THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME III SEPTEMBER, 1927 NUMBER 5

STUDIES ON ENDOTHELIAL REACTIONS

X. ON THE ORIGIN OF THE PULMONARY "DUST CELL" *

NATHAN CHANDLER FOOT, M.D.

(From the Department of Pathology, University of Cincinnati College of Medicine and Cincinnati General Hospital, Cincinnati, O.)

INTRODUCTION

It would be very inadvisable to attempt to present the results of the experimental work to be reported in this paper without first reviewing the subject as a whole. So much controversy has centered about the mononuclear phagocytes in general and the pulmonary dust cell in particular that the reader would probably be bewildered by a discussion concerning the latter, were he not permitted to familiarize himself with the views of the various disputants. It will, therefore, be the purpose of this article to afford him this opportunity by presenting a full review of the present status of the doctrine of the dust cell; after which, he may proceed to an enlightened consideration of the value of the data that constitute the original portion of the paper.

Seven years ago, Permar ('20) and Foot ('20), experimenting independently and using somewhat different methods, came to the conclusion that the pulmonary dust cell was derived from the capillary endothelium of the lung. We believed that the capillary endothelium became swollen, its cells proliferated and then migrated into the alveoli, there to become free phagocytes and to multiply still further by mitotic division. We suggested that the phagocytes already present in the alveoli under normal conditions (not called out by a special crisis, such as the injection of dyes or bacteria) had

* Received for publication June 22, 1927.

the same origin, presumably migrating from vascular endothelium from time to time in order to form a sort of scavenging reserve force. We both described cells, which Lang ('25) has subsequently named "septum cells," that are normally present in niches or recesses in the alveolar wall and considered that they had the same origin.

Our theories have been critically studied. They have been accepted either entirely or partially by some and rejected by others. Many investigators agree as to the mesenchymal origin of these cells, although questioning their being derived from the capillary endothelium. A number have put forth well substantiated claims that they arise in the circulating blood as monocytes, migrating to the alveoli as occasion arises. M. R. Lewis ('25) and her pupil Eliot ('25) have employed vital staining to show that the phagocytes are at least of mesenchymal origin, whereas Wislocki ('24) agrees, but remains in doubt as to their precise derivation.

Thus we might be drifting back toward the monocytic origin of these cells, as formerly postulated by Metschnikoff ('07), were our regression not impeded by numerous papers, chiefly by German investigators, who claim that they are merely somewhat altered, desquamated alveolar epithelium. Metschnikoff 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, microörganisms and other foreign bodies. In reality these elements are nothing more than white corpuscles that have immigrated into the alveoli and bronchi." The most recent article to support the epithelial side of the argument is by Gross ('27) from Aschoff's laboratory. It will be well to quote at some length from his paper, for in this way one may present the subject in an unbiased fashion, as the statement emanates from the opposing camp and will, for that reason, stress their views rather than ours.

Résumé by Gross: "The ancient dispute concerning the origin of the epithelioid cells has not come to rest since the time of Baumgarten ('01) (fixed tissue cells) and Metschnikoff ('88) (leukocytes). Herxheimer ('03) has injected into the debate, as a third possibility, the participation of the alveolar epithelium. The Japanese school (Kiyono, '14) traces them to histiocytes. Töppich ('25), whose experiments were performed to evaluate Herxheimer's work, champions the endothelial derivation. He sees, as early as one hour after infection, a migration of swollen capillary endothelial cells through

the reticulum of elastic fibrils into the alveolar lumen and their transformation into large mononuclears and epithelioid transitional cells. 'There can be no question of alveolar epithelium, so far as the great majority of the cells is concerned, for disregarding transition-pictures, exactly the same cell types may be recognized as lying definitely within the capillaries.' He explains the circumstance that he found no alveolar epithelium at all by the fact that these cells, 'probably under the direct action' of the large doses of bacilli, had been totally destroyed. Kageyama ('25) found bovine and avian tubercle bacilli, injected in massive doses into the peritoneal cavity, appearing in the pulmonary blood as soon as two hours thereafter. The excretion of these bacilli into the alveoli followed very promptly. After eighteen hours, changes were first noted in the lung tissue, consisting of a proliferation of the alveolar epithelial cells which phagocytosed the bacilli and desquamated into the alveolar lumen as in desquamative catarrh. He never found bacilli in the endothelial cells.

"Tissue cultures have also been drawn upon to solve the problem. Timofejewsky and Benevolenskaja ('25) discovered that tubercle bacilli inhibit the growth of rabbit lung tissue cultures. Despite the fact that they established that the particularly active elements in the cultures were the epithelial cells, they do not venture to exclude monocytes, endothelial cells and fibroblasts from the category of bacillary phagocytes, but believe that the epithelioid cells of an organism develop rather from the connective tissue than from the alveolar epithelium. Lang ('25), who denies the existence of alveolar epithelium, finds exudative and productive processes in similar experiments. Pagel ('25) has attempted to answer the question as to the origin of the exudate cells in caseous pneumonia on morphologic grounds. 'In the guinea pig, unmistakable transition pictures as well as structural identity, permit us to trace the exudate cells to the alveolar lining.' Proof in the case of the human subject has fallen flat. ('Ist gescheitert.')."

Further on Gross states: "In the case of the rabbit, I must hold fast to the theory of a primary epithelial reaction." His reason for this is based on the flimsiest of morphologic evidence obtained from the examination of experimental animals a month to six weeks after the injection of tubercle bacilli or dust into the trachea. His argument might be epitomized in the statement: "The dust cells look like epithelium, therefore they are epithelium."

In his summary there is one misstatement that should be corrected; Lang does not deny the existence of alveolar epithelium. He says: "The epithelium is of no importance either, as the alveolar wall, according to my previous investigations, is not provided with any special 'respiratory' epithelial layer, *except the non-nucleated plates.*" The italics are mine. Kageyama's work is not entirely relevant to the discussion, as tuberculosis in the lungs of rats and mice presents an altogether different picture from that which is observed in the case of other mammals.

Here, then, is a statement from the "epithelial camp," whose adherents antedated Herxheimer's "injection" of the epithelial origin of the dust cell into the debate by a number of years, as is indicated by Herxheimer himself. This older literature will be found listed in his paper, in Permar's and in mine ('20).

The Experiments of Westhues and Westhues: Gross merely touches upon the work of the Westhueses ('22, '25), who also adhere firmly to the epithelial origin of the dust cell and have done careful and excellent experiments in an endeavor to prove their case. It has been considered in a former paper (Foot, '25), but it should be mentioned here. In view of the fact that Wislocki ('24) has stated that "Chinesische Tusche" is a colloidal and not a mechanical suspension of carbon as I had thought, it would be well to withdraw my criticism of the work of H. Westhues, wherein I stressed the point that particulate carbon would not mark capillary endothelium.

Westhues noted that there was a decided predilection on the part of the alveolar phacogytes for carbon, carmine being taken up sparingly and slowly. From this he concludes: "As phagocytosis in the lungs takes place more rapidly and energetically than histiocytes could phagocytose, it follows that the engorged (vollgefressenen) cells in the alveoli are not emigrated histiocytes, but could only be alveolar epithelial cells." But, has it been proved that the alveolar epithelium is so energetically phagocytic? Have not the Kupffer cells and splenic phagocytes marked phagocytic properties? More recently H. and M. Westhues ('25) performed clever and painstaking experiments to prove this point. They believed that Permar's inferences, drawn from lung sections that had necessarily been cut in many planes, were "very daring." They attempted to narcotise the phagocytes, but without success. Next they replied to my earlier criticism by using colloidal Elektrokollargol for intratracheal

and Elektroferrol for intravenous injection. No phagocytosis was observed on the part of the capillary endothelium. This would appear to be an important point, were it not for the fact that the endothelium was presumably healthy and would therefore scarcely phagocytose foreign material. They then perfused rabbit lung with normal saline solution to wash the circulation clear, injected 1: 150 India ink into the trachea and suspended the lung in warm saline solution in an incubator for half and hour. The ink was taken up avidly by the dust cells. Reversing the process, they flooded the capillaries with ink and the alveoli with normal saline solution, observing no phagocytosis of the ink by the endothelium or the dust cells. In the first instance no ink was shown to have been phagocytosed by the respiratory epithelium *in situ*, either in the description or illustrations; in the second, although it is stated that there was no phagocytosis of the ink by the endothelium, the colored illustration depicts black granules all through the walls of the alveoli and embedded in the violet tinted cytoplasm of their cells. It is difficult to reconcile this unfortunate discrepancy with the text of the article.

Theories of Mesenchymal Origin of the Dust Cells: So much for the epithelial origin of the dust cell, let us now consider the other side of the argument. When Permar and I published our experimental results we were content to avail ourselves of the evidence at hand; perhaps it was insufficient. My line of deduction was as follows: Cells were found in the alveolar spaces in tuberculous inflammation containing carbon that had been administered intravenously. It appeared as fine particles and coarser clumps in what was interpreted as capillary endothelium which was swollen and which showed mitotic figures in what were supposed to be its lining cells. Therefore the intra-alveolar phagocytes were emigrated cells from the capillary walls. Experimentation with meningeal tuberculosis (Foot,'22) subsequently proved that there was little change in the local capillary endothelium in that case, yet tubercles formed near the vessels and consisted of carbon-marked cells. Similar cells were found within the lumina of the pial vessels, often in mitosis and mingled with the other blood cells. Obviously something was wrong with the earlier theory, which should have applied here as well, or this was a notable exception to the rule. It is possible that what Permar and I interpreted as vascular endothelium in mitosis,

was, in reality, mitosis in monocytes adhering to the capillary walls on the inside.

