STUDIES ON ENDOTHELIAL REACTIONS.

III. The Endothelium in Experimental Pulmonary Tuberculosis.*

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Plates 87 and 88.

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In two preceding papers of this series (Foot, 1919, 1920) the reaction of the endothelium to a foreign body, agar, was discussed, as well as its reaction to the tubercle bacillus when introduced subcutaneously. It is the purpose of this communication to consider the reaction to this organism when injected through the trachea into the lungs, with special reference to the origin of the epithelioid cell of the pulmonary tubercles. It has been shown in the second article of this series (Foot, 1920) that it is the endothelial cell which forms this component of the subcutaneous tubercle. Wechsberg, writing in 1901, apparently considers the endothelium to be the source of these cells in the pulmonary tubercle, but both Watanabe and Sewell have taken the stand that the alveolar epithelium plays an important part in their formation. It is in an attempt to answer this question conclusively that the present experiment has been undertaken. A general discussion of the theories on the subject will be found in the second paper (Foot, 1920) and will, therefore, be omitted here. There is strong evidence that the endothelial cell is called out in response to foreign bodies and to the presence of bacteria of a low grade of virulence. That this is true in the case of the liver has already been shown. There does not seem to be any reason why there should be a different response in any other part of the body.

Wechsberg injected tubercle bacilli into the lung by way of the trachea and studied the reactions in sections stained mostly with a view to getting an idea of the damage done to connective tissue. He found that the elastic fibers were destroyed within 6 hours after the injection and that there were defects in the endothelium of the capillaries, through which polymorphonuclears and endothelial cells migrated into the alveoli. He summed up his findings thus: (1)

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There is first a destruction of fixed tissue cells. (2) New formed or migratory cells are injured only in as far as the formation of new vessels is hindered and connective tissue growth is hampered and incomplete. Giant cells are formed. (3) There is a second phase of destruction with caseation. In view of his evidence as to the endothelial origin of the tubercles, it is unfortunate that he does not commit himself more definitely on this head.

Watanabe used the same methods and killed the animals 12, 24, 48, and 96 hours after injection. He found bacilli in "swollen epithelial cells" of the alveolar walls, in the epithelium of the bronchi, and in cells which might be endothelial in origin—"mononuclear leucocytes." He found nothing to indicate that the fibroblast took them up.

Sewell was the first to use vital stains, in the modern sense, in connection with experiments on pulmonary tuberculosis. In his paper he quotes an experiment done by Slavjansky in 1869. This investigator injected suspensions of indigo into the trachea of rabbits and after 2 or 3 days introduced 5 to 7 cc. of a fairly thick "solution" of cinnabar into the jugular vein. 2 days later he killed the animals and found some cells lying free in the alveoli and containing indigo, others containing cinnabar, and a third group free from either. The alveolar epithelium contained indigo and was otherwise unchanged. If, however, he injected the cinnabar into the blood immediately after introducing the indigo into the trachea, he could find cells in the alveoli and bronchial mucosa containing granules of both pigments. He concluded that, in the first case, the cells containing the cinnabar had wandered out of the vessels (thus indicating the possibility of their being of endothelial origin) and finding no indigo free, remained unaffected thereby; while in the second case, they arrived before the supply of indigo had been exhausted and therefore contained both kinds of pigment.

Tchistovitch repeated these experiments in 1889, substituting bacteria for the indigo in the tracheal injection and using carmine intravenously. The animals were killed in 24 hours. Carmine-laden cells were found in the alveoli, and there was also phagocytosis of the bacteria by small cells which contained no carmine granules; he could find no cells containing both, which is not consistent with my findings, as will be seen later.

Sewell injected a suspension of bovine tubercle bacilli in lithium-carmine into the trachea, using 2 cc. at a time. The rabbits were vitally stained with trypan blue and were killed 24 and 72 hours after introducing the bacilli. In one animal he found cells stained with the blue dye lying in the alveoli. As this rabbit showed an extensive pneumonia and pleurisy, he concluded that these cells migrated in response to the infection at a time prior to the injection of the carmine and bacilli, but after the trypan blue staining was complete. Some of these cells contained trypan blue granules, carmine, and tubercle bacilli. The other animal, however, showed only carmine in the free cells. He injected a fine suspension of India ink into the trachea of rabbits vitally stained with lithium-carmine and killed them 3, 6, 15, and 24 hours later. Cells stained with car-

mine did not appear until after 24 hours, but cells containing carbon were found before this and appeared to originate from the epithelium near the alveolar angles. Carmine injected into the trachea gave excellent staining reactions in the alveolar epithelium after 3 hours and trypan blue after 6 hours. He injected 10 cc. of a mechanical suspension of India ink into the ear vein of a rabbit and found none whatever in the lungs.

