# BACTERIOLOGY

Chairman: HAYWARD CAMPBELL, JR., Eli Lilly and Co.

D. S. WEGENER, I. U. Medical School, was elected chairman for 1969

There was actually no meeting of the Bacteriology Section this year. The paper by Petersen et al. was presented in the Cell Biology Section, but is printed here. Normally the Bacteriology Section of the Academy meets with the Indiana Branch of the American Society for Microbiology, because of the great overlapping of membership. This year the Indiana Branch met with the Ohio and Kentucky-Tennessee branches of the ASM. The abstracts of papers presented by the Indiana members at that meeting are included here.—Ed.

## ABSTRACTS

**Development of a Modified Antibody Plaque Technique for the Detection of Single Cells Making Anti-viral Antibody.** B. H. PETERSEN, Z. BRAHMI, J. S. INGRAHAM, and A. S. LEVINE, Indiana University Medical School.—Hemolytic plaque techniques detect the antibody produced by individual antibody-forming cells against various species of red blood cells (rbc), or even against haptens or proteins coupled chemically to rbc. As certain viruses adsorb spontaneously to rbc it was suggested that cells making anti-viral antibody might also be detected by a hemolytic plaque technique. Rabbits were given I. V. injections of Influenza virus strain WS-A. Suspensions of spleen cells obtained 3 days after the final injection were examined for hemolytic plaque formation against Chicken rbc and Pigeon rbc coated with virus. These virus-coated rbc spontaneously agglutinated in our system. By use of our plaque assay in liquid medium (Fed. Proc. 26, 641, 1967) it was possible to observe both hemagglutination inhibition and hemolytic plaque formation. Anti-WS-A antibody was demonstrated in the serums by hemagglutination inhibition. Specific plaques were also obtained from a rabbit immunized with Influenza strain Lee-B. These results demonstrate the feasibility of detecting anti-viral antibody produced by single cells utilizing virus-coated rbc.

**Amber Streptomycin-resistant Mutants of** *Escherichia coli.* KAREN CARLSON and RICHARD BOCHRATH, Indiana University Medical Center.—A specific genotypic defect such as the nonsense *amber* codon can cause premature termination of protein synthesis, at the site of a UAG codon, during translation. This defect may be mitigated by suppressor mutations which lead to the synthesis of specific altered transfer-RNA's. Streptomycin-resistant ($Sm^r$) mutants of *E. coli* WWU were isolated and characterized. Some $Sm^r$ mutants appeared to be *amber* streptomycin-resistant mutants: they were sensitive to streptomycin whenever certain suppressor mutations were introduced. The existence of these mutants suggests that streptomycin resistance can be conferred by an incomplete protein. Experiments are in progress to determine whether this partial polypeptide is a ribosomal protein.

**Immune Response to *Streptococcus faecalis* in the Rat.** B. PERI and M. WAGNER, University of Notre Dame.—Rats immunized by various methods with a formalinized suspension of *Streptococcus faecalis* (S.f.) strain ND547 were compared for agglutination titers in serum and saliva. Low levels of agglutinating antibody to streptococci are commonly found in serum and saliva of rats under the usual laboratory conditions since these organisms are part of the normal microflora. Response to parenteral immunization was studied in rats of three categories: germfree, conventional and monoassociated with *S. faecalis.*

High serum titers were found in all (S.f.) monoassociated, but nonimmunized, rats, indicating a strong immune response to gastrointestinal microflora alone. Immunized, monoassociated animals usually showed higher antibody levels than the nonimmunized monoassociates in both serum and saliva, but in some cases, high serum antibody level was not accompanied by high salivary antibody. Young rats show more antibody in saliva than in serum. It would seem that salivary antibody is not directly related to serum level.

Germfree rats gave no immune response to immunization procedures which produced responses in both the conventional and monoassociated animals. In this case, the germfree animal, which represents removal of the antigenic competition of viable microorganisms, did not show the enhanced response anticipated. This phenomenon is being investigated further.

**Enzymatic Effect of Cobra Venom on Rauscher Leukemia Virus (RLV).** A. C. RAITANO and A. S. LEVINE, Indiana University School of Medicine. —The inactivation of RLV by *Naja naja atra* and *Naja naja* venoms was studied and an attempt made to identify the active virucidal factors in venom. *Naja naja atra* venom (150, 500, and 1000 $\mu$g/ml sodium citrate) was incubated with RLV at 37°C for ½ hour. The latter concentration significantly decreased RLV infectivity. The RLV lipoprotein envelope contains 68% lecithin. Cobra venom contains a potent phospholipase A (PLA) that hydrolyzes lecithin to lysolecithin and a fatty acid. Therefore, several characteristics of PLA were used to identify its activity against RLV. PLA is heat stable at 100°C for 10 minutes (pH 5-6). *Naja naja* venom (1.5 mg/ml PBS) boiled for 10 minutes significantly inactivated RLV. *Naja naja* venom containing inactivated (boiling for 20 minutes) PLA did not inactivate RLV. PLA is also antigenic; therefore, *Naja naja* venom neutralization with specific antisera was studied. *Naja naja* venom (1.0 mg/ml PBS) inactivated RLV but neutralized venom (1.0 mg/ml PBS) did not. Thin layer chromatograms of venom on plasma phospholipids showed inhibition of PLA activity (lecithin hydrolysis) by antisera. Lysolecithin (100 $\mu$g/ml $H_2O$) itself was also found to inactivate RLV while lecithin (100 $\mu$g/ml $H_2O$) did not. The levels of other venom enzymes were also examined. The results suggested PLA is an important factor in RLV inactivation by cobra venom.

