STUDIES ON ENDOTHELIAL REACTIONS.

V. THE ENDOTHELIUM IN THE HEALING OF ASEPTIC WOUNDS IN THE OMENTUM OF RABBITS.

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PLATES 51 TO 54.

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A study of the reaction of the capillary endothelium to various pathological conditions would be incomplete if the part played by its cells in aseptic wound healing were not considered. The fact that fibrils were demonstrated in the epithelioid cells in tuberculosis (Foot, 1921) makes it imperative to ascertain whether cells of like origin take part in the formation of fibers in the process of simple tissue repair after aseptic injury. As stated in previous papers in this series of studies on endothelial reactions (Foot, 1919, 1920, a, b, 1921), it may be assumed that the endotheliocyte after leaving the vascular endothelium, may be transformed into larger forms, known as macrophages and by many other names. They may also fuse with like cells to form syncytia, or giant cells. What is their function in the formation of granulation tissue? We have seen that they may be vitally stained in situ by means of a 50 per cent mixture of Higgins' waterproof drawing ink and distilled water, administered intravenously; under these conditions they may be followed after they leave the parent tissue and migrate to other parts. Thereafter any cell in the omental lesion found to contain these ink globules is.

1 The name endotheliocyte has recently been applied to the small mononuclear endothelial cell, in contradistinction to the macrophage, or histiocyte, the larger form of this cell. It will be employed throughout this paper in connection with the endothelial cell in its small form, as seen shortly after emerging from the vessels.
presumably of endothelial origin, irrespective of its position in the tissue.

It must be borne in mind that this appears to be the only reliable means for identifying the endothelial cell extra situ. The use of the more diffusible colloidal stains, particularly those of the benzidine type, is not alone sufficient; they usually fail to stain the endothelium in situ, although they appear in its derivatives in the tissues outside of the vessels and also stain cells that were never a part of the endothelium. They are, therefore, only reliable as adjuncts to the colloidal carbon used in this connection. Recent articles, quoting this series of papers, seem to have missed this point; unless colloidal carbon be employed, accurate comparisons cannot be drawn. Aside from the liver epithelium, in which this pigment can be found after forced, prolonged administration, it is taken up only by cells which conceivably could have originated in the capillary endothelium. It is probably not phagocytosed by cells outside of the vessels, since it is never found escaping through their walls in a free state, unless there be hemorrhage; since it does not appear to be transferred from cell to cell, except when dead phagocytes are, in turn, phagocytosed by younger cells of similar origin; and since it apparently disappears from the circulating blood in a short time after its administration, being withdrawn by the endothelium of the liver, lungs, and bone marrow, or lying in the splenic sinuses or their endothelium. This localizes the carbon granules principally in the endothelium. Furthermore, the endothelium of peripheral capillaries in the connective tissues does not seem to take up the ink readily unless it is somewhat inflamed and swollen; this accounts for the fact that ink is so sparingly found in this situation in the earlier lesions of the series to be described later. When injected directly into the subcutaneous tissue, the carbon particles are taken up almost entirely by phagocytes and not by the sessile cells; fibroblasts, when part of the fixed tissues, seem to have so limited a capacity for incorporating these carbon granules, even when coated with them, that they may be considered as having no phagocytic function.\(^2\)

\(^2\)It seems probable that foreign particles are phagocytosed by these cells while they are wandering about and retained by them after they become sessile; the reasons for this assumption will be made evident further on in this paper.
Another point that should be kept in mind is that cells containing carbon and other vital stains seem in no way to be hindered from performing their vital functions; they can wander freely, divide by mitosis when almost filled with these pigments, and phagocytose other substances, such as fat, cellular debris, or microorganisms. Fig. 1 shows such a cell in mitosis, with large granules of carbon and Niagara blue grouped at both poles. Such cell division explains the rapid dispersal of carbon particles through foci of active cell proliferation, each successive division resulting in a corresponding diminution in the number of pigment granules per cell.

In order to answer some of the objections that could be raised against deductions based upon the study of fixed and stained tissues, the behavior of the ink particles has been observed in vivo in two widely separated groups of animals, the frog and the rabbit.

