Studies on Endothelial Reactions.*

(First Paper.)

The Macrophages of the Loose Connective Tissue.

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Of recent years a decided advance has been made in the study of various cells in the mammalian body, by the employment of vital stains and dyes. Administered during life and vital in the true sense of the word, since many of them are so harmless as to interfere but little with the vital functions of the organism, they can be regarded as indicators of the activity of the cells into which they have become vitally incorporated. This is not only true of dyestuffs — particularly those of the benzidine series — but also of various inert substances, the use of which has long been understood but as yet only incompletely appreciated.

It is fortunate that these dyes and substances enter into a group of cells concerning whose origin and activities there has been much uncertainty and even more dispute. These cells, the "macrophages" of Metchnikoff, have a definite affinity for the benzidine and other dyes of a colloidal nature, as well as for certain inert substances (carbon, India-ink, cinnabar), and this fact has reawakened not only the old interest in their origin and function, but the old disputes as well. Any one who teaches normal or pathological histology is repeatedly confronted with the problem of explaining the identity and origin of the "large mononuclear cell," "endothelial cell," "macrophage," "clasmatocyte," "wandering connective-tissue cell" and almost as many more. Are these cells all entities? Do they represent several groups, or are they one and the same cell masquerading now under one name, now under another?

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F. A. Evans[2] has given a very good idea of the prevailing confusion regarding the origin of these cells in an article published in 1917. He gives a list of the various names given and the authors responsible for them. Combining his paper with that of Goldmann, to be quoted later, one gets a very good idea of the whole subject.

It was with a view of coming to some conclusion on this point that this series of experiments was undertaken. The means employed is a combination of methods used by other experimenters, but by them used singly. Goldmann,[8] in 1909, published his first article on the action of the benzidine dyes *intravitam*, and followed this by another three years later.[9] In the latter he discusses many and varied topics, — so many, in fact, that much of value has been buried in its hundred-odd pages. Vital staining of the macrophages is one of the more important subjects, combined with the behavior of these cells in miliary tubercles, trichiniasis and carcinoma. One finds, however, that the origin of the cell is still far from certain; the author makes conjectures and very shrewd ones, but leaves much unproven. H. M. Evans[3, 4, 5] and Downey,[1] have continued along these lines and added a great deal to our knowledge of the behavior of various cells to even more varied dyes.

To McJunkin[12] belongs the credit of introducing the use of a colloidal lampblack-gelatin solution as a method of labeling cells of endothelial origin; while Forbes,[7] under the guidance of Wolbach and Mallory, did a valuable experiment in connection with the formation of giant-cells from the fusion of phagocytic mononuclears, which he claimed to be of purely endothelial origin. His article will serve as a foundation for the hypothesis I wish to build up in the following paper; for I have omitted repeating much of his work, as his findings dovetail with my own and are perfectly clean-cut, making repetition unnecessary. For the same reason a long series of animal experiments was deemed unessential.

In most of the work done on the origin of these cells there is, however, a valuable link missing, — that which will definitely trace the cell from its point of origin to its final destination.

McJunkin's work is complete in so far as the origin of the endothelial leucocyte is concerned. The work on the macrophages of the liver-sinusoids (this includes Goldmann, Evans-Winternitz and others) is likewise complete; but these observers consider the sinusoidal endothelium specialized, and hence different from the vascular endothelium elsewhere. This leaves the parentage of the macrophage still in doubt, so far as they are concerned. Forbes admitted in his paper that, while he had demonstrated the origin and destination of the cells, the intervening link was still lacking. He says, "Whether these cells are derived mostly from those which existed in the tissue-spaces before the lesion or mostly from those which migrated through the vessel walls, it is impossible to ascertain."

This being the case, it seemed best to me to combine these methods and see what the result would be; i.e., inject trypan blue intraperitoneally, agar subcutaneously, and, if necessary, lampblack emulsion intravenously. If the mononuclears were of omental or connective-tissue origin they would stain with the benzidine dye; if endothelial, they would ingest the lampblack.