It is apparent that one can neither prove nor disprove much in this connection unless the process be observed *in vivo*, or many methods be applied simultaneously. M. R. Lewis ('25) has observed carbon-laden monocytes leaving the pulmonary capillaries to enter the alveoli of the lungs of living frogs; she mentions, however, that this animal does not normally possess dust cells. Herzog ('24) has also noted carbon-laden sessile cells in the capillaries of a frog's tongue detaching themselves and floating off into the circulation, or migrating through the vessel walls and wandering to points quite distant therefrom. Of course, these "sessile cells" may have been clinging monocytes and only apparently connected with the capillary endothelium.

That even observations *in vivo* are subject to misinterpretation is shown by the fact that Stilwell ('26) has repeated Herzog's experiments in Maximow's laboratory and has been unable to confirm them. She finds that the vascular endothelium does, indeed, store ink for a time, indulging in what she calls "passive phagocytosis," but this ink is gradually transferred to the perivascular tissue while under direct observation. Furthermore, the ink-laden cells that leave the vessels in the frog's tongue are, according to her views, monocytes; she did not observe any rounding-up of the vascular endothelium to produce monocytes or polyblasts. This is of interest not only because it shows Herzog to be in error, but because it entirely refutes my former claims as to the specificity of ink for vascular endothelium and the argument that the cells seen to migrate from the vessels were therefore detached vascular endothelium. In the face of such observations, made *in vivo* and therefore quite different from deductions derived from the study of fixed tissue, one can only retract one's claims and admit that they are unsound.

Thus we come to a point where the matter seems to require some method other than the mere observation of sections of fixed tissue, be they ever so carefully made, for its solution. Wislocki ('24) has considered the origin of the dust cell to be impossible of detection by this means. He says: "We find it impossible from sectioned material or by the methods employed for its identification, to determine its origin." Employing supravital staining with neutral red and Janus green and dyeing the scrapings of fresh lung from

rabbits and a cat, after a preliminary intravenous injection of Higgins' ink, he found that the various pulmonary elements could be identified and classified. The leucocytes, erythrocytes and monocytes were easily recognized; the dust cells were found to be roughly divisible into three classes: Type (1) large cells with oval nuclei and a variable number of large neutral red vacuoles, Type (2) slightly smaller cells with many carbon granules and a smaller number of red vacuoles, Type (3) large cells which, in addition to a few specks of carbon and the neutral red vacuoles, contained large, greenish, refractile granules arranged about the periphery of the cells. The carbon charge was always in inverse ratio to the number of neutral red vacuoles, the inference being that the cells preferred carbon to neutral red; just as in Westhues' ('22) experiment it was found that they had a greater affinity for carbon than for carmine. The epithelium was often found in sheets or cords, it was partly of the non-ciliated cuboidal and partly of the ciliated cylindrical type. Its cytoplasm was filled with minute red granules. None of the epithelial cells contained carbon. Transitions between "clasmatocytes" (dust cells) of Types 1 and 2 were observed.

Wislocki concludes: "The carbon deposited in the capillaries of the lung is gradually eliminated probably by way of the circulation, the respiratory tract and the lymph channels. After the initial phase of deposition of carbon particles in the lungs, phagocytic cells play a prominent part in its storage and elimination. By studying the fresh, living cells of the lungs in a warm-box, it has been shown that the carbon particles are phagocytosed principally by clasmatocytes. The origin of these cells in the lungs is discussed." As to this point, he says: "Three possibilities suggest themselves. The first is that it (the dust cell) arises by mitosis from the endothelium of the pulmonary capillaries. The second is that it arises in the liver and spleen and is carried by the circulation to lodge in the lungs. The third possibility is that it arises in the stroma of the lungs from clasmatocytes normally present there." In explanation, it may be said that "monocytes" are Aschoff's "blood-histiocytes," the "large mononuclear leucocytes" of clinical parlance; while "clasmatocytes" are the "tissue histiocytes" of Aschoff, "endothelial leucocytes" of Mallory, "polyblasts" of Maximow, etc.

An article by Masugi ('27) appears in the same number of "Ziegler's Beiträge" as does Gross' paper; it describes the tinctorial char-

acteristics of the monocyte and histiocyte when treated supravitally with neutral red and Janus green or stained with other dyes. The beautiful color plates correspond very closely with Wislocki's description of the cells observed in lung scrapings. As Masugi's work was undertaken "at the suggestion and under the direction of" Professor Aschoff, it would seem that Wislocki had satisfactorily complied with the criteria for identifying monocytes and histiocytes as laid down by the latter. This will be of great importance later on in this article.

Fried ('27) has just published a report in which he apparently denies the presence of the alveolar epithelium altogether, but a careful perusal of his paper brings out the fact that he, too, concedes the existence of non-nucleated epithelial plates. According to him, what appears to be respiratory epithelium is, in reality, masses of histiocytes that are attached to the alveolar wall near the angles and which, when stimulated by the presence of foreign material, become detached to give rise to the typical dust cell, or "Herzfehlerzell." His most striking results were obtained in rabbits by injecting large doses of pyrrhol blue in 1 per cent concentration in Ringer's solution intratracheally, either in one massive (acute) dose, or in five smaller (chronic) doses of 5 cc. each. Because the cells attached to the alveolar wall gave a typical picture of vitally stained histiocytes, he believes that they are of mesenchymal and not of epithelial origin. His conclusions are necessarily based upon morphology alone. The epithelium of the bronchi, although drenched in the dye, remained unstained; the vascular endothelium of the pulmonary capillaries was also unstained; he could demonstrate no respiratory epithelium and therefore he disbelieves in its existence. He rules out the monocytes as parent cells because of the total lack of histiocytic proliferation in the other organs. Well founded though these conclusions may be, they leave loop-holes for the critic in so far as they fail to explain the histogenesis of the groups of mural histiocytes, and overlook the possibility of independent propagation of the monocytes while circulating in the blood stream. His failure to demonstrate respiratory epithelium was due to his technic, for it may be easily demonstrated with silver impregnations.

Having surveyed the literature in a fairly comprehensive fashion, one must admit that the subject is still unsettled, as both Gardner ('26) and Sacks ('26) remark in recent reviews on this topic. If there

be a way in which it may be settled, it would appear to lie along the lines of: (*a*) Supravital staining, (*b*) intravital or supravital injection of dyes, (*c*) by the discovery of a specific method for staining the dust cell and its parent cell in fixed tissues. Therefore the work detailed in the remainder of this paper has been undertaken along these three lines. For a more general consideration of the ramifications of the reticulo-endothelial system the reader is referred to reviews by Aschoff ('24), Foot ('25), Gardner ('26), Sabin ('22) and Sacks ('26).

Report of Original Experimental Work

In order to attack this problem from several angles at once, the following experiments were performed. Rabbits were given intravenous and intratracheal injections of various dyes and of milk, the cells that responded in the alveoli were examined *in vivo* in supravital films, the lungs were sectioned after freezing or after embedding in paraffin, and examined from the morphologic standpoint. A specific method for identifying monocytes, histiocytes and dust cells on the one hand and distinguishing them from epithelium or from pleural mesothelium on the other, was fortunately found. Material from scraped human lungs was examined supravitally as soon as permissible postmortem and the sputum from a patient with aortic insufficiency was investigated in supravital films. Lung tissue from similar cases was sectioned in paraffin and studied with routine stains and the specific silver tannate technic. The findings with the latter were checked up with material from fresh human spleen and a case of tuberculous meningitis. Fresh human spleen was also examined in supravital films and the findings confirmed those obtained with the silver technic.

Experiments on Rabbits

Technic (Experiment No. 1). The first experiment combined the technics of Eliot ('26) and Wislocki ('24). Two-tenths gm. of carmine rubrum optimum (Coleman & Bell) were ground in a mortar until fine and stirred with 6 cc. of distilled water, added little by little, until the mixture was smooth. It was centrifugated for ten minutes to throw down the coarser particles, boiled and cooled to body temperature. Four cc. were then injected into the ear vein of

a rabbit and 25 cc. of its heart's blood were withdrawn after a wait of fifteen minutes into a syringe containing 2 cc. of 10 per cent aqueous sodium citrate. After mixing this well, 8 cc. were injected intravenously into each of three rabbits. These next received intratracheal injections of from 3 to 4 cc. of pasteurized milk containing 0.5 per cent of saturated alcoholic Sudan III. The milk was administered slowly through an aspirating needle introduced directly through the skin into the trachea, an influx of air bubbles into the syringe indicating that the needle had penetrated to the lumen. The rabbits stood the injection well; there was transient dyspnea with some regurgitation of milk, but after a minute or two they were on their feet again and quite alert. By the next day they seemed perfectly well.

One rabbit was killed 24 hours, the next 48 hours and the third 90 hours after the injection, by introducing air into the circulation. Necropsy revealed lungs of an almost normal external appearance in the case of the first two rabbits, but section showed some consolidation at the bases, and slices from these sank in water. Nevertheless, the consolidated lung lacked the congestion and granular appearance of the usual pneumonia. The lungs of the third rabbit were in no way grossly remarkable.

