STUDIES ON ENDOTHELIAL REACTIONS.

IV. THE ENDOTHELIUM IN EXPERIMENTAL GENERAL MILIARY TUBERCULOSIS IN RABBITS.

BY NATHAN CHANDLER FOOT, M.D.

(From the Department of Comparative Pathology, George Fabyan Foundation, Harvard Medical School, Boston.)

(Received for publication, September 21, 1920.)

It is the purpose of this paper and the others of the series (Foot, 1919, 1920, a, b) to draw attention to the important part taken by cells, originating in capillary endothelium, in various types of inflammation. In the first paper (Foot, 1919) their reaction to the presence of a foreign body (agar-agar) was discussed; in the two succeeding their behavior in experimental subcutaneous and pulmonary tuberculosis in the rabbit was described. By means of intravenous injections of a colloidal suspension of carbon it was shown that the cells forming the foreign body tubercles, as well as those found in lesions of experimental tuberculosis, were traceable to the endothelium of the neighboring capillaries. The present paper is a report of the histogenesis of the cells forming the tubercles produced in rabbits by the intravenous injection of tubercle bacilli. Such a general infection as this simultaneously produces lesions in various organs, and the question arises whether the cells found in all these are traceable to the capillary endothelium.

The literature on experimental tuberculosis has already been discussed at length in the earlier articles of the series (Foot, 1920, a, b), but it may be well to review it briefly. Sewell, Watanabe, and Wechsberg have experimented upon the lung, studying the lesions produced by the insufflation of tubercle bacilli through the trachea, with or without the use of a vital staining method.1 The

1 A recent article by Permar (Permar, H. H., J. Med. Research, 1920, xlii, 9) has appeared since the present paper was submitted for publication. It is interesting to compare his findings in experiments on intratracheal injections of carmine in guinea pigs and rabbits, coincident with intravenous administration of isamine blue, with those of this paper and of Paper III (Foot, 1920, b). His conclusions as to the endothelial origin of the phagocytic cells of the lung tissue coincide in every way with mine and his emphasis of the non-participation of the epithelium in these phenomena of phagocytosis seems well supported by facts.
Liver has been extensively studied because of the readiness with which exact amounts of bacilli can be introduced into the intramesenteric circulation and thence into the portal vein and its radicles in the liver. Among others Kockel, Miller (1902), Oppenheimer, Goldmann, Evans, Bowman, and Winternitz, Kiyono, and more recently Soper have investigated the histogenesis of tubercles produced by the intraportal injection of tubercle bacilli. Kostenitsch and Wolkow in 1892 studied tuberculous lesions produced in the cornea and kidney of the rabbit by means of local injections. So far as can be ascertained there are no reports on experimental work on miliary tuberculosis, in which methods of vital staining have been brought to bear.

Since the consensus of opinion has not been in favor of the endothelial origin of the so called epithelioid cell, particularly in the case of the lung and kidney, the following experiments were performed which confirm the theory that the tubercle in the lung and kidney is not of epithelial origin.

Two vital stains were employed, one a benzidine dye—Niagara blue 3 B—\(^2\) the other a colloidal suspension of carbon in the form of Higgins' waterproof India ink, as in the preceding experiments. This is similar to the suspension devised by McJunkin and more easily prepared. The former gives a granular vital staining reaction, quite similar to that obtained with trypan blue, in the macrophages of Metschnikoff (a class of phagocytes designated by a large variety of names, but undoubtedly of endothelial origin), the lymphoid reticulum, the endothelium of the liver sinusoids, which is known as Kupffer cells, the fibroblasts, and the epithelium of the convoluted tubules of the kidney and, to a slight degree, that of the liver. It also appears in the polymorphonuclear neutrophils under conditions of extravascular existence or circulatory stagnation. Necrotic tissue and occasionally elastic fibers take on a diffuse blue color in its presence. Higgins' ink is chiefly taken up in situ by the endothelium of the smaller capillaries in certain organs, but it is also phagocytosed by the endothelium anywhere in the presence of inflammation. The free endothelial phagocytes likewise take it up in large quantities; if a benzidine dye is also present granules of carbon and this dye are taken up by these cells at the same time.