A series of experiments was accordingly performed, with the results hereinafter recorded.

First, a female guinea-pig was saturated with trypan blue, by administering four or more doses of five cubic centimeters of a one per cent solution of the dye in distilled water intraperitoneally. Sterile agar was then injected in a fluid state into the left gastrocnemius muscle. The trypan blue was continued at intervals, the animal killed after twelve days had elapsed and the muscle hardened in ten per cent formalin and examined. Beautiful foreign-body syncytia had surrounded the agar-mass, but they contained trypan blue in but small amounts and this quite diffusely, which confirms the findings of F. A. Evans in connection with lycopodium injections and carmine staining.

A series of mice was next injected in a similar way, excepting that the agar was mixed with a little lampblack. The

injections were so arranged that, when the last mouse was killed, there was a series of lesions representing all the stages from one to fourteen days under the skin, and in muscle on the 2d, 5th, 7th, 9th, 10th and 12th days. The trypan blue was given in frequent doses of one cubic centimeter of a one per cent solution intraperitoneally. The reaction was surprisingly slight and wholly unsatisfactory for study.

Finally a large, white, male rabbit was saturated with trypan blue, several doses of ten cubic centimeters of a one per cent solution being given intraperitoneally; then this was continued almost daily and daily injections of an emulsion of five per cent lampblack with one per cent gelatin in normal salt-solution were given into an ear-vein in quantities varying from 1.5 to 2.0 cc. (trypan blue given on 1st, 2d, 3d, 4th, 6th, 8th, 10th and 11th days). Sterile agar was melted and injected daily under the skin of the back over a period of thirteen days, omitting the sixth. The idea was to keep on hand a supply of both trypan blue and lampblack in the tissue fluids of the rabbit, as well as a substance that would call forth an exudate of phagocytic cells. This method gives a series of such reactions covering a period of from one to twelve days at daily intervals, with the exception just noted. Agar was also injected into the fleshy part of a leg on the 2d, 4th, 6th and 8th days, and into the muscle of the back on the 10th, care being taken to get it deep into the muscle. This gave a series of lesions eleven, nine, seven, five and three days old respectively.

On the thirteenth day the animal was anesthetized, blood-smears were made from an ear-vein, and he was killed by injecting ten per cent neutral formalin into the heart. He was then autopsied and his organs placed in the same fixative. The subcutaneous lesions were fixed separately, as were those produced in the muscles, ten per cent neutral formalin being used.

There was nothing grossly abnormal found at autopsy, save for the effects of the coloring-matters used. The lungs and spleen were a sooty, brownish blue; the other organs deep to

light blue according as their affinity for trypan blue varied. The retained tissues were fixed, washed and one-half counterstained in bulk with alum cochineal, before embedding in paraffin, the other half being embedded and counterstained after sectioning with Mayer's carmalum (aqueous). Sections were cut as thin as possible, the cedar-oil method of embedding being employed to prevent undue hardening, those stained with the alum cochineal giving by far the most precise results. Mallory's phosphotungstic acid hematoxylin and Van Gieson's connective-tissue stains were used when needed.

Before describing what is found at the site of the agar injections, it will be necessary to state several facts concerning the general microscopical appearances and point out what becomes of the injected carbon in the body. Blood smears, stained by Wright's method, show seventy per cent of the circulating mononuclears containing carbon particles, to thirty per cent containing none; a result obtained by actually counting one hundred mononuclears. A differential count of four hundred leucocytes shows fifty per cent polymorphonuclears, forty-two per cent lymphocytes, seven per cent endothelial leucocytes and one per cent basophiles; which is an approximately normal count for a rabbit, according to the table given in Weidenreich's monograph, "Die Leukocyten und verwandte Zellformen."[14] The carbon in the endothelial leucocytes appears sometimes as one or two granules, sometimes a few more, and less often in large quantities, so that the cell is literally stuffed with them.