**Action of Ink in Vivo.**—0.2 cc. of Higgins' ink and distilled water in equal parts, injected into the heart of a pithed frog; can be found circulating in a free state in the capillaries of the mesentery and toe-web for about an hour. After this it is chiefly contained in cells which, in Giemsa films, prove to be mononuclear leucocytes. (This refers to the distribution of the ink in the mesentery and toe-web only.) These often adhere to the endothelium, particularly at the sharp angles of anastomosing vessels, and sometimes penetrate their walls and appear in the tissues. Little or no ink is taken up by the endothelium so long as the circulation continues moderately brisk.

In the rabbit omentum, however, the ink circulates in a free state for less than an hour, in a urethanized animal injected intravenously with 5 cc. of a 50 per cent mixture of Higgins' ink in distilled water and observed in a warm chamber. After $\frac{1}{2}$ hour the endothelium is liberally dotted with black granules, even in vessels where the circulation is rapid. During 24 hours no free ink was seen to leave the vessels of either species of animal; apparently the pigment was always carried out by cells. The results of these observations on two amphibian subjects and one mammal would indicate that there are marked differences in the physiology of their endothelium, just as there is great dissimilarity in their connective tissue cells. It is unsafe to draw conclusions from observations based upon a study of inflammation in Amphibia and then to attempt to apply them to the same process in Mammalia. More work will be done on this subject and reported later.
After experiments with various rabbit tissues, the fatty omentum was chosen as the most suitable for the purpose. The results were compared with those obtained in wounds of the ear, abdominal wall, and skull; a discussion of these will have to be deferred for the present, as it would make this article too unwieldy. The fatty omentum contains between its two mesothelial layers little else save fat cells and vessels (Fig. 2); fibrous tissue is so scanty as to be almost negligible and the lymphoid taches laiteuses are not encountered in this fatty portion of the organ. The tissue is, therefore, very simple and any added element is quickly detected.

A series of animals was anesthetized and the omentum looped up by through and through sutures into a bunch about 1 cm. in diameter. Rice's No. 100 white sewing silk was always used. The omentum was then dropped back and the abdomen closed by suture. The aseptic lesions thus obtained were removed, either after death by the intravenous injection of fixative under anesthesia, or at operation under ether, at such intervals as would produce lesions representing periods from 2 hours to 5 weeks (2 hours; 1, 1½, 2, 3, 4, 5, 6, 7, 9, and 12 days; 2, 3, 4, and 5 weeks).

The tissues were fixed either in Helly's or in Zenker's fluid, cut to 5 microns in paraffin and stained with Mayer's aqueous carmalum, Van Gieson's stain, and Mallory's aniline blue and his phosphotungstic acid-hematoxylin stains. In the Van Gieson procedure care was taken to use only freshly prepared Weigert's iron-hematoxylin (the iron chloride solution deteriorates rapidly) and to soak the sections for ½ hour or more in water, before counterstaining with Van Gieson's picric acid-acid fuchsin solution. This brings out the nuclear details and the fibroglia fibrils in a way that is impossible to obtain if the counterstain is applied immediately after the hematoxylin. All the animals were given 10 cc. of 1 per cent Niagara blue in distilled water intraperitoneally daily for three doses and thereafter thrice weekly. They received 50 per cent Higgins' waterproof drawing ink in distilled water intravenously; the injections were begun at the time of operation. Up to 1 week they were given 5 cc. daily, those living longer were injected with 2 to 3 cc. three times a week.

Several of the lesions representing critical periods in the series were produced in duplicate or triplicate, on separate animals; especially the 1, 4, and 5 day lesions. None of them showed evidence of infection except one of the 5 day set, in which there was more exudate than could be explained by trauma alone. This infection was, however, insufficient to cause gross changes.

The Niagara blue was kindly furnished by Dr. George B. Wislocki, of the Department of Anatomy of Johns Hopkins Medical School.
Macrosopic Examination.

Macroscopically the omental lesions were swollen and hemorrhagic up to 3 days, thereafter diminishing in size, but increasing in firmness. Their surface was covered with a light coating of fibrin in most cases; in some, where there was slight capillary oozing, it was encased in a thick, whitish envelope. This was purposely produced, as will be seen later. On section the lesions were like the normal fatty omental tissue, with hemorrhagic areas in the early lesion; in the older specimens they appeared pale and fleshy and finally fibrous.

Microscopic Examination.

