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 After any desired interval of time, the cover glass with the attached sleeving is carefully withdrawn from the Kahn tube, and uniform suspension of the cells remaining in the supernatant and on the walls of the Kahn tube is obtained by vigorous shaking for 1½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.)

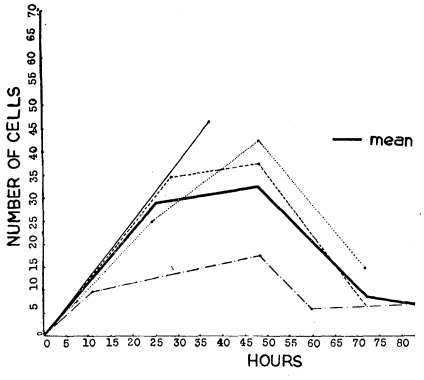

Fig. 1. Counts of migratory cells in supernatant as a function of time. Each curve represents the results from a series of 12 separate cultures, each point being the average of the measurements from three cultures. The heavy line represents the mean of the curves.

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.

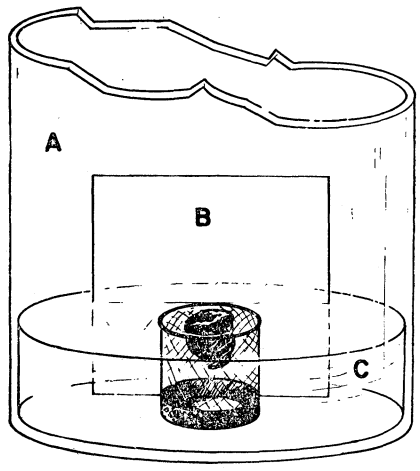

Fig. 2. Simplified diagram of culture assembly. *A*, Kahn tube; *B*, cover glass; *C*, Fiberglas sleeving with piece of spleen.

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1. This work was supported in part by a research grant (H 2249) from the National Institutes of Health.
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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

studied the chemical and physical properties of this venom and have found that it does not resemble the venom of any stinging insect previously studied.

Venom was collected from major workers taken in the field during the fall and winter. The ants were held by the petiole with a forceps while the tip of the abdomen was stroked with a fine capillary until the sting was everted. Droplets of venom issuing from the tip of the sting were collected in the capillary. The procedure was carried out conveniently under low magnification with a dissecting microscope.

The venom is water-insoluble, being less dense than water, in which it disperses as fine milky-colored globules. The absence of ninhydrin-positive reactants indicates it is nonproteolytic. The venom consists of two phases, primarily being composed of an alkaline carrier which suspends fine droplets of a greater density. The alkalinity of the mixture is not due to metal ions. These were determined to be absent by emission spectrographic examination in the Jarrell-Ash 4.8-meter grating spectrograph. The venom is soluble in most organic solvents, but least soluble in ethanol.

Ultraviolet spectrophotometric examination of the venom (in ethanol) in a Beckman DU spectrophotometer showed no peaks, absorption being strongest at the lower wavelengths. Infrared examinations (*3*) were made on a Perkin-Elmer model 21 spectrograph either as a carbon tetrachloride solution or as a film of venom applied directly to the rock salt prism. Only aliphatic C—H stretching was found (3.4 μ), demonstrating the nonaromatic nature of the venom. A carbonyl group (5.70 μ) is present which does not appear to be an open chain, simple ketone (*4*). Both methyl (7.25 μ) and methylene groups are present as well as a possible ether linkage (8.6 μ). The C—H/C=O ratio was found to be much higher when the sample contained small amounts of suspended globules. This indicates that the globular component contributes most or all of the carbonyl-containing compound.

Insecticidal activity was examined by exposing insects to residues, or by topically applying the venom as obtained from the ants. Samples for residual determinations were prepared as acetone or ethanol solutions. The venom was found to be highly toxic to the fruitfly, *Drosophila melanogaster* Meig., the housefly, *Musca domestica* L., a termite, *Kaleotermes* sp., the boll weevil, *Anthonomus grandis* Boh., and the rice weevil, *Sitophilus oryza* (L.). In addition, two species of mites, *Tetranychus telarius* L. and *T. cinnabarinus* Boisd., were highly susceptible. Interestingly, the fire ant is not highly susceptible to its own venom.