\(^2\) The Niagara blue 3 B was kindly furnished by Dr. George B. Wislocki, of the Department of Surgery of Harvard Medical School.
Therefore in an experimentally produced miliary tuberculosis in which both of these dyes are present, the epithelioid cells will contain ink only if they are the product of the only tissue that takes it up; that is, the endothelium. It was of particular interest to determine whether this would be true in the lesions produced in the spleen, lymph nodes, and omentum, where many phagocytes are present and where the reticulum, on the one hand, and the mesothelium, on the other, have been considered the parent tissues of the epithelioid cells. It would be well to know also whether or not the renal epithelium contributes to the tubercles in the kidney, as claimed by Kostenitsch and Wolkow.

By using the two dyes together it is possible to obtain a relatively exact differential staining reaction; the macrophages, lymphoid reticulo-endothelial cells, and the mesothelium, and the convoluted tubules of the kidney show a marked affinity for Niagara blue. Although many of these cells take up ink the renal tubular epithelium never does so, and the others always show more blue than black granules in the presence of both dyes.

Soper has quoted the experience of other observers, as well as himself, to show that the epithelioid cell loses its affinity for benzidine dyes once it has taken up tubercle bacilli, or has come under their influence. In studying the formation of subcutaneous tubercles I (1920, a) have found this to be in a measure true, as the cells seem to become vacuolated and the dye granules dispersed and lost after tubercle bacilli are phagocytosed. It does not seem that cells loaded with these dyes, for example lymphoid reticular cells or omental macrophages, would immediately lose all the dye in the presence of tubercle bacilli, nor do the findings of this experiment indicate that such is the case.

Rabbits have, therefore, been injected intravenously with bovine tubercle bacilli and at the same time been vitally stained, the resulting lesions being studied to determine the origin not only of their epithelioid cells, but also of the so called tubercle reticulum. By combining the vital and the usual connective tissue stains it is hoped that new light may be shed on the origin of this fibrillar structure, particularly by observing its formation in organs normally poor in collagen fibrils. Kon, and Yoshida, working with Rössle, and more recently Warren have demonstrated the possibility of its being produced by the endothelium. Downey has discussed the literature on this
subject in connection with the formation of the fibrils of the lymphoid reticulum. Hueck's recent communication further strengthens this view which is discussed more fully below.

**Technique.**

Four rabbits were given intraperitoneally 10 cc. of a 1 per cent solution of Niagara blue 3 B (twice purified) in distilled water. The next day this injection was repeated, with a 1 per cent solution of the commercially pure dye, which is less concentrated. The 3rd day the injection with the weaker dye was repeated and 5 cc. of Higgins' waterproof ink and distilled water, in equal parts, were introduced into an ear vein. The animals were now, by this means, vitally stained; their skin and mucosa were a deep ultramarine. On the 4th day each was given 1 cc. of a suspension of bovine tubercle bacilli intravenously, the strain employed being one of those used and tested out in previous experiments (Bov. XIV), and the dilution such that 1 cc. equaled 1 mg. of the bacilli. The suspension was made by rubbing up a weighed amount of culture from glycerol agar slants in normal salt solution, by means of a glass bulb pestle, shaking in a machine, and diluting and rediluting with normal saline solution until the desired concentration was reached. Intraperitoneal injections of 10 cc. of 1 per cent twice purified Niagara blue and intravenous injections of 5 cc. of 50 per cent Higgins' ink were instituted 3 days later and administered twice a week until the animals died or were killed. This kept all the rabbits vitally stained, with one exception. Rabbit 1 was killed 7 weeks after the experiment was begun, but the injections were only continued for 4 weeks, in this instance, in order to see what would become of the dyes accumulated in the tissues during that time. Later two other rabbits were given one intravenous injection of 1 mg. of the same strain of tubercle bacilli and the dyes were withheld until the 3rd week, when they were administered in exactly the same way as in the first set and continued until the animals were killed. The object was to begin supplying the dyes to the tissues at the approximate onset of the disease, instead of simultaneously with the inoculation. This lot of six rabbits gives a series of potential cases of miliary tuberculosis representing stages of 1, 2, 2½, 4, 5, and 7 weeks development. All except one were killed by injecting 4 per cent neutral formaldehyde into the beating heart under anesthesia. Rabbit 2 died of an intercurrent coccal infection after 2 weeks, but showed a few pulmonary tubercles. The tissues were fixed both in neutral 4 per cent formaldehyde and in Helly's fluid (potassium bichromate 2.5 gm., corrosive sublimate 7 gm., water 100 cc., and neutral 40 per cent formaldehyde 10 cc.) in order to compare the action of these fixatives on the intracellular granules of Niagara blue. Helly's fluid is found to be in every way superior to neutral formaldehyde alone; the granules are somewhat paler than when only formaldehyde is used, but as they are apt to be very dark in the latter case, this is a distinct advantage as it enhances the blue color and prevents confusion between
this dye and the ink. Sodium hyposulphite should not be used to remove the iodine, before the final staining, as it tends to bleach the dye completely; 95 per cent alcohol, although slower, is preferable, as it does not affect the vital stain in any way.