The description of the microscopic changes will be confined as far as possible to the consideration of the behavior of the endotheliocyte and its derivatives, and to that of fiber formation. The other features of aseptic wound healing are too well known to merit further discussion. As there are two fields of study in the experiment, the lesion in the omentum and that in the immediate vicinity of the stitches, they will be considered separately.

Changes in the Omental Tissue.—At first there is the usual hemorrhage and exudation of fluid, fibrin, and polymorphonuclears; the capillary endothelium becomes swollen very early in the process (Fig. 3) but does not show much ink at first, as this was administered at the time of operation when the capillary endothelium was as yet unaffected. Numbers of endotheliocytes surround the smaller vessels, and single cells can be found penetrating their walls, one group migrating through the tissues, another tending to become sessile and to form a network of anastomosing cell processes. These groups will be followed separately. By the 2nd day mitotic figures are abundant in the endothelium of the capillary blood vessels and, to a lesser extent, of the lymphatics; they are also found in the wandering, rounded, or spheroidal cells at some distance from the vascular areas. Some of these cells lie just outside of the capillaries, others at a distance, and many contain from one or two, up to many granules of carbon. Very few mitotic figures are found in sessile spindle cells; there are more division figures in the vascular endothelium and immediately outside of the vessels than elsewhere in the sections. The prolifera-
tive activity of the injured tissue is found chiefly in or near vessels. Lymphocytes and plasma cells appear by the 3rd day (Fig. 4). At the end of 24 hours the wandering group of endotheliocytes begins to surround the damaged fat cells and form a wall about them; but this is more striking on the 3rd day (Fig. 5), when syncytia, or foreign body giant cells, are formed. These are easily found on the 4th day (Fig. 6). Many of the phagocytes, now grown large enough to be called macrophages, show mitotic figures, and mitoses can be found in the protoplasm of the syncytia, as shown in Fig. 7, taken from a 6 day lesion. In one instance a multiple mitosis is seen in one of these giant cells (Fig. 8), resembling those observed in the true tumor giant cells. There is a monaster at the center, lying at the equator of a diaster (seen slightly diagonally); a second diaster, much smaller, is situated in one corner of the cell. This is the first time that mitotic figures have been observed in the syncytia formed in this series of experiments. Phagocytes also invade the pyramidal spaces between fat cells and multiply freely, some of them becoming sessile and intimately connected with a delicate fibrous network (cf. Fig. 6), thus joining the sessile group.

By the 4th day wandering phagocytes begin to appear in increasing numbers near the periphery of the omentum, lying in remnants of fibrin which was deposited during the more acute stages of the inflammation. Niagara blue, in granular form, is now very evident in these cells. Plasma cells and lymphocytes continue to increase in number, but are not vitally stained and do not seem to play a formative part in the process. By the 6th day the injured fat cells are reduced in size and largely replaced by syncytia, and there is a definite zone of vitally stained, vacuolated phagocytes at the periphery of the omental sections. Apparently they have removed much of the fat that was liberated by operative trauma. New capillaries are found branching among the fat cells, where they were formerly few in number. The number of plasma cells and lymphocytes decreases.

By the 9th day phagocytes are found penetrating the surface of the omentum and entering the peritoneal cavity, where they lie in fibrin or coagulum (Fig. 9). They may also enter lymphatic spaces in the tissue and form large, pyramidal plugs of granular, blue-stained macrophages. Their task is apparently finished, they have removed the
debris. Many of them degenerate and die, death being indicated by the vital staining of their nuclei with the Niagara blue. Although the morphology of nucleus and cytoplasm is apparently normal, the color (in carmalum sections) is blue instead of the normal carmine, upon which the blue is superimposed. Niagara blue, like trypan, isamine, or pyrrhol blue, or trypan red, is valuable as an indicator of cell death, for it marks the onset of death before the morphology of the cell has changed beyond the degree shown in degeneration. These cells then disintegrate, liberating their contained ink and Niagara blue not as a group of dispersed granules, but as black or blue globules, often as large as erythrocytes. One or more endotheliocytes often lie near such dead phagocytes and appear to pick up their debris.

