effect is related to growth.

The effect is not apparently due to blockage of a capillary by a leucocyte, so that the injection mass is excluded from a small portion of the capillary. In preparations stained for nuclei the majority of these spaces are unrelated to a nucleus. Moreover, the size even of a leucocyte or endothelial nucleus is much larger than the average size of these spaces, so that the whole cell of the leucocyte must be larger still.

More significant evidence comes from the injected partner lung which has been distended to maximum capacity by intratracheal injection. It would appear that distension has not had the effect of enlarging these minute spaces and thus of reducing their numbers. It appears, indeed, that their frequency is about the same and that they are of the same order

of size, namely, 2 to 3μ. If therefore such minute spaces are found in the distended lung, what, it may be asked, do they represent in the undistended partner? For this effect there can only be two possible explanations. Either that 'maximum distension' is not of uniform distribution in the distended lung or that these minute spaces have been newly formed as a result of distension of the lung, which has enlarged those already present.

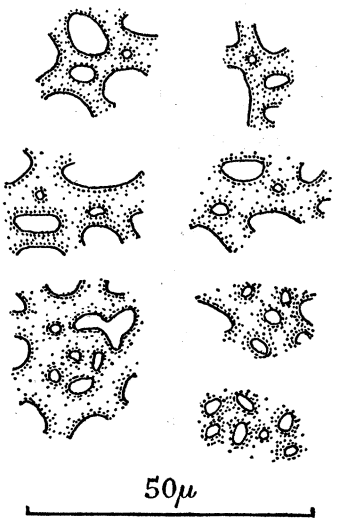

FIGURE 17. Minute intercapillary spaces in distended partner lungs. Term (left) and 24 hr. of life (right).

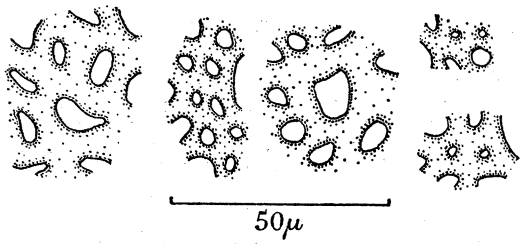

FIGURE 18. Capillary plexuses in distended lung. 48 hr. of life.

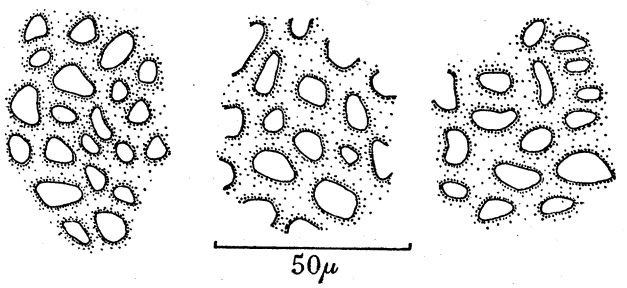

FIGURE 19. Capillary plexuses in distended lung. Adult rabbit.

It is probably significant that the range of size of the intercapillary spaces shows greater uniformity in the adult (when enlargement of the capillary bed can be assumed to have ceased) than in the embryo or newborn rabbit (see figures 18, 19). Moreover, figure 20 seems to show that the range of size of intercapillary spaces is rather less in the undistended lung. Distension would seem to have enlarged the largest spaces whilst scarcely altering, perhaps even reducing, the size of the smallest. The smallest spaces in the distended lung may therefore represent potential spaces in the undistended partner: minute areas of adherence between opposite walls of a capillary, unrecognizable by ordinary methods. Lastly, between the 23rd day of embryonic life and the 28th day, growth of the capillary plexus is accompanied by a well-marked narrowing of the constituent vessels (figures 21, 22, 23). This is some evidence that growth takes place by multiplication of intercapillary spaces than by multiplication of the capillaries themselves.

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These observations, though not conclusive, suggest the possibility of an undescribed method of capillary proliferation by multiplication of the intercapillary spaces. Large areas of the capillary bed of the lung are kept physiologically closed (Wearn *et al.* 1934). The mechanisms of closure and re-expansion are unknown, but, during the period of growth, closure suggests capillary collapse and the possibility of adherence of the endothelial lining. Re-expansion may result in the appearance of new intercapillary spaces. By increasing the numbers of intercapillary spaces, a larger surface area could be vascularized without the loss of complexity of the vascular bed which would otherwise occur from stretching. Further evidence of this method must be sought from direct observation of the living lung during the period of active growth.

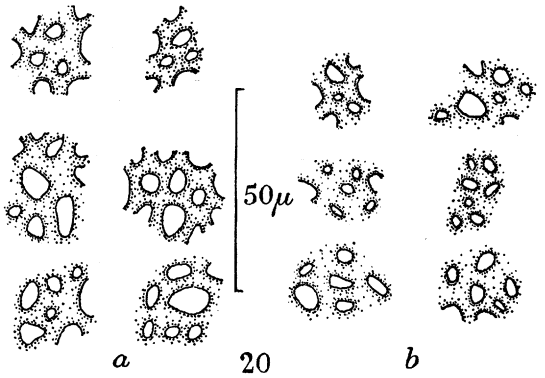
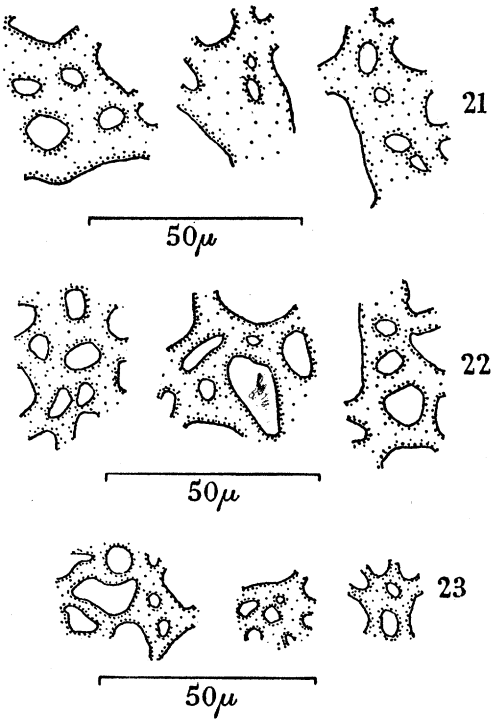


FIGURE 20. Effect of distension of lung upon capillary mesh size from a lung fixed *in situ* (*a*) and from its distended partner (*b*). 24 hr. of life.



FIGURES 21 to 23. Comparison of capillary diameter at 23 days (figure 21), 26 days (figure 22) and 28 days (figure 23) of embryonic life.

HISTOGENESIS OF THE EPITHELIUM OF THE RESPIRATORY TREE (RABBIT EMBRYO)

The description which has already been given of the stages leading to desquamation of epithelium between the 24th and 26th days of the rabbit's embryonic life requires further elaboration. The following sections contain further evidence which supports the view that the surface of the alveolus is not covered by epithelium in the normal rabbit after the 26th day of embryonic life.

Quantitative data are given first and are followed by analysis of the effects of silver impregnation in the foetus and adult rabbit. The interpretation of silver impregnation, undoubtedly, is the heart of the problem of alveolar epithelium which would not have been supposed to exist in the adult lung had silver nitrate not been discovered to impregnate cement lines. The results of counts of nuclei in the alveolar septum of the adult rabbit and

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of dissociation of its structure are next described. The results of these four methods of investigation provide strong evidence that the alveolar septum is not covered by any sort of epithelium after the stage of epithelial desquamation in embryonic life is passed.

Estimate of volume of epithelium

If drawings of lung fields are made with a camera lucida between the 18th and 24th days, the percentage area* occupied by epithelium increases whilst the height of its constituent cells falls (see table 5). In relation to total lung volume,* the total volume of epithelium is therefore increasing. The epithelium succeeds in keeping pace with the ever-increasing internal surface area by stretching laterally with consequent loss of height of its constituent cells. Unless cell multiplication occurs, this continual increase in cell volume, together with the lateral stretching of the cells, must ultimately lead to cell rupture. Rupture occurs between the 24th and 26th days, and is accompanied by extrusion of nuclei into the respiratory lumen which has already been described.

TABLE 5. VOLUME OF EPITHELIUM AND HEIGHT OF EPITHELIAL CELLS

day	% of lung field occupied by epithelium	height of epithelial cells (μ)
18	8	17·5
21	6	—
22	11	7·5
24	14	5·0
26	0	0

TABLE 6. ESTIMATE OF CELL DIVISION IN EPITHELIUM

day	lung volume (ml.)	$\sqrt[3]{}$(lung volume)	ratio increase of $\sqrt[3]{}$(lung volume)	counted number of cells per 100 μ	calculated number of cells per 100 μ if no growth
	(1) In epithelium of distal tubes which desquamates				
18	0·038	0·336	1	$32 \pm 1{\cdot}93$	32
22	0·194	0·580	1·72	$16 \pm 1{\cdot}73$	19
24	$0{\cdot}347 \pm 0{\cdot}07$	0·703	2·03	$12 \pm 2{\cdot}06$	16
	(2) In epithelium of proximal tubes which persists				
18	0·038	0·336	1	$32 \pm 1{\cdot}93$	32
22	0·194	0·580	1·72	$24 \pm 1{\cdot}37$	19
24	$0{\cdot}347 \pm 0{\cdot}07$	0·703	2·03	$26 \pm 1{\cdot}64$	16

It is possible to show that cell division is non-existent. If a length of wall covered by epithelium is stretched to twice its original length, the number of cells per unit length will be reduced to half the original number. From the curve of lung volume, the increase by growth in linear measurements may be calculated ($=\sqrt[3]{}$(lung volume)). The number of cells actually found per unit length are very close to those expected if the original number of cells per unit length at the 18th day remained the same (see table 6). These results from the distal epithelium, which desquamates, are contrasted with similar measurements from the proximal part of the respiratory tubes, where epithelium persists. If the calculation for the 24th day is repeated for the lung volume represented by three times the standard devia-

* The complete data upon which the mean figures of lung volume and its percentage composition are based will be found in the section on lung growth.

tion (lung volume $= 0{\cdot}347 \pm 0{\cdot}07$), upper and lower values for the calculated number of cells are 20 and 13. Therefore 26 cells per 100 μ is significant.

The embryological development of the epithelium and its desquamation have already been described. Cell rupture and extrusion of nuclei between the 24th and 26th days are in agreement with the conclusions based upon quantitative data. Taken together they give evidence of degenerative changes in the epithelium which culminate in desquamation at or about the 26th day of embryonic life in the rabbit.

Review of literature concerning epithelial desquamation

The observation that the epithelium of the distal portion of the respiratory tree undergoes degeneration is not new. In the earlier stages of its history, the observation was entangled with the theory of pre- and post-natal flattening of epithelium elaborated by Kuttner (1876) and his followers. The interesting feature is the relatively late appearance of the view that it constitutes evidence for desquamation of an epithelium which is never replaced in the normal adult.

Jalan de la Croix (1883) described nuclear disappearance in the flattened epithelial cells of late pre-natal life and regarded the process as maturation of the 'non-nucleated plate'. In his illustrations of the lung of the foetal pig, Flint (1906) shows clearly the epithelial transformation which occurs at the 22 cm. stage. From being clearly visible cuboidal epithelium, the lining becomes invisible to ordinary methods of investigation and his conclusion is that the cells flatten. Bremer (1904) and Miller (1913, 1932, 1937), whilst admitting the difficulty of seeing it, consider that an epithelium must be present to allow of continued growth of the lung from birth to maturity. Ogawa (1920) described and figured nuclear pycnosis, karyorrhexis and chromatolysis of foetal epithelium and satisfied himself of the existence of non-nucleated plates by the discovery of a single example.

Quantitative evidence of the amount of epithelium which disappears from the lung of the foetal sheep is given by Fauré-Fremiet & Dragoiu (1923). The change was associated with increase in the fatty-acid and total nitrogen content of the lung and with a sharp decline in glycogen, which accumulates in the degenerate epithelium in the sheep (and also in the rabbit). The contemporary work of Stewart (1923) on the rat embryo showed that this critical period in epithelial development was associated with fatty change in the cytoplasm of both epithelial cells and of stroma cells in the septal wall. He also described sloughing of the terminal epithelium associated with hydropic (suggesting increased volume of epithelium) and fatty changes in the cytoplasm and with degenerative changes in nuclei of epithelial cells. Though he is very cautious in drawing conclusions as to the lining of the adult alveolus his description of 'flanged epithelium' in the 20-day rat embryo seems to be a more acceptable alternative than to admit 'that capillary endothelium may be in contact with air'. Bremer (1939) also records fatty change in the epithelium and connective tissue of the chick's lung.

Of modern workers, Policard (1926) seems to be the first to strip the alveolus of its disputed epithelium. His argument is based less on interpretations of minute structure than on the derivation of the septal cell from the reticulo-endothelial system. The alveolar septum of the adult is thus of mesenchymal origin since all traces of epithelium have disappeared during embryonic life. Supporting evidence is adduced from Carleton's (1925) results of tissue

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culture of foetal lungs (to produce epithelial sheets showing fibrinolytic activity, fibroblasts and few active macrophages) and adult lungs (no epithelial sheets but many fibroblasts and numerous, actively phagocytic macrophages).

Following Policard's review, subsequent workers have turned their attention to the capillary bed of the alveolar wall. Chiodi (1928) describes its increase during the 5th to 6th month in the cow foetus, when the epithelium desquamates. Palmer (1936) regarded epithelial desquamation in the human embryo as the result of capillary proliferation. In this interpretation he was followed by Clements (1937), Barnard & Day (1937) and the more recent work of Norris, Kochendorfer & Tyson (1941) and of Ham & Baldwin (1941). These authors, however, do not describe the development of the capillary bed whose double character, produced by two plexuses laid back to back, is described in the older work of Schultze (1871). Their evidence from the human embryo rests upon the appearance of capillaries projecting into the alveolar lumen or penetrating between adjacent degenerate epithelial cells. This may partly be due to increased prominence following desquamation of epithelium but, in the rabbit, does not appear to be due to active capillary proliferation by the usual method of buds or loops.

Results of silver impregnation

No single factor has exercised a greater influence upon the history of alveolar epithelium than has von Recklinghausen's (1860) announcement of impregnation of epithelial cement lines by silver nitrate. The silver is deposited at the cell periphery where it may be blackened by sunlight or by a photographic developer (the latter used by Jeker 1933; by Ritter, quoted Cowdry, 1943; Bensley & Bensley 1935). The deposit is soluble in sodium thiosulphate and in potassium ferricyanide, which serve as useful differentiators in dilute solutions.

In perfectly successful preparations no more than these black lines are visible. Very commonly, however, a granular deposit is found at or round the nucleus which serves as a useful landmark. Unless precautions are taken to avoid folding of the membrane, a granular deposit is likely to occur in the folds and thus to mask, though not to obliterate, an otherwise clear picture. In transverse sections of such impregnations, the silver is seen to have been largely deposited at the luminal surface of the cement line (cp. Robinow 1936, 1938), though a granular deposit may be observed to extend towards its deeper extremity. In such cases, a weak differentiating solution may remove the granular precipitate whilst it leaves the cement line untouched. It was probably by such means that Gustav Mann obtained his very perfect results (figures, 24, 25, plate 1).

