

BACTERIOLOGY

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ABSTRACTS

The Application of Immunology in Testing Anti-Allergic Agents for Clinical Use. HARRY L. MURRAY, PAUL S. PRICKETT, HOWARD R. WILLIAMS, JR. and PAUL M. LISH, with the technical assistance of Russel L. Stratman, Mead Johnson Research Center, Evansville.—Four basic immunological models, of which one is *in vitro* and three are *in vivo*, are applied to several drugs, such as aspirin, phenylbutazone, and aminopyrine to evaluate their anti-allergic action. In each model the guinea pig is the test animal. A battery of ancillary tests are also employed to differentiate true anti-anaphylaxis from some other undesired action. Ancillary tests are necessary only for those drugs showing apparent anti-anaphylaxis in the basic tests. Details of the types of action of the drugs mentioned will be described and compared with previously published information. We will attempt to define the usefulness of this group of tests for consideration of utility in inflammatory diseases with an allergy component.

Possible Influence of Salivary Antibody on Experimental Dental Caries in the Gnotobiotic Rat. MORRIS WAGNER and FRANK J. ORLAND, University of Notre Dame and University of Chicago.—Definitive studies in caries etiology have not been possible because of the ubiquitous and varied microorganisms present in the oral cavity of animals and man. Gnotobiotic techniques have made it possible to elucidate upon specific bacteria capable of producing carious lesions in experimental animals. Germfree rats brought into gnotobiotic association with pure strains of certain streptococci developed typical carious lesions. Similar findings were reported at the National Institute of Dental Research.

Recent work has shown that gnotobiotic rats harboring a single homofermentative strain of lactobacillus could also develop caries. Germ-free rats inoculated orally at weaning developed only an average score of 2.0 in gross caries intensity. Their progeny (2nd generation) showed an average score of 8.6 and the 3rd generation had an average score of 14.5. Coincident with these findings was the observation that while the first generation had serum agglutinins against the homologous organism, 2nd and 3rd generation animals did not. Furthermore, salivary antibody titers in the first generation animals were highest in the animals showing the lowest caries scores. It appears that presence of salivary antibody may have affected cariogenesis.

Infection of Cortisone-Treated, Germfree Mice with Mouse Hepatitis Virus. PHILIP P. VELLA and THEODORE J. STARR, University of Notre Dame.—The Gledhill strain of mouse hepatitis virus (MHV-1), free from

the enhancing agent *Eperythrozoon coccoides*, produces a fatal infection in newborn Swiss mice and is usually innocuous in weanlings and adults. The disease in newborns appears initially as focal lesions in the liver and gradually becomes diffuse. The infection in weanling and adult mice is enhanced in the presence of cortisone.

Swiss-Webster germfree mice and their genetically related conventional counterparts were inoculated with MHV-1 and cortisone. The inoculum usually administered to adult mice (0.1 ml, intraperitoneally) titered $10^{-3.0}$ newborn ID₅₀s. Cortisone acetate administered (IP) in single doses varied from 1.25-5.0 mg. Mice in the germfree, cortisone-virus group showed significantly fewer deaths. No deaths were observed in the cortisone and virus control groups. Surviving mice were sacrificed at 1, 2 and 3 weeks and autopsied for macroscopic liver damage. If mice survived the virus challenge in the presence of cortisone for 7 to 9 days recovery usually was inevitable.

Chemical Induction of Germfree Tumors. MORRIS POLLARD, University of Notre Dame.—Tumors have been induced in germfree mice and rats by chemical carcinogenic agents. Subcutaneous inoculation of sterile methylcholanthrene (MC) into newborn mice of Swiss-Webster strain induced lung tumors; in older mice transplantable sarcomas appeared in the inoculated area. Inoculation of MC into rats resulted in sarcomas at the inoculation area. One feeding of sterile 7, 12 dimethylbenzanthracene (20 mg) in sesame oil to 50 day old Sprague-Dawley rats resulted in cancer of the breast in all of them. Examinations of animals and tumors for viral flora by conventional tissue culture and electron microscopy were negative. The tumors resembled in gross and histological character the tumors observed in conventional animals. The results suggest that while viruses are involved in specific oncogenic processes, they were not of concern in those described above. The effects of oncogenic viruses and physical factors on germfree animals are being studied.

Colchicine-induced, Micronucleated, Tissue-cultured Cells. THEODORE J. STARR, University of Notre Dame.—The effect of colchicine on mitosis of a tissue-cultured cell line was followed by fluorescence microscopy of acridine orange-stained preparations. The formation of micronucleated cells is described at the chromosomal level. DNA synthesis was followed by autoradiography with tritiated thymidine. Despite the gross structural changes involved, micronucleated cells maintained its functional capacity to incorporate thymidine and to support the predictable maturation process of the agent of psittacosis.

Inhibition of Macromolecular Syntheses in *Escherichia coli* by Phenethyl Alcohol. RONALD W. TREICK and W. A. KONETZKA, Indiana University.—When *Escherichia coli* is inhibited by phenethyl alcohol an unbalanced synthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein is observed. This report deals with the relationship between the concentration of phenethyl alcohol and the physiological state of the cells on the inhibition of macromolecular synthesis in *E. coli*. Concentrations of inhibitor ranging from 0.25% to 0.35% (v/v) do not allow cell division during a period of at least 4 hours, but the

cells remain viable. Increasing concentrations of phenethyl alcohol added to exponentially growing cells causes a progressively greater inhibition of the rate of RNA and protein synthesis. DNA synthesis is completely inhibited after an increase which is never greater than double the amount of DNA initially present. Moreover, the physiological state of the cells at the time of addition of the inhibitor markedly influences the patterns of syntheses of DNA, RNA, and proteins. Upon removal of phenethyl alcohol the syntheses of the three polymers is resumed and is followed by cell division. The results indicate that phenethyl alcohol can be employed to inhibit specifically DNA synthesis during the synthesis of RNA and proteins.

Multiple Nitrate Reductases in *Bacillus subtilis*, R. J. DOWNEY and H. POLLARD, University of Notre Dame.—A particulate nitrate reducing system has been studied in *Bacillus subtilis* grown in a peptone-nitrate medium. This enzyme system catalyzed the reduction of nitrate to nitrite by NADH under aerobic and anaerobic conditions. Oxidation of NAD-linked substrates by the soluble enzyme proceeded at a rate which greatly exceeded that of the particulate system. The latter was observed to require a vitamin K-like naphthoquinone for activity whereas only menadione (K_3) was capable of restoring the former. Nitrate reduction via the particulate system was greatly stimulated by catalytic amounts of FAD.

Spectral analyses of a chromatographically purified soluble system disclosed no detectable hemeproteins. Carbon monoxide, antimycin A and gramicidin inhibited only the particulate system while azide, cyanide and dicumarol strongly inhibited both. Although nitrite in high concentrations only partially inhibited the soluble reductase, it completely inhibited the bound enzyme. The study suggested that nitrate reduction in *Bacillus subtilis* is accomplished by two related but essentially dissimilar mechanisms. The soluble complex transports electrons through a quinone reductase while the particulate complex appears to utilize the conventional flavin-heme sequence.