THE PHAGOCYTIC POWER OF CONNECTIVE TISSUE CELLS.

By F. S. JONES, V.M.D., and PEYTON ROUS, M.D.

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

Plates 22 and 23.

(Received for publication, October 25, 1916.)

Suspensions of individual, living cells from the fixed tissues can be obtained by digesting with trypsin the clot of proliferating tissue cultures.[1] Certain of the cells thus freed, especially those of connective tissue and the sarcomata, will survive in Locke's solution for many hours, and proliferate when reimplanted in plasma. The method has made possible direct tests of the phagocytic power of fibroblasts.

In connective tissue *in vivo* phagocytosis of bacteria or particles of unorganized matter occurs not infrequently. It can sometimes be seen about bacterial foci and it is common in the neighborhood of old blood extravasations. Lambert and Hanes[2] have shown that carmine particles are ingested by some of the cells of *in vitro* cultures of sarcomatous tissue. But we need scarcely point out that in even the simplest of these instances the tissue is a congeries of cells of at least two sorts; namely, fibroblasts and endothelial cells from the blood and lymph channels. The latter are known to have phagocytic power. It is possible that their activity, with that of wandering cells, may account for the positive findings cited.

Method.

For our experiments bits of the heart and skeletal muscle from embryo chicks in the 3rd week of incubation, from embryo rats near to term, and from rats 2 to 5 days old were implanted in plasma of the appropriate species. Special care was taken to rule out the

[1] Rous, P., and Jones, F. S., *J. Exp. Med.*, 1916, xxiii, 549.

[2] Lambert, R. A., and Hanes, F. M., *J. Exp. Med.*, 1911, xiii, 495.

presence of blood, since some of the white cells might, by their phagocytic activity, have introduced confusion into the results. The tissue was washed free of blood by perfusing the animal with Locke's solution[3] injected into the heart, and the effectiveness of the washing, as well as the normality of the tissue, was controlled histologically. The plasma was centrifugalized at high speed, and the central portion drawn off for use through a fine pipette. The microscope showed it to be cell-free.

The cultures were made in small Petri dishes. Sometimes the undiluted plasma was used, and at other times it was mixed with from one to three parts of Locke's solution. After 24 to 48 hours' incubation of the cultures they were submitted to trypsin, and the freed cells were separated from the tissue fragments by filtration through gauze, were twice washed with Locke's solution by means of the centrifuge, and suspended in this fluid. 24 hour cultures yielded the best results. After longer periods the cells were apt to contain fat droplets.

In cultures made as described the muscle did not proliferate but there was an abundant outgrowth of connective tissue which, on digestion of the clot, furnished a thick suspension of living cells. The ability of these to take up particles of carmine was tested as follows: Finely ground carmine was added to the cell suspension, incubation carried on for 1 or for 2 hours, and the preparations were examined in the fresh, and in fixed films stained with methylene blue. Or the carmine was mixed directly with the plasma medium of the original culture, and when growth had taken place the cells were liberated with trypsin and examined for phagocytosis.

Experiments involving the phagocytosis of bacteria proved more troublesome, owing to difficulty in staining the preparations so that the presence of intracellular organisms and the character of the cells should both be brought out. A Gram-staining organism, *Staphylococcus pyogenes albus*, finally was selected, and the tissue cells were counter-stained with lithium carmine. This gave excellent results. Suspensions of freed cells were employed for the tests. With the cells of chicken connective tissue an antistaphylococcus serum was used,

[3] Locke's modification of Ringer's solution, but without sugar.

derived from chickens injected with cultures of the organism, and with rat cells, rat serum obtained in the same way. Leukocytes of the appropriate species were employed to determine the mixture of cell suspension, bacterial suspension, and serum, optimum for phagocytosis. In all the experiments control preparations without serum were made. Locke's solution was used throughout as a diluent. Incubation was for 2 hours, after which the cells were thrown down with the centrifuge, suspended in a little serum, and films taken.

The tests were many times repeated. The results were the same in all, irrespective of the species furnishing the cells, and whether they came from embryos or new-born animals. Both carmine particles and bacteria were phagocyted (Fig. 1), but always to a very small extent. Even in the best preparations it was necessary to go over many cells in order to find one phagocyte. The result cannot be attributed to unfavorable conditions in the mixtures, such for example as insufficient concentration of cells or bacteria, for the conditions were varied through a wide range, and in control preparations with leukocytes phagocytosis was profuse. Nor can it be laid to injury and death of the cells during the tryptic digestion and the subsequent incubation. Such treatment failed to lessen noticeably the phagocytic activity of leukocytes. The majority of the connective tissue cells were still alive at the conclusion of the tests, as shown by the refusal of the nuclei to stain with trypan blue.[4] Furthermore, as already stated, connective tissue cells freed with trypsin will proliferate when plated anew in plasma. The conclusion seems warranted that our technique was not at fault and that the phagocytic power of the cells derived from proliferating connective tissue is actually slight.