Sections of 5 microns were cut in paraffin and stained with Mayer's aqueous carmalum (ammonia alum 20 gm., carminic acid 2 gm., and distilled water 400 cc.), with Mallory's phosphotungstic acid-hematoxylin and aniline blue connective tissue stain, and with Delafield's hematoxylin in conjunction with the Ziehl-Neelsen carbol-fuchsin stain. It is, of course, necessary to rely upon a red nuclear stain for the routine procedure, in order to get the best contrasts with the blue vital dye; there is too little difference in color between this and hematoxylin or methylene blue to render them available except for topography or for photographic purposes. Sections were also impregnated by the Bielschowsky-Maresch method. Six organs were chosen for detailed study as described below.

**Pulmonary Lesions.**

Since bacilli, after being introduced into the circulation, are first swept into the pulmonary capillaries and, as Miller (1919-20) has shown, into the intrapulmonary lymph nodules, lesions are found developing in this organ before they can be found elsewhere. After 1 week two types of tubercle are distinguishable. The first consists of small aggregations of deeply ink-stained cells, 70 microns in diameter, which in the light of their further development, seem to be merely an agglomeration of phagocytic cells in response to the presence of the ink. The second type is composed of true, specific tubercles, few in number and only found after some searching. They show no Niagara blue, but their epithelioid cells contain ink globules. After 17 days development larger tubercles, about 375 microns in diameter, can be found. They show a few syncytiata, or giant cells, and contain a uniform amount of ink in the cells composing them. Polymorphonuclear leucocytes now appear at the periphery of the tubercles. The latter are obviously interstitial in origin and are situated near the blood vessels of larger caliber; one occasionally finds crescentic thickenings of the endothelium of these vessels, the crescents being deeply impregnated with ink globules. Similar lesions, which are not crescentic, are found in the intrapulmonary lymph nodules, near bronchioles. The alveolar spaces are not involved until surrounded by the tuberculoid infiltrate, when they begin to fill up with the large, emigrated endothelial cells described in the preceding paper (Foot,
These cells, like those of the tubercles, contain fine granules of carbon.

