ON THE NATURE OF THE PULMONARY ALVEOLAR LINING

and

THE ORIGIN OF THE ALVEOLAR PHAGOCYTE

by

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C O N T E N T S .

INTRODUCTION.

It would be inadvisable to attempt to present the results of the experimental and pathological work to be reported in this thesis without first reviewing the subject as a whole. So much controversy has centred around the nature of the alveolar lining and the origin of the alveolar phagocyte that any fresh discussion on this subject must for the sake of clarity be prefaced by a summary of the views of the various disputants.

SECTION I.

ON THE NATURE OF THE PULMONARY ALVEOLAR LINING.

EVOLUTION OF IDEAS.

In order to understand properly a man, an art, or a science, a knowledge of their development and past history is essential; what embryology is to the study of man's structure and evolution, history is to the comprehension of an art or a science. It is therefore reasonable to follow in outline the evolution of ideas regarding the nature of the pulmonary alveolar lining.

According to W. Addison, Malpighi (1661) was the first to describe the air-vesicles of the lungs, and the air-tubes ending in them.

In 1822 Reisseisen, in his work, "Uber den Bau der Lungen," described the air-cells as the blind extremities of the air-tubes, and postulated that, like the latter, they are lined by the same kind of epithelium.

Thomas Addison (1843), in a paper read before the Physical Society of Guy's Hospital, disagreed with Reisseisen, He said "But, notwithstanding the most careful investigation, aided by the use of magnifying glasses, I must confess myself unable to arrive at any positive conclusion, either as regards the elementary tissues which compose the air-cells of the /

the lungs, or the exact construction and arrangement of the cells themselves. These are questions which, there is reason to hope, will ere long receive a satisfactory solution, from those who have already distinguished themselves as successful cultivators of microscopic anatomy. In the meantime, without venturing to decide whether the innumerable, minute, irregular, and manifestly elastic air-cells, constituting an individual lobule, partake more of the character of areolar tissue or of a serous membrane, I am fully persuaded that, pathologically, they present none of the attributes of a mucous membrane, as Reisseisen and others would lead us to believe."

In his study of the ultimate distribution of the air-passages and the formation of the air-cells of the lungs, William Addison (1842) found that "They are (the air-cells) also marked by numerous delicate lines, which are, no doubt, uninjected vessels of the capillary network. They possess an epithelium in the form of large round nucleated scales, and from one to fifteen or more nuclei may be counted in a single scale. A great many nuclei without any epithelium envelope may be seen upon them, but I have never satisfied myself that they possess the ciliated cylinder epithelium so abundant in the trachea and the bronchi."

In 1855 Rainey of St.Thomas Hospital published a paper in the British and Foreign Medical and Chirurgical Review in which he stated that the air-cells of the /

the human lungs are not lined on their internal surface by any kind of epithelium whatever.

To this, Thomas Williams (1855) has quoted Kölliker, Schrader van der Kolk, Harting, Schultz, Adriani, Paget, Sharpey, and Andrew Clark as opposed to the views of Rainey. "Mr Rainey stands alone in the opinion as to the absence of epithelia from the covering of the pulmonary cells. Those who hold the same view are passive followers, teaching merely what they have been taught." He confirmed the observation of William Addison regarding the human lungs and partly based his conclusion as to the presence of epithelium on the air-cells from the analogy afforded by the lungs of reptiles, newts and Salamandridae; "I have then shown that there exist parts on the comparatively smooth walls of the pulmonary sac of the newt, which form the structural analogues of the cells of the mammal lungs, that these parts coincide precisely with the capillary areas, and that they contrast with the neighbouring portion of the pulmonary surface, by their smooth, non-ciliated, hyaline epithelium. In the lungs of the Salamandridae, the tracts coinciding with the course of the larger arteries and veins are invested by a flocculent cylinder, ciliated epithelium, which, where it is prolonged over the capillary area, undergoes a change of structure; the ciliated epithelia are supplanted by the pavement scales."

In /

In 1862 Munk attempted the technique then recently advocated by Von Recklinghausen (1862) for studying cellular tissues by means of impregnation with silver nitrate. He failed to demonstrate any lining cells and denied the presence of alveolar epithelium.

Eberth (1862) and Hertz (1863) described islands of epithelium that occupied the mesh of the capillary network, but did not extend over the capillaries, the latter being covered by a structureless membrane.

Chrzonszczewsky (1863) varied the silver technique somewhat and immersed inflated lungs in weak silver nitrate solutions. His results showed a continuous lining for the respiratory surface consisting of polygonal squamous cells completely covering the alveolar walls. In criticising the technique of those investigators who denied the presence of an epithelium lining the alveoli, he says "in all these instances the fault evidently lies, not in the lung, but in the method."

A year later, Elenz (1864), by intratracheal injections of silver nitrate solutions into the lungs of experimental animals, went a step further. He described the same islands of epithelium in the capillary network as Eberth (1862), but found that the structureless membrane described by Eberth as extending over the capillaries was made up of large, irregular, membraneous, non-nucleated plates.

It is, thus, evident that by this statement Elenz became the predecessor of Kölliker (1881) in ascribing to the alveolar walls two types of cell: small cubical nucleated cells and large flat non-nucleated squames. Kölliker's views have since been supported by many writers and have come to be regarded as classic for the finer structure of the respiratory portions of the lung.

Much later Ogawa in 1920 conducted an extensive study of the comparative histology of the alveolar spaces and confirmed the views of Elenz and Kölliker.

Five years later Lang (1925) denied the presence of any epithelium, except the non-nucleated plates, "we were unable to find any respiratory epithelium on the wall of the alveoli, except the well-known non-nucleated plates, the limits of which can easily be made visible through the use of silver nitrate."

Maximow-Bloom (1930) doubted whether the lines seen on the alveolar walls in silver impregnations of lungs should be interpreted as cell limits bounding non-nucleated plates.

Lastly in a recent publication Josselyn (1935) repeated the observations and reasserted the views of Maximow-Bloom, "the black lines seen on alveolar septa in silver nitrate impregnations of lungs are susceptible of other explanation than the view that characterises them as outlines of lining epithelial cells. The /

The alveolar walls, in addition to the capillary vessels and cells, consist of a membrane composed of reticular and elastic fibres and a homogenous transparent ground substance. It is upon the latter that the continuity of the membrane depends."

It will be noted from the above survey that the presence or absence of an epithelium lining the walls of the pulmonary alveoli is a much debated subject. While its presence is asserted by one group of authors (Reisseisen 1882, W.Addison 1842, Thomas Williams 1855, Eberth 1862, Hertz 1863, Chrozonszezewsky 1863, Elenz 1864, Kölliker 1881, Ogawa 1920), it is strenuously denied by Rainey 1855, Munk 1862, Lang 1925, Maximow-Bloom 1930 and Josselyn 1935.

THE DEVELOPMENT OF THE LUNG.

There is a unanimous agreement amongst embryologists (Keith 1933, Frazer 1931) that the lung arises in the embryo as a medial diverticulum of the fore-gut; it extends caudally and divides into two branches. The medial diverticulum is the primordium of the future larynx and trachea, and the first two lateral branches form the two main bronchi of the adult lung. These two branches divide repeatedly; becoming surrounded by a relatively dense mass of mesenchyme, so that throughout most of the embryonic period the lung has a suggestively gland-like structure. Thus, Schafer compares the lung directly to a "compound alveolar gland," in which trachea and bronchial ramifications correspond to duct systems and the air vesicles to glandular alveoli.

The primitive bronchi are at first lined with low cylindrical or cuboidal epithelium. They branch dichotomously and are capped by end buds, called pneumoneres. The pneumoneres are lined by several layers of cylindrical epithelium which differentiate them from the primitive bronchi. After ten weeks, the epithelium of the end knobs becomes a single layer. During the third and fourth months the pneumoneres, which until now had been dividing dichotomously with regularity, begin to branch irregularly. At six to seven /

seven months, the small bronchi are lined in part with ciliated epithelium which flattens toward the peripheral ends of the bronchi. These branch and end in the terminal ducts capped with the end knobs or pneumoneres. The young pneumoneres finally become alveoli. In this connection Keith (1933), p.390, says "the wall of the pocket is lined by a mass of entoderm, which ultimately forms the epithelial lining of the whole respiratory tract, from the ciliated epithelium of the trachea to the pavement epithelium lining the alveoli of the lungs."

Regarding the process of flattening of the cells there are two schools. Küttner (1876) asserted that the respiratory epithelium remained cuboidal until flattened and distended by the first respiratory movements. His method of investigation consisted in first silvering the epithelium by instilling silver nitrate, and subsequently injecting gelatin. Upon examination it was noted that the portion of the lung uninjected with gelatin showed a cuboidal epithelium, whereas in the injected region the epithelium was flattened. Küttner was later supported by Jalan de la Croix in 1883, who expressed a devout hope that he had once for all settled the question, and very recently by Bensley and Groff (1935) in the alveolar epithelium of the rat. On the other hand Colberg 1867) Laguesse (1886), Stieda (1878), Ogawa (1920), Stewart /

Stewart (1923) and others have maintained that the process of thinning out of the respiratory epithelium is a gradual one and occurs in the final embryonic stages. In addition to other material Ogawa (1920) investigated the lungs of embryo rabbits and concluded "the respiratory epithelium of the rabbit embryo in early stages consists of a single kind of cuboidal cell, and as development proceeds and comes nearer to the final stage, some of them become flatter. In the final stage the respiratory epithelium of all the alveoli consists of a mixture of the two kinds of cells without respiration. The flat cells become flatter at the beginning of respiration. Disappearance of the nuclei of the flat cells takes place in the final embryonic stages and occurs, not suddenly, but gradually by processes of pyknosis, Karyorrhexis, etc."

Recently very novel views have been advanced by Policard (1926) who claims that the epithelial lining of the air spaces undergoes degeneration during the latter part of intra-uterine life, and that a new lining is furnished by the mesenchyme cells of the lung stroma "contrairement à la description classique, le revêtement des alvéoles pulmonaires, chez les Mammifère et les Oiseux, n'est pas formé de cellules epitheliales mais de cellules d'origine mésenchymateuse, d'histiocyte, disposés en une couche plus ou moins /

moins continue. Le revêtement alvéolaire est un endothelium, non un epithelium." According to this theory the air spaces come to be lined secondarily by mesenchymal cells of histiocyte type which produce fine protoplasmic extensions to cover the alveolar surface while the cell body and nucleus lie in the intercapillary spaces. The non-nucleated squames are then said to be mere processes of the histiocytes, and the "dust cells" derived from the alveolar lining are thus brought into line with the other phagocytic cells of the body and are of mesenchymal origin. Rose (1928) claims that embryologically the lung is of dualistic origin, the bronchi being developed from endoderm and the alveoli from mesenchyme into which the bronchi grow. He concluded "Mesodermal cells form the capillary system and other mesodermal cells persist as septal cells. The septal cells are probably not epithelial cells, but persistent mesodermal cells spread irregularly among the capillaries. These mesodermal cells should be classed as part of the reticulo-endothelial system. Evidence was not found that the alveoli develop as an outgrowth of the bronchial tree."

It may be concluded, then, from the above histogenetic study that while the majority of authors (Keith, Frazer, Schafer and others) believe that the alveolus is lined with an entodermal layer of cells, others (Policard and Rose) believe that the alveolus is /

is entirely mesordermic. This has an important bearing on various pathological conditions, especially upon the disputed question regarding the existence or not of a primary lung cancer arising from the alveolar lining - alveolar carcinoma. Indeed, this cannot be answered with certainty until the nature of the alveolar lining is definitely ascertained.

THE ALVEOLAR LINING.

The view which was first put forward by Elenz (1864) and Kölliker (1881) that the alveolar lining was composed of two types of cell, small cubical nucleated cells and large flat non-nucleated plates, has since been supported by many writers and appears to be generally accepted (Veraguth 1880, Fleiner 1888, Foster and Clarkson 1896, Ziegler 1897, Ogawa 1920, Piersol 1916, Jordan and Ferguson 1916, Stöhr's Lehrbuch der Histologie 1930, Sobotta-Piersol 1930, Hartridge and Harnes 1930, Jordan 1927, Sir E. Sharpey-Schafer's Essentials of Histology 1934). According to this view the alveolar lining is believed to consist of (i) groups of small nucleated cubical cells which occupy chiefly the intercapillary spaces and while not strictly confined to the alveolar angles are most frequently met with in this situation. Granel (1919), Fauré-Fremiet (1920) and Stewart (1923) believe that these cuboidal cells contain large numbers of fatty globules; michiochemical tests led Fauré-Fremiet to believe the globules were cholesterol and Stewart suggested that these lipoid-containing cells may play a part in relation to lipoid metabolism generally; (ii) extremely thin non-nucleated squames which form an extremely delicate layer, separating the blood capillaries from the air within the alveoli. According to Ogawa (1920) these cells are formed in the final embryonic stages by the gradual /

gradual disappearance of the nuclei of the cubical cells. According to Stewart (1923) they are formed by segmentation of plate-like flanges from the cubical cells. Carleton (1934) draws a functional analogy between them and the red blood corpuscles. "It is possible that the absence of nuclei in these elements (as in the red blood corpuscles) is in relation to a purely respiratory function." In addition to the above two types of cell, some authors (Foot 1920, Stewart 1923, Cappell 1929, Miller 1932) believe that a third variety exists representing a transitional form. This transitional form is represented by (iii) flattened nucleated cells very closely applied to the capillary walls and separated from the latter only by the reticular and elastic fibrils. These cells possess flattened protoplasmic processes which join with those from similar cells and with the non-nucleated plates. Cappell believes that the non-nucleated plates form a relatively inconsiderable part of the alveolar lining. The latter in his opinion is composed chiefly of nucleated cells, the majority of which are stretched and flattened on the alveolar walls. Miller holds views similar to Cappell but is inclined to deny the existence of the non-nucleated plates.

The other view which was first advocated by Rainey (1855) and recently reasserted by Lang (1925), Fried /

Fried,(1927), Rose (1928), Maximow-Bloom (1930) and Josselyn (1935) that the air-cells are not lined by any kind of epithelium whatever, suggests that the walls of the alveoli in the adult lung are quite simple and that they consist primarily of a very dense network of anastomosing capillaries. These are accompanied by scattered perivascular cells of mesenchymal origin morphologically identical with the connective tissue histocytes all over the body. The capillaries and their perivascular cells are contained in an exceedingly thin-walled, ground membrane in which the supporting reticular and elastic fibres run. This thin, moist membrane readily permits of the diffusion of oxygen from the air into the blood and of carbon dioxide from the blood into the air. According to this view neither the non-nucleated plates nor the flattened nucleated cells, 'transitional cells,' exist and the cubical cells which occupy chiefly the intercapillary space and occur most frequently in the alveolar angles (designated septal cells by Lang 1925) are of mesenchymal origin and form part of the reticulo-endothelial system.

THE ALVEOLAR PHAGOCYTE.

In practically every section of a lung, free macrophages or alveolar phagocytes can be found in the alveoli. They are indistinguishable from the macrophages in other parts of the body. They usually contain a varying number of black granules and hence are called "dust cells." In certain cardiac diseases they become filled with granules of haemosiderin and are then called "heart lesion" or "heart failure" cells. The origin of these cells is much debated, there being four possible primary sources, namely from the lining epithelium, from the blood monocytes, from the local histiocytes and from the vascular endothelium. It would serve no useful purpose to abstract all the literature upon the subject. I shall mention only some of the more important communications.

(a) Epithelial Origin.

Some of the early investigators (Küttner 1876), Dreschfield 1876, Ruppert 1878, Arnold 1885, Fleck 1886, Ribbert 1887, Laehr 1887, Herxheimer 1903, Briscoe 1907-8) believed that the alveolar phagocytes were derived from the alveolar epithelium and this view has been recently again upheld by Sewell (1918) Westhues (1922-25), F.Cross (1927), Kageyama (1925), Paget (1925), Carleton (1927), Cappell (1923 & 1929) and others. Briscoe (1907-8) injected intratracheally /

ally laboratory animals with physiologic sodium chloride solution, and blood corpuscles of the pigeon, potato bacilli, staphylococci and Freidländer's pneumobacillus. The animals were killed at different intervals of from 30 minutes to 75 hours after injection. The conclusions drawn were that the phagocytic cells of the lungs are an essential part of the alveolar lining and that these cells are probably derived from the epithelial elements. Among recent experiments those of Sewell and Cappell are of interest. Sewell (1918-19) was the first to apply the method of vital staining to the study of the origin of phagocytic cells in the lungs. His experiments can be divided into two groups: (a) intravenous injections of a solution of lithium carmine followed by the intratracheal injection of Chinese ink, pigeon's blood and staphylococcus pyogenes aureus, and spores of oidium albicans; (b) the intratracheal injection of solutions of lithium carmine and trypan blue, respectively, in normal guineapigs. As a result of his experiments he states "the alveoli of the lungs are provided with a highly specialised system or tissue of phagocytic cells, independent of the wandering macrophages and that these cells play a very important active part in the early stages of inflammatory conditions in the alveoli." He argues in favour of the origin of these cells from the alveolar epithelium as follows: "It might /

might be said that when the epithelial cells become so specialised as to possess cilia, they lose the strong phagocytic properties they possess when more simple in construction." Cappell (1929-30) conducted an extensive and complete study of the intravitam and supravital staining of all the organs including the lungs. His experiments on the origin of the alveolar phagocytes are as follows: (a) He subjected mice to intense intravitam staining, introducing 5 to 12 injections of isamine blue at weekly intervals and after a varying number of days exposing them to a sooty atmosphere in order to produce anthracosis. The animals were killed at varying intervals. (b) He produced pulmonary irritation in guinea-pigs by intratracheal injection of saline suspensions of insoluble particles such as carmine and india ink and then killed the animals at intervals varying from 30 minutes to 96 hours. (c) He gave intratracheal injection of soluble dyes - neutral red and trypan blue, and finally (d) Intratracheal injection of carbon suspensions etc. to animals which have been vitally stained by repeated intraperitoneal or intravenous injections of soluble dyes - trypan blue. He concluded "The reacting cells are derived from those of the alveolar lining . . . . The flattened nucleated squames and the cuboidal nucleated cells (septal cells) both take part in the process."

(b) /

(b) Monocytic Origin.

As far back as 1869 Slavjansky and others (Von Ins 1876, Friedländer 1876, Tchistovitch 1889, Metschnikoff 1901,) believed that the large mononuclear phagocytes which serve to remove from the airpassages and pulmonary alveoli, foreign particles contained therein, were derived from the circulating mononuclear leucocytes of the blood or monocytes. This view has been strongly supported by Maximow (1926) and Foot 1927. Slavjansky (1869) injected a suspension of indigo into the trachea of a rabbit, following this 2-3 days later by injections of 5 to 7 c.c. of a fairly thick solution of cinnabar into the jugular vein. Two days later the animal was killed. He found cells in the alveoli containing indigo, others containing cinnabar, and others free from pigment. The alveolar epithelium contained indigo and was otherwise unchanged. If, however, he injected the cinnabar into the blood immediately after the indigo was introduced into the lung, he could find cells in the alveoli and in the bronchial mucus, containing granules of both kindsof pigment. He concluded that in the first case the cells containing the cinnabar had wandered out of the vessels, and finding no indigo free they remained unaffected thereby, while in the second case they arrived before the indigo was taken up /

up and therefore contained both sorts of granules. Tchistovitch (1889) repeated these experiments, injecting carmine into the blood stream and bacteria into the lungs. He arrived at the same conclusion as Slavjansky, e.g. that the phagocytes in the alveoli were derived from the macrophages of the blood. Metschnikoff (1907) said: "For long, the large 'dust cells' of the respiratory channels were looked upon as being epithelial cells which were capable of taking up carbon particles, micro-organisms and other foreign bodies. In reality these elements are nothing more than white corpuscles that have immigrated into the alveoli and bronchi." Of particular interest is the recent work of Foot (1927) whose methods I have employed without achieving the same results. In 1920 Foot had published a paper in which he concluded that the pulmonary dust cells were derived from the capillary endothelium of the lungs. However, in 1927, he changed his views as a result of finding a specific method by means of which he was able to identify the monocytes, histiocytes and dust cells on the one hand and distinguish them from epithelium or from pleural mesothelium on the other. His method consisted of impregnating thin sections with silver tannate in which case the dust cells became reddish brown, with brownish black to sepia granules. These granules were usually grouped in rosettes or balls as in typical /

typical monocytes. No other cells except the polymorpho-nuclear leucocytes showed similar characteristics; these had similar, but rather finer granules. Careful scrutiny failed to demonstrate any coarse granules in the epithelium, mesothelium or vascular endothelium. He therefore concluded "the dust cells are probably larger forms of monocytes or blood histiocytes."

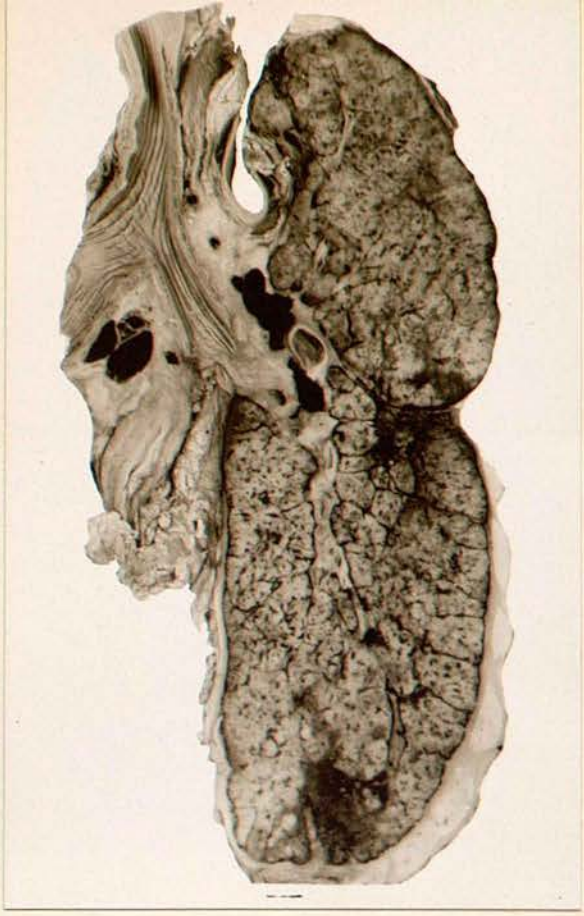
(c) Histiocytic Origin.

The histiocytic nature of the alveolar phagocytes has been advocated by the Japanese School (Kiyono 1914), Lang (1925), Policard (1926), Fried (1927), Gardiner and Smith (1927) on the evidence supplied by vital and supravital staining of the lung.

(d) Vascular Endothelial Origin.

The theory of origin of the alveolar phagocytes from the interalveolar capillaries emanated chiefly from America. Haythorn (1913), Permar (1920), Foot (1920) and Töppich 1925. Haythorn (1913), in a study of anthracosis, demonstrated that the cells constituting the lining of the lung alveoli are not concerned in phagocytosis. He believed that the phagocytic cell is a wandering cell, of endothelial type, and suggested the endothelial lining cells of blood and lymph channels as its possible origin. Permar (1920) and Foot (1920), experimenting independently and using somewhat different methods, came to the conclusion that /

that the pulmonary dust cell was derived from the endothelium of the lung. This view, however, is being less universally maintained.



ig. I. Primary Alveolar carcinoma. Note the resemblance to the stage of grey-hepatisation of lobar pneumonia.

ig. II. Primary alveolar carcinoma.

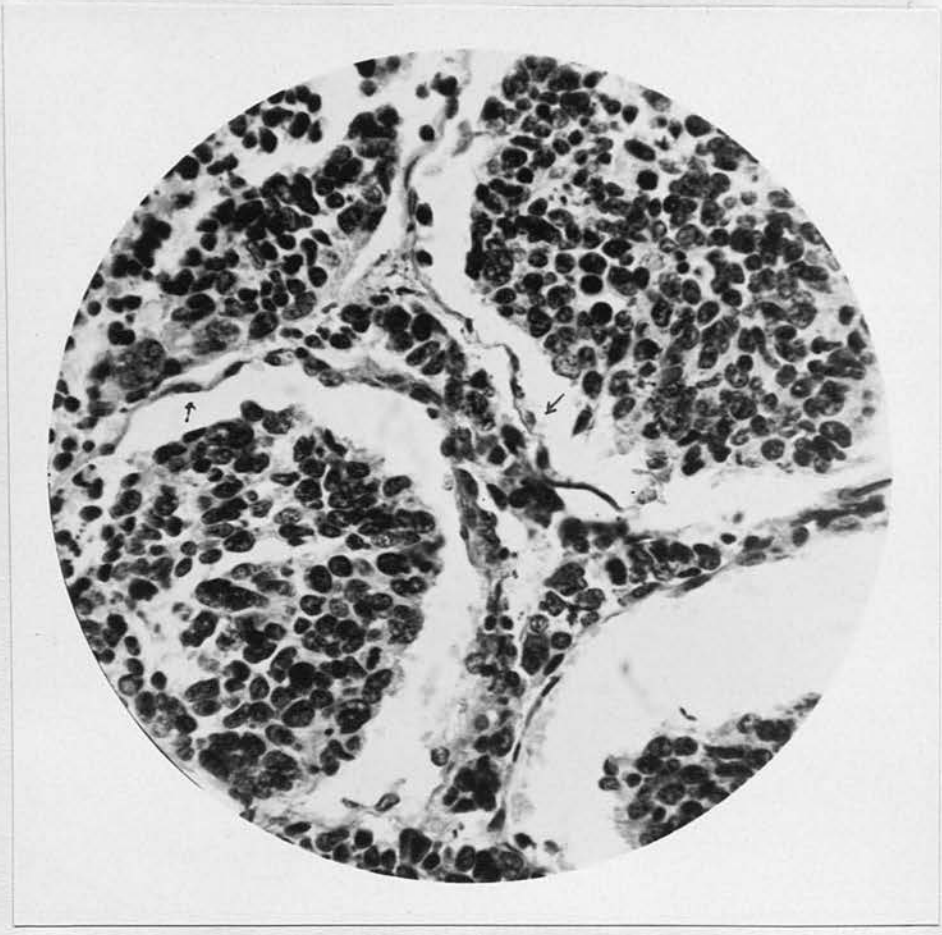


Fig. III. (X 500) A case of "oat-cell" bronchial carcinoma. Note the flattened nucleated cells of the alveolar lining.