This investigator did a number of experiments with various dyes injected alone into the trachea of rabbits and guinea pigs, which give an idea of the lesions produced by the injection of such dyes, unmixed with bacteria, while they also serve as valuable control experiments. Quoting Briscoe as to the three possible sources of the mononuclear cells found in the alveoli in pulmonary inflammation, he says that these may be (1) the macrophages in the interstitial tissue near the alveolus, (2) the alveolar epithelium, and (3) specialized epithelium not concerned in respiratory function. His frequent allusions to the similarity of the epithelial and endothelial phagocytes and the fact that he admits that the only means of ruling out the latter in these experiments is the use of intravenous dyes which do not immediately appear in these cells, but require the lapse of a day or more to make their presence known, all indicate the difficulty that is encountered in drawing conclusions from such experiments. Moreover, the carmine that he used intravenously is not specific for the vascular endothelium *in situ* and the mechanical suspension of India ink, upon which he based his final conclusions, did not show in the lungs at all; whereas, had he used a colloidal suspension of carbon, which never fails to show, his conclusions might have been different. In summing up he says: (1) The alveolar cells are endowed with the full phagocytic powers of the histiocyte. (2) Their behavior under the action of a stimulus is similar. (3) When certain dyestuffs are offered to them in colloidal solution, they store up granules of the dye in their protoplasm in the same manner as the histiocytes. He clearly indicates his belief that, in the case of experimental pulmonary tuberculosis, the epithelioid cells are really epithelial cells which are the first to respond to the stimulus of the infection.

Method.

In the experiments which are the basis of this paper I have used two pigments, lithium-carmine and Higgins' water-proof ink, which is a colloidal suspension of carbon in water, with an emulsifying agent and a little camphor. The former was employed solely for intratracheal injections, the latter for the intravenous; both of them are colloidal suspensions. The carmine suspension is prepared by mixing 4 gm. of Grübler's carmine rubrum optimum in 100 cc. of a saturated solution of lithium carbonate, boiling, and filtering through filter paper. The ink suspension is a mixture of equal parts of commercial

Higgins' water-proof ink and distilled water. The carmine suspension is sterilized before use; the ink mixture need not be sterilized if sterile distilled water is used, as the ink contains enough camphor to keep it free from bacterial growth.

The fundamental principles involved are the supplying of two substances of different color, one through the air passages, the other through the circulation; that used in the latter instance must be one that will be taken up by the endothelial cells *in situ* and I know of only one such, a colloidal suspension of carbon, either in the ready made form just stated, or in that recommended by McJunkin. The latter is a 4 per cent suspension of finely triturated lampblack in normal salt solution, with 1 per cent of gelatin added to it. In this way the alveolar epithelium will be offered a red dye, the endothelium of the capillaries a black one, the origin of the cells that respond to the stimulus of the tubercle bacilli being readily traced by their color. The question of the specificity of the carbon for the vascular endothelium has been presented at length in the two preceding papers of this series, so any further discussion will be omitted here.

Histology of the Alveolar Epithelium.

Before proceeding to the consideration of the experiments done along these lines, it will be necessary to discuss the histology of the alveolar epithelium, for much error may result from misconceptions as to its structure. Once a definition of a typical alveolar epithelial cell has been accepted, the investigator will know what to look for when searching for such cells and will realize that not all are globular, with the bean-shaped nucleus and other characteristics of the endothelial leucocyte.

