THE MONONUCLEAR PHAGOCYTES IN EXPERIMENTAL PNEUMONIA *

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A few years ago the writer undertook a series of experimental studies of lung lesions with the view to determining definitely, if possible, the nature, source and behavior of the mononuclear phagocyte of the lung in various pathologic conditions. It was planned to conduct the work as three separate but related problems. These were (1) a study of the origin, development, migration and fate of the mononuclear phagocyte of the lung; (2) a study of this cell type in its relation to pulmonary tuberculosis and to the spread of the tuberculous infection in the lung; and (3) a study of this cell in relation to the acute inflammatory reaction seen in the lungs in pneumonia.

About the time the first division of the work (Permar [1, 2, 3]) was completed, Foot [4] brought out his very thorough study of experimental pulmonary tuberculosis, demonstrating clearly the endothelial nature of the mononuclear phagocytic cell characteristic of tuberculous inflammation regardless of its situation, but especially evident in pulmonary tuberculosis. He also showed the endothelial nature of the so called "epithelioid" cells of the tubercle, and he excluded the alveolar epithelium from any participation in the specific tissue reaction to the tubercle bacillus. Furthermore, he showed that the mononuclear cell of endothelial origin was phagocytic for tubercle bacilli and that it carried these organisms into other alveoli, into the lymphatics and to the lymph nodes, just as these cells had been shown to transport carmine granules in our own work mentioned above. It should be noted here that the fundamental ideas underlying the work of Foot and myself had been not only advanced but actually worked out in preliminary experiments by Haythorn in 1913.[5]

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The origin and nature of the large mononuclear phagocytic cells of the pneumonic exudate are questions which up to the present have not been investigated experimentally by the India ink method of vital staining. It is this phase of the general problem of the activities of the mononuclear phagocytic cells of the body in relation to pathologic processes in the lung that will be dealt with in this article.

It may be stated here at the outset that it has been possible to show a definite endothelial origin for the phagocytic cells of the pneumonic exudate, with evidence pointing to great activity of the pulmonary vascular endothelium in their production. Conversely, it has not been possible to show that the alveolar epithelium is ever active in producing phagocytes or that vascular endothelium of other parts of the body does not contribute largely to their origin.

In presenting a review of the literature, it will be convenient to group together papers bearing on different phases of the subject.

Literature on the nature of the mononuclear cells found in various pathologic processes in the lung. The time honored view regarding the large mononuclear cell, which appears in the exudate in acute pneumonia, was that they were desquamated epithelial cells from the alveolar lining. This opinion still appears in certain text-books on pathology. Mallory has long taught the endothelial nature of these cells and as experimental work accumulates, his views are becoming more firmly established. In his text-book on the Principles of Pathologic Histology, Mallory[6] states that endothelial leucocytes appear in the early alveolar exudate, usually in small numbers, while in the stage of resolution they are more numerous. No mention is made of desquamated epithelium as a distinctive feature of the exudate, although he notes the regenerative activity of the alveolar epithelium.

MacCallum[7] in his Text-book of Pathology takes the view that both desquamated epithelial cells and mononuclear wandering cells, which he holds are related to the lymphocytic group, are present in the early exudate. He states that as the

pneumonic process goes on the desquamated epithelial cells increase in number and come to contain fat droplets and pigment granules. In a word, he believes the alveolar epithelium to be capable of producing phagocytic cells.

Haythorn [5] reported a new staining method which was particularly useful in defining the alveolar epithelium. It consists of a combination of Heidenhain's haematoxylin with Mallory's anilin blue connective tissue stain. By using this stain on a long series of anthracotic lungs, he was able to report that in no instance had he seen fixed alveolar epithelium showing phagocytic properties. When the epithelial cells were desquamated he found them very difficult to distinguish on a morphological basis alone from the mononuclear phagocytes of the lung alveoli; but he noted that the free cells, which could be identified as epithelial because they lay in small strips attached end to end, were never found to contain pigment. He also employed the same stain in a series of pneumonic lungs, in which lesion he found that desquamation of alveolar epithelium was not a constant feature. It occurred only in some alveoli. Instances were observed in which the reaction had reached the stage of resolution and yet practically all the epithelium was intact. Klotz [6] referred to epithelial cells as well as phagocytic mononuclears of the macrophage type in the acute exudate of influenzal pneumonia.

Blake and Cecil [9] followed Mallory in the use of the term "endothelial leucocytes" for the phagocytic mononuclear cells of the acute pneumonic exudate. They also described desquamated epithelial cells in the affected alveoli. The numerous earlier investigators of experimental pneumonia have generally paid little attention to the mononuclear cell of the exudate. It was usually classified in their reports as a desquamated epithelial cell from the alveolar lining. Wadsworth,[10] for example, used this terminology and noted that desquamated epithelial cells were more numerous in the later stages of the reaction.

Literature on the alveolar phagocytes or dust cells. The mononuclear phagocytic cells commonly found in the lung alveoli

and frequently termed "dust cells" have attracted the interest of many experimental pathologists. Although their phagocytic activities toward particulate matter, other cells and cell débris, and various types of bacteria have been thoroughly investigated, their association with acute pneumonic inflammation has not been stressed or even especially studied by more modern methods such as the vital staining technique.