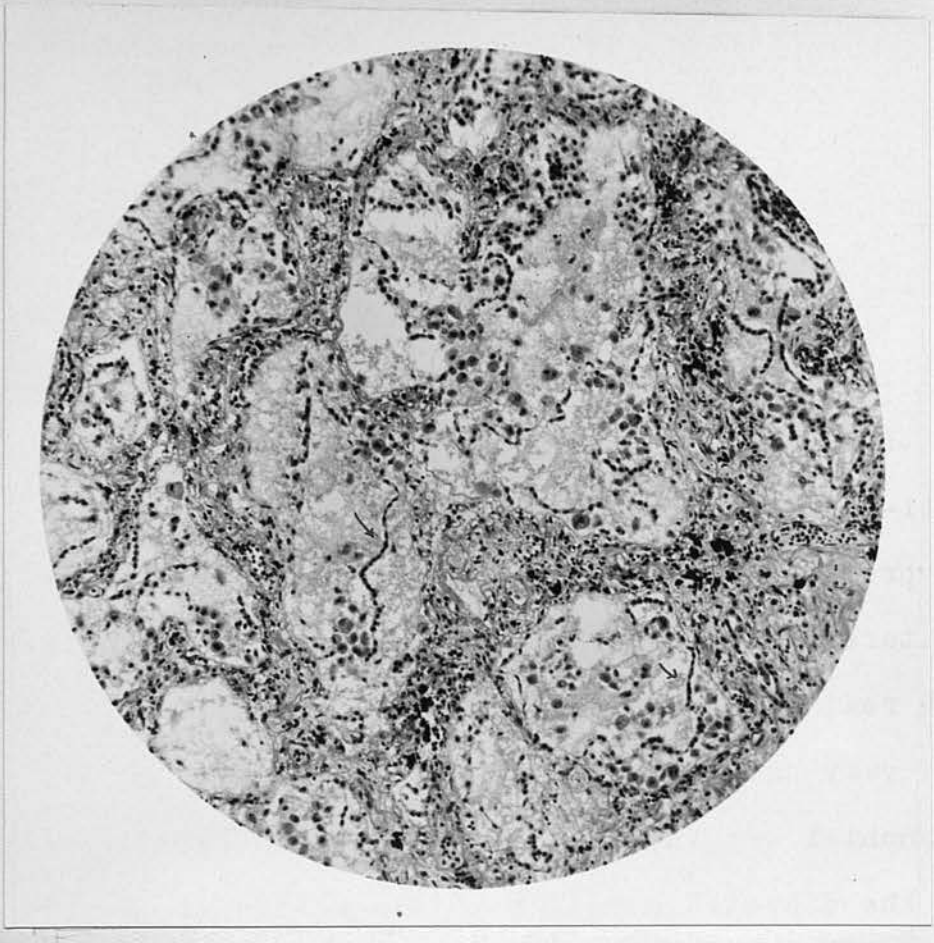


Fig. IV.(X 150) From a case of bronchiectasis. Note the flattened and low cubical cells lining the alveoli.

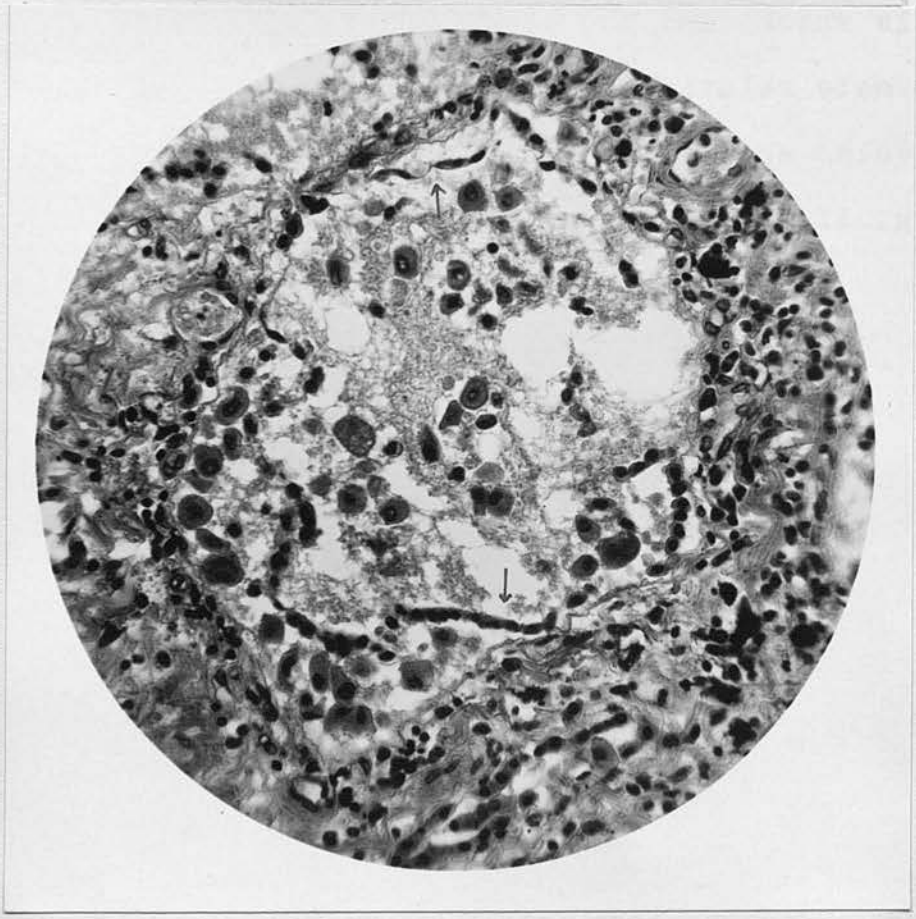


Fig. V. (X 450) Higher magnification of alveolus marked X in figure IV.

SECTION II.

Introductory Statement.

In a previous Ph.D. Thesis, I examined lung sections from 94 cases of primary lung cancer. Two of these cases differed remarkably from the ordinary well-known bronchial carcinomata. I described them as primary alveolar carcinomata on account of certain features which separated them almost entirely from the rest of the cancers (Fig.I and Fig.II). Yet I was very careful to state that they might be of bronchial origin, simultaneously drawing attention to the disputed question of the nature of the alveolar lining - entodermic or mesodermic. I was struck by the presence of certain flattened nucleated cells which line the alveolar walls, sometimes in intimate relationship to the capillaries of the alveolar septa or slightly raised from their surfaces (Fig. III). Also, in all cases of bronchiectasis examined I came across the same flattened cells, partly attached to the alveolar walls and partly separated from them (Fig.IV and Fig.V.). The constant finding of those cells in such a large number of cases stimulated me to investigate their origin. Are they the lining cells of the alveoli as is asserted by some authors? What are they? was my first question, and indeed this was the starting point of my inquiry into the whole problem of the alveolar lining and the origin of the alveolar phagocyte. Therefore, in my Ph.D. Thesis I concluded that no definite /

definite assertion of the existence or not of a primary alveolar carcinoma can be furnished until the nature of the alveolar lining is definitely established.

In Fig.III the appearances were typical of alveoli and to leave no doubt regarding the character of the spaces seen in Fig.IV in case they were sections of bronchioli, I have cut the block into serial sections (300 sections) and applied various stains, such as Von Gieson's stain, to demonstrate muscle fibres. The sections were carefully searched and I satisfied myself that the spaces belong to air-cells as no muscle fibres could be demonstrated in their walls and the same appearance was repeated in every one of the 300 available sections. Having accomplished this, slides from 400 autopsy cases were collected. Some showed various diseases of the lungs such as pneumonia, tuberculosis and bronchiectasis, while others were normal from the point of view of the lung.

I then turned to the various histological techniques specially devised to demonstrate the alveolar lining. By the courtesy of Dr. A.Macgregor of the Royal Sick Childrens' Hospital I secured the lungs of three children where death was due to no pulmonary involvement. The lungs were moderately distended with /

with 2 per cent solution of silver nitrate. The trachea was tied and the lungs placed in a large volume of the same solution. They were left therein for 24 hours as recommended by Carleton. Then the lungs were sliced and small pieces placed in a fresh change of silver nitrate. After 24 hours the pieces were washed in many changes of distilled water until they became brown. They were dehydrated, cleaned and embedded in paraffin. Thin and thick (20-40 u.) sections were cut. The results may be summarised as follows:- Sections at different angles showed an irregular distribution of a darkly stained network, from which no definite conclusions could be drawn. I did not find the method satisfactory and decided to go no further with it, particularly in view of the fact that it is greatly criticised and its fallacies are abundant. It was, therefore, necessary to tackle the problem indirectly by means of animal experiments and to continue the study of the various sections collected from the post mortem.

Experimental Study.

Ribbert (1904), Goldman (1912), Aschoff (1913 & 1923), Cappell (1929) and other workers have shown that by repeated injections of various solutions of dye-stuffs into living animals a selective staining of certain tissues occurs. The process is one of phagocytosis. The dye appears in the cell in the form of granules./

granules. Whether these granules are simply particles of dye or stained preformed elements in the cell, is not known. The staining is confined to the cytoplasm; no staining of the nucleus has ever been observed. By this means the cells composing the reticulo-endothelial system have been studied. All the cells of the system are derived from the primitive embryonic mesenchyme and the cell constituents include the clasmatocytes of Ranvier found in the loose connective tissue, the adventitial cells of Marchand, the pyrrol cells of Goldman, the polyblasts of Maximow, the histiocytes of Aschoff and Kiyono, the reticular cells and the cells of the sinuses in the lymph-nodes, in the spleen and bone-marrow, and finally the Kupffer cells in the liver.

By the use of intravital staining, then, one is able to recognise a special variety of cell which in the earliest stages of ontogenesis is derived from the embryonic mesenchyme.

EXPERIMENT I.

(Intraperitoneal injections of Trypan Blue.)

In Experiment I, three rabbits and two guinea-pigs were employed. The vital stain used was trypan blue, in the form of a 1.0 per cent solution in distilled water. The solution was boiled and filtered when cool. The animals were injected intraperitoneally and the dose has been calculated on the basis laid /

laid down by Cappell, namely, 5 c.c. of a 1.0 per cent solution of the dye per kilogram body weight for rabbits and 1 c.c. of the same solution per 100 grms. weight for guinea-pigs. After a preliminary trial with Zenker's fluid, corrosive sublimate and formaline, the latter was found to be the most suitable fixative. Sections were prepared by the paraffin method, as the dye once fixed is relatively resistant to decolorisation by alcohol. The staining methods used to show up the cell structure were 1.0 per cent neutral red, 1.0 per cent Fuchsin and Hoematoxylin and eosin.

Animal No.	Individual dose.	No.of injections I.P.	Period of survival after 1st injection.	Naked-eye and Histological Findings.
Rabbit A 36	10 cc.	2	5 days.	The skin was coloured blue. The abdominal organs particularly the liver and omentum were deeply stained. The lungs were of a normal pink colour, only the walls of the bronchi being stained blue. Microscopically, the liver and spleen showed the usual characteristic staining of the reticulo-endothelial system (reticular cells and the endothelial cells of the sinuses in the spleen and Kupffer cells in the liver). The lung parenchyma showed practically no staining of any of the constituent cellular elements except for a few spindle-shaped histiocytes in the walls of the large vessels and bronchi. The blueness of the bronchi was due to the fact that the elastic and cartiloginous tissues have taken up the stain.

Rabbit /

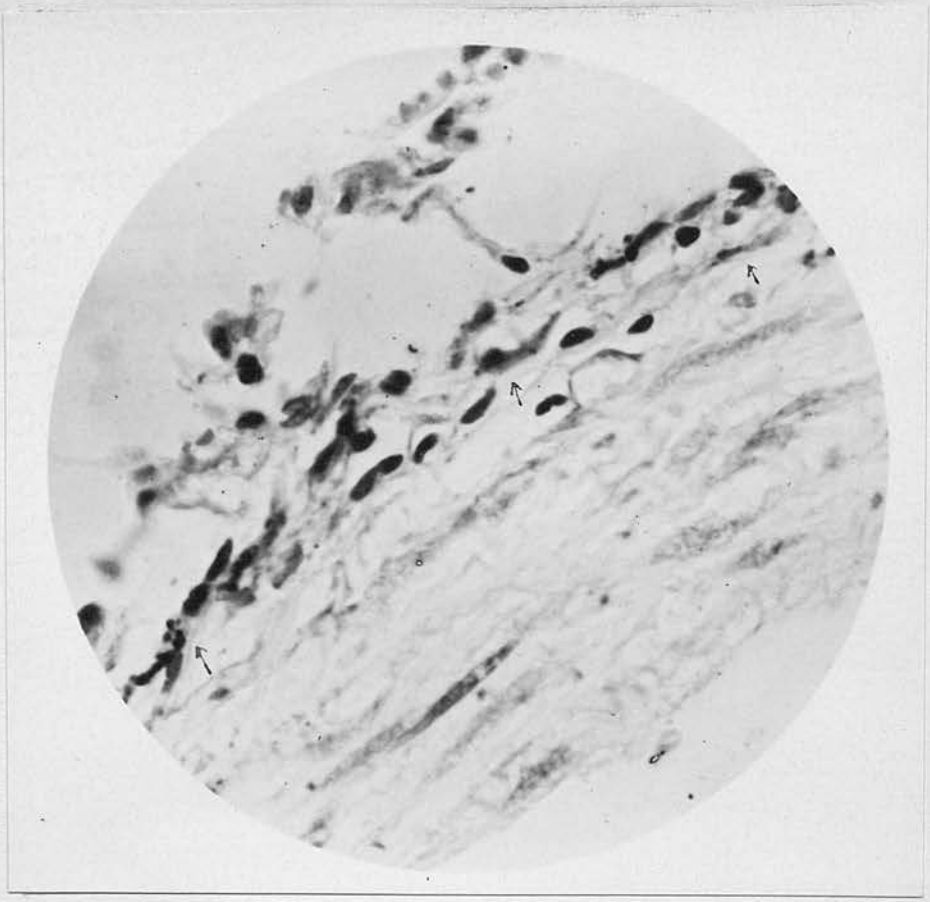


Fig. VI.(X 730) Note histiocytes containing trypan blue granules in the outer wall of a blood vessel.

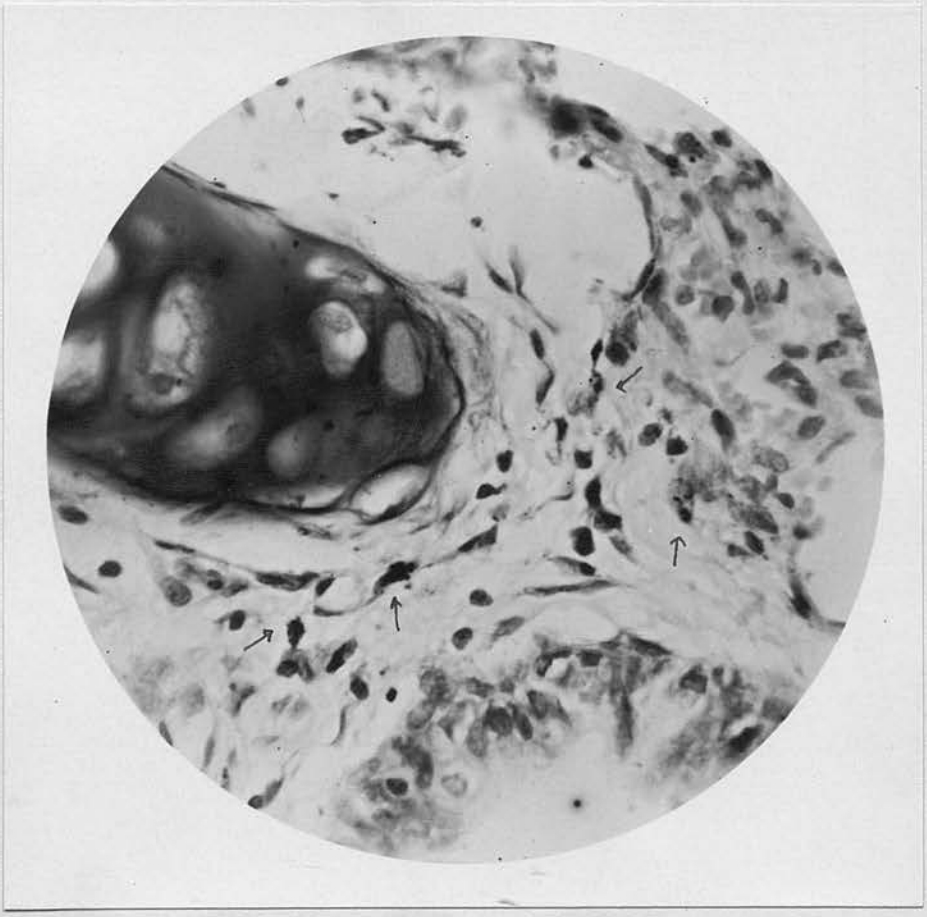


Fig. VII (X 620) Note histiocytes containing trypan blue granules in the wall of a bronchus.

Rabbit R.96	10 cc.	eleven daily injections.	12 days.	The skin and viscera were markedly blue. The abdominal organs, liver, spleen, kidneys, omentum, etc. were deeply stained. The lung substance showed a faint blue colour and the walls of the bronchi and vessels were deeply stained blue. Microscopically, the reticulo-endothelial system of the liver and spleen showed the characteristic staining. The histiocytes in the walls of the vessels, bronchi and interstitial tissue of the lungs were deeply stained and certain cuboidal cells in the alveolar angles contained trypan blue granules. Some of the cuboidal cells contained in addition granules of carbon pigment. The free "dust cells" showed trypan blue staining.
Rabbit A 103	10 cc.	eleven	12 days.	Naked-eye and histological findings are the same as in rabbit R 96.
Guinea-pig I	4 cc.	four	5 days.	Naked-eye and histological findings are the same as in rabbits A 103 and R 96.
Guinea-pig III.	4 cc.	one	2 days.	The animal died on the third day. The skin was coloured blue. The liver and omentum were stained. The lungs were of a normal pink colour, only the walls of the bronchi were slightly blue. Microscopically the lungs showed no histiocytic staining.

The conclusions to be drawn from Experiment I. are quite simple. By repeated intraperitoneal injections of trypan blue one could outline clearly the cells forming the reticulo-endothelial system of all the organs. In the lungs the following cells take up the dye:- (1) The spindle-shaped histiocytes in the walls of the vessels, bronchi and interstitial tissue (Fig. VI. /

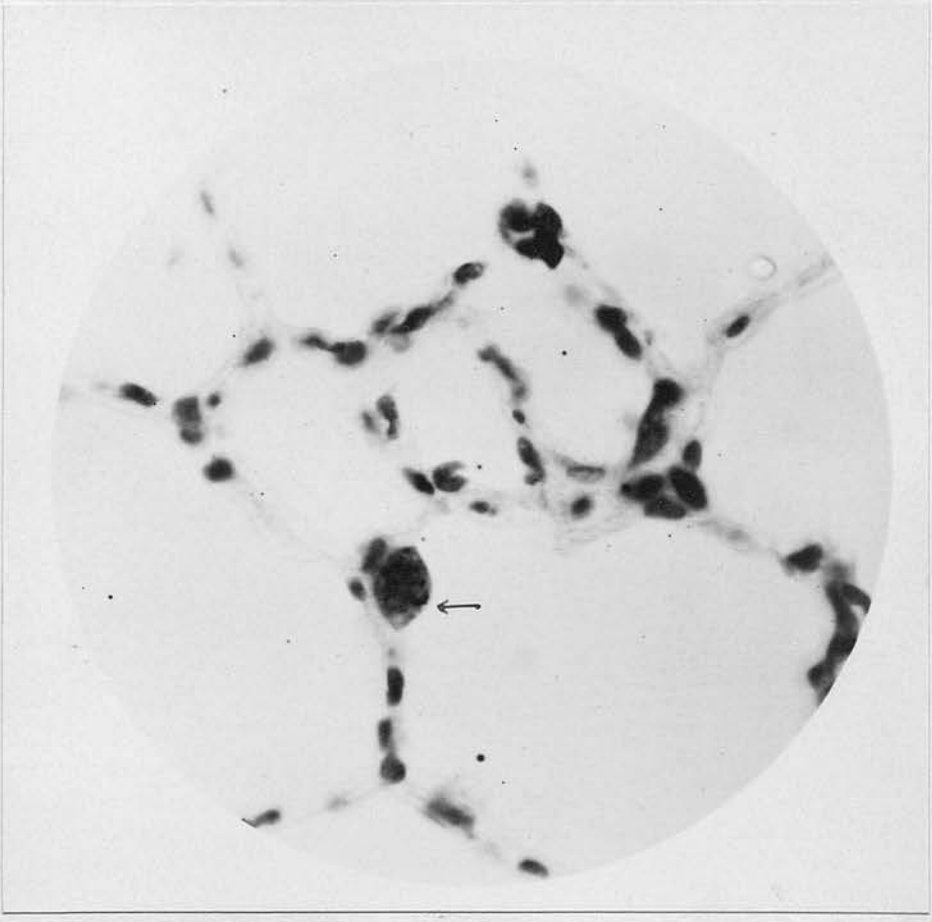


Fig. VIII. (X 700) Note trypan blue granules in the cuboidal cells which occur most frequently in the alveolar angles.

VI and Fig.VII). (2) Certain cuboidal cells found chiefly in the alveolar angles (Fig.VIII). Some of these contain in addition granules of naturally occurring carbon pigment. (3) The free "dust cells." The flattened cells lining the alveoli show no staining.

EXPERIMENT II.

(Intraperitoneal injections of trypan blue and intratracheal injection of saline suspensions of insoluble particles - carmine and india ink.)

Two rabbits and four guinea-pigs were used in this experiment. The animals were first of all subjected to intense vital staining with 1.0 per cent trypan blue in the same way as in Experiment I, and then a small quantity of carmine or india ink suspended in saline was injected into the trachea. The best method of intratracheal administration was found to be as follows:- The experiments were done under ether anaesthesia. The trachea just below the larynx was exposed by a short incision and blunt dissection; the suspension of carmine or india ink was drawn up into a record syringe and with a fairly long needle of moderately large gauge, the tissues of the trachea were pierced. The needle point was gently carried down to the bifurcation of the trachea and the suspension was forced as far as possible down the bronchial tree by a sudden pressure on the plunger. In this way it /

it was possible to get a good distribution of the injected material throughout the lung, with fair quantities reaching the air-sacs. The animals were killed at short intervals. In all cases the lungs were fixed in formalin, and paraffin sections were prepared.

Animal No.	No. of Trypan blue injections.	Material injected intra-tracheally.	Survival period after intra-tracheal injection.	Naked-eye & Histological Findings in the lungs.
Rabbit A 13	Eleven injections	2 c.c. 1% carmine.	1 hour.	The lungs showed a definite, though pale, blue tint and a fair amount of the injected carmine was visible underneath the pleura. The histiocytes in the walls of the vessels, bronchi and interstitial tissue of the lungs were deeply stained with trypan blue. The cuboidal cells noted in Experiment I. contained trypan blue granules and some of them contained in addition carmine particles. A large number of free phagocytes were noted in the alveoli. These contained both trypan blue and particles of carmine. A large quantity of carmine was lying free in the bronchi and air-sacs.
Rabbit A 439	Eleven	2 c.c. 1% carmine.	2 hours.	Naked-eye and microscopic appearances were identical with those of Rabbit A 13.