After 4 weeks development the tubercles are visible to the naked eye, being 0.3 to 0.7 mm. in diameter and occupying the walls and air sacs of the lobules affected. The tubercles often include larger vessels and cause their obliteration by overgrowth of the lining endothelium, which proliferates not only into the crescentic masses just described, but becomes a plug of anastomosing, rather stellate cells, not unlike the embryonal mesenchyma in appearance. Whether these lesions start within the vessels and work outward, or whether they surround and involve the vessels from without, is sometimes difficult to determine. As the infection is presumably intravascular, in this experiment, the first hypothesis is the more plausible. All the cells composing the tubercles are dotted with ink globules and a few now show granules of Niagara blue. Caseation begins at the center of the larger lesions, the caseous mass taking on a diffuse bluish stain. Polymorphonuclear leucocytes penetrating the tubercles also show a granular blue vital stain, but no ink. This indicates that the blue dye is available here, as elsewhere in the body and that it has no particular affinity for the epithelioid cells, whereas the ink has. As in the experiments on intratracheal infection no activity is seen among the epithelial cells, other than regenerative phenomena. A light vermilion blush now suffuses the tubercles in the Van Gieson preparations, while a similarly faint bluish tinge appears in those stained with Mallory's connective tissue method. This can be seen to be due to the appearance of delicate fibrils in the lesions, not only among fibroblasts but also in and between the ink-containing epithelioid cells. These fibrillae are also demonstrable as pinkish threads with the phosphotungstic acid-hematoxylin stain. While they are always red with the Van Gieson process, they may be red or reddish with Mallory's connective tissue stain, or blue or purplish with the phosphotungstic acid-hematoxylin. This would, presumably, indicate that they were gradually becoming impregnated with collagenous material.

After 5 weeks all these processes are more striking. The fibrous reticulum is now well formed and easily seen, the caseation more advanced and a deeper blue. After 7 weeks the lung is so widely
involved as to leave little normal tissue. In this case, as noted under Technique, the use of the dyes was discontinued in the 4th week; consequently the newer tubercles are almost devoid of ink and Niagara blue, while the older ones show plenty of both, the latter in diffuse form in the caseous areas. Older tubercles are sometimes centrally stained and peripherally free from the vital dye, the younger portions of the lesion having apparently formed after the supply of available dye was exhausted. There are a great many intravascular, crescentic, and deeply ink-stained tubercles in the sections from this rabbit, as well as a few of the obliterator, retiform type.

Thus it is found in the lung that the tubercles develop in and near larger vessels, that they contain much ink, but little granular Niagara blue, and that the reticulum is formed chiefly in the epithelioid cells. Groups of migrating endothelial cells lying free in the alveoli can be seen in the 5 and 7 week preparations, in which delicate fibrils are demonstrable. There is no question of any relation between these cells and the alveolar walls, as their origin has been worked out and reported in the preceding paper (Foot, 1920, b). The fibrils first form about the periphery of the cells by consolidation of their cytoplasm and cell processes (ectoplasm); next there is vacuolation of the endoplasm and a consolidation of the walls of the vacuoles, with the production of fibrillar circles and lines, which gradually seem to twist and break into fibrilla. The process is similar to that described by Hueck in his article on the mesenchyma. This is not strange in view of the embryonal origin of these cells.

Sections from the lung after 5 weeks were impregnated with silver by the Bielschowsky-Maresch method with the view of obtaining an idea of the distribution of the reticulum fibers among the epithelioid cells. Fig. 1 shows that they are readily demonstrable in cells that contain carbon particles. The majority of the epithelioid cells were found to be free from reticulum fibers, but there are many places in the sections where the fibers can be found running in and among these cells and forming a fairly close network about them. Of course, many fibers run out from the alveolar walls into the exudate, but there are instances in which no connection between these and the smaller fibrils in the epithelioid cells is demonstrable. This method of impregnation, then, bears out what has already been noted with the connective tissue stains.
**Hepatic Lesions.**

