THE CHARACTERS OF KUPFFER CELLS LIVING IN VITRO

By J. W. BEARD, M.D., and PEYTON ROUS, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

Plates 43 and 44

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Methods whereby the large phagocytes of the liver sinuses can be procured and propagated outside the body have been described in an accompanying paper (1). The present communication deals with their identification as Kupffer cells, their morphology and general characteristics. Their physiological potentialities under the conditions of life *in vitro* will be dealt with later.

The Kinds of Cells Washed from the Liver

The method of washing out the cells has already been described. The first Tyrode solution put through a normal liver at low pressure (10 cm. Tyrode) removes only the blood. The erythrocytes come away far more readily than the leukocytes. Even long washing, with pressure gradually raised to 105 cm., does not remove all of the latter, sections of the washed liver showing an occasional polymorphonuclear cell in the sinuses and not infrequent lymphocytes and monocytes. It has seemed possible that these leukocytes might have settled out of a slowed blood stream, though the method of cannulation involved no interruption of the hepatic circulation; but they are found even when the washing is done under pressure, through a large trochar thrust into the portal vein while the circulation is intact. The possibility of a settling out of cells is still not excluded, since the blood current may have slowed as a result of the etherization. However this may be, there is no doubt that the washings from the normal liver contain far more leukocytes than can be accounted for by the blood content of the organ at any one moment; and during infections or after the injection of nucleic acid immense numbers of them can be flushed from the hepatic sinuses. The washings from a 6.25 kg. dog with pneumonia

yielded several cubic centimeters on centrifugation. The findings support the old assumption that the leukocytes may dally on occasion in some of the abdominal viscera, thus affecting counts made on the peripheral blood; and they corroborate the histological observations of numerous authors who have reported that lymphocytes and monocytes are normally present in considerable numbers in the liver sinuses. No evidence has been found, however, that the organ serves as a graveyard for white cells. Those washed out have regularly proved to be in excellent condition, when studied in the warm box.

Flushing the liver at 30 cm. to 105 cm. pressure straightway after the blood has been removed brings out not only leukocytes in progressively lessening numbers but a multitude of globular, colorless, slightly refractile bodies of various sizes. These have many of the characters of red cells, being laked by water, bile, and other hemolysins, notably serum hemolysins when complement is present. They show a reticulum with cresyl blue, are agglutinated by specific agglutinins, and leave shadows when laked. Yet, as a previous paper has shown (2), they are products of the liver parenchyma extruded during the first minutes of perfusion.

In most cases these bodies and a greater or less number of leukocytes and platelets are the only yield of the late washings from the normal liver. Very occasionally though one encounters a spherical element 30 to 40 mm. in diameter which flattens on contact with a glass surface and puts out a great circular membrane. The washings from animals injected with India ink or ferromagnetic iron oxide several days previously yield such cells in enormous numbers, each now containing the particulate matter (Fig. 1). They differ greatly from the blood cells, and it is plain that they are inhabitants of the liver sinuses.

Numerous experiments were done to find the conditions which would give the most abundant yield of the large cells. These have shown that not only the number but the kind of phagocytes varies with the interval elapsing between the last injection of particles and the washing out. When only 1 day has elapsed polymorphonuclear leukocytes containing several small particles are fairly frequent, and there are considerable numbers of typical monocytes that have phagocyted particles, in addition to the peculiar cells above mentioned. The sinus cells, as we may call these latter, are much bigger

than the blood elements, nearly always contain numerous particles, and frequently are so crammed with them as to be greatly enlarged. In washings procured 3 to 5 days after the last injection the polymorphonuclear phagocytes have disappeared and there are fewer of the monocytic type. This is the period at which the large sinus cells are most abundant. Washings obtained 2 to 3 weeks after the last injection of particles contain relatively few sinus cells, but phagocytic monocytes are more infrequent still, their proportion as compared with that of sinus cells being much reduced. Sections of the liver at this time show most of the phagocytes gathered into large clumps here and there, with fusion and giant cell formation in pockets in the liver parenchyma. On backward washing through the hepatic vein to the portal an occasional giant cell comes away; but most are so large and so well ensconced as not to be dislodged. Sometimes a giant cell comes away so large that it can readily be seen with the unaided eye. One having a diameter of 540 μ was washed out of the liver of a dog repeatedly injected with iron oxide; and cells measuring 200 to 300 μ are procured with relative frequency (Fig. 2). That they are living is shown by their prompt segregation of neutral red into the numerous vacuoles; and they survive in cultures.

