Technical Papers

Lack of Bactericidal Effect of Mouse Serum on a Number of Common Microorganisms*

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Study of the bactericidal effect of the blood serum of the adult white mouse (albino *Mus musculus*) was undertaken as part of an investigation of the relative role of antibiotics and of nonspecific defense mechanisms in resistance to experimental infection. Various substances and procedures (such as x-irradiation and adrenalectomy) were to be tested for their effect on the bacterial power of mouse serum acting against suitably susceptible bacteria. The method was standardized using rabbit serum because of the relative difficulty of obtaining mouse serum.

In the method used, 0.25 ml of a saline dilution of an 18-hr nutrient broth hour culture of *Bacillus subtilis* (ATCC PCI 220) was added to a tube containing 2.0 ml of a saline dilution of fresh rabbit serum. The mixture was incubated at 37°C. The effects of varying the size of the inoculum, the dilution of serum, and the time of incubation were studied. It was found that when the inoculum was about 2000 organisms (as determined by plate count), rabbit serum diluted 1:10 would render nonviable about 99 percent of the bacteria in 2 hr.

Since this method showed rapid killing of *B. subtilis* by rabbit serum, this organism and also a strain of *Escherichia coli* were tested using mouse serum which was obtained from the pooled blood of eight mice. No evidence of bactericidal action of the fresh mouse serum during 1- or 2-hr incubation was noted with either organism with a 1:3 and 1:10 dilution of serum and inocula of 2000 and 10,000 organisms. A number of other organisms were tested on three different days against fresh pooled mouse serum (four to eight mice) diluted 1:3, inocula of the order of 10,000 bacteria and an incubation time of 2 hr.

The organisms used in these experiments, with the exception of *B. subtilis*, were obtained from the stock culture collection of the Department of Bacteriology, University of Utah. The test organisms were *B. subtilis, E. coli, Pseudomonas aeruginosa, Ps. fluorescens, Proteus vulgaris, Aerobacter aerogenes, Alkaligenes fecalis, Vibrio comma,* and *Salmonella ballerup.*

With none of the aforementioned organisms was there evidence of killing by mouse serum under the experimental conditions described. Indeed, the bacteria were not even inhibited, since in every instance the final bacterial concentration exceeded the initial concentration. Sterility controls showed the serum itself to be free of bacteria in every case.

In addition to the experiments cited, 13 additional experiments have been carried out with serum pools from three or four mice (five experiments with albino *Mus musculus,* six with CBA strain mice, and two with wild *Mus musculus* captured at the Salt Lake City Zoo). The test organism in these later experiments was *B. subtilis.*

The mouse has received very little attention with respect to this type of study. Zinsser, Enders, and Fothergill (*1*) have reviewed numerous studies on the bactericidal action of serum from a variety of common laboratory and domestic animals with the exception of the mouse. Since the organisms employed in the present experiments were species ordinarily susceptible to the bactericidal action of serum from other animals (*2*), the results suggest that mice occupy a unique position among mammals in having serum devoid of bactericidal power against these organisms.

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Plant Tissue Cultures Produced from Single Isolated Cells*

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The value of plant materials for fundamental studies of growth was increased by the successful cultivation of plant tissues *in vitro* (*1–4*). Although this development was an important advance, the desirability of producing such cultures from single isolated cells has long been recognized (*5*). Plant tissue cultures display a degree of physiological and morphological variability that may be troublesome in analytical investigations. Although the tissue of a culture is derived from one plant, the potentialities of the cells may differ. This seems especially true of cultures derived from pathological growths, such as crown

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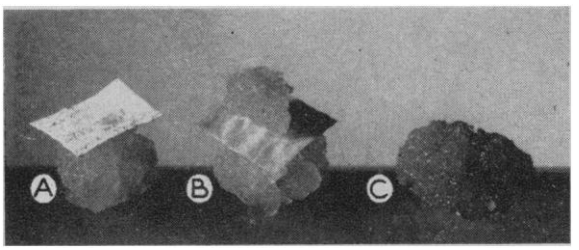

Fig. 1. *A*, filter paper, 8 × 8 mm, over sunflower culture. *B*, marigold culture of single-cell origin growing on filter paper over sunflower. *C*, marigold culture of single-cell origin, growing independently.

gall, where chromosome numbers vary (*6*) and normal and abnormal cells of several types may exist together. Cultures derived directly from single isolated cells might show less variation and prevent confusion from mixtures of cell types.