The endothelium of the smaller capillaries and lymphatics contains moderate amounts of this carbon, and even medium-sized vessels show some lampblack granules in their intima. The cells of the omentum are often loaded with lampblack, but as they are all well stained with trypan blue, in the form of coarse granules of uniform size, it is sometimes difficult to distinguish mesothelial from endothelial cells in this organ. It is evident that there is more lampblack in the neighborhood of the vessels than further out. Some cells, rather smaller than the mesothelial cells, are loaded with it and are probably migrated endothelial cells. The lung and spleen, and the

Kupffer cells of the liver sinusoids to a lesser degree, are particularly active in taking up the lampblack. The endothelial cells of the lung are far blacker than in most cases of advanced anthracosis. The phagocytic cells in the spleen are likewise loaded down with carbon and the sinusoids of the liver are, in some places, almost blocked by cells filled with this substance. It is necessary to emphasize all these points, for therein we have an explanation for the comparatively small amount of carbon found elsewhere. The lungs get the first chance to take it up, as it is here that the lampblack encounters the first narrowing of capillary lumina in its transit from the ear vein. The liver, spleen and omentum, as well as some of the lymph-nodes, have a definite phagocytic function to perform, and therefore take a heavy toll. After running the gauntlet of these organs, the circulating carbon particles are markedly reduced in numbers.

We now come to a discussion of the microscopical findings in the subcutaneous and intramuscular lesions, where agar has been injected. This has already been too well described by Forbes, from the morphological standpoint, to need much description other than that of the distribution of the carbon and trypan blue. I shall depend largely on his paper for support where the question of intravascular endothelial proliferation comes up.

Besides the acute exudate on the first day after injecting agar, one finds many mononuclear cells which are of two types: (*a*) A compact, dense cell, with bean-shaped nucleus and rather opaque cytoplasm, most frequently found near the vessels of the panniculus carnosus and corium; and (*b*) a large vacuolated mononuclear, the "clasmatocyte," found singly and sparingly in the loose connective tissue. The smaller type measures on the average seven microns, about the same as the polymorphonuclears in these slides. They lie in dense masses about the vessels, penetrating the muscle and resemble crowds of people leaving a theater, as they fan-out or deploy into the deeper and looser subcutaneous tissue. They contain no trypan blue, but about one third

of them exhibit varying amounts of lampblack; some of them a granule or two, some of them many, just as was seen in the endothelial leucocytes of the circulating blood. The larger cells or clasmatocytes measure 7.5 by 12 to 15 microns, they contain granular trypan blue around the edges of vacuoles and in the cytoplasm and they also contain carbon. Although the agar has been in the lesion for only twenty-four hours, carbon has been present in the blood for twelve days — a statement that will be readily understood if the reader will refer to the description of the technic employed. The inference is, therefore, that these larger cells have taken up their lampblack at a time probably several days prior to the injection of agar at this point. Fig. 1 shows these two types of cell.

The vascular endothelium of the vessels of the corium and subcutaneous tissue also show lampblack granules in some of their lining-cells, as shown in a vessel in Fig. 2. Although a few particles may be found here and there in polymorphonuclears and free in the tissue, this is only occasional and in quite negligible quantities. It is in larger amounts in the vessels near the epidermis, near the hair follicles, than it is deeper down, — a fact that needs explanation. There is a marked increase in the number of cells in the intima of the vessels of the corium, as well as a swelling of their cytoplasm; the lumina of some of the vessels seem much narrowed by this increase in number and size, which is in accordance with Forbes's findings.

The two days' lesion shows a continued migration of the mononuclears, with an increase in the number of cells in the vicinity of the blood vessels. The cells nearer the agar-mass are swelling and tending to form groups or clumps, and their cytoplasm is beginning to take on a diffuse, light bluish stain with trypan blue. Carbon is present in about one third of the cells, the same proportion as seen on the first day.