Even forcible washing and massage bring out only a small proportion of the phagocytic sinus cells from the liver,—which remains black or rusty brown, according as it contains ink or iron oxide. Inspection under the microscope of scrapings of the cut surface shows numerous large phagocytes scattered within the tissue fragments, many of them with processes that extend into crannies between the parenchymal cells. In stained sections the sinuses appear swept clean save for these securely fastened phagocytes and the endothelium proper. The loosely attached Kupffer cells have been swept out practically *en masse*, a fact the more evident when the section is compared with one from a piece of the same liver snared off before washing was begun. Search shows, however, both monocytes and lymphocytes in and about the sinuses. A little ink or iron is present here and there within the sinus endothelium.

Characters of the Dislodged Kupffer Cells

The sinus cells have been studied in deep preparations made by putting a few drops of the washings within a ring of vaseline on a slide coated with dry neutral red in the usual way (3). The cover-glass is put on slantingly to expel the air from within the ring and the preparation seals itself with the vaseline. It is then turned upside down in a warm box surrounding the microscope and, supported at its ends, is left undisturbed for about 20 minutes. During this period the phagocytes settle to the cover-slip where the greater proportion become attached. When the slide is turned right side up most of the other elements and the debris fall away, and the adherent cells can be studied through an oil immersion lens, without any complicating pressure upon them. That they are in the main Kupffer cells is certain not only from their morphology but from the fact that these have been largely swept from the liver. At the period when they are most abundant in the washings they are so easily dislodged as to come away in considerable number with the blood of the first washing. Those then obtained are mostly smaller than the ones got later at higher pressures, but they are practically all alive, segregating neutral red promptly. The living Kupffer cells are spherical or ovoid when in suspension, whereas dead ones may be shaped like blunt casts or cigars, or be star-shaped, or flattened, or show twig-like projections, sometimes combined with forking so that they resemble elk horns. Frequently they are like fused spherules of several different sizes, the projecting portions of each spherule consisting of clear cytoplasm. In all these dead cells the outline of the nucleus is sharp cut; and they fail to segregate neutral red though the nucleus stains readily with trypan blue. Some have ragged protoplasmic processes as if they had been forcibly torn from the capillary wall; and that this actually happens, with death in consequence before the cell has had time to change shape, has been shown by counts of the living and dead elements in specimens of the successive washings at higher and higher pressures. In the final washings at 105 cm. pressure nearly all of the Kupffer cells have bizarre shapes and are dead, and many of them show shredding. Washing at 30 cm., immediately after the blood removal, has proved most favorable in yield both for numbers and for proportion of living cells.

The morphology of the Kupffer cells is the same whether they have been washed out with Tyrode or with pooled homologous serum; and those of the rabbit and the dog have the same general features. Their size is largely conditional on how much particulate matter they contain. Those having little are from 30 μ to 40 μ across when spherical, whereas those crammed with particles may be 100 μ in diameter. Very occasionally one is seen with no particles, as rarely indeed as in the washings from normal livers. The cells that have taken up little or no material show a granular cytoplasm in which lie nucleus, vacuoles, and particulate matter, surrounded by a thick layer of transparent cytoplasm. The two sorts of cytoplasm grade into each other in the case of rabbit phagocytes, whereas in those of the dog the clear outer layer is as sharply demarcated as the rind of an orange. If the cells die while in the spherical form the granular cytoplasm often comes to lie at one side like a crescent moon, with the "old moon" of clear cytoplasm in its arms (Fig. 1). The nucleus is in the middle of the crescent, with the ink or iron particles to either side. Under such circumstances one sees that the proportion of transparent cytoplasm is great.

Within a few minutes after living Kupffer cells containing a moderate number of particles have settled on a glass surface at room or body temperature a zone of clear cytoplasm is seen to be extending out around some of them (Figs. 1 and 3) and they slowly flatten, the granular cytoplasm yielding last. Soon this lies in the midst of a great circular sheet of pellucid, glassy, apparently structureless material (Fig. 1, and Fig. 1 of the accompanying paper). None of the pictures that we have been able to obtain of this membrane gives a just idea of its proportions, or of its smoothly curving, circular outline. Always it has been retracted partially and irregularly as result of the stimulus of light. During the outward extension of the membrane, lava-like flows can be seen on its surface, when the light is cut down, and at its edges fimbriated or "petaloid" extrusions, at times appearing whip-like, which are in constant slow motion. Their edges can easily be mistaken for flagella if resolution with the microscope is poor. They are still present and active after the membrane has been completely extended. It then appears almost perfectly round, the slightly raised central hummock of granular material at its center making up only one-fourth

to one-third the entire diameter of the cell. This may amount to 150 μ or more, but usually from 40 μ to 80 μ with an average of about 60 μ. Before the granular portion of the cell has flattened it looks, under the low power, like a black or brown specked marble in the center of a clear disc of glass,—the specks being the phagocyted material and the disc the membrane. The greater the amount of material that has been phagocyted the less proportionately is the amount of membrane. Its refractility is so slight that pictures of it are difficult to obtain. In homologous serum it can scarcely be seen.