G.P. /

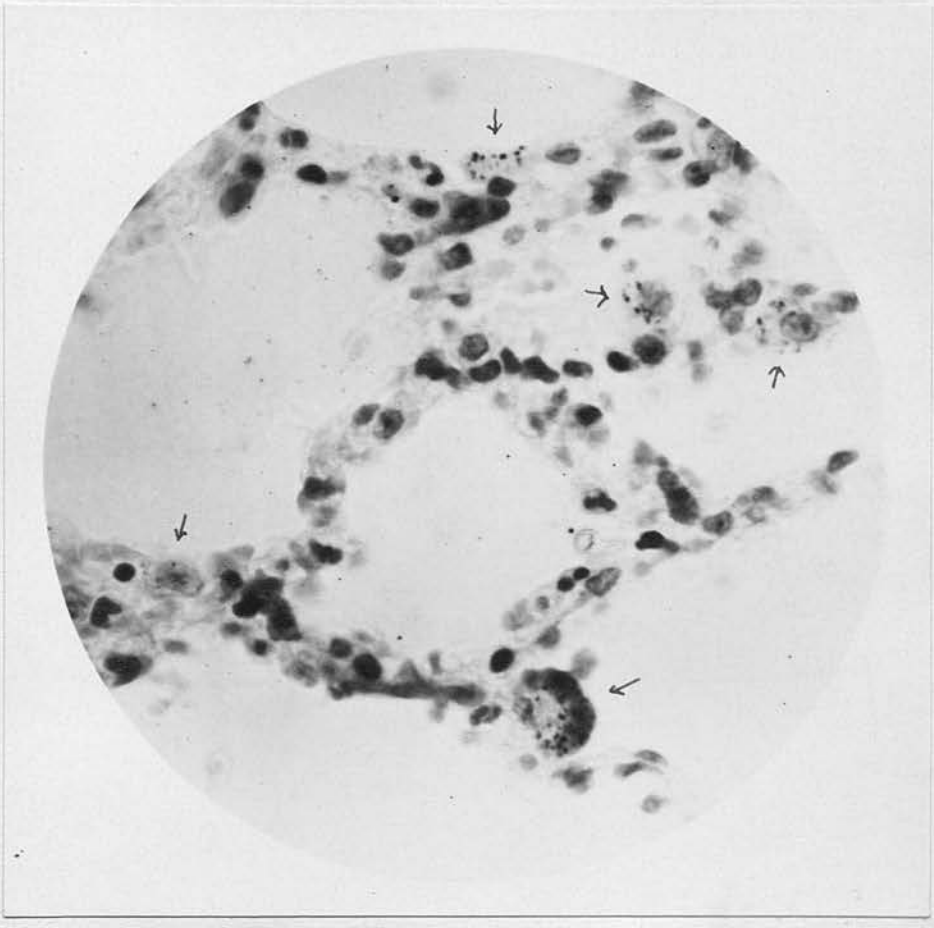


Fig. IX (X 750) The cuboidal cells contain pale and darker granules of carmine and trypan blue respectively.

G.P. VI.	3	1.5 c.c. 1% india ink.	2 hours.	The results were the same as in rabbits A 13 and A 439. The cuboidal cells and the free phagocytes contained trypan blue and particles of india ink. Up to 19 hours the quantity of free ink in the alveoli did not diminish to any appreciable extent, but the bronchi contained a large number of free "dust cells" containing both trypan blue and india ink.
G.P. IV.	4	1.5 c.c. 1% ink.	3½ hours.	
G.P. V.	4	1.5 c.c. 1% ink.	4½ hours.	
G.P. II.	3	1.5 c.c. 1% ink.	19 hours.	

The conclusions drawn from Experiment II were:-

(1) As a result of the intratracheal injection of carmine or india ink a large number of new mononuclear phagocytes appeared in the air sacs and alveoli of the experimental animals. The majority of these contained trypan blue and in addition particles of ingested carmine or india ink.

(2) The cuboidal cells contained granules of trypan blue and some showed in addition particles of carmine or india ink. Some of them were seen to be swollen and others were almost on the point of being detached into the luminae of the air-cells. (Fig.IX).

(3) The flattened cells lining the alveoli contained neither trypan blue nor carmine or india ink. They remained flattened and inactive.

(4) The histiocytes in the walls of the vessels, bronchi and interstitial tissue of the lungs contained granules of trypan blue only.

The /

The above findings suggested that the source of the alveolar phagocytes might be from the cuboidal cells which occur most frequently in the alveolar angles. Therefore Experiment III was undertaken.

EXPERIMENT III.

(Intratracheal injection of saccharated oxide of iron.)

In Experiment III 5 rabbits were employed. The material used for intratracheal injection consisted of a suspension of 2 per cent saccharated oxide of iron in distilled water. The dose was about 2 c.c. The animals were killed at short intervals, 10 minutes, 20 minutes, 30 minutes, 1 hour and 2 hours respectively, in order to study the early stages of cellular response to the injected iron. The lungs were fixed in formalin and after sectioning in paraffin the prussian blue reaction was developed as follows:-

Reagents (A) 2 per cent potassium ferrocyanide (Freshly prepared,)

(B) 2 per cent hydrochloric acid.

For use a mixture containing one part of (A) and three parts of (B) was heated and poured on the slides to act for one to two minutes. The slides were then washed in water and counter-stained with one per cent neutral red for half a minute. This is a method which has been found useful by Mr J.C.Sommerville in the Laboratory of the Scottish Asylums Board.

Animal No.	Survival period after intra-tracheal injection.	Histological Findings in Lungs.
Rabbit A 469	10 minutes	The bronchi and alveoli showed a fair quantity of free iron in their luminoe. There were also present a few monuclear phagocytes loaded with iron and lying free in the cavities of the alveoli. The cuboidal cells occurring chiefly in the alveolar angles contained iron; some were greatly enlarged and others exhibited an amoeboid appearance. The flattened cells lining the alveoli contained no iron. In one or two of the cuboidal cells a suggestion of early mitosis was visible.
Rabbit A 468	20 minutes	The cuboidal cells were swollen, loaded with iron and exhibited a variety of amoeboid appearances. The number of free monuclear phagocytes had increased. Some of the iron-containing cuboidal cells showed definite mitosis in situ. The cells lining the alveoli remained flattened and contained no iron.
Rabbit A 467	30 minutes	The appearances were almost identical with rabbit A 468. Mitosis in the iron-containing cuboidal cells was visible.
Rabbit A 466	1 hour.	The number of free phagocytes had increased enormously. Abundant iron was still lying free in the luminoe of the alveoli. Mitosis was visible.
Rabbit A 102	2 hours.	Free mononuclear phagocytes containing iron were now seen in the luminoe of the bronchi. The attached cuboidal cells contained iron and mitosis was present in some of them. The cells lining the alveoli contained no iron and remained flattened.

Conclusions:

As a result of the intratracheal injection of a suspension of saccharated oxide of iron, a large number of new mononuclear phagocytes appear in the air sacs /

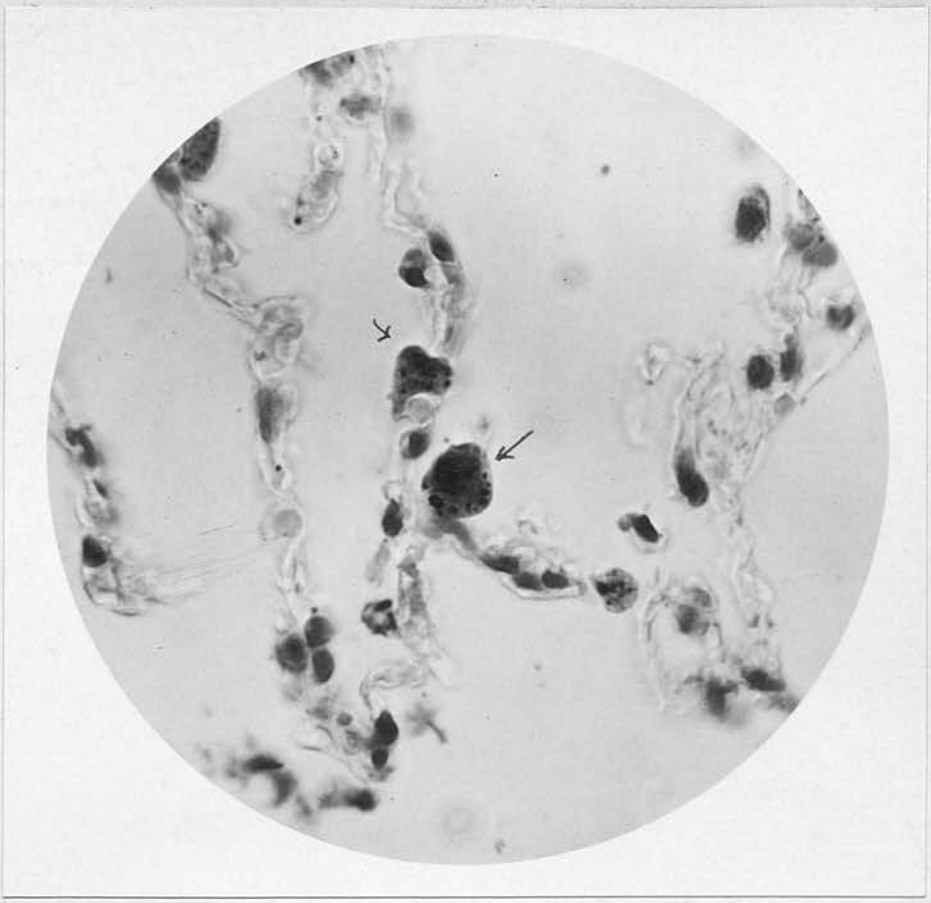


Fig. X. (X 775) Note within 10 minutes particles of iron are actively taken up by the cuboidal cells

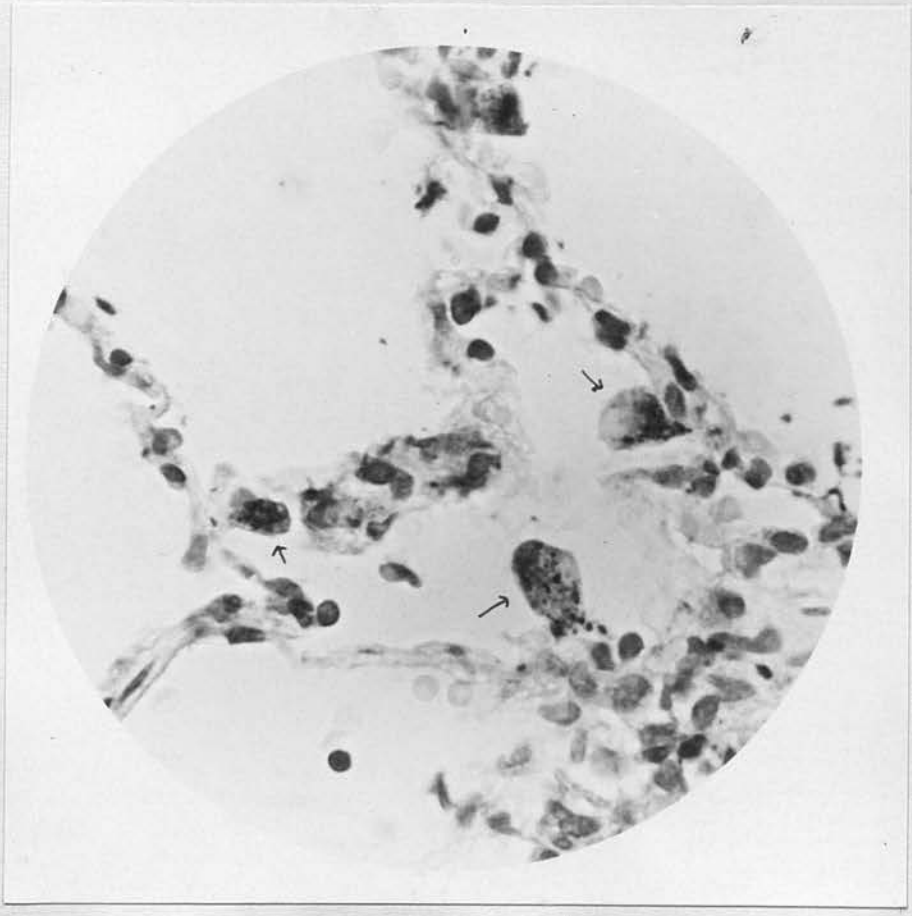


Fig. XI. (X 750) Note within 20 minutes the cytoplasm becomes loaded with iron which pushes the nucleus to one side and the cells become very conspicuous by their size and irregular shape.

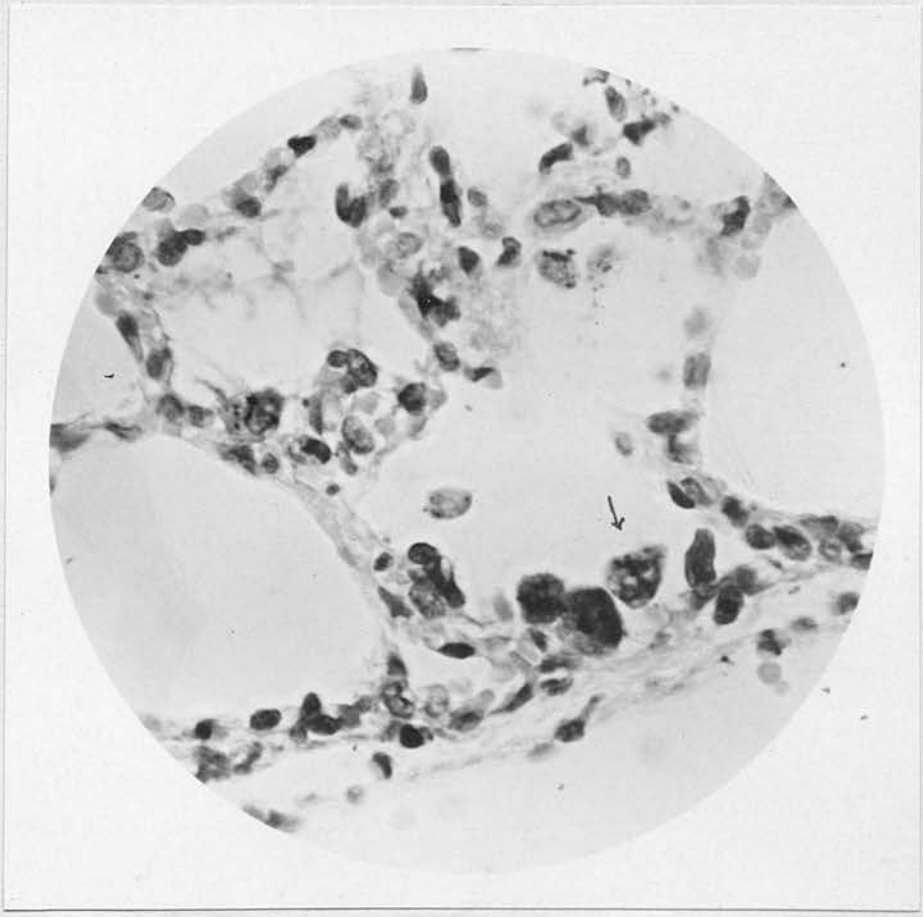


Fig. XII. (X 750) Note the newly shed triangular cell from a rabbit killed 20 minutes after the intratracheal injection.

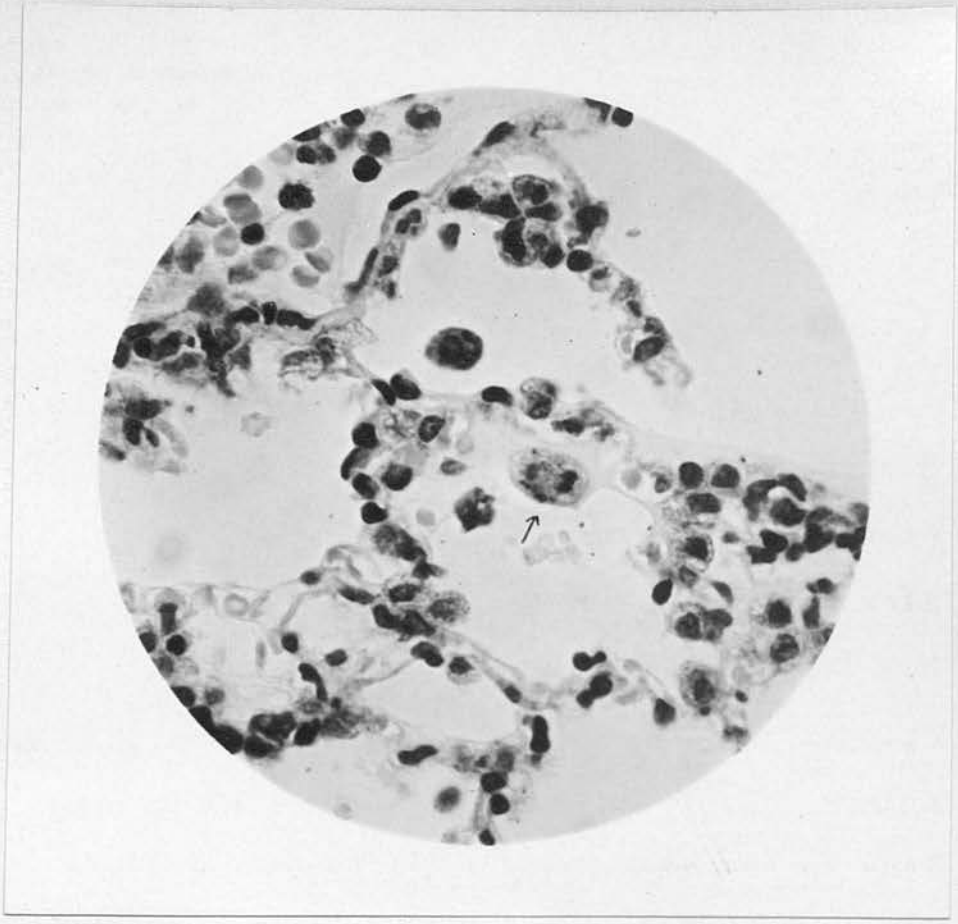


Fig. XIII. (X 700) Note mitosis of a cuboidal cell containing iron.

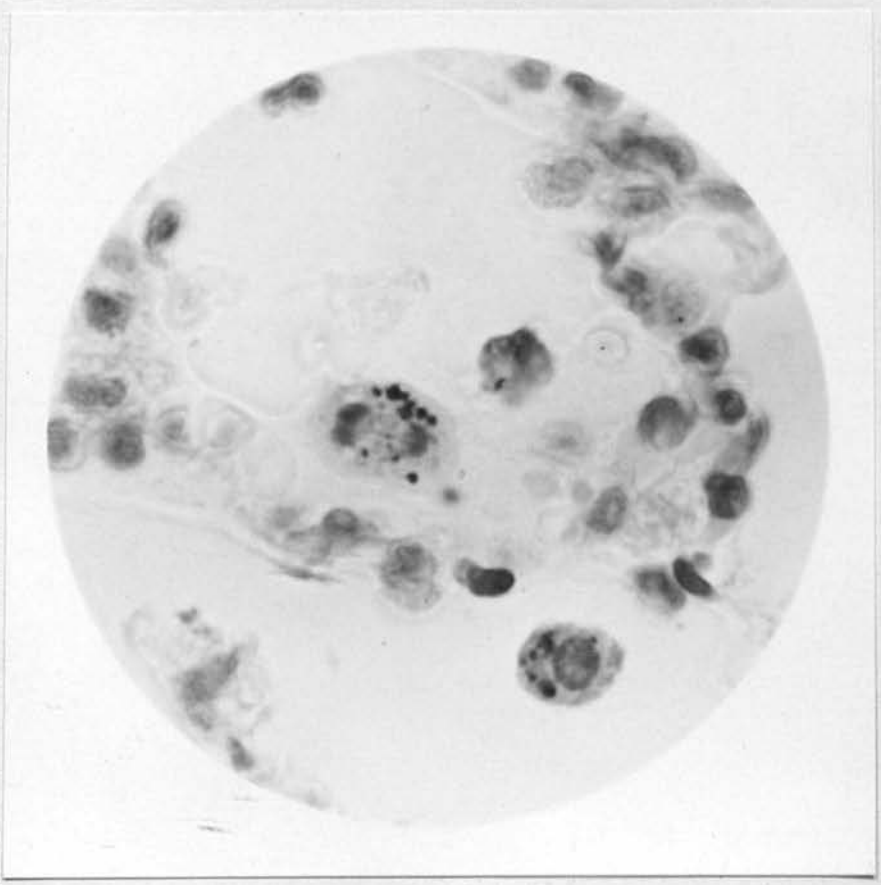


Fig. XIV. (X 1300) Higher magnification of figure XIII to show division by mitosis of a cuboidal cell.

sacs and alveoli of the experimental animals. The active cells are clearly derived from the cuboidal cells occurring chiefly in the alveolar angles. The flattened cells lining the alveoli take no part in the process. The steps appear to be as follows:- Within ten minutes particles of iron are actively taken up by the cuboidal cells in situ. They become swollen and exhibit an amoeboid appearance (Fig.X). Within twenty minutes the cuboidal cells become very conspicuous by their size and irregular shape. The cytoplasm becomes loaded with iron which pushes the nucleus to one side (Fig. XI.). Very soon they separate themselves from the alveolar walls and come to lie free in the luminoe of the air cells. The newly shed cell is more or less triangular in shape and shows a nucleus displaced to one side (Fig.XII.). The cuboidal cells divide by mitosis to provide the alveolar phagocytes (Figs. XIII & XIV.).

EXPERIMENT IV.

In spite of the convincing evidence afforded by the previous experiments that the alveolar phagocytes are clearly derived from the cuboidal cells a further attempt was made to ascertain whether the circulating monocytes participate in their production. A rabbit was intensely stained by repeated intraperitoneal injections of trypan blue. It then received an intravenous /

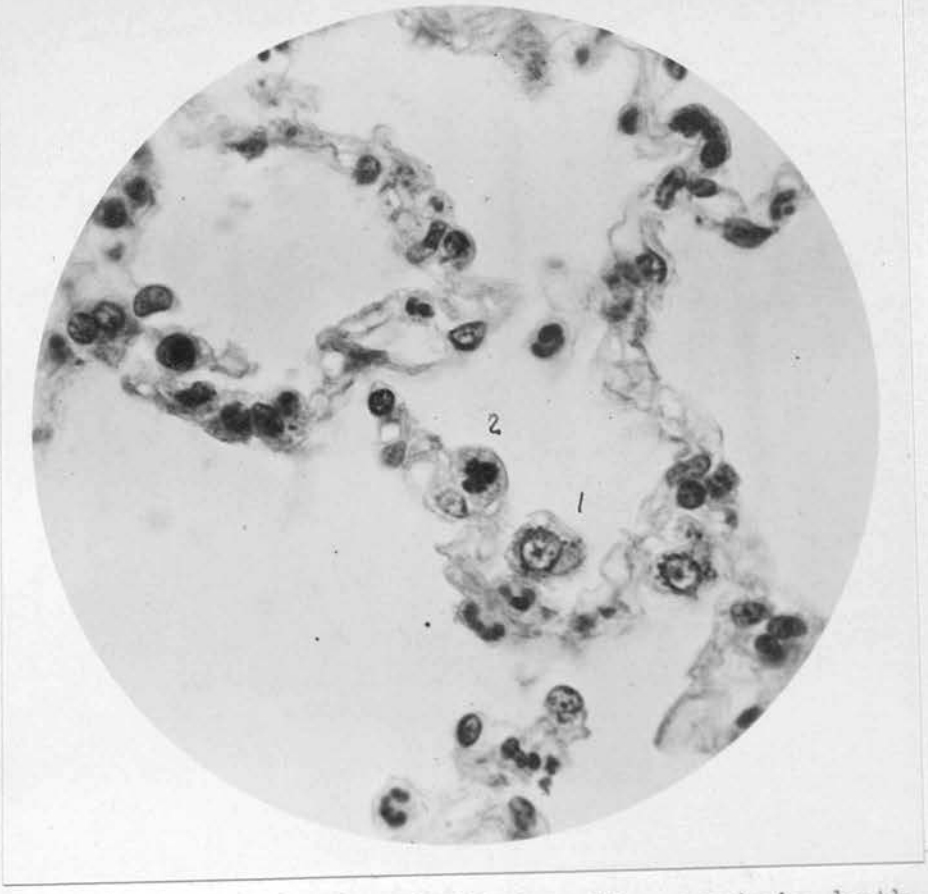


Fig. XV. (X 750) The cuboidal cells contain both trypan blue and carmine (1) and divide by mitosis (2) to provide the alveolar phagocytes.