The epithelium of the alveoli, as well as that of the infundibula, is not a continuous layer of nucleated cells, but a membrane of varying thickness, composed of several types of cells, non-nucleated plates, and flanges, or processes, of nucleated cells. There are areas where one finds groups of nucleated cells very similar to those of the endothelium and practically indistinguishable from them. There are more or less cuboidal nucleated cells which extend almost through the alveolar wall and around which the capillaries twist, but whose alveolar

surface is more or less flared out to become continuous with adjacent cells. Thirdly, there are large, flat cells with broad plates extending from them, in which the nucleus is situated in a small mass of protoplasm more dense than the rest. These extending plates may represent similar cells that have lost their nuclei, or the processes, or flanges, of the flat, nucleated cells; both theories are advanced, the former being the most commonly encountered.

A cross-section of one of these plates is extremely difficult to see, as the cytoplasm has a refractive index that approaches that of Canada balsam very closely; but they can be made out under suitable conditions. It is possible to demonstrate them by injecting the fresh lung with a 0.25 per cent solution of silver nitrate, when the demarcation becomes evident. In my preparations they are best seen in tangential planes of section in material fixed in neutral formaldehyde; Helly's fluid shrinks them and causes fenestration of the cytoplasm. How they are formed from the cuboidal epithelium which precedes them in the fetus is still a matter of dispute. Bremer's work on the opossum lung would indicate that they are, in reality, flanges, or processes, of epithelial cells that are stretched out thin over capillaries. The reader is referred to such text-books as those by Ellenberger, Minot, Stöhr, etc. That most of these cells do not resemble the endothelial leucocyte can be seen at a glance; that they desquamate in the forms just described is readily demonstrable.

The group of roughly cuboidal cells is the most difficult to classify in regard to origin. Without ink injections the question seems unanswerable, as they look exactly alike; with ink in the cytoplasm of some of them, we can assume that these are endothelial. It would seem advisable to study the question of cell types in animals that are vitally stained, using a tridimensional wax reconstruction technique. That some of the cells in the groups which so closely resemble the endothelium may really be such, must be kept in mind. They might migrate from the capillary walls and collect in these situations, just as they do in the other tissues in the body, there to act as potential phagocytes in an organ where phagocytosis is very necessary. This is not a new idea; that many endothelial phagocytes are normally present in the lung is well recognized; their origin in the capillary walls and storage in the clumps of cells already described would, it seems, be perfectly natural.

Reaction to Pigments Alone.

Before describing the experiments in which tubercle bacilli were injected into the trachea, it would be well to know the reaction to pigments alone. In one rabbit ink was injected intravenously, and the animal died about an hour later while going under ether. Two rabbits were injected intravenously with 5 cc. of Higgins' ink and distilled water in equal parts and intratracheally, under an anesthetic, with 2 cc. of the lithium-carmine preparation. One was killed 24 hours later, the other 6 days later. The lungs and bronchial tree were removed *en bloc* and the former distended to three-quarters their normal expanded volume by injecting 4 per cent neutral formaldehyde. They were then sliced thin and fixed either in formaldehyde or Helly's fluid. They were stained with Delafield's hematoxylin, eosin-methylene blue, and Mallory's phosphotungstic acid hematoxylin.

In the animal in which the ink was used alone, the carbon is distributed in the endothelium of the interalveolar capillaries, and to a slight degree in the circulating mononuclear leucocytes, while some of it lies free in the circulating blood. It is also found in large cells in the alveoli, as described below. In the two other animals the ink distribution is the same, more appearing in the large cells in the alveolar spaces after 6 days than after 24 hours. There is a slight polymorphonuclear leucocytosis, with a scanty exudate of these cells after 24 hours; this is not seen in the 6 day preparations.

The large intraalveolar mononuclears resemble macrophages in every way; they are of irregularly globose, or obtusely angulated outline and have vesicular reniform nuclei and may contain vacuoles. They lie in the lymph spaces just below the epithelium, or free in the alveolar sac, or fitted into indentations in the alveolar surface of the epithelium, which surrounds them on three sides. Sometimes they seem to be interposed between two epithelial plates, as though penetrating the lining, and they can be seen, not infrequently, leaving the capillaries and lying athwart the lymphatics, with a portion of their cytoplasm penetrating the epithelium, in the act of migration. These cells are all important in the work at hand, for they play the principal part in the reaction to the tubercle bacillus in the air-borne infection and are evidently the cells which Sewell thought to be epithelial.