It is singular that the more recent work on the nature and origin of the dust cell is no more perfectly in agreement than the earlier studies. Slavjansky [11] indicated a haematogenous origin and Tchistovitch [12] agreed, though specifying the lymphocytic series as the probable source of derivation of the large mononuclear phagocytes. Wechsberg [13] studied experimental pulmonary tuberculosis particularly from the standpoint of injury to connective tissue but he also mentioned the probable endothelial origin of the large mononuclear cells found in the cellular reaction to the tubercle bacillus. Watanabe [14] believed the alveolar epithelium produced some of the mononuclear cells which he found acting as phagocytes for tubercle bacilli in the lung alveoli, while others were of possible endothelial origin. Neither of these writers was quite definite regarding the endothelial origin of the mononuclear phagocytes. Briscoe [15] studied the dust cells in their reaction to particulate matter and to tubercle bacilli and other bacteria and concluded that they arose from alveolar epithelium which produced them by proliferation as they were required.

Haythorn,[5] employing particulate carbon inhalations and the injection of suspensions of carbon particles and tubercle bacilli, was able to induce miliary tubercles in which the mononuclear ("epithelioid") cells contained both carbon and tubercle bacilli. In other words, it was shown in his work that the essential cell of the tubercle and the pigment phagocyte or ordinary dust cell were identical. Since he was unable to demonstrate an epithelial origin for the dust cell, from a comparative morphological and functional basis Haythorn concluded that it was probably an endothelial cell.

Sewell [16] followed the vital staining method to identify the dust cells and employed materials for intratracheal injection

similar to those used by Briscoe. He was convinced of the epithelial origin of the dust cells and even advanced the view that the so-called epithelioid cells in experimental pulmonary tuberculosis were epithelial in origin.

Permar [1, 2, 3] employed vital stains intravenously and a particulate substance intratracheally in the study of the mononuclear phagocytes of the lung alveoli. He showed the endothelial nature of these cells which took their local origin from the capillary and subcapillary vessels in the lung. After their migration into the alveoli they were actively phagocytic. They wandered into lymphatics after becoming more or less loaded with particulate material, finally reaching the lymph nodes at the hilus of the lung. In these experiments the alveolar epithelium was not found to be phagocytic *in situ* or to produce free phagocytic cells.

Foot's papers [4, 17, 18] on endothelial reactions in experimental pulmonary tuberculosis and on general miliary tuberculosis, clearly indicated the endothelial nature of the essential cell of the tubercle regardless of the location of the lesion, thereby extending the observations of Evans, Bowman and Winternitz[19] on experimental miliary tuberculosis of the liver. Foot demonstrated the origin of the endothelial phagocytes from the capillary endothelium and showed that they not only engulfed the tubercle bacilli but carried them to other alveoli or, by wandering into and along lymphatics, to distant locations such as the lymph nodes. As to the alveolar epithelium, he found it took no active part in the process and that its proliferative activity was directed only toward the repair of denuded surfaces on the alveolar walls.

Mallory [20] discusses in his text-book the essentials of the activities of the endothelial phagocytes. He describes the widespread wanderings of the endothelial leucocytes with various types of phagocyted materials, including bacteria, and states that tubercle bacilli are slowly distributed in the body by the ameboid movement of the endothelial leucocytes as these cells progress into and along blood and lymph vessels and epithelial-lined spaces.

Haythorn [21] has recently published a study of tuberculosis

and other reactions in which suspensions of both India ink and carmine were used after the manner of vital stains to mark the phagocytic mononuclear cells. This work amplified and extended his earlier observations, making possible certain definite conclusions in the important question of the spread of tuberculous infection in the tissues. He showed that the intracellular transportation of tubercle bacilli has a wide application in the finer interpretation of tuberculous processes. The organisms are thus carried to neighboring tissues and through lymphatic channels to lymph nodes, where they give rise to new lesions. Less commonly the infected cells gain entrance to the circulation and are transported for considerable distances. Haythorn also found that by making local injections of pigment suspension about a subcutaneous tuberculous nodule, he could demonstrate pigment in most of the endothelial cells making up metastatic tubercles in distant lymph nodes. For example, he produced pigmented tubercles in the substernal lymph nodes of guinea pigs by local injections of India ink about a subcutaneous tuberculous lesion in the groin.