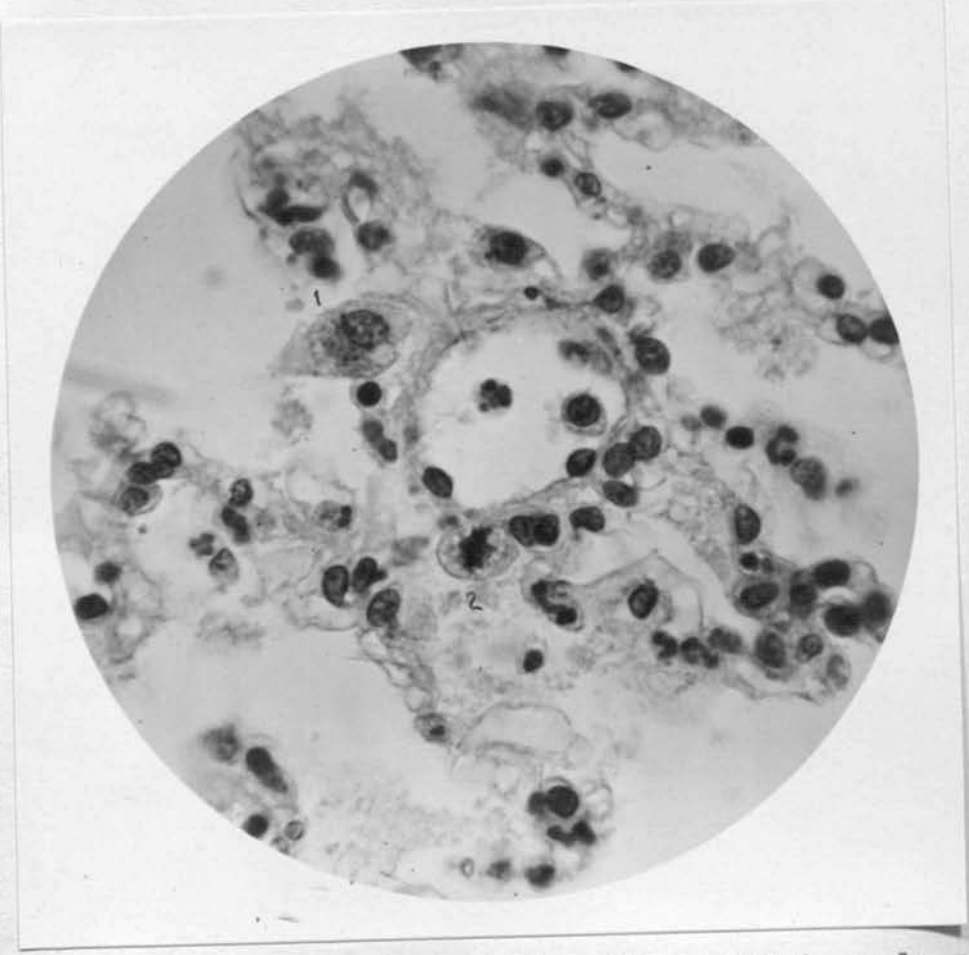


Fig. XVI. (750) The cuboidal cells contain pale and darker granules of carmine and trypan blue respectively (1) Note mitosis in one of them (2).

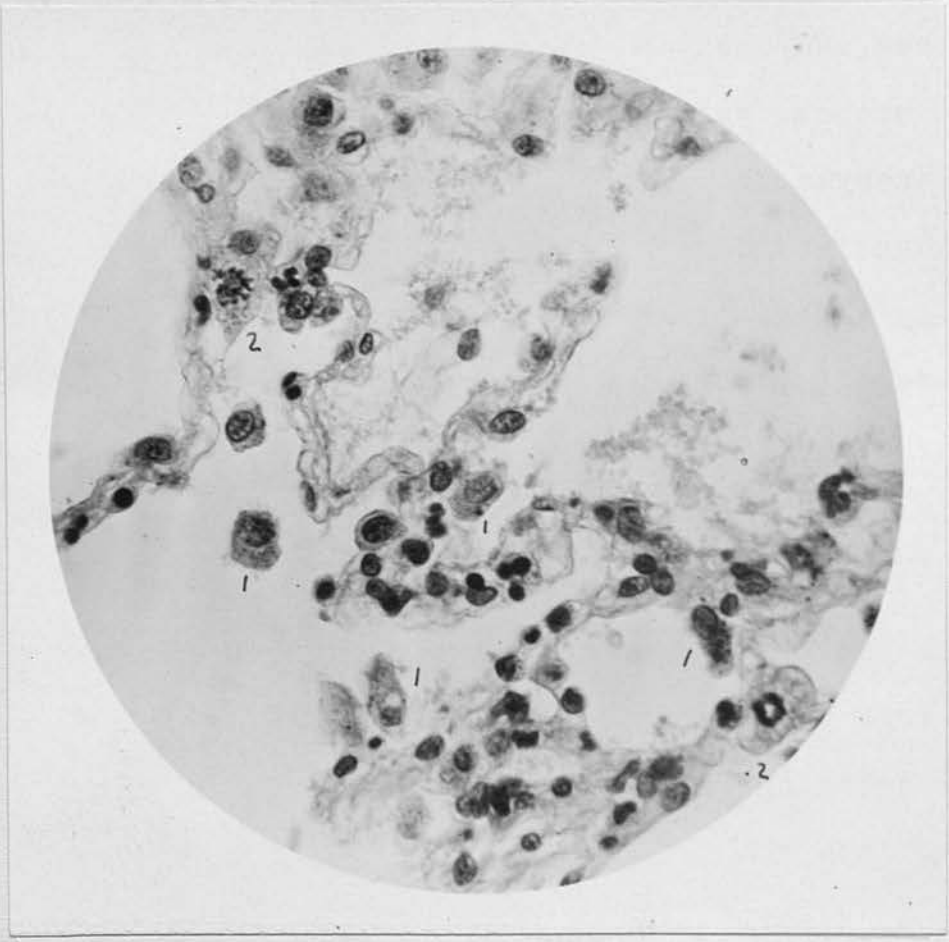


Fig. XVII. (X 500). Note mitosis of the cuboidal cells (2) and the granules of carmine and trypan blue in them (1)

venous injection of india ink and immediately thereafter 2 c.c. of fine carmine suspension in saline was injected into the trachea. The rabbit was killed after 5 hours. The skin and viscera were markedly blue. The abdominal organs, particularly the liver, spleen and omentum, were deeply stained. The trachea and bronchi were deeply coloured blue and the lung parenchyma showed a faint blue colour and reddish discolorations due to the injected carmine. Microscopically the greater part of the carmine was already intracellular, being contained within large phagocytes, many of which showed in addition granules of trypan blue. No phagocytes containing ink were observed. The alveolar phagocytes appeared to be derived entirely from the cuboidal cells. All stages in the formation of free phagocytes could be seen, the cells swelling and becoming more irregular before finally desquamating into the alveoli. Mitosis in the cuboidal cells was abundant. In one slide over twenty mitotic figures could be counted (Figs. XV, XVI & XVII). The intravenously injected ink was distributed irregularly throughout the lungs, some areas being free while in others the ink formed heavy deposits on the capillary walls. No actual phagocytosis by the capillary endothelial cells had occurred and no blood monocytes containing ink passed through the capillary walls to gain entrance into the alveoli.

To /

To draw conclusion from the previous experiments that the flattened cells lining the alveoli do not share in the production of the alveolar phagocytes and that this is restricted entirely to the cuboidal cells which occur most frequently in the interalveolar angles "septal cells" seemed premature. The reason for this statement is that the lining cells are so flattened as to be almost invisible. Therefore it was decided at this stage to find some method by means of which these flattened cells could be rendered more visible in order to subject them to another series of experiments.

In 1928 and 1930 J.S.Young produced active proliferation in the epithelial cells lining the marginal alveoli of the lung of the rabbit by the intrapleural injection of an emulsion of liquid paraffin and bile salts and a variety of neutral salts, viz., NaCl, $CaCl_2$, $SrCl_2$, $AlCl_3$, etc. He gave a detailed physico-chemical interpretation of the phenomenon and his histological findings can be summarised briefly as follows:- Within the first twenty-four hours, the epithelial cells lining the marginal alveoli of the lung become swollen and more spherical; during the next twenty-four hours their nuclei become hyperchromatic; and active proliferation is evidenced on the third day by more or less numerous mitotic figures. On the fourth day mitotic figures are scanty and the epithelial /

epithelial cells have assumed a cubical or columnar shape; thereafter a process of involution ensues indicated by increasing nuclear pyknosis and reduction of size, so that the normal appearances of the lung are largely restored by the seventh or eighth day. The phase of active epithelial hyperplesia is usually accompanied by proliferation of fibroblasts in the subserosa and these become organised later, forming thin plaques of fibrous tissue.

EXPERIMENT V.

Three rabbits were employed and the material used for intrapleural injection consisted of an emulsion of 100 c.c. liquid paraffin and 10 c.c. of a 5 per cent aqueous solution of sodium cholate.* The resulting mixture was autoclaved. Each rabbit was anaesthetised in turn and 10 c.c. of the emulsion were injected into its right pleural sac with the usual aseptic precautions. The injections were performed postero-laterally, near the inferior angle of the right scapula with an ordinary record syringe. Two rabbits were killed by a blow on the back of the neck on the third day and one on the fourth day. The trachea /

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* I am indebted to Dr C.P.Stewart, Biochemistry Dept., Royal Infirmary, for the preparation of the inoculum as follows:- The bile salts were purified by solution in alcohol and filtration; ether was added to the point of producing a turbidity, after which the salts were crystallised out at 0° c.c.

trachea was ligatured before cutting the chest wall to reduce collapse of the lungs to a minimum. Next the pericardial sac was opened and the heart excised. The left bronchus was ligatured before removing the left lung. The right lung was then fixed as a whole in Zenker's fluid for three or four hours before the organ was cut into segments for complete microscopical examination.

Animal No.	Survival period after intrapleural injection.	Naked-eye and histological Findings.
Rabbit A 478	3 days	The right pleural cavity contained a very small quantity of the injected emulsion. The visceral pleura was somewhat rough and congested. Microscopically, the subserosa was thickened and contained proliferating fibroblasts. The epithelial cells lining the marginal alveoli had become swollen and more or less cubical with hyperchromatic nuclei.
Rabbit A 477	3 days	The appearances were identical with those of Rabbit A 478.
Rabbit A 481	4 days	The injected emulsion had been completely absorbed as there was none left to be seen in the right pleural sac. The visceral pleura was wrinkled and congested. Microscopically the subserosa was much thickened by proliferation of fibroblasts and the formation of new capillaries. The epithelial cells lining the peripheral alveoli had become cubical and columnar with hyperchromatic nuclei. No actual mitotic figures were observed.

The results confirm the brilliant work of Young (1928-1930) and render the flattened cells conspicuous and therefore susceptible to further experiments and more thorough study. Within four days the marginal alveoli /

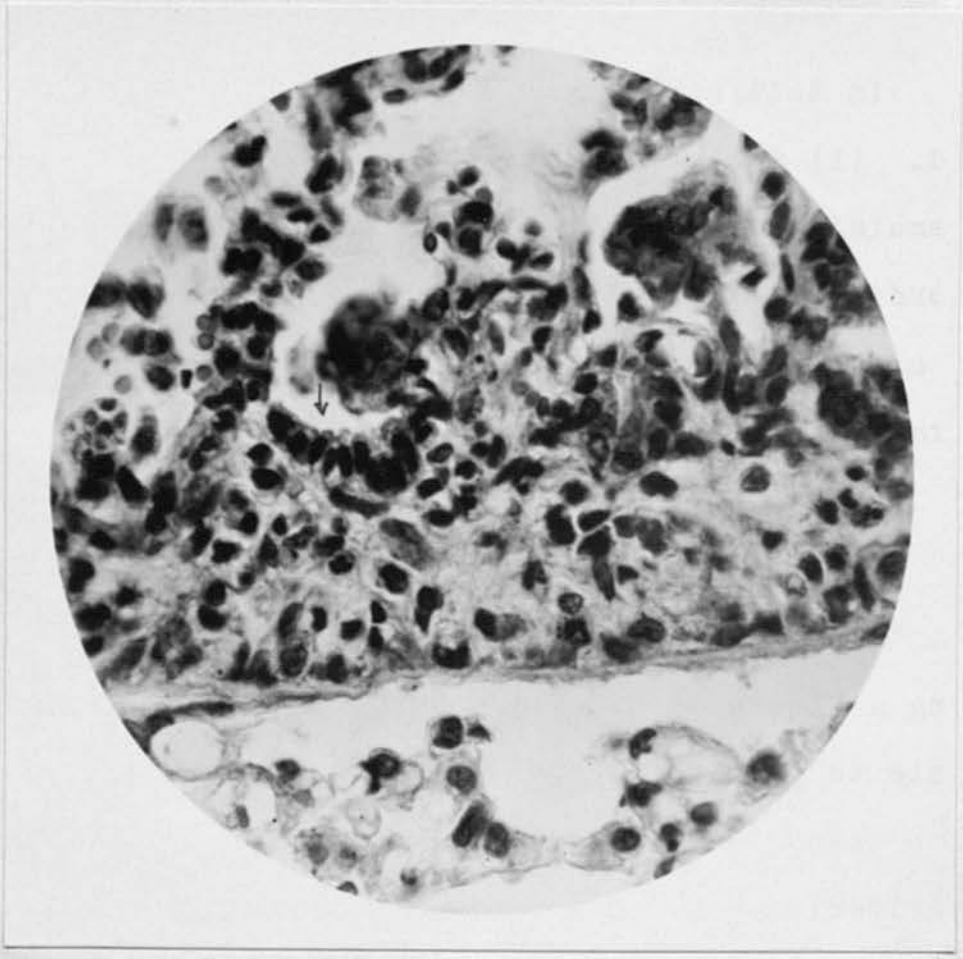

Fig. XVIII. (620) Rabbit A 481. Note within 4 days the marginal alveoli are lined by cubical and columnar cells possessing hyperchromatic nuclei. The subserosa is thickened by a layer of granulation tissue.

alveoli are lined by cubical and columnar cells (Fig. XVIII) and all stages in the formation of the latter from the flattened cells can be seen.

EXPERIMENT VI.

In this experiment two series of rabbits were used. (1) One group were injected intrapleurally with an emulsion of liquid paraffin and bile salts prepared according to the method described in Experiment V, and after a varying number of days were injected intratracheally with trypan blue or india ink. (2) In a second set of rabbits a solution of 4.3 per cent NaCl (Young 1928) was employed instead of the paraffin emulsion. This is equally effective, and being a liquid is quickly absorbed and therefore not liable to produce collapse of the lung, a feature which was present to some extent in the previous experiments. The dose of NaCl was 5 c.c. The material used for intratracheal injection consisted of a suspension of 2 per cent saccharated oxide of iron in distilled water. In all cases the lungs were fixed in formalin and in group (2) the prussian blue reaction was developed.

Animal /

Animal No.	I-Pleural injection	Time of I-Trach. injection after I.Pleural.	Survival period after I-Trach. injection.	Histological Findings.
Rabbit A 480	10 c.c. emulsion	3rd day. 2 c.c. 1% Trypan blue	7 hours.	The marginal alveoli were lined with cubical and columnar cells. The hypertrophied lining cells contained no trypan blue granules, whereas the "septal cells" were deeply stained.
Rabbit A 483	10 c.c. emulsion	3rd day 2 c.c. 1% Trypan blue	8 hours	
Rabbit A 476	10 c.c. emulsion	5th day 2 c.c. 1% Trypan blue	6 hours	The epithelial cells lining the peripheral alveoli showed a slight degree of hypertrophy. These contained no trypan blue granules. The "septal cells" were stained.
Rabbit A 482	10 c.c. emulsion	7th day 2 c.c.1% India ink.	2½ hours	There was slight swelling of the epithelium lining the marginal alveoli. Ink particles were seen only in the "septal cells."
Rabbit A 441	5 c.c. 4.3% NaCl	1st day 2 c.c. 2% iron	3 hours	There was slight swelling of the epithelium lining the marginal alveoli. Iron was present in the "septal cells" but not in the swollen lining epithelium.
Rabbit A 443	5 c.c. NaCl	1st day 2 c.c. iron	6 hours	
Rabbit A 442	5 c.c. NaCl.	2nd day 2 c.c. iron	2 hours	There was clear evidence of the hyperplasia of the epithelium lining the peripheral alveoli, characterised by swelling and by nuclear hyperchromatism. The swollen lining cells contained no iron, whereas the "septal cells" were deeply stained.
Rabbit A 445	5 c.c. NaCl.	4th day iron	3 hours	Iron particles were seen in the "septal cells" but not in the hypertrophied lining epithelium.

The conclusions to be drawn from Experiment VI were:-

1. Following the intrapleural introduction of both an emulsion of liquid paraffin and bile salts and 4.3% solution of NaCl, there was progressive proliferation of the marginal epithelium during the first four days of experiment.

2. It has not been possible to demonstrate any trypan blue, india ink or iron within the proliferating cells, whereas, as in all cases, the "septal cells" were deeply stained.

EXPERIMENT VII.

In this experiment it was decided first of all to subject the animals to intense intravitam staining with trypan blue, in order to outline the cells composing the reticulo-endothelial system of the lungs; secondly to render more conspicuous the cells lining the alveoli by means of intrapleural injections of strontium chloride; and thirdly to continue the intraperitoneal injections of trypan blue during the period of hypertrophy in order to maintain an adequate supply of dye in the pleura while the cellular reaction is taking place. Strontium chloride is one of the substances recommended by Young (1928). The dose is 5 c.c. of 9.94 per cent aqueous solution. It was autoclaved prior to injection into the right pleural sac.

Animal No.	Survival period after I.Pleural injection.	Naked-eye and Histological Findings.
Rabbit A 29	2 days	The right pleural sac was dry. The visceral pleura was slightly congested. The cells lining the marginal alveoli were swollen. Trypan blue granules were found in the "septal cells" and the histiocytes in the walls of the vessels, bronchi and interstitial tissue of the lungs but not in the hypertrophied lining cells.
Rabbit A 440	3 days	The right pleural sac was dry. The visceral pleura was wrinkled and opaque. The lungs were of a pale blue colour. The marginal alveoli were lined with cubical and columnar cells free of trypan blue staining. The latter was present in the "septal cells" and the other histiocytes of the lungs.
Rabbit A 6	4 days	The hypertrophied lining cells of the alveoli which were columnar contained no trypan blue granules. The dye was present in the "septal cells" and histiocytes of the lung.

From these results it may be concluded that in spite of the previous intense vital staining of the animals and the continued administration of the dye so that the plasma contained a large amount of stain throughout the experiments, the hypertrophied cells lining the alveoli failed to stain, whereas the "septal cells" and the other histiocytes contained numerous trypan blue granules. However, it was determined to test the behaviour of the lining cells still further.

EXPERIMENT VIII. /

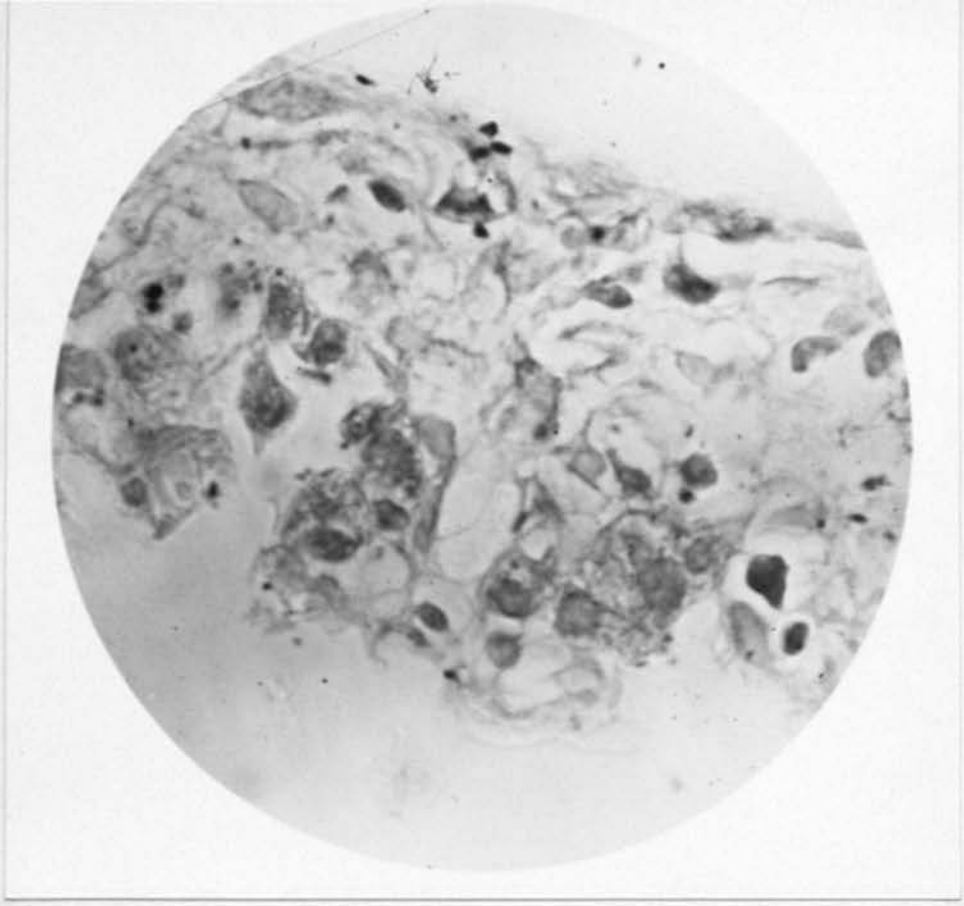

Fig. XIX. (X 930) Rabbit A 8. The marginal alveoli are lined by columnar cells containing no trypan blue or carmine granules.

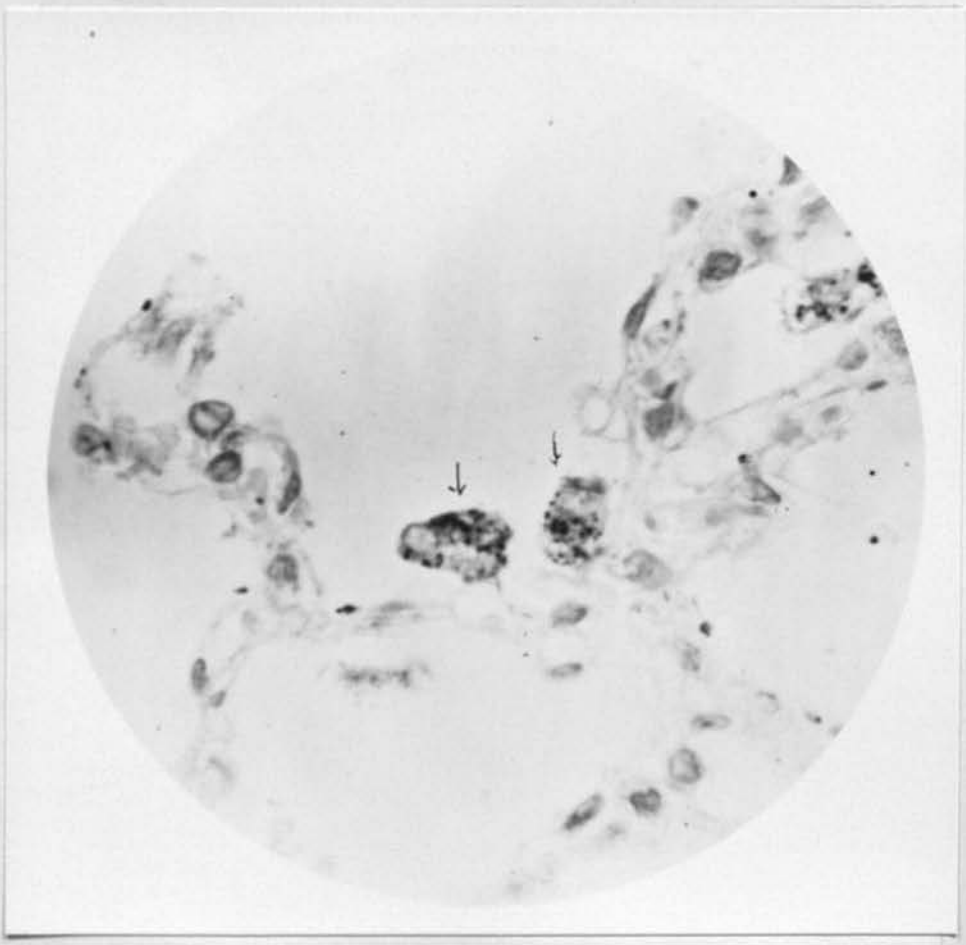

Fig. XX (X 930)Rabbit A 8. The cuboidal cells contain trypan blue and particles of carmine. They are enlarged and ready for desquamation into the alveoli as phagocytes.

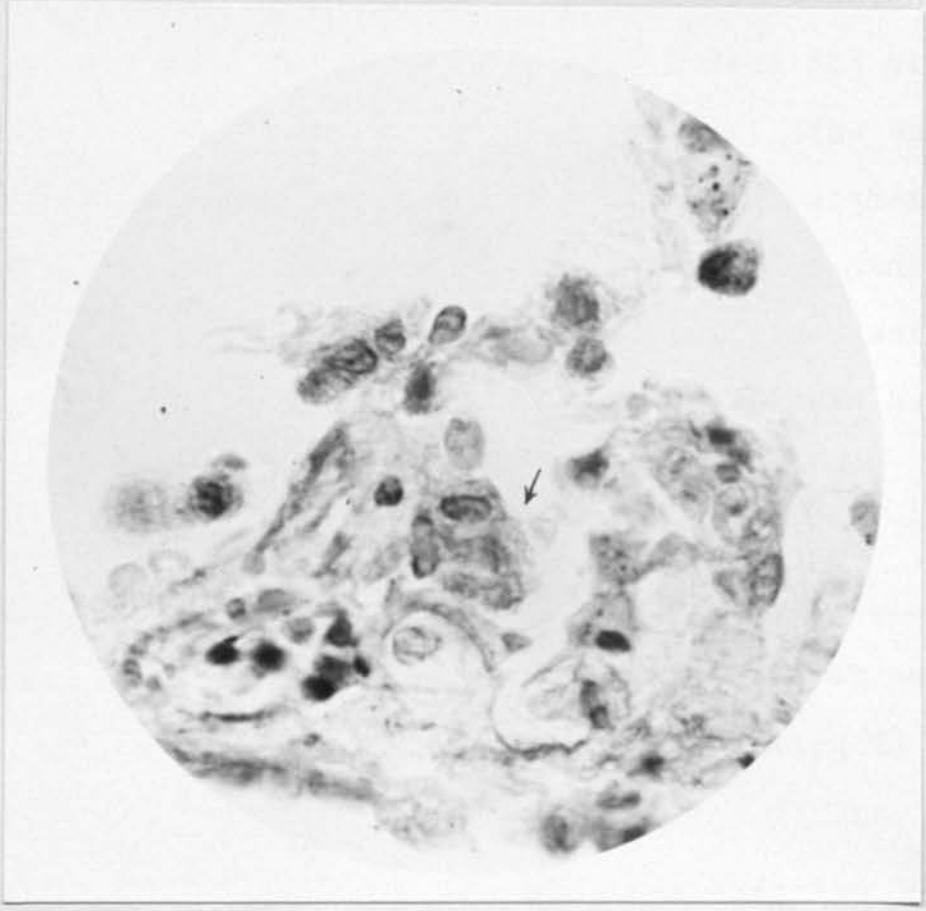

Fig. XXI. (X 1070) Rabbit A 101. The marginal alveoli are lined by columnar cells which contain neither trypan blue nor carmine particles whereas the cuboidal cells contain both dyes.