The phagocytosis of bacteria by the connective tissue cells occurred only in the presence of serum.

Nature of the Phagocytes.

The nature of the phagocytic cells remained to be decided. A study of stained films threw light on this question. In our previous paper[1] mention is made of the striking change in the form of cells which

[4] Rous and Jones, *J. Exp. Med.*, 1916, xxiii, 601.

occurs when they are freed from the clot in which they have been growing. The cells of connective tissue cultures, even those with an attenuated spindle shape, all take an approximately spherical form, and their processes merge in the general cytoplasm. But despite this approach to uniformity two sorts of cells, or rather two cell series, can be distinguished in stained preparations (Figs. 2 and 3). They have been figured in color in the paper referred to. With Wright's stain the cells of the smaller sort, which are much the more numerous, are characterized by a round or oval, pyknotic nucleus and a relatively scanty cytoplasm, while those of the larger kind have an oval, vesicular nucleus and abundant cytoplasm. The cytoplasm of both is somewhat basophilic, staining pale blue, and the smaller cells usually divide before it has reached any considerable bulk. Many of the latter closely resemble the lymphocytes of the blood, although in stained sections of the tissue from which they are derived lymphocytes are not evident. The observation may have a bearing on the long discussed problem of the origin of the cells resembling lymphocytes which appear in granulation tissue.

The differences between the two kinds of cells are striking and are still evident to some extent when a mixed suspension of them is plated out in plasma. The small kind then assume a slender, spindle form with long, and often attenuated processes, and the nuclei become oblong or indeed almost rod-shaped; whereas the large cells remain compact in form, as a rule irregularly stellate, with a few short processes, and the shape of the nucleus does not change. These differences are not always well maintained. The trypan blue test indicates that cells of the large kind die much more quickly when kept in Ringer's solution than do the small ones.

It is possible that here are young and old elements of a single sort; but the morphological evidence is against this and in favor of the assumption that the large cells are endothelial, and the more numerous, smaller ones are fibroblasts. Attempts to demonstrate fibroglia in the latter were unsuccessful, as was to have been expected because of their youth. The large cells, and these only, have in our experiments shown phagocytic activity,—a fact which is in keeping with the known character of endothelial cells.

SUMMARY.

By the tryptic digestion of cultures *in vitro* of avian and mammalian connective tissue, suspensions of individual, living cells have been obtained. Their ability to phagocyte carmine and bacteria has been tested. The great majority of them fail to take up either, but a few large cells are able to do so. They will ingest bacteria only when serum is present; that is, they require the interaction of opsonins. There is good reason to suppose that the phagocytic cells are endothelial in nature. Should they prove to be fibroblasts, like the other elements present, the fact will remain that the phagocytic power of fibroblasts is practically negligible. Their failure to ingest foreign matter *in vivo* is to be laid not to the obstacles offered by the solidity of the tissue they compose, but to an inherent lack of ability on their part. The phagocytosis of blood pigment, bacteria, etc., which takes place in granulation tissue *in vivo* is probably carried on wholly by endothelial cells and wandering cells.

EXPLANATION OF PLATES.

PLATE 22.

FIG. 1. Phagocyted staphylococci in cells derived from the connective tissue outgrowth from bits of the heart and skeletal muscle of young rats, cultivated *in vitro*. The cells belong to the larger of the two sorts figured in the illustrations which follow. Gram's stain followed by lithium carmine.

FIG. 2. The two sorts of cells liberated from connective tissue cultures by digestion with trypsin. Bits of the heart of a young rat were used for the cultures. Wright's stain.

PLATE 23.

FIG. 3. The same two sorts of cells but derived this time from cultures of the skeletal muscle of a young rat. In these cultures the intermuscular connective tissue alone had proliferated. Some of the large cells show fat droplets. Wright's stain.

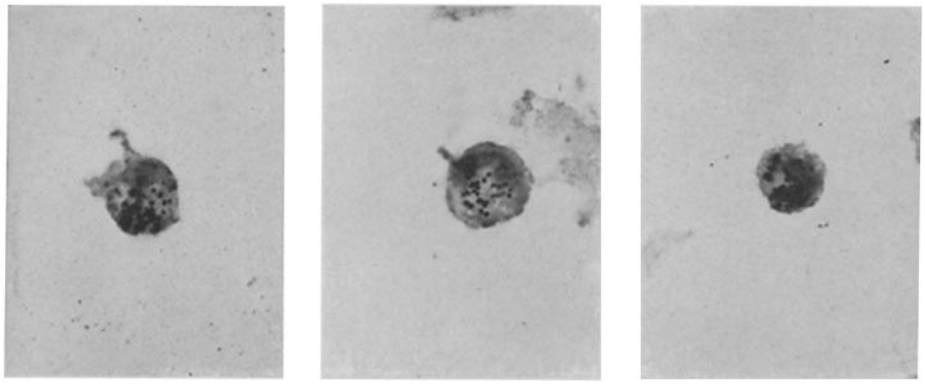

FIG. 1.