The third day one sees that these cells have increased still further in size, until they measure 7.5 microns in width and from 10.5 to 13.5 microns in length; they continue to take up the trypan blue. Vacuolization is common, and many of

them have become indistinguishable from the clasmatocytes of the first day, both in size, shape and staining-properties. Trypan blue is distributed around and in their vacuoles, and about a third of them contain carbon particles also. There are many of the smaller, younger endothelial cells about (for we can call them by this name from now on), and they, too, show carbon in the same percentage. Clumping of these cells has begun, and several are found with two or more nuclei.

On the fourth day there is still carbon in one third of the endothelial cells, both large and small. There seems to be some fibroblastic growth also, these cells standing out rather sharply on account of their perinuclear granules of trypan blue, gathered in two groups, one at each nuclear pole. New blood vessels are growing into the exudate and their walls contain lampblack particles, just as was the case in the preformed vessels; while in their lumina are many smaller, circulating endothelial leucocytes, numbers of which contain lampblack grains. Out in the exudate the vacuolated endothelial cells are not only clumping and forming syncytia, but they are also beginning to anastamose and form delicate reticula, which will be described later. Carbon particles are found sprinkled through the cytoplasm.

By the fifth day, shown in Fig. 3 of the plate, the vacuolization of the protoplasm of the larger endothelial cells has still farther increased and there is less carbon in evidence; only about a quarter of the cells show it. The anastamosing processes of the cells and the reticulum formed by the vacuolization of their cytoplasm all stain light orange-red with Mallory's phosphotungstic acid hematoxylin. Some of the fibers have beaded blue granules threaded along on their filaments, but none stains blue throughout. Fibroglia and collagen fibers are abundant in places, but seem to be survivors from the connective tissue that was torn apart by the agar-injection mass. The endothelial cells work their way into these spaces and then anastamose or fuse: whichever they do, carbon particles are found scattered through their cytoplasm. The picture is quite analogous to that seen in tissue-cultures of bone-marrow or spleen on the fifth day of their

growth in vitro, and does much to explain why these organs make so much more successful cultures than do others, since they are extremely rich in endothelium. I have described such growth in a paper published in 1912.[6] A new phase of the development of the endothelial cells in the exudate of the present experiment is the finding of mitotic figures in the endothelial cells which have already migrated. Whether by coincidence or not, they have not been found in carbon-containing cells; but, as McJunkin has often found them in dividing carbon-carriers in vessel walls, it is probable that they could be demonstrated here. The vessels of the corium and the cells in its spaces show much less activity than in the earlier stages of the experiment; the corium is much more normal in appearance.

The migration of endothelial leucocytes comes almost to a standstill on the seventh day, and mitoses are easier to find in the now sessile cells of the exudate. The syncytia here all contain carbon granules, although they are becoming more and more thinly spread out in the cytoplasm. The eighth-day lesion proved unsatisfactory, as it overlapped that of the ninth.

On the ninth day the syncytia are more compact, and mitoses are found in the single cells in their vicinity. The corium sometimes shows giant-cells, or syncytial masses, as the agar may be deposited under the epidermis as well as deeper down, while the needle is being withdrawn or if it does not penetrate far enough. In the nine- and twelve-day lesions of this series this occurred. In the former one finds much more carbon in the syncytia formed near the hair-roots than in those more deeply situated; I cannot explain this, but it is in accordance with the findings described under the first day. The cells in this situation are loaded with lampblack.

The preparations made from ten-, eleven- and twelve-day lesions do not differ materially from those of the ninth. The syncytia are either in the form of compact "giant-cells" (which are really cell-groups and hence neither giants nor cell-entities, so why not drop the term?), with nuclei arranged peripherally; or they are composed of many anastamosing

cells, which are rather less intimately blended with one another. They contain a small amount of trypan blue, a larger amount of carbon, and still more refractile droplets of agar, contained in vacuoles. The cells of the looser syncytia are often found in mitosis; the dividing cells almost always give indications of a cell-boundary. As the migration of carbon-bearing cells has practically stopped and the cells forming the syncytia have become actively mitotic (except in the denser groups), no new carbon is brought in. What is left of the original supply has become disseminated by the growth and division of the cells that contain it. It takes but little calculation to realize that cells containing from one to ten granules of lampblack will, after they have increased from three to five diameters, seem to contain much less. Likewise the division and consequent increase in number of these cells will still farther reduce the number of granules per cell. Why the trypan blue decreases may be explained by the rarification and thinning-out of the cytoplasm and by the extrusion of the dye-granules.