The tendency of the endotheliocytes to form fixed tissue is not limited to the first 24 hours of the process under observation. They appear to do this throughout the entire series of lesions, so that new tissue is formed first near the vessels, gradually spreading among the fat cells, or along the denuded surface of the omentum (Figs. 10 and 11). At first cells lie free on this surface, surrounded by strands of fibrin. They gradually send out more and more protoplasmic processes, and, as time goes on, fibrous tissue forms in their neighborhood and is penetrated by new capillaries, which in turn furnish more endotheliocytes. There are normally very few fibroblasts present in this tissue; that some of them proliferate and form new fibrous tissue is, of course, probable, but the impression gained from an intensive study of a great many sections from these lesions is that the new tissue is the result of a migration of cells and subsequent proliferation, rather than of the proliferation of cells already present. This impression is intensified by the presence of carbon in many typical fibroblasts.

The changes seen between the end of the 1st week and that of the 2nd are to be ascribed to absorption and fibrosis. The phagocytes remove much of the fat, leaving in place of the large, pale omental fat cells, areas of much smaller fat vesicles. Between these, fibrous tissue is formed (Fig. 12).

Stitch Absorption.—After 1 day the stitches are found surrounded by bands of coarse fibers, apparently collagenous in nature. This could be accounted for by the stitch cutting through several layers of
fat cells and gathering the interstitial fibers that lie between them into one strand of connective tissue, or by a coagulation of some material or substance around the stitch. As these fibers often appear rather granular, and as similar fibers, to be discussed later, appear in areas of hemorrhage, the latter explanation seems the better. The only cells present at this time are erythrocytes and polymorphonuclears, which lie between the silk fibers in a coarse, granular coagulum that also gives the color reaction of collagen, though faintly (Fig. 13).

Endotheliocytes invade spaces between the silk fibers on the 4th day; many of them contain ink and show fibroglia fibers. Were it not for the ink, they would pass for fibroblasts from the surrounding connective tissue; but it must be remembered that the surrounding connective tissue is very scanty, while these cells are quite numerous. They next crowd together and form syncytia (Fig. 14), which are well developed by the 6th or 7th day. Among these, numerous discrete cells can be seen, with and without ink granules, and numerous fibroglia and collagen fibrillae of a delicate, well defined type can be demonstrated. At the same time the coarse, fibrous band surrounding the stitch as a whole is invaded by wandering cells and teased out into a dense network of delicate collagen fibrillae. This is the first evidence of cellular activity on this particular band of fibers. Some of the cells that penetrate it show ink granules.

By 1 week the syncytia in the stitch have become very large and surround the silk fibers with sheaths of protoplasm, within which lie fibroglia fibrils, ink granules, and Niagara blue. A few days later some of the elongated syncytia begin to show peculiar fringes of very coarse fibroglia fibers, which project from their narrow extremities (Fig. 15). These fibers stain blue with Mallory's phosphotungstic acid-hematoxylin, red with his aniline blue connective tissue stain, and faintly gray with Van Gieson's stain. After 3 to 5 weeks much of the silk is disintegrated and the syncytia are filled with the fragments (Fig. 16). Collagen fibers run in all directions between the remaining silk fibers, and fibroglia binds the cellular elements closely together. The silk is sometimes completely absorbed, and the syncytia, their work completed, break up into masses of epithelioid cells (Fig. 17) after their nuclei have rearranged themselves. Cell boundaries appear between the latter, where none existed in the earlier
lesions. The coarse fibroglia fibers disappear. In place of the fields of syncytia, there are masses of more or less closely packed cells and small syncytia, with few instead of many nuclei.\(^4\) Capillary vessels grow into these areas and are surrounded by numerous endothelio-290cytes. Ink particles are retained throughout this process by the cytoplasm of the syncytia and the cells into which they resolve. Coarse collagen fibers are interwoven with these cells (Fig. 18).

**Fiber Formation.**—In contrast to normal omentum, in which there is so little fibrous tissue as to be almost negligible (cf. Fig. 2), fibers are found soon after inflammation sets in. Their formation is difficult to follow step by step. They are scattered through small masses of fibrin in distended tissue spaces; coarser and more undulating than fibrin threads, they lack the nodal thickenings of these and give a faint collagen reaction. The fibrin is partly removed, partly consolidated into coarser bands, like the torn shreds of a spider-web (Fig. 19) and, by the 4th or 5th day, it is abruptly replaced by a delicate felting of crinkled collagen fibrils (Fig. 20).