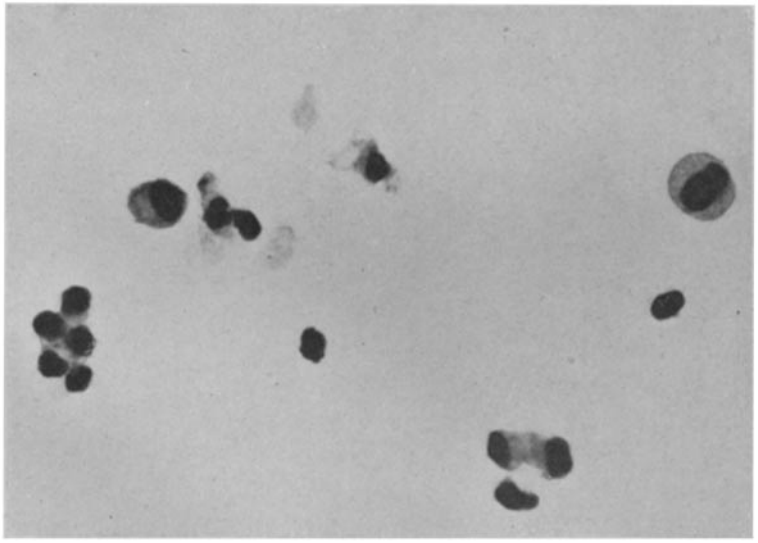

FIG. 2.

(Jones and Rous: Connective Tissue Cells.)

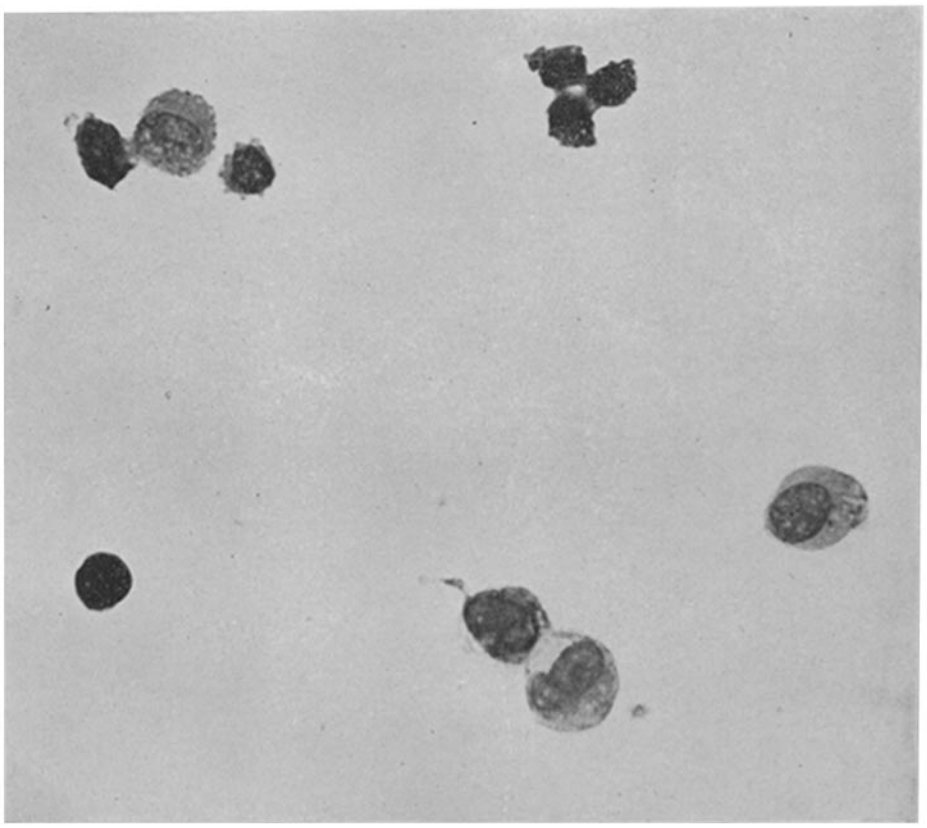

FIG. 3.

(Jones and Rous: Connective Tissue Cells.)