The process in the case of the muscle injected with agar is essentially the same as that in the subcutaneous tissue. In one preparation, that of the fifth day, there is definite increase in small mononuclear cells normally found in the intermuscular connective tissue, granular cells which stain intensely with trypan blue. These are often found near the blood vessels, and many also contain carbon. Whether they come from the vascular endothelium is very uncertain, as the latter does not usually stain deeply with trypan blue, nor does it take a granular stain. (The sinusoidal cells of the liver and spleen are exceptions to this rule.) Sometimes a few particles of this dye are found in intimal cells in vessels, but they are never as abundant as in the type now under discussion.

These blue-staining cells become intimately incorporated in the syncytia formed chiefly by the endothelial cells, and give rise to mottled blue areas in the resulting cell-complexes. Their nuclei are so similar to those of the endothelium that they would be indistinguishable one from another were it not for the blue granules. They are oftenest found in the

deeper injections of agar; shallow ones under an aponeurosis do not seem to bring them out. If we consider that the trypan blue is most in evidence near the panniculus carnosus in the skin preparations (a fact which I have not hitherto emphasized, but which is strikingly evident in all the slides) and in the deep intramuscular injections, we will be led to seek some connection between the chemistry of the stain and muscular metabolism. The muscle fibers do not stain with trypan blue, they are peculiarly free from it; but it seeks out these small, ovoid cells of the intermuscular supporting tissue.

A lymph-node, from the popliteal space of one leg, shows interesting lesions. There are groups of syncytial cells in its substance, usually near the marginal sinus, composed of fused endothelial cells containing much lampblack. The lesion is similar to a tubercle, but lacks the caseation at its center and is evidently more or less filled with particles of agar, which have probably been brought thither by phagocytes.

The results of this experiment seem to point to four things: (1) that the macrophages of the connective tissue spaces are, in reality, of endothelial origin; (2) that they are not derived from the omentum, nor (3) from lymphocytes; (4) that a few seem to be of doubtful origin, exhibiting characteristics common to endothelium and connective tissue. This is true of the small, granular cells of the intermuscular connective tissue.

This still leaves two questions to be answered: How do the emigrating and emigrated endothelial cells behave to the oxydase reaction and is the carbon they contain taken up at the time of emigration (as would seem to be the case) or later? In order to answer them, a supplementary experiment was performed, the technic being as follows: A rabbit was injected with a mass of melted agar under the skin of the nape of the neck and two cubic centimeters of gelatin-lampblack solution was given intravenously as before. The lampblack injection was repeated the following day, after which the animal was left unmolested for ten days, when it was killed and its affected tissues fixed in the manner already described.

Frozen sections from the lesion proved that while the polymorphonuclears present gave excellent oxydase reactions, the endothelial cells of the lesion and syncytia were free from granules. Paraffin sections showed very beautiful syncytia and were morphologically the same as those of the same period in the preceding experiment. Carbon was present in the syncytial groups and in some of the discrete cells in their neighborhood, but the latter were in the main free from it, just as one would expect. The walls of the blood vessels in the neighborhood of the lesion showed no carbon particles, the presumption being that all the carbon-containing cells had migrated and become syncytia. Evidently the lampblack is taken up by the cells shortly before, or at the time of, their migration, and retained in their cytoplasm after they become sessile in the syncytia. In other words, it may be used as a means of labeling and tracing these cells from source to destination. (August 6, 1919.)