They show a few carbon granules as early as 1 hour after injection and take it up steadily for 6 or 7 days, when the experiments terminated. A few may be loaded with the ink particles, especially when in the alveolar wall; sometimes they are filled with carbon when free in the air space; usually, however, those that migrate contain less than those that do not. The foreign matter may hinder their motility to some extent. A little carbon is found in occasional polymorphonuclear leucocytes, but as a rule it is confined to the mononuclear cells. It is never found in the bronchial epithelium, or in any cells of clearly epithelial type in the alveoli. That it could be taken up by the epithelium from the blood stream seems highly improbable in the light of our present knowledge of the selectivity of the endothelium for this substance. The ink never seems to lie free in a position where the epithelial cells could phagocytose it, and the idea that it could be passed from the endothelial to the epithelial cells is not worth serious consideration.

The mononuclear migrating cells take up the carmine diffusely at first; after 5 hours, as seen in later series of sections, only one or two granules appear; after 24 hours the granular stain is complete and very beautiful. In the 6 day control animal these cells are found scattered in twos and threes in the alveolar sacs and bronchial lumina, or grouped in small masses in the alveoli and infundibula. They do not tend to form syncytia and show an intense granular carmine stain. One can also find them in the lymphatics and lymph nodes of the lung tissue and mediastinum. The epithelium of the bronchi and alveoli does not take the carmine except in a very faint, irregular, and diffuse manner. Thus the tissue reaction to the pigments alone is of a very slight and transitory character.

Experimental Pulmonary Tuberculosis.

In the actual experiment on the reaction to the tubercle bacilli the following procedure was carried out. Two stock strains of bovine tubercle bacilli were utilized and two methods were employed in preparing the suspensions used. In the first case one or two loops of dry culture on glycerol agar were rubbed up as finely as possible with a flat platinum needle and gradually suspended in 10 cc. of the lithium-carmine preparation used in the control tests, to be mechanically shaken for from ½ to 1 hour. The bacilli were first rubbed into a paste, more fluid was added, and the needle was bent into a J and rapidly spun between the

thumb and index finger until a fairly uniform cream was obtained. In the second instance a glass bulb pestle was used to triturate the same amount of culture into a thin film on the walls of a test-tube, fluid being added gradually and the whole shaken as before.

A series of rabbits was then injected intravenously with dilute Higgins' ink as in the controls; they were then anesthetized and 2 cc. of the suspended tubercle bacilli in lithium-carmine introduced into the trachea, just below the larynx, by means of a hypodermic syringe and needle. After a lapse of 1 hour, 5 hours, 1, 2, 3, 4, 5, 6, and 7 days the rabbits were killed by anesthetizing and injecting neutral 4 per cent formaldehyde into the beating heart. The lungs were then removed and treated as in the controls. The 5 and 24 hour lesions were produced in duplicate, one pair of rabbits receiving one strain, the other the second strain of tubercle bacilli.

The lesions resulting in all cases varied slightly with the individual and with the type of suspension used, the coarser type causing changes more like the usual tubercles and tuberculous bronchopneumonias, while the finer caused lesions that resembled caseous lobar pneumonia and were accompanied by fibrinous pleuritis (*cf.* Sewell's case).

The development of the experimental lesions, observed at the intervals indicated, proceeds as follows: First there is a widespread desquamation of the epithelium of the affected alveoli, often with destruction of their entire wall, including the capillary; large, flat cells, plates, and the debris thereof are found lying free in the air sacs and the lumina of the bronchi. Sometimes there is swelling of the epithelial plates, which peel off at their edges or gradually disintegrate. They may appear as a broad, hyaline, amorphous band at the periphery of the alveolus. The elastic fibers, as shown by Weigert's stain, also swell and undergo fragmentation as the process goes on. Many polymorphonuclears appear in the capillaries and migrate into the air vesicles, and with them comes an ever increasing number of large, ovoid, or slightly polygonal cells, which contain carbon and carmine at first to a slight, later to a marked degree (Fig. 1). They are in no way different from the mononuclear migratory cells described under the control experiment; at first they contain little or no ink, as they were presumably free in the alveoli before it was administered, but later they take it up in the vessels and appear in the alveoli with many granules in their cytoplasm, as can be seen in the photomicrographs.