In less successful impregnations, the cement outlines may show a tendency to be granular, and a fine granular deposit may occupy the cytoplasm of the cell. Such areas may exist with others in the same section where impregnation is perfect. In the study of a capillary bed, for example, such imperfect areas have the advantage of outlining the capillary bed in one portion and its constituent endothelial outlines in another. It may, indeed, be impossible to reconstruct the extent of a capillary bed from a successful impregnation of its endothelial outlines alone.

Impregnation in the foetal rabbit

At the 23rd day of development when ordinary histological examination shows a continuous nucleated epithelium, intratracheal injection of silver nitrate reveals the cell outlines

as a regular meshwork (figure 26). In the alveolar ducts the cell outlines are distinctly larger than is the case in the bronchioles or in the extreme tips of the tubes. This is probably the result of distension by the injected solution. In all outlines nuclei are clearly visible either as a finely granular deposit of silver or stained by haematoxylin or neutral red.

At the 25th to 26th days, the silvered outlines present the classical picture of small, nucleated cells and large non-nucleated plates (figure 27). The changes are not distributed uniformly in the lung. Some lobules show the appearance whilst others do not. The most

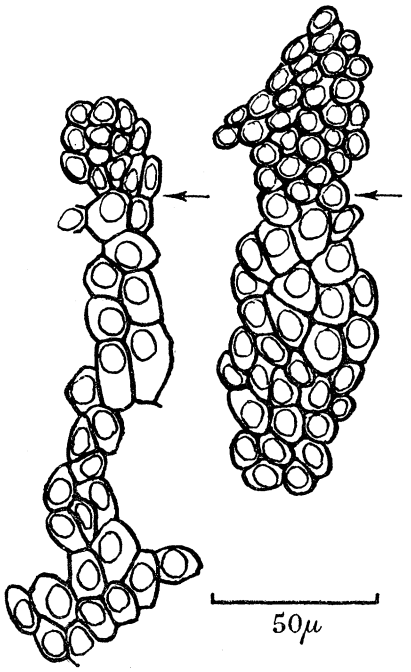

FIGURE 26. Silver impregnation of lung at 23rd day. Termination of bronchiolar epithelium is marked by an arrow in this and subsequent figures.

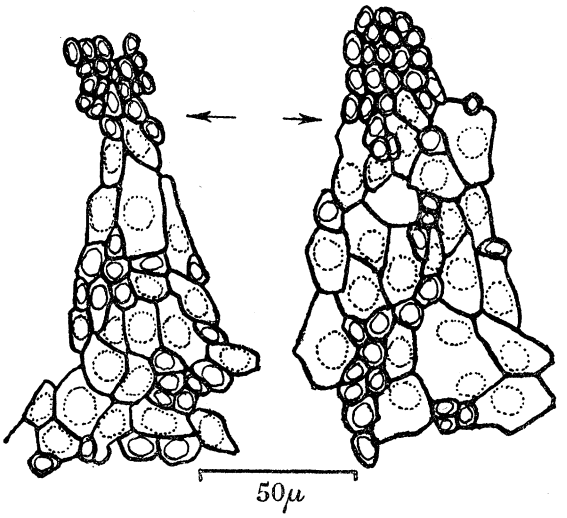

FIGURE 27. Silver impregnation of lung at 25th day. This is the classical picture of small nucleated cells and 'non-nucleated plates'. Of the latter, however, most are nucleated.

advanced portions show a wide-meshed network of lines among which lie smaller areas. Both types of outline contain nuclei, the larger areas being sometimes bi-nucleate though the smaller never have more than one. Occasionally the larger areas are non-nucleate. The nuclei of the larger areas are themselves larger than are those of the small cell, and they do not stain so intensely with a nuclear counterstain. The larger nuclei are certainly to be identified with the swollen, degenerate nuclei of the unsilvered haematoxylin preparation where the same cell is of smaller size. The larger size of these cell areas is probably therefore an effect of distension by injection of silver nitrate. The termination of bronchiolar epithelium is now clearly marked, and its close-meshed, nucleated network undergoes a sudden change into that just described.

By the 27th day, the contrast between large and small cell outlines is maximal (figure 28). Without a knowledge of the previous stages it would not be suspected that the largest areas were the result of distension of degenerate cells in the original epithelium. Degenerate nuclei may or may not be found. Still more significantly, the pattern of the mesh shows early signs of irregularity. Lines may be found which no longer link up with their neighbours and thus give rise to larger areas of very irregular outlines. Nevertheless, a tolerably regular

mesh is still present over large areas, and it is probable that these minor degrees of irregularity would have been overlooked if attention had not been specially directed to finding them. Owing to the thickness of the septa, no difficulty is found in recognizing beyond any doubt that these meshworks are true surface meshworks. Such a picture in the distal portion of the lung renders the termination of bronchiolar epithelium even more distinct than was found at the 25th to 26th day.

28*th to* 31*st day*. The surface impregnations have now become so exceedingly irregular that considerable difficulty is found in drawing them (figure 29). The lines are now fragmentary, incomplete and irregular, and thus contrast with the small regular mesh of bronchiolar

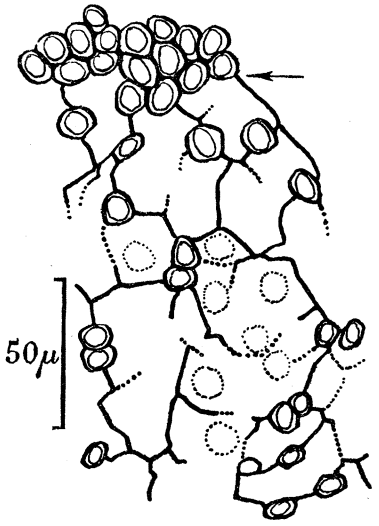

FIGURE 28. Silver impregnation of lung at 27th day. Early irregularity of the impregnated meshwork is seen. Several cells have lost their nuclei.

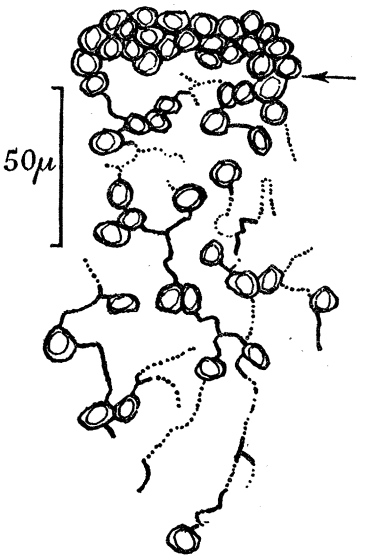

FIGURE 29. Silver impregnation at 31st day. The impregnated outlines have become exceedingly irregular. Nuclei have disappeared.

epithelium. The only trace of former regularity is found in alveolar septa exposing one surface to a lumen in which a few lines connecting the relatively numerous small nucleated cells give rise to patterns of tolerable regularity. It is in these situations that small, regular portions of the mesh persist after birth and may even be dimly recognized in the adult. This description is in harmony with Jeker's (1933) findings in the kitten, with the qualification that what the kitten accomplishes by the second or third week of life, the rabbit has accomplished by term.

A point worthy of emphasis is the size of the small nucleated cell. It appears to be distinctly smaller at term than were the corresponding structures at the 25th day. Since the small nucleated cell of silver impregnations has been regarded as identical with the alveolar phagocyte, the slight difference in size raises the question whether the small nucleated cell (apparently of epithelial origin) of the 25th day persists to become the alveolar phagocyte. The smaller size of the corresponding cell at term suggests an alternative possibility that certain more deeply situated histiocytes may reach the surface during the process of epithelial desquamation and may there round themselves off. No clear evidence of the origin of the alveolar phagocyte is given by this material.

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Impregnation in the adult rabbit

As thus described there is no difficulty in tracing the stages of development between the pattern of the foetal mesh and that of the adult. At almost all periods the impregnation can be related to the more ordinary histological picture of haematoxylin and eosin. An exception is found in the impregnation of subpleural alveoli or those septa which expose but one surface to a lumen.

Following intratracheal injection of silver nitrate into the lung of the adult rabbit, absolutely no surface impregnation can be recognized over most of the lung surface. At the most a granular deposit is found which defines folds and irregularities in the film-like septa. Where this has occurred evenly it may be possible to define intercapillary spaces as pale areas outlined by a granular border bearing a superficial resemblance to true impregnation of epithelium.

It is, however, possible to recognize traces of true epithelial impregnation in subpleural alveoli (figure 30, plate 1) or in those which abut against an interstitial structure (figures 31, 32, plate 1). In these situations, the picture of small nucleated cells and large non-nucleated plates may be so clear as to recall the 26th day stage in the foetus. In silvered preparations the size of the large areas is between 600 and 800 sq.μ or more. The smaller areas clearly nucleated, are rather larger than an average intercapillary space. The lines joining them are not limited to the area of the capillary bed, since they do occasionally cross an intercapillary space and they appear to be limited to the surface.

Such observations have stimulated fruitless search in the rest of the lung, and by multiplying modifications of technique have sought to obtain unequivocal results in the largest part of the lung surface. No such evidence has been obtained from the rabbit. Much of the criticism of the silver method is based upon its inconstant or capricious results, and the locally successful results in the rabbit were for long attributed to such behaviour. Impregnations of foetal lung clear, fresh and entirely convincing were a constant challenge to further search in the adult. Such a situation is not easily resolved. Recognition of stages leading to irregularity of impregnation in the embryo showed that further search in the adult rabbit was equivalent to looking for something which did not exist. It has already been suggested that the inequality of size between the large and small impregnated areas of the foetus is the result of stretching of degenerate cell envelopes by injection of silver nitrate solution. Similar reasoning will account for the preservation of traces of the original epithelium in the subpleural alveoli of the adult rabbit. By far the largest part of the surface area of the lung is subjected by growth to considerable deformation as the result of deformation by septa (to be described in detail later). Epithelial remnants may therefore be expected to be distorted beyond recognition, and none, indeed, can be recognized over the largest part of the surface. The mean size, however, of subpleural alveoli, or of those abutting against an interstitial structure, is considerably larger (as noted by Schultze 1871; Ogawa 1920). In the central parts of the lung 25 to 28 septa can be counted over 1 mm. length—in subpleural alveoli only 15 to 18 are found. This suggests that their manner of growth is different. Whereas 'normal' alveoli distend and subdivide at the same time by evocation of septa, subpleural alveoli are probably distended by growth to a greater degree with less accompanying subdivision. There may therefore be less distortion of their luminal surface.

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Review of the literature concerning silver impregnation

The earliest signs of controversy concerning the lining of the pulmonary alveolus appear in 1843, when Thomas Addison wrote that he was 'fully persuaded that pathologically they (manifestly elastic air cells) present none of the attributes of a mucous membrane as Reisseisen and others would lead us to believe'. The choice evidently lay between a continuous nucleated epithelium or bare capillaries. Rainey (1855) adopts the latter alternative and declares that proof depends upon seeing the epithelium *in situ* and detached and following one into the other. Publication by von Recklinghausen (1860, 1862) of a method for impregnating cell outlines by salts of silver had the effect of adding a third interpretation of structure of the alveolar wall. Passing through the hands of Kolliker (1854, 1881) it became the commonly accepted teaching. At the same time the unsuccessful use of the method (perhaps partly dependent on species differences) gave encouragement to the two existing schools of thought whose subsequent development is most easily considered after toe new interpretation has been analyzed.

In the field of comparative anatomy, Elenz (1864) and Eberth (1864) obtained results of such importance that Kolliker, who had previously (1854) described a continuous nucleated epithelium, changed his opinion (1881). He accepted the doctrine of small nucleated cells lying singly or in small groups between the larger non-nucleated plates which together formed a complete lining to the alveolus. As a rule the amphibian lung showed an epithelium formed by large nucleated squames whose nuclei lay in the intercapillary spaces. Other species showed an intermixture of small cells with the squames. In certain reptiles, the small nucleated cells were numerous and sometimes appeared to have become separated from what was interpreted as the non-nucleated, film-like extension. A similar condition was described in man. In birds, no epithelial investment was demonstrable in the distal (respiratory) portions of the lung. Elenz, Oppel (1905) and Schultze (1871) considered nevertheless that an epithelium was present, and Eberth thought he recognized an occasional flat cell. Ogawa (1920) considered that an alveolar epithelium does not exist in birds. He modified certain conclusions of Elenz, but in general confirmed the conclusions of Kolliker. In addition, therefore, to producing very convincing preparations, Elenz and Eberth derived strength from the discovery of a series of stages whereby the epithelium of the mammalian lung could be derived through reptiles to an amphibian ancestor by modification of the lining cells of its terminal (respiratory) portion. This evidence, together with the fundamental desire to preserve in the lung 'the regulator effect usually exerted by epithelium on subjacent tissue',* 'satisfied tidy minds'† (an important action), convinced Kolliker and is to-day accepted as current teaching.

Doubts, however, persisted, in spite of ingenious modifications by the Bensleys (1935) of the silver technique. Three modern workers using silver have reached the conclusion that the alveolar wall is bare (Josselyn 1935; Loosli 1935, 1937; Clements 1937). Their conclusions are in part derived from a scarcely veiled mistrust of silver impregnation. To anyone who has seen the preparations (see figures 24, 25, plate 1) made by Gustav Mann in the Histological Department of Oxford University, it is clear that the appearance must be due

* The phrase comes from my friend Dr N. M. Hancox of the Department of Physiology, Liverpool University.

† Professor G. R. Cameron, F.R.S., *Lancet*, 11 January 1947.

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to impregnation of some sort of cell outline. The possibility of artifact, or impregnation of folds, fibres or red blood cells is not seriously to be considered. Policard's (1938) off-hand dismissal* of their identity with any sort of cell boundary seems to me to be unsubstantiated by examination of such preparations.

However, in many of the illustrations of the older workers, a peculiar irregularity of the surface meshworks is seen. Kolliker's figure shows it (1881, figure 10) and similar irregularity is found in the drawings of Sobotta (1903) and Merkel (1902). Often attributed to technical error or to the capricious results of silver (Miller 1937; Carleton 1938; Policard 1938), Jeker (1933) was able to trace the development of such irregularity in the cat embryo. For a satisfactory discussion to be possible this effect must be taken into account. Bargmann (1936), whilst supporting alveolar epithelium, relates this irregularity both to the classical theory of Kolliker and to the diametrically opposed theory of naked capillaries of Seemann (1931) by supposing that the non-nucleated plates survive to the adult in a modified form. If non-nucleated plates may lose their outlines to persist in a modified form, how much more need they lose to suffer total annihilation?