EXPERIMENT VIII.

Two rabbits, A 101 and A 8, were intensely stained by repeated intraperitoneal injections of trypan blue and received intrapleural injections of strontium chloride, as in the previous experiment (VII), but in addition 2 c.c. of fine carmine suspension in saline were injected into the trachea. Microscopically the marginal alveoli were lined by a continuous layer of columnar cells which contained neither trypan blue nor carmine particles (Figs. XIX & XX). Only the spindle-shaped histiocytes in the walls of the vessels, bronchi and interstitial tissue of the lung, were stained with trypan blue. On the other hand, the "septal cells" contained both trypan blue and carmine and all stages in the formation of free phagocytes from them could be seen (Fig.XXI).

It is, therefore, evident that the lining cells of the alveoli behave quite differently from the cubical cells which occur most frequently in the interalveolar angles, "septal cells." They possess no phagocytic properties, whereas the septal cells are characterised by the ingestion of particles of carmine, iron, ink, etc., if these are injected intratracheally. In addition they show granules of trypan blue when the latter is administered intraperitoneally. In other words, they become stained in the same way as the Kupffer cells in the liver, the cells lining the sinuses /

sinuses of the spleen and the other members of the reticulo-endothelial system.

The reticulo-endothelial system is recognised by its phagocytic power and its faculty to furnish a defence system distinct from that afforded by the blood leucocytes. From the previous experiments we have observed how the "septal cell" became stained with trypan blue and how it ingested particles of iron, ink, carmine, etc. Also, we have seen it dividing by mitosis to furnish the alveolar phagocyte. Therefore we are justified in classifying it as a member of the reticulo-endothelial system and as a matter of fact we have still further evidence to show that it is really a reticulo-endothelial cell.

EXPERIMENT IX.

The work of Jaimenez de Asúa (1927), Belezky (1931), Dunning and Stevenson (1934) and Dunning and Furth (1935), suggested that the reticulo-endothelial system may be selectively stained with the silver carbonate method. These authors, using Hortega's method, stained cells in the normal liver, spleen and kidney of the rabbit, which, on account of their morphological properties, their reaction following injury to these organs and their ability to store trypan blue, they concluded were identical with the micioglia.

Material and Methods.

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Three rabbits were employed in this experiment. One was used as a normal histological control and the other two were injected intratracheally with 2 c.c. 2 per cent saccharated oxide of iron in distilled water. The brain, lungs, liver and spleen of the three rabbits were stained with silver carbonate according to Penfield's modification ( ) of the method of del Rio-Hortéga for the demonstration of microglia (with slight variations). The tissues were fixed in Formol-ammonium-bromide solution. Frozen sections 10 to 15 microns thick were cut. They were received in distilled water, transferred to distilled water containing a few drops of strong ammonia (2-3 minutes) and washed in distilled water. They were then placed in Hortéga's silver carbonate solution:-

Silver nitrate 10% ..... 10 c.c.)
Sat. Lithium carbonate ..... 12 c.c.)

mixed and strong ammonia water added, just enough to dissolve the precipitate. Solution filtered and stored in a dark bottle. The sections required about three minutes in the silver solution. Silver was reduced in the sections by blowing them about forcibly in a 5 per cent solution of formalin. They were then washed in distilled water for 3-4 minutes, toned in 1-500 gold chloride solution, fixed in 5 per cent sodium hyposulphite, washed in distilled water, blotted with /

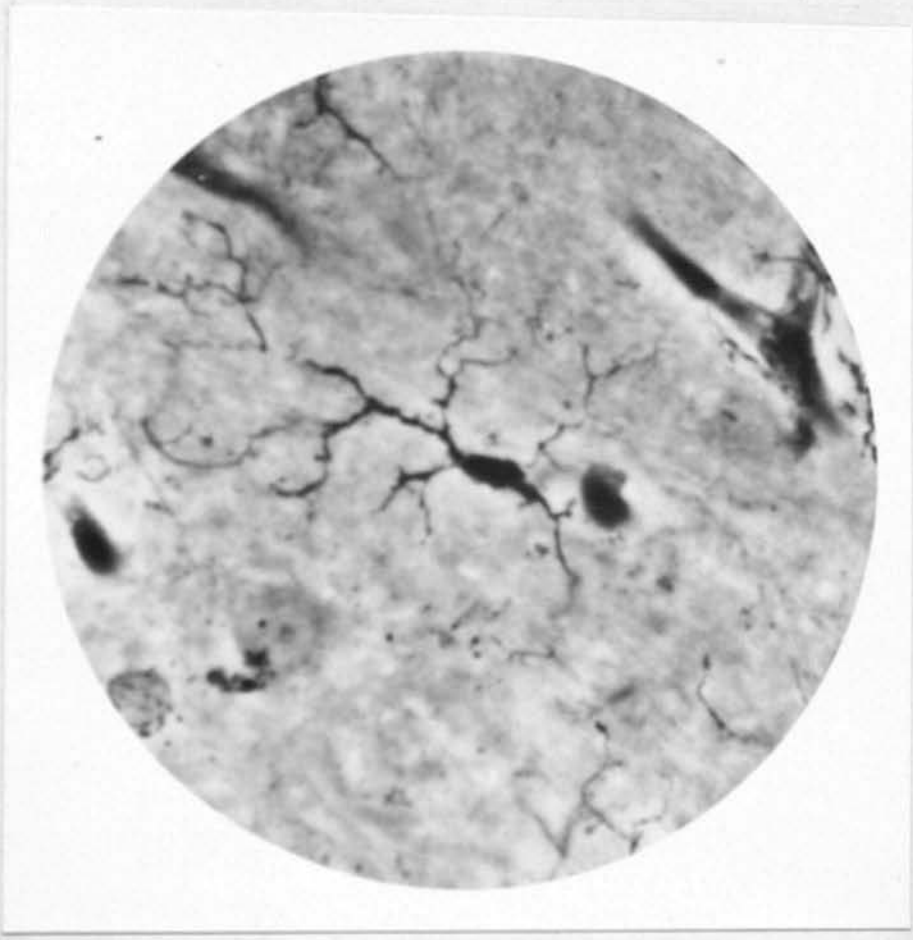


Fig. XXII. (X 1070) Note the silver impregnation of the microglia.

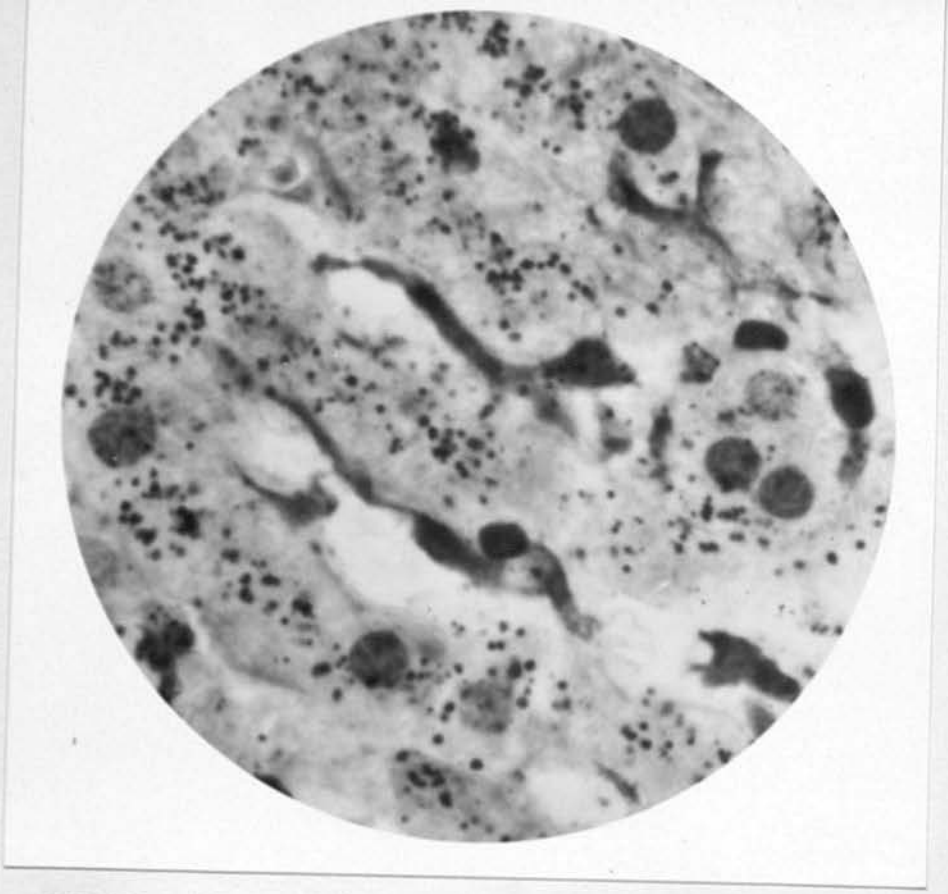
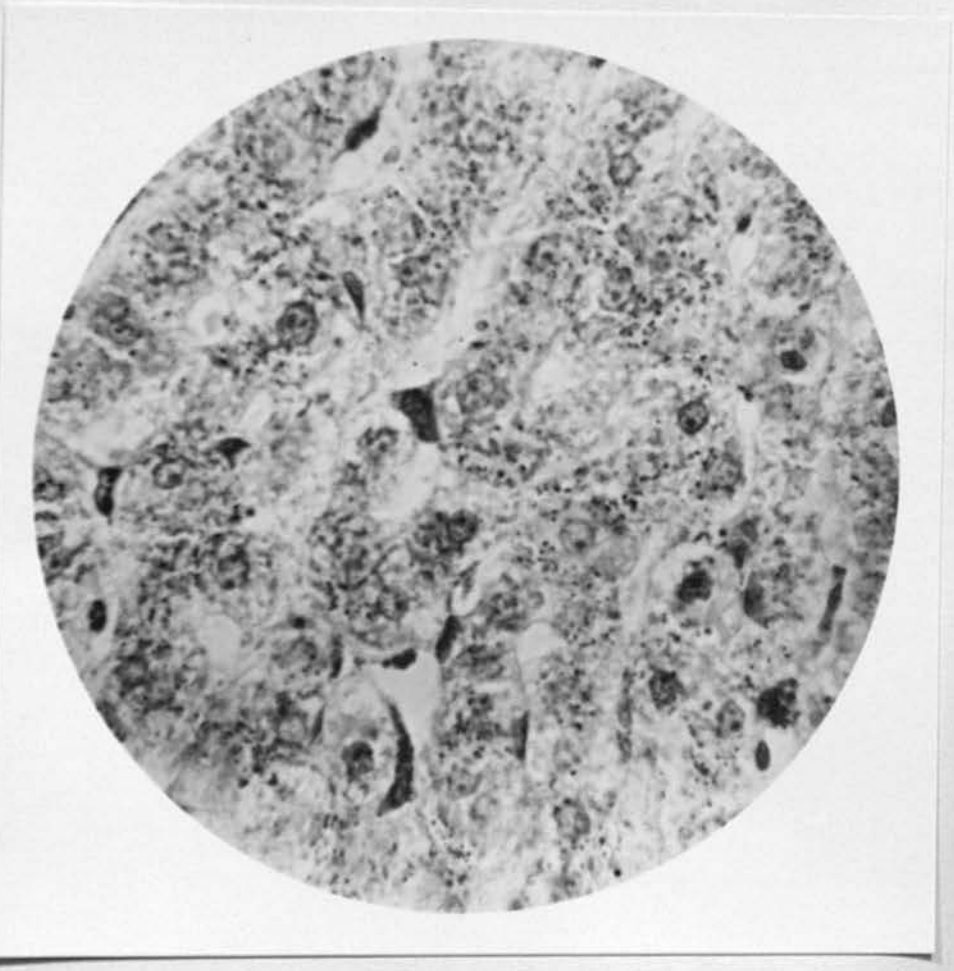


Fig. XXIII. (X 1070) Note the silver impregnation of the Kupffer cells.



ig. XXIV. (X 620). From a rabbit stained with trypan blue to illustrate the dye in the Kupffer cells. Compare with figure XXIII.

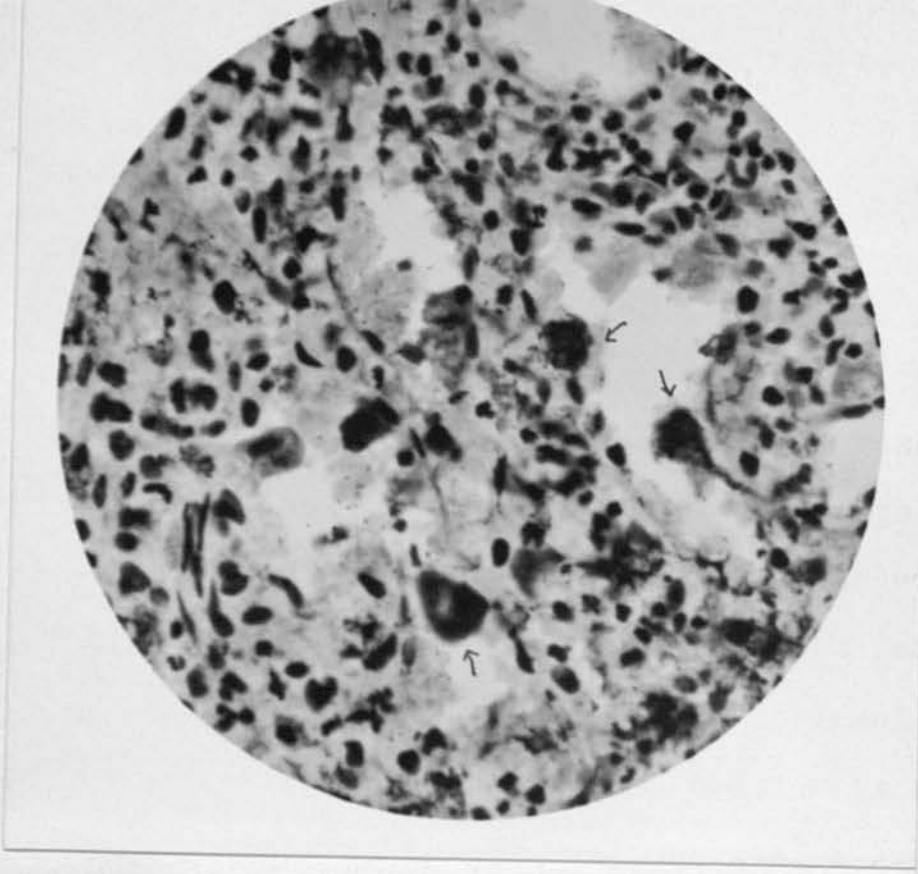


Fig. XXV. (X 500) The phagocytic cells in the sinuses of the spleen are heavily impregnated. Compare with fig. XXVI.

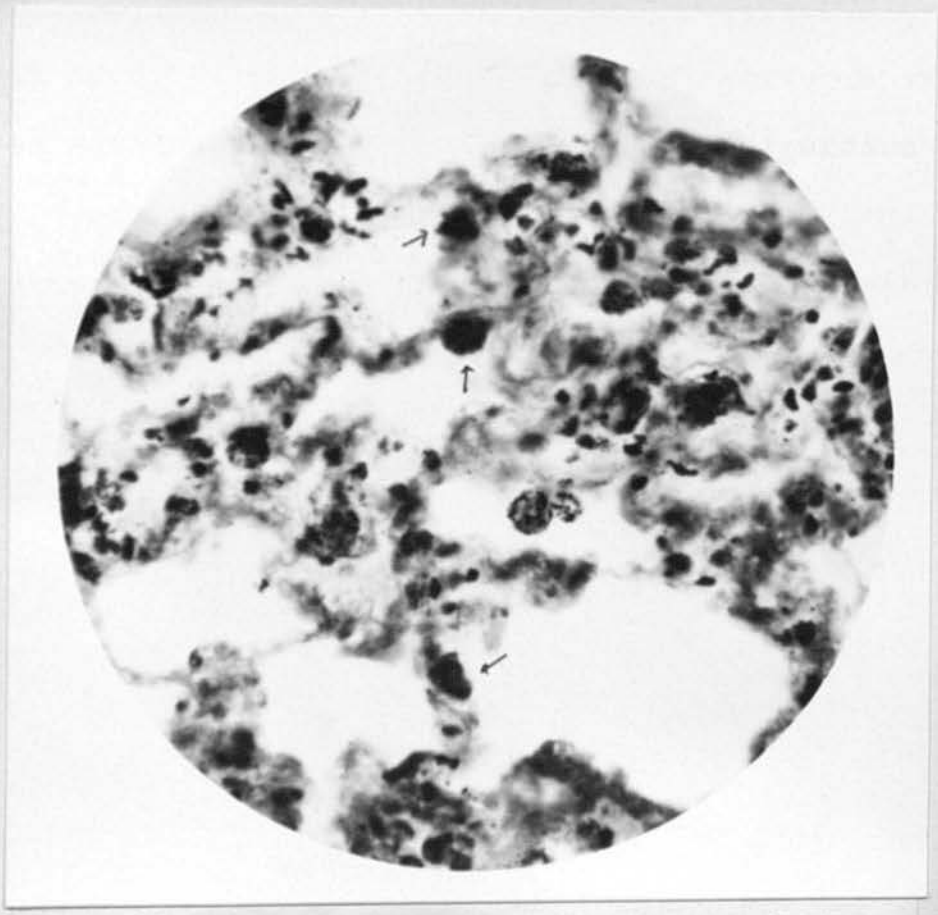


Fig. XXVI. (X 500) The cuboidal cells which occur most frequently in the alveolar angles are impregnated with silver. Compare with spleen. (Fig. XXV).

with filter paper, dehydrated with absolute alcohol, cleared in zylol and mounted in Canada balsam.

The results were striking. The microglia was impregnated (Fig.XXII); as were also the Kupffer cells (Fig.XXII & XXIV) and the phagocytic cells in the sinuses of the spleen (Fig.XXV), and scattered throughout the lung there were many cells selectively impregnated and conspicuous against the gray-coloured septal walls (Fig.XXVI). These are the "septal cells." The lungs of the rabbits whose alveolar phagocytes were stimulated by intratracheal injection of iron showed a large number of fine phagocytes which were also silver-stained. The alveolar lining cells were not stained.

Briefly, then, we have accumulated from the above experiments sufficient evidence to state that the cuboidal "septal cells" are members of the reticulo-endothelial system. On the other hand the cells of the alveolar lining possess no power of phagocytosis when normally flattened or when they are made to hypertrophy. In addition they do not become impregnated with silver, and in this respect as well as in their behaviour towards vital stains they resemble the bronchial epithelium.

Let us, now, turn to the normal and pathological human material, and study it in the light shed from these experiments.

PATHOLOGICAL STUDY.

The 400 cases collected from the post-mortem consisted of lung sections from cases of pulmonary tuberculosis, lobar and broncho-pneumonia, pleurisy and empyema, bronchiectasis, nephritis, blood diseases, septicaemia, arsenical and carbolic poisoning, malignant disease of stomach, pancreas, kidney, ovary and colon, endocarditis, mitral stenosis, myocardial infarction and cerebral hemorrhage, osteomyelitis, and cirrhosis of liver. In addition 30 paraffin blocks from lungs belonging to still-births were collected through the help of Dr A.Macgregor and the kind permission of Professor S. Smith who, also, on certain occasions provided me with fresh material belonging to infants that died in utero.

Haematoxylin and eosin was used as a routine staining in every case, and the following special stains were employed when necessary:-

(a) Heidenhain's iron-hoematoxylin

(b) Heidenhain's Azan

(c) Verhoff's elastin stain,

(d) Wiegerts' elastin stain,

(c) Foot's stain for reticulum.

The findings may be summarised as follows:- In the healthy adult lungs, when there is no pulmonary involvement by any kind of disease, the alveolar wall appears /

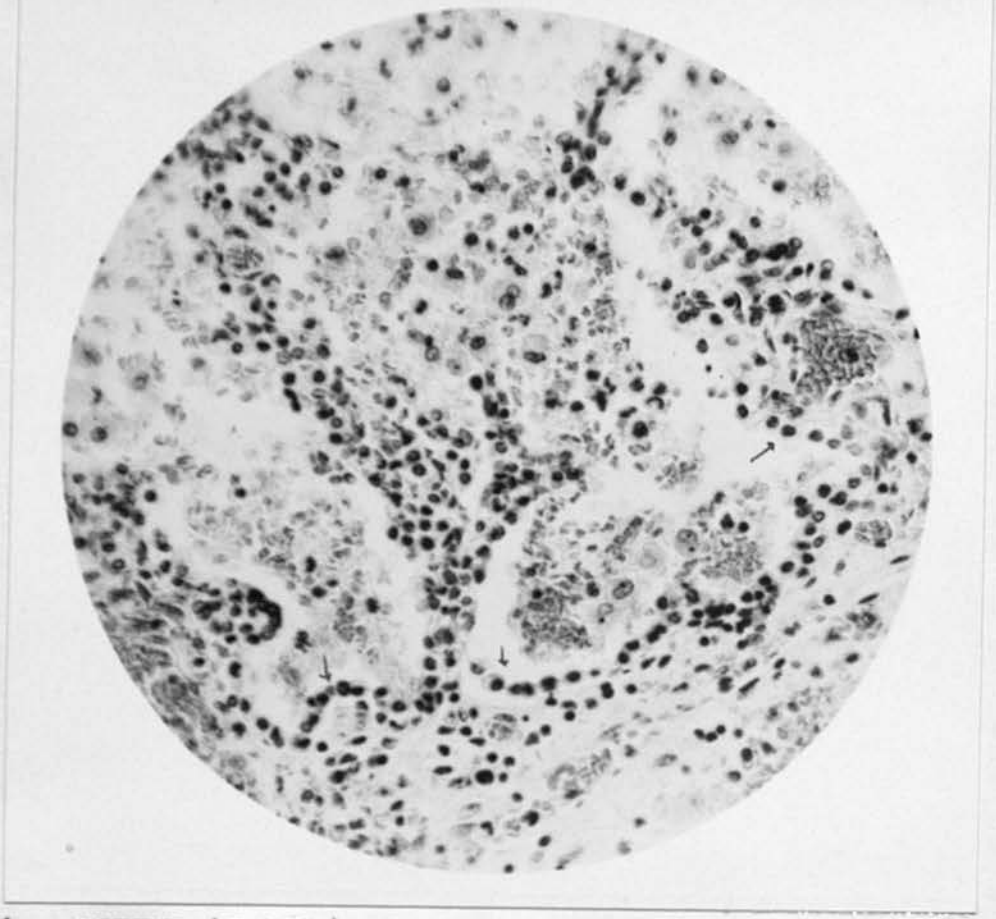


Fig. XXVII (X 450) Pleurisy. The marginal alveoli are lined by cuboidal cells.