Cultures of animal tissue recently have been grown from single isolated cells (*7*). The present report describes the production of tissue cultures from single isolated cells of higher plants.

Single cells were obtained from tissue cultures of marigold (*Tagetes erecta* var. Sunset Giant) of crown gall origin (*8*) and of tobacco (*Nicotiana tabacum* var. Havana 38) from the normal stem. These tissues had been maintained in culture for several years (*9*). Their friable growth on agar media suggested that single cells might be obtained more readily than from firm cultures.

An abundant, diffuse type of growth consisting of single cells and small clumps of cells resulted when the marigold and tobacco tissues were grown in liquid culture on a reciprocal shaker (*10*). Suspensions of cells were easily removed and spread over an agar medium. It was more difficult to obtain single cells from tissue grown on agar.

Single cells, deposited on an agar medium, were located under a dissecting microscope and removed with a flattened needle under aseptic conditions. They were placed on 8 by 8 mm squares of sterile filter paper (Reeve Angel, crepe surface, No. 202). These squares had been resting for two or more days on the top surface of young tissue cultures (about 7 mm in diameter) of marigold, sunflower, or tobacco growing on an agar medium. Each filter paper square was then returned to the upper surface of the "host" culture from whence it came (Fig. 1). These operations required speed to avoid injury from excessive light and desiccation. At no time was the upper surface of the filter paper in direct contact with the underlying host tissue. Double layers of paper were used in numerous trials, including some that were successful. More than 1500 cells were isolated. Marigold and sunflower cultures were "hosts" for single marigold cells, because both grow well on the same synthetic medium (*9*). Tobacco cultures were "hosts" for single tobacco cells.

After precision was achieved, about 8 percent of the marigold and tobacco cells isolated grew and divided. Marigold cells grew on filter paper over either marigold or sunflower tissue. It was necessary to transfer the filter paper to a fresh host piece one or more times; for as a host culture became old and senescent, the young culture it supported also lost vigor. After reaching a diameter of 4 mm, which usually required 6 to 10 wk, cultures resulting from single cells were transferred directly to agar medium, where they grew well independently (Fig. 1). Several stocks of single-cell origin have been carried through four or more agar transfers, each of approximately 5 wk. The tissues were subdivided to a diameter of 4 mm each time, and no diminution in growth rate occurred.

The usefulness of plant tissue cultures of single-cell origin is apparent, for example, in studies on biochemical, morphologic, and genetic differences between normal and gall tissues (*11–13*), on virus-free and virus-infected tissues (*14*), on the habituation process for plant growth regulators, and on mutation to diseased or to healthy tissue. The single cell eliminates the possibility of culture change due to variation in the proportions of the cell types present in an original mixture.

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Till-like Deposits on Natapoc Mountain

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About 11 mi north of the town of Leavenworth, in north-central Washington near the center of the Chiwaukum quadrangle, is Natapoc Mountain (Fig. 1), an outstanding topographic feature that has been carved from Eocene sandstones (*1*). The summit of the mountain is 2 mi west of the Wenatchee River and is 4235 ft in elevation, rising 2530 ft above the river. The mountain lies within the Wenatchee River drainage area and is entirely surrounded by deep well-defined valleys. On the west is the crest of the Cascade Mountains and on the east are the Entiat Mountains, marginal ridges composed of granitic and metamor-