The hypothesis that the alveolar wall is lined by a continuous nucleated epithelium received support from Chrzonszczewsky (1863) using the silver technique. Modern adherents of this view have come to rely less and less on silver impregnation, and the main weight of the argument has ceased to be derived from normal lungs. Miller (1937) thus relies upon pneumonia, Cowdry (1925) upon Jagziekte, a disease of sheep, and Young (1928) upon experimental collapse of the lung. In relation to the silver nitrate method, the difficulty of this school is to explain away the appearance of non-nucleated plates. Having done so (Miller, 1937, pp. 59, 66) they are faced with a far more serious difficulty. The number of nuclei in the normal alveolar septum is not sufficient to provide the highly cellular epithelium which Miller illustrates (1937). Josselyn (1935) had already called attention to the fact that nuclei, other than those of endothelial cells, 'are so few in number in most cases that they appear quite incidental'. This aspect is considered in detail in the next section.

Nuclear counts of the alveolar septum of the adult rabbit

In thick sections of the lung where it is possible to study the whole extent of an alveolar septum, it is a striking fact that the distribution of nuclei is not regular. In material of which the capillary bed has been injected by weakly coloured carmine-gelatin, and subsequently counterstained by haematoxylin, surprisingly large areas are devoid of any nuclei whatever (see figure 33).

Assuming that the capillary bed of the alveolus is invested by endothelial cells, it should be possible to discover the size of these cells by counting endothelial nuclei in measured areas of the septum in which the capillary bed has been defined. It should also be possible to discover whether the total number of nuclei is sufficient to provide a complete investment for the capillary bed and also to provide epithelium for both surfaces of the alveolar septum. By excluding leucocytes and by identifying nuclei of endothelial cells and alveolar phagocytes, the remaining nuclei could be regarded as belonging to epithelium. No such nuclei were found.

* 'Mais on peut affirmer qu'ils n'ont rien de commun avec des limites cellulaires', loc. cit (p. 61).

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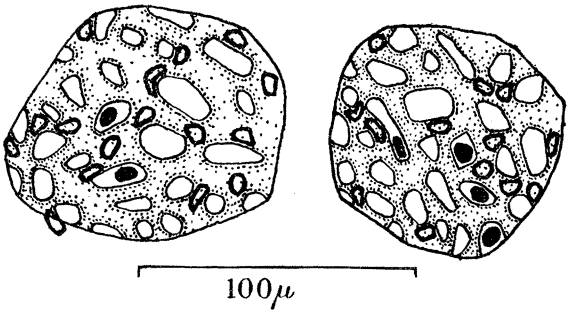

FIGURE 33. Nuclei of endothelial cells (black outline) and of alveolar phagocytes (solid black) in alveolar septa viewed in plan, in which the capillary bed has been injected and is represented by stippling. Adult rabbit.

TABLE 7. EXTENT OF CAPILLARY BED IN ALVEOLAR SEPTA. ADULT RABBIT

example	area of septum (sq.$\mu = S$)	area of capillary bed (sq.$\mu = C$)	$\frac{C \times 100}{S}$ (%)	surface of capillary bed (sq.$\mu = \pi \times C$)
1	8,260	6,500	79	20,400
2	7,280	6,220	85	19,600
3	6,530	5,080	78	15,900
4	6,650	5,180	78	16,300
5	7,100	5,680	80	17,800
6	5,770	4,320	75	13,600
7	7,740	5,900	76	18,500
8	5,770	3,930	68	12,300
9	4,000	2,700	67	8,480
10	4,180	2,780	67	8,750
11	2,830	1,790	63	5,630
12	2,780	1,960	70	6,150
13	3,450	2,220	64	7,000
14	2,390	1,920	80	6,030
15	3,600	2,710	75	8,500
mean	5,220	3,920	75	12,300

TABLE 8. NUMBERS OF NUCLEI IN ALVEOLAR SEPTA. ADULT RABBIT

example	numbers of nuclei: endothelial	numbers of nuclei: alveolar phagocytes	total	size of endothelial cell (sq.μ)
1	33	4	37	620
2	34	6	40	580
3	25	5	30	640
4	16	2	18	1010
5	16	1	17	1110
6	13	4	17	1040
7	16	4	20	1160
8	14	6	20	880
9	13	5	18	650
10	10	5	15	875
11	9	5	14	630
12	11	3	14	560
13	7	4	11	1000
14	10	4	14	600
15	13	2	15	660
mean	16	4	20	800

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Tables 7 and 8 summarize the observations on fifteen septa in a rabbit's lung. The capillary bed occupies about 75 % of the total area of the septum. By multiplying the area of the capillary bed by π, an approximation can be obtained to the total surface area of the capillary bed. Dividing this estimate by the total number of endothelial nuclei, the average size of an endothelial cell is of the order of 800 sq.μ. Four cells, identified as alveolar phagocytes, remain, and if it is assumed that they are capable of forming a complete epithelial investment to both surfaces of the septum, their size would need to be 2600 sq.μ. If allowance is made for folding of the cells round the capillary bed the average size would be almost 4000 sq.μ. Even if we assume that nuclear identification is incorrect and that all types of cell may be called upon indiscriminately to cover the capillary bed and both surfaces of the septum, the average size of these twenty cells would need to be 1360 sq.μ. We may conclude confidently that a continuous nucleated epithelium does not exist in the normal adult rabbit.

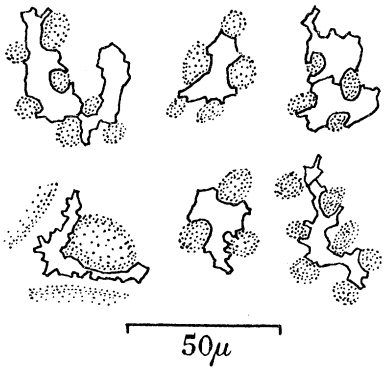

FIGURE 34. Impregnated outlines of endothelial cells from the capillary bed of alveolar septa. Intercapillary spaces are stippled. Adult rabbit.

This estimate of endothelial cell size is seen to be much larger than that of the isolated cells shown in figure 34. This is due to two causes: a greater degree of distension was deliberately obtained in the lungs from which counts of nuclei were made in order to facilitate their study, and allowance has been made for curvature of the wall of the capillary. Both these factors will therefore reduce the calculated size of the endothelial cell. The volume of lungs used for nuclear counts is about 1·5 times the volume of the silvered lungs, making measurements of area too high by 1·3. An approximation to the second factor is given by $\frac{1}{2}\pi$ or 1·77, since not more than two endothelial cells enclose the capillary wall, though one cell may sometimes do so. The total amount therefore by which area measurements in tables 7 and 8 are too high is 2·4, and division by this factor gives the mean cell area of flattened capillary endothelial cells as 310 sq.μ. This figure is in harmony with measurements of area of capillary endothelial cells which I have made in the skin of the adult rabbit.

Results of dissociation of the alveolar septum

Several attempts were made to dissociate the structures composing the alveolar wall of the rabbit's lung by perfusion through the pulmonary artery at pressures of 50 mm. Hg. Using normal saline as perfusate, heavy oedema occurred within 15 min., but histological examination showed no more than widely dilated capillaries. Using formol-Müller solution,

histological examination showed that three grades of dissociation had been produced in three rabbits. A similar effect following perfusion of fixative seems to have been noticed by Schultze (1871). The first rabbit showed heavy oedema with slight evidence of rupture of the alveolar septa. In the second considerable rupture with oedema was present. In the third, a generalized interstitial dissociation with no rupture and a minimal oedema within the alveoli had been produced, a condition which could be described as interstitial oedema.

Histological examination of this lung showed that the effect had been to dissociate the capillaries with the protoplasmic films separating them from the lumen of the alveolus and thus to separate the constituent structures of the alveolar wall by accumulation of fluid within its substance. The most striking feature was the thickness of the septum. The typical appearance of congested capillaries of the normal alveolar wall, bulging into the lumen to either side of the delicate septum, had completely disappeared. Instead, the dilated capillaries were situated between parallel lining membranes of extreme delicacy. The continuity of the lining membrane was occasionally interrupted by a septal cell, but over long tracts no nuclei were to be seen on the luminal surface (figures 35, 36, 37, plate 1). Endothelial nuclei, recognizable by their minute speckling with Heidenhain's iron haematoxylin, lay more often in the substance of the alveolar septum than in contact with the surface. Nowhere was there an orderly nor numerous arrangement of nuclei to suggest the presence of a nucleated epithelium. In many alveoli not one nucleus was to be seen in contact with the lumen.

Where they were in closest apposition to the alveolar lumen, the capillaries appeared to be separated from it only by an almost structureless membrane containing reticulum fibrils. Not once was this membrane found to be lifted off or separated from the endothelial cytoplasm, and normally both appeared to be in direct continuity. It was not possible to be certain whether this lining membrane was merely the stretched cytoplasm of endothelial cells, but appearances frequently suggested that this was the case. If such is the case, the lining membrane is that part of the endothelial cell excluded from contact with the vascular lumen. The distribution of mitochondria in the cytoplasm of endothelial cells was thought to show that similar bodies in that portion of the lining membrane nearest to the capillary were also mitochondria.

Close attention was paid to the points at which bronchiolar epithelium came to an end to be replaced abruptly by the lace-like structure of the alveolar septum (figure 38, plate 1). A capillary was often in direct contact with the lateral wall of the last epithelial cell. Elsewhere a basement membrane of reticulum intervened between the capillary plexus and the epithelial cells.

It appeared, therefore, that the capillary bed was everywhere ensheathed by a delicate membrane containing reticulum fibrils. The impression was formed that the membrane was continuous with the cytoplasm of endothelial cells. No evidence was obtained of the existence of a continuous nucleated or non-nucleated epithelium which could be detached from the capillary.

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PART 2. LUNG GROWTH

Introduction

The histological picture of lung development is everywhere consistent with the view that alveolar epithelium ceases to exist after a certain stage in the embryonic life of the rabbit. It is, however, easily recognized that the complexity of the lumen of the foetal lung increases as the result of branching and rebranching. It might be supposed that the invasive activity of epithelium was displayed to great advantage. After the 26th day further subdivision occurs and the complexity of the lumen increases still further. However, at the 24th day, a continuous epithelium has ceased to exist. Yet the lumen has continued to grow and to become more subdivided.

The growth of the lumen can only be investigated when quantitative data for total lung volume are known. When lung volume is known, the percentage distribution of lumen in stained sections of the lung can be applied to total lung volume. Volume data are therefore the point of departure in this second part of the investigation of lung development.

These results can next be compared with quantitative estimates of 'complexity' of subdivision of the lumen. A simple method, capable of interesting mathematical development, has been used to measure internal surface area of the lung. Moreover, it has been found possible to distinguish increase in surface area resulting from simple distension from that which must occur as a result of increasing complexity of branching. It is found that internal surface area increases as a result of increased subdivision long after desquamation of epithelium has occurred.

Methods

The numbers of animals are shown in table 9.

Table 9. Series of embryo and post-natal rabbits

day	numbers of animals	observations of: lung weight	observations of: lung volume
18	2 (mean)	2	—
20	4 (mean)	4	—
23	2 (mean)	2	—
24	3 (individual)	3	—
26	7 (mean)	7	—
	5 (mean)	5	—
	11 (individual)	11	—
29	2 (individual)	2	—
30	15 (individual)	15	15
31	3 (individual)	3	—
	3 (individual)	3	3
	3 (individual)	3	3
	4 (individual)	—	4
(birth)			
3 hr.	5 (individual)	—	5
4 hr.	1 (individual)	—	1
6 hr.	5 (individual)	—	5
1 day	4 (individual)	—	4
	10 (individual)	—	10
2 days	2 (individual)	—	2
4 days	2 (individual)	—	2
5 days	3 (individual)	—	3
10 days	3 (individual)	—	3
3 months	3 (individual)	—	3
adult	4 (individual)	—	4

The mother was anaesthetized intravenously with 1·0 ml. nembutal per kg. body weight, the abdomen was opened and the uterus rapidly incised. The foetuses in turn were removed from the uterus and strangled without opening the amniotic sac. Care was taken to avoid the occurrence of premature respiratory movements caused by rough handling. After death, the trachea was tied with cotton. The body weight of the foetus was then determined after removal of the placenta and membranes.

In the larger lungs at term and in those which had breathed, lung volume was measured directly (as described below) in lungs which had been fixed for 48 hr. within the unopened thorax. In the smaller, normally fluid-filled lungs of the embryo, direct measurement of volume was found to be impracticable and volume was calculated from lung weight and lung density.

Shrinkage of the lung produced by fixation

Fixation of the embryo lung in 10 % formol saline affords so much protection against trauma to the jelly-like consistence of the lung when fresh, that all manipulation in these experiments has been delayed until fixation was complete. This procedure has the disadvantage that measurements of weight must be made on fixed lungs. It becomes necessary to establish whether the effect of fixation upon lung weight can be regarded as a constant and if not, to prepare a calibration curve between fresh and fixed lung weight.

In the following preliminary experiments the fresh thoracic contents were removed as carefully as possible. Their fresh weight was determined and the whole was then fixed in formol saline. Weight was determined at 1, 3, 24, 48 hr. and 3 days after fixation. To remove the thoracic contents is not a difficult matter, and can be performed in the confidence of having avoided trauma to the jelly-like lungs and of preserving their fluid contents. To separate the individual fresh lungs is very much more difficult though very easy after they have been fixed.

When fixed in formol saline shrinkage of the lungs is found to be variable, and the weight of the fresh embryo lung is reduced by an amount which depends upon three factors:

(1) The time after immersion in fixative. An initial fall, maximal between 1 and 3 hr. after immersion, is succeeded by a slow rise to a figure somewhat less than the weight of the fresh lung. Measurements of weight have been made in all cases at 48 hr. after immersion of the embryo lung in formol saline.

(2) The age of the embryo. At the 24th day of the rabbit's 32-day gestation period, the effect of 48 hr. fixation in formol saline is to reduce the weight of the fresh lung by 1 %. At the 26th day about 5 % is lost, and at the 28th and 30th day about 10 and 15 % respectively. The effect is probably due to loss of water following shrinkage of the lung, and the size of the effect is due partly to the degree to which the lung contains a lumen. Earlier than the 24th day when about 25 % of total lung volume is occupied by lumen, shrinkage is slightly below 1 %. Moreover, in the completely collapsed lung of the adult rabbit loss of weight by fixation amounts also to no more than 1 %. It is therefore justifiable to regard shrinkage of pulmonary interstitial tissue as a constant of negligible importance.

(3) Respiration *in utero*. If half the embryos in a litter older than the 26th day are strangled before respiration has occurred, lung weight of these foetuses is reduced by the amounts shown in the preceding paragraph. Intra-uterine respiration is then encouraged

in the remaining foetuses by tapping the unopened uterus. Loss of weight is found to be considerably greater in most of the members of this group. It is no doubt due to the increased stretch (and thus to increased capacity to shrink) resulting from inhalation of liquor amnii. A similar effect was noted in the control experiment of the collapsed lung fixed in formol saline. The control lung was placed in normal saline and gained 6 % of its original, fresh weight in 4 hr. It was then placed in formol saline for 48 hr., by which time it had lost 1 % of its original fresh weight—the same loss as was found in the collapsed lung which had been fixed at once. If, however, loss of weight is calculated as loss of greatest weight (as must be the case in embryos which have inhaled liquor amnii), loss of weight in the control collapsed lung is 7·0 %.