In the hepatic lesions also, distinction should be made between small pseudotubercles, apparently due to the presence of ink, and the true tubercles resulting from the infection. During the first 3 weeks there are slight changes in the sinusoidal endothelium, in part probably due to the ink, in part possibly the forerunner of true tubercles; but it is not until the 4th week that fully developed, specific lesions appear. They seem to begin as syncytia and to be situated in sinusoids lying in the mid-zone, between the hepatic and portal systems, usually nearer the former. The cells forming them are so heavily laden with ink globules that it is difficult to make out their histology (Fig. 2). The ink is usually arranged peripherally in the syncytia and single cells, forming a dense black rim that occupies much the same portion of the cytoplasm in the former, as that in which the nuclei are generally grouped, while in the latter it forms a ring about the single nucleus. In no organ is it so plentiful in the cells as in the liver. There is no indication that the syncytia are formed by mitotic nuclear division, without cytoplasmic division, as claimed by Soper; it seems more likely that they are the result of a fusion of several single cells. This is the rule in the subcutaneous lesions, and there is no apparent reason why there should be an exception in the liver endothelium. After 5 weeks the tubercle reticulum is demonstrable in the sections stained by appropriate methods. The fibers are best seen if green or greenish brown filters are interposed between the condenser and slide, when they stand out sharply. Here, as in the lung, they can be definitely demonstrated in the epithelioid cells, as well as in the few connective tissue cells present.

**Splenic Lesions.**

In the preparations representing the first few weeks development nothing definite can be distinguished. The pulp and its phagocytes are intensely blackened with ink, and the splenic reticular cells are deeply stained with the blue dye, taking up the ink to a much less degree. In the 4 week preparations the tubercles are mature and situated at the periphery or near the center of the Malpighian corpuscles. Their epithelioid cells contain a varying amount of ink.
but far less than heretofore encountered in experimental tubercles of this type. Only a few of them contain Niagara blue granules. Small tubercles forming in the secondary nodules of Flemming show much ink in their cells. The tubercle reticulum is beginning to form.

After 5 weeks, caseation is found and the caseous material is stained a diffuse blue. There is the same scanty deposit of ink in the tubercles (Fig. 3) of the Malpighian corpuscles but a moderate amount in the secondary nodules. Although the splenic reticular cells are deep blue, only a few of the epithelioid cells show this stain. After 7 weeks, however, the tubercles are deeply stained with ink. Besides the tubercles, one finds groups of ink-bearing cells in the lymphoid nodules; they show no tubercle reticulum, while the true tubercles exhibit a fully formed network of fibrils. In this way the two are readily distinguishable. The splenic sinuses are widely dilated and almost free from ink-bearing cells; it seems as if these had migrated to the lymphoid tissue and, possibly, into the tubercles as well. The reticular cells of the splenic tissue are still deeply stained with Niagara blue.

Thus, although the splenic sinuses and their free cells are so heavily laden with ink whenever it is administered intravenously, relatively little of the ink seems to be found in the cells forming the tubercles located in the lymphoid tissue of the Malpighian corpuscles, while a moderate amount is present in the smaller, or secondary nodules. This is not readily explained; the subject will be discussed further in the consideration of the lesions in the lymph nodes, where a somewhat similar condition exists.

Renal Lesions.

Definite tubercles are first found in the sections from the 4 week rabbit although there are indefinite changes in the glomerular tufts and the walls of the vasa recta before this time. The tubercles vary from 75 to 750 microns in size and appear to be of two types, a moderately diffuse infiltration of the interstitial tissue between the cortical tubules, and more localized collections of epithelioid cells, with an underlying glomerular topography—one can make out the remains of Bowman’s capsule with swollen cells surrounded by the tuberculous lesion. In the first type, there are many small endothelial
leucocytes, similar to those seen in the subcutaneous lesions described in the first and second papers of this series (Foot, 1919, 1920, a). These are compact cells, with a bean-shaped nucleus and rather dense cytoplasm, about half the size of fully developed epithelioid cells, many of which are found in these tubercles. These cells often contain ink globules, as do their syncytial derivatives. The tubular epithelium, on the other hand, takes up no ink, although that of the proximal convoluted tubules is vitally stained with Niagara blue. It is significant that the tubercles show ink and that their cells take on no blue stain, except in scattered places where degeneration is obvious. One can find mitotic figures in the epithelium of the surrounded tubules, but this is probably an attempt at regeneration, as they are found only in cells that are still in situ in the tubules. In some of these diffuse tubercles it is possible to see the tubular epithelium degenerating and disintegrating, with endothelial cells, containing ink, pressing in upon its thinned out portions and apparently producing pressure atrophy.