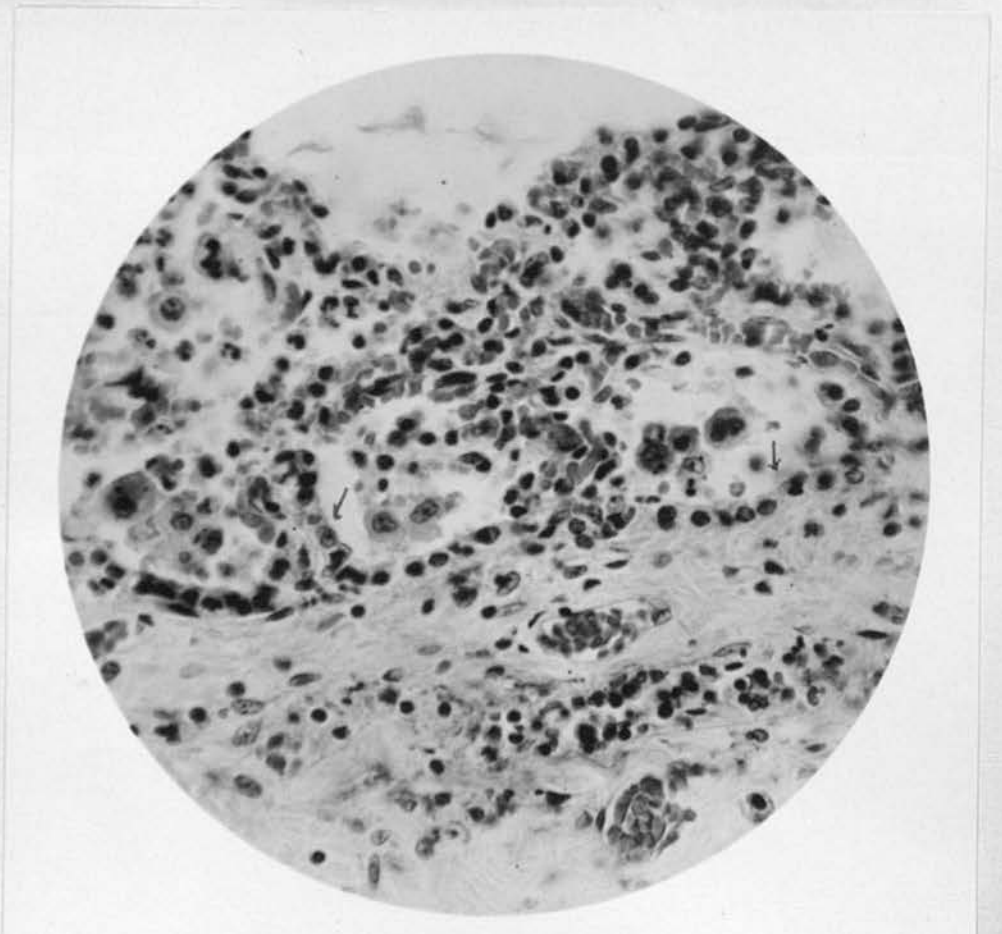


Fig. XXVIII (X 450) Pleurisy. The peripheral alveoli are lined by cuboidal cells.

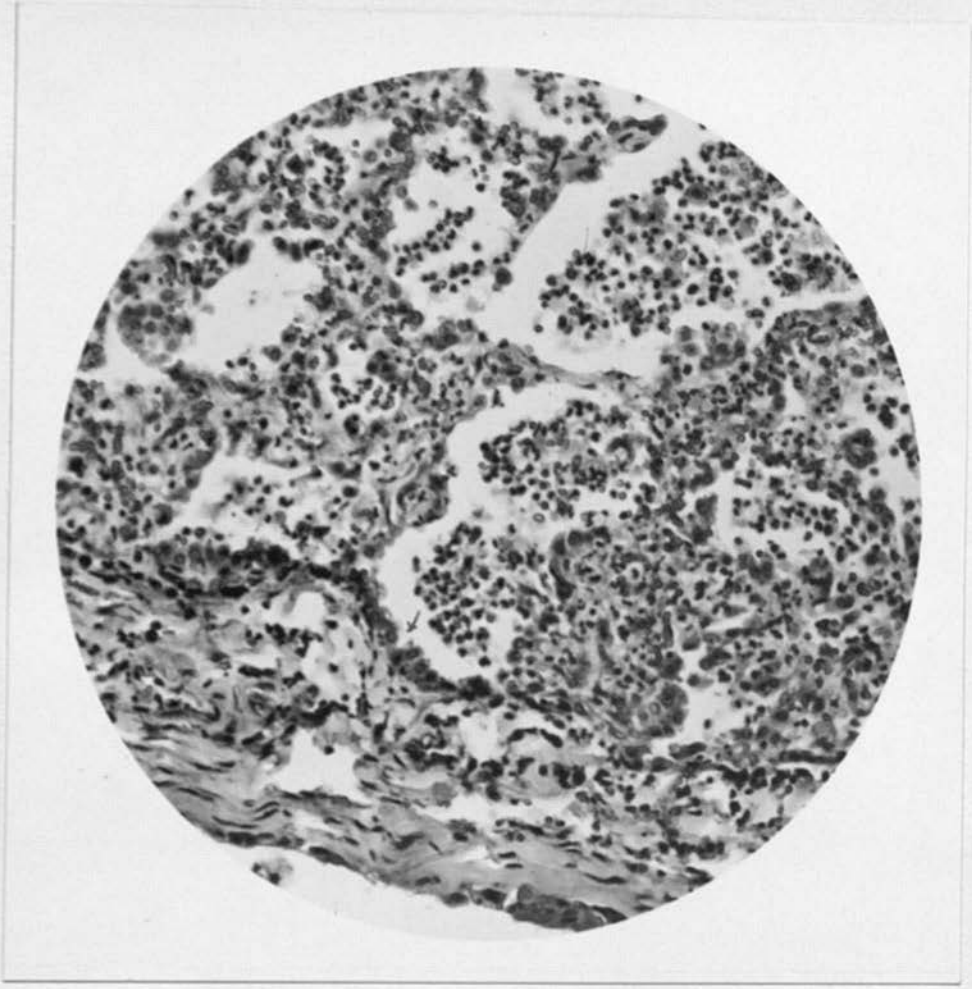


Fig. XXIX (X450) Pleurisy. The marginal alveoli are lined by columnar cells.

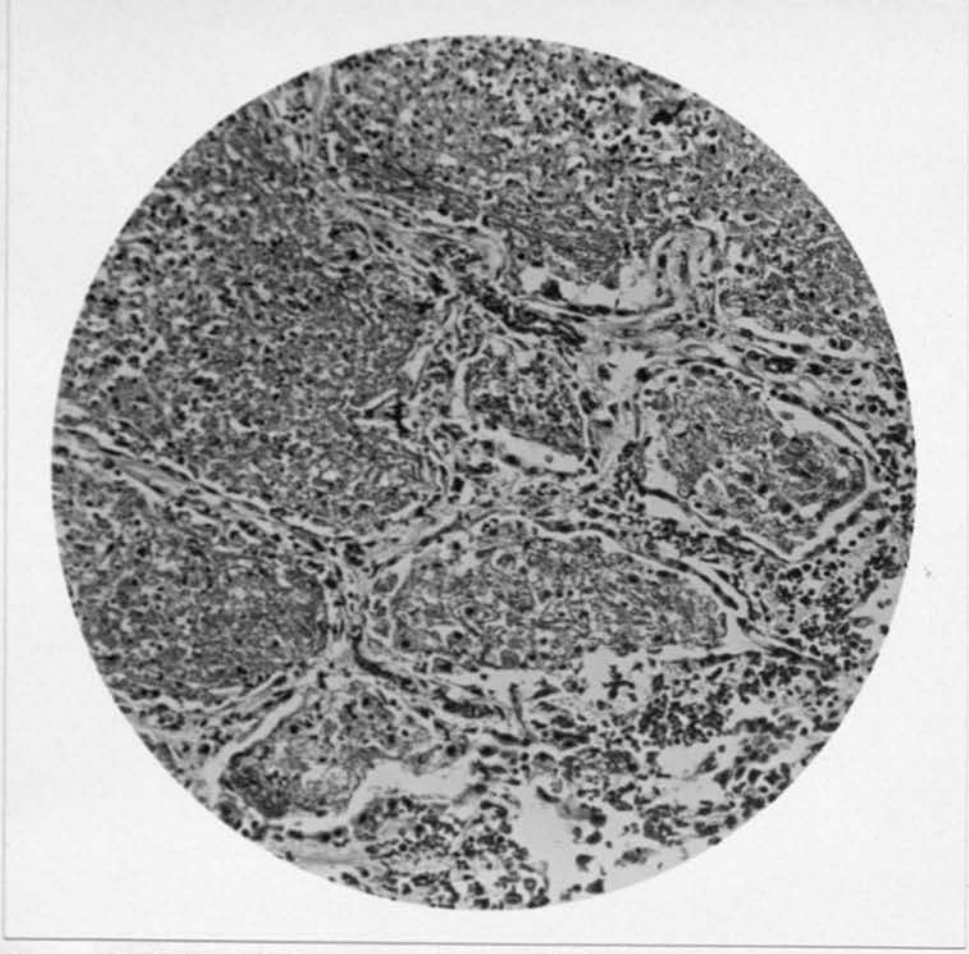


Fig. XXX (X 175) Pulmonary tuberculosis. The lining epithelium is slightly raised from the surfaces of the alveolar walls.

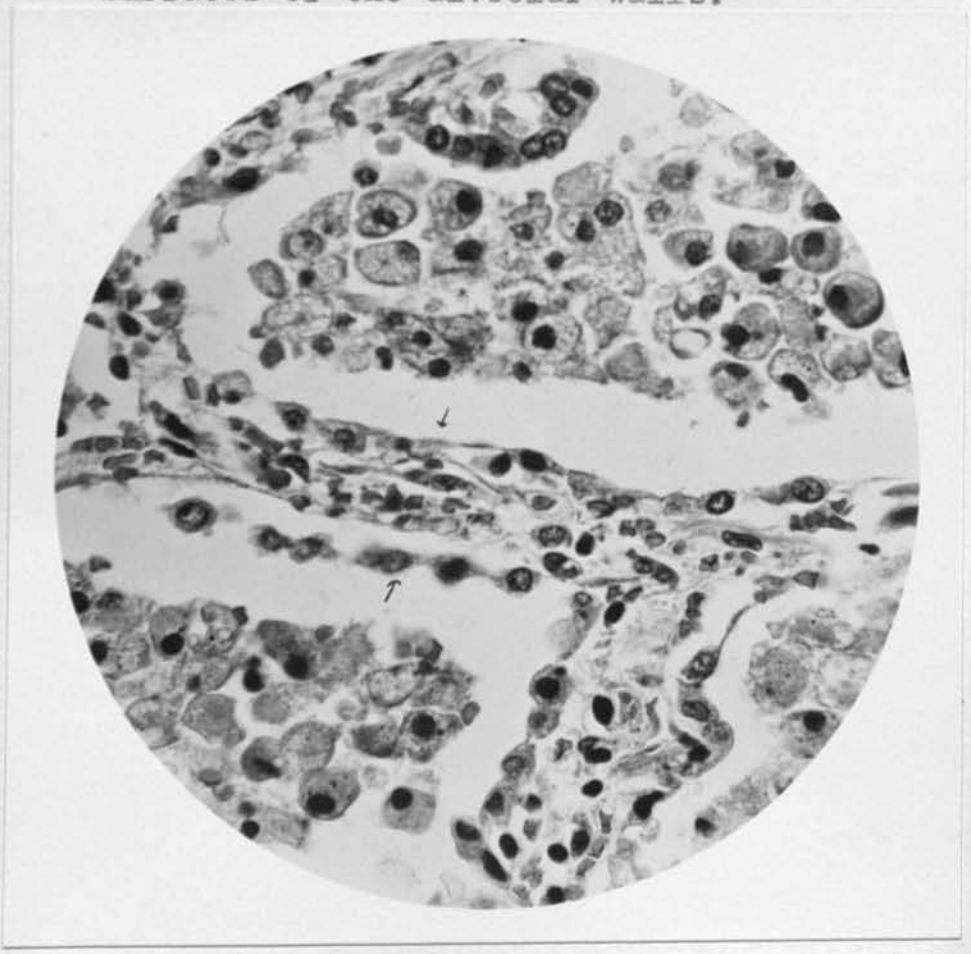


Fig. XXXI (X 1000) Higher magnification. Note the continuous alveolar epithelium.

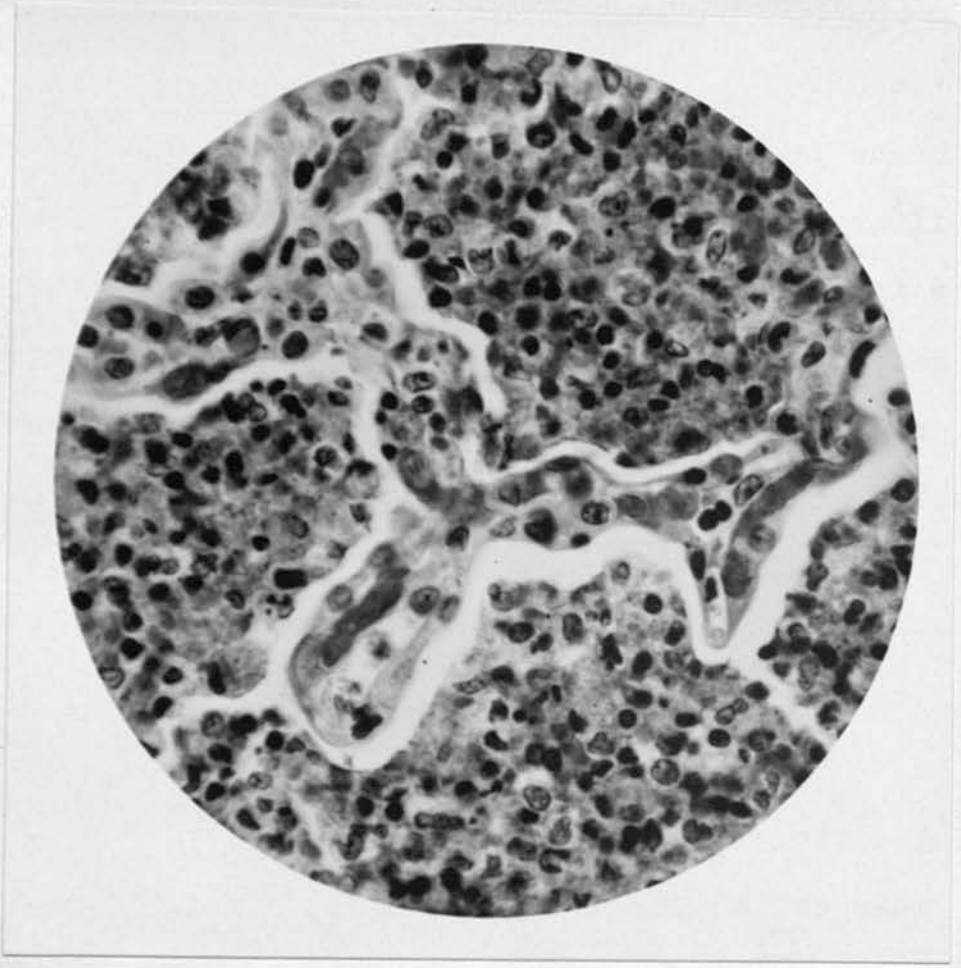


Fig. XXXII. (X 600) Pulmonary tuberculosis. Note the flattened nucleated alveolar cells.

appears to consist of a centrally placed capillary of variable diameter. Over the capillary stretches a thin membrane whose nuclei are irregularly spaced and at some distance from each other. In most places the membrane is so intimately apposed to the underlying capillary wall and is so thin as to be indistinguishable from the latter. In other places the membrane bulges to enclose some elastic and reticular fibres and occasional nuclei of various connective tissue cells.

On the other hand, in cases where the lungs are diseased, there is no difficulty whatever in demonstrating the lining cells of the alveoli.

In all cases of pleurisy and empyema, the marginal alveoli are found to be lined by cubical and columnar cells (Figs. XXVII, XXVIII & XXIX). Comparison between these and Figure XXIII reveals the close similarity between the effect of disease and the results obtained from animal experiments. In the two conditions, the subserosa is thickened by a layer of granulation tissue and the peripheral alveoli are lined by proliferating cells.

In cases of tuberculosis it is common, particularly in the vicinity of foci of disease, to see the lining epithelium slightly raised from the surfaces of the alveolar walls (Figs. XXX, XXXI & XXXII). Also, the alveoli may become lined by cubical cells, the structure /

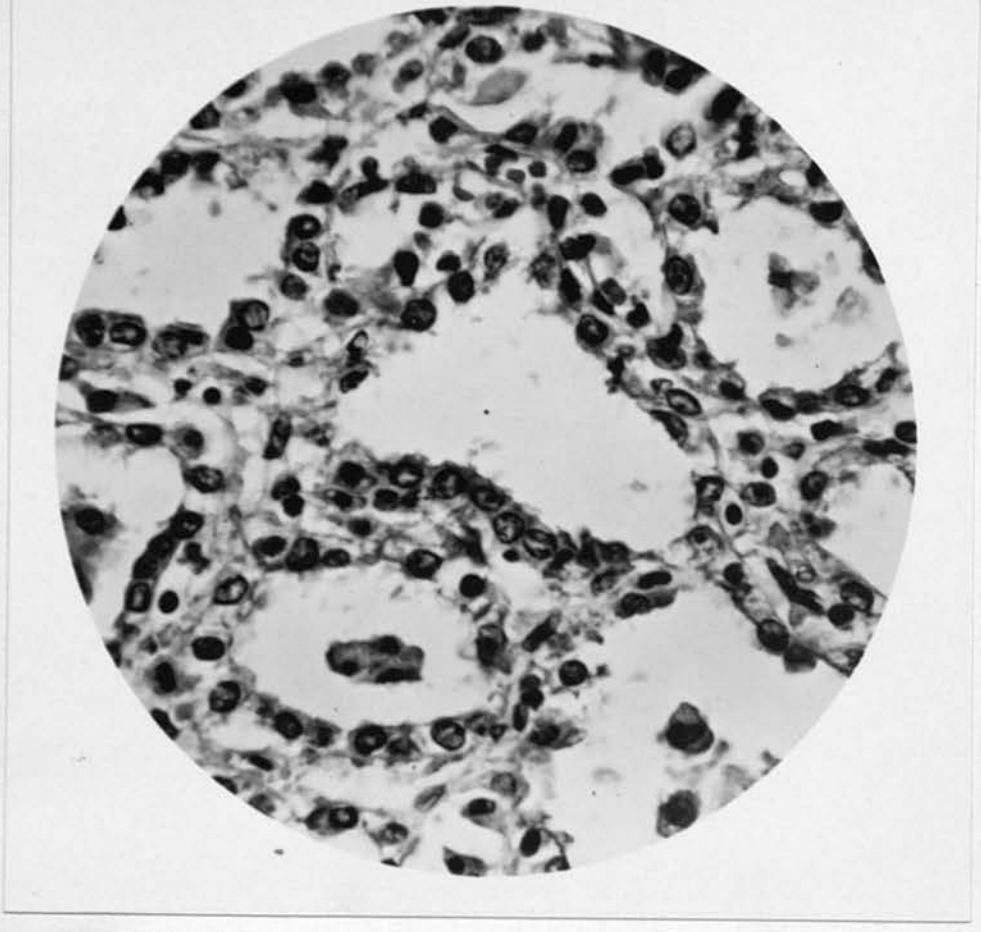


Fig. XXXIII (X 630) Pulmonary tuberculosis. The alveoli are lined by cubical cells. Note the gland-like structure.

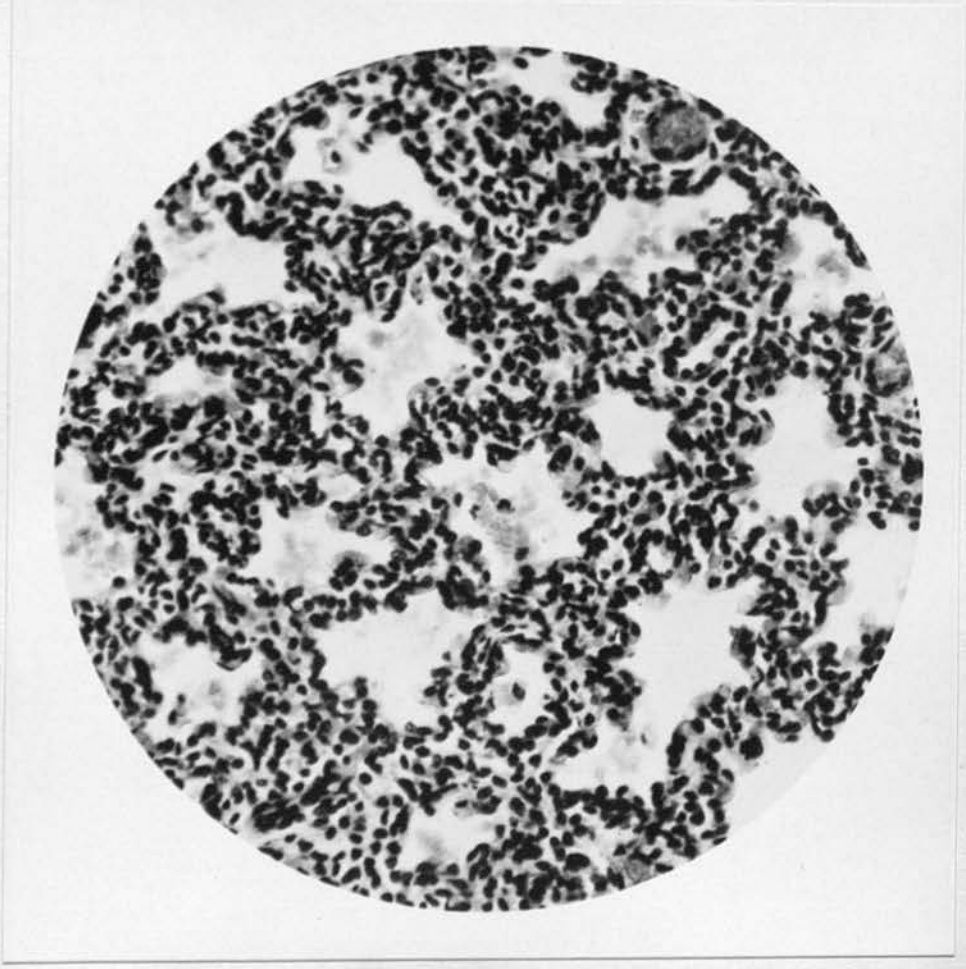


Fig. XXXIV (X 250) Foetal lung. The alveoli are lined by cubical cells.

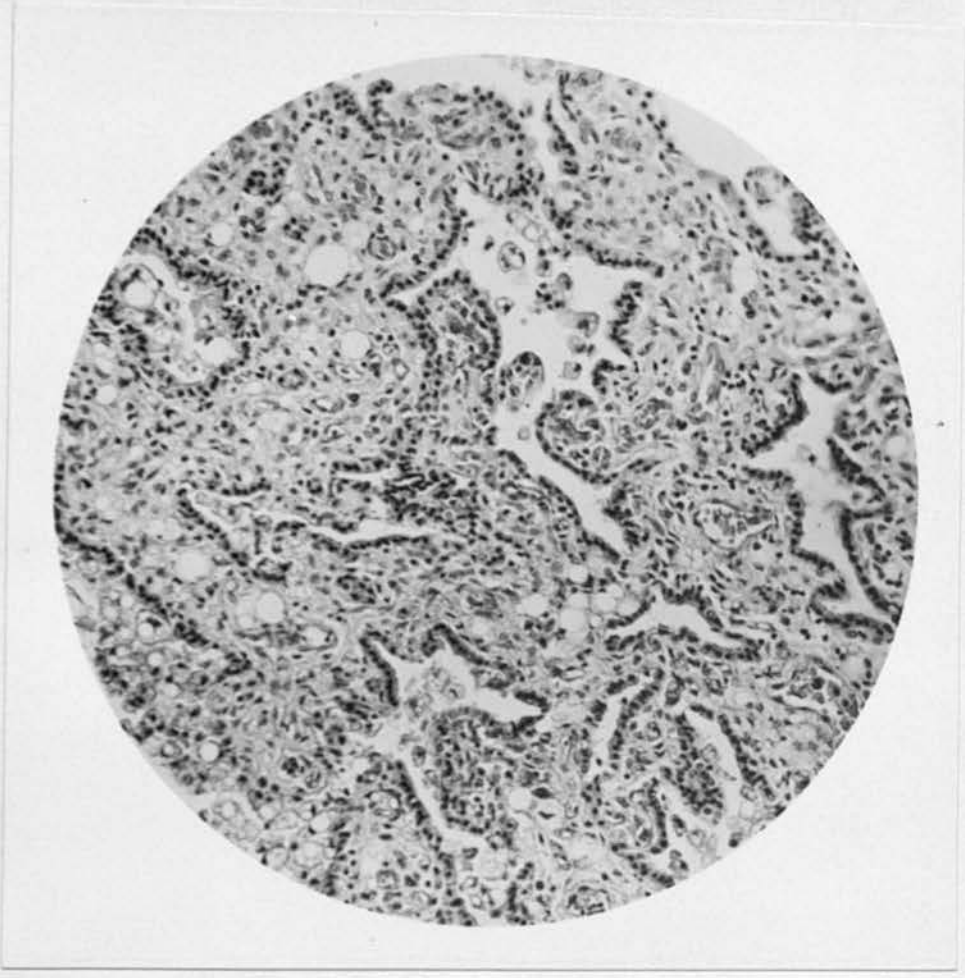


Fig. XXXV. (X 190) Lung of a syphilitic still-born child. The alveoli are lined by cubical cells.