Premature respiration will therefore increase the amount by which the embryo lung shrinks during fixation. Though it is possible to discard those foetuses which show intra-uterine respiration during the experiment, it is not possible to affirm that no respiration has occurred in the remaining foetuses before the experiment was started. Therefore the factor by which fresh lung weight differs from fixed lung weight has been calculated from the mean loss of weight of lungs from both groups and not merely from that group in which respiration *in utero* was not observed.

TABLE 10. SHRINKAGE OF THORACIC CONTENTS DURING 48 HR. FIXATION

	percentage of original weight lost		
day	no respiration	mean of both groups	premature respiration
24	0·2	1·5	
	1·0		
	3·3		
26	3·4	8·5	12·8
	4·5		14·0
	5·0		14·6
	5·9		
	7·8		
28	9·8	14·2	16·7
	9·8		17·6
	11·6		20·3
	13·5		
	13·8		
30	12·0	15·5	17·5
	14·2		20·2
	14·2		
	14·9		

TABLE 11. CORRECTION FOR SHRINKAGE DURING FIXATION. RABBIT

day	correction for weight	correction for volume*	correction for length†
24	1·015	1·014	1·005
26	1·09	1·040	1·013
27	1·14	1·08	1·025
28	1·16	1·10	1·033
29	1·175	1·12	1·037
30	1·182	1·125	1·04
31	1·19	1·135	1·043

* Obtained by dividing the correction for weight by lung density = 1·05.
† The cube root of corrections for volume.

Shrinkage of air-filled lungs

The volumes of air-filled lungs have in all cases been measured after intravenous perfusion of fixative whilst the lung was contained within the unopened thorax. Attempts have been made to discover whether or not such air-filled lungs fixed *in situ* are shrunk by fixation.

Table 12 shows that no significant shrinkage is found between the volume of a wax cast of the thoracic cavity and the volume of the fixed thoracic contents in pairs of animals of the same body weight. These measurements were made upon 10-day-old and adult rabbits.

TABLE 12. SHRINKAGE OF AIR-FILLED LUNGS

age	body weight (g.)	volume of cast (ml.)	volume of thoracic contents (ml.)
10 days	138	9·8	10·3
	140	15·0	12·7
		mean 12·4	11·5
adult	1840	65·0	71·5
	1840	79·0	85·5
		mean 72·0	78·5

Treatment of results

The fitting of curves to growth data has been the subject of many discussions, and no agreement has been reached as to the most suitable type of curve. In view of this situation and of the purpose of the present investigation it has seemed unnecessary to attempt to fit a curve. Since successive groups of observations were made at relatively short intervals it has been deemed adequate to intrapolate linearly between adjacent means in order to obtain, when required, an estimated mean value at intermediate times.

Fixed lung weight. Embryos fixed in 10 % formol saline after tying the trachea and after opening the thorax in order to obtain rapid penetration of fixative. After 48 hr. the lungs and heart were carefully removed from the thorax and the lungs were separated after tying the hila. Excess fluid was gently removed from the surface with cotton-wool, and fixed lung weight was determined in a weighed watch-glass.

Lung volume. Determinations of lung volume at the 30th day were carried out as described below after weighing fifteen lungs which had been fixed for 48 hr.

In all other cases a different procedure was adopted since the lungs had breathed. The newborn or older rabbit was given 0·5 ml. nembutal by intraperitoneal injection. When respiration had ceased, the abdomen was opened and 2 ml. of 10 % formol saline was slowly injected into the inferior vena cava. The whole foetus was then immersed in formol saline. After 48 hr. the trachea was tied and the lungs and heart carefully removed from the thorax. After tying the hila, the lungs were gently separated and their volumes determined by displacement of water. Smaller lungs were placed in a burette reading to 0·1 ml. The larger lungs were placed in a burette of larger calibre, and after re-levelling the volume of displaced fluid was collected in the smaller burette reading to 0·1 ml., which was placed below. By 'lung volume', therefore, is meant volume of the lung fixed *in situ*.

Percentage of lumen and interstices. From histological material, whose preparation has been described in part 1, ten fields, including as far as possible only the terminal lobules, from each lung were drawn with a camera lucida using a sixth-inch objective. Two sets were

prepared. In the first, only lumina were drawn; in the second, only the basement membranes. The spaces were then cut out and weighed together. The remaining parts were weighed, added to the spaces and both expressed as a percentage of the total. The assumption was made that the composition of an average volume of the lung was the same as that of the average of the ten fields. The figures were then applied to lung volume to obtain the data in tables 21 and 23, which therefore represent the composition of a lung lobule of the same size as the lung or the composition of an imaginary lung from which all the larger structures (arteries and bronchi) have been removed.

Counts of septa. Using a sixth-inch objective, five fields from each lung were drawn on squared paper and the intersections of the lumen with eighteen lines were counted. Two sets of drawings were prepared, counting intersections with the lumina in one and with the basement membranes in the other. Each line measured 308μ. The total grid length was therefore 5550μ or 0·555 cm. So far as possible, fields were chosen where the larger structures (arteries and bronchi) were absent.

For a better understanding of the implications of this method, I am indebted to Mr C. A. Rogers of the Department of Mathematics, University College, London. I quote, with his permission, from a letter in which he draws attention to a formula by which the surface area of a branched lumen can be related to its complexity of branching. He says:

'Suppose that the total volume of a lung or of a lobe of a lung is V and that the surface area of the lumen is S. Suppose that a histological section is taken at random from this lung or lobe and that it is drawn upon a system of lines (squared paper) of total length l. Suppose further that the lines of total length l cut the surface of the lumen n times. It can be proved (the proof is appended) that the average value of n obtained by repeating this process a number of times will be given by the formula

$$n=\frac{lS}{2V}. \tag{1}$$

So if l is the length of lines of the two systems of the grid reduced to the appropriate scale for the magnification factor of the microscope and n is the average of the number of intersections obtained from the study of a section, then an estimate of the ratio of the internal surface area S to the volume V of the lung or lobe is given by

$$\frac{S}{V}=\frac{2n}{l}. \tag{2}$$

There are two quantities of biological significance which may be calculated. The first is the surface area S given by

$$S=\frac{2nV}{l}. \tag{3}$$

The second quantity of significance is the non-dimensional ratio

$$\frac{S}{V^{\frac{2}{3}}}=\frac{2nV^{\frac{1}{3}}}{l}. \tag{4}$$

This is a well-known criterion of the complexity of the convolutions of a surface of area S packed into a volume V.

Proof of formula (1) *by C. A. Rogers*

Consider a volume V of lung. Let S be the surface area of the lumen contained in the volume V. Let G be a grid of lines of total length l, the size of the grid being small in comparison to V (see figure 39). We show that if G is moved by a simple translation to a random position in the volume V

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(each position being equally likely) then, on the average, the number of intersections of the lines of G with the surface S will be

$$n = \frac{lS}{2V}.$$

We first consider the case when all lines of G are parallel to some fixed direction. For convenience we choose an origin O and rectangular Cartesian co-ordinate axes OX, OY, OZ, with OX in the direction of the lines of G. Consider an element of length δl of one of the lines of G. Let P_0 be the end-point of this element with the lesser x co-ordinate. We suppose that the element is moved by a translation to a random position in V and show that, provided δl is sufficiently small, the chance that the element has an intersection with S is

$$\frac{S\delta l}{2V}.$$

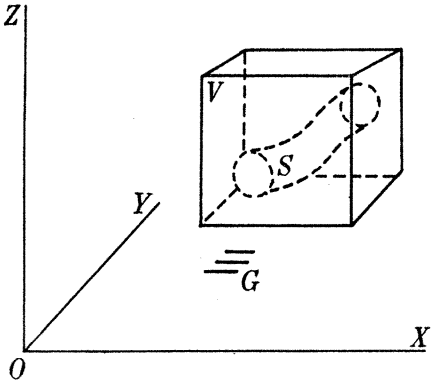

FIGURE 39. In this diagram, V has been taken to be a cube, S to be a cylinder and G to be a system of three equal lines.

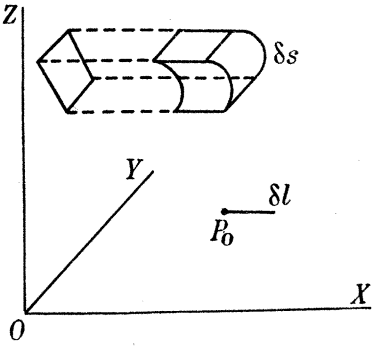

FIGURE 40.

Consider an element with area δs of the surface S. We suppose that the element is so small that it may be regarded as flat, and we suppose that θ is the acute angle between the normal to the element and the OX axis. Now when the element δl of G is moved into V it intersects the element δs of S if, and only if, the point P_0 is moved into the skew prism generated by moving the element δs by a translation in the direction from X to O through a distance δl (see figure 40).

The volume of this prism is the product of δl and the area of the projection of the element δs on the plane OYZ, i.e.

$$\delta l\, \delta s \cos\theta.$$

As P_0 is equally likely to be at any point in the volume V, its chance of being in this prism of volume $\delta l\, \delta s \cos\theta$ is

$$\frac{\delta l\, \delta s \cos\theta}{V}.$$

This is consequently the chance that the element δl of G intersects the surface element δs. We may assume that δl is so small that the chance of intersecting the surface S more than once is negligible.

Then the chance that δl intersects the surface S at some point is obtained by summing the chances of δl intersecting surface elements such as δs or by evaluating the integral

$$\int_s \frac{\delta l \cos\theta}{V}\, ds = \frac{\delta l}{V}\int_s \cos\theta\, ds,$$

taken over the surface S. As the surface S is highly convoluted, and as the normals to the surface are equally likely to point in any direction, we have

$$\int_s \cos\theta\, ds = cS,$$

where c is the mean value of $\cos\theta$. To find c we consider a sphere centre O with unit radius. The surface area of this sphere is 4π; the area of the projection of the surface on the plane OYZ is 2π (π from the hemisphere on one side of the plane OYZ and π from the hemisphere on the other side). The mean value for $\cos\theta$ is the ratio of these two areas. Thus $c = \frac{1}{2}$, and the chance that the element δl intersects the surface S at some point is $\frac{S\delta l}{2V}$. On the average, the element δl will intersect the surface $S\frac{S\delta l}{2V}$ times. So, summing over the whole length of the grid G, the grid will intersect the surface $S\frac{Sl}{2V}$ times on the average. Although we have for convenience assumed that all the lines of G are parallel to the x-axis, it is clear that this assumption is not necessary. This completes the proof.'

Results of counts of intersections are recorded as n. Since n varies directly with length of the grid or, which is the same thing, inversely with the cube root of changes in volume, a correction for shrinkage must be applied by dividing by the cube root of shrinkage of lung volume. N therefore represents the slightly smaller number of intersections which would have been counted if lung volume had not shrunk during fixation. To be comparable with results obtained by using other lengths of grid, results are calculated as $2N/l$, an estimate, as C. A. Rogers has shown, of internal surface area per unit volume of lung.

Results

Weight of the fixed lung

From tables 10 and 11 showing the loss of weight of the lung consequent upon fixation, a correction may be applied to weight of the fixed lung shown in table 13, in order to restore its loss.

Density of the fixed lung

Density of the fixed lung was measured at four stages during the embryonic period. Variation is probably the result of experimental error, and it is assumed that density of the fixed lung is a constant at 1·05. This is in some agreement with the statement of Fauré-Fremiet & Dragoiu (1923), that in the sheep foetus it is of the order of unity.

Volume of the fixed lung

Except in the few embryos where volume of the fixed lung was measured directly for the purpose of estimating density, volume in all other cases between the 18th and 28th days has been calculated from lung weight and density. The mean figure from the data in table 16 can be corrected for shrinkage during fixation, and this is used as the basis for subsequent calculation.

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TABLE 13. LUNG WEIGHT OF THE RABBIT EMBRYO

day		weight of fixed lung (g.)		mean weight corrected for shrinkage (g.)
18		$(0{\cdot}04)^2$		0·04
20		$(0{\cdot}072)^4$		0·072
23		$(0{\cdot}271)^2$		0·271
24		0·33		
		0·335		
		0·455		
		0·275		
		0·347		
		0·418		
	mean	0·36	(+1·5%)	0·365
26		$(0{\cdot}74)^7$		
		$(0{\cdot}94)^5$		
		0·560		
		1·100		
		0·590		
		0·655		
		1·125		
		0·745		
		0·955		
		0·715		
		0·990		
		0·720		
		0·705		
		0·633		
		0·553		
		0·600		
		0·555		
		0·415		
		0·525		
		1·132		
		0·590		
		0·670		
		0·675		
	mean	0·656	(+8·5%)	0·715
28		1·021		
		1·008		
		1·065		
		0·778		
		0·885		
	mean	0·960	(+14·0%)	1·115
30		1·910		
		1·425		
		1·415		
		1·267		
		1·267		
		1·247		
		1·172		
		1·167		
		1·784		
		1·386		
		1·212		
		1·235		
		1·197		
		1·182		
		1·032		
		1·538		
		1·438		
		1·309		
		1·530		
		1·146		
		1·205		
	mean	1·335	(+15·5%)	1·58
31		0·980		
		0·955		
		0·990		
		0·980		
		0·870		
		1·055		
		1·385		
		1·355		
		1·550		
	mean	1·125	(+16%)	1·34

Figures for weight within brackets are mean values for the numbers of animals shown outside the bracket.

TABLE 14. DENSITY OF THE FIXED LUNG. RABBIT EMBRYO

(From measurements made on the same lungs)

age (days)	mean weight of fixed lung (g.)	mean volume of fixed lung (ml.)	density of fixed lung
24 (3 lungs)	0·346	0·333	1·04
26 (5 lungs)	0·546	0·533	1·025
28 (5 lungs)	0·951	0·92	1·035
30 (3 lungs)	1·36	1·265	1·075
	1·29	1·2	1·07
	1·36	1·31	1·03
			mean 1·046

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Experiments described earlier show that shrinkage of air-filled lungs by fixation *in situ* is probably negligible.

TABLE 15. MEAN LUNG WEIGHT: RABBIT EMBRYO; AND CALCULATED MEAN LUNG VOLUME

day	lung weight (g.)	density = 1·05 calculated lung volume (ml.)
18	0·040	0·038
20	0·072	0·068
21*	0·138	0·131
22*	0·204	0·194
23	0·271	0·258
24	0·365	0·347
26	0·715	0·680
28	1·115	1·100
30	1·580	1·500
31	1·340	1·275

* Values obtained by linear intrapolation between means.