While recent work in this country on the nature of the dust cell has been pretty generally in agreement, certain French investigators have again taken an opposite view. Guieysse-Pellissier [22] reported a study of the finer lung structures following the intratracheal injection of olive oil. He used osmic acid to stain the intra- and extra-cellular particles of oil which remained in the lung tissue. He was of the opinion that both the free dust cells and the alveolar epithelium took up oil globules; and he described a process of enlargement and specialization of the alveolar epithelium very like that published by Sewell in the same year. Gravel [23] followed with a paper describing fat-containing alveolar phagocytes, regarding which he inclined to the opinion expressed in 1905 by Gilbert and Jomier,[24] namely, that the dust cells and the fat-containing phagocytes were identical and that they were derived from the wandering mononuclear phagocytes of the blood. This stimulated still another study of the alveolar phagocytes by Fauré-Fremiet,[25] who remarked that since the subject had been reopened, he desired to support unreservedly the epithelial theory, arguing

on the basis of chemical studies of the alveolar epithelium. He stated that the nucleated type of alveolar lining cell contained globules (considered by him to be cholesterin) which were soluble in acetone. When epithelial proliferation was produced, as by a mild irritant, the newly formed cells showed this type of globule, as did also certain of the free cells in the alveoli. Although the larger, actively phagocytic cells did not show acetone-soluble globules, the writer thought this was due to a gradual loss of them when the cells became actively phagocytic for other substances.

A recent publication from one of the German laboratories also points to the alveolar epithelium as the source of the dust cells. Westhues [26] studied the phagocytic action of the dust cells for two different types of colloidal preparations, namely lithium carmine and a colloidal suspension of Chinese ink. The one is a colloidal sol and the other a suspensoid. They are only remotely comparable in their vital staining reactions on certain tissues, yet Westhues used them interchangeably, without regard to the fact that lithium carmine is taken up by the cells more slowly in every instance. It is not clear why he failed to get phagocytosis of carbon granules in the capillary endothelium of the lung after the intravenous injection of suspensions of Chinese ink. That lithium carmine did not vitally stain the capillary endothelium is less strange. Sewell used it with negative results and the writer had the same experience. As noted before, the benzidine dyes such as isamine blue and pyrrhol blue are less effective than carbon suspensions for this purpose. Westhues, therefore, failed to show the origin of the alveolar phagocytes from vascular endothelium. While he described mononuclear phagocytes in the interstitial tissues, he did not realize the endothelial origin of these cells, nor did he appreciate that they wandered into the alveoli to become dust cells. His work was in large part a repetition of Briscoe's and he reached the same conclusions.

Literature dealing with a more general discussion of the free mononuclear cells of the blood and tissues. Papers by several authors have recently appeared in which the whole problem of

the mononuclear cells of the blood and tissues is dealt with in a broader manner. It may be serviceable at this point to review briefly the varied terminology which has been applied to the free mononuclear cells. Maximow used several terms, derived largely from their activities in inflammation. He called them resting or active wandering cells and polyblasts. Ranvier used the term clasmatocytes. A second group of terms was given by various workers who had employed vital stains in the study of this cell group. Renaut termed them "cellules rhagiocrines"; Aschoff and Kiyono, "histiocytes"; Goldmann, "pyrrhol cells"; Marchand, "adventitial cells"; and H. M. Evans, "macrophages." Mallory has consistently used the term endothelial leucocytes to designate this group, while others have adopted one or other of the above names, or have merely used all or part of the descriptive phrase "large wandering mononuclear phagocytic cells."

Sabin,[27] whose theme was primarily the origin of the cells of the blood, presented an extensive study taken up largely from the embryological standpoint. By studying the living chick embryo, she has been able to demonstrate that vascular endothelium produces mononuclear cells of the blood (monocytes and the so-called transition forms), which proliferate and drop off into the lumen, and also clasmatocytes (histiocytes, adventitial cells, macrophages or endothelial leucocytes), which are proliferated in the same way but in the opposite direction, coming to lie in the tissues outside the vessel. Regarding the studies of Mallory and a group of others on the endothelial leucocyte, Sabin pointed out that they all center around the important question as to whether new clasmatocytes can differentiate from endothelium in the adult under normal conditions or can be made to do so by abnormal stimuli. It is stated as her belief that "this will ultimately be proved conclusively."

Bunting,[28] writing on the leucocytes in general, was not ready to admit the identity of the mononuclear leucocytes of the blood with the phagocytic endothelial cells freed from lymph sinuses and blood vascular walls. He was only dealing with the mononuclear cells of the blood, but was unwilling to assert

the common origin and interchangeability between the "monocyte" type in the blood and the "clasmatocyte" or macrophage of endothelial origin found in the tissues. He agreed with Simpson[29] in her statement that "if there is a conversion of monocytes into macrophages it at any rate is limited in time and space and does not occur indiscriminately in the general circulation. The evidence at present available indicates a close biological relationship, but there is not a proven identity of these two types of cell."

Cunningham[30] published a study of the origin of the free cells of the serous exudates, dealing with inflammatory reactions in the peritoneal cavity. He was able by the use of trypan blue to distinguish between the clasmatocytes and the mesothelial cells arising from the serous membranes. He concluded that while a few slightly phagocytic cells might be produced by the mesothelium, the great majority of the mononuclear phagocytes in the exudate were clasmatocytes. This was practically in agreement with the standpoint originally taken by Marchand[31] who stated that the bulk of the mononuclear phagocytes in peritoneal exudate were derived from the adventitial cells. In a more recent contribution to this subject Marchand[32] maintained the same view, though here he emphasized the belief that the serosal cells also produced actively phagocytic wandering cells. Cunningham made no definite statement as to the origin of the clasmatocytes save that in his experimental lesions there was no evidence of proliferative activity on the part of the vascular endothelium of the omentum and mesentery. Regarding the proliferative activity of capillary vascular endothelium, it may not be out of place to recall here that this reaction is quite common in certain types of inflammation. Mallory[33] described it first in typhoid fever, and his text-book descriptions of the same process, as one of the essential features of tuberculous and syphilitic inflammation, are generally accepted. Further, in their recent article on the skin reactions in measles, Mallory and Medlar[34] published not only descriptions but beautiful photographic plates of this endothelial reaction. Cunningham cited some personal observations leading to the hypothesis that if the circulation be