It is difficult to interpret the part played by cells in the production of this felting; that the polymorphonuclears remove, or dissolve much of the fibrin seems evident. Sometimes there is a moderate number of spindle cells in such an area, sometimes but two or three (Fig. 21). They may or may not contain ink and are always surrounded by a delicate, cocoon- or sheath-like network of fine collagen fibrillae; but as fibrils of the same type, or coarser, may be found at a distance from any cells, these sheaths might represent a local reaction to the cells on the part of the intercellular substance, or a product of the cells themselves. The theory that these filaments are formed either from rearranged fibrin, or by a filamentous coagulation of tissue fluids, to be impregnated later with collagen, should be reconsidered. Coarse bands of collagen fibers appear where none are found in control sections, without any obvious association with fibroblasts. Furthermore, they appear near masses of fibrin (Fig. 22) or in vascular areas (Fig. 23). Fig. 22 shows a mass of coarse, almost hyaline fibers that formed beneath a hemorrhagic effusion purposely produced.

\(^4\) This breaking up of giant cells has been observed in vitro in tissue cultures of bone marrow, in which they form on cotton threads, or the cover-slip itself.
over the omental surface, which was of 4 days standing. It will be seen that there are practically no fibroblasts near these fibers. The latter stain red in Van Gieson sections, blue with Mallory's aniline blue method, and brownish red with phosphotungstic acid-hematoxylin; either they are collagen fibers, or the three best known collagen stains are worthless as such. Similar fibers are found in areas where there has been infarction due to strangulation by the stitches. They do not appear in clots formed outside of the tissue in the peritoneum, fibrin alone apparently does not produce them. Some fluid, either secreted by living cells, or released from them by crushing, coming in contact with another fluid in the clot might combine with it to form a fibrous coagulum, after the manner of a chemical reaction. The resulting fibers would then become impregnated with collagen, or might in themselves represent a collagenous precipitate. Judging from their gradually becoming more and more intense in color reactions the first hypothesis would seem more probable.

That fibroblasts alone do not form fibers of this type is to be inferred from the fact that they invade areas of hemorrhage and grow luxuriantly, forming a dense network of anastomosing cells (Fig. 24). In these masses there are no collagen fibers whatever, and it is also significant that there is little or no fibrin present here. As far as fibroglia fibrils are concerned, there is nothing in this experiment to alter existing theories; that they apparently appear and disappear with readiness is shown in two instances: when fibroblasts undergo mitotic division there is no trace of fibroglia in their cytoplasm, and when syncytia that have apparently contained heavy fibroglia fibers break up into smaller cells, they leave no trace of these fibers as such. It is of some interest that fibroglia is readily demonstrable in these syncytia, which are generally admitted to be of endothelial origin, but in which heretofore only reticulum has been demonstrated by other writers.

The literature on fiber formation is too voluminous to be extensively quoted here; ample discussion and references can be found in any standard text-book on histology or pathology. What has been said here bears out the findings of Baitsell (1915, 1915–16, 1916) to a striking degree, although his papers had not been read at the time of writing this section. While he was unable to obtain a successful red staining
reaction, using Van Gieson's stain, with the collagen fibers he described, and although they stained blue with Mallory's aniline blue method, it will be seen that in the case of the rabbit tissue now under discussion not only these two stains, but also phosphotungstic acid-hematoxylin gave a successful collagen reaction. For a résumé of the literature on this immediate topic the reader is referred to an article by Lewis (1917).

Mesothelial Reactions.

There is very little to say concerning the reaction of the mesothelial covering cells of the omentum; they become detached very early in the process of inflammation and regenerate during the first 3 or 4 days. Areas of local proliferation are found, the cells tending to form small clumps, or heaps, on the denuded surface. The peritoneum thus formed is very readily detached during the process of fixation, particularly over areas where there has been extensive scar formation, so that they appear to be completely denuded. A few cells of the mesothelial type may be found here and there, often partially detached, indicating that the covering layer has been rubbed off by manipulation. The vital staining of the mesothelium is peculiarly scanty; although bathed in a large amount of aqueous 1 per cent Niagara blue, most of this appears to go through the cells without leaving very noticeable traces beyond a few, faintly blue granules. The phagocytes immediately beneath the peritoneum, however, are deeply pigmented both with blue and black granules. This is true of the normal as well as of the injured portions of the omentum after fixation with Helly's or Zenker's fluid.