TABLE 16. MEASURED VOLUME OF THE FIXED LUNG: RABBIT EMBRYO

day		volume of fixed lung (ml.)		corrected lung volume (ml.)
30		(1·200)[7]		
		(1·310)[8]		
	mean	1·255	+14·8 %	1·47
31		1·0		
		0·8		
		1·0		
		1·3		
		1·3		
		1·5		
		1·5		
		1·2		
		1·4		
		1·4		
	mean	1·24	+15·2 %	1·46

These values are included with those of the preceding table in table 17.

TABLE 17. MEAN LUNG VOLUME: RABBIT EMBRYO

	lung volume (ml.)		
day	direct measurement	by calculation	
18	—	0·038	
20	—	0·068	
21	—	0·131	
22	—	0·194	
23	—	0·258	
24	—	0·347	
26	—	0·68	
28	—	1·10	
30	1·5		
	1·47		
mean	1·49	1·49	
31	1·275	—	mean 1·426
	1·46	—	
mean	1·375	1·367	

It will be noted from table 18 that the increase in lung volume during the first 24 hr. following birth amounts to 40 % of lung volume before respiration has occurred.

TABLE 18. VOLUME OF THE FIXED LUNG. POST-NATAL PERIOD (RABBIT)

age	volume of fixed lung (ml.)		age	volume of fixed lung (ml.)	
3 hr.	1·9		48 hr.	3·1	
	1·1			2·5	
	1·2			mean 2·8	
	2·6		5 days	4·5	
	2·2			5·0	
	mean 1·8			4·5	
4 hr.	1·8			mean 4·66	
6 hr.	2·2		10 days	6·5	
	2·5			7·0	
	1·7			8·0	
	2·0			mean 7·2	
	2·5	mean = 1·956	3 months	19·2	
	mean 2·2			21·3	
24 hr.	1·35			28·1	
	1·7			mean 22·9	
	1·9		adult	38·0	
	2·0			46·4	
	2·1			51·4	
	2·6			47·2	
	1·5			mean 45·75	
	2·1				
	2·5				
	2·5				
	mean 2·025				

TABLE 19. PERCENTAGE COMPOSITION OF SECTIONS OF LUNG: RABBIT EMBRYO

	measured to lumen (%)		measured to basement membranes (%)		
day	lumen	mean	lumen	mean	% occupied by epithelium
18 (2 foetuses)	5	5	10	13	8
	5		16		
21 (2 foetuses)	10	16	18	22	6
	22		26		
22 (3 foetuses)	17	22	27	33	11
	22		29		
	26		39		
24 (3 foetuses)	26	30	38	44	14
	28		41		
	35		49		
26 (4 foetuses)	34	44			
	41				
	46				
	57				
27 (3 foetuses)	42	50			
	52				
	58				
28 (3 foetuses)	45	51			
	56				
	54				
30 (3 foetuses)	54	57			
	56				
	61				

Growth of the respiratory lumen

The percentage area occupied by lumen (see table 19) as measured in a series of fields requires a correction for shrinkage, for it has been shown that the lumen shrinks whilst shrinkage of the interstitial tissues is negligible. Since the extent of lumen is measured in terms of volume, the percentage loss of weight shown in table 11 requires to be divided by density and the quotient added to the figure for percentage of lumen. This will involve recalculation, since the correct figure for the lumen added to that of the interstices must exceed 100. The percentages are recalculated from this total and the steps are indicated in table 20.

TABLE 20. MEAN PERCENTAGE COMPOSITION OF SECTIONS OF LUNG. EMBRYONIC PERIOD

day	percentage of lumen in fixed lung	percentage of interstices	percentage of lumen lost	percentage of lumen lost $\left(=\frac{\text{weight}}{1{\cdot}05}\right)$
18	9	91	—	—
21	19	81	—	—
22	27·5	72·5	—	—
24	37	63	1·5 by weight	1·43 by volume
26	44	56	8·5 by weight	8·1 by volume
28	51	49	14·2 by weight	13·5 by volume
30	57	43	15·5 by weight	14·8 by volume

day	corrected % of lumen	percentage of interstices	total	true % lumen	true % interstices	total
18	9	91	100	9	91	100
21	19·00	81	100	19	81	100
22	27·50	72·5	100	27·5	72·5	100
24	38·43	63	101·43	37·4	62·6	100
26	52·10	56	108·1	48·2	51·8	100
28	64·50	49	113·5	57·0	43·0	100
30	71·80	43	114·8	62·7	37·3	100

TABLE 21. COMPOSITION OF LUNG VOLUME. EMBRYONIC PERIOD

day	lung volume (ml.)	volume of lumen (ml.)	volume of interstices (ml.)	$\frac{\text{lung volume*}}{\text{interstitial volume}}$
18	0·038	0·0034	0·0346	1·10
21	0·131	0·025	0·106	1·24
22	0·194	0·053	0·141	1·38
24	0·347	0·130	0·217	1·60
26	0·680	0·327	0·353	1·93
28	1·100	0·630	0·470	2·34
30	1·490	0·930	0·560	2·67

* This ratio is calculated here for later use when the principles underlying subdivision of the lumen are considered.

Up to the 24th day, the presence of an easily visible epithelium must be taken into account when estimating the volume of lumen and of interstitial substance. Owing to the great height of the epithelium in the early stages of development, the size of the lumen is often very small. It may be non-existent in the extremities of tubes. Nevertheless, a luminal space will be defined by the basement membranes. Two parallel sets of measurements have therefore been prepared, the difference between which represents the volume of epithelium.

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Between the 18th day of gestation and term, branching of the lumen occurs at a rate which exceeds that at which mesenchyme is being laid down. Consequently the lumen enlarges until by term rather more than half the area of any lung field is occupied by lumen.

TABLE 22. PERCENTAGE COMPOSITION OF LUNG. POST-NATAL PERIOD

age	percentage of lumen	mean
1 day	65	68·6
	68	
	73	
2 days	68	76
	74	
	81	
5 days	76	80
	78	
	85	
10 days	82	85
	84	
	89	
3 months	83	88
	87	
	92	
adult (18 months)	88	90
	89	
	91	
	92	

TABLE 23. COMPOSITION OF LUNG VOLUME. POST-NATAL PERIOD

age	lung volume (ml.)	volume of lumen (ml.)	volume of interstices (ml.)	lung volume / interstitial volume
1 day	2·0	1·37	0·63	3·18
2 days	2·8	2·12	0·68	4·12
5 days	4·66	3·72	0·94	4·96
10 days	7·2	6·1	1·10	6·54
3 months	22·9	20·2	2·7	8·5
adult	45·75	41·18	4·57	10·0

Subdivision of the respiratory lumen: foetal period

A series of lung fields, excluding so far as possible the larger structures and limited to the terminal portions of lobules, shows clearly the increasing complexity of branching of the respiratory tubes. By drawing ten such fields on squared paper and by counting intersections of the drawing with the ruling, a quantitative estimate can be made of the internal surface area per unit volume ($2N/l$) of the lung or of total internal surface area ($2NV/l$). Before the 26th day, the presence of a complete epithelium interferes with the estimate. No lumen will exist in the extremities of many tubes which are filled solid with epithelium. On the other hand, a basement membrane is still present. Two sets of data have therefore been prepared. In the first, intersections with the basement membrane were counted. In the second, only those septa have been counted which enclose a lumen however minute, and in this series the numbers of intersections are smaller than in the first. After the 26th day epithelium now invisible by ordinary methods causes no further trouble. It is to be noted that massive desquamation of epithelium occurs between the 24th and 26th days without influencing the slope of the line. These data are shown in table 24.

TABLE 24. SEPTA COUNTED PER UNIT AREA. RABBIT EMBRYO

	counted to basement membrane				counted to lumen			
day	septa			mean	septa			mean
18 (2 foetuses)	20	26		30	18	16		18
	28	32			18	17		
	30	33			18	17		
	30	34			19	20		
	32	35			20	20		
21 (2 foetuses)	48	58		65	53	57		61
	56	60			60	58		
	62	74			63	62		
	68	78			63	63		
	70	79			66	63		
22 (3 foetuses)	83	74	87	89	69	70	72	79
	84	78	89		70	78	83	
	84	86	95		70	81	84	
	90	87	101		70	81	86	
	95	90	104		89	84	96	
24 (3 foetuses)	111	119	124	126	91	92	92	109
	116	122	125		97	98	106	
	118	126	130		105	106	115	
	124	131	136		106	117	126	
	126	132	138		127	124	128	
26 (3 foetuses)	135	120	118	137				
	136	130	130					
	144	140	133					
	149	146	134					
	151	146	140					
27 (2 foetuses)	118	169		158				
	120	177						
	131	178						
	139	188						
	154	202						
28 (2 foetuses)	174	173		177				
	174	173						
	174	174						
	175	186						
	180	185						
30 (2 foetuses)	198	198		208				
	199	209						
	201	220						
	206	221						
	218	224						

From embryonic material these counts will require correction for shrinkage due to fixation. It is clear that too many intersections have been counted, since the area of lung has shrunk in proportion to the shrinkage of lung volume. Since the numbers of intersections are proportional to grid length, the counted number divided by the cube root of volume lost by shrinkage will reduce the counts of intersections to the extent required by the same lung field in which no shrinkage had occurred. This correction has been applied in table 25.

Subdivision of the respiratory lumen: birth and neo-natal period

During the first 24 hr. following birth, the effects of aeration show an irregular distribution in the lung. Certain lobules contain no air and may be collapsed, others are well expanded. Depending upon the extent of collapse, the aerated lobules are often over-

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TABLE 25. NUMBERS OF SEPTA. (BETWEEN THE 18TH AND 24TH DAYS MEANS OF BOTH VALUES ARE SHOWN)

day	septa counted ($=n$)	$\sqrt[3]{}$(shrinkage) (see table 11)	corrected for shrinkage ($=N$)	$2N/l$
18	24	—	—	87
21	63	—	—	227
22	84	—	—	302
24	117	1·005	116	418
26	137	1·013	135	485
28	177	1·033	172	620
30	208	1·04	200	720

distended, an effect which slowly disappears as more and more lobules expand. It is, in my experience, uncommon to see a lung in which aeration is uniform during the first 24 hr.

At 48 hr., aeration is uniform. Spaces previously over-distended, compressing the adjacent architecture, are now represented by regularly spaced ducts in whose septa a remarkable change has occurred. Septa everywhere are now minutely indented and show numerous small septa drawn up between the indentations. The expansile stress of respiration, originally merely distensive, has become redistributed to produce regular indentations of the septa as a result of increase of tension in the elastic fibres in their free edges. The walls of the alveolar ducts thus become minutely honeycombed by new formation of polyhedral chambers (the alveoli) whose septa are drawn up by elastic fibres into the lumen of the alveolar duct. This is a very different process from an expansile formation of alveoli by budding from the alveolar duct; budding must occur into interstitial tissue, and such a mechanism would require a far larger amount of interstitial tissue than actually exists in the lung before this stage is reached.

In consequence the pattern of lung architecture shows the first post-natal signs of increasing subdivision. The subdivisions are smaller than have been seen in the earlier stages and gradually exhibit a very clear pattern within the lobule, being situated as a double row on each side of the septa between adjacent alveolar ducts. There is no objection to calling these subdivisions 'alveoli', providing it is realized that the alveolus of one stage is destined to become the alveoli of the next. The size of the smallest subdivisions at term is the same as that at 10 days of life, and when it is remembered that lung volume has been multiplied 7 times, it is obvious that there is no foetal homologue for the 10-day alveolus.

Some attempt can be made to decide how far the formation of septa depends upon vital processes of growth and how far upon the purely mechanical effects of distension by increasing tension in elastic fibres. The possibility that active contraction of plain muscle in the free edges of alveolar ducts may play a part in determining lung architecture may be considered, though it is difficult to investigate further. The results of artificial distension of an excised lung, however, will not depend upon vital processes, and may be expected to intensify the effects produced by increase of fibre tension.

It is found that artificial distension of the excised lung at term reproduces the picture of the normal lung at 5 days after birth. The mean cross-sectional area of the smallest spaces in the distended lung is smaller than those in the undistended lung (see figure 41). The effect of distension upon the excised lung at term is therefore to subdivide existing spaces by drawing up new septal divisions. Yet evidence of distension is seen in the longer and wider

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alveolar ducts and in the larger lobules. Parallel with these changes, the effect of distension is to produce a marked narrowing of the septa. Stretching cannot account for all of it; part is due to a redistribution of the capillary bed within the newly formed septa, and this has already been described.

By applying the method for estimating internal surface area per unit volume of lung ($2N/l$), it is seen in table 26 that the effect of respiration for 24 hr. is to reduce the numbers of intersections with the ruling. This is due mainly to enlargement of the septal mesh. Though a few existing septa may be flattened by hyperdistension, the numbers of septa cannot be seriously reduced. By dividing the numbers of septa at term ($N=200$) by the

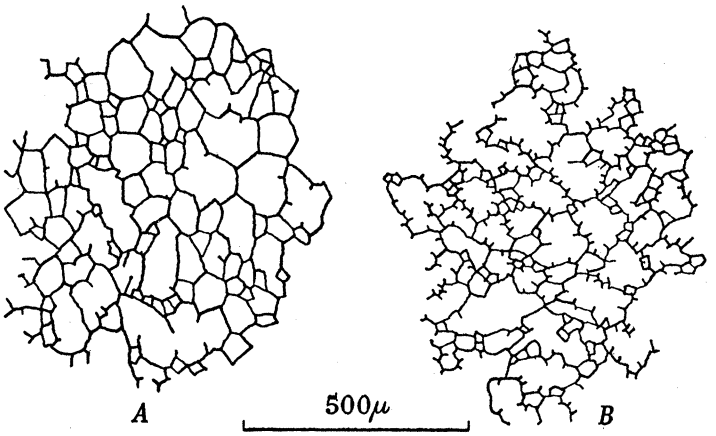

FIGURE 41. Effect of artificial distension upon the size of the smallest subdivisions in a pair of lungs, 2 days after birth. *A*, natural size; *B*, distended.

cube root of the enlargement of lung volume ($\sqrt[3]{(1{\cdot}4)}=1{\cdot}12$) which has occurred by 24 hr. respiration, an estimate may be made of the pattern at term subjected to distension. The result, $N=179$, is in tolerably good agreement with the figure for 24 hr. ($N=164$). However, many lobules remain collapsed, and the whole picture is so varied that quantitative estimates can only be made in those parts where reasonably uniform conditions are present. Results for the post-natal period are shown in table 26.

By 48 hr., in spite of the fact that lung volume has increased, the number of intersections is of the same order as the figure at term. By 5 days it is exceeded and more so by the 10th day. When it is remembered that lung volume was increased by a factor of almost 2 at 48 hr., by 3·2 at 5 days and by 5 at the 10th day, it is obvious that considerable new formation of septa is taking place. It would otherwise be impossible for the counted number of intersections to increase, for unless the pattern changes, distension of the septal mesh by respiration must of necessity reduce the numbers of intersections, in proportion to the cube root of the increase of lung volume.

