BACTERIOLOGY

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ABSTRACTS

Pathogenesis of mouse hepatitis virus in germfree and conventional mice. GEORGE LAVELLE and THEODORE J. STARR. University of Notre Dame, Lobund Laboratory.—Triolein-protected mice against an otherwise lethal challenge of cortisone and mouse hepatitis virus (MHV-1). In addition, triolein stimulated the reticuloendothelial system as measured by the clearance of colloidal carbon from the blood. The growth of MHV-1 in mouse livers was studied under conditions of triolein and cortisone pretreatment. Virus titers of conventional anmals were significantly higher than germfree animals when mice were challenged with virus alone. Cortisone increased titers in germfree mice but these remained lower than conventional mice. After triolein pretreatment, however, germfree titers were greatly elevated and comparable to that obtained with virus alone in conventional animals. It is suggested that the enhanced ability of germfree animals to support virus growth is associated with triolein stimulation of the reticuloendothelial system. The mechanism whereby triolein protects against lethal doses of cortisone and virus is currently under consideration. The problem considers interferon production and carbon clearance.

Inhibitory Effects of Human Indigenous Bacteria on *Corynebacterium diphtheiae* and *Streptococcus pyogenes*. PAUL C. LEAVIS, Forsyth Dental Center, Boston, Massachusetts.—Twenty-two strains of bacteria indigenous to the oral cavity of man were isolated from throat swabs in order to determine whether they could inhibit growth of the pathogens, *C. diphtheriae* and *S. pyrogenes*. This effect was tested *in vitro* by seeding petri plates containing Trypticase Soy Agar (with 0.25% glucose added) with the two pathogens and then spotting the plates with the human indigenous bacterial strains. Six strains of the 22 tested were found to inhibit growth of the *C. diphtheriae*, when grown under anaerobic conditions. However, none of the strains affected *S. pyogenes*. The inhibitory organisms included 2 *Staphylococcus* strains, 3 strains of *Streptococcus salivarius* and one of *Streptococcus mitis*. The most effective inhibitor among these was a *Streptococcus salivarius* strain which produced a distinct inhibitory zone of approximately 1.53 cm^2 in area. The other *S. salivarius* strains produced inhibitory zones of 0.94 cm^2 and 0.39 cm^2, the *S. mitis*, 1.05 $cm,^2$ and the *Stpahylococcus* strains, 0.63 cm^2 and 0.57 cm^2 in area respectively.

The most effective inhibitor was then tested *in vivo* using gnotobiotic mice. Duplicate broth cultures of *C. diphtheriae* sealed in glass ampules were surface sterilized with peracetic acid and introduced into germ-free isolators containing the test animals. One ampule was used to inoculate the test animals, and the other was removed from the isolator and cultured in T. Soy Broth. The establishment of the pathogen

was determined by culturing fecal samples on T. Soy Agar plates. When the pathogen became established in the animals, the same procedure described above was used to introduce the inhibitory organisms into the animals, and after 72 hours, fresh feces were again cultured. The results indicated that the inhibitor produced an approximate 50% decrease in the numbers of *C. diphtheriae* previously present as a monocontaminant in the animal.

Subsequent investigations are now being directed toward elucidation of the mechanisms involved in the inhibitory reaction.

Bacterial phagocytosis in the dorsal lymph sac of *Rana pipiens*. MELVIN E. EVERLY and ROBERT J. HANSON, Valparaiso University.—A literature search revealed little information on the process of bacterial phagocytosis in the common laboratory frog, *Rana pipiens*. Massart observed that a fine capillary tube containing *Staphylococcus pyogenes albus* placed in the large lymph sac of frogs induced a swarm of leukocytes to enter the tube within 12 hours. Nobel showed that an intravenous injection of bacteria caused an increase in monocytes in the blood of frogs. Further study of bacterial phagocytosis by frog leukocytes seemed warranted.

Killed *Staphylococcus epidermidis* were injected into the dorsal lymph sac of urethan anesthetized *Rana pipiens*. Drops of lymph were made of the fluid on slides, stained with Wright's stain, and examined for the types of cells in the fluid and for intracellular cocci.

The number of leukocytes varied appreciably from animal to animal. On the average there were 80 percent lymphocytes, 12 percent monocytes and large lymphocytes, and 8 percent granulocytes. After injection of the bacteria, there was a 2-fold increase in the number of monocytes in the lymph fluid, and this occurred as early as one hour after the injection. Phagocytosis, as evidenced by intracellular cocci, occurred as early as 15 minutes after bacterial injection. Intermediate lymphocytes, the stage prior to cell maturation into monocytes, were the most active in the uptake of the bacteria, although the number of intracellular cocci was not large and only 20 percent of the cells participated. Some phagocytosis of cocci also occurred with monocytes and with polymorphonuclear leukocytes.

In order to have some comparison for the phagocytic activity in lymph fluid, cocci were injected into the peritoneal cavity of frogs, fluid samples taken periodically for smears, and the same staining and examining procedures followed. Less phagocytic activity by intermediate lymphocytes was observed while more polymorphonuclear leukocytes had phagocytosed cocci. Through the 4 hours of the study there was no significant increase in the numbers of cells in the peritoneal fluid.

These studies show that phagocytosis of killed staphylococci occurs in the frog, *Rana pipiens*, primarily by intermediate lymphocytes in the lymph fluid and by polymorphonuclear leukocytes in the peritoneal cavity.