Cells that have crammed themselves with particles are more frequently dead when washed out. Only a thin, pellucid skim of cytoplasm can be seen when they are in suspension; but living ones soon flatten out after they have settled on a glass surface, and then one sees that they have the same general characters as the others, but only a narrow outer zone of clear cytoplasm. They segregate neutral red promptly, showing that their immense burden of foreign matter has not "blocked" them, in this respect at least.

The nucleus of the Kupffer cell is large, oval, and eccentric, its vacuoles of highly various size up to that of an erythrocyte, scattered irregularly through the granular cytoplasm (Fig. 3). The cells promptly segregate neutral red in supravital preparations, then varying in color from reddish orange to a slightly crimson red. Some of them contain small phagocyted particles, but most of these lie separate in the cytoplasm, which occasionally contains leukocytes or red cells.

It will be seen that the general characters of the Kupffer cells place them with the clasmatocytes. They have an evident relation to the giant cells encountered with them. In fixed smears in serum, which have been colored with Wright's stain, one sees that both have a light blue cytoplasm and their nuclei a darker reticulation; but the nucleus of the Kupffer cell is oval and relatively dense whereas those of the giant cell are spherical and much larger, with an open network so that they appear spongy. The giant cells contain relatively little particulate matter. One can recognize potential ones by these features while they are still mononuclear (Fig. 4), and can find all gradations between them and Kupffer cells on the one hand and immense multinucleate elements on the other (Fig. 2).

In washings obtained from the liver at the time when the yield of

Kupffer cells is greatest, some are found in process of division. Paired cells joined by a flat face across a region of constriction are fairly frequent, and there are occasional tetrads and even larger masses. The cells of any such pair or group are of about the same size and contain about the same amount of particulate matter, as is not usually the case with individuals that have accidentally stuck to each other. We have several times observed paired cells lying within a single membrane, and in cultures have noted division by fission within a membrane which as yet appeared homogeneous. The great variation in size and particle content of the individual Kupffer cells is doubtless referable to their relative opportunity for phagocytosis and to the vicissitudes of proliferation.

Comparison with the Phagocytic Monocytes from the Liver

The discrimination of Kupffer cells from the phagocytic monocytes present with them in greater or less number in liver washings is ordinarily easy, the monocytes having the characteristic indented or saddle nucleus and many little granules of even size located in a central "*Hof*," all taking the same color with neutral red. They are much the smaller cells, their diameter in the dog averaging only about one-third that of the Kupffer cells when both are in spherical form. In the rabbit this difference is less marked. Practically all of the Kupffer cells contain phagocyted particles whereas most of the monocytes have none, and the generality of the phagocytic ones but little, which is true as well of those found in exudates and in scrapings of the spleen. On the other hand they are far more actively motile than the Kupffer cells. They put forth a relatively small, circular membrane, and nearly all possess mitochondria staining with Janus green, few being found in the Kupffer cells.

A statistical listing of the features of the individual mononuclear phagocytes encountered in neutral red preparations from liver washings has disclosed rare intermediate elements having some of the characters of the monocyte and others of the Kupffer cell (clasmatocyte). The latter is known to be a specialized derivative of the endothelium of the liver sinuses, all stages in its differentiation being seen in fixed preparations of the hepatic tissue. Hence the question has arisen of whether some of the monocytes at least may not represent transition

forms from the endothelial syncytium. The evidence would appear to negate this possibility. The number of "transition forms" is never considerable, being no greater than in exudates containing both clasmatocytes and monocytes. The longer the liver is washed the greater is the preponderance of Kupffer cells over monocytes amongst the elements coming away. The change should be in the opposite direction if the monocytes represented early stages of differentiation from the endothelium, since they should be more difficult to dislodge than the Kupffer cells which are more or less sessile upon it. Portions of the endothelial syncytium as such never appear in the washings. Fragments of the well-washed liver, examined in some cases after enough digestion with weak trypsin to dislodge the phagocytes without killing them, have been found to yield many that are of clasmatocytic type, few that are monocytic, and no transition forms. The occasional monocyte much larger than those of the blood, rivalling the Kupffer cell in diameter, has always a large content of phagocyted matter, thus accounting for its size.

