# SELECTION WITH THE MAGNET AND CULTIVATION OF RETICULO-ENDOTHELIAL CELLS (KUPFFER CELLS)

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PLATES 40 TO 42

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The existence on the walls of the small blood vessels within certain organs of multitudes of cells endowed with remarkable phagocytic ability has long been recognized. To the older students of body processes these cells appeared to have a sufficient task in the removal and disposal of bacteria, effete corpuscles, and other particulate matter. Recent observations and the growing realization that cells of a single sort may do several kinds of work have led to a renewed scrutiny of the capabilities of the fixed phagocytes. They are now supposed by many investigators to constitute a distinct physiological system, the reticulo-endothelial system, so-called; and functions in great variety have been ascribed to them.

The reticulo-endothelial cells may very well carry out important tasks besides scavenging. Much evidence points to this. But it has been obtained under complicated conditions and hence has remained inconclusive. The innumerable attempts to produce a functional blockade of the cells by inducing them to gorge themselves with particulate matter have inevitably disturbed the organism in other ways, as have also the tests wherein the cells have been led to take up particles which poison and kill them. The need to procure and maintain living reticulo-endothelial cells for study *in vitro* is an obvious one. By the methods here to be described, this has been done for the reticulo-endothelium of the liver (the Kupffer cells).

## *Flushing the Cells from the Liver*

Forcible washing out of the normal dog or rabbit liver through the portal vein, or backwards through the hepatic vein, even when com-

bined with intermittent distention under pressure and kneading, causes few or no Kupffer cells to be dislodged, and the yield is equally poor whether the fluid be serum, or Tyrode containing $\frac{1}{8}$ per cent of gelatine, or ordinary Tyrode, at room or body temperature. Into chilled fluid the Kupffer cells do not come away at all, and attempts to shock them off the wall of the liver sinuses by adding a trace of formalin to the wash fluid, to digest them off with trypsin, or to anesthetize them into letting go their hold by means of perfusion with a fluid containing ether, have alike proved unavailing.

Von Kupffer (1) noted that after the cells had taken up particulate matter some of them came away into the venous blood; and later students of vital staining have described "showers" of dye-laden Kupffer cells in blood procured from the right heart. A major difficulty in the attempts to "block" the cells with India ink and other materials has been their rapid proliferation with result that great numbers of new, unblocked ones soon come into being. These observations have led us to inject animals several times intravenously with particulate matter, washing out the liver forcibly a few days later when one might suppose that the cells could be dislodged from the capillary wall.

The initial experiments were carried out with India ink. Rabbits were injected intravenously at intervals of a few days with 5 to 10 cc. of a half strength preparation of Higgins American Drawing Ink, a non-waterproof suspension, which had been dialyzed in the cold for 10 days against several changes of sterile Locke's solution and filtered through paper. Three or four injections of 20 cc. were made into dogs, and of 5 to 20 cc. into rabbits.

At various periods after the last injection the animals were anesthetized, the inferior cava was ligated above the kidney, and the liver was perfused with warm Tyrode solution through the portal vein, with collection of the washings from the superior cava. Flow was begun at a pressure of 10 cm. Tyrode, and when the washings no longer contained blood the pressure was raised to 30 cm. and finally to 60 cm., with intermittent obstruction to the outflow, and kneading of the distended liver as it emptied itself after the obstruction was removed. The fluid first collected on raising the pressure was a cloudy, dark gray, owing to the presence of myriads of ink-laden cells. These phagocytic cells were of several sorts, many being polymorphonuclear leukocytes. When the flushing out was done on the day after the last ink injection, as much as $\frac{1}{2}$ cc. of them could be obtained from the liver of a 2000 gm. rabbit, though the yield was usually less. If on the other hand washing was done after an interval of a week or more the yield of phagocytes was very small.

The tests showed that when the conditions were rightly chosen great numbers of phagocytes could be obtained. Not a few were present with blood in the first perfusate at low pressure. The morphology of the cells and their significance is considered in an accompanying paper. Many were enormous as compared with the accompanying leukocytes, and there was no difficulty in identifying these as Kupffer cells. On coming in contact with a glass surface, in the warm box, they put forth a broad membrane and then were often as much as 100 $\mu$ across (Fig. 1).

The ink-containing cells, though sedimenting rapidly, could not be separated entirely from the associated elements by differential centrifugation. It was found, though, that if iron had been taken up instead of ink, selection could be accomplished with the magnet.

For the first attempts minute spherical iron particles (alcoholized iron, Merck), selected from the commercial preparation by differential sedimentation, were injected in suspension in a solution containing 7 per cent gum acacia and 0.9 per cent NaCl. They were taken up by Kupffer cells, some of which were found in the liver washings; but the heavy material so weighted the cells that they were incapable of movement. Resort was now had to the gamma ferric oxide of Baudisch and Welo (2), a relatively light substance of strong magnetic properties.[1] The pure material was suspended to the amount of 4 per cent in 14 per cent gum acacia made up in distilled water, and ground in a colloid mill (No. 1 High Speed Laboratory Mill of the U. S. Colloid Mills Corporation)[2] until the particles were 1 $\mu$ or less in diameter. Distilled water was then added to make a 2 per cent suspension of the iron oxide in 7 per cent gum acacia, and the suspension was autoclaved in test tubes under washed paraffin oil to rule out oxidative changes. The larger particles tended to sediment during storage in the ice box, but were readily resuspended for injection. We have gained the impression that preparations made within 2 or 3 weeks of use yield the best results in terms of free Kupffer cells. The iron oxide, as seen within these cells, consists of roughly spherical, amber-brown particles. They persist unchanged within the cells. The liver of a rabbit killed 19 months after injection yielded phagocytes containing the characteristic, irregular, brown particles, and these obeyed the magnet and gave a positive potassium thiocyanate test.