**Suppression of Rauscher virus-induced Murine Leukemia by L-Asparaginase.** W. F. CAMPBELL and A. S. LEVINE, Indiana University Medical

Center.—L-asparaginase from extracts of *E. coli* has been demonstrated to cause regression of several transplantable murine leukemias and lymphosarcomas. The leukemia cells, which apparently cannot synthesize L-asparagine, depend upon an exogenous supply of the amino acid. Normal cells have high asparagine synthetase activities, and do not require asparagine. Apparently, asparaginase treatment depletes the plasma asparagine level, and deprives the leukemic cells of their exogenous supply of the amino acid.

Investigations in our laboratory demonstrated that asparaginase treatment significantly suppressed splenomegaly in mice infected with Rauscher leukemia virus (RLV). The enzyme significantly increased survival time, but did not suppress viremia of RLV-infected mice. In small, multiple doses asparaginase altered the histological picture as seen in spleen sections, but did not completely inhibit the leukemic process. In apparent contradiction to the asparagine deprivation hypothesis, was our observation that asparagine treatment suppressed splenomegaly and prolonged survival. The results warrant a reappraisal of the asparaginase mechanism of antileukemic activity in RLV-infected mice.

**Physiological Studies of the Incorporatsion of 5-Bromouracil During Growth and Sporulation in *Bacillus subtilis* -168.** PATRICIA C. MORGAN and ROBERT F. RAMALEY, Indiana University.—Prior to an investigation of DNA synthesis and segregation during sporulation, studies were conducted on the physiological consequences of 5-bromouracil (5-BU) and 5-bromodeoxyuridine (5-BUdR) incorporation in a thymine requiring strain of *Bacillus subtilis*-168. The results are summarized as follows: (1) The deoxynucleoside forms of thymine and bromouracil were incorporated more rapidly and to a greater extent than were the free bases. (2) 5-bromouracil incorporation was stimulated by the addition of a small amount of thymine. This stimulation was also true for the deoxynucleosides of thymine and bromouracil. (3) Both thymidine and 5-bromodeoxyuridine were incorporated when they were both supplied to growing cells at a final ratio of BUdR/TdR + BUdR = 0.9. (4) Incorporation of 5-BU or 5-BUdR for periods less than that inducing thymineless death (30-60 minutes) resulted in fully viable cells. (5) Under these conditions the 5-BU incorporated was not selectively removed from the cells upon resuspension in medium containing thymine. This suggested that the 5-BU incorporated was not in false growing points or "unusual" DNA. (6) 5-BUdR incorporated during late vegetative or very early sporulation was included in the developing spores. Purified BU-labeled-spores showed the same viability as untreated spores. Thus, conditions have been obtained that allow the synthesis of apparently biologically active 5-BU DNA which is incorporated into the developing spores and does not decrease the viability of the completed final spores.

**Biosynthesis of Thiadiketopiperazine Antibiotics.** D. R. BRANNON, M. GORMAN, B. B. MOLLOY, W. M. STARK, and J. MABE, Eli Lilly and Company, Indianapolis.—We have investigated the biosynthesis of the aranotins, a new group of thiadiketopiperazines from *Arachniotus aureus*. The aranotins are structurally related to the microorganism metabolites

gliotoxin and sporidesmin. Carbon-14 labeled amino acids were incubated with *A. aureus* to determine the extent of their incorporation into BDA-aranotin and to determine the biosynthetic relationship between the different aranotins and gliotixin. Experiments were conducted to determine the source of sulfur in the thiadiketopiperazines and the relationship between disulfide and thio methyl analogs.

**Mycophenolic Acid: Studies on Biological Activities.** ROBERT H. WILLIAMS, JOHN C. CLINE, RICHARD E. HOLMES, and MARTIN J. SWEENEY, Eli Lilly and Company, Indianapolis. The fermentation broth of a strain of *Penicillium stoloniferum* was found to possess reproducible *in vitro* antiviral activity. The active component was isolated by chloroform extraction of filtered broth at pH 3.0 followed by column chromatography on silica gel and crystallization. Comparison of the physical properties of the crystalline substance with those of mycophenolic acid led to its identification. Mycophenolic acid is one of the oldest known biologically active mold metabolites, first isolated by Gosio in 1896, and since then reported to have weak antimicrobial activity. The present study includes some aspects concerning interesting new biological activities of this compound recently discovered in our laboratories, including antiviral and antitumor activity. Information concerning mechanism of action and the effects on modification of the basic structure to biological activity will also be presented.