As early as 5 hours after injection one can see marked proliferation of the endothelial cells of the capillary wall (better seen after 24 hours as in Fig. 2). Mitotic figures and ink globules are easily found. The endothelium is swollen and the cells are often in layers, or form nodal thickenings. Many figures of eight nuclei are present, and some swollen and distorted forms can be demonstrated, suggesting amitosis. The epithelium, on the other hand, does not show regenerative activity until the 3rd or 4th day, when it begins to proliferate, chiefly in the neighborhood of the bronchioles (Fig. 3). Here too, nodal heaps of cells occur, but they lie on the epithelial surface and project into the alveoli. Their cells are paler, more reticular, and contain no carbon, though they may take up a few bacilli. After 2 or 3 days the epithelium forms the familiar sheets of cuboidal cells on the alveolar wall and shows less activity. These sheets not infrequently grow over and enclose the masses of endothelial migratory cells which form in the alveolar spaces, sometimes almost entirely covering them with an epithelial envelope.

By the 4th day tubercles are found in the interstitial tissue, at the site of small collections of lymphoid cells (Fig. 4). They are composed of mononuclear cells containing ink, a few with the morphology of these but without carbon, and a few of the lymphocytes already present in the nodules, with a scattering of polymorphonuclears which have been attracted to the lesion. A day or so later the mononuclears in the alveolar spaces, which are now present in large numbers, fuse to form anastomosing masses of tubercles and syncytia, or giant cells (Fig. 5). In the series resulting from the injection of the coarser suspension of bacilli these areas are isolated and patchy (Fig. 6); in those in which the finer suspension was used the entire section is solid with the mass of mononuclear migrants, in many of which are mitotic figures, as in Fig. 3, showing that they are still actively growing. Numerous syncytia, commonly miscalled giant cells, are present and contain ink, carmine granules, and tubercle bacilli (Fig. 5). They first appear on the 3rd day after injection. The mononuclear endothelial cells of the exudate are often loaded with tubercle bacilli, as well as with ink and carmine (Fig. 6). While the intraalveolar tubercles have a predominatingly red color, that of the interstitial variety is black. On the 5th day these two types tend to fuse with one another,

including all the tissue between them, and large conglomerate areas result, which represent localized foci of tuberculous bronchopneumonia. As will be seen in Fig. 7, the fibroblasts that are included in these fusions begin to grow through the masses of endothelial cells and form interlacing bands of young connective tissue.

The reaction where a widespread lobar type of pneumonia resulted is essentially the same; there are more polymorphonuclears in the exudate and many erythrocytes are present. Both of these, with the endothelial mononuclears, fill whole sections of a lobe and, becoming caseous, form the exudate which gives this type of tuberculous pneumonia its name. The gelatinous exudate also appears and is found in alveoli where there is less active inflammation and cellular reaction and whose lymphatics are always widely dilated. Undoubtedly this is formed from coagulated lymph and a certain amount of very delicate fibrin, which can be demonstrated by using phosphotungstic acid hematoxylin either as a fine network or in a finely granular form, almost always without cellular admixture. The destruction of the elastic fibers is most marked in this type of inflammation; the fibrillæ are broken into very short, curly structures, not unlike large bacilli in their appearance. Here, then, the process is more diffuse and acute than in the cases in which the bacilli were less finely and evenly suspended and hence apt to enter the lung tissue in clumps.

The large phagocytic endothelial cells can be found in the lymphatics in this experiment, as they were in the controls. They are recognizable by their carmine and by the fact that tubercle bacilli can be demonstrated in their cytoplasm. That they contain ink in this case would, alone, prove that they came originally from the vascular endothelium; but the presence in these cells of the carmine and bacilli, which were introduced intratracheally, indicates that the cells come from the alveolar spaces. Hence we have pigmentary evidence that they have originated in the capillary walls, migrated to the air spaces, taken up bacilli and carmine, and, reentering the lymphatics, have carried these to the lymph nodes. Why they do so is not evident. This throws an interesting light on the spread of tuberculosis by means of migrating phagocytes; moreover, it furnishes additional evidence as to the endothelial origin of these cells, for such behavior is not characteristic of epithelium as we usually understand it.