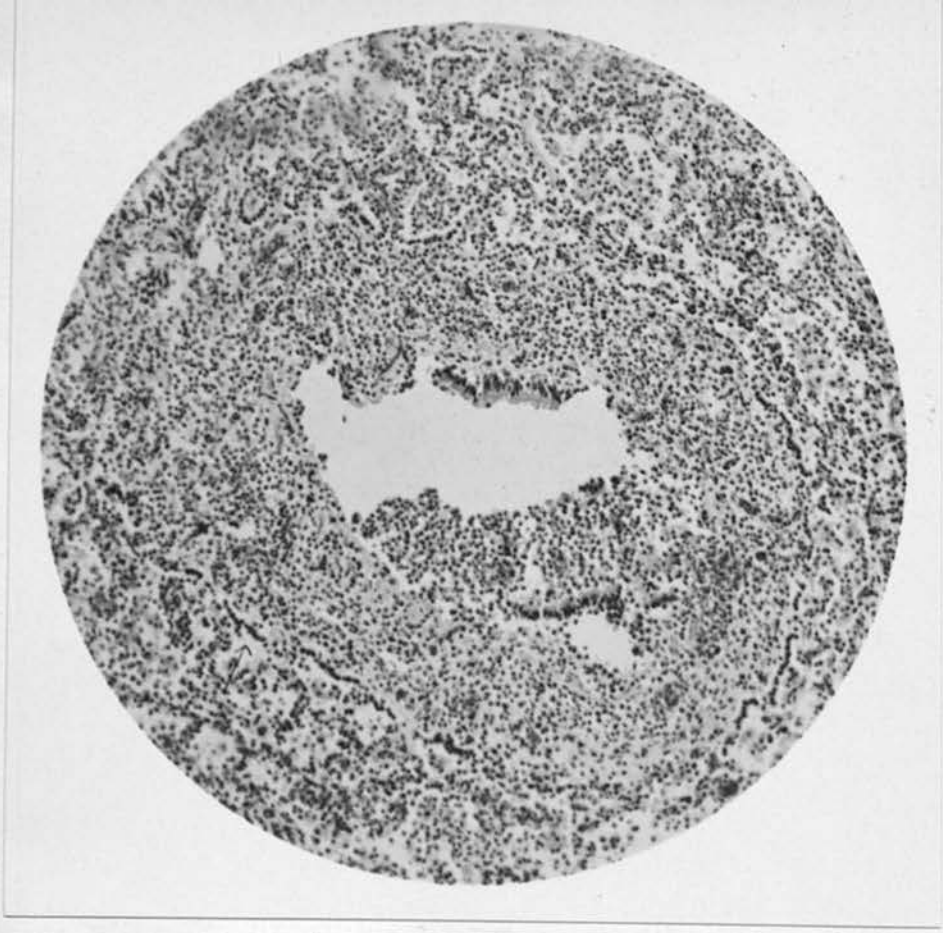


Fig. XXXVI (X 120) Bronchitis. The surrounding alveoli are lined by cubical and columnar cells.

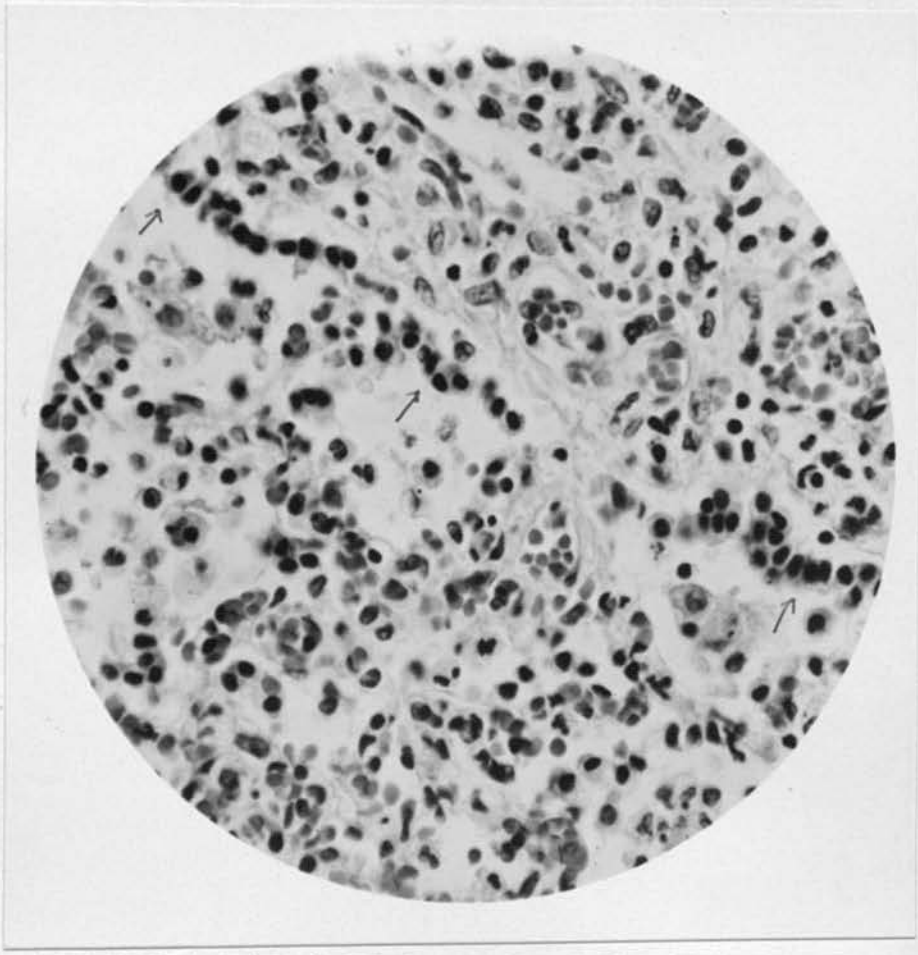


Fig. XXXVII (X 450) Higher magnification of fig. XXXVI

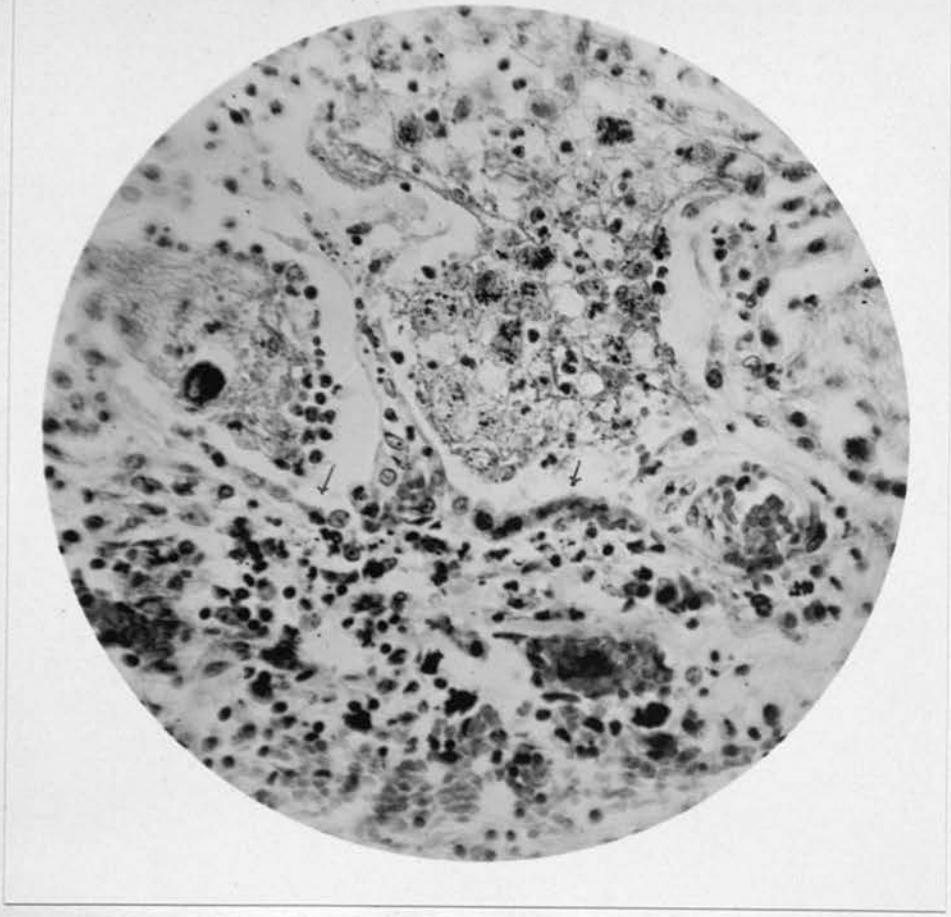


Fig. XXXVIII. (X 450) Lobar pneumonia. The alveoli are lined by cubical and columnar cells.

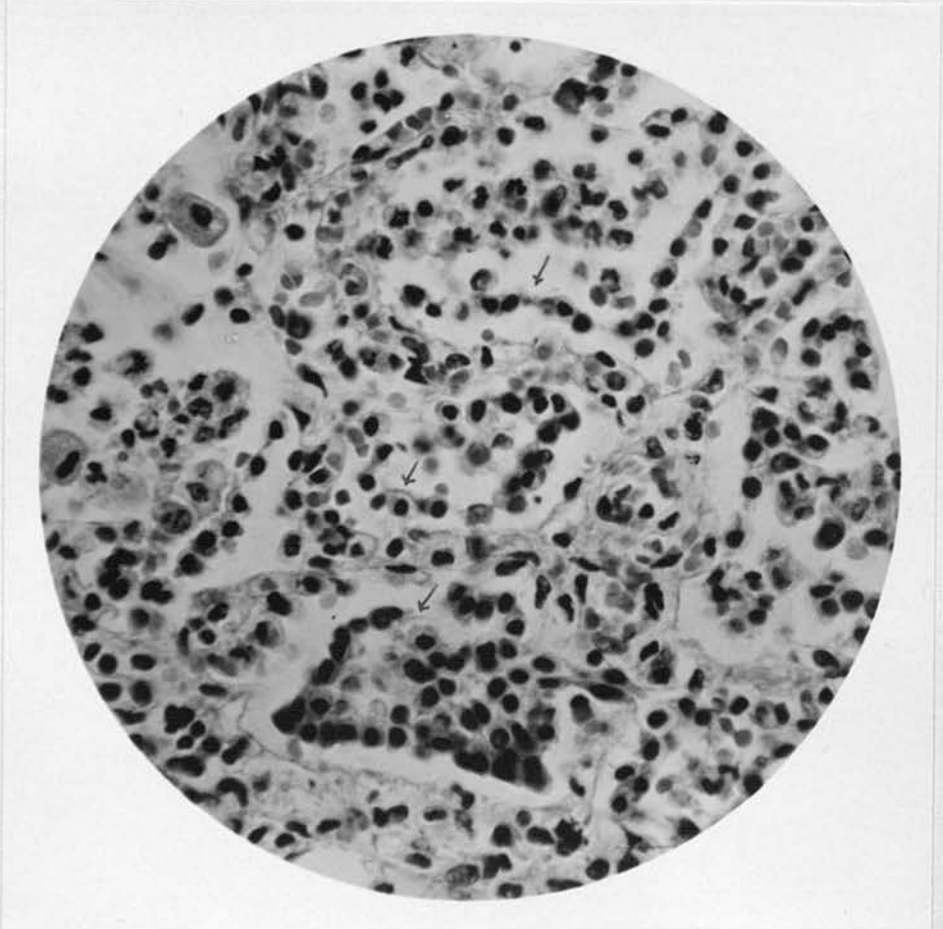


Fig. XXXIX. (X 450) Lobar pneumonia. The hypertrophied lining epithelium is shed into the alveoli

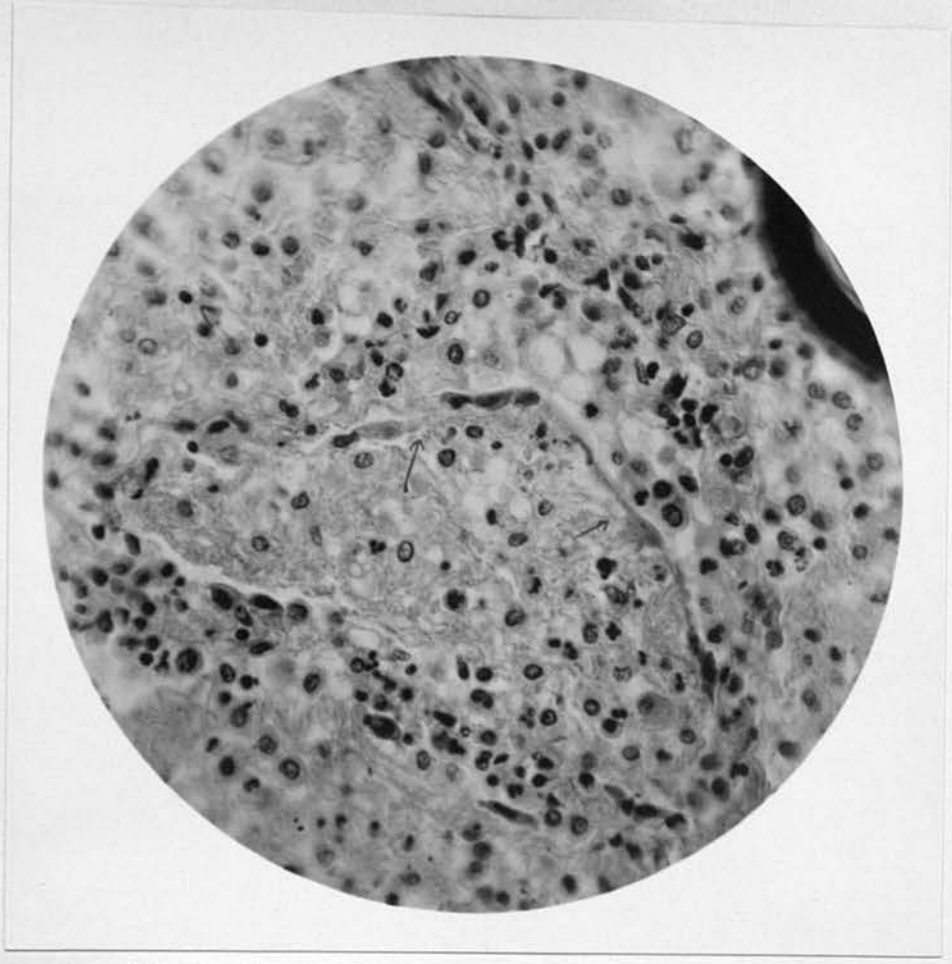


Fig. XL (X 350). Broncho-pneumonia. Note the desquamation and degeneration of the lining epithelium.

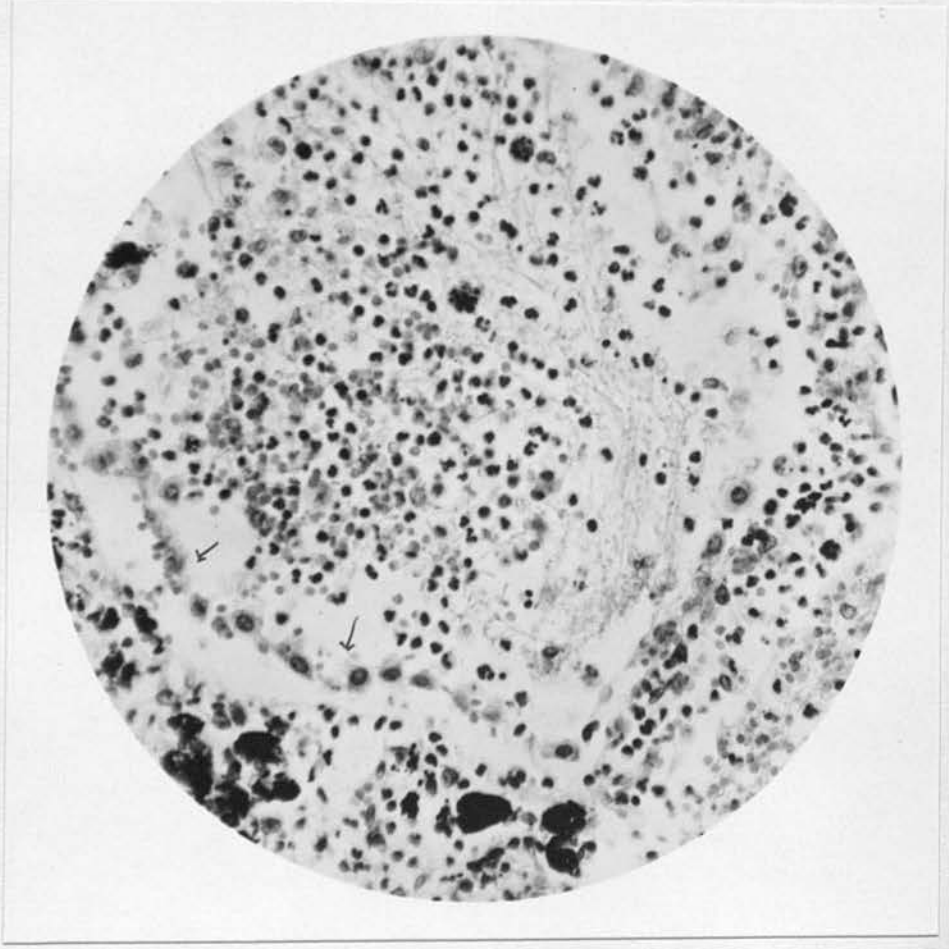


Fig. XLI. (X 325). Lobar-pneumonia. Note degeneration of the desquamating cells.

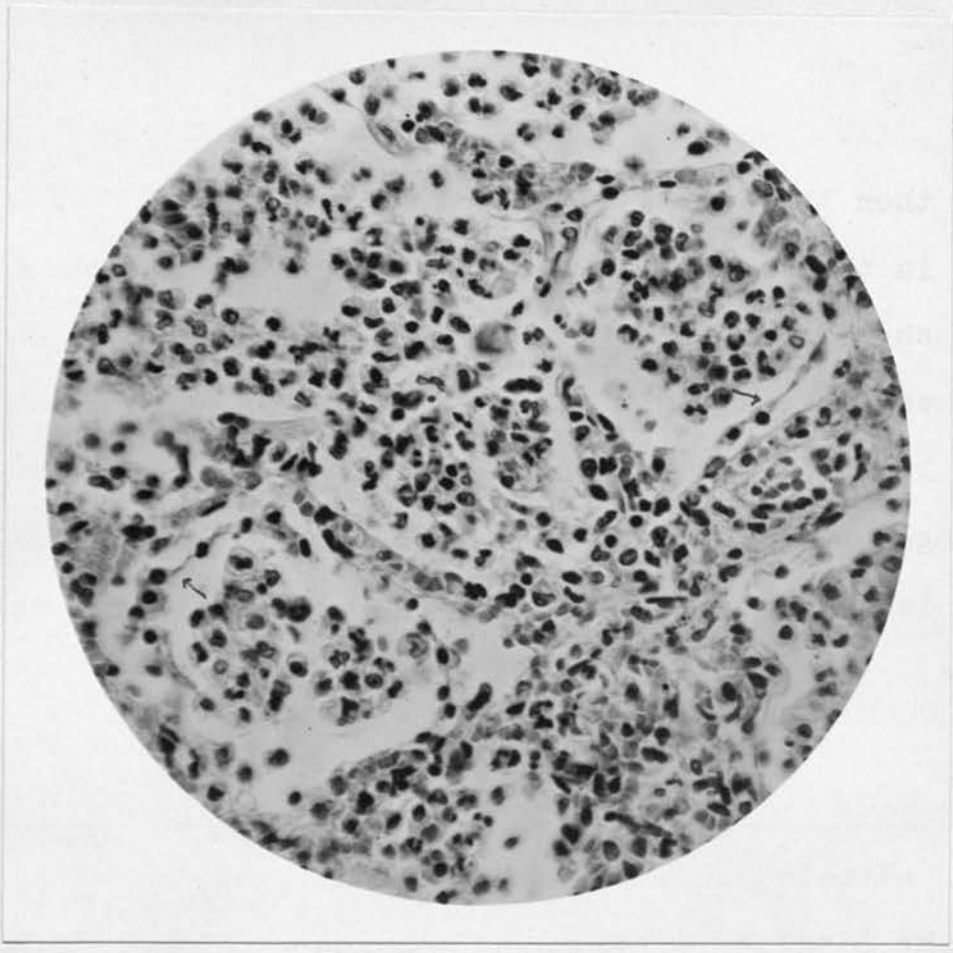


Fig. XLII (X 450) Lobar pneumonia. Note desquamation and degeneration of the lining epithelium.

structure then acquiring a gland-like appearance which seems to be a reversion to the embryonic type (Fig. XXXIII) and should be compared with Fig.XXXIV which represents a section from a healthy fœtal lung and Fig.XXXV from a syphilitic still-born child. In all of them the septal walls are thickened (particularly so in the syphilitic lung) and lined by cubical cells which have clear cytoplasm and dark staining nuclei. Since the still-births varied from 6 months to almost full term I have concluded from the thirty foetal lungs examined that the alveolar lining cells remain cubical till the later stages of foetal life. In this respect I am in agreement with Stewart (1923) and the recent work of Bensely and Groff (1935).

In cases of bronchitis and broncho-pneumonia, the alveoli surrounding the inflamed bronchi are seen to be lined by cubical and columnar cells (Figs.XXXVI & XXXVII).

In pneumonia, the following observations can be made. In some cases the cells lining the alveoli are hypertrophied (Figs.XXXVIII & XXXIX) and in others (probably in severe toxic cases) the lining cells are shed into the alveoli and show signs of degeneration. The Figures XL, XLI &XLII, show degeneration of the desquamating cells after a preliminary stage of hypertrophy.

In cases of chronic venous congestion the lining cells show a certain degree of hypertrophy and desquamation. Figure XLIII illustrates this point and also the /

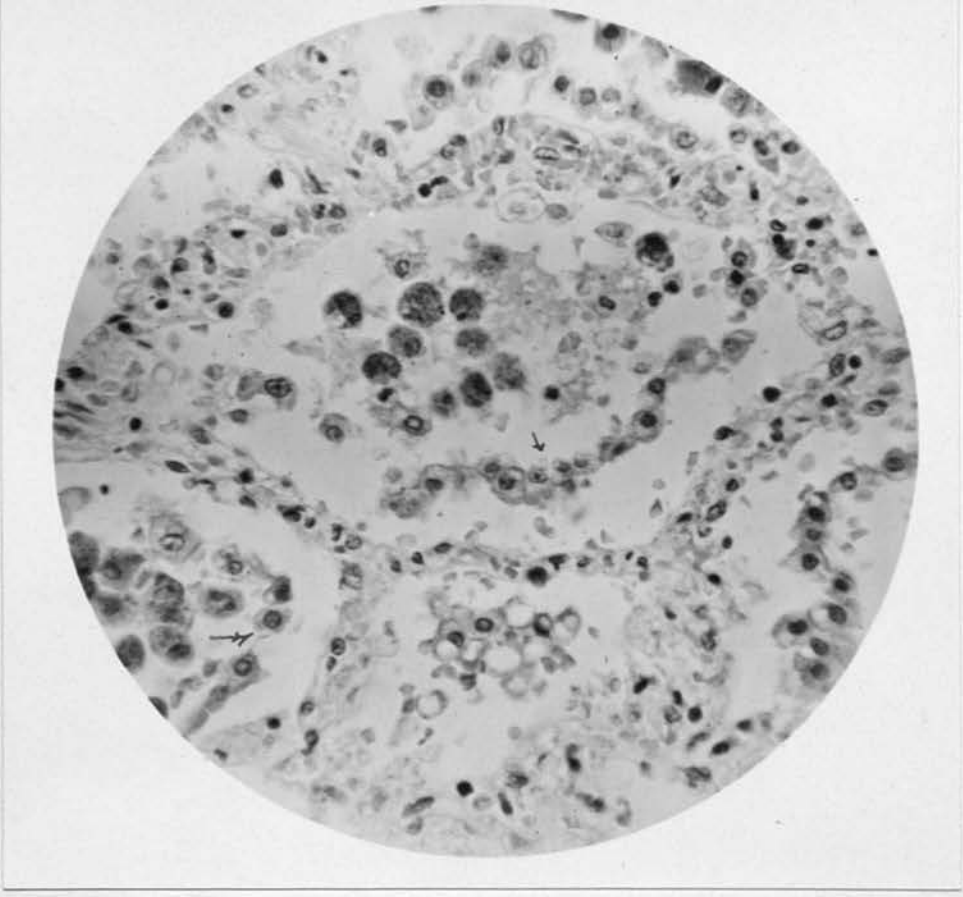


Fig. XLIII (X 400) Mitral Stenosis. The desquamated lining cells do not contain hoemosiderin whereas the alveolar phagocytes are loaded with the pigment.