cut off from a capillary bed which at the same time continues to receive sufficient nourishment to prevent death of the capillary endothelium, these cells may undergo a reversion to embryonic endothelial type with subsequent differentiation into clasmatocytes. In this connection he quoted Macklin and Macklin,[35] who found that in new formed capillaries certain areas of endothelium appeared to become transformed into clasmatocytes. However, Cunningham states that "While much evidence has been presented on both sides in the question of the formation of free cells from the general vascular endothelium, there does not, as yet, seem to be sufficient to define the developmental potentialities which these cells retain in their adult state."

The work of Karsner and Swanbeck,[36] who studied the phagocytosis and removal of lampblack and carmine pigment from the pleural cavities in cats, indicated that the mesothelial lining cells of the pleura were capable of actively proliferating to form phagocytic cells which subsequently wandered along the lymphatics to the regional lymph nodes. This is quite the reverse of Cunningham's results in similar work done on the peritoneal mesothelium in rabbits and cats. Karsner did not mention any evidence that blood vascular endothelium was associated with the development of the wandering mononuclear phagocytes described in the pleural cavity and lymphatics, though the endothelium lining the lymphatic channels was found to contain particles of the materials introduced into the pleura.

Foot,[37] in discussing aseptic inflammation in the omentum of the rabbit, proposed the hypothesis that the endothelium of the capillaries, the fibroblasts, and the new cells produced by both types are all mesenchymal in origin, and that under the conditions of the reaction of the tissues to injury the embryonal characteristics of these cells become reëstablished. Therefore, he argued, they form a local temporary mesenchyma at the site of injury, the cells of which are truly polyblastic, though he in no sense agreed with Maximow in considering a lymphocytic derivation for any of the elements of this temporary mesenchyma.

Literature on the Histology of the Alveolar Epithelium. The recent literature contains no significant addition to our knowledge of the histology of the alveolar epithelium. Ogawa [38] described this structure in the rabbit as arising primarily as a cuboidal cell which later assumes a flattened form. He pointed out that even before respiration has occurred two distinct cell types appear in the alveolar lining. One retains the cuboidal form and the nucleus, while the other is a large non-nucleated plaque-like structure. These latter become still wider and more flattened after birth, and cover the greater part of the alveolar wall. The nucleated cells remain in small islets which Ogawa believed to be the source of new cells in the regenerative process. Bremer's work [39] on the lung of the opossum indicated that the non-nucleated masses are in reality wide cytoplasmic processes of nucleated cells which stretch out thinly over the capillaries of the alveolar wall. Miller [40] described the alveolar epithelium in the human in practically the same terms as were given later for the rabbit by Ogawa. Foot [4] was in agreement with the accepted view as far as the presence of nucleated and non-nucleated cells is concerned, but he believed that not all the nucleated cells lying in small clumps in the alveolar wall may actually be epithelium. He noted that the administration of India ink resulted in the pigmentation of certain of these apparently typical epithelial cells, which led him to support the idea that extravascular but as yet inactive endothelial phagocytes are a normal component of the alveolar wall. He remarked that in thin sections stained by ordinary methods alveolar lining cells are not readily distinguished. Permar [2] had noted the same difficulty in identifying the alveolar epithelium; he found that practically all the nuclei visible in cross sections of the alveolar wall appeared to belong to cells forming the walls of capillaries and, therefore, to vascular endothelial cells. The small clumps of nucleated cells in the alveolar wall were also observed by him occasionally to show granules of the vital stain injected intravenously; such cells were interpreted as phagocytes recently developed from vascular endothelium but still occupying an interstitial position.

Summary of the literature. To sum up this résumé of the literature, it appears that the prevailing opinion still assigns considerable importance to desquamated alveolar epithelium as a source of the mononuclear phagocytic cells of the pneumonic exudate, although some observers, notably Mallory, Klotz, and Blake and Cecil were convinced of the importance here of the endothelial leucocyte. Haythorn held the same view and showed in the human that desquamation of the alveolar epithelium was not a constant feature of the reaction in acute pneumonia.

The origin of the dust cells and their relation to certain mild or chronic types of irritation in the lung are matters which have given rise to a voluminous literature. As the question now stands, it is probably fair to say that there is very good evidence pointing to the capillary endothelium as the source of the phagocytic mononuclear cells characteristic of lungs exposed to dust and also appearing in the reaction to mild irritants and to tuberculosis. The arguments of those who believe the alveolar epithelium gives rise to the dust cells and the allied group of mononuclear phagocytes of the lung do not seem essentially sound.