The lymph nodes of the peribronchial lung tissue, as well as those of the mediastinal peribronchial group, show progressive increase in reticular cells and decrease in lymphocytes and lymphoblasts; small tubercles develop in the pulmonary nodes on about the 6th day. Most of these tubercles seem to be caused by an extension of the infection from tubercles in their immediate neighborhood. If bacilli carried to the nodes by phagocytes cause a development of tubercles in them, such a process would take place later, as there is little direct evidence thereof in this experiment, which represents a lapse of but 7 days after the introduction of the bacilli. Lymphocytes appear in the tubercles on about the 6th day; they seem to play no part in the primary formation of these lesions.

Another series of experiments is now in progress, a full report of which will appear in the near future. In these each rabbit receives in an ear vein 0.01 mg. of tubercle bacilli, of the same strain as that used in half the animals of the foregoing experiment. Niagara blue 3 B, an American form of trypan blue, is given intraperitoneally and Higgins' ink intravenously until the animals are vitally stained. The tubercles that result in the lung are of the miliary variety, almost all interstitial in location, apparently originating in, or near blood vessels and lymphoid tissue in the vicinity. They are deeply stained with ink and do not appear to take on much of the blue. There is little or no intraalveolar exudate or tubercle formation, in sharp contrast to the preceding experiment, in which the intratracheal infection resulted in striking exudation into the alveoli. In some places where the tubercles have become well developed and caseation is in an advanced stage, there is a spread to the alveoli (7 weeks), which gives rise to a reaction slighter than, but similar to that in the air-borne infection. This will be described in detail in the next paper, in connection with the lesions in the liver, spleen, kidney, etc., which, of necessity, result from a generalized hematogenous infection with tubercle bacilli. There is no doubt that there is a striking difference between the two types of infection when experimentally produced. All the hematogenous tubercles are composed of cells which contain more ink than do those found in the tubercles in the intratracheal infections. If the ink is administered for a while and the injections are then stopped, the tubercles are found to be deeply pigmented in

the older portions, while the newer zones of these lesions and the more recently formed lesions show very little carbon. For this reason it is necessary to continue the ink injections at least twice a week until the animal is killed.

Comparing the results of the experiments on the two types of infection, we find that the interstitially formed tubercles of the air-borne type compare well with those that predominate in the hematogenous type; they probably originate in the lymphatics in the first case and hence present this striking similarity. In the experiments on hematogenous infection we have no difficulty in tracing the origin of the epithelioid cell to the vascular endothelium; one can find tubercles produced as crescents in the lumina of medium sized capillaries, composed of cells that are so loaded with ink that their architecture is almost obscured. These tubercles can also be found in intratracheally infected series, but are more difficult of demonstration, for in this case we are dealing with secondary lesions, caused by bacteria which have escaped from the air vesicles, a condition which does not occur very frequently. The marked indifference of the epithelium to the hematogenous infection is another point against the theory that this tissue is the primary respondent to the tubercle bacillus.

It will be seen from what has been said that the findings in these experiments do not differ materially from those of Wechsberg, Watanabe, or Sewell; owing to the intravenous use of a colloidal suspension of India ink, however, the conclusions are decidedly different from those of the last two observers. If this material is not specific for the vascular endothelium, or at least selective enough in its action to be considered practically specific, this work must be discarded and new lines of approach devised before the question can be settled definitively. It appears that a specific agent has finally been found for marking the endothelial cell *in situ* and following it through its wanderings.

SUMMARY.

1. The injection of a colloidal suspension, or sol, of carbon into the veins of a living animal, as recommended by McJunkin, furnishes an apparently reliable means of tracing the so called epithelioid cell of the pulmonary tubercle from its origin in the vascular endothelium to the lesion.

2. Experimental tubercles are formed in the lung, as in the liver, primarily by cells originating in the capillary endothelium. These cells are probably present in small numbers in the normal lung, lying free both in the alveolar wall and the air vesicles. In response to infection they proliferate in the capillary walls in the vicinity of the invading organisms, migrate in steadily increasing numbers, and, arriving at the site of the infection, further multiply and to some extent fuse to form the syncytia known as giant cells.