Mallory[10, 11] has for a long time maintained, in the face of much opposition, that the phagocytes of the body were chiefly of endothelial origin, basing his claim on a chain of evidence which, suggestive as it was, many anatomists and pathologists refused to accept without many reservations. H. M. Evans,[5] while giving him due credit for having pointed out the endothelial origin of many of the macrophages of the body, which he admits in some cases, takes exception to Mallory's claims as being altogether too sweeping. McJunkin, as already stated, has strengthened Mallory's position materially, by his work with lampblack emulsion and his findings in connection with the circulating endothelial leucocytes. It would seem that the experiment just described, in which his methods were combined with those of the "benzidine school," would clear up this dispute quite satisfactorily, in so far as the loose connective tissue of the body is concerned.

Since lampblack particles are found in the walls of the smaller vessels and in the circulating endothelial leucocytes (as already described by McJunkin and confirmed by my experiment), it is evident that cells of the same morphological

appearance, containing carbon and therefore capable of being traced from the vessels to the exudate at daily intervals, came from one of these sources. Seventy per cent of the circulating cells contain carbon, while but thirty per cent of those in the exudate show it. Were they derived from the circulating blood alone, we would expect to find the same percentage of carbon-carriers in the exudate, which is not the case. One would also expect to find the vascular endothelium in general (in other parts of the body) in a state of proliferative activity, which is again not so; and lastly, one would look for an increased percentage of circulating endothelial leucocytes in the blood smears, which we do not find to be true in this experiment. On the other hand, there is great activity in the vascular endothelium near the agar lesions, there are more endothelial leucocytes in the lumina of these vessels and a great many in the tissue near them. Therefore we would suppose that these cells originated chiefly in the walls of the capillaries in the immediate vicinity of the lesion, which is perfectly logical, as the effects of the agar injection are, after all, purely local. F. A. Evans[2] finds that most of the circulating mononuclears give the oxydase reaction, while most of the phagocytic cells in exudates do not. This fits in very well with the findings here, as proof of the local origin of the phagocytes in this experiment.. It will be noted that no claim is made, in this paper, that all macrophages are of endothelial origin; the cells of the serous membranes and possibly some epithelial cells cannot be placed in this category; the point I wish to make is that the cells of the loose connective tissue, which are generally known as " macrophages," are descended from the endothelium.

The cells of the exudate containing no carbon might be pointed out as " not proven "; but they are identical in every respect with the carbon-carriers save one: they lack carbon. This is easily explained: if they originate in the vascular endothelium near the lesion, we cannot expect them all to contain lampblack particles, as the endothelium of the vessel which produces them contains carbon in some of its cells, many being carbon-free. I have already explained why

carbon-carriers diminished in numbers in the later stages of the experiment; on account of the increase in the number of cells by division, and increase in their size. It also seems probable that the sessile " clasmatocyte " is of like origin, as I have already indicated, and as Ranvier supposed when he first described them. Under normal circumstances these cells probably represent a steady migration of a few endothelial leucocytes, which become fixed and await eventualities; as they die off, or are used up, other similar cells take their place.

Reynaud[13] claimed the omentum as the parent organ of the " cellules rhagiocrines," — his name for the cell under discussion. I have pointed out that the mesothelial cells of the omentum — which are undoubtedly phagocytic and probably perform the same function for the serous cavities as the endothelial cells do for the loose connective tissue — are deeply stained by trypan blue. Reynaud thought that they reached their destination in distant parts of the body through the circulation, via the thoracic duct and subclavian vein. Were this so the circulating mononuclear leucocytes would contain a granular stain, similar to that seen in those of the omentum in this experiment; or at least a large number would. This is not the case in my smears. One per cent of the cells counted showed deep blue granules somewhat analogous to the trypan-blue granules, but they were unmistakable basophiles. The endothelial leucocytes were filled mostly with carbon, a few showed a granule or two that was blue, but there was nothing comparable to the blue mulberry-cell of the omental tissue. We can dismiss the omentum, therefore, when it comes to subcutaneous inflammations.