At some time between the 10th day and 3rd month of the rabbit's life, the formation of septa ceases. The counts of septa therefore fall slowly as the result of distension by growth of the existing lung architecture. By dividing the counts from term onwards by the cube roo of increase in volume at the same times an estimate may be made of the resulting complexity when the original pattern is subjected to simple distension by growth and enlargement of the thoracic cavity. Results of such calculation are shown in table 27.

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TABLE 26. COUNTS OF SEPTA—POST-NATAL PERIOD

age	septa counted			mean ($=N$)	$2N/l$
24 hr.	128	149	151	164	590
	129	152	159		
	131	178	168		
	154	197	178		
	186	200	200		
48 hr.	161	194	203	211	760
	178	198	218		
	183	224	231		
	198	228	246		
	200	245	247		
5 days	213	214	217	245	880
	220	230	227		
	222	249	251		
	231	264	253		
	237	269	275		
10 days	243	254	249	259	935
	250	257	266		
	250	259	268		
	261	260	270		
	286	261	272		
3 months	179	188	198	200	720
	184	197	201		
	190	200	212		
	195	208	217		
	198	211	222		
adult	119	121	134	140	503
	125	130	145		
	128	137	154		
	139	146	159		
	149	148	167		

TABLE 27. POST-NATAL GROWTH OF THE LUNG

age	lung volume (ml.)	ratio increase	cube root of increase	septa (N)	$\frac{N \text{ of previous stage}}{\sqrt[3]{(\text{increase})}}$
term	1·49	1	1	200	—
10 days	7·2	4·85	1·69	259	118
3 months	23	3·2	1·47	200	176
adult	46	2	1·26	140	159

If, therefore, the lung at term ($N=200$) is distended without altering the pattern of architecture, 118 intersections would be found. Actually 259 are counted. Septa have therefore been formed. If the 10-day lung is distended to the volume at 3 months 176 septa would be counted. Actually 200 are counted, but the standard deviation of the order of ± 20 shows that the estimated count is probably the same as that actually found. It would therefore appear likely that alteration in pattern of lung architecture had been accomplished by the 10th day of the rabbit's life. Thereafter distension enlarges the pattern, and it is found that the pattern at 3 months ($N=200$) subjected to distension yields a result ($N=159$) not significantly different from that actually found ($N=140$).

Elastic fibres

Though essentially histological, this section has a close relation with the quantitative data under consideration. The position of the inter-duct or inter-alveolar septa is seen to depend entirely upon the distribution of elastic fibres. One or more elastic fibres are present in the

free edges of all septa, however small they may be. The rule is invariable and applies to embryo and to the adult. It is also interesting that the numbers of elastic fibres appear to be increased as a result of expansion of the lung by respiration at birth.

Owing to the weak staining properties of elastic fibres in the embryo, their distribution is not so easy to determine as it is in the adult. The larger bundles are situated in the free edges of the alveolar ducts. Smaller fibres encircle the walls of the ducts, producing a slight constriction of the lumen, but owing to their weak staining properties it may be difficult to trace their relation to adjacent ducts or to trace the complete circle. Such is the arrangement at term. 48 hr. of respiration produces striking changes both in the staining qualities and, apparently, in the amount of elastic tissue which appears to have increased considerably.

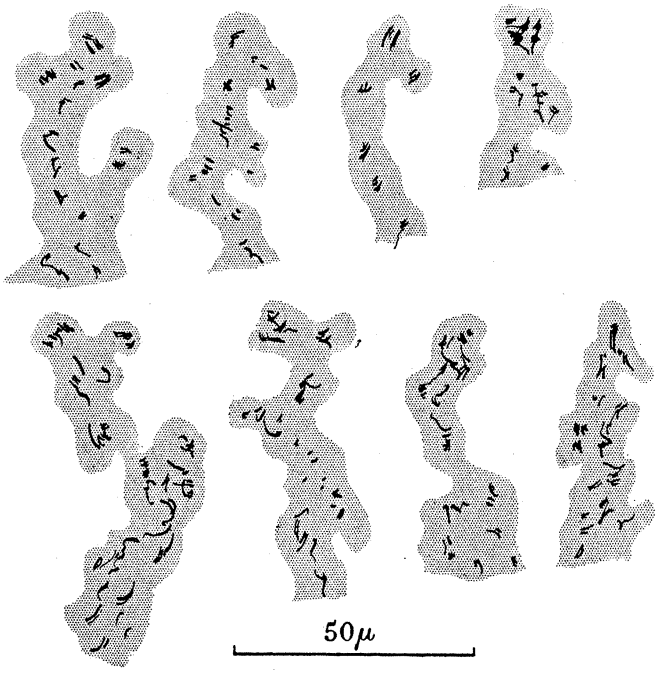

FIGURE 42. Comparison of numbers of elastic fibres in septa of comparable size in normal sized (upper row) and distended lungs (lower row). Rabbit foetus at term.

The increased subdivision and angularity of the alveolar ducts has evidently been evoked by a parallel response in the numbers of elastic fibres. The main strands sweep round the alveolar ducts at regular intervals, drawing up folds into the lumen, as may be seen by looking into the mouth of an alveolar duct in a thick section. Respiration has stretched the fibre and distorted the unresisting, probably inelastic, reticulum scaffold of the alveolar walls. A fold is thus formed by the elastic fibre in its free edge. The pictures of Orsos (1907, 1933, 1936) show how the reticulum framework of the septum is braced on to the elastic fibres by a splicing of fine reticulum fibres. Between these folds the alveolus, as it were, spreads sail and the pressure of adjacent lumina may impress a faceted contour.

An exactly parallel effect can be produced by inflation with air or with formalin in excised foetal lungs at term. Figure 42 shows that a larger number of elastic fibres is present in the distended lung. Most of this effect is probably due to splitting and separation of fibres from the main bundles, of which more appear thicker in the undistended lung and become thinner and more numerous after distension. I cannot convince myself that

stretching (whether by respiration or by artificial distension) produces any enhancement of staining either by resorcin-fuchsin or by orcein. It is preferable to attribute the effect to splitting, and it may be that the lapse of 48 hr. before subdivision of alveolar ducts occurs is due to the time required for splitting of the main bundles. The purely distensive effect at 24 hr. is therefore to stretch the main bundles; subdivision of the lumen at 48 hr. is the result of redistribution of tension by thinner fibres split from the main bundles.

The minute arrangement of fibres at the points where bundles appear to cross suggests how increase of tension will evoke a new septum and permit also of further formation of septa. At such points, close examination shows that many fibres do not cross (see figure 43). Opposite limbs of one loop are arranged so as to separate in the same direction when

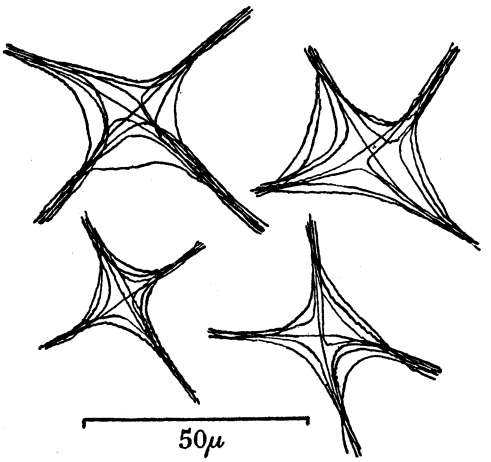

FIGURE 43. Arrangement of elastic fibres at points where bundles appear to cross. Rabbit at term.

tension is applied. The space between two such loops forms the anlage of the alveolus of the next generation. At such places, rudiments of two or three future generations of alveoli may be recognized. It may be assumed that new formation of alveoli stops when the disposition of elastic fibres no longer permits of their splitting to form a new series of spaces. When this occurs, further growth of the thorax is accompanied by dilatation without subdivision of existing alveoli, but other evidence from the rat (Hilber 1934) and the dog (Longacre & Johansman 1940) suggests that species differences are of great importance in deciding when new formation of septa ceases.

The weak staining properties and late appearance of elastic fibres in the foetus have been noted by Lenzi (1898), Linser (1900) and by Dubreuil, Lacoste & Raymond (1936), who described their earliest appearance in the walls of the pulmonary artery. Following birth, its improved staining qualities were regarded by Ottolenghi (1903) as the result of contact with air and therefore as having an application to forensic medicine. His results in man were re-examined by Ogawa (1920) in the rabbit, who was unable to satisfy himself of any significant alteration in staining at birth. However, a similar conception seems to have been held by Keibel & Mall (1912), who write that: 'The tissue [elastic fibres] does not stain as deeply as it does later on and is to be regarded as young, immature elastic tissue which becomes mature a few weeks after birth as the result of use.' Foerster (1932) and Bohmer (1933) consider that staining is improved by birth. Policard (1938) remarks that: 'La mise en

fonctionnement de ces fibres [elastiques] sous l'influence de...la respiration aerienne, modifie probablement leur constitution colloidale intime.' It is therefore a matter for regret that the present investigation does not support the view that the staining of elastic fibres depends upon their having been stretched.

Principles of subdivision of the lung lumen

Superficial inspection of a series of lung fields from the later stages of embryonic life would appear to show an increase of lumen at the expense of the surrounding mesenchyme. Measurement, on the other hand, suggests that mesenchyme increases in order to provide a mantle for a lumen which is becoming more and more complex; to provide, in Goodrich's (1930) phrase, 'the building material for future differentiation'. In this section, the principles are examined which underlie this differentiation.

The force required to produce an enlargement of lung volume increases with the number of elastic fibres and therefore with the tension evoked by stretching them. Though there are no quantitative data concerning numbers of elastic fibres, it is clear that they increase as the numbers of septa increase both before and after birth. It is also clear that both elastic fibres and septa increase, whilst the percentage of interstitial substance continually decreases. Hence the number of elastic fibres increases with the reciprocal of the percentage of interstitial volume, i.e. with the ratio of lung volume to interstitial volume. Probably the fibre tension also increases with this ratio. The increasing values of the ratio of lung volume to interstitial volume in table 28 show that lung volume increases more rapidly than interstitial volume both before and after birth. Since the interstices contain elastic fibres the effect of this inequality of growth rates may be to stretch these fibres. Tension is thus maintained in elastic fibres, and it is this factor which is here regarded as underlying the formation of septa and which itself may be a consequence of the unequal rates of growth of lung volume and interstitial volume. If it is possible to show that a simple relationship exists between the numbers of septa ($2N/l$) and the values of the ratio, some evidence of a causal relation between fibre tension and septa will have been obtained.

Table 28. Data for figure 44

age	$\frac{\text{lung volume}}{\text{interstitial volume}}$ (from tables 21, 23)	counts of septa $= 2N/l$ (from tables 25, 26)
18 days	1·1	87
21 days	1·24	227
22 days	1·38	302
24 days	1·6	418
26 days	1·93	485
28 days	2·34	620
30 days	2·67	720
birth		
1 day	3·18	590
2 days	4·12	760
5 days	4·96	880
10 days	6·54	935
3 months	8·5	720
adult	10·0	503

In figure 44 the values for the ratio of lung volume to interstitial volume are plotted against septa ($2N/l$). The graphical relationship is linear, though the slope changes three times between embryonic and adult life. The four periods so defined may be considered in turn.

The first period ends with the first signs of desquamation of 'alveolar' epithelium. This can be recognized at the 24th day in the rabbit. During this period more subdivision of the lumen takes place for a given increase in the ratio than is found at any subsequent period.

The second period starts with desquamation of epithelium and ends at birth. The slowing of subdivision for a given increase in the ratio may be the result of desquamation of epithelium, but it is difficult to see how desquamation of epithelium affects the issue one

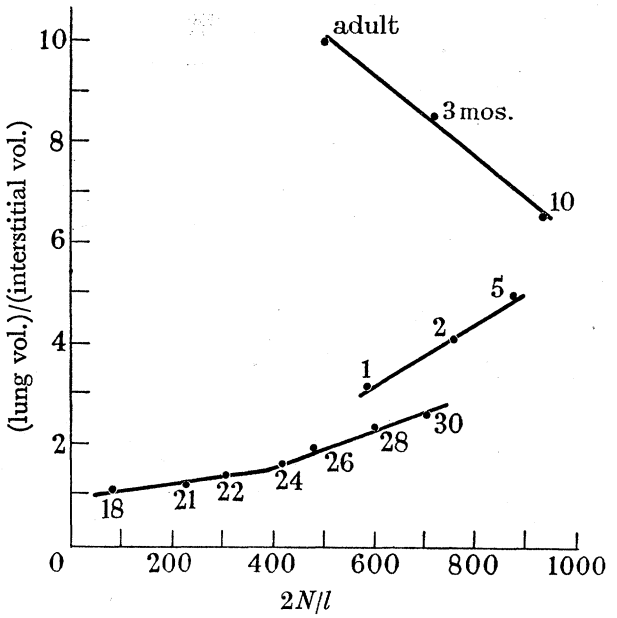

FIGURE 44. Linear relation between the ratio of lung volume to interstitial volume and $2N/l$. Four phases with characteristic slopes are seen (see table 28).

way or the other. Having in mind the experiment by which subdivision of the lumen was accomplished by simple inflation of an excised lung, the effect of epithelium upon subdivision under these circumstances can clearly be dismissed. Its desquamation, moreover, cannot be interpreted as removal of a factor which hampers the growth of lung volume; lung volume shows signs of a slower rate of growth after the 26th day. On the other hand, the slowing may be another effect of the cause which also promotes desquamation. During this period the ratio increases more rapidly than subdivision. Increase in the ratio may be the result either of a relative increase in lung volume or of a relative decrease in interstitial volume. It seems possible to separate these two factors by plotting the ratio against its numerator and denominator separately. This has been done in figure 45, which shows that an increase in the ratio during the first period is associated with almost as much increase of interstitial volume as of lung volume. In the second period (after the 24th day), on the other hand, increase in the ratio is accompanied by marked slowing in interstitial volume as compared with the total volume of the lung.

It is therefore probable that desquamation of epithelium is the earliest manifestation of the factor by which growth of the interstitial tissues of the lung is slowed. This factor may

well be fibrinolysis, direct evidence for the existence of which was found in tissue culture of foetal lungs by Carleton (1925). It has already been pointed out in the histogenesis of the respiratory epithelium that the reticulum basement membranes disappear when the epithelium desquamates.

The third period commences with birth. The initiation of respiration causes a greater increase in lung volume than in volume of the interstitial tissues. Consequently the slope changes though the trend remains linear. During this phase, which probably lasts as long as 10 days, considerable increase in complexity of subdivisions of the lumen is found.