As each rabbit was killed, the chest was opened, the lungs and heart removed *in toto* and bits of lung were either squeezed out or scraped off on slides coated with neutral red and Janus green or Nile blue sulphate. Coverslips were sealed over the drops with paraffin-vaseline, a little Ringer's fluid being added if the material was too scanty to cover the whole coverslip, and the preparations were incubated for a few minutes at body temperature before being examined microscopically on a warm stage. The slides were prepared by adding 40 drops of 1 per cent neutral red in absolute alcohol and 30 drops of Janus green in like solution, to 10 cc. of absolute alcohol, flooding clean slides and allowing them to dry. The Nile blue sulphate was used undiluted in 1 per cent solution in absolute alcohol. Before the blood from the donor rabbit was injected into the others, it was examined to determine that there was no free carmine in its plasma.

After these films were completed, the lungs were injected through the trachea with 10 per cent neutral formalin until moderately distended and dropped entire into that fixative. Part of the material

was sectioned on a freezing microtome and part embedded in paraffin and subsequently sectioned at 7 microns.

(Experiment No. 2.) This practically duplicated the first, but five cc. of the carmine suspension were injected intravenously into the donor rabbit and its blood was withdrawn five, instead of fifteen minutes later. Only two rabbits received this blood intravenously and only 1 cc. of milk (this time diluted one-half with Ringer's fluid) was injected into their tracheae. The supravital examinations were omitted in this case and the lungs were removed *in toto* 24 and 48 hours after they had been injected with the milk. In neither case was there anything abnormal to be noted, the organs being of normal color, consistence and general appearance.

Donor Rabbits: The donor for the first experiment died about a week after giving its blood. No gross evidence for its death was manifest at necropsy. Its lungs were removed and injected with neutral formalin, as in the other cases; its spleen was also fixed for microscopic examination.

The donor for the second experiment was killed by air-embolus 96 hours after the bleeding and its lungs (which appeared in no way abnormal, although in this case the donor rabbit had also received a milk injection) were fixed as above. Bits of spleen and liver were also secured for examination as to the distribution of the carmine.

Purpose of the Experiments

By introducing "marked monocytes" from another animal, it was hoped that the blood origin of the intra-alveolar phagocytes might be proved or disproved; if these contained carmine it is obvious that they would most probably represent marked cells from the donor animal — unless polymorphonuclear leucocytes should have emigrated and been engulfed by the dust cells, in which case uncertainty would result. The milk was used as a mild irritant, to excite the emigration of macrophages into the alveolar sacs; incidentally, the neutral fat would serve as a dye if it retained the Sudan III, if not, use could still be made of it by staining with more of that dye or using Nile blue sulphate. Ballon and Ballon ('27) have used this method for tracing the fate of lipiodol in the lung. The purpose of the supravital stains was to determine the behavior of the dust cells, the epithelium, vascular endothelium and mesothelium toward

these stains. Nile blue sulphate would also act as a fat stain and indicate whether the neutral fat underwent chemical changes after becoming incorporated in the cells.

Results in Supravitally Stained Films

These confirmed Wislocki's findings in every particular, his three types of "clasmatocytes" were recognized and their striking similarity to Masugi's illustrations was at once noted. The results with Nile blue sulphate were as satisfactory as those with neutral red; this dye gives more rapid impregnation of the granules and the pictures are sharper. Reviewing the types of cells noted in both stains, but omitting a description of the neutral red pictures, — as Wislocki has already covered this, — we find the following:

Dust Cells: These were large, rounded or ovoid cells with numerous dark blue to greenish granules which varied not only in color, but also in size and shape. There were fine, sharply stained granules and large, variably stained vacuoles which sometimes contained a speck or two of dust. Some of these cells showed uniform dark blue granules with a pale, yellowish, unstained nuclear space; others showed the variable characteristics noted for dust cells in general, while a third type — the largest of the three, contained large, polyhedral, refractile pieces of yellowish or brownish material as well, sometimes almost as large as erythrocytes. Frequently there were large vacuoles in the third type that contained either erythrocytes or drops of neutral fat, which stained rose with the Nile blue sulphate. The mitochondria seen in the neutral red-Janus green technic were not prominent in this case. Sometimes one occasionally observed carmine-colored granules, but as they were also present in films from controls that had received no carmine they probably represent metachromatic staining. Attraction-spheres were not prominent. The carmine injected in the first experiment could not be found in the blood cells of the animals that received the transfused blood. This is not readily explained, for the only differences in the technic, as compared with Eliot's, were the use of sodium citrate instead of heparin and a slightly longer delay in withdrawing the blood. The second experiment demonstrated that this delay could have made little difference in the results.

Monocytes: Small monocytes were frequently encountered in the stained films; a little larger than polymorphonuclears, they were

usually filled with blue granules, uniform in size and brilliantly stained, with the untinted nucleus crowded to the periphery. A large proportion of these showed a cluster of granules in the concavity of the reniform nucleus, with a more thinly distributed line of granules extending like the horns of a crescent to the nuclear poles; a comparatively unstained zone separated them from the cell periphery (Figs. 1 and 2). The appearance of these cells, when stained supravitally with neutral red and after fixation with Sudan III and silver tannate, is shown in Figs. 4, 5 and 6. Figure 3 shows a monocyte with a phagocytosed fat droplet, transitional between Figs. 1 and 2 and Fig. 7, which represents a "Type 3" dust cell. Figure 8 is a similar transitional type impregnated with silver tannate and containing a fat vacuole.

Polymorphonuclears: These stained a diffuse light blue and showed yellowish granules, with a few that were blue.

Lymphocytes: They took on an even, diffuse light blue color though unstained as to nucleus and granules.

Vascular Endothelium: Bits of capillaries were included in the scrapings (Fig. 10), with a few erythrocytes in their lumina. Nothing indicated that they stained specifically, nor were the fibroblasts at all granular.

Epithelial Cells: Three types were recognized:

(1) Large, flat or slightly curled plates with no nucleus or merely a shadow of one (Fig. 9).

(2) Groups of interlocking, flanged cells, some of them showing denser nuclear shadows and resembling the descriptions of Bremer ('04), Ogawa ('20) and Stewart ('23).

(3) Non-ciliated cuboidal, or ciliated cylindrical cells from the bronchial mucosa.

None of these cells stained deeply, the larger cells showed a diffuse light blue, but no granules (very fine granules were seen with neutral red), while the small bronchial cells either stained not at all, or only faintly with an occasional minute granule of Nile blue sulphate. Some of the groups showed a drop or two of rose-colored neutral fat in, or on, their cytoplasm. Branching, Y-shaped streaks were observed in the large epithelial plates, corresponding to Stewart's pictures of epithelial mitochondria. Sometimes, where there were extensive sheets of epithelium which had separated from the alveoli, one might observe vitally-stained, rounded cells lying

in small fenestra in the membrane; these were probably "septum cells" seen from above.

Mesothelium: Sheets of pleural tissue were quite unstained with Nile blue sulphate, although very fine, closely arranged and refractile granules which stained faintly with neutral red could be observed in their cytoplasm.

Free Fat: This was present in the form of larger or smaller drops of light orange or rose, according to the stain. Apparently most of the Sudan III in the milk had disappeared.

Frozen Sections; Sudan III Stain

(First Experiment)

These showed that the dyed milk injection had caused a mild lobular pneumonia in which the exudate was composed of polymorphonuclear leucocytes and macrophages in about equal numbers; the greatest reaction was seen in the lung of the two-day rabbit. After four days the lungs had practically returned to normal, but there was still a large number of dust cells in the alveoli. As we are interested chiefly in the fat in these sections, let us confine our attention to that substance. It lay free in the air-sacs at first and was then taken up by the phagocytes, the epithelium being merely dusted with fine particles. The large cells protruding from the alveolar septa (considered to be epithelium by Aschoff, Gross and Westhues, and histiocytes by Kiyono, Fried and Gardner), contained much fat. In the frozen sections there was little, if any clue as to the origin of these cells. The monocytes in the capillaries, the polymorphonuclears to a lesser degree and the vascular endothelium all showed intracellular fat and there appeared to be some of it free in the blood plasma, although this may have been scattered out in the process of sectioning. Most of it was contained in dust cells and monocytes. Occasionally globules were found between the bronchial epithelial cells, but their cytoplasm was practically free from Sudanophil material, although diffusely "rusted" by the stain. There was a moderately heavy deposition of fat in the peribronchial lymph nodes, some within phagocytes and some apparently free.

The areas of bronchopneumonia in the first two rabbits showed such distortion of the normal pulmonary architecture that very little could be judged as to what had taken place; apparently the walls

of the alveoli had become thickened by engorgement of the capillaries and proliferation of the stroma, and exudate had filled the air-sacs here and there. There were fields of large, pale cells liberally dotted with fat, closely resembling those in Fried's photomicrographs, but there was little evidence to show whence they came. These might have been histiocytes, proliferated adventitial cells, thickened capillary endothelium or migrated monocytes — that they were epithelium, however, was most improbable as they lay outside of the air sacs, rather than within them.

The only assistance obtained from the use of Sudan III, then, is the fact that it is taken up by the phagocytes in a manner that stains them in the same way that either neutral red or Nile blue sulphate does. The granules, vacuoles and foreign material are found to correspond accurately in all three methods, therefore there must be a striking similarity in the chemistry of these stains within the cytoplasm.

Paraffin Sections

(*First Experiment*)

These differed very little from the frozen sections. As the fat had been extracted by the chloroform, the pictures were somewhat less complicated. No carmine could be definitely identified, although many leucocytes and dust cells contained minute granules that appeared to give off a reddish luster under the oil-immersion lens. The experiment, then, completely failed to corroborate Eliot's observations.