Many authors have described monocytic accumulations in and about the liver sinuses of animals injected with bacteria, foreign proteins, and other material. The considerable literature has been recently reviewed by Swift (4). De Haan and Hoekstra (5) injected, into a mesenteric vein of rabbits, monocytes derived from peritoneal exudates and marked by a content of trypan blue. They found some of these cells later, living along the liver sinuses; and in consequence they advanced the view that the Kupffer cells are merely a monocytic colony derived from the blood. Our findings render this view untenable. The Kupffer cell, as washed from the liver, has the typical characters of a clasmatocyte, and is far larger than any blood cell.

Comparison of Kupffer Cells with Other Reticulo-Endothelial Elements

The Kupffer cell is generally supposed to be essentially identical in its characters and functions with the clasmatocytes present in other organs, notably spleen and bone marrow. Some authorities go so far as to suppose that it may be merely a migrant from the spleen. We have found that the yield of Kupffer cells is not less than usual when rabbits have been splenectomized prior to the injections of ink or

iron;[1] while furthermore the cells have morphological features that distinguish them from splenic clasmatocytes. The cells obtained by scraping the splenic pulp of rabbits several times injected with India ink or with alien red cells have been studied both in Tyrode and in serum, by the inversion technic. Cunningham, Sabin, and Doan (6) encountered two types of phagocytes in splenic scrapings, the one monocytic and only about 15 μ in diameter, the other obviously clasmatocytic and sometimes measuring 30 μ. These latter are as large as some Kupffer cells; but we find that in the warm box they put forth a membrane on one side only, a broad, irregular tongue of cytoplasm like ground glass, never having an area larger than that of the granular, vacuolated portion of the cell. Injections of ink and of ferromagnetic iron oxide into the peritoneal cavity of rabbits results in exudates containing many clasmatocytes which put forth a membrane similar to but in general smaller than that of the Kupffer cells. Sabin, Doan, and Cunningham in their detailed description of the clasmatocyte of peritoneal exudates in the rabbit (7) make no mention of any such extensive membrane as that of the Kupffer cell.

Peculiarities of the Kupffer Cell

Except for the immense circular membrane, no feature of the Kupffer cell is more striking than its stickiness,—which is equally remarkable whether the enveloping medium is serum or Tyrode solution. It has much interfered with the study of the cells *in vitro*. Whatever touches their surface tends to adhere, whether it be red or white cells or "bodies" that are floating by; and hence in selecting them from the washings with the magnet some of these elements are inevitably included in the collection. If the liver circulation is interrupted for a few minutes prior to the washing out, many of the Kupffer cells coming

[1] The production of hemochromatosis in rabbits by daily transfusions of rabbit blood over long periods of time results not only in the characteristic pigmentation of the liver parenchyma with hemosiderin but in pigmentation of the splenic clasmatocytes and Kupffer cells as well (Rous, Peyton, and Oliver, J., *J. Exp. Med.*, 1918, **28**, 629). In order to learn whether the latter had perhaps migrated from the spleen this organ was removed from a number of rabbits prior to the transfusion period. When they were examined, after 3 to 6 months of transfusions, the same liver findings were obtained as in animals still possessing the spleen.

away will be covered with leukocytes which cannot be dislodged. When they contain ink or iron they sediment rapidly out of suspension and they soon become fixed so firmly on the bottom of the container that a considerable proportion resist even a forcible jet of Tyrode. Always some loss occurs from this cause on the sides of the taper flask during selection with the magnet, even though the suspension is frequently agitated; and there is a more considerable loss on the collodion membrane to which the cells are attracted. Centrifuging at the lowest speed that will bring them down in a few minutes in a flat-bottomed tube results in a lumping together that can be only partially broken up by vigorous pipetting; and despite it a thick skim of cells regularly remains on the glass. In slide and cover-glass preparations one frequently sees erythrocytes or leukocytes which had appeared merely to touch the extended membrane of a Kupffer cell, dragged across the field with this membrane when it retracts. A sticky surface is evidently one of the special characters enabling the cell to perform its task of phagocytosis. The stickiness of leukocytes from the blood and from exudates,—with both of which we have had much experience,—is in comparison negligible.