Tschaschin, quoted by Downey and F. A. Evans, made some experiments very similar to my own, coming to the conclusion which, in the light of the evidence just presented in this paper, seems to be erroneous, that the macrophages under these conditions originate from migrating lymphocytes, which later swelled and grew until they became " polyblasts " Maximow's name for this much-named cell). None of the

lymphocytes in the smears made from the circulating blood of my rabbit contained carbon particles, nor was their number increased. Furthermore, the cells in the neighborhood of the vessels had nothing in common, morphologically speaking, with the lymphocyte series; nor were lymphocytes very abundant in the exudate. Do lymphocytes possess the power of phagocytosis? I think not.

The greater number of authorities, too numerous to quote, but available in Goldmann's bibliography, have maintained that these cells were of connective-tissue origin; Goldmann, Pappenheim, H. M. Evans, and others maintaining that they represent a special "line," or cell-race. Until I completed this experiment I was inclined to take the same view, so that it cannot be said that I have gone at this problem with a preconceived bias. As already noted, in the case of the intramuscular injections, there were cells which were of doubtful origin and seemed to resemble either connective-tissue cells, or specialized endothelium, like the Kupffer cell. These are not proved to be one or the other — they need further study. The phosphotungstic acid hematoxylin specimens of my experiment showed no production of fibroglia fibers by the syncytial cells; these, when found, were apparently formed exclusively by fibroblasts which lay between the syncytial masses, or were included by them.

Domenici, quoted by Goldmann, says: "En un mot, cellules endotheliales, cellules connectives, macrophages de Metchnikoff sont modalités d'une même espèce cellulaire, la cellule conjonctif." We might add a great many names to this list, and whether we accept his statement or not the fact remains that we must endeavor to simplify our nomenclature in the case of these cells and if we cannot call them by one name we must try to at least reduce the number of useless ones to a minimum. Although it might be well to adopt a new name to cover all of them, as Domenici suggests, it seems to me that it would be better to call these phagocytes simply "endothelial cells," as this retains one of the old names, implies their origin and is already used by many men who have accepted this view. We come to the conclusion, then,

that the greater majority of phagocytes in the body are either endothelial or mesothelial in origin. I have, in this paper, frequently used the term "clasmatocyte" for the large, vacuolated and sessile phagocytic type of endothelial cell; the more or less descriptive term, first used by Ranvier, was employed simply for the sake of convenience in description.

Summary. — 1. The phagocytes, or macrophages, of the "cellular" or loose connective tissue of the rabbit are of endothelial origin.

2. They do not originate either in the omentum or the connective tissue cells, or from lymphocytes.

3. They are probably derived from the proliferating vascular endothelium in the immediate vicinity of the lesion which calls them forth rather than from the vascular endothelium in general.

4. They do not appear to come entirely from the circulating mononuclear leucocytes, as McJunkin has suggested.

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More complete bibliography in Goldmann's second and in F. A. Evans's article.

DESCRIPTION OF PLATE V.

Fig. 1. — Exudate on first day after injecting agar. Both types of endothelial leucocytes shown; the larger clasmatocytic is marked "*c*" in the figures. Carbon particles in many of the cells of each type. Polymorphonuclears and connective tissue fibers here and there.

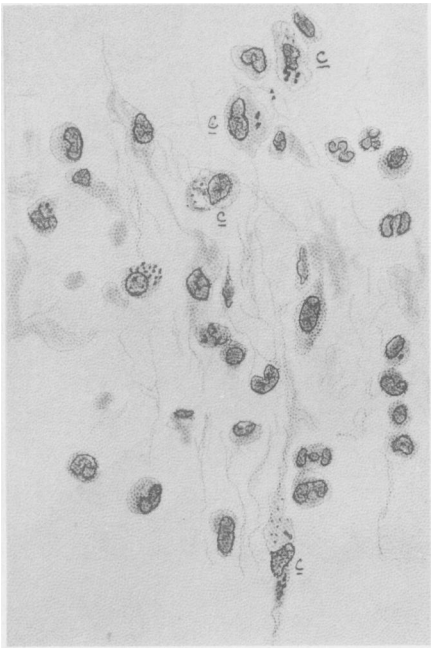
Fig. 2. — Exudate on third day; a capillary is seen surrounded by migrated endothelial cells. One of the lining cells contains carbon, and many of the migrated cells show it. Note increasing size and number of these cells.