Silver Tannate Impregnations of Paraffin Sections: (First experiment.) As cell granules were well demonstrated by silver tannate impregnations used in experimenting with the Rio de Hortega technic (Foot, '27), it was supposed that this method might aid in solving the dust cell problem. As a result, a very satisfactory means of identifying monocytes and polyblasts was discovered. The sections impregnated with silver tannate showed that the dust cells became reddish brown, with brownish black to sepia granules that corresponded in every way with those observed in the supravital films and the Sudan III sections. Furthermore, these granules were usually grouped in rosettes or balls as in typical monocytes. No other cells in these sections except the polymorphonuclears showed similar characteristics; these had similar, but rather finer granules,

and in the fat-treated lungs they tended to take on a somewhat reddish tinge.

Here, then, is a means of impregnating dust cells specifically. As there was a possibility that this depended upon the presence of ingested fat, lungs from six other rabbits were sectioned and impregnated in the same way, invariably showing the same thing although presumably fat-free. The dust cells, monocytes and polymorphonuclears were the only cells that became specifically impregnated. By combining supravital intratracheal staining with neutral red, Niagara blue 3b or Nile blue sulphate injected at the time of death from air-embolism and incubated *in situ* in the dead rabbit for fifteen minutes, as recommended by Gardner ('27), slight variations in the color reactions of the impregnation were effected, but the granular impregnation remained the same. As the fixation was ordinary neutral formalin and the routine paraffin technic was used, instead of Gardner's more elaborate procedure which requires an adjustment of the pH of the fixative necessitating special color indicators or a potentiometer, the results with neutral red were inferior to his. The cells showed the typical granular stain seen in supravital films, however, but the bright red of these had paled to a dull brick-red.

In order further to check up on the specificity of the silver tannate method, smears of rabbits' blood were made, fixed in neutral 10 per cent formalin and impregnated. The monocytes and polymorphonuclears showed the specific dark brown cytoplasm and sepia granules seen in the sections, although the monocytes tended to be paler in the smears. The lymphocytes were pale and showed no granular stain, their cytoplasm was almost colorless and their nuclei stood out distinctly, as with iron hematoxylin. The erythrocytes were either slightly and diffusely brownish, or where they had dried and laked somewhat, showed blackish reticulation. This reticulation was observed in some of the sections also.

Observations in Silver Tannate Sections: In the sections from the first milk-injection experiment, monocytes were found crowding the capillaries, undergoing karyokinesis while within their lumina, and dividing within the alveoli after emerging from the vessels. A monocyte is shown in a large vein in Fig. 11. Careful scrutiny of all the slides failed to demonstrate any coarse granules in the epithelium, mesothelium or vascular endothelium. The large cells that

are attached to the alveolar septa appeared in the rôle of greatly swollen, clinging monocytes; smaller monocytes might be seen congregated in the capillaries and apparently emerging therefrom (Fig. 12). There was a general dotting of the various pulmonary elements by a light silver precipitate, but this was totally different from the sharply defined and definitely grouped granules in the monocytes or dust cells (Fig. 13). In properly impregnated sections the epithelium of the alveoli could be observed as it lined the extremity of an air-sac and one could discern the epithelial cells and non-nucleated plates, and the capillaries and reticulum beneath them (Fig. 14). The latter was an immense help in keeping the topography of the lung clear in the infiltrated areas. The epithelium could be better brought out in the sections where neutral red had been injected at the time of death. It was seen to correspond accurately with the descriptions of Ogawa and Stewart, except that my preparations showed fenestrations in the epithelial membrane, often occupied by intercalated dust cells. Of course, the empty fenestra might have been fixation artifacts, but the presence of dust cells in some of them, both in these sections and the unfixed scrapings observed *in vivo*, makes this quite unlikely.

Where the alveolar walls had become much thickened in the areas of lobular pneumonia, one noticed two things: The capillaries were distended with monocytes and polymorphonuclears and their endothelium had become vague and merged with the stroma, so that wide fields of pale cells might be observed, with here and there a monocyte lying in the tissue spaces. The cells of these fields resembled vascular endothelium, adventitial or connective tissue cells, — pale, swollen, with ovoid and vesicular nuclei and vacuolated, reticulated cytoplasm. They showed no definite granules other than an occasional blackish grain that might have been precipitate (Fig. 15). On more than one occasion dust cells were observed to be enveloped in veil-like epithelial plates, as though these had been lifted off by them and carried into the alveolar space, where they remained folded about the cell that had detached them.

Findings in Second Rabbit Experiment

Attention was focussed upon paraffin sections stained with hematoxylin alone or impregnated with silver tannate. The observations tallied in the main with those of the preceding experiment, but the

mixture of equal parts of pasteurized milk and Ringer's solution, injected in much smaller quantities, caused only a local reaction in the bronchi. There was no pneumonia. No carmine-marked cells were found in any of the sections, it seemed as though none had been transferred from the donor rabbit, despite the fact that it had a larger intravenous injection of carmine suspension than did the first donor.

Findings in Donor Rabbits

The donor rabbit of the first experiment showed relatively little carmine in the lungs and a good deal in the spleen. In the former it was contained in monocytes and polymorphonuclears within the capillaries and very occasionally within a dust cell in which it appeared as a group of carmine particles that occupied about as much room as would an erythrocyte. In the spleen the carmine was in large macrophages which, owing to the fact that the rabbit was found some time after its actual death, also contained the oxydase granules that one finds in somewhat "spoiled" formalin-fixed tissue. These were brownish black and corresponded in their arrangement with the vital granules seen in supravital, Sudan III and silver preparations. These were also present in the monocytes and polymorphonuclears of the capillary sinuses and larger vessels, where more or less carmine was also encountered.

Two points are brought out in this case: The spleen had apparently acquired most of the injected carmine. Certainly it contained more than the lungs, and the fact that dust cells were found in the air-sacs, laden with carmine that had been introduced intravenously, strongly indicated that this particulate material had been carried out of the vessel within the cytoplasm of emigrating monocytes, rather than transferred from the circulation to intra-alveolar dust cells. If the latter hypothesis were true, the carmine would have to traverse the vascular endothelium, stroma and alveolar epithelium. Furthermore, no carmine was found free in the alveolar spaces.

The donor rabbit in the second experiment, killed by air-embolism four days after bleeding, showed some carmine in macrophages in the spleen and a trace in the Kupffer cells of the liver. None was found in the lung. Although this animal had some milk injected into the trachea, the lungs were practically normal.

Silver tannate impregnations of the liver and spleen showed the

specific granular stain in the Kupffer cells of the former and in monocytes circulating in the sinusoids; the spleen was sprinkled with monocytes, also showing the specific granules. These findings further strengthen the supposition that this method marks monocytes specifically, and they point out the fact that the Kupffer cell is, indeed, different from ordinary endothelium. It takes a granular impregnation quite similar to that seen in the monocyte, which apparently indicates a close relationship between the two. Are Kupffer cells, for instance, merely monocytes anchored to the sinusoidal endothelium by one or more pseudopods?

Examination of Human Material

Supravital Films of Lung Scrapings: Scrapings made from lungs in cases of chronic passive congestion were treated exactly like those from the rabbit lungs; the cases chosen for examination were necropsied as soon after death as permissible. In lungs removed as long as eight and fourteen hours postmortem, the results resembled in every detail those obtained in the case of rabbits. The cells were still viable in both instances and were observed on a warm-stage, the staining of the nuclei being considered a criterion of cell death. The same types of epithelium, stroma, blood cells and dust cells were observed; the latter were plainly marked with the carbon that abounds in Cincinnati atmosphere.

Supravital Films of Sputum: Fresh sputum from a case of cardiac decompensation was obtained from the wards on two occasions and examined by the supravital method; again the results were quite similar, although the copious and tenacious mucus interfered rather noticeably with the efficacy of the neutral red. Nile blue sulphate, however, still gave good results, and the check-up on the observations of necropsy material was perfectly satisfactory.

Paraffin Sections from Human Lung: Specimens of lungs showing chronic passive congestion were sectioned and stained with hematoxylin and eosin as a control, and the silver tannate impregnation was used for critical observation. They showed, even more strikingly than the rabbit lung, the specific character of this impregnation for dust cells. These were very dark, filled with small, uniform black granules and they stood out in bold relief in comparison with the desquamated epithelial cells whose cytoplasm showed no granules and was rather vacuolated and violet-gray. This is well shown

in Fig. 16. Here again, one found the epithelial plates wrapped about the dust cells. Comparing the hematoxylin and eosin controls with the silver impregnations, one noted that the former showed dust cells heavily laden with hemosiderin and carbon ("Herzfehlerzellen"), but totally lacking the smaller, uniform black granules that were so prominent in the silver impregnations (Fig. 17). Another case of chronic passive pulmonary congestion with infarcts was examined and the granular impregnation of the dust cells was far less striking; this was in material that had been fixed some time postmortem and proves that one should be sure of the freshness of one's material before drawing conclusions adverse to this technic. It has been found to be quite worthless in brain tissue that has remained unfixed for a day or more. Even shorter periods are unfavorable to the specificity of the stain in warm weather.

Spleen and Brain: Paraffin sections from a human spleen and from the meninges in a case of tuberculous meningitis show that the monocytes, polymorphonuclears and polyblasts in these become impregnated exactly as in the case of lung tissue. The splenic endothelium of the capillary sinuses differs absolutely in its staining properties from these cells. The reticulo-endothelium is so vacuolated, so intimately associated with fibrils, and its granules so indefinite, that it also differs from them to a certain extent.