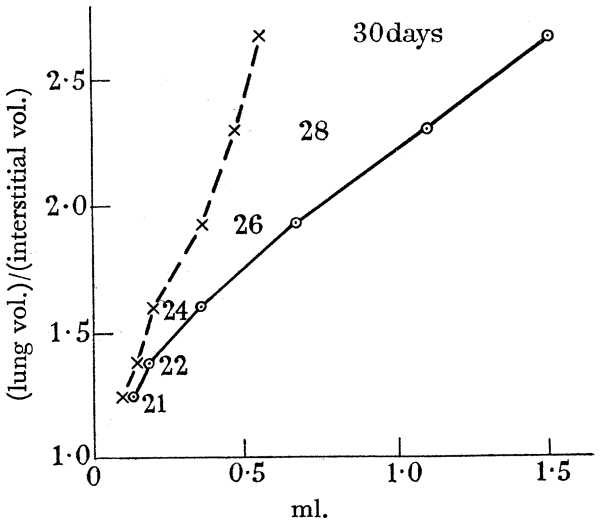

FIGURE 45. For explanation see text. ——, lung volume; – – –, interstitial volume.

During the fourth or terminal phase, increase in lung volume is no longer accompanied by subdivision, and in consequence the existing lung architecture is subjected to simple distension by growth of the whole organ. The trend is still linear but its direction has become re-entrant.

A simple relation has been demonstrated between numbers of septa ($2N/l$) and the ratio of lung volume to interstitial volume, but it must be remembered that graphical relationship, however suggestive it may be, is not proof of a causal relationship. However, when one factor may be equivalent to tension and the other is septa with elastic fibres in their free edges, the suggestion of causal relationship is undeniably strong.

This method of growth postulated for the Amphibia by Marcus (1928), Heiss (1923), for certain Mammalia by Hilber (1934) and Willson (1928), is the result of subdivision of space by centripetal formation of septa. In the amphibian or reptilian lung, the free edges of the septa, in addition to elastic fibres, contain large muscle bundles (Oppel 1905; Schultze 1871), no doubt capable of exerting traction on the septum to increase or diminish the area of capillary bed according to the demand. It will make no difference to the argument whether a positive pressure exists within the lung lumen to distend the lung (as in Amphibia) or whether the lumen is enlarged by pressures lower than atmospheric outside the lung (as in Mammalia). Both mechanisms will evoke elastic recoil, assisted in Amphibia by contraction of plain muscle in the edges of the septa. Available evidence shows that the facts of lung growth in the rabbit can be fitted more easily into this scheme than into

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the alternative scheme of alveolar budding supported by Bremer (1935). Whether or not the desquamation of epithelium from the distal portion of the respiratory tree be accepted as a fact (and strong evidence has been produced to show that this occurs in the foetus and persists in the adult), epithelial growth cannot account for the quantitative facts of growth of the lung lumen and its subdivision. Nor can it account for the fact that the structure of a 5-day-old lung can be reproduced by simple inflation of one that has never breathed.

One further step can be taken. The number of septa is clearly a function of internal surface area of the lung which is therefore a linear function of the rates of growth of lung volume and interstitial volume. This relation expresses 'the phenomenon...which is visible throughout the whole field of morphology...the tendency (referable doubtless in each case to some definite physical cause) for mere bodily surface to keep pace with volume, through some alteration of its form'. Illustrating this principle with cases of organs furnished with complicated luminal subdivision, D'Arcy Thompson (1942) proceeds: 'the vast increase in respiratory surface in the air sacs and alveoli of the lung,...all these and more are cases in which a more or less constant ratio tends to be maintained between mass and surface, which ratio would have been more and more departed from with increasing size, had it not been for such alteration in surface form.'

The total internal surface area of the rabbit's lung can be calculated from the formula $2NV/l$, and its ratio with body weight is shown for the span of the rabbit's life in table 29. Except for the earliest period of growth and for the period of maximum evocation of septa after birth, the ratio is probably to be regarded as a constant.

TABLE 29. LUNG SURFACE-BODY WEIGHT RATIO

age	$2N/l$	lung volume (ml.)	area of internal surface of lung $=2NV/l$ (sq.cm.)	body weight (g.)	internal surface / body weight
18 days	87	0·038	3·3	1·5	2·2
21 days	227	0·131	29·7	4	7·42
22 days	302	0·194	58·7	5	11·7
24 days	418	0·347	145	13	11·2
26 days	485	0·68	328	25	13·1
28 days	620	1·10	680	40	17·0
30 days (birth)	720	1·49	1,040	54	19·2
1 day	590	2·0	1,180	50	23·6
2 days	760	2·8	2,120	80	26·6
10 days	930	7·2	6,700	140	48
3 months	720	22·9	16,400	900	18·2
adult	500	46·0	23,000	1,500	15·3

It is interesting that D'Arcy Thompson speaks of 'some definite physical cause'; in the lung there is evidence of the importance of tension in elastic fibres, shown most clearly when the lung starts to breathe but apparently in existence during intra-uterine life.

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Description of plate 1

Figures 24, 25. Silver impregnation of lung (? kitten) by Gustav Mann, from a preparation in the Department of Histology, Oxford University (by permission of Dr H. M. Carleton). Magn. × 63, × 77.

Figure 30. Traces of impregnation of epithelium in subpleural alveolus. Adult rabbit. Magn. × 800.

Figures 31, 32. Traces of impregnation of epithelium in alveoli adjacent to pulmonary artery. Note the cement lines crossing the intercapillary spaces and a small nucleated cell in figure 32. Adult rabbit. Magn. × 800.

Figures 35, 36, 37. Dissociation of alveolar septa produced by perfusion of fixative. The absence of epithelial nuclei and the presence of a delicate surface membrane are to be noted. Adult rabbit. Magn. × 530.

Figure 38. The point of termination of bronchiolar epithelium from the same preparation. Distally, only the delicate membrane is seen. Adult rabbit. Magn. × 530.

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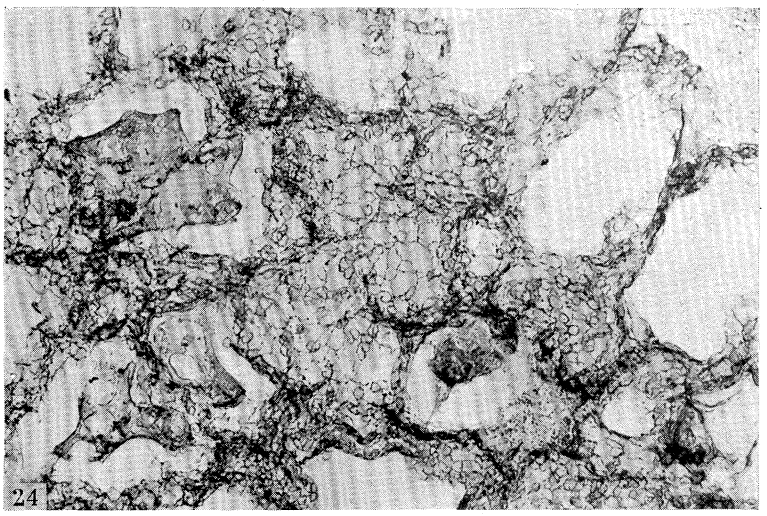

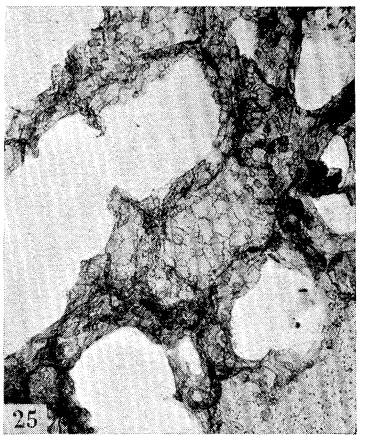

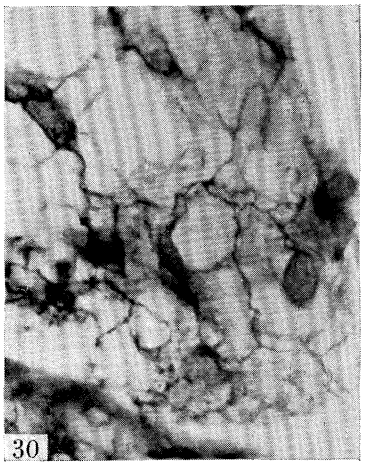

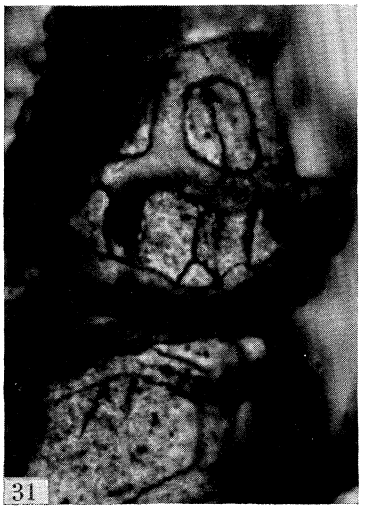

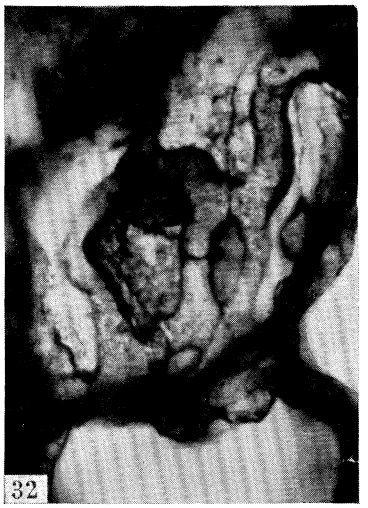

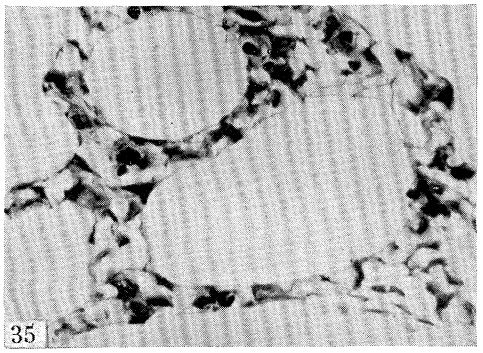

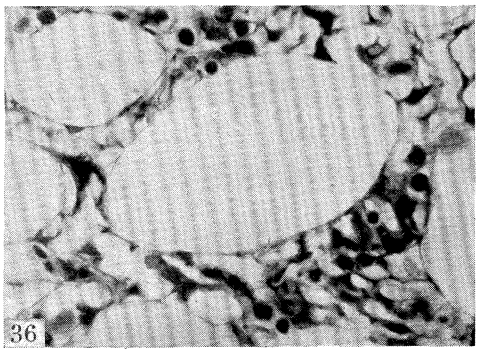

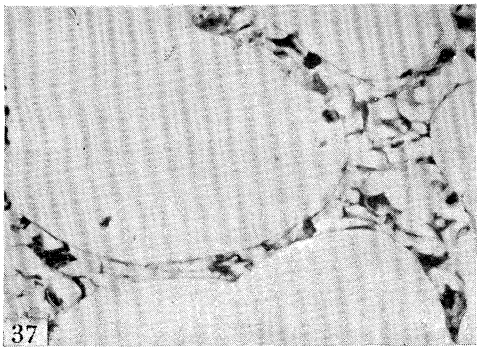

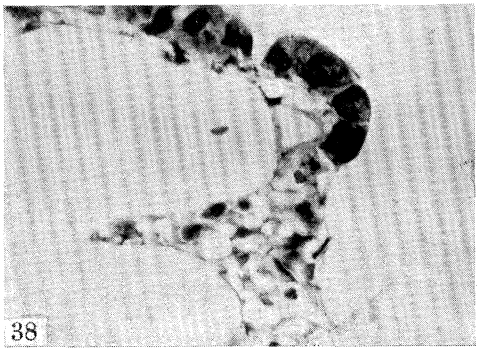

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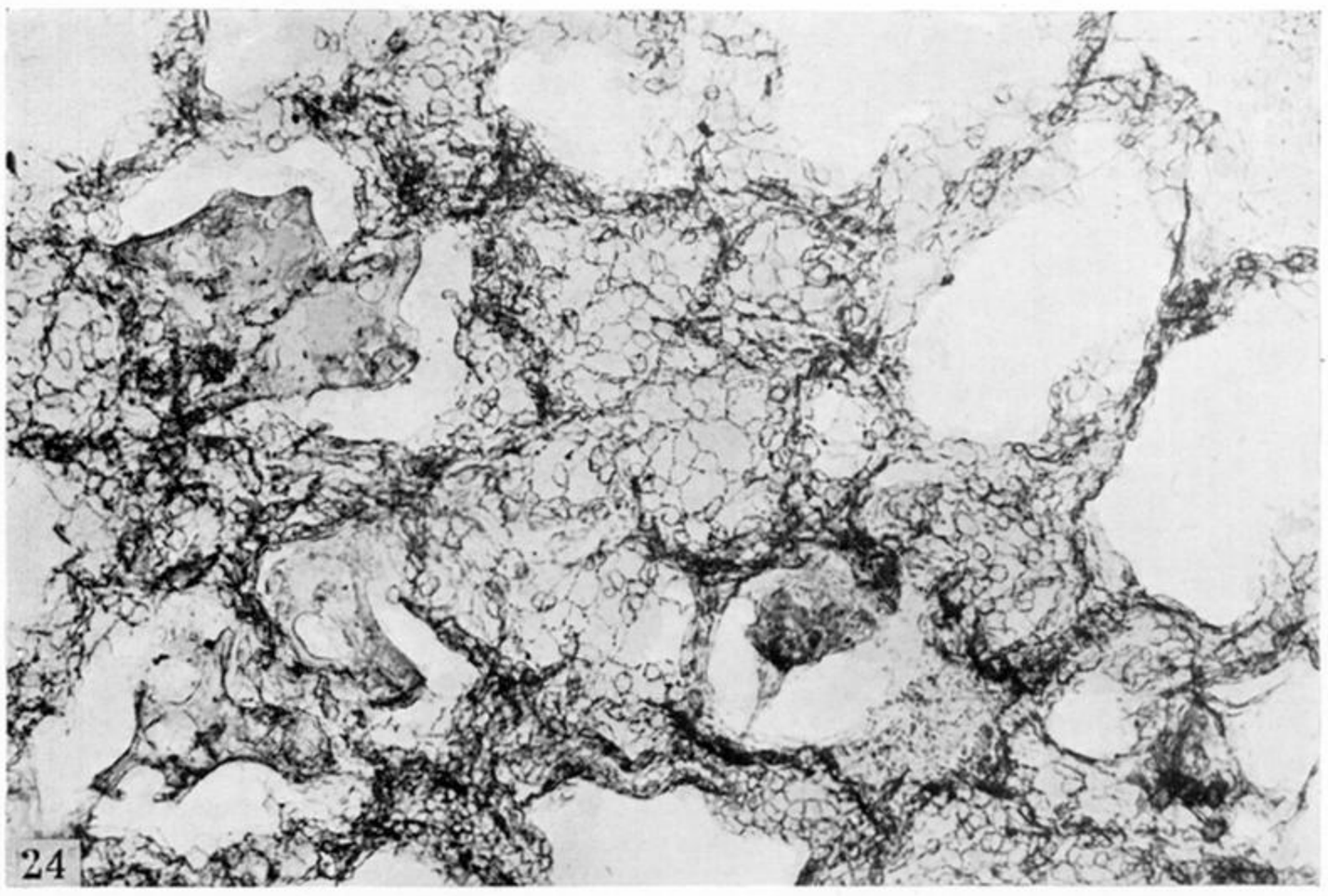