[1] We would stress out great indebtedness to Dr. Oskar Baudisch for his repeated generous gifts of material and for his interest in the work.

[2] The material was ground for us through the kind interest of Mr. M. P. Hofmann of the Corporation.

### *Method to Procure Kupffer Cells*

On the basis of these orienting observations the following method was developed:—

A rabbit of approximately 2000 gm. is slowly injected by way of an ear vein with 10 cc. of a sterile 2 per cent suspension of gamma ferric oxide in 7 per cent gum acacia, and the injection is repeated twice at intervals of a day. Albinos should not be used since their cannulated portal and hepatic veins sometimes rupture under the perfusion pressure. The washing out is done 3 days after the last injection. The animal should be fasted in the interval to reduce the size of the liver and render it less friable.

Under ether the shaved abdomen is opened widely in the mid-line, the right renal and adrenal veins are ligated together, and the inferior vena cava is freed from about 2 cm. below the entrance of the right renal vein to where the liver is attached to it. Two threads are placed at either end of the freed segment but left untied. With due care not to interrupt the blood flow, about 3 cm. of the portal vein is freed in turn, just below where it forks before entering the liver masses, the tributaries to the segment are tied, and two ligatures are carried under the portal but left loose. A stout thread is tied down on the gastrohepatic omentum to close off the hepatic artery.

The lower of the ligatures around the inferior cava is now tied, the anterior one is lifted to obstruct the vessel temporarily, preventing a back flow of blood, a slit is made just above the posterior ligature, and the cannula connecting with the perfusion apparatus is inserted toward the diaphragm. The obstructing ligature is relaxed so that the cannula can pass it, and is then tied down. At once fluid is allowed to enter at 10 cm. water pressure, and then the chest is widely opened by cutting transversely across the sternum and between the ribs in the 5th interspace; a thread is thrown about the superior cava about 2 cm. below the heart; a cut made in the vessel to allow the segment of cava back of the liver to be flushed free of blood by the wash fluid; and the ligature is tied below the cut, and the heart and the portal vein are opened wide with scissors. In this way circulation through the liver is maintained until the moment comes for the backward flushing out with the wash fluid. All operating is done as rapidly as possible, but with due precautions for asepsis. If there is a delay in the washing, many of the Kupffer cells will have leukocytes clumped upon them.

The portal vein can now be cannulated at leisure, the upper ligature upon it serving for this purpose and the lower to block off blood flow from the intestines.

The wash fluid is Tyrode solution[3] sterilized by Berkefeld filtration and warmed to approximately 40°. With this as good a yield of Kupffer cells is obtained as with homologous serum. Tyrode tends to become more alkaline on standing.

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[3] $\frac{1}{8}$ per cent gelatine Tyrode was used in the earlier separations (3); but experience has shown the gelatine to be unnecessary.

For this reason phenol red should be added to it before filtration, in the proportion of 4 cc. of a watery 0.3 per cent solution for every 4 litres, and $CO_2$ bubbled through until the color becomes orange-pink, indicating a reaction of about pH 7.3. The warmed wash fluid flows to the liver through a syphon from a 6 litre Florence flask. The washings are collected from the portal vein through a rubber tube with a glass connection projecting through a stopper into an inverted funnel with cut-off stem. The flange provided by the funnel serves to protect the fluid from contamination during collection.

At 10 cm. pressure the fluid flows but slowly through the liver, and while it is flushing out the blood the suspensory ligaments are cut through and the gall bladder is slit and mopped out. All is now ready for the flushing away of the Kupffer cells.

To aid in the removal of the last blood, the liver lobes are gently lifted from time to time, and when the wash fluid comes away practically clear collection is begun, at first of samples into 10 cc. cylinders in which 0.1 cc. of a sterilized 1 in 1000 heparin solution has just been placed. Then the pressure is raised to 30 cm. and the further washings are received in a pyrex vessel of about 300 cc. capacity, ("taper flask") shaped like a narrowed separation funnel (Text-fig. 1), with straight sides to minimize the accumulation of sedimented cells. The flask contains 1 cc. of 1 in 1000 heparin solution in saline. Just before the pressure is raised the inflow is stopped and the right hand is thrust palm up under the liver so that the first and second fingers lie on the left side of the cava and the fourth and fifth on the right side. The fluid is then turned on and, as it flows through, the liver is gently kneaded. Soon the outlet tube is clamped, the liver is allowed to swell moderately, the outlet is opened again, and massage is done gently but firmly with the left hand to aid in expelling the fluid. This alternate swelling and massage of the liver as it empties is repeated several times with short waits between, during which as the findings show, the Kupffer cells tend to loosen. The entire procedure from the start of the operation requires about 20 minutes. A final sample is taken from which, with the earlier, one can learn whether iron-containing cells are present in quantity.

The same procedures are used with dogs but they are given much more iron, 30 cc. each day for 3 days, with washing out on the 3rd day after the last injection. The dog liver yields far more phagocytes than the rabbit and it can be massaged with less danger of rupture. Furthermore the Kupffer cells survive longer in Tyrode.