**Genetic Evidence for Resistance of *Cephalosporium* to Specific Compounds.** PAUL A. LEMKE, Eli Lilly & Co., Indianapolis.—One hundred compounds, antibiotics, antimetabolites and organic toxicants, have been surveyed for their toxicity to the antibiotic-producing fungus, *Cephalosporium acremonium*. This survey was designed to obtain compounds suitable as selective agents for resistant mutations and of potential use in the detection of somatic recombination through homozygosity of recessive mutations for resistance.

Toxicity levels for several compounds were determined by a gradient-plate method, and mutations for resistance to certain compounds, principally antimetabolites, induced. Selections for resistance were obtained from among survivors of 90% mortality after treatment with a chemical mutagen, nitrosoguanidine. Resistant cultures were selected on gradient plates, and mutation frequencies for resistances, spontaneous as well as induced, were calculated. Frequencies for induced mutations for resistance to specific compounds varied but were demonstrated to be of the order of $10^{-4}$ to $10^{-6}$.

Among selections for resistance to antimetabolites no evidence for cross-resistance has been obtained, and markers for resistance to antimetabolites have proved to be recessive by heterokaryotic tests. Data on selections for resistance to other compounds—specifically, actidione, acriflavin, endomycin, and hydroxylamine—were less consistent. Cultures selected as resistant to these compounds grew poorly even in the absence of toxicant.

In addition to the *Cephalosporium* organism seven other fungi *(Penicillium chrysogenum, Emericellopsis glabra, Aspergillus nidulans, Saccharomyces cervisiae, Sistotrema brinkmanni, Schizophyllum commune, Coprinus lagopus)* and an actinomycete *(Actinoplanes utahensis)* were compared for sensitivity to each of the hundred compounds. The prokaryotic nature of the actinomycete is implied by its reaction to specific compounds. The fungi examined were heterogeneous in response to the assembled compounds.

**Effect of Oxygen on the Synthesis of Nitrate Reductase in *Bacillus stearothermophilus*.** JAMES H. NUNER and RONALD J. DOWNEY, Lobund Laboratory, University of Notre Dame.—We are investigating the biosynthesis of the membrane-bound electron-transfer enzyme, nitrate reductase (NaR). The oxygen-repressible synthesis of this enzyme in resting cells has been observed to begin shortly after the addition of nitrate. Cell division in cultures not originating in nitrate medium can be delayed for 10-12 hours.

We have observed dramatic differences in the effect of imposing derepressing oxygen tension ($pO_2$) on cells exposed to nitrate, with and without previous histories of exposure to nitrate. Cells without previous contact with nitrate die much more rapidly than those exposed to the same $pO_2$ in the absence of nitrate. Cells with previous contact with nitrate lose viability during the first four hours and then recover. Turbidity of such cultures does not increase until recovery is complete. Other evidence also suggests that two populations of cells are present in the culture: one nitrate-adapted, capable of using nitrate as a terminal oxidant, and the wild type, capable of using only oxygen.

Oxygen is shown to permit growth of a nitrate-adapted culture and to promote the degradation of nitrate reductase to a basal level.

The effect of different oxygen tensions on the synthesis of nitrate reductase in an adapted population was studied. A repressing tension of about 20 mm Hg was found.

**The Serum Profiles of Certain Reptile Sera and preliminary Observations on Antibody Formation in Snakes.** SYLVIA H. KENDALL and S. A. MINTON, Indiana University Medical Center.—Serum samples from several families of snakes, rock iguana (*Ctenosaura*) and rabbit were fractionated by zone electrophoresis (Pevikon) and gel filtration (Sephadex G-200). Zone electrophoresis of snake sera gave protein profiles differing from the mammalian pattern and showed variations among the different snake families. No cathodic protein was detected in snake sera and the largest peak was not always the fast anode-migrating one, characteristic of the mammalian pattern. In addition, the electrophoretic profile of iguana serum differed from rabbit serum and all snake sera. No antigenic relationship between iguana and snake sera could be shown by capillary precipitin tests. Gel filtration of rabbit serum gave a profile of three progressively larger peaks. Two out of three snake sera differed in that the first peak was largest and the third intermediate. *Python* serum gave two peaks of comparable size with a third peak suggested by

a shoulder on the last peak. Peaks from fractionated snake sera, concentrated and used in gel diffusion and immunoelectrophoresis, were found to contain multiple components. Immunoelectrophoresis of whole snake sera resolved 7 to 11 components; 3 to 5 detected on the cathode side of the origin. Immunization of fox snakes (*Elaphe vulpina*) with bovine serum albumin (Pentex Fraction V) in Freund's adjuvant produced no antibody response one month after primary injection. Two weeks after secondary injection, snakes showed evidence of antibody response as measured by agglutination of erythrocytes coupled to BSA.