After 5 weeks the tubercles are more numerous, larger, and show beginning central necrosis, with the usual diffuse blue stain in the caseous areas. The two types of tubercles, glomerular and interstitial, can still be distinguished. Whereas the tubercles were formerly all cortical, there are now a few of the interstitial type among the collecting tubules and Henle's loops in the medulla. They do not differ from the cortical interstitial type and their cells show ink (Fig. 4), but no Niagara blue. After 7 weeks the picture is not noticeably altered, but is merely more pronounced. The tubercle reticulum is demonstrable after the 4th week and is best seen in the 7 week lesions, differing in no way from that of the lesions in other organs.

That many of the tubercles originate in the glomeruli is indicated by the following facts. (1) Remains of glomerular structure are present in some of them. (2) The early tubercles are often rounded and of the same diameter as the average glomerulus. (3) The tubercles occur chiefly in the cortex. (4) The glomerulus is the chief renal structure showing ink in its cells; moreover, there is none in the tubules. Nevertheless, there are other lesions showing no evidence of glomerular architecture which occur near the vasa recta and may be found well down in the pyramids where no glomeruli are located. Ink is found in the endothelium of the vasa recta in controls. It
seems probable, then, that the tubercles originate from two sources, the endothelium of the glomerular capillaries and that of the capillary branches of the vasa recta, the former remaining near their site of origin, the latter migrating into the interstitial connective tissue between the tubules. There is no evidence that the epithelium takes an active, formative part in the process, although a few epithelial cells may be included in the tubercles and survive there for a time. Such cells, which have their origin in the vitally stained convoluted tubules, may contain granules of Niagara blue, and their nuclei often stain a bright blue, which is pathognomonic of degeneration and indicates early karyolysis. The ink globules prove to be an invaluable indicator of the origin of the component cells of the tubercles in the kidney as elsewhere.

Omental Lesions.

The omentum, bathed as it is in a copious supply of Niagara blue from the intraperitoneal injections, shows deeply stained mesothelial cells on the surface, some staining of the reticulum of the taches laiteuses, and a double staining of the wandering macrophages in its meshes. These macrophages contain some ink as well as the blue granules of the benzidine dye. The vascular endothelium of the complex capillary network, however, is dotted with black spots alone. The tubercles appear early, the first being found in the 17 day specimens, beginning as single syncytia and gradually enlarging as time goes on, until miliary tubercles are formed. The simplest of these lie within and not on the omentum, between the peritoneal layers; their syncytia contain ink alone. After 4 weeks they are, of course, more abundant, and the ink still predominates, although a few blue granules are demonstrable in some of the single cells near the periphery which are doubtless wandering macrophages. The taches laiteuses are free from ink and from tubercles. After 5 weeks tubercles can be found penetrating the peritoneal covering, which is broken through and shows dissociated and swollen cells. The general impression obtained is similar to that gained from a study of an ulcer of the intestine; the mesothelial cells are apt to be thickened and piled up at the edges of the lesion, which is composed of larger, paler cells.
containing ink and forming a mass that extends well into the connective tissue of the omentum. The mesothelial cells are blue-stained and readily distinguishable from the epithelioid cells as long as they remain well preserved; once they begin to degenerate they are more difficult to distinguish, as they lose the blue granules. It seems probable, from what has just been described, that the tubercles here are formed primarily from the endothelium of the omental capillaries, near which they are first found. The macrophages probably join in the process as it progresses, while the mesothelial tissue appears to be, at the most, sympathetically involved.

Lesions in Lymph Nodes.

