Simple Tissue-Culture Technique for Quantitating Free Migration of Reticulo-endothelial Cells

This paper (*1*) describes a method for short-term, reproducible, quantitative measurement of the effects of experimental procedures on the migratory powers of reticuloendothelial cells in culture. Although splenic tissue was used in the preliminary work, the technique is adaptable for observations on mature and precursor motile cells in bone marrow and other organs.

A 5-mm length of woven Fiberglas sleeving is prepared by repeated washing in xylol, alcohol, water, dilute HCl, NH_4OH, and double-distilled water. Its lower 1.5 mm is made into a well-type receptacle by filling its interstices and the lower end with Diatex. (Diatex is a toluol solution of a transparent acrylic acid, plastic compound which hardens on drying and which can be autoclaved or sterilized in a heat oven. It has proved to be nontoxic in over 1200 tissue cultures.)

The prepared sleeving is attached along its length to a piece of cover glass (Fig. 1). In the developmental stage of this project, half of a spleen from a 12-day chick embryo, removed with the usual aseptic precautions, was used as test material. This piece of tissue is slipped into the open end of the Fiberglass sleeving, which with its attached cover glass is then placed in a Kahn tube containing 0.7 ml of supernatant, which consists of 0.25 percent human serum protein in Hanks' balanced salt solution. The Kahn tube is closed with a sterile serum-type rubber stopper containing a No. 20 hypodermic needle (fitted at its upper end with a cotton-plug bacterial filter).

Any free, nonmigratory, dead or damaged cells from the fragment of tissue fall into the well-type receptacle at the lower end of the Fiberglas sleeving, where they are retained. Any living cells with migratory powers move actively through the interstices and enter the supernatant in the Kahn tube. After any desired interval of time, the cover glass with the attached sleeving is carefully withdrawn from the Kahn tube, and uniform suspension of the cells remaining in the supernatant and on the walls of the Kahn tube is obtained by vigorous shaking for 1½ minutes. (We assumed that the number of cells remaining on the sleeve and cover glass bears a constant relationship to the total number counted. No attempt was made to identify these cells. In the future, siliconing of the tube will eliminate this difficulty.) Enough cell suspension is removed to charge the counting chamber of a hemocytometer, and the number of cells per square millimeter is counted under phase-contrast microscopy. Alternatively, aliquots of supernatant can be removed from the tube at any time for counting (with subsequent restitution of the total volume of 0.7 ml of supernatant in the Kahn tube.)

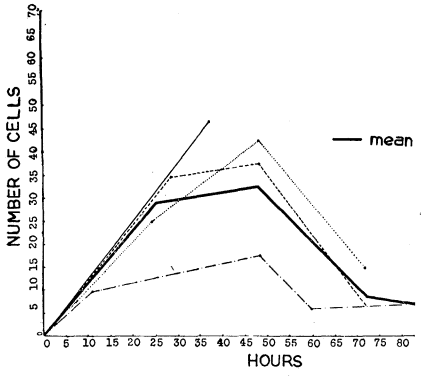

Fig. 1. Counts of migratory cells in supernatant as a function of time. Each curve represents the results from a series of 12 separate cultures, each point being the average of the measurements from three cultures. The heavy line represents the mean of the curves.

Figure 2 shows the results of cell counts done at 12-hour intervals on over 50 such cultures. In the first 24 hours there is a sharp increase in the number of cells in the supernatant; during the second day there is much more gradual increase in the cell count; and during the third day in culture there is a sharp decline in the cell population. The curves for the individual cultures were similar to the composite one shown in Fig. 2.

Smears of the centrifuged supernatant, when stained by Wright's method, showed large lymphocyte-like cells and polymorphonuclear cells containing either eosinophilic or neutrophilic granules. Erythrocytic or other nonreticuloendothelial cells were not seen.

Much work has been done on the tissue culture of migratory cells (*2, 3*). In the past, changes in the numbers of these cells in culture have been followed by counts on all cells present (including damaged cells) or by complex histological or biochemical studies. Our method is simple, speedy, and economical. It can be used to measure the effects of experimental procedures (including screening of pharmacological agents) on a function of reticuloendothelial cells. It has all the advantages of previous techniques. In addition, whole organs can be used, and the anatomical structure of the organ is maintained. Experiments in which this system is used will involve a representative section of the whole reticuloendothelial system. Most important, the cells measured by this technique have shown themselves to be healthy in that they are capable of normal migration. This work also confirms the ability of cells in the spleen to migrate in a fluid medium.

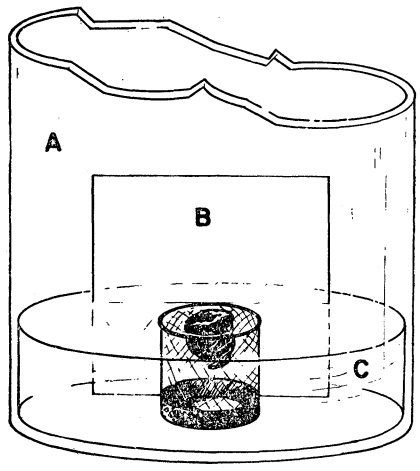

Fig. 2. Simplified diagram of culture assembly. *A*, Kahn tube; *B*, cover glass; *C*, Fiberglas sleeving with piece of spleen.

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References and Notes

1. This work was supported in part by a research grant (H 2249) from the National Institutes of Health.
2. E. E. Osgood, *J. Lab. Clin. Med.* 24, 945 (1938).
3. ———, *Ann. N.Y. Acad. Sci.*, 59, 806 (1956).

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Chemical, Insecticidal, and Antibiotic Properties of Fire Ant Venom

The imported fire ant (*Solenopsis saevissima* var. *richteri*) has become an insect of considerable economic importance in the southeastern United States. It has been reported to cause damage to a variety of crops and to attack livestock (*1*). This ant also attacks human beings. The reaction caused by the sting varies with each individual but is generally limited to the area surrounding the wound. An umbilicated pustule develops which is surrounded by a red halo or an edematous painful area (*2*). In some individuals, febrile and allergic systemic reactions have been reported. In at least one case, and possibly in two, reactions to ant stings have been fatal (*2*).

The nature of the venom of the imported fire ant has not been described. The necrotic activity and the characteristic pustule at the site of the sting indicate that the venom is different from any reported insect venom (*2*). We have