Culture of Mononuclear Cells from Rat Peritoneal Exudate. (20149)

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The need for a convenient source of macrophages in opsonic studies on chicken malaria led to the development of a technic for growing such cells from chicken blood agranulocytes(1). In attempting to adapt this technic to studies of rodent malaria we found it technically impossible to obtain enough rodent blood to provide agranulocytes in the required quantities, and have therefore developed an alternative method for obtaining similar agranulocytes for culture from peritoneal exudates sterilely induced in rats.

The method is here briefly described since it may be of some value to other workers who require cultures of mammalian macrophages or of fibroblasts developing from them, spread in a single layer, which may be exposed to known concentrations of drug, antigen, antibody, etc. in a supernatant phase. Such cultures can be fixed and stained as whole mount preparations, and require no sectioning as do many types of culture employing tissue fragments. Thus the entire contents of each cell rather than sections of a cell are available for study. The described method is further preferable to those using tissue fragments in that contact of the constituents of the liquid phase with each cell is ensured, whereas such contact in the interior of a tissue fragment is at best open to question. *Peritoneal exudate* induced in adult white rats by the intraperitoneal introduction of sterile paraffin oil according to the method of Bloom *et al.*(2) was collected as follows. Two days after injection of the paraffin oil, an anesthetised experimental rat received an intraperitoneal inoculation of 5 to 6 ml of Tyrode's solution modified by omitting $CaCl_2$ to avoid blood clotting, and also containing 1:50,000 Connaught heparin. Following brisk abdominal massage, the abdominal muscles were cut along the midline with aseptic precautions, and the contained fluid, consisting of a milky suspension of cells and paraffin oil in the modified Tyrode's solution, was sterilely collected into a centrifuge tube. Following centrifugation for 5 minutes at 2,000 r.p.m., the plug of semi-solidified paraffin oil was pushed aside, and the cell sediment was transferred to fresh modified Tyrode's solution. After 3 such washings by means of centrifugation, the pooled cell sediment obtained from 2 rats treated as described above was finally suspended in 10 ml of a medium consisting of the following components: 20% normal rat serum; 7% dilute aqueous chick embryo extr.; 73% Tyrode's sol. without $CaCl_2$; 5 units crystalline penicillin g/ml medium; 0.1 ml 0.1% phenol red/ml medium. The *cell sediment* consisted of large mononuclear cells with relatively abundant cytoplasm, as described by Bloom *et al.*

Six 10 mm^2 coverslips were cemented to the floor of each of a series of Carrel flasks with plasma clots. Each flask then received 5 ml of the fluid medium containing the suspension of mononuclear exudate cells. The flasks were closed with rubber stoppers and incubated at 37°C. When cultures were to be maintained for extended periods, the supernatant phase was renewed every 4 or 5 days. The effete supernatant fluid was removed, the cultures were washed for 15 minutes in modified Tyrode's solution at 37°C, and fresh supernatant medium, 5 ml per flask, was added after the washings were discarded. Hypertrophied cells adhered to the floor of the flasks and to the coverslips, and were not disturbed by the rinsing. *Serial observations* on events in each flask were possible, since a single coverslip could be detached and removed without disturbing the rest of the cultures in the flask. In this study cultures were fixed in Zenker-formalin and stained with hematoxylin-eosin-azure stain.

Results. Fourteen-day cultures consisted chiefly of a network of elongate or stellate fibroblasts with long, filiform processes, frequently arranged in a syncytium (Fig. 1). Such cells were often more than 100 μ long, not including the processes, by about 20 to 40 μ wide. Mitotic figures observed in them (Fig. 1) demonstrated that they were actively

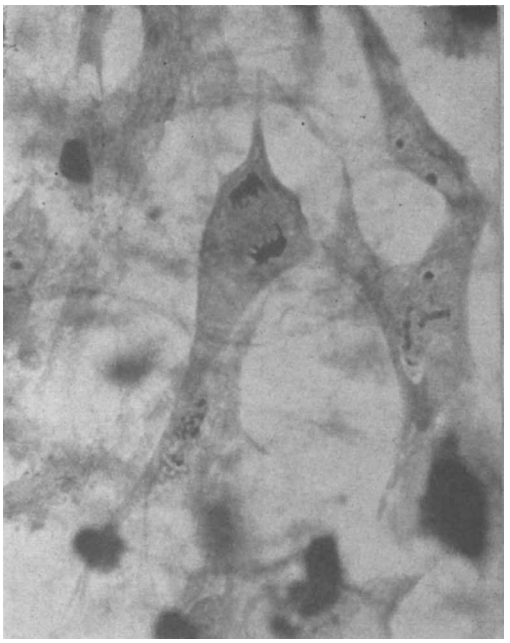

FIG. 1. Fibroblast syncytium developing from mononuclear cells of rat peritoneal exudate 12 days after explantation. Note mitotic figure in central fibroblast.

functioning and not merely surviving. Fibroblasts of this type were suitable hosts for obligate intracellular parasites of connective tissue cells, as may be seen in Fig. 2, which depicts colonies of the leishmaniform stage of *Trypanosoma cruzi* developing in such cells 7 days after flagellated crithidial forms had been introduced into the supernatant phase of the culture. Mitoses were still seen in such cultures, even in infected cells, when extensive areas had already been damaged by the parasites. The morphology of the parasites and their relation to the host cells is essentially similar to that described by Hawking(3) and Meyer(4) for cultures of *T. cruzi* in a variety of cell types. Muniz and Freitas (5) grew *T. cruzi* in a medium containing peritoneal exudate, but host cells were not stressed in their study, since their medium supported growth even in the absence of cellular elements.