Enlarged lymph nodes were regularly found in the axillae and groins of the rabbits, as well as the peribronchial groups, and were always excised and examined. During the first 3 weeks the medulla is very edematous and its sinuses are filled with large cells of the macrophage type which are deeply stained with Niagara blue and contain phagocytosed cellular debris. They increase in number and size and, in the 17 day specimens, fuse to form free lying syncytia. They do not form tubercles, and carbol fuchsin shows them to be filled with short, plump, reddish rods, totally unlike tubercle bacilli and possibly representing mitochondria. The lymphoid reticulum is stained deeply with Niagara blue, and there is no ink present, except in or near vessels of the cortical nodules.

Tubercles are first found in the 4 week specimens. They are composed of large, pale, anastomosing cells with an evenly distributed amount of carbon granules, which are very fine, but still perfectly evident (Fig. 5—after 5 weeks). Small dilated capillaries are usually demonstrable in their immediate vicinity, five with ink in the endothelium and blood corpuscles in the lumina being found at the periphery of one such tubercle. The tubercle reticulum is suggested in the 4 week specimens, and is well formed and easily demonstrable in the 5 and 7 week preparations. Although the lymphoid reticulum is everywhere deeply stained with Niagara blue, little or none is found in the tubercles. In the 7 week preparations the medulla is nearly free from the large, free cells, and the tubercles show much more ink than those of the earlier stages.
Thus less carbon is deposited in the tubercles formed in the lymphoid tissue of the spleen and lymph nodes than in those of the other organs, with the exception of the kidney. This is probably due to the fact that these tissues have little affinity for carbon and, in the case of the lymphoid tissue, are poorly supplied with blood capillaries. Where the latter abound, plenty of ink is found, as in the lungs, liver, and splenic pulp. It is interesting that, in both spleen and lymph nodes, the large, free lying phagocytes of the pulp or medulla appear to migrate into the lymphoid tissue, for as soon as the administration of the dyes ceases, they leave the sinuses, while the lymphoid tissue becomes full of carbon-bearing cells. That the blue granules are not seen in the lymph nodules in this case is possibly due to the fact that they are more diffusible and can be excreted by the cells that contain them and then be eliminated through the blood and urine, the latter always being of a bluish green color. The carbon seems to be more difficult to remove and is probably retained until the disintegration of the cells containing it sets it free for other phagocytes to take up.

**DISCUSSION AND SUMMARY.**

A review of the findings in this series of experimental inoculations brings out clearly two points: (1) the specific lesion of the miliary type is composed chiefly of cells of endothelial origin, apparently coming, for the most part, from the walls of the small capillaries near it; and (2) these cells are capable of forming the reticulum of the tubercles and hence collagen. In Hueck's paper, which has already been referred to, the origin and development of the vascular systems are discussed as a subdivision of the mesenchyma. Hueck states that he considers mesenchymal cells of the endothelial type capable of forming collagen fibrils, even in adult life, but indicates that he would limit this attribute more or less strictly to the endothelium of the capillaries. He points out that no "silver lines" can be demonstrated in the capillary endothelium, while they are readily shown in the endothelium of the larger, more specialized vessels; the capillary endothelium is more syncytial, less differentiated, and hence capable of assuming rather varied forms. Hueck's conclusions are in agreement with the findings of the present paper which emphasizes a point that
has been stressed in each of the reports of the series; namely, the importance of the capillary endothelium as distinguished from the endothelium in general.

Rössle and Yoshida several years ago claimed that endothelial cells could produce collagen fibrils by a process of metaplasia. It seems unnecessary, in the light of what has just been said, to apply the term metaplasia to this phenomenon. These cells evidently represent a cytological element in the body that is intended for the process of repair and is unusually plastic, being able to assume varied functions. It is, as it were, a persisting mesenchyma; if this was not so and there were no polyblastic cells present, the process would be slower and less effective. Hueck's ideas on the impregnation of "indifferent fibrillae" with collagen, elacin, or other substances are also in accord with the staining phenomena observed in the formation of the tubercle reticulum. There is at first a fibrillar or retiform structure to the cytoplasm of the epithelioid cells, without a definite collagen staining reaction. The fibrils can be seen but their color at first does not differ materially from that of the cytoplasm. Gradually, however, they take on a faint pink stain with acid fuchsin or phosphotungstic acid-hematoxylin, or a faint turquoise-blue with aniline blue. These colors increase steadily in intensity as the fibrils become thicker and more evident. The changes from black to brown in the Bielschowsky-Maresch technique are analogous.