DISCUSSION.

For the sake of convenience the endothelial cells under discussion may be considered as belonging to three types, or groups; but it must be understood that these groups are quite artificial. The endothelial cells of the end capillaries appear to retain non-specific or embryonal characteristics, or to acquire them under the influence of injury; they can then take on one of three types of growth, each of which passes readily into one of the others.
Group A.—These behave exactly like those cells observed in the first paper of this series (Foot, 1919), on the reactions to agar injections. Arising from the capillary endothelium, they leave the vessels and wander about in the tissues as phagocytes, and may persist as such to the end of the experiment. They are capable of further proliferation in the tissues, of forming syncytia, which are probably temporary in character, by fusion with one another and by further multiplication of their nuclei after this fusion (cf. Figs. 7 and 8). They can remove debris of various sorts, either by digesting it, or by rendering it inert by surrounding it with an "insulation" of protoplasm. They either migrate to the peritoneal surface or enter lymphatics, at the termination of the process that calls them out.

Group B.—These cells do not function as phagocytes after leaving the vessels, but become sessile almost immediately and produce new tissue. They differ from fibroblasts in no respect, excepting that they contain ink, and they exhibit all of the characteristic functions of that cell. It is probable that these two groups are freely interchangeable, phagocytes (Group A) becoming sessile, and fixed cells (Group B) rounding up and becoming free phagocytes.

Group C.—This group remains specific in as far as the cells of which it is composed are concerned with the production of new capillaries by budding. The endothelium of these new capillaries takes up ink with the same avidity as does that of its parent vessel, provided that the ink be supplied frequently enough throughout the experiment. Its component cells may then undergo the changes which characterize the preceding groups.

Injury or inflammation causes dilatation of the end capillaries, stagnation of the blood stream, and swelling and proliferation of the essentially unstable or non-specific endothelial cells. These can be found in the act of penetrating the vessel walls and leaving the capillaries, each of which becomes surrounded by a distinct zone, or collection of spheroidal cells that are not present in this situation in controls. Many of these cells can be found in mitosis and numbers of them contain ink globules. Despite this proliferation, new vessels are not formed to any extent before the 5th or 6th day of the process. The distribution as shown by a count of 100 unmistakable monasters and diasters, in sections from 5 day lesions and three counts of 50 from 2, 3, and 6 day lesions, is presented in Table I.
The second column includes mitoses found in cells in situ in the vascular endothelium; the third, those in cells in the immediate neighborhood of the vessels; the fourth, mitoses found in cells not apparently near any vessel; the fifth, peritoneal cells; and the sixth, cells of spindle shape in various situations. The count of 100 mitoses was made by two observers, to avoid personal error. The variations in the count from day to day are not very marked, if we except that of the 6th, which shows a marked increase in perivascular figures and a diminution in those found in spindle cells. From the average percentages it is clear that 59 per cent of the total number of mitotic figures are in or near the capillaries, 31.7 per cent in cells that are at a distance from vascular areas, these cells being rounded and some of them containing ink, so that they might well be migrated endotheliocytes; only 3 per cent mesothelial cells, or, more properly, 6 per cent, seen in the younger lesions only; and but 6.5 per cent in cells which, from their morphology alone, would be classed as fibroblasts. As previously stated, some of these, too, contain ink.