### *Selection of the Cells with the Magnet*

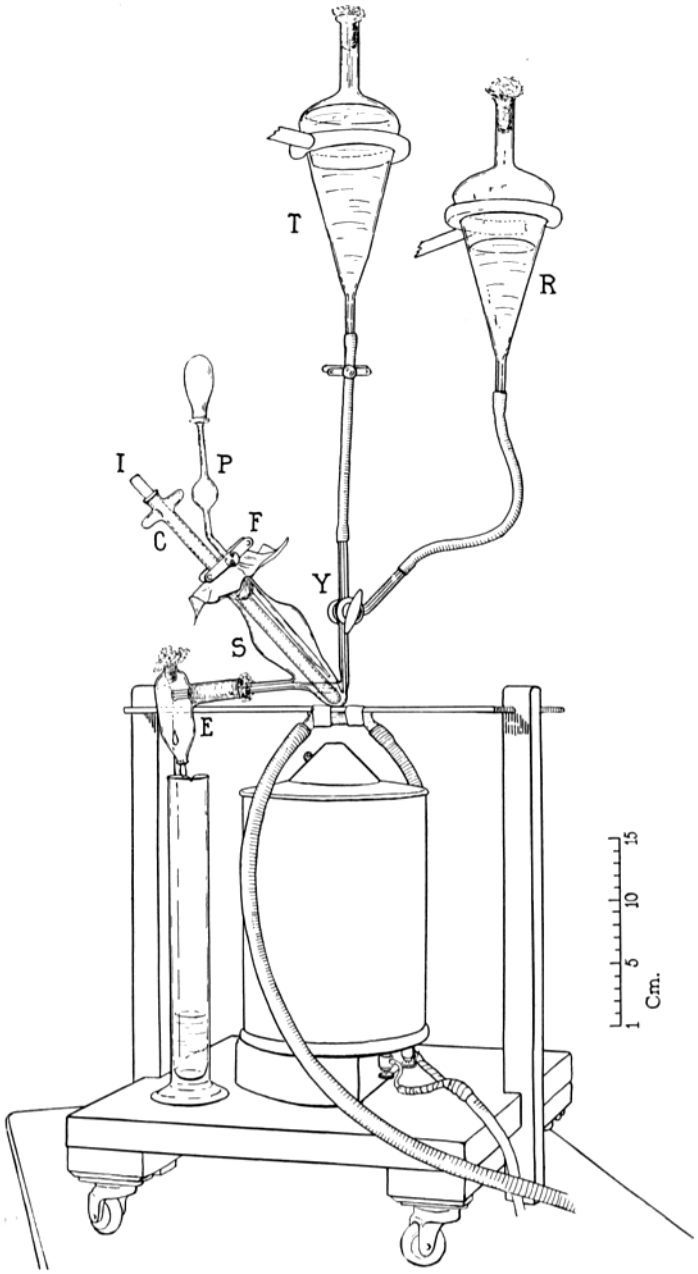
It was essential for the ultimate objects of the work that the cell suspension should be handled under conditions excluding infection, that the phagocytes containing iron should be drawn upward by the magnet,—to avoid an admixture with sedimenting elements of other

sorts,—and that after collection they be held in place while Tyrode flowing past removes all traces of blood. A further essential not foreseen as such was to collect the cells on a surface from which they could be readily dislodged. For when they had been attracted to a glass surface a strong stream was required to bring them away and a considerable proportion were killed during the process. After many modifications, a satisfactory apparatus has been developed (Fig. 2 and Text-fig. 1).

A large unipolar electric magnet (Giant Eye Magnet of the General Electric X-Ray Corporation) is fixed vertically beneath a glass chamber into which the cell suspension is run. It flows in a thin layer under and around a long glass tube having on the outside a collodion membrane and inside a rod of soft iron. When the current is passed through the electric magnet, this rod becomes magnetized secondarily and if the distance between the two has been properly adjusted, its pull upwards is considerably greater than the downward one, sufficing to attract and to hold practically all of the iron-containing cells present in the layer of slowly moving fluid round about. They collect on the collodion membrane and are washed *in situ* with Tyrode solution, run through with enough rapidity to flush out and remove all sedimented cells from the separation chamber. The tube and iron core are then taken out together, the cells remaining attached because the core has now some magnetism of its own; the end of the tube is dipped in rabbit serum; the iron core is withdrawn; and the cells are shaken off into the fluid. They can then be cultured.

Details of the apparatus are given in Fig. 2 and Text-fig. 1. The neck of the taper flask (*T*) connects with a rubber tube which is closed off with a screw-clamp until it is slipped over the end of the tube which leads to the separation apparatus. The latter (*S*) is set up in a room with air freed of bacteria by spraying and air filtration. The mouth of the taper flask is closed with a tight-fitting cotton plug, so that it can be inverted at will to keep the cells in suspension, and it rests in a ring stand with an opening at one side of the ring to facilitate removal. The *Y* tube with which it connects vertically,—to give less chance for sedimentation,—has a three-way stop-cock at the fork. The slanting limb of the *Y* connects with a second flask containing Tyrode solution. Flow into the separation chamber is regulated with the stop-cock, and its rapidity is gauged by the outflow tube which is protected by a glass shield (*E*).