The broader question of whether or not vascular endothelium is capable of producing extravascular phagocytes has been discussed at some length in the recent literature. The work of Sabin leaves little doubt that this is possible in certain embryos. While there has been some hesitancy to accept the facts regarding capillary endothelium which Mallory years ago demonstrated in pathological states as physiological, even in a limited sense, the trend of recent experimental work is in that direction.

So far as the available discussions of the microscopic anatomy of the alveolar epithelium are concerned, there would seem to be nothing to indicate that this tissue is actively phagocytic in character. It is a highly specialized structure and has never been conclusively shown to be capable of giving rise to wandering phagocytic cells.

The comparative inactivity of the mesothelium of the peritoneal cavity in producing phagocytic cells, as shown by Cun-

ningham, is interesting as another example of a degree of specialization in an apparently simple and undifferentiated type of cell.

Experimental. The materials upon which this paper is based consisted of a group of twelve rabbits in which "vital staining" was carried out in conjunction with experimental pneumonia, and four control animals with vital staining alone. These all formed a portion of the larger series of experimental pneumonias described in another paper (Permar).[41] The experimental pneumonia was produced by the intratracheal injection of small doses of type I pneumococcus after which the animals were sacrificed at intervals ranging from one-half to seventy-two hours. The vital staining method employed was the intravenous injection of a particulate carbon suspension, as recommended by McJunkin[42] and Foot. In our experience the best results were obtained by the use of a 25 per cent mixture of Higgin's India ink with physiological salt solution, in 3 to 5 c.c. doses.

There was some variation in the amount of ink received by the different animals. Those destined to show the earliest stages of pneumonia received one or more doses before, and at least one dose after, the pneumococci were injected; while those intended to illustrate the reaction after a period of hours received one dose of ink before, and two or more after, the introduction of the pneumococci. In the experiments of longer than twenty-four hours' duration, one or two doses of ink mixture were given each day. Because no point was made of giving the last dose of ink at a specified time before the death of the animal, there was variation in the amount of ink found free in the vessels, but this had no apparent influence on the results of the experiments. Nor did the intravenous injection of a carbon suspension appear to have any influence on the development of experimental pneumonia. The latter point was controlled by the closely comparable incidence of pneumonia in another series of rabbits in which the experimental procedure was the same, save that the "vital stain" was omitted. Gross and microscopic studies of the two groups showed no important

differences in the nature of the inflammatory reaction or in its rate of progress.

In the gross, the lungs of the series receiving ink mixture intravenously showed various degrees of pigmentation, depending on the amount of free ink present in the vessels at the time of death. And in the first three rabbits of this series, which had received a 50 per cent ink mixture, there was also some pulmonary edema, due to a widespread plugging of the capillaries by the ink. In one of these, death resulted a half hour after the injection of 5 c.c. of the 50 per cent mixture. Edema was not found with the use of a 25 per cent ink mixture though ink emboli appeared microscopically, especially in the animals killed shortly after an injection of ink.

The tissues from both the vitally stained and the unstained animals were studied microscopically. They were fixed in Zenker's fluid and stained by hematoxylin and eosin and by Haythorn's modification of Mallory's anilin blue stain. The latter is an excellent high power stain and is especially valuable in the study of the lung, since it colors the alveolar lining cells more sharply than the ordinary stains do. The unstained series included several animals with a degree of anthracosis. From these it was possible to study the reactions of the carbon-bearing dust cells during an acute inflammation in the lung.

The study of the control animals injected intravenously with India ink suspension brought out the point previously made by Foot [37] regarding the superiority of colloidal carbon suspensions over benzidine dyes, lithium carmine, etc., in vitally staining capillary endothelium and the free cells developed from it. And although the degree of anthracosis naturally present in most laboratory animals may be slightly confusing when one is studying the normal lung or the effect of a mild irritant introduced into a healthy lung, this is no longer true when one is dealing with more active irritants such as the tubercle bacillus or the pneumococcus, since the endothelial reaction is quantitatively so much greater in these instances. The control animals showed ink granules in vascular endothelium, in mononuclear leucocytes free in the blood stream, in extravascular mononuclear cells interstitially located within the alveolar wall

and in similar mononuclear cells which appear to be penetrating and to lie upon the alveolar lining, and finally, in free alveolar phagocytes. This is entirely in agreement with Foot's[4] account of the cellular reactions to the intravascular injection of India ink. Although it is not of primary interest here, it may be noted that the polymorphonuclear leucocytes of the circulating blood also contained small quantities of carbon granules.