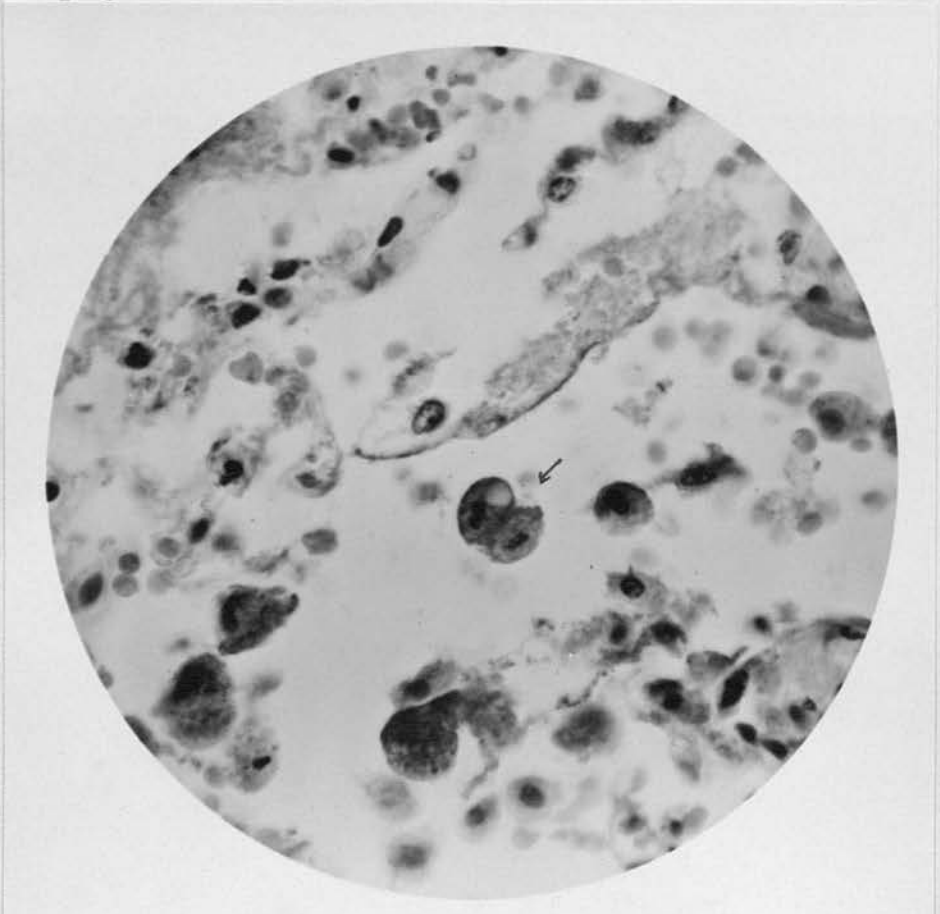


Fig. XLIV (X 620) Mitral stenosis. It shows division by mitosis of an alveolar phagocyte containing hoemosiderin.

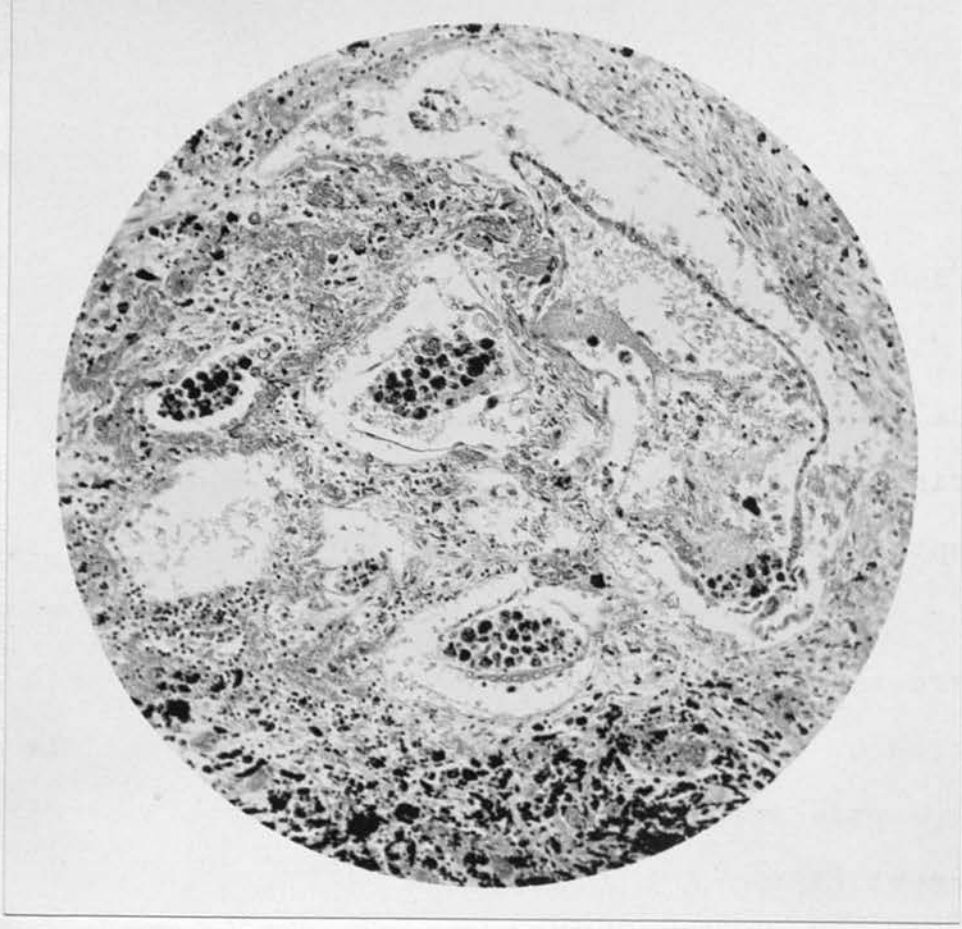


Fig. XLV (X 120) Silicosis and anthracosis.

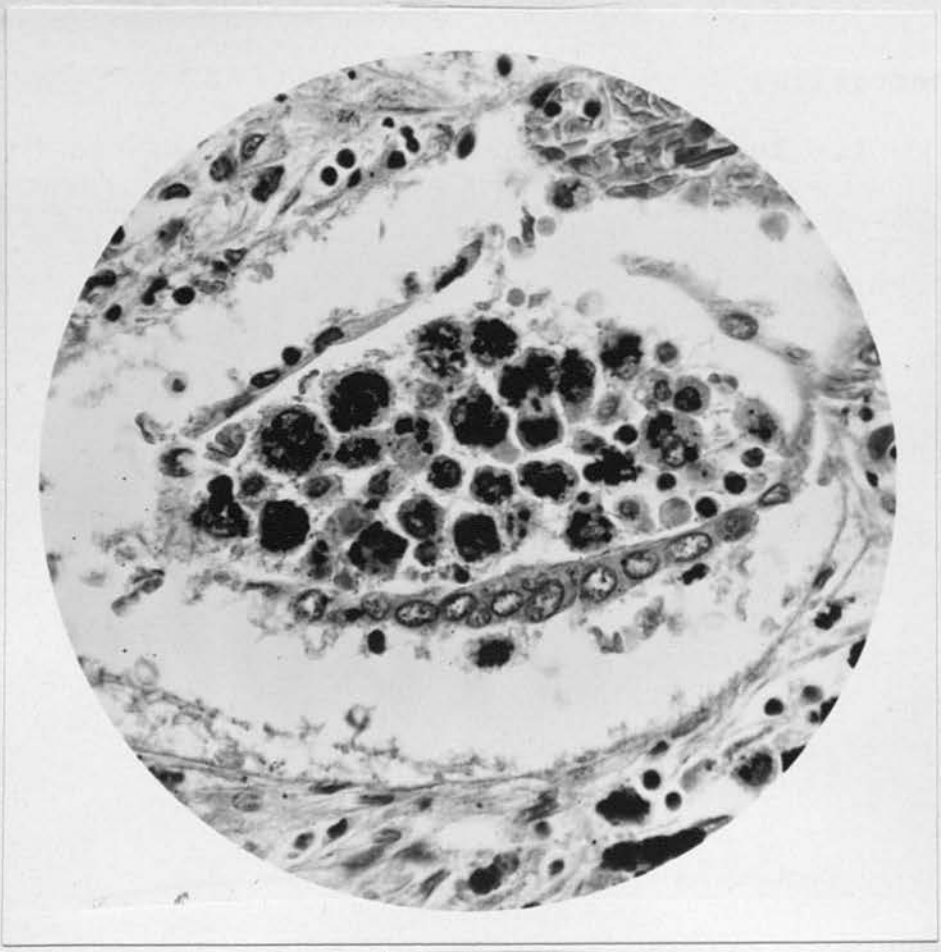


Fig. XLVI.(X 500) Higher magnification of figure XLV The lining cells contain no carbon whereas the alveolar phagocytes are packed with the pigment

the fact that the desquamated lining cells do not contain hæmosiderin, whereas the alveolar phagocytes are loaded with the pigment. Figure XLIV is from a case of mitral stenosis. It shows division by mitosis of an alveolar phagocyte containing particles of hoemosiderin and should be compared with the division of the "septal cell" in the experimental work.

In cases of bronchiectasis and silicosis where there is much fibrosis, the lining cells are shed in strings. They contain no carbon, whereas the alveolar phagocytes are packed with this naturally occurring pigment (Figs. XLV & XLVI).

From these findings it may be concluded that the experimental work has been amply confirmed by numerous observations drawn from human material.

1. In the healthy animal and in the healthy human adult lung, it is difficult, if not impossible, to demonstrate the alveolar lining.

2. Following the introduction of various substances into the pleural sac, there occurs proliferation of the cells lining the marginal alveoli during the first four days of experiment, and thereafter (when the effect of the irritant ceases) involution ensues, so that the normal appearances of the lung are restored by the seventh or eighth day. The same results are obtained from human cases of pleurisy, empyema, pneumonia, tuberculosis, chronic venous congestion /

gestion etc.; the lining cells particularly nearby the focus of infection become cubical and columnar. Young (1930) gives a detailed physico-chemical interpretation of the phenomenon of proliferation in the experimental animal and it would appear from the number of human cases studied that toxins, metabolic upsets and oxygen deficiency may act in the same way as the various substances injected intrapleurally, and when the patient recovers and the causal factor ceases to act, involution sets in and the normal appearances of the alveolar lining are restored.

3. Neither the proliferating epithelial cells in the experimental animal nor the hypertrophied cells in human material show evidence of phagocytosis.

4. On the other hand the "septal cells" are phagocytic and reproduce by mitosis, as has been amply confirmed by numerous observations in human cases.

Before entering into a brief discussion there is one more experiment to be recorded.

EXPERIMENT X.

Having examined in the previous experiments the site and mode of origin of the alveolar phagocyte and its manner of desquamation into the air-cells, it was decided to consider its path of exit. The experimental work /

work, in so far as this phase of the study is concerned, was extremely simple, consisting merely in intratracheal injections under ether anaesthesia of 2 c.c. of a suspension of 1% saccharated oxide of iron, allowing the animal to live for varying periods of time up to eleven days following the injection. The tissues were fixed in formalin and the prussian blue reaction was developed.

Animal No.	Survival period after intratracheal injection.	Naked eye and Histological Findings.
Rabbit A 44	2 days	A fair amount of the injected iron was visible underneath the pleura. A peribronchial lymph-gland was slightly enlarged. It measured about 2 mm. in diameter. A large number of free phagocytes were seen in the alveoli and bronchi. These contained iron. The lymphatic system of the lung showed large mononuclear phagocytes containing iron. The lymph node showed one or two collections of phagocytes loaded with iron.
Rabbit A 43	4 days	The injected material was still visible. The lymph-gland at the bifurcation of trachea was slightly larger than that of Rabbit A 44. The alveoli and bronchi contained a large number of free phagocytes. The lymphatic system contained iron-loaded phagocytes.
Rabbit A 41	6 days	The naked-eye and microscopic appearances were identical with those of rabbits A 44 and A 43 except that the peribronchial lymph-gland was much larger and microscopically showed a larger number of phagocytes containing iron.
Rabbit A 42	7 days	The appearances were almost identical with those of Rabbit A 41.

Rabbit	8 days	The lymph-node at the bifurcation of trachea was about 5 m.m. in diameter. Microscipically, iron-containing phagocytes were seen in the alveoli, bronchi, lymphatic channels of the lung, and in the peribronchial lymph-gland.
Rabbit A 40	11 days	The naked-eye and microscopic appearances were the same as in rabbit A 45.

It was quite evident that the paths of exit were

(a) directly through the bronchial tree since the iron-loaded cells were seen to collect in the atria, alveolar ducts and bronchi and were caught in plugs of mucus.

(b) along the lymphatic channels of the lung to be deposited in the peribronchial lymph nodes. The iron-containing phagocytes were seen passing between the cells lining the respiratory tract to gain entrance to the lymphatic channels. They were sometimes seen wedged into the narrow tissues of the interalveolar septa; but they were found more frequently where the stroma is a little wider as in the walls of the atria. Here, according to Miller the lymphatics of the lung have their beginning.

The path along the lymphatics is very rapid since in two days groups of phagocytes contining iron were already seen in the peribronchial lymph glands.

Up to and including a period of eleven days the alveoli were still seen to be equally crowded by heavily /

heavily loaded phagocytes. The actual time period required to effect complete clearing of the alveoli could only be estimated by experiments covering long periods of time, possibly even running into months. Mavrogordato (1918) followed his animals for long periods of time and concluded that after being exposed to a moderate "dusting." the lungs are practically cleared of dust granules at the end of a year.

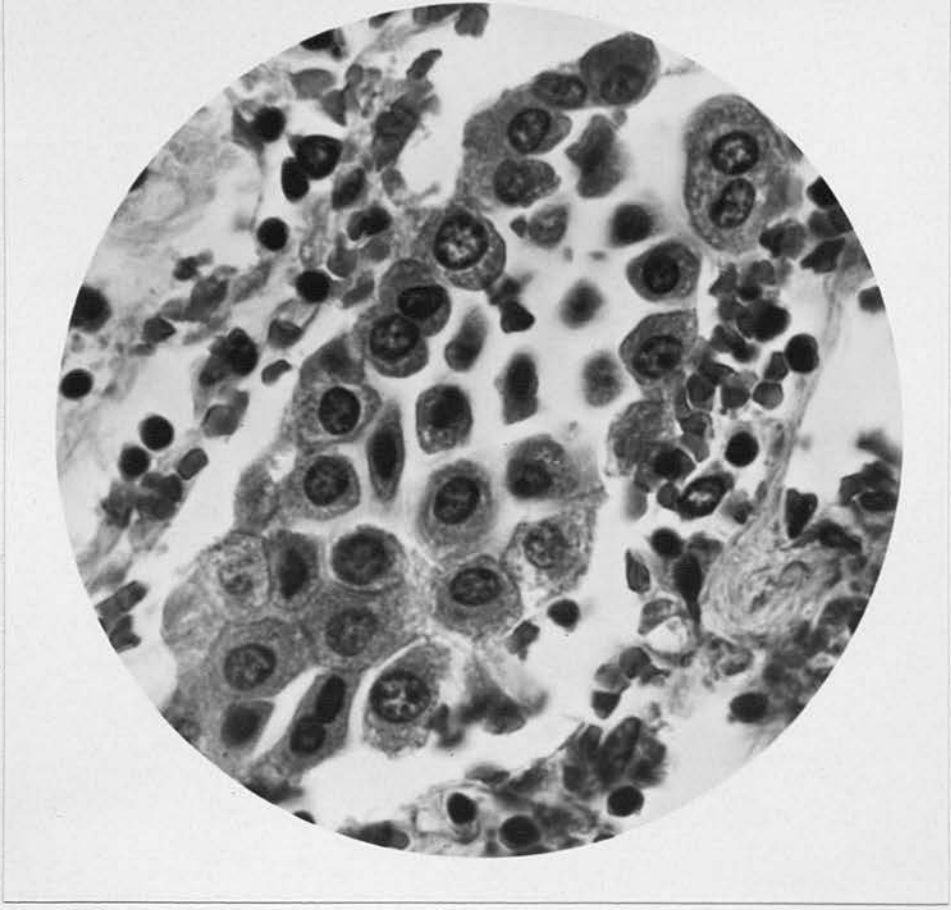


Fig. XLVII (X 1000) Pulmonary tuberculosis. Note the polygonal nucleated cells of the alveolar lining.

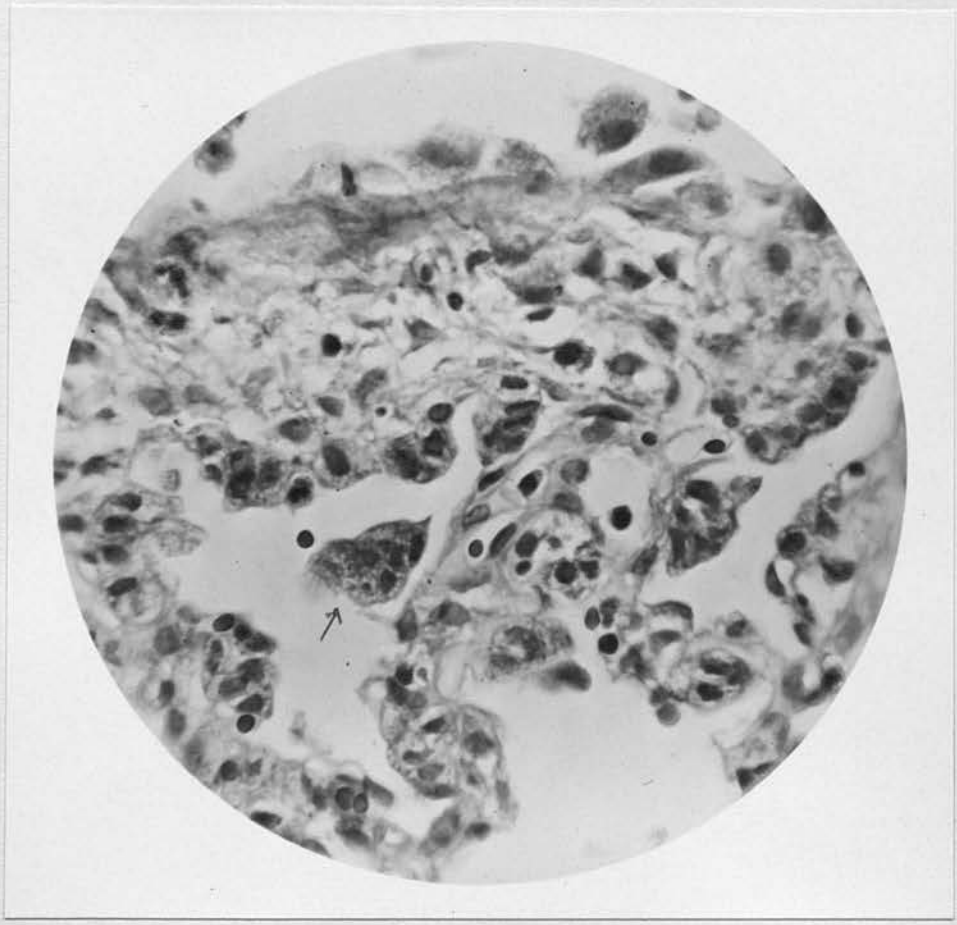


Fig. XLVIII (X 600) Rabbit A 8. Following an intrapleural injection of $Sr Cl_2$ the marginal alveoli are lined by columnar cells. Note the group of desquamated polygonal cells.

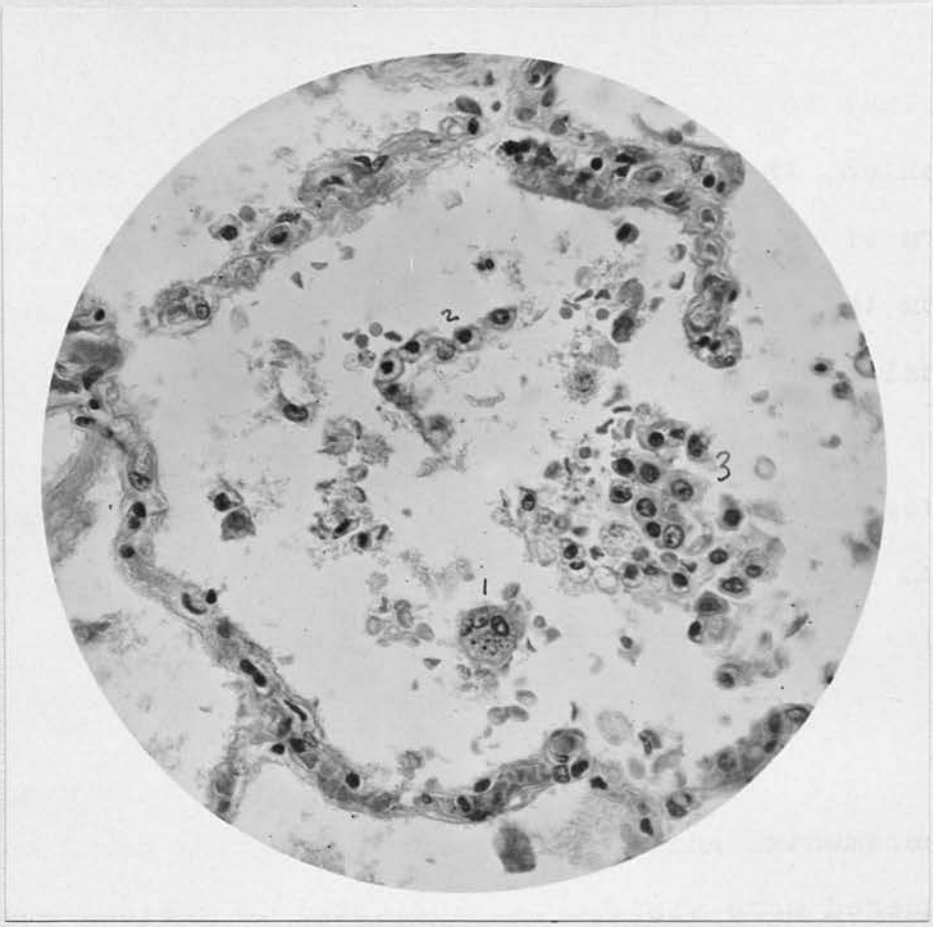


Fig. XLIX. (X 390) Mitral stenosis. Note an alveolar phagocyte containing hoemosiderin (1); a string of desquamated lining cells free of pigment (2); a group of polygonal lining cells free of hoemosiderin (3).

DISCUSSION.

In view of the fact that the literature has already been surveyed in a fairly comprehensive fashion, the following brief discussion will be in the form of an attempt to analyse the results obtained from the experiments recorded above and the patholo-lical material studied.

The nature of the lining of the pulmonary alveolus has been the subject of debate because it is such a delicate structure that it is difficult to see under normal conditions in man and animals. It has been found that by using Young's (1928-30) method of injecting various substances into the pleural sac of experimental animals, the alveolar lining could be rendered more visible and subjected to various studies. It was also found that disease accomplishes the same results which enable us to understand the nature of the alveolar lining. The observations made from the experimental work and the vast amount of pathological material go to show that the normal lining consists entirely of flattened polygonal nucleated cells. (Fig. XLVII, XLVIII, XLIX). This view is different from the opinions expressed by the majority of authors; the most similar to it is that of Miller (1932) who is inclined to deny the existence of the non-nucleated plates. It differs from the view expressed by Cappell (1929) in that though this author believes that the

greater /

greater part of the alveolar lining consists of nucleated cells, yet he admits the existence of the non-nucleated plates and describes them as being derived from the nucleated cells. He also regards the cuboidal cells which occur most frequently in the interalveolar septa as part of the alveolar lining, whereas my observations demonstrate clearly that these cells are essentially phagocytic and that they differ totally from the cells of the alveolar lining. The experimental evidence was conclusive in demonstrating that these cells located in the angles of the alveolar spaces give rise by mitosis to the free alveolar phagocytes. It must be emphasised that in all the literature reviewed the animals were killed at a comparatively long interval (the shortest period recorded is 30 minutes - Briscoe 1907-8 and Cappell 1929), whereas in the present research the animals of one experiment were sacrificed at very short intervals after the introduction of the foreign element into the lung (10 mins., 20 mins., 30 mins., 1 hour and 2 hours respectively). This procedure enabled us to follow the ingestion of the injected substance by the cuboidal cells, their subsequent swelling and desquamation into the alveoli and their mitotic division to provide the alveolar phagocytes. Their morphology, their phagocytic avidity and their silver staining peculiarities definitely indicate that they belong /

belong to the reticulo-endothelial system, in other words that they are of mesenchymal origin. In this point I am in agreement with Lang (1925) who designated them "septal cells" but differ from this author in ascribing to the alveolus a continuous nucleated epithelium, he denying the existence of any lining except the presence of the non-nucleated plates. On the other hand the flattened cells constituting the lining of the lung alveoli in man and animals are not concerned in phagocytosis. None of them ever showed the presence of intracellular trypan blue, india ink, iron or carmine particles. In the earlier stages of intratracheal administration of carmine and iron, some free granules were noted lying upon, or in contact with the flattened cells, but definitely not within them. This was substantiated in the experiments in which the lining cells were first of all rendered more visible by the intrapleural injection of salts. In addition strong evidence was collected from the pathological material to show that the desquamating lining cells do not contain carbon pigment or hoemosiderin. Moreover, they fail to stain with Hortega's silver carbonate, and in this respect as well as in the fact that they possess no phagocytic properties, they resemble the bronchial epithelium - a structure which is universally accepted to be of entodermal origin.

To /

To sum up, then, the alveolus is lined by an entodermal structure consisting of extremely flattened polygonal nucleated cells, with here and there a more prominent special cell (septal cell) belonging to the reticulo-endothelial system. These cells are prepared to deal with foreign particles which gain entrance into the air sacs, hence they frequently contain particles of naturally occurring carbon pigment.

C O N C L U S I O N S /

C O N C L U S I O N S.

1. The alveoli possess an epithelial lining.

2. This epithelium is composed of flat nucleated cells. The existence of the non-nucleated cells has not been demonstrated.

3. In addition to the purely epithelial cells, certain cuboidal cells, "septal cells," are found which have been demonstrated to have a phagocytic function and to stain specifically with Hortega's silver carbonate. These are part of the reticulo-endothelial system and give rise by mitosis to the free alveolar phagocytes.

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