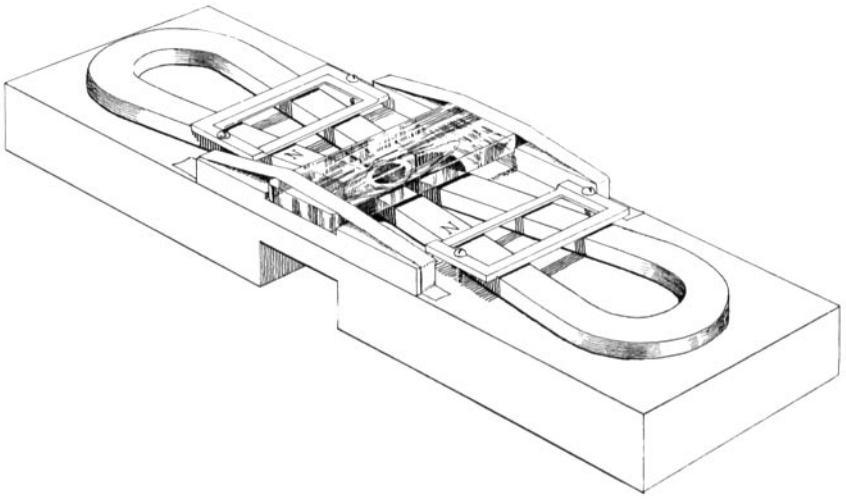
The separation chamber is shaped like an elongated pear. It has a broad mouth




TEXT-FIG. 1

closed with cotton covered with Chinese silk to prevent fibres from falling into the fluid. Two tubes pass through the stopper, the glass "core tube" through its center, and, through its uppermost side, a narrow pipette (*P*) provided with a bulb and a large rubber nipple. The pipette is used to draw off all possible fluid from about the core tube, prior to removal of the latter from the apparatus. Until that time the point of the pipette is not pushed down into the fluid.

The core tube (*C*) has a cross-piece near the top for ease in handling. Around its lower portion is a collodion sheath not shown in the drawing, made by dipping this portion into a concentrated sucrose solution and, when this has dried, dipping a little more deeply into a collodion solution. Before the latter has completely



TEXT-FIG. 2

dried the tube is immersed in several changes of water to dissolve out the sugar. The result is a sheath attached at its top to the tube, but not elsewhere, and hugging the glass closely after sterilization. When the separation chamber has been assembled it is autoclaved, with a little water in it to prevent the collodion from becoming brittle. Before the liver washings are run in, the core tube is adjusted and held with its lower end about 2 mm. above the bottom of the separation chamber, by means of the rubber-covered screw-clamp (*F*). Two pointed glass studs (not shown), projecting into the separation chamber a little way up from the bottom, prevent it from being pulled against the lower side of the apparatus when magnetization is begun. A soft iron rod (*I*), which fits it snugly and has a rounded end, is then inserted.

The separation chamber rests on a heavy glass plate held in cleats between two uprights. Under it is fixed a glass chamber in which water circulates, to prevent heating when the magnet gets warm.

After the taper flask has been connected with the apparatus the water in the separation chamber is replaced with Tyrode's solution by turning the three-way stop-cock, and then the magnet is brought into play and the cell suspension is slowly run in. It flows under and around the bottom of the core tube. A rate of about 1.5 cc. per minute has proved best for collection. When iron-containing cells are plentiful a brown skim, soon thickening to a fur, collects on the core tube. If the magnet is allowed to get very hot its pull falls off greatly. To prevent this we have regulated the current by means of a rheostat in such wise that the magnet exerts a pull of 1.13 kg. at a distance of 4.75 cm. on a cylinder of cold rolled steel shafting 5.06 cm. in diameter and 18.25 cm. long, held vertically over its pole. With this amount of current our magnet does not overheat and the strength of the pull is maintained.

When the collection of the cells is finished,—a matter of 2 to 3 hours, depending on the amount of material,—the separation chamber is flushed out with a brisk current of Tyrode solution from the reservoir (*R*), thus washing the cell material while still in place. Then by means of the pipette as much fluid as possible is drawn off. This is important since the higher the meniscus the greater is the likelihood that some of the fur of iron-containing cells will be lost when the core tube is withdrawn. It is now lifted out, stopper, core, pipette, and all, without turning off the magnet; and its cell-coated end is at once submerged in sterile rabbit serum. Transfer without loss is possible because secondary magnetization of the iron rod holds the cells in place after removal from the neighborhood of the large magnet. The rod is now slipped out of the collecting tube, and the bulk of the cell material falls away immediately into the serum. Most of that which still adheres to the collodion can be dislodged by cutting this up and gently agitating and pipetting the fragments. Several tenths of a cubic centimeter of Kupffer cells can be obtained from the liver of a 2000 gm. rabbit.

### *Cultivation of the Cells in Vitro*

Attempts were first made to grow the cells in a plasma clot according to the usual technic. They were dislodged from the collecting tube into Tyrode solution, and 3 parts of the resulting cell suspension were mixed with one of rabbit plasma and plated in amounts of 2 cc. in Carrel dishes. In cultures thus made the cells sedimented to the glass before clotting occurred, and their morphology could be readily studied. Few put out membranes during the succeeding days of incubation, though many survived as large spherical, or slug-shaped, entities. When first plated they were frequently in aggregates, as collected by the magnet, but in the course of 48 hours at 37°C. these broke up, the cells slowly moving apart (Fig. 3).