From the preceding paragraph it will be plain why many of these cells are considered to be endothelial in origin. Aside from the presence of ink there are (a) the predominance of intravascular and perivascular proliferation without a corresponding production of new vessels; and (b) the presence of many new cells of a rounded form, with vesicular nucleus and generous cytoplasm in places where none are found under normal conditions, together with insufficient evidence of proliferation in the preexisting, fixed tissue elements in these localities.
It is said that the perivascular collections of cells arise from the proliferation of fibroblasts closely applied to the outside of the capillaries and not readily visible under normal conditions. It is admitted that if these become detached and rounded up they cannot be distinguished from endotheliocytes, until they again become spindle-shaped and form fibrils. How does the ink get into their cytoplasm? Since it does not pass the vessel wall as such, it must be transferred from cell to cell, which, in this case, would merely fix a foreign body in the tissue, instead of leaving it in a cell that might migrate to the lymphatics and remove it as in anthracosis. In short, we must assume a cell that is so scarce as to be barely demonstrable; we must have it proliferate enormously in situ, which cannot be demonstrated in this experiment; and we must have inert particles of material transferred from cell to cell; all this before the theory that these are all connective tissue cells can be maintained. Is it not more probable that the cell becomes laden with ink while it is a part of the vessel (demonstrable in vivo) where there is plenty of that substance, and migrates from the vessel, retaining this ink? That these cells can form fibrils of reticulum is well known (Corner, 1920); that the reticulum undergoes transformation into fibroglia and is associated with the production of collagen fibers around the liver sinusoids has also been shown (Kon, 1907-08, Rössle and Yoshida, 1909). In fact, fibrils can be demonstrated readily in the capillary endothelium with Mallory's aniline blue connective tissue stain (red) and with his phosphotungstic acid-hematoxylin method (blue); the differences in color observed in fibers stained by the Bielschowsky-Maresch method may be attributed largely to their size and density, rather than to definitely specific differences.

SUMMARY.

The following hypothesis may be deduced from the evidence just submitted: The endothelium of the end capillaries, the fibroblast, and the immediate derivatives of these two are all mesenchymal in origin. Injury produces a reaction wherein there is stasis and hemorrhage, with the exudation of cells and fluids that contain some element or elements that affect these mesenchymal derivatives in such a way as to reestablish their embryonal characteristics. As a result, they form
a local, temporary mesenchyma at the site of injury, proliferate, migrate, and differentiate according to the needs of the case until the injury is repaired. They may form connective tissue, phagocytes, polyblasts, new vessels, etc. Phagocytosis and absorption gradually withdraw the exciting agents from the seat of injury, the temporary mesenchyma returns to adult type, and all that remains is the products of the process that are necessary for scar formation. It is apparently erroneous to think of the mononuclear cells of young granulation tissue as fibroblasts, endotheliocytes, etc., they should be considered as representing various stages in the differentiation of a local mesenchyma until the process of healing is completed, when they return to the adult types represented by these more specific names. The term polyblast, originated by Maximow, is particularly applicable to these cells, for under the conditions they are truly polyblastic; but that they are derived from lymphocytes is not indicated by results of this experiment—the lymphocytes and plasma cells appear to play a part that is unconnected with the formation of new tissue.

No definite statement can be made as to the nature of the stimulating substance, or substances, that bring about this reversion to embryonal type. As indicated, they are associated in some way with the blood, for the most marked cellular metaplasia is noted in connection with areas of hemorrhage, or in proximity to vascular areas. That the exciting substances are contained in the erythrocytes is furthermore indicated by the fact that the most metaplastic areas are found where the mesenchymoid cells are growing among masses of red corpuscles, with little visible fluid or fibrin present (cf. Fig. 24). Whether these substances are enzymes or not is purely problematical.

There is very little, in the several hundred sections studied for this and later papers, to indicate that wound healing is an orderly procession of specific cells to designated positions in these wounds. The only tissues retaining their continuity in growth to a degree in any way comparable with the descriptions in some text-books, are the surface epithelium and the vascular buds, or branches. The majority of mononuclear cells of mesenchymal type appear to fill in the injured area by migration, rather than by growth in continuity. Once they have reached a certain point, they react in the various ways just described. There is, of course, a migration of differentiated cells,
such as macrophages, polymorphonuclears, lymphocytes, etc., from the healthy tissue; but the framework, or structural foundation, of the granulation tissue appears to be composed of the practically undifferentiated derivatives of the connective tissue and capillary endothelium. In other words, it is conceivable that cells originating from connective tissue might become phagocytes and vice versa, both types temporarily losing all specificity under the influence of some chemical or enzymatic excitant in the exudate. Until we understand the physiology and chemistry that determine the developmental and retrograde changes of the normal, embryonal mesenchyma, as well as those of this temporary mesenchyma, we can never fully comprehend wound healing.

CONCLUSIONS.

1. The proliferation of the capillary endothelium of rabbit omentum, in aseptic inflammation, may result in the production (a) of new vessels, (b) of phagocytic endotheliocytes, and (c) of cells which appear to take on the functions of, and to be indistinguishable from fibroblasts.