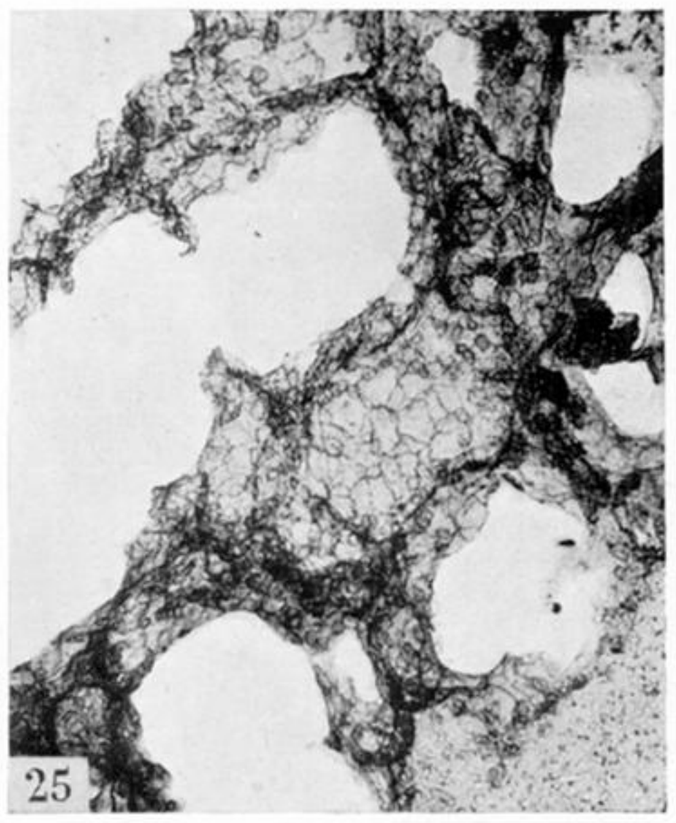

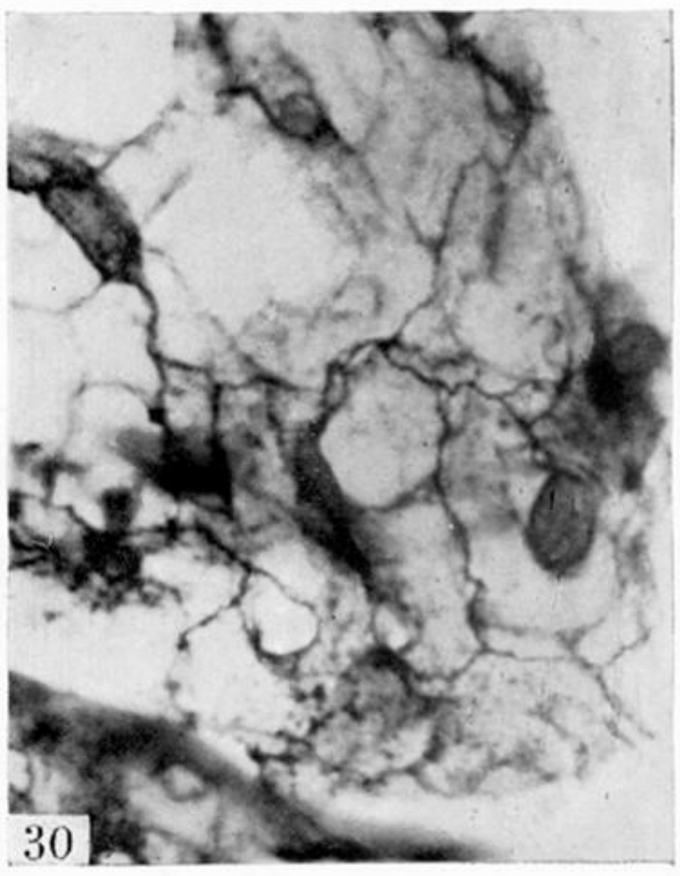

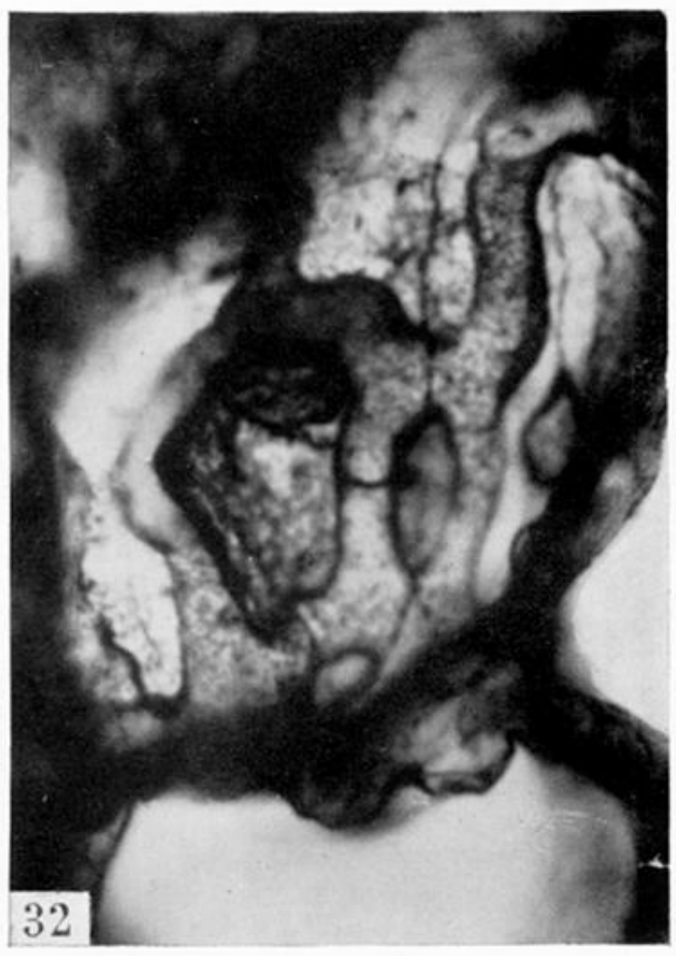

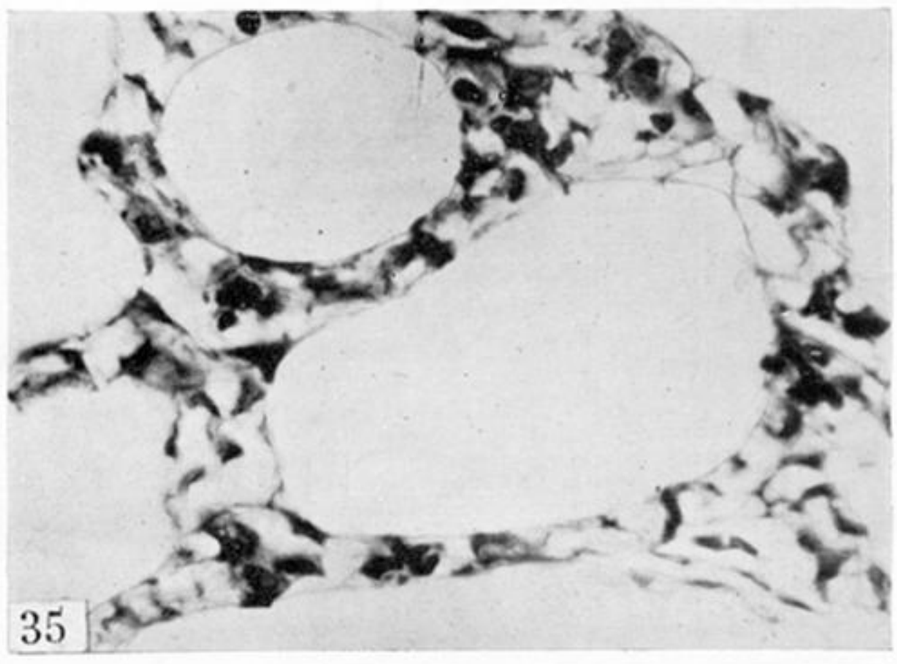

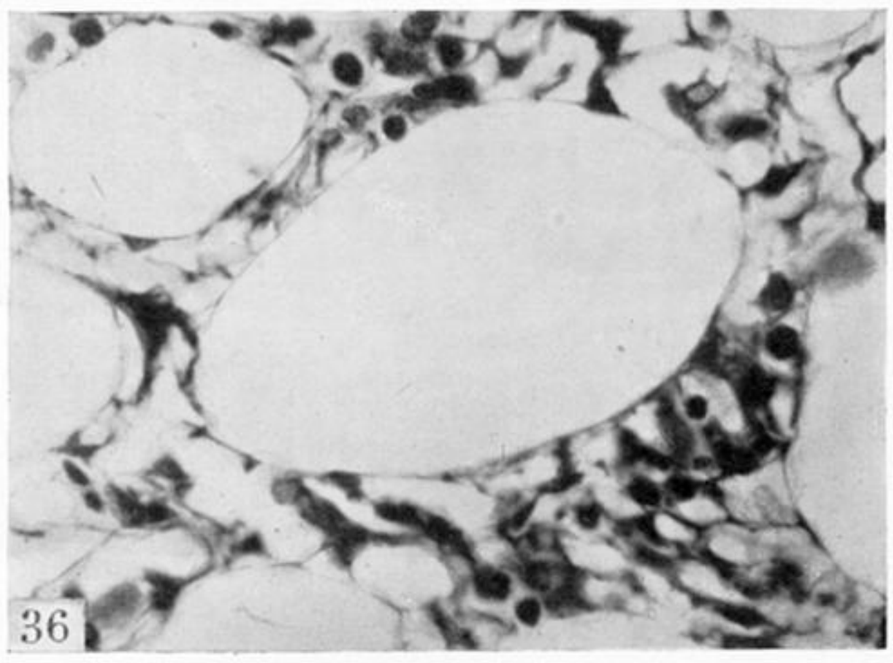

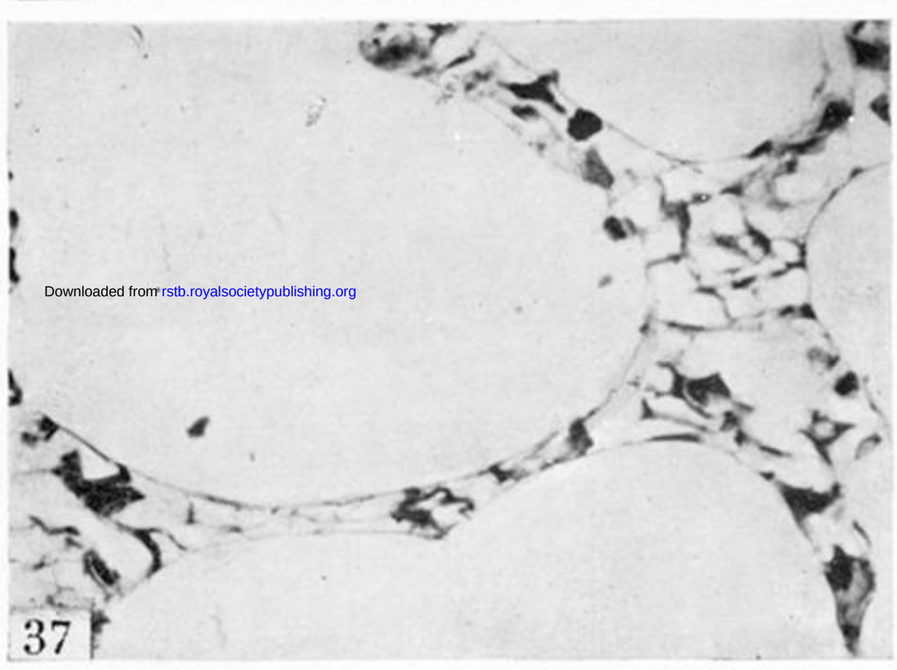

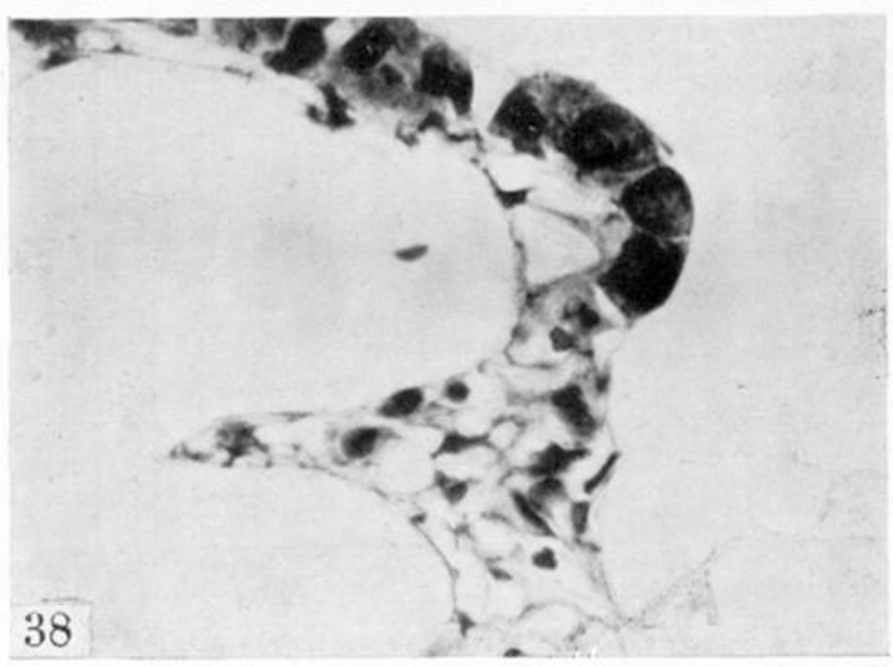

Description of plate 1

Figures 24, 25. Silver impregnation of lung (? kitten) by Gustav Mann, from a preparation in the Department of Histology, Oxford University (by permission of Dr H. M. Carleton). Magn. × 63, × 77.

Figure 30. Traces of impregnation of epithelium in subpleural alveolus. Adult rabbit. Magn. × 800.

Figures 31, 32. Traces of impregnation of epithelium in alveoli adjacent to pulmonary artery. Note the cement lines crossing the intercapillary spaces and a small nucleated cell in figure 32. Adult rabbit. Magn. × 800.

Figures 35, 36, 37. Dissociation of alveolar septa produced by perfusion of fixative. The absence of epithelial nuclei and the presence of a delicate surface membrane are to be noted. Adult rabbit. Magn. × 530.

Figure 38. The point of termination of bronchiolar epithelium from the same preparation. Distally, only the delicate membrane is seen. Adult rabbit. Magn. × 530.