Kupffer cells are notably sensitive to injury, as evidenced by the number that die within a few minutes after they have been washed from the liver. Most of those procured from the rabbit survive only 4 or 5 hours in Tyrode at room temperature. They are rapidly killed by the slight alkalinity which develops in this fluid on storage in the ice box; and its reaction must be brought to pH 7.2–7.4 by bubbling CO_2 through it prior to flushing out the liver if the cells are to survive during the 2 to 3 hours required for selection with the magnet. When they are transferred immediately afterwards to serum in a culture flask many lie as if dead for a day or more before putting forth a membrane and beginning to move about. Kupffer cells of the dog are relatively resistant, many surviving for 24 hours in Tyrode at 4°C. and a few even for 48 hours, at which time, however, most of the monocytes and polymorphonuclears associated with them are still in excellent state.

The Kupffer Cells in Cultures

On *a priori* grounds it had been feared that most if not all of the Kupffer cells coming away in the washings would be over-mature or else rendered incapable by their content of foreign matter, with result that they would not long survive in cultures. This has not proved to be the case, though it is true that cells which have taken up coarse lumps from a poorly-ground preparation of iron oxide die very soon. Those that have taken up the material as fine particles flourish under proper *in vitro* conditions and proliferate, sharing said particles almost equally, with result that the individual cells soon come to contain but few.

The iron particles turn black when the cells die *in vitro*, but while these are doing well they remain yellowish brown, persisting without apparent change. By their appearance and response to the magnet they have been recognized within Kupffer cells washed from the liver 19 months after the injection into the blood stream, as stated in the paper on method (1).

On first removal to cultures in plasma or serum most of the Kupffer cells are still aggregated in coarse lumps which have resisted pipetting. These usually remain as such for a day or more and then the individuals composing them disengage themselves and move slowly away (Fig. 3 of the accompanying paper). The first step showing that the cell is alive is the extrusion of the characteristic large, clear, circular membrane. This happens only on a surface. The cell may remain where it is or begin to creep about very slowly, hours of observation being often required to discern a change in position. The petaloid edge of the membrane, though, is in constant, rather quick, wavy motion, with "lava flows" over its surface and slight, gradual withdrawals and re-extrusions. In even the thinnest plasma clot the cells are greatly hampered and move but little in the course of days, leaving in some cases a clear track behind them, doubtless of digested fibrin web. When moving they frequently elongate, becoming slug-shaped. They progress only along the glass, never penetrating into the over lying plasma layer. When they are about to divide the particles of iron become massed in approximately equal quantity on opposite sides of the cell, and occasionally are ranged in two roughly parallel

lines as if they were intrinsic elements. As soon as division has occurred the cells move apart. It is possible to maintain them for a few days within a thin layer of clot overlaid by serum, if it is frequently washed with Tyrode and the serum changed; but they tend to die off in a week or two, despite some initial multiplication, even when proteolytic products are furnished of the sort on which Baker has shown (8) that chicken monocytes thrive.

When transferred in serum to Carrel dishes containing lens paper the cells distribute themselves upon this, as already described, and cling so firmly that the serum can be pipetted off and replaced daily with but slight loss, more than compensated for by proliferation. A few of the cells coming away at the daily replacement are dead; but with neutral red most are found to be in good condition, an incidental wastage. When the cultures are crowded and the cells contain much iron or ink, some secondary formation of giant cells may take place; but the general tendency of the Kupffer cells is to scatter and live apart (Fig. 5 of the accompanying paper). This tendency is curiously instanced when they mount from the bottom of the dish along a fibre of lens paper. They then space themselves on the fibre at almost regular intervals, with no clustering back of an obstruction or clambering over one another (Fig. 6 of the accompanying paper). Yet despite what would appear to be a negative chemotaxis as concerns one another, the cells do poorly and soon die off when widely separated, like those of the other kinds thus far cultivated. Efforts to prevent this by regulating the pH with a mixture of nitrogen, oxygen, and carbon dioxide have proved unavailing.

When living on strands of lens paper, the Kupffer cells lie with membrane spread, and the edges of its glassy expanse can be discerned only imperfectly (Fig. 6). It thickens somewhat toward the center and here the granular cytoplasm containing nucleus, vacuole, and particulate matter projects as a mound or lump or more or less tangential sphere (Figs. 5 and 6). The cells appear stationary but prolonged observation shows that they move slowly along the fibre. When this is very slender they may wrap themselves about it, then appearing spindle-shaped. Only occasionally is a cell found to be connected by a protoplasmic process with a neighboring fibre (Figs. 7 and 8). These facts, the difficulty of dislodging normal Kupffer cells from the

liver, their tendency to spread like pancakes without extrusion of pseudopods worthy the name, all lead one to doubt the current view that many of them normally lie athwart the blood current, moored to the sinus walls by slender protoplasmic processes. This view is based on fixed, and perhaps distorted, histological preparations.