Some division of the cells took place (Fig. 4), yet the fact soon be-

came plain that in even the thinnest clot they could not be propagated in quantity nor could reliable tests of their functions be carried out. Consequently a nearer approximation to natural conditions was sought.

Within the liver the Kupffer cells live on the walls of small channels with a current of fluid flowing past them; and histologically they appear to be fixed on the walls by a broad membrane. They put out such a membrane on coming in contact with a flat glass surface when they are first procured. Normally it is extended on the inner side of the curve of the capillary wall; but there seemed some likelihood that extension would also occur on the outer side of a curve or in other words that the cells would fix themselves and live on the outside of fibres bathed in fluid, in much the same way as on the interior of small vessels. This has proved to be the case. When a suspension of the cells in serum, as first procured, is poured upon a layer of washed sterile lens paper in a Carrel dish, they fall through the interstices of the paper to the bottom of the dish. Here some of them flatten out and remain. The majority, however, swarm up the fibres within the first 48 hours of incubation (Fig. 6) and scattering, fix themselves here and there. Their arrangement may be likened to that which would exist if the liver capillaries were turned inside out. Immense numbers, distributed on the fibres, can be kept in flourishing condition if the serum is replaced each day (Fig. 5). The following technic has been developed:—

Leitz lens paper, non-ribbed, is washed in several changes of 5 per cent hydrochloric acid, rinsed in distilled water until acid-free, dried, and cut into discs that will cover the bottom of Carrel dishes 3 cm. in diameter. Two discs are introduced into each dish, spread flat with a glass spatula and autoclaved. The Kupffer cells selected with the magnet are shaken or pipetted away into serum,—in amount regulated by the number of cultures desired,—and 2 cc. of the suspension is distributed to each dish. The latter are closed with rubber stoppers and incubated 24 to 48 hours, that is to say until the cells have put out membranes and fixed themselves on fibres or glass. The serum is changed daily thereafter by tipping the dish and drawing off as much as possible, usually about 1.8 cc., or nine-tenths of the whole. Enough remains between the fibres for the cells to remain undisturbed. They have been successfully maintained for as long as 10 days, during which period an active multiplication of them took place. We have not tried to keep them longer since it seemed best to study their functions as soon as possible.

When the cells were propagated in serum diluted with Tyrode they showed many small granules of fat after the first few days, and tended to round up and fall from the lens paper. Successful cultures in undiluted serum showed practically no fat, and the cells remained more or less flattened.

### *Effect of the Magnet as Such*

Under the influence of the magnet most of the cells collect along the lines of force and the intracellular iron particles undergo a regrouping, with result that cells and iron, on suspension in serum, may appear like cherries skewered on a stick, the iron particles of the cells being ranged in a line to form the stick. It was noted that the cells of such configurations seldom survived in culture. To test whether magnetization, as such, kills cells containing ferromagnetic iron oxide, the following experiment was done:—

Two small horseshoe magnets were screwed to a board, with the north poles opposite each other at a few millimeters distance (Text-fig. 2). Under aseptic conditions a few drops of a freshly procured suspension in serum of iron-containing cells were placed within a deep ring of vaseline in the well of a large hollow-ground slide, a cover-slip was sealed on, and the slide was turned over and fixed on raised cleats to either side of the magnet, so that the vaseline ring,—which extended from slide to cover, retaining the fluid,—lay between the magnet poles, with the cover-glass just clear of these. A hole through the middle of the wooden block on which the magnets were set permitted study of the cells by transmitted light. Incubation was done as usual. The iron-containing cells promptly fell out of suspension onto the cover-glass. The majority were drawn into the lines of force within the first minutes, and the intracellular iron particles were seen to shift position gradually until they had become arranged in apparently continuous lines (Fig. 7). Not a few cells which happened to have fallen between the lines of force fixed themselves there, and the arrangement of the iron they contained did not indicate any influence by the magnet. These served as controls. In preparations incubated for 4 days without change of the medium they fared no whit better than the ones skewered on the lines of force. Both put out membranes (Figs. 8 and 9), and underwent gradual fatty changes to the same degree. Preparations that were removed from the influence of the magnets after 1 or 2 days, and further incubated, showed a prompt breaking up of the linear fixation of the cells, these moving off in all directions along the glass.

The experiment shows that sufficient magnetization of the intracellular iron to cause alignment of it and a grouping of the cells along the lines of force causes no harm. It is possible, of course, that strong magnetization is lethal in effect. However a sufficient explanation

of the cell death we observed as result of it is to be found in the brusque rearrangement of the intracellular particles that it causes. Chambers and Fell (4) have demonstrated that tearing the cytoplasm of a mammalian cell with a micro-needle suffices to kill it. The Kupffer cells are stabbed from within so to speak. That they are extremely sensitive to trauma is shown by the fact that most of those dislodged by very forcible liver washing are dead when obtained.