Numerous macrophages were scattered among the fibroblasts in cultures like those described above. These were generally more or less rounded, about 20 to 40 μ in diameter,

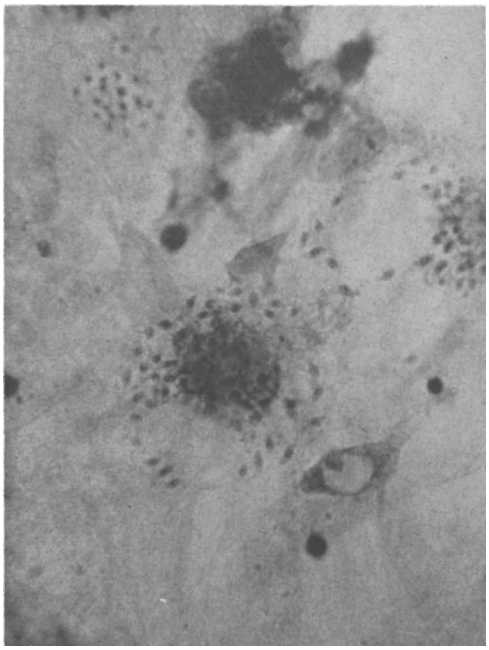

FIG. 2. Leishmaniform stages of *Trypanosoma cruzi* in fibroblasts developing from rat peritoneal exudate 12 days after explantation and 5 days after addition of crithidia of *T. cruzi* to supernatant phase. Parasites in intercellular bridges as well as in the body of infected cell.

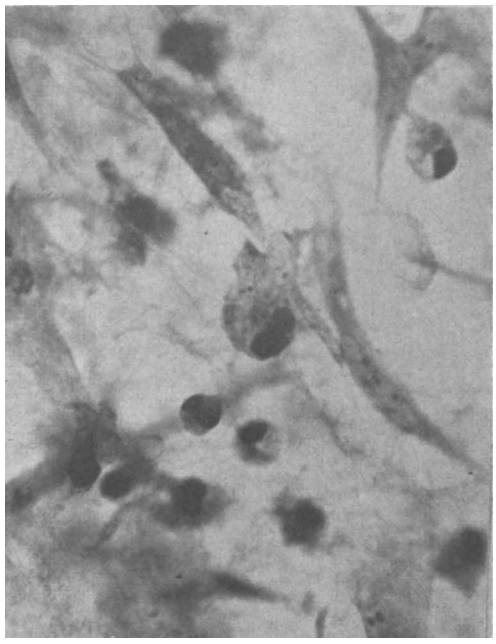

FIG. 3. Phagocytosis by a macrophage in a 12-day culture of rat peritoneal exudate, previously exposed to rat blood infected with *Plasmodium berghei*. Several erythrocytes and a number of rod-shaped granules of malarial pigment have been ingested by the macrophage.

with deeply staining, irregularly oval nuclei. Such cells are capable of phagocytosis, as illustrated by the rounded central macrophage in Fig. 3, taken from a culture previously exposed to rat erythrocytes infected with *Plasmodium berghei,* suspended in the liquid phase. The illustrated macrophage contains several erythrocytes in varying stages of digestion, as well as a number of rod-shaped granules of malarial pigment. No phagocytosis was seen in fibroblasts of 2-week cultures in which the macrophages were actively phagocytic.

Summary. A method for culturing a single layer of mononuclear agranulocytes from rat peritoneal exudate is described. Two-week cultures of this type consist chiefly of a network of fibroblasts interspersed with numerous macrophages, and are suitable for the maintenance of obligate intracellular parasites (ex.: *Trypanosoma cruzi,* leishmaniform stage). They may conveniently be used for drug testing, opsonic studies, or other tests requiring the exposure of a single layer of cells, normal or parasitised, to a supernatant liquid phase.

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Influence of Oxygen upon Genetic and Nongenetic Effects of Ionizing Radiation on *Paramecium aurelia.** (20150)

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It has been shown for a variety of organisms that X irradiation under low oxygen tension is less effective in producing biological damage than X irradiation under high oxygen tension. Hollaender, Baker, and Anderson(1) and Giles(2) may be consulted for review. It has been repeatedly suggested that this effect is caused by the reduced quantity of the active materials HO_2 and H_2O_2 produced at low oxygen concentration, implying that a considerable part of the biological effect of X rays must be due to these substances. It is important to find out how far this generalization is true and so to examine carefully any cases in which there is little or no oxygen effect. The present paper reports a series of experiments with *Paramecium aurelia* which show that under certain circumstances oxygen has little or no effect on the production of certain kinds of radiation damage, although under other circumstances it has a marked effect. It seems possible to interpret these findings in terms of changes in the importance of H_2O_2 in producing these effects. Evidence is also presented that oxygen may influence radiation damage in other ways than its effect upon H_2O_2 production.

Materials and methods. Stock 90 of variety 1 of *P. aurelia* was used throughout. The paramecia were selected to be in the first half of the interval between divisions. Shortly after treatment, a number of paramecia, usually 30, were isolated and continued as daily isolation lines.

The technique for measuring the genetic effect at doses of a few kiloroentgens has been described(3). In the present paper, the results are expressed as the percentage of normal exautogamous clones (clones which reach a maximum population in a limited quantity of culture medium in 4 days). This percentage becomes too small to be useful at about 10 kr. However, the percentage of exautogamous clones which survive 4 days, regardless of the

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