In the rabbits showing early pneumonia, as at intervals of six and eight hours after the introduction of pneumococci into the trachea, the vital staining phenomena were identical with those seen in the control animals, the only difference being the greater activity of the vascular endothelium in taking up carbon granules. This was because more active proliferation of endothelial phagocytes was stimulated by the presence in the lung of an acute inflammatory process. Very often the fixed endothelial cells containing ink particles were considerably enlarged, though such was not always the case. This corresponded with our results in previous work[1, 2, 3] where benzidine dyes were used; but in the present instance the reaction was not only greater but more distinct, due to the more active stimulus afforded by the presence of an acute infectious agent in the alveoli and to the superiority of colloidal carbon suspension as a stain for endothelium. Not only was the activity of the vascular endothelium in taking up carbon particles more marked in the presence of an acute pneumonia, but also numbers of carbon-bearing mononuclear cells appeared in the pneumonic exudate which had certain features distinguishing them from the dust cells ordinarily present in the alveoli. They were more irregular in size and shape and as a rule contained but few carbon particles which were either very fine or rather coarse, and without definite distribution in the cytoplasm. The dust cells were fairly constant in size, showed a rounded form and were usually uniformly stippled with particulate matter, though the zone immediately about the nucleus was often more heavily loaded. In the pneumonic exudate both types were found phagocytic for nuclear debris and red blood cells as well as carbon. Large carbon-bearing phagocytic mononuclear cells also appeared in the exudate in the finer air

passages. The fixed epithelial cells of the alveoli and of the air passages showed no evidence of carbon.

After ten hours the pneumonic lungs showed an extension of the inflammation to the interstitial tissues with the development of an increasingly intense acute lymphangitis. The cells of the interstitial and lymphatic exudate consisted largely of polymorphonuclear leucocytes with a certain number of mononuclear cells. Some of these contained moderate quantities of carbon pigment, though, as a rule, there were only a few scattered granules in their cytoplasm. The non-pigmented cells, however, were identical in morphology with those containing carbon granules; there was little doubt as to the endothelial nature of both forms. The main characteristics of the endothelial reaction in the later stages of pneumonia were in all respects like those described for the early exudate save that the vascular endothelial reaction and the proliferation of endothelial phagocytes became more marked with the development of the exudate.

Carbon-bearing mononuclear phagocytes also appeared in the lymph nodes at the hilus of the lung. In the earlier stages of the acute pneumonia it was impossible to determine whether the injected ink suspension was responsible for the carbon pigment in these cells or whether it was due to simple anthracosis; and the same question came up regarding the pigment bearing cells in the alveoli in the early stages of the reaction. It is most probable that in each instance the more heavily loaded cells represented the effect of anthracosis, while the more lightly stippled ones contained carbon because of the intravenous ink injections. This was no doubt the case, since the more lightly loaded type of cell was also varied in size and shape, and became more numerous in the alveoli, in lymphatics and in the lymph nodes as the acute pneumonic process developed. There was no evidence to indicate that any considerable development of mononuclear cells took place within the lymph node, though it is possible that they were proliferated locally from the capillary endothelium. However, the presence of numerous carbon-bearing mononuclear phagocytes in the larger lymphatics of the lung stroma indicated that the pneumonic exudate was the

source of the greater part of those found in the lymph nodes at the hilus.

The epithelial cells lining the alveoli showed no evidence of proliferative activity nor did the free nucleated cells identified as derived from the alveolar lining ever contain phagocyted material of any type. The alveolar epithelium was difficult to identify satisfactorily, though the tissues were obtained fresh, were well fixed and well stained. Moreover, the lungs showed inflammatory edema, which should have made these cells more readily visible, since edema is claimed to cause swelling of the alveolar epithelium (Westhues).[26] The alveolar lining appeared as a palely stained, irregular, film-like line with here and there an occasional rounded or oval nucleus. Demarcation of the cell limits in the region of the nuclei was indistinct. The epithelial lining was best seen where the air spaces lay close to the wider stroma and in larger air spaces such as the atria. Desquamated epithelial cells could be identified with certainty only when they appeared in short strips or small sheets. These were rare in the pneumonic exudate and never showed any signs of phagocytic properties.

Discussion. The foregoing is a brief description of the results of carrying out "vital staining" by the India ink suspension method on a series of rabbits suffering from experimental pneumococcus pneumonia. It offers experimental proof of a fact which is pretty generally accepted at the present time, namely, that when phagocytic mononuclear cells of the clasmatocyte or macrophage type occur in the lung alveoli and interstitial tissues, it is because the capillary vascular endothelium has been stimulated to produce them. Further, it corroborates the statements previously made regarding the specificity of colloidal carbon suspensions for vascular endothelium and the free cells derived from it.

Whether the vascular endothelium of the smaller venules and capillaries of the lung alone is active in producing phagocytic cells, or whether the ordinarily more active endothelium of the liver and spleen or other tissues shares or even takes a major part in this activity is a matter about which no dogmatic

statement can be made. One cannot rule out extrapulmonary sources. There were certainly many mononuclear phagocytes to be found in the blood stream, as the blood examined in stained films and in lung vessels in the sections clearly showed. At the same time, there were many developing locally in the capillaries of the lung. It would appear that the greater number of those found in the alveolar exudate arose locally, since those lying free in the vessels were, as a rule, more heavily loaded with carbon than those which appeared in the pneumonic exudate. This is probably not intrinsically because the monocytes of the blood contained larger quantities of carbon. Although it has been claimed that much phagocyted material hampers the mobility of the endothelial phagocyte, a study of pneumonia in anthracotic lungs reveals great activity on the part of interstitially placed, heavily pigmented mononuclear cells. It was not possible to say whether or to what extent the monocytes of the general circulation contributed to the mononuclear phagocytes of the exudate.