*Infection as a Complication*

The vascular arrangement whereby blood coming from the intestines must pass by the Kupffer cells, and the known special ability of these cells to take up and destroy bacteria, suggest that they are constantly engaged in removing bacteria which have entered the circulation from the gut. Many experiments have been reported which have been taken to support this conception. In the material utilized by us for cultivation there were always Kupffer cells that had died during the manipulations; and *a priori* it seemed all too likely that bacteria recently phagocyted by some of these, but not destroyed prior to their own death, would overgrow the cultures, especially when the bactericidal action of the serum had been lessened by heating it at 56°, as was frequently the case. Such overgrowth did occur on occasion, the bacteria rapidly multiplying throughout the fluid medium. This happened almost regularly when the cells were obtained from the livers of animals that had appeared to be ailing. In the attempt to obtain a larger yield of iron-containing cells than ordinary, some rabbits and dogs were given one or several intraperitoneal injections of a sterile solution of nucleic acid, while others received a suspension of killed cultures of *B. prodigiosus* intravenously. They had fever in consequence and the washing out of the liver was done while it was still present. The yield of Kupffer cells under such conditions was extraordinarily abundant, but the cultures were promptly overrun with bacteria. On the other hand cultures from normal rabbits and dogs remained uninfected when there had been no slips in technic. It was clear that either the bodily derangements caused by the injections so affected the Kupffer cells that they could not cope with incidental bacterial invaders phagocyted by them, with result that these were carried with them into the cultures, or else that blood

invasion by bacteria capable of growing in cultures containing Kupffer cells in serum (whole, or inactivated, or diluted with 3 parts of Tyrode) occurs to but a slight extent if at all normally. The latter alternative seems the more likely, since the Kupffer cells washed out of the animals with fever, instead of appearing to be adversely affected, were notably more active than usual, swarming up the strands of lens paper and scattering upon it after only a few hours of incubation instead of the usual 24 to 48,—within which period, however, they died in consequence of profuse bacterial growth. In rabbits with fever, whether produced by nucleic acid, killed bacteria, or by infection, living and active Kupffer cells could be washed from the liver when there had been no preliminary injection of particulate material. An animal washed out at the height of vaccinial infection gave a notably large yield. As already stated washings from normal animals contain only an occasional Kupffer cell.

These facts indicating that the Kupffer cells are markedly influenced in their activity by the general body state, as further, perhaps, that non-specific disturbances favor the entrance of bacteria into the blood stream, have as yet not been followed up experimentally.

## SUMMARY

Methods and apparatus are described wherewith living Kupffer cells can be procured from the liver of the rabbit and the dog for study and cultivation *in vitro*. Almost none of these cells can be dislodged from the normal liver by forcible perfusion; but after they have taken up finely particulate matter (India ink, iron oxide), they come away in great numbers. When they have phagocyted ferromagnetic iron oxide they can be selected with a magnet from amongst the blood elements present in suspension with them; and they are obtainable in quantity by this means. They do poorly when plated in a thin plasma clot, failing to multiply or to assume their characteristic shape; but they flourish when allowed to attach themselves to strands of lens paper bathed in serum that is frequently changed.

Bacterial infection of serum cultures of Kupffer cells from normal rabbits and dogs occurs only as the result of secondary contamination of the materials, whereas it regularly develops in cultures from animals with fever induced by the injection of nucleic acid or of killed *B.*

*prodigiosus*. Kupffer cells obtained under such conditions are abnormally active, and some can be washed out of the liver of sick animals in the absence of any preliminary phagocytosis of particulate matter. The facts have a bearing both on the conditions conducing to blood invasion and on the response of the Kupffer cells in the emergency.

The characters of the isolated Kupffer cells and the results of tests of their presumptive functions will be described in later papers.

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## EXPLANATION OF PLATES

All of the photographs were made from fresh preparations.

### PLATE 40

FIG. 1. Slide and cover-slip preparation of the liver washings of a rabbit repeatedly injected with India ink,—to show the relative size of erythrocytes, nonphagocytic white cells, and Kupffer cells with membranes extended on the glass. The photograph was taken slightly out of focus since otherwise the membranes would not be seen. Their edges are crinkled as result of partial retraction induced by the strong light. × 400.

FIG. 2. The separation apparatus ready for use.

### PLATE 41

FIG. 3. A clump of iron-containing Kupffer cells from the dog, breaking up as the cells slowly disperse. Photograph taken after 48 hours incubation in a thin plasma clot. × 180.

FIG. 4. Part of the same culture at a higher magnification. Three pairs of cells can be seen, resulting from *in vitro* division. × 360.

FIG. 5. A culture of iron-containing rabbit Kupffer cells on lens paper in serum, after 4 days incubation. × 115.

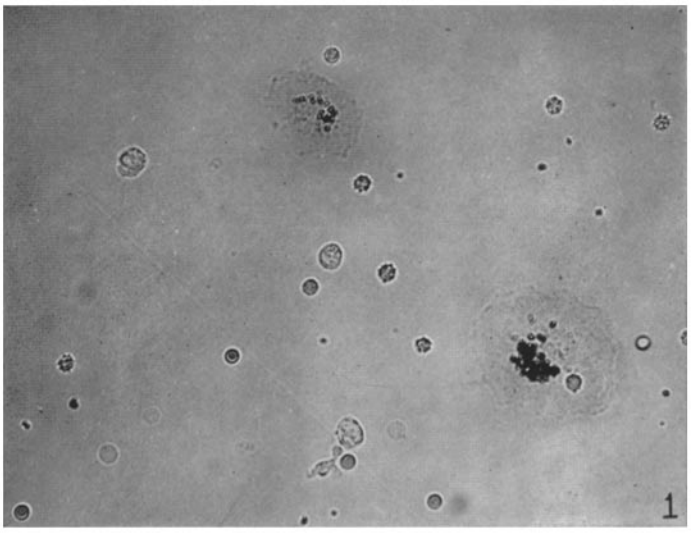
FIG. 6. Iron-containing, rabbit Kupffer cells climbing a fibre of lens paper in a serum culture. Photograph taken through the bottom of the dish. The cells