2. The last two of the above types may be identified by their selective action for colloidal carbon, taken up while they are part of the vessel wall and apparently retained by them throughout the experiment.

3. The three types appear to be freely interchangeable and not distinctly specific groups.

4. Cellular proliferation is almost equally abundant in or near vessels and in free cells at some distance from them, but mitoses in fixed tissue cells constitute only 6.5 per cent of the average total count.

5. Carbon contained in the endotheliocyte does not affect its vital functions appreciably, particularly that of mitotic division.

6. Mitoses may be found, in this experiment, in syncytia (foreign body giant cells); sometimes multiple mitoses, such as occur in tumors, are found in true giant cells among the syncytia.

7. In this experiment collagen fibers are formed, apparently independently of cellular activity, from fibrin or some substance associated with fibrinous clots. The mechanism of this phenomenon is not yet evident.
8. Coarse fibroglia fibers may be formed within the cytoplasm of foreign body syncytia, which are of endothelial origin.

9. The reaction of the omental mesothelium, under the conditions of this experiment, is similar to that of the epithelium in skin wounds; aside from covering denuded surfaces it apparently takes no further part in the process of scar formation.

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EXPLANATION OF PLATES.

Figs. 9, 11, 14, 15, 16, 17, and 23 were prepared with Mallory’s aniline blue connective tissue stain, the rest are from slides stained by the Van Gieson method. This explains the pallor of the silk fibers in Fig. 13. Wratten filters G and H were used on all Van Gieson photomicrographs, filters B and E used on most of the aniline blue connective tissue stain photographs.

PLATE 51.

**Fig. 1.** Mitosis in spindle cell containing carbon and Niagara blue. Note condition of endothelium in neighboring capillary and distribution of ink. 3rd day. × about 780.

**Fig. 2.** Normal fatty omentum for control purposes. × about 390.

**Fig. 3.** Perivascular proliferation at end of 1st day. × about 390.

**Fig. 4.** Distribution of carbon and plasma cells in upper right corner. 3 days. × about 390.

**Fig. 5.** Endothellocytes surrounding fat cells. Note ink and diminution in size of fat cells. 3rd day. × about 390.

**Fig. 6.** Same on 4th day. Syncytia and fibers forming. × about 390.
PLATE 52.

Fig. 7. Same after 6 days. Mitosis in syncytium at left. Further diminu-
tion in size of fat cells. × about 390.

Fig. 8. Multiple mitosis in giant cell at edge of fat. (The black structures
are chromosomes and not carbon.) 6th day. × about 780.

Fig. 9. Vitally stained cells in exudate on surface of omentum. 9th day.
× about 390.

Fig. 10. Fibroblasts containing ink. 6th day. × about 390.

Fig. 11. Organization on surface of omentum. 9th day. × about 390.

Fig. 12. Fibrosis and great reduction in size of fat cells. 9 days. × about 390.

PLATE 53.

Fig. 13. Silk suture after 1 day. × about 390.

Fig. 14. Same after 5 days. × about 390.

Fig. 15. Same after 12 days. Note coarse fibroglia fibers. × about 390.

Fig. 16. Same after 3 weeks. Silk fibers becoming striated and less opaque.
× about 390.

Fig. 17. Same after 35 days. Epithelioid cells developing from syncytia as
these break up; silk almost absorbed. × about 390.

Fig. 18. Silk absorbed; collagen fibers forming at site of syncytia which are
breaking up. × about 390.

PLATE 54.

Fig. 19. Clot after 2 days. Note wavy fibers that appear and compare them
with the fibrin. × about 390.

Fig. 20. Fibrin absorbed; new collagen fibers appearing. 6th day. × about
390.

Fig. 21. Coarse fibers in clot, two or three fibroblasts among them. 3rd
day. × about 390.

Fig. 22. Coarse collagen fibers formed between exudate and fibrin in tissue.
Note comparative absence of fibroblasts. 4th day. × about 390.

Fig. 23. Fibers in vascular area; cell leaving neighborhood of capillary just
below center. 6 days. × about 390.

Fig. 24. Metaplastic fibroblasts growing in a fibrinous hemorrhage. × about
390.
(Foot: Endothelial reactions. V.)
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