Further suggestive evidence that the phagocytes occurring in the alveoli might have come from distant tissues is to be found in the singular reaction encountered by Haythorn [21] in his study of experimental tuberculosis. He noted repeatedly that tubercles made up largely of ink-marked endothelial cells developed in the substernal lymph node following the subcutaneous injection into the groin region of cultures of tubercle bacilli mixed with India ink. In this instance, then, it would appear that the greatest production of phagocytic endothelial cells was at the site of the most intense irritation, i. e., in the subcutaneous nodule, with the result that numbers of them were free to take part in a secondary reaction in the lymph node. Although, as has been stated, it is impossible to say in what degree extrapulmonary sources contributed to the mononuclear cells of the pneumonic exudate, the proliferative activity of the pulmonary capillary endothelium offers at least some evidence pointing to the largely local character of their production.

The subsequent wanderings of the endothelial leucocytes of the exudate may include a return to the vascular lumen as was

shown by Haythorn [21] who injected carbon subcutaneously in animals suffering from trinitrotoluene poisoning and later found a few carbon-bearing mononuclear phagocytes in the general circulation; and Sabin [27] has described the indiscriminate wanderings of the monocyte-clasmatocyte group of cells in her embryologic preparations. The route by way of the lymphatic channels to interstitial locations is common in anthracosis. In these experimental pneumonias, and to a greater degree in pneumonic inflammation in the more anthracotic lungs of the human, numbers of free and fixed dust cells are set in motion, going from interstitial locations and alveolar spaces to the widened lymphatics and eventually to the lymph nodes at the hilus. In case the lung and lymph nodes are the seat of an acute edema with marked widening of the lymphatics and sinuses, as in pneumonia, acute gassing, or cardiac decompensation, the nodes may fail in their filter-like action and carbon-loaded cells, or free carbon from the disintegration of such cells, may pass directly to the circulation by way of the thoracic duct. This indicates that there is another source beside the intestinal tract for the carbon deposits occasionally found in the liver and spleen, since the endothelium of these organs readily removes particulate substances from the circulation. A pulmonary edema, moreover, is the simplest mechanism by which anthracotic deposits may be dislodged from the lung stroma; and if the edema is a part of acute inflammation of the lungs, much of the liberated pigment is probably lost to the body in the expectorated exudate, although some of it reaches the lymph nodes and possibly the circulation, as detailed above.

Some points bearing on the endothelial as against the epithelial origin of the mononuclear phagocytes of the lung are brought up by the foregoing review of the facts regarding the migratory activity of the dust phagocytes and the mononuclear endothelial cells of the pneumonic exudate. If these cells are actually epithelial in origin, then we must admit that the alveolar epithelium is a tissue of a low degree of differentiation, with qualities that are ordinarily associated only with mesenchymal structures. Further, we must take the difficult position that when an alveolar epithelial cell becomes a free

phagocyte, it is no longer an epithelial cell; for the only other instance in which epithelium infiltrates the tissues and gains entrance to lymphatics is in carcinoma, and there is, of course, no possible analogy between the action of the alveolar phagocytes and that of the cells of a cancerous new-growth.

Regarding the possible phagocytic power of the alveolar lining cells, there would appear to be no reason why they might not exhibit this property; but the fact remains that epithelial cells which were definitely to be identified as such did not show it in the experimental materials, or in any human tissues that we have examined. It is true that Nagao [43, 44] claimed to have found in a few instances that the alveolar epithelium of the rabbit contained ink granules following intravenous injections of Chinese ink. While one would scarcely wish to make an absolute statement on the question, it is most probable that the answer lies in the interpretation placed on the microscopic findings. When one administers a colloidal carbon suspension intravenously to a normal animal, the occasional mononuclear phagocytes which are proliferated and migrate to the air spaces contain carbon granules. And if they are encountered in the act of emerging into the alveolus, they appear as irregular, flattened cells somewhat resembling the alveolar epithelium, but usually larger and more prominent. There is, therefore, little evidence and no proof for the statement that alveolar epithelium is phagocytic.

Conclusions

1. The mononuclear phagocytic cells appearing in the exudate in acute experimental pneumonia are of vascular endothelial origin. They arise in part at least, if not predominantly, from the fixed endothelial cells of the capillary and subcapillary vessels in the walls of the atria, sacculi alveolares and alveoli pulmonis.

2. Cells of endothelial origin proliferated in other organs, as the spleen and liver, may contribute in some degree to the total number of phagocytes in the exudate.

3. Dust cells of similar origin already present in the alveoli also act as phagocytes in the inflammatory reactions. Intersti-

tially placed dust cells become reactivated and through liberation by the inflammatory edema are enabled again to appear in the exudate.

4. This entire group of cells, including the newly produced phagocytes and the dust cells, appear in the exudate in the finer air passages, in the alveoli, in the lymphatics and diffusely in the stroma, and in the sinuses of the regional lymph nodes.