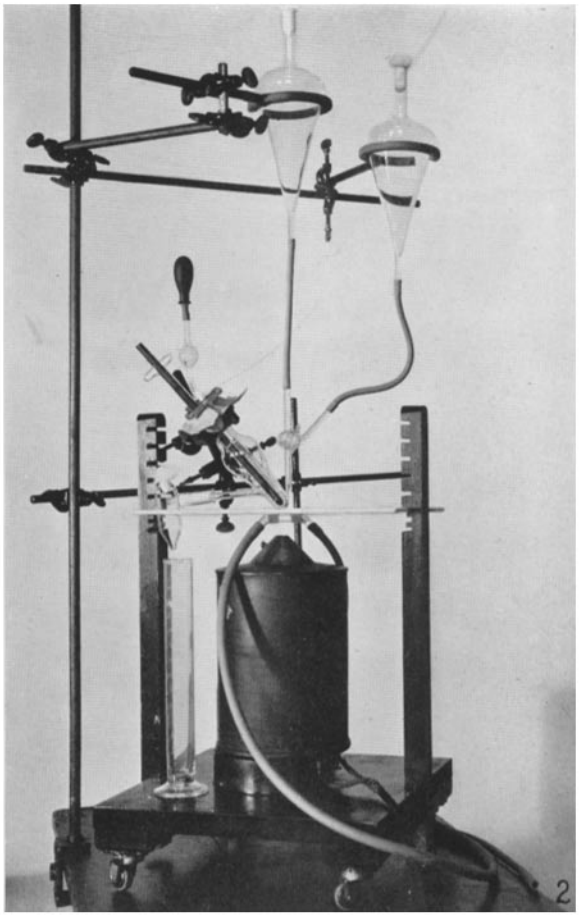
at either end of the fibre lie spread upon the glass. Their membranes cannot be seen. × 400.

PLATE 42

FIG. 7. Dog Kupffer cells living in the field between two horseshoe magnets. Many of them have been drawn into the lines of force as is indicated by the linear arrangement of the iron they contain. Impure culture, containing many blood leukocytes; photographed on the 3rd day of incubation. × 135.

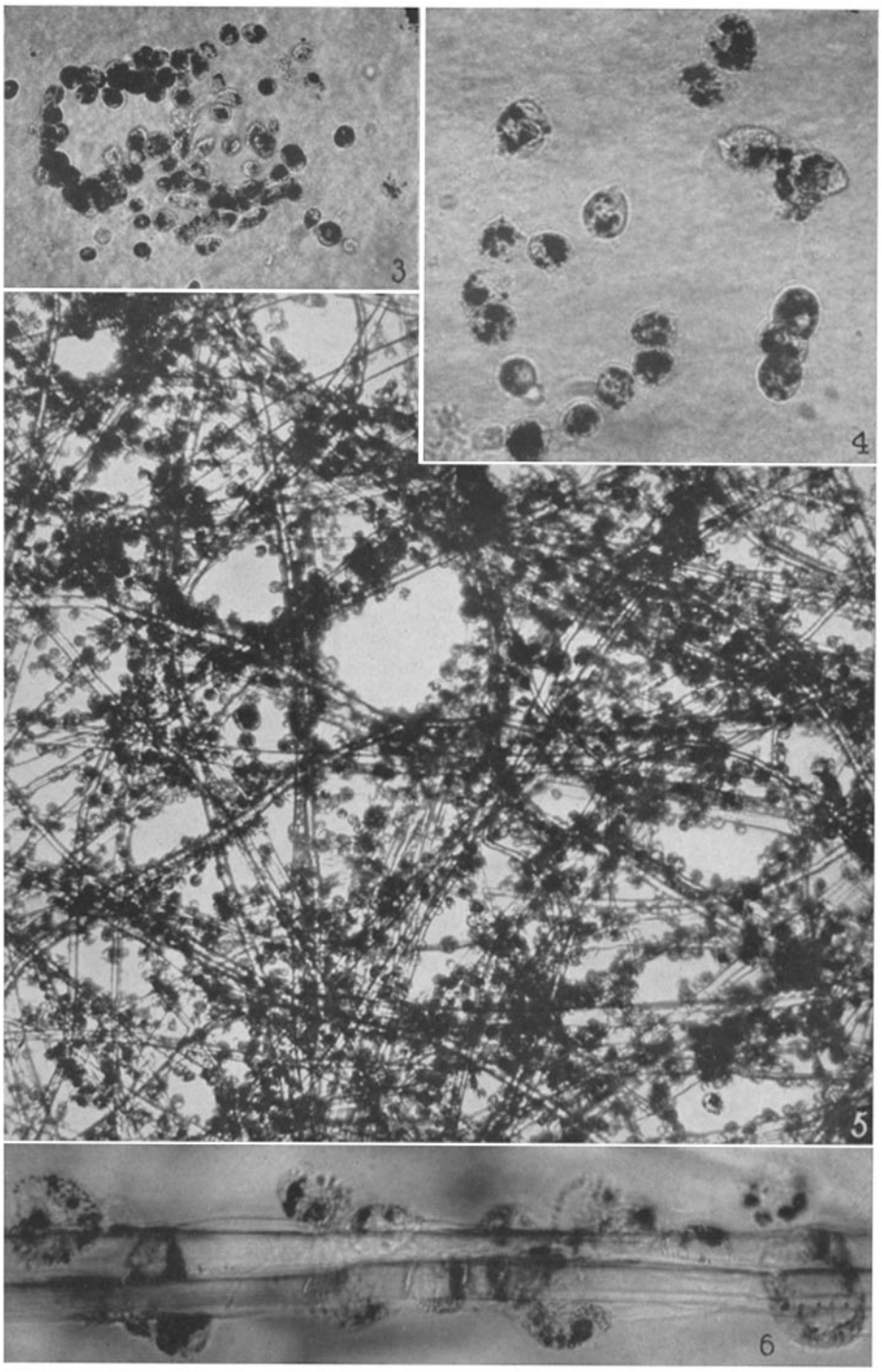
FIGS. 8 and 9. Two cells of the same preparation, photographed on the previous day. One lies in a line of force, as shown by the linear arrangement of the intracellular iron, the other outside this influence. They are in equally good condition. × 1100.