In every magnet collection of Kupffer cells some monocytic elements containing iron are to be found as well. Phagocytes of this sort generally contain but little and in consequence most of them escape the magnet, the proportion being much reduced from that present in the washings: sometimes almost none reach the cultures with the Kupffer cells, and again they contribute one-fourth to one-fifth of the cell population. They survive as such during the first days of life *in vitro* and can be readily recognized. Their ultimate fate is not known. A single cell type, the result of repeated division, is found in cultures that have been propagated for a week. This cell type contains little or no iron,—for the particles have been shared by the multiplying elements; it is not quite so large as the "adult" Kupffer cell, though possessed of a similar membrane and nucleus; and the granules are smaller and not so various as in this latter, though they are still of unequal size. In other words the general characters of the clasmatocyte have been retained, though they are not as pronounced as in the original Kupffer cell. We have in general discarded the cultures before they have reached this stage.

The iron-containing giant cells which come over into the cultures on lens paper live to all appearance unchanged (Fig. 9). They tend to fix themselves where many fibres touch one another, spreading out irregularly in various directions; but not infrequently they change place, gliding along a fibre, which they envelope, as if it were a wire. When on the bottom of the dish, giant cells containing little iron put out a membrane even larger than that of a Kupffer cell; but those stuffed with the foreign material have no more than enough pellucid cytoplasm to cover the great mass of granular cytoplasm with a thin coat. Such crammed cells remain roughly spherical while fixed on a fibre.

SUMMARY

The Kupffer cells procured from the liver of the rabbit and dog for culture *in vitro* have the typical characters of clasmatocytes. They are readily discriminated from the monocytes washed from the liver with them; and they have certain peculiar features which suffice to differentiate them from some at least of the clasmatocytes of other organs. Their surface is extraordinarily sticky,—far more so than that of blood leukocytes or of the clasmatocytes found in peritoneal exudates; and in consequence they are exceedingly difficult to handle *in vitro*. They put forth enormous, pellucid, circular membranes resembling those of exudate clasmatocytes but larger. Splenic clasmatocytes, on the other hand, put forth rather small, one-sided ground-glass membranes like broad tongues. On comparing them with Kupffer cells and exudate clasmatocytes one perceives that they are not wholly identical in their characters, but have secondary peculiarities. However, there exist good morphological reasons for grouping them together and terming them all reticulo-endothelial.

Kupffer cells are notably sensitive to injury, surviving in Tyrode solution for a much shorter time than blood leukocytes. However, they can be readily cultured on lens paper in serum. Under such circumstances they scatter on the fibres and live separately, presenting the same general aspect as when in the liver; but in the course of proliferation they soon lose some of their pronounced characters, retaining such as are common to clasmatocytes in general.

A considerable population of ordinary leukocytes exists in the hepatic sinuses over and above those circulating in the blood. During infection, their number may greatly increase. Several cubic centimeters of packed white cells have been obtained from the liver of a sick dog. The fact has been realized that leukocytes may stop a while in the liver, yet the extent of the accumulation which sometimes takes place seems deserving of stress.

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EXPLANATION OF PLATES

PLATE 43

FIG. 1. Washing from the liver of a rabbit injected intravenously with India ink repeatedly: fresh slide and cover-glass preparation. Two Kupffer cells are putting forth membranes along the slide surface, but these have retracted irregularly as result of the strong light. A third Kupffer cell is dead. The ink in it lies in a crescent at one side of an "old moon" of pellucid cytoplasm. The nucleus is midway in the crescent. × 350.

FIG. 2. Giant cell washed from the liver of a rabbit repeatedly transfused with incompatible rabbit's erythrocytes and injected intravenously with India ink shortly before the washing. The many large, rounded nuclei lie scattered in a granular cytoplasm containing ink, red cell debris, and phagocyted white cells. Wright's stain. × 650.

FIG. 3. Rabbit Kupffer cell containing ink particles, and stained with neutral red; slide and cover-glass preparation of fresh liver washings. The black particles are the ink, the gray spots dye-stained vacuoles. The membrane is as yet only half extended. To render it visible the photograph has been taken with the light coming from one side. The cell is of great size as compared with the lymphocyte and red cells associated with it in the washings. × 1000.