Silver Tannate Technic *

Cut thin paraffin sections from formalin or Zenker-fixed material and remove the paraffin in the usual way. If Zenker's fluid has been used, remove the mercury by treating the sections for five minutes in mahogany-brown, alcoholic iodin solution. Bleach in 5 per cent aqueous sodium thiosulphate. Wash. Remove the chromium salts by five minutes treatment in 0.25 per cent potassium permanganate and ten minutes in 5 per cent oxalic acid, washing between solutions. Wash and re-wash in distilled water. Mordant for fifteen minutes in a solution of 0.15 per cent pure tannic acid, 3 per cent ammonium bromid and 10 per cent neutral formalin; this should be done in an incubator, first heating the mordant to 55° C. Treat for thirty

* It is advisable to summarize this procedure here, although it is described in full elsewhere (Foot, '27).

seconds with three drops of strong ammonia to 100 cc. of distilled water, while the sections are still warm. Impregnate for five minutes in silver-ammonium oxid prepared as follows: To 10 cc. of 1 per cent silver nitrate add one drop of 40 per cent sodium or potassium hydroxid, dissolve the precipitate in five drops of strong ammonia (which should leave a few grains still out of solution), dilute up to 200 cc. with distilled water. Use two baths if the first becomes turbid after two or three minutes. Wash in distilled water and "reduce" for two minutes in 20 per cent neutral formalin. Wash at the tap. "Tone" for two minutes in a 1:500 solution of Merck's "brown, acid" gold chlorid, in which 0.5 per cent bichloride of mercury has been dissolved with the aid of heat. Wash at the tap and fix in 5 per cent sodium thiosulphate (Hypo) for two minutes. Wash and dehydrate in the usual manner with alcohol of increasing percentages, xylol, and mount in Canada balsam.

If the sections be too dark, they may be lightened by immersion in strong potassium cyanid solution (aqueous), but it is better to run through a new set of sections and increase the strength of the ammonia wash, used after the mordant, to ten drops instead of three to 100 cc. of distilled water. Tissues vary a good deal and one must do a certain amount of experimentation on each batch of slides in order to produce the best results. The depth of impregnation may be largely controlled by the strength of the ammonia wash — a weak wash producing dark sections, a strong one lighter impregnation.

Discussion

We come, finally, to a consideration of this subject as a whole in the light of the evidence of others which is, perhaps, somewhat intensified by the additional data supplied by the experiments just described. The easiest way to undertake this task will be to set down *seriatim* the various hypothetical sources of the dust cell and to discuss each in turn.

Epithelial Origin

As the arguments in favor of this have been set forth at the beginning of the paper, let us consider those opposed to it. There are several valid reasons for rejecting the epithelial theory.

1. Supravital staining with neutral red and Nile blue sulphate and the apparently specific silver tannate impregnation not only

fail to demonstrate any similarity between epithelium, whether alveolar or bronchial, and the dust cell, but actually separate them into two distinctly morphologically unrelated groups.

2. Dust cells often appear in the lymphatics and lymph nodes of the lung laden with carbon, tubercle bacilli, dyes, fat or other material that has come in through the trachea or circulation. There they remain. Permar ('23), Haythorn ('13), Sewell ('18) and Foot ('20) have all reported this phenomenon. Is it more likely that these are cells of mesenchymal origin, or strangely metamorphosed epithelium? Sewell manifested a great deal of difficulty in reconciling the latter view with his findings and his explanation was not, even then, at all convincing. He was forced to regard these as renegade epithelial cells that had become transformed into leucocytes.

3. Macrophages or polyblasts are found in large numbers in a variety of processes in epithelial organs, but apparently it is only in the case of the lung that their presence is ascribed to epithelial proliferation and desquamation.

4. It has been proved that the epithelioid cells of hepatic tuberculosis are formed directly from the Kupffer cells (Evans, Bowman and Winternitz, '14; Goldman, '09, '12; Oppenheimer, '08 and others. To-day no one would consider the bile duct or hepatic epithelium as a source of epithelioid cells in tuberculosis of that organ. As the Kupffer cell appears to be somewhat different from the ordinary vascular endothelium, its participation in the formation of tubercles may be misleading to those who maintain that the vascular endothelium produces the macrophages, as I once believed.

5. Tubercles identical with these arise in the spleen and lymphoid tissue where phagocytes in every way similar to dust cells abound and where there is not epithelium to produce them.

6. Typical multinucleated syncytia, or giant cells, are formed from the dust cells in the alveoli; this is not characteristic of epithelium, which forms multinucleated cells usually under neoplastic conditions, but these differ materially from the typical foreign body giant cell.

7. Stewart ('23) describes the mitochondria of alveolar epithelium, when stained by the Altmann method, as rod-like, often branching or Y-shaped. Such structures may be seen in the epithelial cells in supravital films of scraped lung. I have pictured them in Figure 9. Stewart also shows a mature epithelial cell "about

to desquamate" into the alveolus; the striking change in its granules is at once apparent; instead of rod-like mitochondria, we see spheroidal granules of varying size. The cell corresponds accurately with a swollen monocyte or dust cell; was he not picturing one of these in this instance? No Y-shaped or rod-like mitochondria are seen in the dust cells, which differentiates them sharply from epithelium.

8. Finally, the arguments of the supporters of the epithelial origin of the dust cells are based exclusively on morphologic similarity, chiefly in hematoxylin and eosin preparations or similar sections. This similarity, under these circumstances, is certainly striking; is it, however, sufficient evidence to advance in the face of that of the proponents of the other theories, whose data are the result of observations on a variety of stains and technics?

If we put any faith whatever in our conceptions of tissue specificity, the idea that the pulmonary epithelium is alone capable of producing cells which, to all intents and purposes, not only resemble, but actually become indistinguishable from mesenchymal derivatives (when observed by a number of vital, supravital and fixed-tissue methods) is, to say the least, irrational. Were it established beyond a doubt that the epithelium could become transformed into connective tissue or adult mesenchyma — and *vice versa*, we might accept such an hypothesis with complacency.

Monocytic Origin

The interesting work of the Lewises ('23, to '25), Wislocki's ('24) findings and Maximow's ('26) long series of investigations, as well as the experiments detailed in this paper all point very strongly to the monocytic origin of the dust cell. Cunningham, Sabin and Doan distinguish between monocytes and clasmatocytes on a technical basis, depending upon the respective supravital staining characteristics. That this distinction is entirely warranted is still disputed by authors like Masugi, who either regard these types as phases of the same cell or take issue with the interpretation of their origin. Masugi considers that there are two types, monocytes ("Bluthistiozyten") and histiocytes proper; the Lewises regard these as different forms of the same cell; Maximow ('26) agrees with Masugi's interpretation in substance, but takes a broader view of the matter.

Personal observation indicates that the finding of typical rosettes, which should mark the monocyte as such, is more or less a matter of chance; they may or may not be present. In fixed tissue they seem to occur rather more regularly than in fresh films. Be this as it may, it seems that the weight of opinion is tipping the scale in the favor of the monocyte as the parent of the dust cell.

A very telling point in favor of the monocytic origin is the great rapidity with which these cells appear in the alveoli. The Germans have used this as proof of their epithelial origin, as desquamation could readily account for such a phenomenon, but it does not account for the radical difference in morphology between epithelium and dust cells when properly stained. Migration of monocytes from the capillaries is not open to this criticism, for not only do they exhibit the same general morphology, but they will (if kept alive *in vitro*) actually become indistinguishable from dust cells. This has been noted by Carrell and Ebeling ('22), by the Lewises, by Gardner and in my latest experiments. Mitosis may be observed in monocytes within pulmonary capillaries and in dust cells, which would add to their rapidity of production and, although not entirely accounting for it, would indicate that two closely related types were multiplying in response to the same stimulus.

The origin of the monocyte is for the embryologist to establish; once in existence, however, it seems that this cell is capable of independent self-perpetuation, without drawing on any particular organ or tissue reservoir to repair the inroads of an inflammatory process on the supply at hand. Removal of the spleen does not lessen the supply of monocytes in any way (Foot, '23) and the hypothesis that they are the progeny of the extremely specialized endothelium of the venous sinuses of that organ is contradicted by the striking difference in the morphology of the two types and, even more so, by the fact that splenic sinus cells do not stain at all in supravital films. This I have just determined by experiment on fresh human spleen.

For years Maximow has been championing the lymphocytic origin of some of the "polyblasts" in the organism. His "polyblast" is essentially the same as the macrophage, clasmatocyte or endothelial leucocyte. His theories have been sharply criticised and I ('25) have been one of the critics. He has recently published experiments ('27) that effectually disarm this criticism, as he reports

the observation of a transformation of lymphocytes in tissue cultures into polyblasts and fibroblasts, while alive and growing. His pupil, Bloom ('27), reports the transformation of lymphocytes, taken from the "water-clear" lymph of the thoracic duct of rabbits, into polyblasts. It is indeed difficult to remain skeptical in the face of this evidence, therefore we must add the circulating lymphocyte to the possible sources of monocytes and, through these, of dust cells.

Histiocytic Origin

That the histiocytes, or reticulo-endothelial cells of the pulmonary stroma share in the production of dust cells cannot be denied, but there is reason to believe that they play a subsidiary rôle. These cells do not become as sharply impregnated with silver tannate as do the dust cells, whereas the monocytes do. This does not prove that they are unassociated with dust cells, however, for they are in a different medium, more or less fixed and possibly correspond to the cells that spread out over the surface of the glass in tissue cultures or films. They were much increased in the experiments with milk injection and they contained much fat, hence they cannot be excluded as possible parents of dust cells.