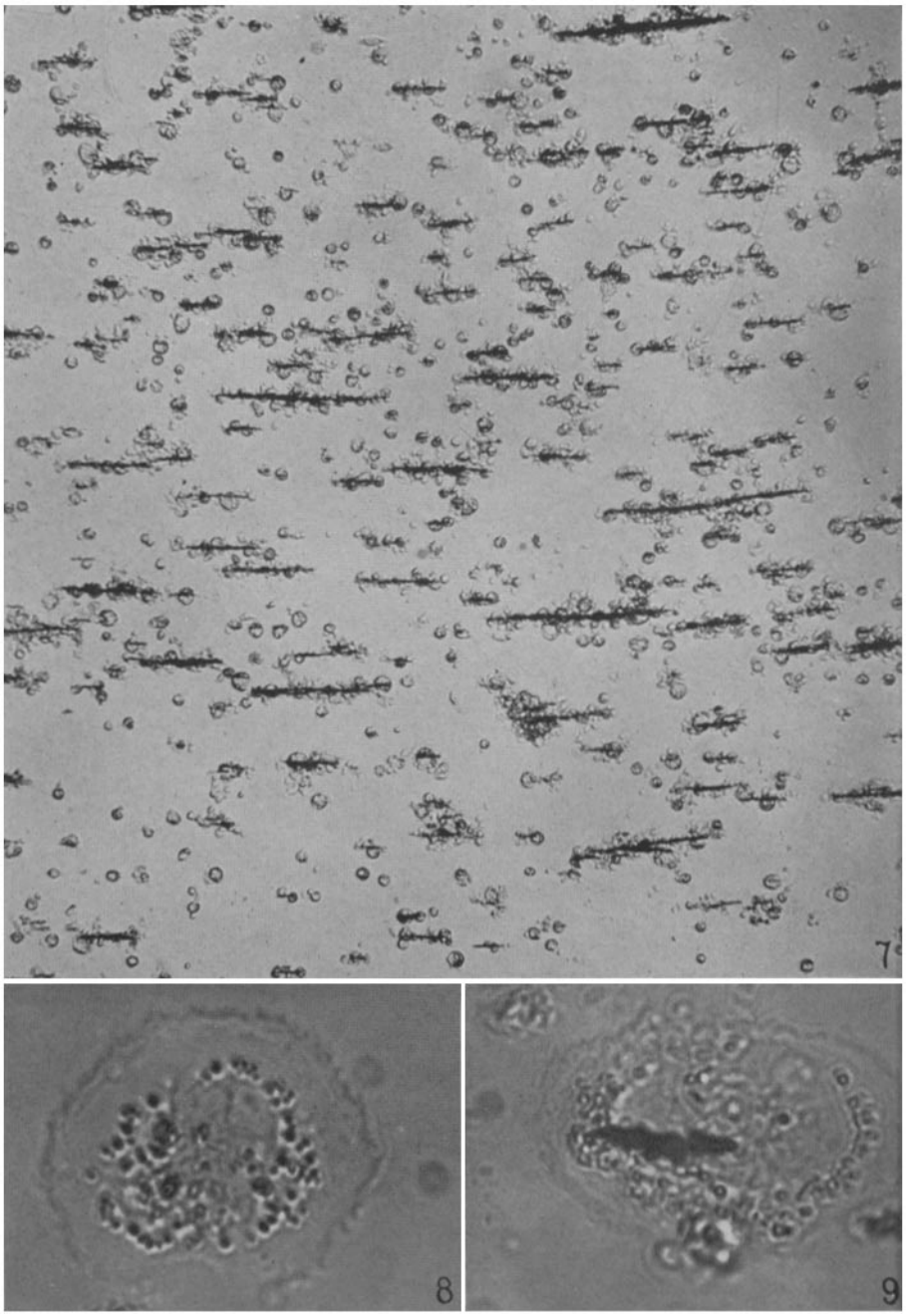

Photographed by Louis Schmidt

(Rous and Beard: Selection of Kupffer cells with magnet)




Photographed by Louis Schmidt

(Rous and Beard: Selection of Kupffer cells with magnet)

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(Rous and Beard: Selection of Kupffer cells with magnet)