FIG. 4. Another portion of the material furnishing Fig. 2, colored with Wright's stain to show a mononuclear giant cell. The nucleus of this cell is large, circular, and spongy in appearance, and it has taken up but little ink. The arrow points to the oval, dark nucleus of a neighboring Kupffer cell so loaded with ink particles that it ruptured when the preparation was made. × 950.

PLATE 44

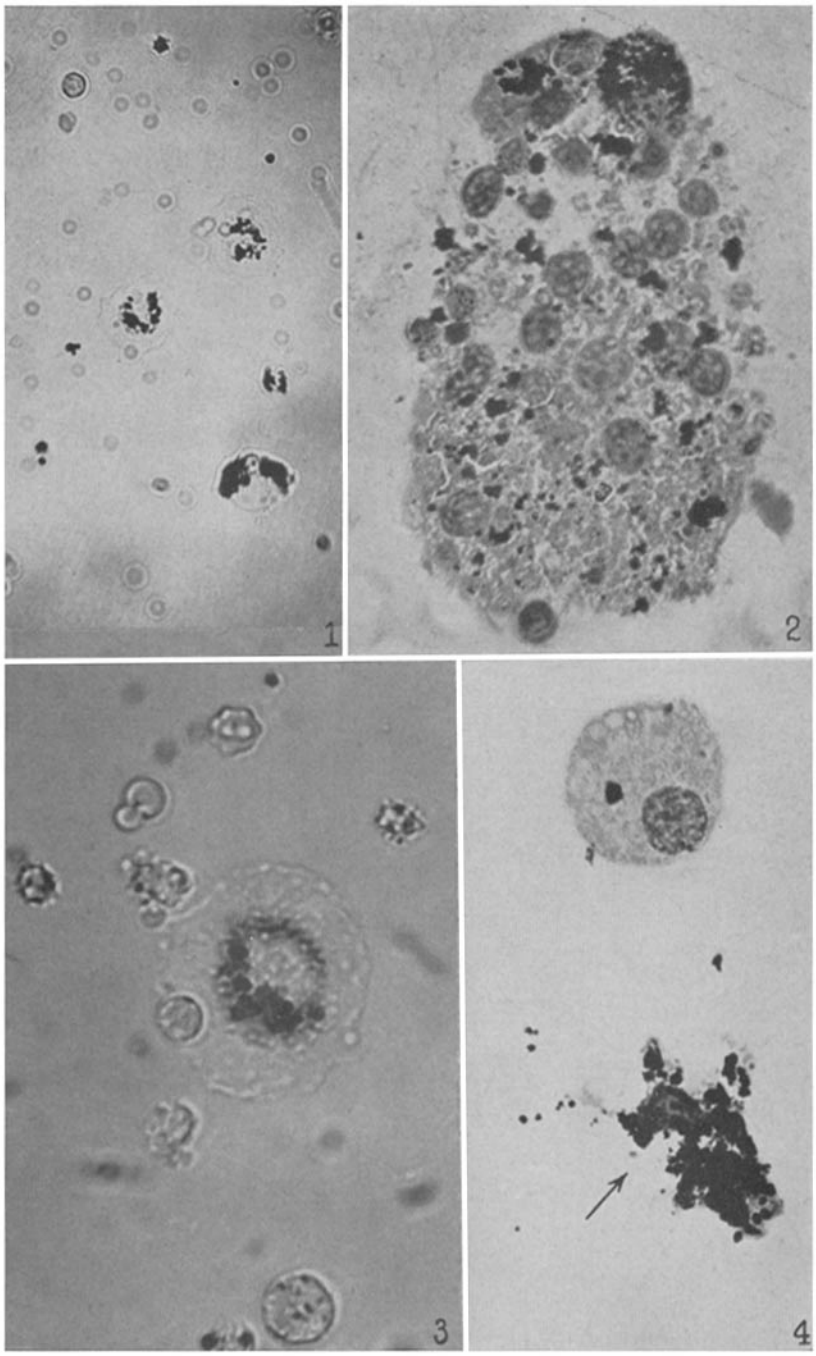
FIG. 5. Side view of an iron-containing dog Kupffer cell fixed on a lens paper fibre; 3rd day of culture. The resemblance is complete to cells of the same sort as seen on the walls of liver sinuses in stained sections of the organ. × 270.

FIG. 6. Dog Kupffer cells living on lens paper. The arrow points to the edge of a cell membrane. The dark particles are intracellular iron oxide. × 400.