Vascular Endothelium

That this tissue produces dust cells seems unlikely. The vascular endothelium does not stain in the same way and it appears to play an entirely passive part in these experiments. Where the reaction to the fat was most intense, the capillary walls seemed to be thickened, but as they were thronged with leucocytes and as the histiocytes and fibroblasts of the stroma were also more numerous, it was very difficult to ascribe to them any importance in the production of free phagocytes. While loth to retract my original claims concerning the rôle of the capillary endothelium in such conditions until convinced that they had become untenable, seven years of further investigation, a critical study of the experience of others, and the results of my recent study of these have all indicated that the time has come to admit that the origin of these phagocytes is more likely to be found in the blood, rather than in the capillary endothelium. In tuberculosis, measles (Mallory and Medlar, '20), typhus (Wolbach, Todd and Palfrey, '22), Rocky

Mountain spotted fever (Wolbach, '19) and a number of other diseases, there is such manifest proliferation of the vascular endothelium that it might be construed as productive of epithelioid cells and phagocytes, but one could consider this change to be limited to the vessel wall and interpret the wandering phagocytes as emigrated monocytes, or histiocytes. Gardner ('26) points out an inherent weakness in the arguments of Permar and myself, when he says that we admit the ingestion of carbon by the monocytes and yet interpret them as being derived from the carbon-laden vascular endothelium. "But this method of demonstration," he continues, "is subject to the objection that within the vessels there are at least two types of cells which take up the vital stain — the endothelial lining cell and also the circulating white blood corpuscles." This is quite true; like McJunkin ('18, '19) I considered that the monocyte was produced by vascular endothelium, but the matter has taken a different turn as data have accumulated to give evidence to the contrary. Moreover, the vascular endothelium is a tissue that will bear further study, — it is not too well understood at the present time.

Lymphocytic Origin

The theory that pulmonary phagocytes, as "polyblasts," are derived from lymphocytes would, at first glance, seem to be susceptible to the same criticism as the epithelium or vascular endothelium. The lymphocytes do not stain in the same way, either vitally, supravitally, or after silver impregnation, but Maximow's and Bloom's observations point too strongly to this possibility to permit its being lightly dismissed. We have no evidence that the lymphocytes become transformed into dust cells in the alveoli, but we cannot deny that they might become converted into monocytes in the circulation and thus enter the alveoli in a new guise and produce dust cells.

Summary

The wealth of evidence adduced from the literature and the experiments here described go far toward proving that the most likely origin of the pulmonary dust cell is from the blood stream and, more specifically, the monocytes thereof. That various reticulo-endothelial elements, such as the supporting cells of the lymphoid tissue of the lung, the adventitial cells of its vessels or even lymphocytes

themselves, share to a degree in the production of dust cells cannot be denied; that they play the chief part in this production, however, seems improbable. That the vascular endothelium gives rise to dust cells under ordinary conditions seems entirely unlikely, in the face of evidence accumulated during the past decade. In view of this fact it will be necessary to readjust our theories of inflammation so that they may more nearly conform with those of Metschnikoff and the Lewises on the one hand and of Maximow on the other. This *volte face* on my part is made only after due deliberation and in the face of what seems to me to be overwhelming evidence.

Conclusions

1. The dust cells or "Herzfehlerzell" are probably larger forms of monocytes or blood histiocytes. While the tissue histiocytes may play some part in their production, it is more likely that their ranks are recruited from the circulating monocytes of the bloodstream. The origin of these is discussed.

2. The alveolar epithelium of the lung does not produce dust cells in so far as can be ascertained; it possesses totally different affinities for silver salts and can, by means of silver tannate impregnation, be readily recognized and differentiated from the alveolar macrophages, which appear to be of mesenchymal, rather than of endodermal origin. Furthermore, as has already been pointed out by other investigators, the reactions of these groups to supravital stains are equally divergent.

3. The assumption that there is no nucleated "respiratory epithelium" does not appear to be warranted, for sections supravitally stained with neutral red and counter-impregnated with silver tannate show pictures in every way similar to those drawn by anatomic investigators. As the refractive index of alveolar epithelium is very close to that of glass, it is imperative that some procedure be used that will render it artificially visible, or that the light in the condenser be arranged so as to utilize the very slightly higher refractive index of the epithelium, otherwise it will escape notice.

The efficient assistance of Mr. J. B. Homan, of our Department of Medical Art, in taking these photomicrographs is hereby gratefully acknowledged. That of Mr. Marvin C. Mènard, in the animal experiments, is also appreciated.

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DESCRIPTION OF PLATE

PLATE 120

FIGS. 1, 2 and 3. These show cells supravitally stained with Nile blue sulphate. Fig. 1 shows the rosette form of granular arrangement and in Fig. 3 (young dust cell) a neutral fat globule has displaced the Nile blue sulphate granules.

FIG. 4. Monocyte stained with neutral red, supravital technic.

FIG. 5. Monocyte stained with Sudan III and hematoxylin, after fixation in formalin.

FIG. 6. Monocyte impregnated with silver tannate, after fixation.

FIG. 7. A typical dust cell, stained supravitally with Nile blue sulphate. It contains (*a*) Nile blue granules and vacuoles, (*b*) neutral fat droplets, (*c*) carbon particles and (*d*) refractile yellowish material. (Wislocki's "Type III Clasmatocyte".)

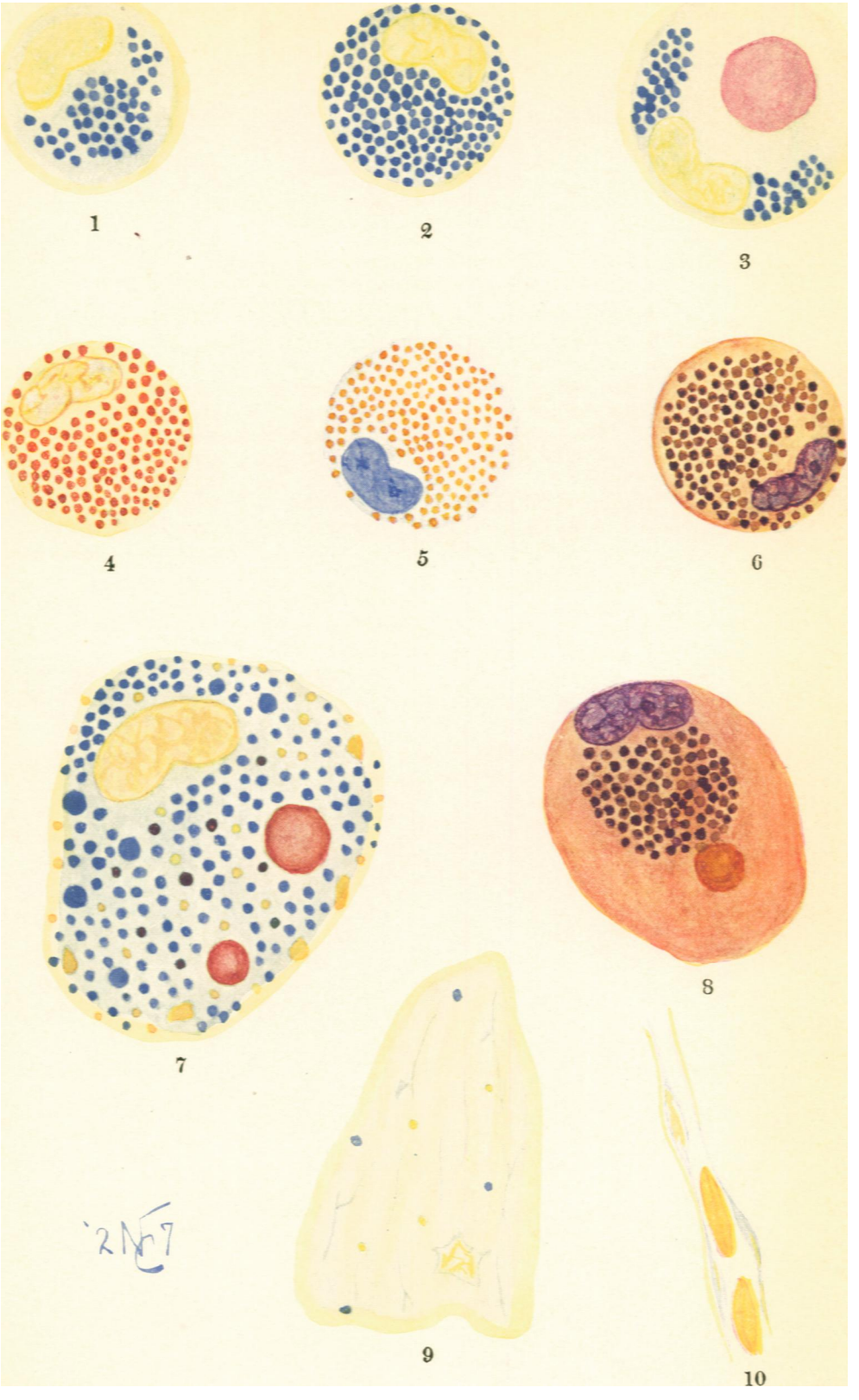
FIG. 8. Young dust cell, impregnated with silver tannate after fixation. Compare with Fig. 3. A fat droplet is present, but has not displaced the granules which form a rosette.

FIG. 9. A desquamated epithelial plate, supravitally stained with Nile blue sulphate. Note the Y-shaped structures and the general pallor of the cell, also the nuclear remnant.

FIG. 10. A bit of pulmonary capillary supravitally stained with Nile blue sulphate and drawn on a smaller scale. The vascular endothelium is free from granules.

The cells were outlined with a camera lucida and drawn in freehand.

Figs. 1 to 6 inclusive are semidiagrammatic drawings of monocytes stained in various ways.



