BACTERIOLOGY

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ABSTRACTS

Visualization of Antibody-binding Sites on the Larva of *Trichinella spiralis* using the Ferritin-conjugated Antibody Technique. DICKSON D. DESPOMMIER, MASAHIRO KAJIMA, and BERNARD S. WOSTMANN, The Lobund Laboratory, Department of Microbiology, University of Notre Dame. —The purpose of this study was to determine the antigenic sites on the larva of *Trichinella spiralis* which stimulate the host to produce humoral antibodies during the course of infection. The ferritin-conjugated antibody technique was used to visualize the antibody binding sites on the larvae.

Three rabbits were infected with 20,000 larvae given via stomach tube. Thirty days later, each rabbit received an additional 20,000 larvae. Forty-five days after the first infection, all three infected rabbits plus one non-infected control rabbit were exsanguinated. Sera from the infected rabbits were pooled. Immune and non-immune serum samples were treated with ammonium sulfate to obtain the gamma globulin fraction. A portion of both gamma globulin solutions were then conjugated with ferritin. Living larvae were incubated in the gamma globulin solutions for 18 hours at 37°C. The larvae were then washed three times in sterile saline, fixed in osmium tetroxide, dehydrated in a graded series of ethanol solutions, and embedded in an Epon-Araldite mixture. After sectioning and staining the larvae with lead citrate, the samples were viewed under the electron microscope. The results showed that only the cuticle of the larva was labled specifically with immune ferritin-conjugated gamma globulin. Non-immune ferritin-conjugated gamma globulin did not attach specifically to any site on the larva. No ferritin particles were seen in the gut tracts of larvae incubated in either immune or non-immune ferritin-conjugated gamma globulin. It is concluded from this study that the cuticle of the larva of *T. spiralis* serves as an effective antigenic stimulus to the host during the course of infection.

A Proposed Universal Biohazards Warning Symbol. C. L. BALDWIN, Pitman-Moore Div., The Dow Chemical Company, Indianapolis.—While working for the National Cancer Institute, the Dow Biohazards R & D Team recognized a need for a signal to warn of biological hazards —"biohazards"—since these hazards are rarely obvious. In November, 1965, the Dow Team conceived, and was authorized to supervise, a project to design a standard biological hazard warning symbol. The first phase of this study showed that scientists and safety groups agreed to the need for a biohazard warning symbol. The second phase established symbol design criteria, the design of candidate symbols, and the use of a professional opinion survey group to test candidate

symbols. Design criteria included the requirements, among others, that the symbol be unique, and that it be easily remembered. Surveys in twenty-five cities allowed rational selection of a symbol embodying these characteristics. The third phase, following acceptance by the National Institutes of Health, will familiarize the public with the design and significance of the biohazards warning symbol. A Use Standard outlining rules for display of the symbol has been derived and will serve as the basis for widespread publicity among scientists and safety people; papers will be prepared for professional journals and meetings. The general public will be reached through feature articles in the lay media.

Oxygen Consumption in the Adult Male Rat. P. LEONARD KNIGHT, JR. and BERNARD S. WOSTMANN, Lobund Laboratory, Department of Microbiology, University of Notre Dame.—Oxygen consumption of adult germfree and conventionalized male rats was determined in a plastic metabolism chamber using a mixture of 22% O_2 and 78% N_2 as an air supply. The 8″ x 8″ x 8″ plastic unit contained soda lime as a CO_2 absorbent. The change of presure in the chamber, as the animal consumed O_2 and as indicated by the difference in level of Brodie's fluid in a U tube connection, was counteracted by buretting mineral oil into the system. The cubic centimeters of mineral oil corresponded to the amount of O_2 consumed by the animal.

Animals were transferred into the chamber, and ½ hour was allowed for adjustment to this new environment. Five or more determinations were made on at least 10 animals of each type. The germfree male rat under these conditions consumed on the average 15.7 liters O_2/Kg/day while its conventionalized counterpart utilized 18.1 liters O_2/Kg/day—a 13% difference between the two groups.