3. The epithelial cell takes no active part in the process; its proliferation tends to repair denuded surfaces and is regenerative rather than combative or phagocytic in nature. This cell is free from carbon and stains only diffusely with carmine, in contradistinction to the endothelial cell which readily takes up both pigments in granular form.

4. The cells of endothelial origin not only phagocytose tubercle bacilli, but carry them into the tissues, for example into lymph nodes, by way of the lymphatics, or into other lung lobules by way of the air passages, in which they are readily demonstrable.

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EXPLANATION OF PLATES.

PLATE 87.

FIG. 1. 5 hours after injection with tubercle bacilli. Note the large endothelial migratory cells in the alveolar spaces and the dilatation of the capillaries and lymphatics. × 1,000.[1]

FIG. 2. 24 hours after injection. The alveolar walls are thickened; polymorphonuclears are more numerous. × 1,000.

FIG. 3. 4 days after injection. Epithelial proliferation and partial fusion of the migrated endothelial cells, one of which shows a diaster. Carbon is not present in the epithelium, but is clearly seen in the intraalveolar mass of cells. × 1,000.

FIG. 4. 4 days after injection. The interstitial type of tubercle, very much richer in carbon than the intraalveolar form. × 1,000.

PLATE 88.

FIG. 5. 6 days after injection. Three well developed syncytia. Note the contained carbon particles. Several smaller cells with carbon are present. × 438.

FIG. 6. 1 week after injection. Part of an intraalveolar tubercle, showing carbon in the cells, as well as tubercle bacilli. × 657.

FIG. 7. 1 week after injection. Low power photomicrograph of an entire lesion, of the conglomerate, intraalveolar type. The streaks are groups of fibroblasts; organization is under way. × 163.

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[1] The figures of magnification are approximate but fairly accurate.

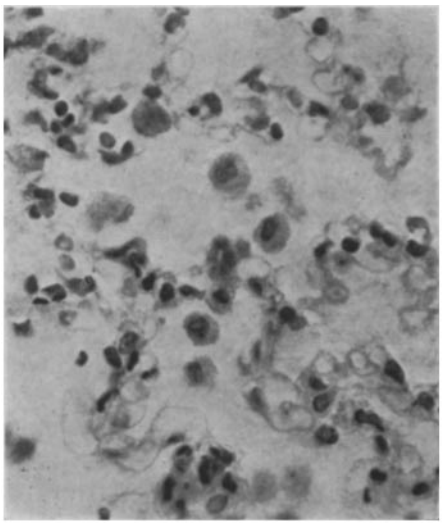

FIG. 1.

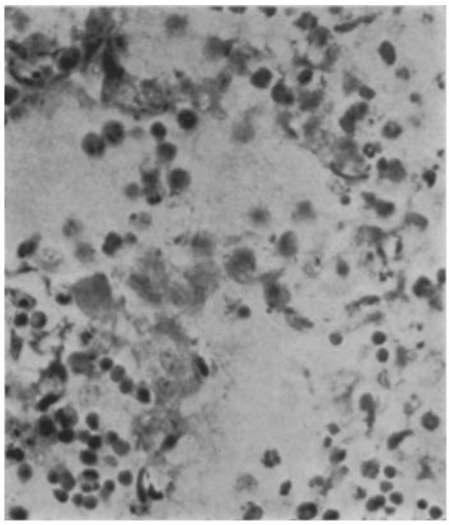

FIG. 2.

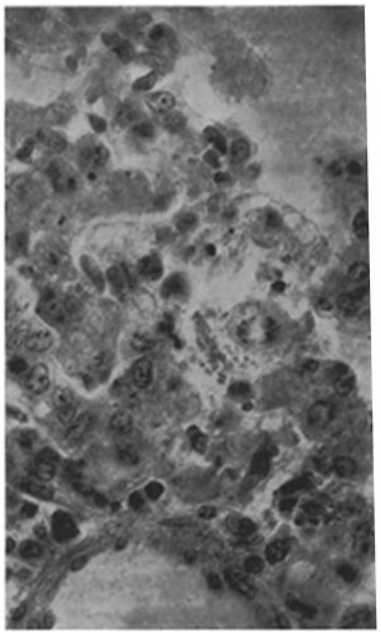

FIG. 3.