That Niagara blue does not appear in the epithelioid cells to any extent must mean one of two things, either the cells do not take it up, or they lose it as soon as they differentiate from the more compact to the larger, paler type. It is certain that they take up and retain the ink through all their phases; it seems doubtful that they have lost many blue granules, as one can seldom demonstrate such a process. If such a reaction did occur the earliest specimens should show groups of cells containing blue granules, while the later stages would show progressive loss of this color. This does not seem to be the case, except in a few instances, when cells with blue granules are occasionally included in tubercles. They are then most often found near the caseous centers.

There may be some question as to the part played by the lymphoid reticular cells in tubercle formation in the lymphoid tissue. If
they do form the epithelioid cells, they must lose their charge of blue
dye in so doing and acquire a heavier load of carbon granules. It
would seem easier to infer that the epithelioid cells are derived from
the endothelium of the scattered vascular capillaries. This would
explain the absence of blue granules as well as the smaller amount of
carbon present in comparison with that found in the epithelioid cells
formed in organs more richly supplied with blood capillaries.

CONCLUSIONS.

1. Miliary tubercles produced in the lung, liver, spleen, kidney,
lymph nodes, and omentum of the rabbit by hematogenous tuber-
culous infection are composed chiefly of cells originating in the capil-
lar y vascular endothelium.

2. These cells have a marked affinity for carbon in colloidal sus-
pension, which makes their identification possible.

3. A benzidine dye like Niagara blue, while acting selectively on
macrophages, lymphoid reticular cells, renal convoluted tubular
epithelium, and free polymorphonuclear leucocytes, is not found in
the tubercles to any appreciable extent, unless there is necrosis,
when the staining is diffuse.

4. The tubercle reticulum is composed of fibrils resembling con-
nective tissue fibroglia and collagen fibers in every way and produced
not only by fibroblasts, but by the endothelial cells themselves, after
these have migrated from the vessels, differentiated into epithelioid
cells, and formed tubercles. This process is well advanced 5 weeks
after inoculation.

BIBLIOGRAPHY.

Evans, H. M., Bowman, F. B., and Winternitz, M. C., J. Exp. Med., 1914, xix,
283.
1920, b, xxxii, 533.
Kiyono, K., Die vitale Karminspeicherung, Jena, 1914.
Kon, J., Arch. Entwicklungsmech. Organ., 1908, xxv, 492.
Kostenitsch, J., and Wolkow, Arch. méd. exp. et anat. path., 1892, iv, 741.

EXPLANATION OF PLATE 12.

Magnification, × about 350. Fig. 1 is from a paraffin section impregnated by the Bielschowsky-Maresch method; the rest are from phosphotungstic acid-hematoxylin preparations.

Fig. 1. Reticulum fibrils and carbon particles in the epithelioid cells of a 5 week tubercle in the lung. It will be noted that the fibrils run in the cytoplasm of cells liberally dotted with carbon.

Fig. 2. A 5 week tubercle in the liver, showing the distribution of carbon particles and the peculiar ring-shaped masses referred to in the text.

Fig. 3. A 5 week tubercle in the spleen with more carbon present than is usual in such tubercles. Rings of ink particles are also found.

Fig. 4. A cortical, 5 week tubercle in the kidney. The carbon is rather sparsely distributed, but it can be made out clearly in many of the epithelioid cells.

Fig. 5. Tubercle in the same stage of development in a lymph node. The carbon is very finely divided and still more sparsely distributed, but several cells have a moderately heavy deposit, and careful search reveals several granules in most of the cells.
PLATE 12.

(Foot: Endothelial reactions. IV.)