A Cinemicrographic Record on the Effect of an Antimitotic Substance Derived from Marine Algae on Animal Tissue-Cultured Cells. THEODORE J. STARR, University of Notre Dame, Department of Microbiology, Lobund Laboratory.—A biologically active substance was extracted from benthic marine algae collected along the coast of Puerto Rico. When added to the growth medium of tissue-cultured cells *in vitro*, specific mitotic anomalies were observed in preparations stained with acridine orange fluorochrome. The cytological abnormalities were associated with the phenomena of amitosis, multiple cytokinesis, and micronucleation. Amitosis was associated with bundles of filaments as seen by electron microscopy. Multiple cytokinesis was associated with the formation of miniature cells and cell fragments. Micronucleation occurred in the absence of cell division and the process appeared similar to that observed after treatment of tissue-cultured cell with colchicine. Events concerned with multiple cytokinesis and micronucleation were recorded by time-lapse phase microscopy.

Effect of Whole-Body Irradiation on Intestinal Disaccharidases of Germfree and Conventional Rats. B. S. REDDY and J. R. PLEASANTS, Lobund Laboratory, Dept. of Microbiology, University of Notre Dame.—Recent studies demonstrated an increase in intestinal disaccharidase activities in germfree (gf) rats and this was attributed partly to longer

life span of epithelial cells in gf animals. To test this hypothesis, 90 day old gf and conventional (conv) rats were irradiated with a whole-body dose of 1000 rads to arrest temporarily the production of intestinal epithelial cells by the crypts, and the disaccharidase activities determined in the intestinal homogenates for 10 days afterwards. The mortality rate in gf and conv rats was 0 and 55% respectively within 10 days following irradiation. After irradiation of gf rats, maltase, invertase, trehalase, lactase and cellobiase activities rapidly decreased to 3-5% of controls at 5 days and then gradually increased to about 45% of controls at 10 days. In conv rats, these activities followed the same pattern, but the minimum values were reached at 3 days and then gradually increased to about 55% of controls at 10 days. These results supported the hypothesis that the longer life span of epithelial cells in gf rats was associated with an increase in disaccharidase activities.

Besides a definite physiological and pathological effect and the reduction of digestive enzymes, there was a decrease in liver glycogen levels, presumably due to decreased food intake. These observations would indicate the possibility of presenting the food to the irradiated animals in an easily absorbable form such as low molecular water-soluble diet developed in our laboratory.

Interferon Production in Gnotobiotic and Conventional Mice. RICHARD G. CONSIDINE and THEODORE J. STARR, University of Notre Dame, Department of Microbiology, Lobund Laboratory.—Serum interferon in both gnotobiotic and conventional mice was inhibited after challenge of animals with high-titered vaccinia. A virus challenge of 10^7 PFU induced the optimum interferon titers when mouse serum was assayed six hours later. Concentrations of vaccinia greater or less than this optimal dose induced less serum interferon. However, when vaccinia virus was heat-inactivated at 60°C for 15 minutes, the serum interferon titers increased at least twofold at all concentrations of vaccinia tested. Animals of both groups responded similarly with minor differences in reaction times and peak titers. The gnotobiote proved to be more sensitive.

A sensitizing dose of vaccinia in a range from 10^6 to 10^9 PFU was injected 12 hours prior to the second inducing dose of virus. Sensitizing doses of vaccinia in the range of 10^7 to 10^9 PFU partially inhibited interferon induction by a second challenge of vaccinia and to a lesser extent Newcastle disease virus. A sensitizing dose of vaccinia less than 10^7 PFU was not inhibitory to the inducing challenge. Sensitizing doses of vaccinia injected 18 hours prior to the interferon inducing dose were much less inhibitory than the 12 hours sensitizing dose.

The interferon was characterized according to species specificity, virus specificity, ultracentrifugation, and also by heat, trypsin and acid liability.

The Possible Role of an Alpha-l-glycoprotein in Phagocytosis. J. C. PISANO and R. J. DOWNEY, Lobund Laboratory, Department of Microbiology, University of Notre Dame.—Phagocytosis of *Staphylococcus aureus* by guinea pig polymorphonuclear leucocytes was enhanced four-fold by

guinea pig serum, both conventional and germfree, and by guinea pig peritoneal exudate fluid. Ammonium sulfate fractionation of the exudate fluid yielded several components, one of which contained the phagocytosis-promoting factor (PPF). This fraction, identified by electrophoresis and immunoelectrophoresis as an alpha-1-globulin, had 25 times the phagocytosis-promoting activity of serum. The PPF (alpha-1-globulin) was not heat-labile and contained no complement activity. Electrophoresis at low pH and histochemical techniques indicated that the PPF may be a glycoprotein. Neither the gamma globulin nor the albumin isolated from the exudate fluid possessed any phagocytosis-promoting activity.