5. Under given conditions carbon-bearing endothelial phagocytes enter the circulation indirectly from the regional lymph nodes by way of the thoracic duct, and it is probable that they may occasionally gain entrance to the circulating blood by direct migration.

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DESCRIPTION OF PLATE I

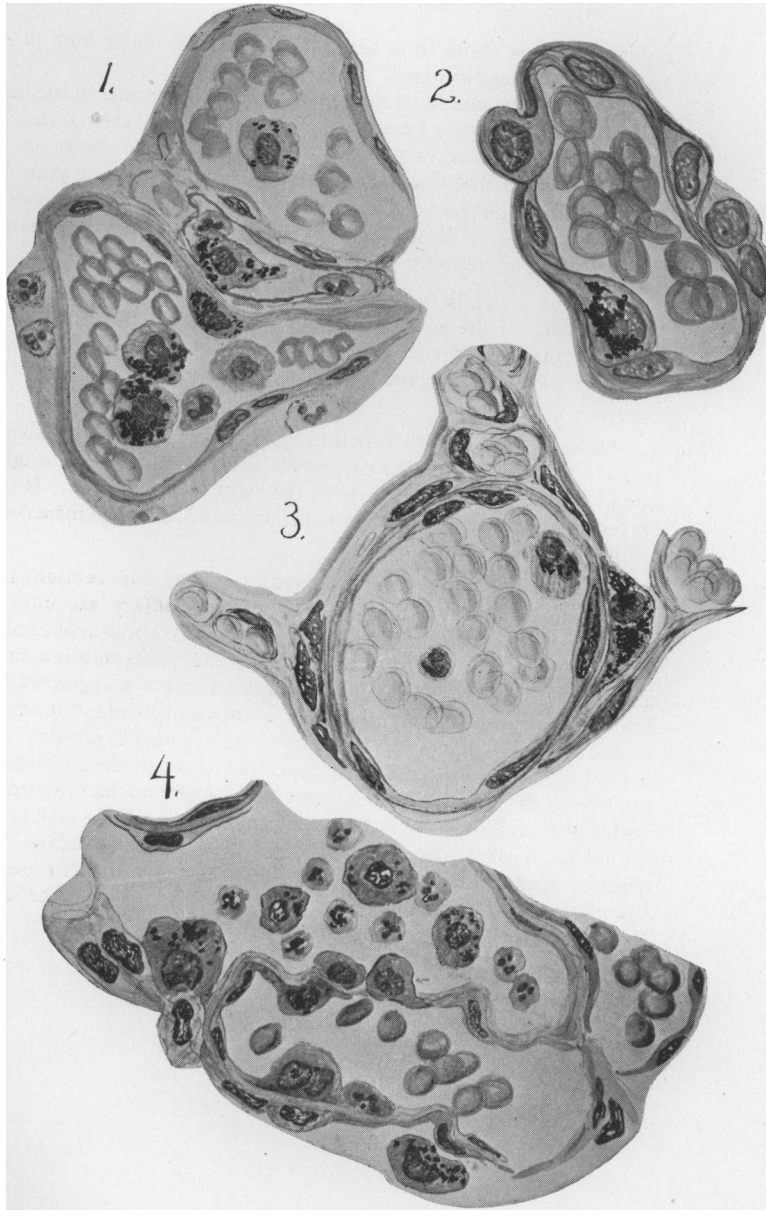
Original drawings made from experimental tissues under high power and oil immersion magnification.

Fig. 1. Showing two small venules and a lymphatic channel in the lung. Within the venules are found free endothelial leucocytes containing variable quantities of carbon granules. One venule shows an enlarged fixed endothelial cell containing phagocyted carbon granules. An endothelial phagocyte characteristically marked by phagocyted carbon granules is also found in the dilated lymphatic channel between the two venules.

Fig. 2. Showing a capillary from an alveolar wall, in slightly oblique section. One of the endothelial lining cells is greatly enlarged and contains phagocyted carbon granules. Another, similar in every way but devoid of pigment, is seen migrating to the interstitial tissues.

Fig. 3. Showing a venule at the point of junction of several interalveolar septa. A large endothelial phagocyte lies flattened in the angle of one of the alveolar spaces, just to the right of the venule. It illustrates the irregular, heavy pigmentation found after the intravenous administration of India ink.

Fig. 4. Showing one capillary in an alveolar wall in cross section, and a portion of another. Above and below the capillary are indicated portions of two alveolar spaces. The capillary endothelium shows enlargement of several of its cells, one of which contains a few carbon granules. One endothelial cell has migrated and now appears almost free in the alveolar space. Another is apparently free, but still lies close to the alveolar wall. Others, with variable amounts of phagocyted ink granules irregularly scattered through their cytoplasm, are free in the alveolar spaces. One polymorphonuclear neutrophile within the capillary contains a small granule of carbon; and several granules appear in one of those free in the alveolar exudate. The alveolar epithelium is visible as flattened nucleated cells in several places on the alveolar walls. At the upper left corner of the sketch there is shown a well defined alveolar epithelial cell.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