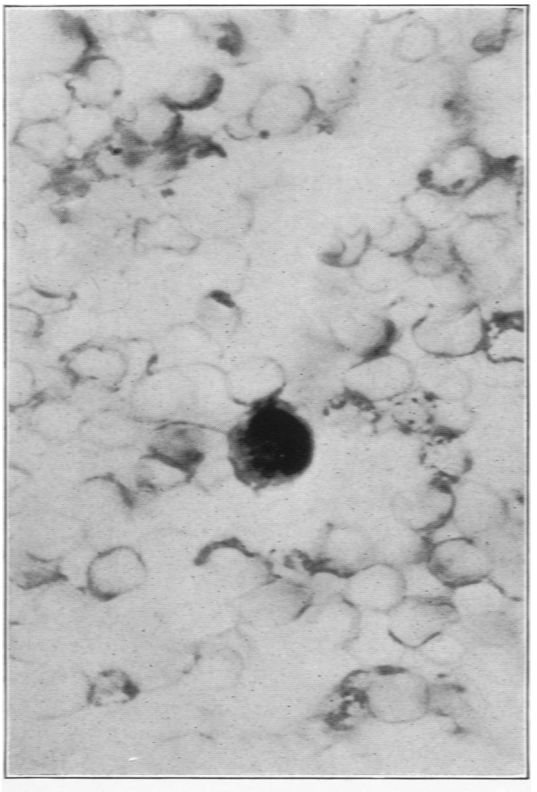
Foot Origin of Pulmonary Dust Cell

PLATE 121

FIG. 11. A monocyte in the blood of a pulmonary vein. Note the granules and the "attraction sphere" in the bight of the nucleus. Silver tannate technic. Oil-immersion photomicrograph, about × 1000.

FIG. 12. A group of monocytes in a capillary knot in a rabbit's lung. One of them has either emerged from the capillary, or is lying in an alveolar "niche." (Fenestrum?) Note the granules in these cells and the total absence thereof in the capillary endothelium. Silver tannate, oil-immersion. × 1000.

FIG.. 13. Dust cells in the alveoli of a rabbit's lung. Note the grouping of the granules and the absence of these in the alveolar epithelium and capillary endothelium. Some of the cells show fat vacuoles. Silver tannate. × 500.



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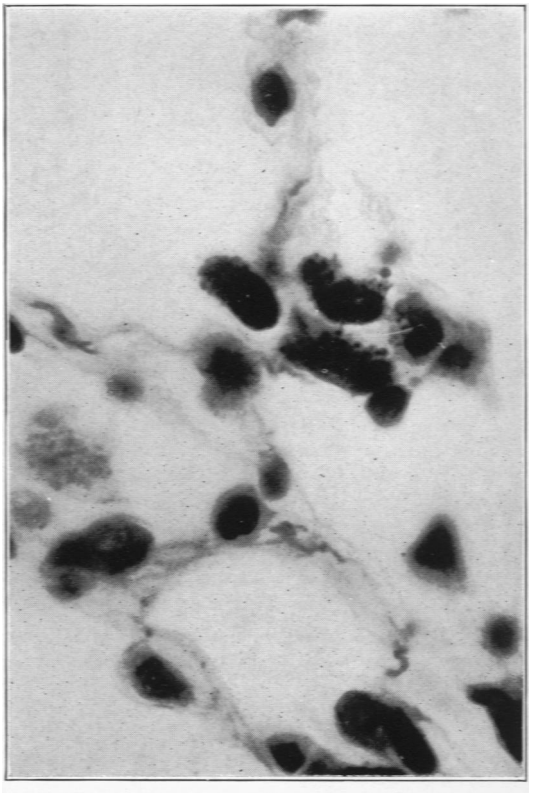

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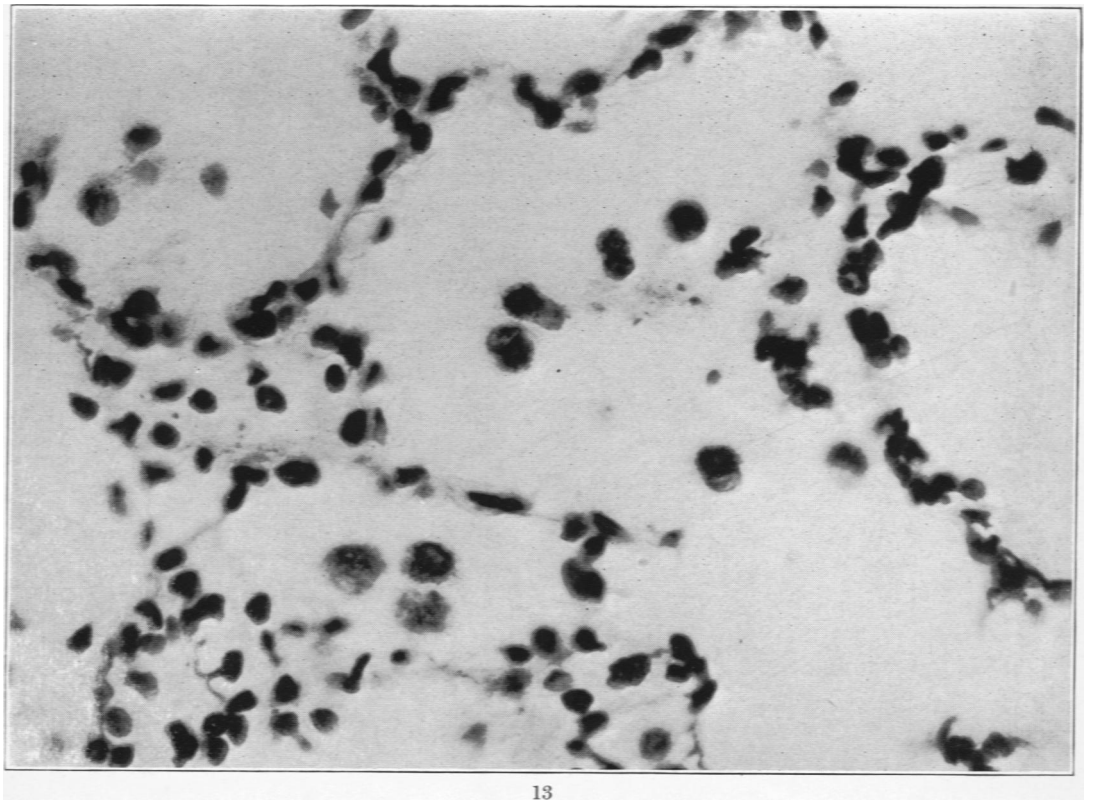

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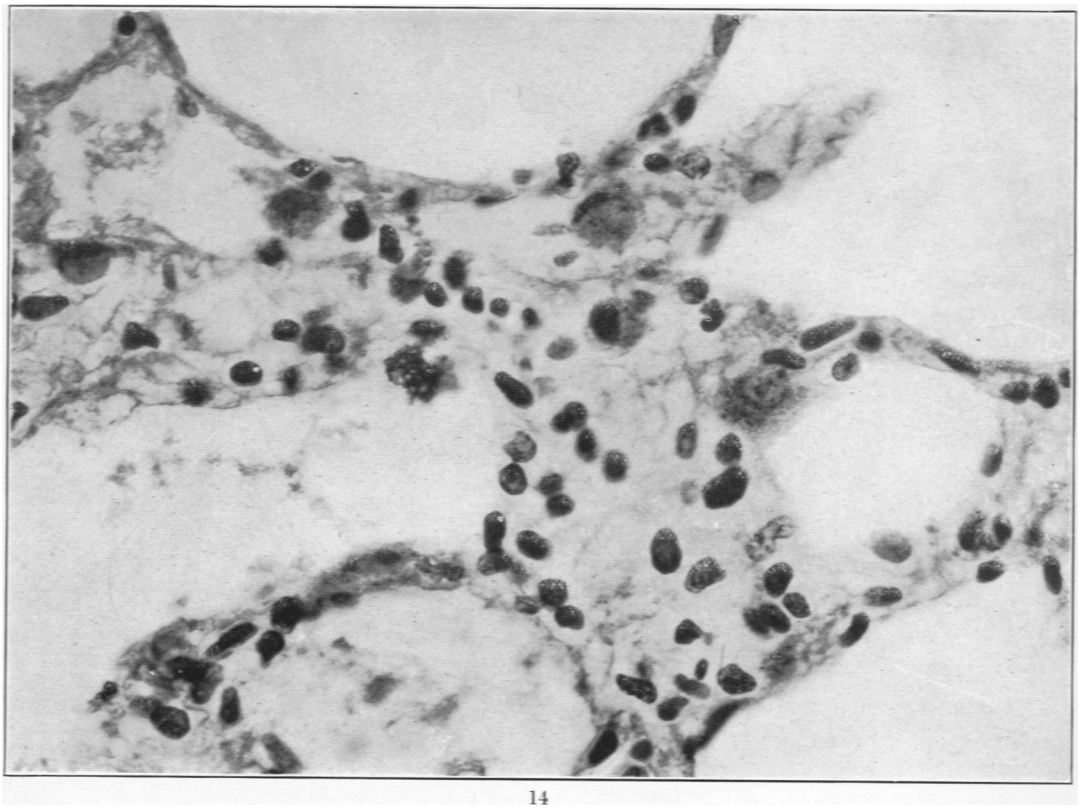
Origin of Pulmonary Dust Cell

PLATE 122

FIG. 14. Surface view of alveolar epithelium. Note the occasional fenestrations, the interposition of dust cells in these and the outlines of the epithelial "flanges" (non-nucleated plates). *Cf.* with Ogawa's drawings. ('20). There are no sizable granules in the epithelium. Silver tannate. × 500.