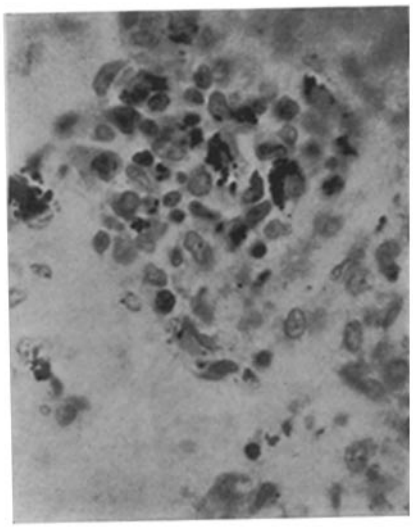

FIG. 4.

(Foot: Endothelial reactions. III.)

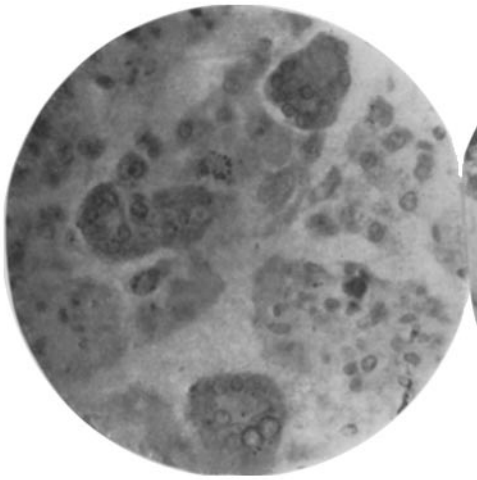

FIG. 5.

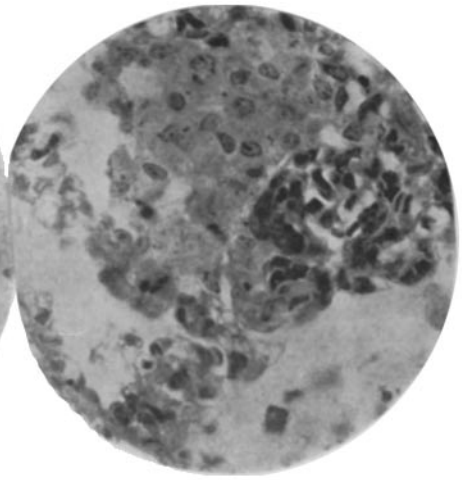

FIG. 6.

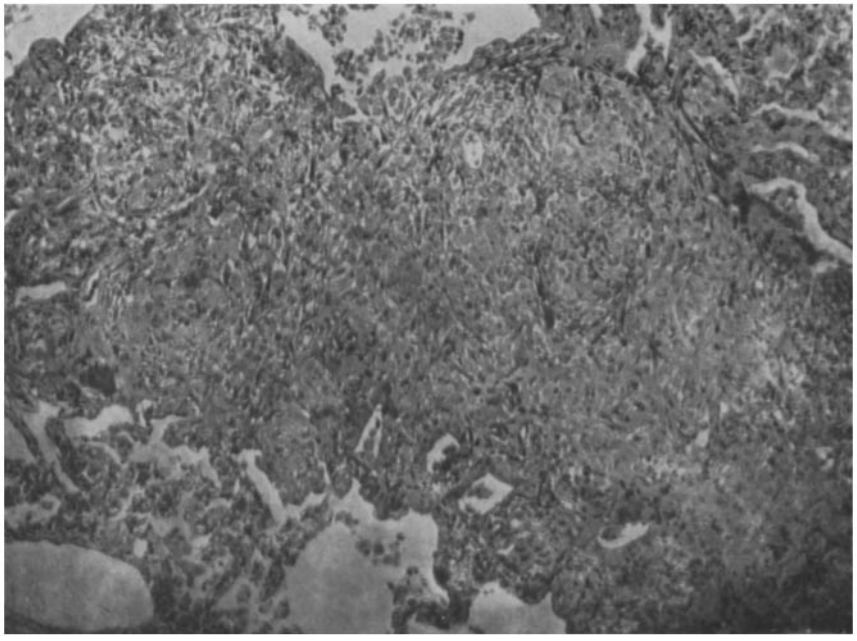

FIG. 7.