MICROBIOLOGY AND MOLECULAR BIOLOGY

Chairman: MORRIS POLLARD, Department of Microbiology, University of Notre Dame, Notre Dame, Indiana 46556

MORRIS POLLARD, University of Notre Dame, was re-elected Chairman for 1973

ABSTRACTS

Some Aspects of Humoral Immunity in Germfree and Conventional SJL/J Mice in Relationship to Age. KATHERINE SEIBERT and MORRIS POLLARD, Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana 46556.——Evidence is accumulating to suggest that there is a close relationship between aging, immunity, and cancerigenesis. Immunological impairment is associated with many neoplasms in man affecting the lymphoreticular system, including Hodgkins' disease, lymphosarcoma, multiple myeloma, and reticulum cell sarcoma. Histologic similarities between the tumors that arise in the SJL/J mouse strain and Hodgkin's disease in man, have raised the question whether this murine tumor is also associated with immune impairment.

In this study, the humoral immune competence of 272 germfree and conventional SJL/J mice was evaluated with increasing age. The spleen cell population of these animals was assayed at 2-month intervals until the age of 14 months for the presence of specific antibody forming cells on day 4, 5, and 6 after intraperitoneal immunization with sheep red cells. Using the direct and indirect hemolytic plaque assay, the number of antibody forming cells producing IgM and γ_1 against sheep red cells was calculated per 10^6 nucleated spleen cells. All animals were autopsied, the tissues were fixed for histological observation, and total body weight, spleen weight, and leukocyte count were recorded.

The results show a similar response to sheep red cells between germfree and conventional animals at all age levels, the peak response for both IgM and γ_1 production occurring at 4 months, with a progressive marked depression with age. The γ_1 response was more severely impaired than the IgM response, however, the latter response showed an age related shift of peak in the kinetics curve. A direct correlation was found between the severely depressed immune response found in most older animals and an abnormally high spleen weight, a reduced leukocyte count, and severe histological lesions characteristic of an advanced stage of SJL/J disease.

These results, indicating a severe depression of humoral immunity with age and progress of the disease in the SJL/J mouse, may be related by either cause or effect, and as is true with human lymphoreticular dyscrasias, many questions regarding the relationship between immunity and disease still remain to be answered.

Oxygen Demand of a Fermenter Medium and its Determination. ROBERT H. L. HOWE, Eli Lilly & Company, Tippecanoe Laboratories,

Lafayette, Indiana 47902.——The oxygen demand of a fermenter medium was explained, the needed mathematical relation derived, and the method of determination illustrated.

The Role of Lysine in Antibiotic Biosynthesis in *Streptomyces lipmanii*. J. R. KIRKPATRICK, L. E. DOLIN and O. W. GODFREY, Eli Lilly & Co., Indianapolis, Indiana 46206.——*Streptomyces lipmanii* produces two β-lactam antibiotics, penicillin N and 7-(5-amino-5-carboxyvaleramido)-u-methoxycephalosporanic acid. Both antibiotics contain α-aminoadic acid side chains. In similar antibiotics produced by certain fungi, the α-aminoadipoyl moiety is derived from an intermediate in lysine biosynthesis. It has been established, however, that in *Sreptomyces lipmanii*, lysine is synthesized via α, ϵ-dieaminopimelic acid—an entirely different biosynthetic route. This finding suggests not only a unique mechanism for the derivation of α-aminoadipate, but also that the system may be particularly amenable to genetic manipulation.

The Elongation of Palmitic Acid in *Penicillium chrysogenum*. JILL K. ASHLEY and ALICE S. BENNETT, Ball State University, Muncie, Indiana 47306.——Results of research on the biosynthesis of long chain fatty acids suggest that palmitic acid is elongated to stearic acid by the acetyl-CoA pathway as well as by the malonyl-CoA pathway.

Cultures of *Penicillium chrysogenum* were incubated with varying amounts of avidin and 1-^{14}C acetate. Avidin, which inhibits the formation of malonyl-CoA from acetyl-CoA, partially inhibited fatty acid synthesis, however, the percentage of radioactivity recovered as C_{18} fatty acids remained relatively constant in cultures which contained avidin and those that did not. Thus, it appears that although avidin decreased the amount of acetate incorporated into fatty acids, it did not inhibit the elongation of palmitic acid. The constancy of the specific activities of the C_{18} fatty acids also lends further evidence that the acetyl-CoA pathway is an important mode of elongation of palmitic acid in *Penicillium chrysogenum*.