FIG. 7. Dog Kupffer cell living on lens paper, with a process attached to a neighboring fibre. × 400.

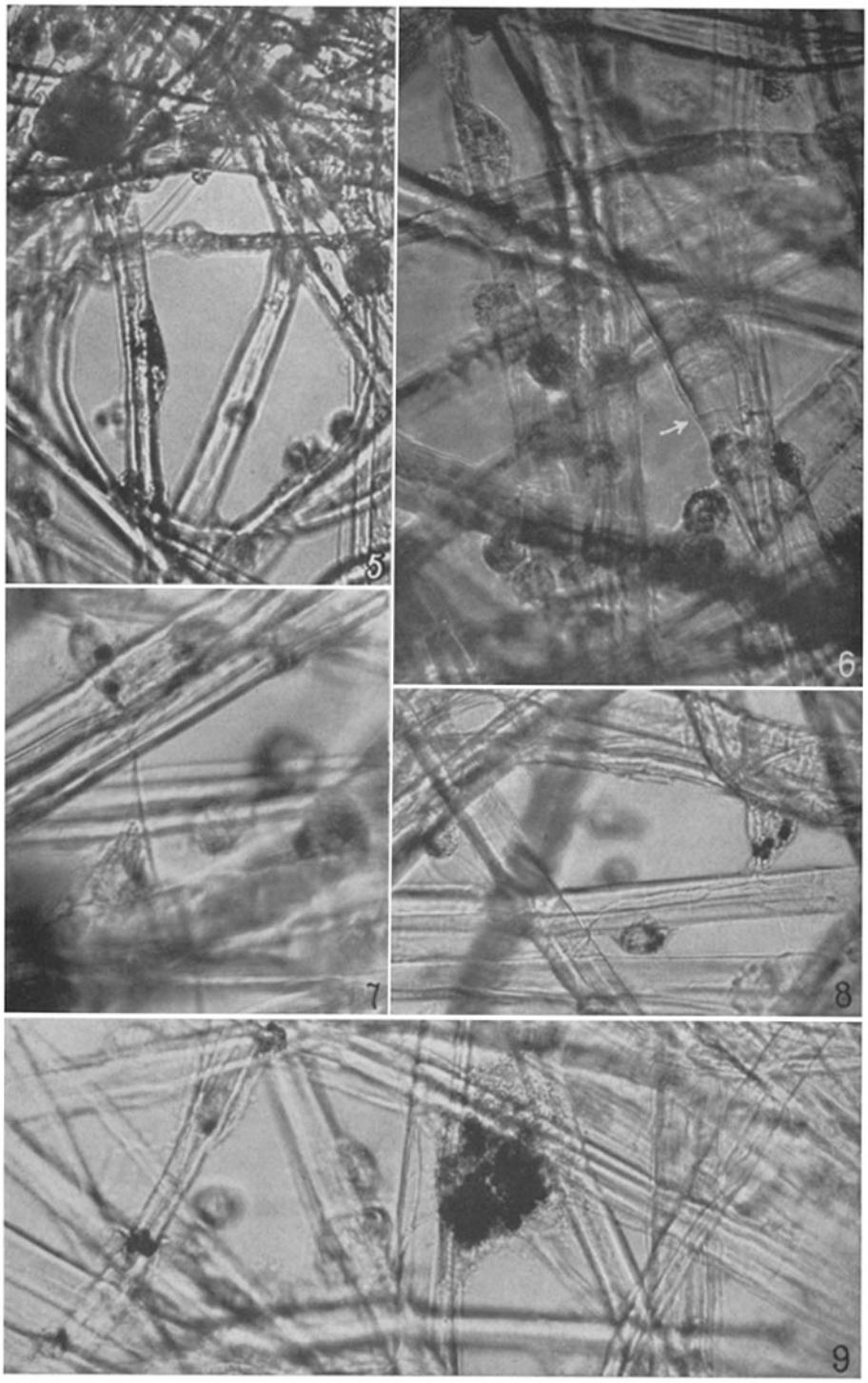
FIG. 8. Another cell with such a process, as also some cells projecting into the serum like hemispheres. The dark particles are intracellular iron oxide. × 400.

FIG. 9. Iron-containing giant cell from a dog liver; 4th day of culture. × 400.



Photographed by Louis Schmidt

(Beard and Rous: Characters of Kupffer cells *in vitro*)



Photographed by Louis Schmidt

(Beard and Rous; Characters of Kupffer cells *in vitro*)