FIG. 15. A thickened area in a rabbit's lung 24 hours after the injection of milk. This illustrates the difficulty experienced in identifying the component cells in such foci of inflammation. Silver tannate. × 500.



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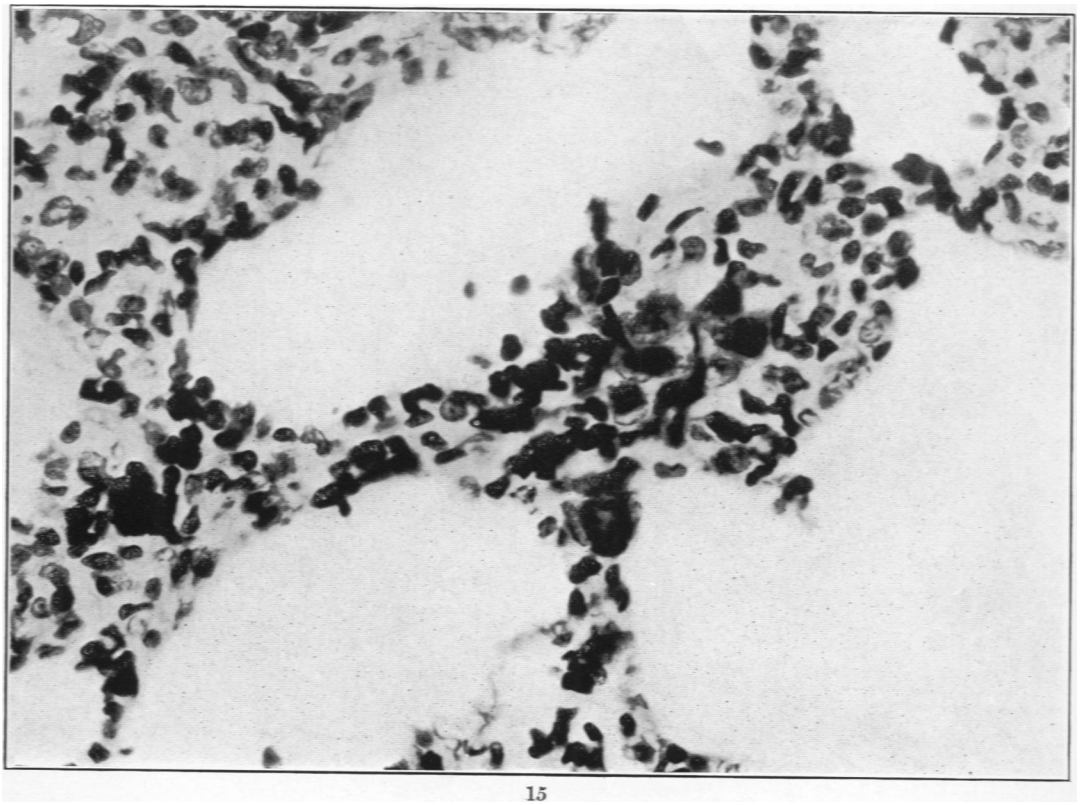

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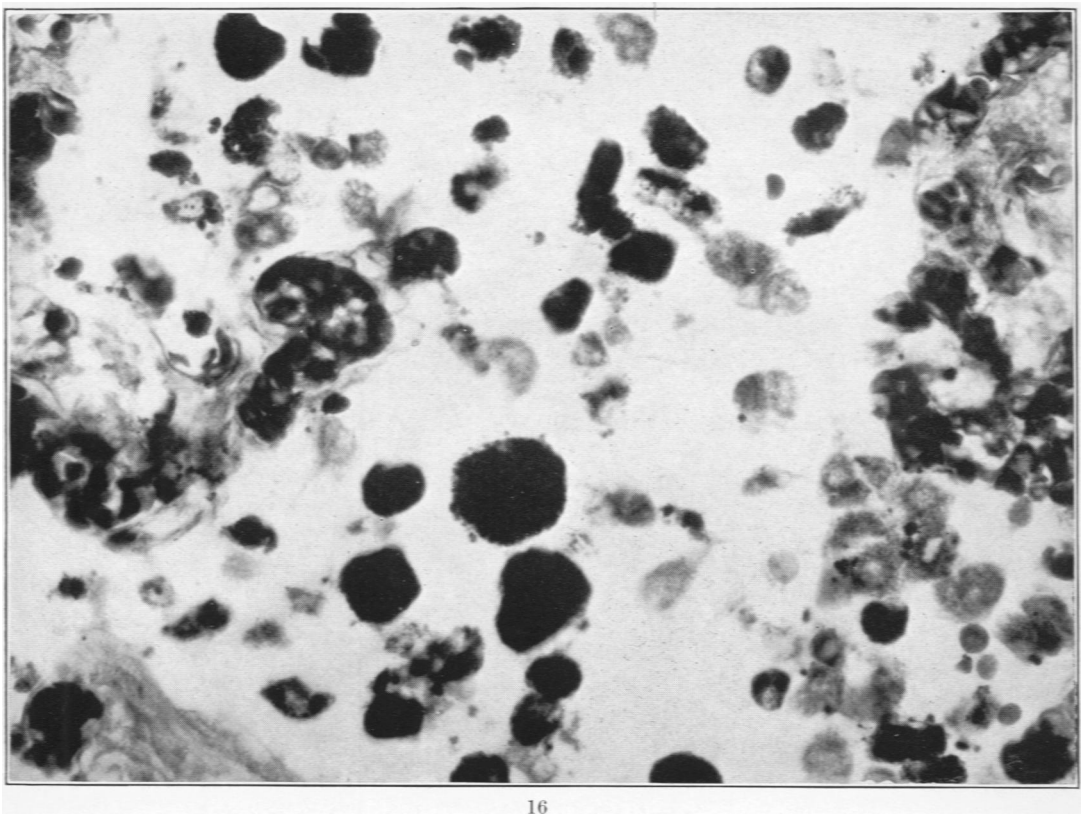
Origin of Pulmonary Dust Cell

PLATE 123

FIG. 16. Alveoli in a human case of chronic passive pulmonary congestion. Note the dense granules in the "Herzfehlerzellen," the comparative pallor of the desquamated epithelial cells, and the monocytes near the alveolar walls. The small, free granules between epithelial cells are coagulated albumen in edema fluid. Silver tannate. × 500.

FIG. 17. Hematoxylin and eosin section from the same block as that in Fig. 16. Note the absence of all granules other than hemosiderin or carbon and the misleading similarity in the appearance of desquamated epithelium and the dust cells, or "Herzfehlerzellen." Compare with Fig. 16. × 500.



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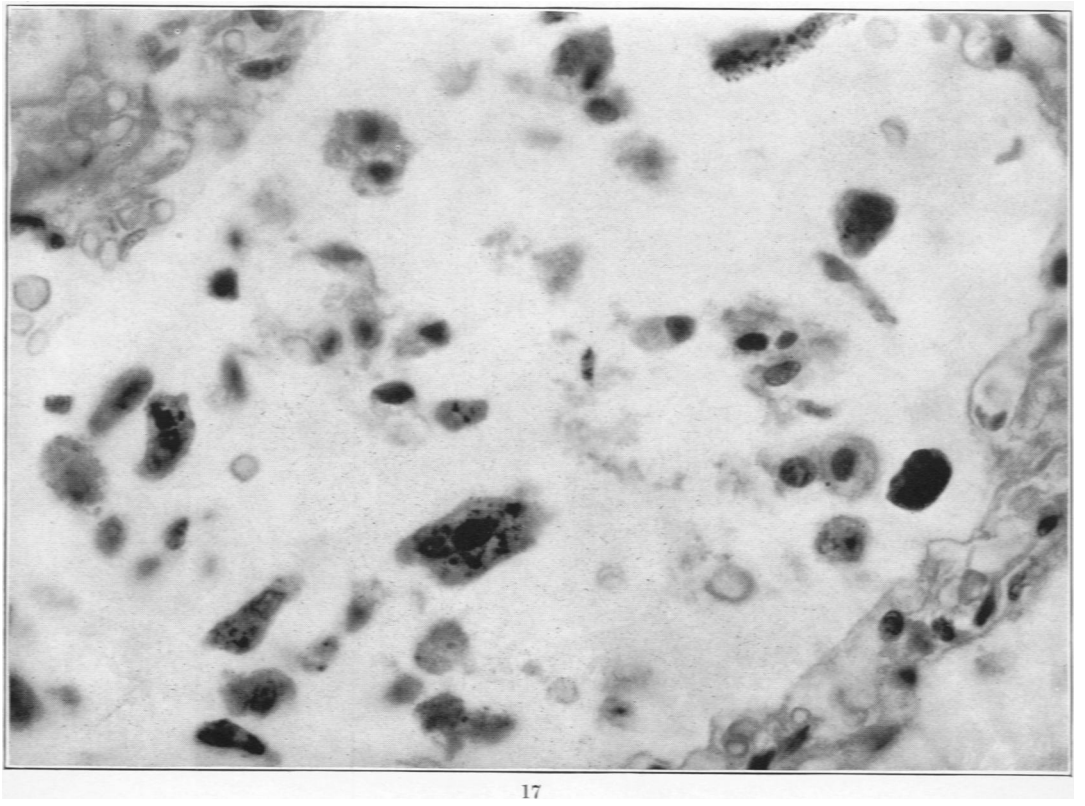

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Origin of Pulmonary Dust Cell