Standardization of Amino-peptidase Profiles for the Identification of Plant Pathogenic Bacteria. K. KRAWCZYK and D. M. HUBER, Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana 47907.——Factors influencing the amino-peptidase activity of three plant pathogenic and one saprophytic bacteria were studied to determine and thereby minimize sources of variation when identifying bacteria. Amino-peptidase profiles were determined fluoremetrically using beta-naphthylamides (10^{-4}M in pH 8.0 Tris buffer) as substrates. *Erwinia amylovora, Xanthomonas campestris,* and *Pseudomonas tabaci* (plant pathogens) and a saprophytic Pseudomonad were used throughout this study. The effects of temperature, incubation time, growth media, inoculum density, salt solution (cofactors), halides, and buffer were evaluated. Peptidase profiles of the four bacteria studied were very different and provided a rapid, specific means of identification. Prior growth media, inoculum density, and incubation time had the

greatest influence on peptidase hydrolysis of the beta-naphthylamides. Temperature, additional cofactor elements, halides, and buffer appeared to have little, if any, general effect on peptidase activity in this study. There appeared to be sufficient latitude in all these conditions for this technique to be easily adapted for routine microbial identification.

Ecology of Thermophilic Fungi in Natural Habitats, with Emphasis on Pathogenic Species. MICHAEL R. TANSEY, Department of Microbiology, Indiana University, Bloomington, Indiana 47401.——The occurrence, growth, and interrelationships of thermophilic fungi in hot springs, geothermal soils, alligator nests, and sun-heated soils has been studied. These naturally heated habitats contain a rich flora of heat-requiring fungi, including several species which are pathogens of warm-blooded animals.

NOTE

Aspects of the Control of Virus Diseases. KLAUS SCHELL, Department of Infectious Diseases, The Dow Chemical Company, Zionsville, Indiana 46077.——During the first 5 years of vaccination against poliomyelitis and measles in the United States more than 50,000 human beings were saved from severe disability and death, and medical expenditures were reduced by $600,000,000. Incidence and mortality of the 20 most important viral and bacterial diseases were analyzed. A reduction in disease incidence was found only where immunoprophylaxis had been available for some time while antibiotic and chemotherapy apparently had little affect on incidence of bacterial diseases.

Aspects of safety and potency were compared for killed and attenuated live virus vaccines. Killed virus vaccines are considered less desirable because of the requirement of virus concentration to obtain sufficiently large antigenic masses to stimulate lasting protective antibody, the need to purify such vaccines to reduce the amount of likewise concentrated viral and cellular byproducts often responsible for undesirable allergenic or pyrogenic side reactions, the large amount of genetic information contained in these vaccines, the difficulty of discovering survivors after "inactivation" of hundreds of millions of infective virus doses, the fact that apparently inactivated virus can retain oncogenic potential, and, the contention that "oncogenes" may be present in the genetic matter of all somatic cells and that cellular genetic information can be transferred by viruses.

Aside from the production of highly concentrated, highly purified, nucleic acid-free antigen vaccines which has not been perfected at this time, attenuated, live virus vaccines are regarded as most desirable. Virus concentration is not necessary, the amount of genetic matter given with the virus is relatively small (10^{3-5} $TCID_{50}$/ dose) and replication remains minimal if compared with that of the wild virus. The type of antibody stimulated is of relatively broad spectrum and long duration, and anamnestic responses are accelerated even after the antibody itself has disappeared.

Other vaccine virus related questions were discussed: The source and development of avirulent mutants, their potential for reversion, development of undesirable tropisms, and the question of under-attenuation. The need for the factorial testing of multiple component vaccines was elucidated on the example of a Dow produced rubella-mumps-measles virus vaccine, where combinations of minimal-maximal component ratios resulted in seroconversion rates of about 90% or more for each of the components regardless of vaccine dose.

The importance of the host response was stressed and the complexity of the body's immune mechanism was discussed in terms of its dependence on a variety of intrinsic and environmental influences, and its expression in beneficial as well as harmful results in response to vaccination.