Fig. 3. — Fifth day. Great increase in size of migrated cells, many of which are identical with clasmatocytes; a good number contain carbon.

Fig. 4. — Foreign body syncytium from ninth day exudates; the vacuoles in the center contain agar particles. Note distribution of lampblack in loosely reticular cytoplasm.

All these figures drawn with an Abbe-Zeiss camera lucida, $\frac{1}{12}$ oil-immersion, 4 compensating Zeiss ocular, drawing table at level of microscope stage. Magnification, 733.33 diameters.

Fig. 1 from Mayer's carmalum preparation, the others from alum cochineal slides. Lightly-dotted areas in the vacuolated cells represent trypan-blue granules.



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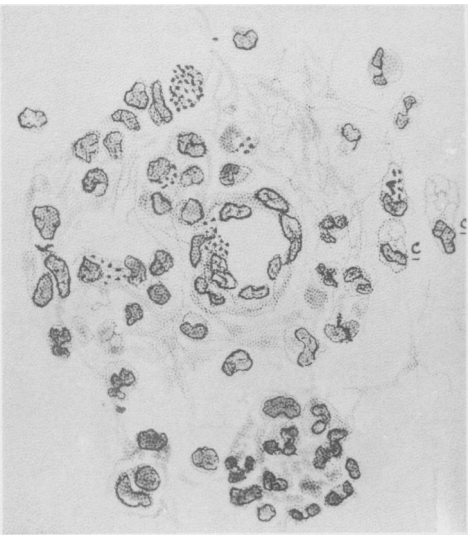

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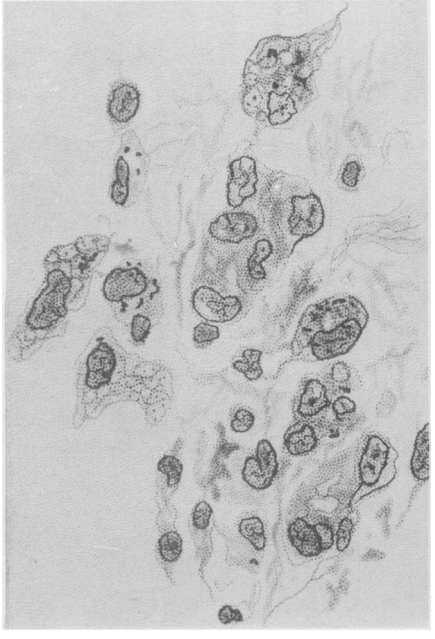
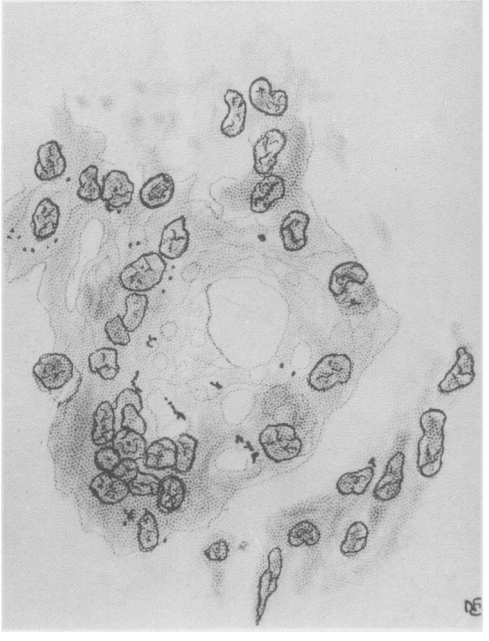


3

Foot.



4

Macrophages.