The antibiotic activity of the venom was investigated, and it was shown that several types of microorganisms were inhibited by a 1/50 dilution. Tests made by the paper-disk method demonstrated the effectiveness of this venom against *Micrococcus pyogenes, Streptococcus pyogenes, Escherichia coli, Lactobacillus casei,* and a variety of molds. The antibiotic activity of fire ant venom probably explains why the pustules arising at the site of the sting are antiseptic (*2*). A thorough study of the antibiotic properties is now being made.

The toxicities of different samples of venom to *Drosophila* have been found to vary, some samples being at least as toxic as DDT. Highly toxic samples of venom produce an instantaneous paralysis highly suggestive of a nerve poison. The most toxic samples contain a large percentage of the globular component, which suggests that this phase represents the toxic principle.

Recent work on the chemistry of ants has demonstrated the presence of a terpenoid lactone, iridomyrmecin (*5*), in various species of ants in the subfamily Dolichoderinae. Although these ants are in a phylogenetically more advanced subfamily than the fire ant (Myrmecinae) and do not have a functional sting, our infrared data suggest similarities in structure to this lactone. Iridomyrmecin also has been shown to have antibiotic and insecticidal activities (*6*). However, whereas iridomyrmecin produces tremors in insects suggestive of DDT poisoning (*7*), fire ant venom produces a sedative reaction, paralysis being unaccompanied by tremors.

The chemical composition of fire ant venom and the effect of it on malignant cells are being studied.

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Methionine Inadequacy of Casein Hydrolyzate as Source of Difficulty in Vitamin Assays

The microbiological estimation of vitamins is undertaken routinely in many laboratories. Despite all efforts to maintain assay procedures under rigid control, it is not uncommon to encounter difficulty suddenly with methods that had been proceeding smoothly. Such occurrences are sometimes attributable to deterioration of one of the solutions used in making the medium, or, less frequently, to mutation of the test organism. In other instances, despite much searching, no reason for the difficulty can be found—then, suddenly, one once again obtains a satisfactory standard curve. Recent experience in this laboratory leads us to suggest that an unsuspected source of difficulty may be the variability in amino acid content of batches of commercial vitamin-free casein hydrolyzate (acid).

The routine analysis of folic acid by the AOAC method (*1*) with *Streptococcus faecalis* 29-21 [isolated by Harrison (*2*)] as the test organism suddenly failed, as evidenced by a very flat standard curve. Although the *assay* medium no longer supported the usual level of growth of *S. faecalis,* the organism still grew well on *nonsynthetic* inoculum broth. Doubling the concentration of certain batches of casein hydrolyzate in the assay medium resulted in improved growth, but the degree of improvement varied greatly from batch to batch.

The effect of supplementation of the folic acid assay medium with the following amino acids was studied: L-arginine · HCl, L-asparagine, L-cysteine · HCl, L-cystine, L-glutamic acid, L-histidine · HCl, DL-isoleucine, L-leucine, DL-lysine, DL-methionine, DL-serine, DL-threonine, DL-tryptophan, and DL-valine. For each amino acid, the amount added was that indicated by Greenhut *et al.* (*3*) to be necessary for the optimal growth of *S. faecalis,* American Type Culture Collection No. 8043. Only two of these amino acids produced any significant effect: the supplement of DL-methionine (5 mg/100 ml of double-strength medium) permitted normal growth of *S. faecalis,* while the supplement of L-leucine slightly improved total growth. The effect of leucine later proved to be due to contamination of this amino acid with 9 percent methionine.

The various batches of casein hydrolyzate were analyzed for methionine by the method of McCarthy and Sullivan (*4*), and the concentration of four lots on hand was found to be 0.15, 0.24, 0.30, and 0.38 mg of L-methionine per milliliter of hydrolyzate (10 percent casein). When the hydrolyzates were assayed by the microbiological method of Steele