

MICROBIOLOGY & MOLECULAR BIOLOGY

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Abstracts

On the Serology of *Bacteroides* species. WILLIAM W. BALDWIN, ALEXANDER A. STEMER and MARSHALL E. LANDAY, Indiana University School of Medicine, Northwest Center for Medical Education, Gary, Indiana 46408 and St. Margaret Hospital, Hammond, Indiana 46320.——Antiserum prepared in New Zealand white rabbits by giving six intravenous injections of formalin fixed whole cells of *Bacteroides fragilis* showed numerous bands when reacted with *B. fragilis* antigen in Ouchterlony plates. This antigen was prepared by breaking *B. fragilis* with glass beads and using the soluble supernatant after centrifugation. The above antiserum also reacted with *Bacteroides thetaiotaomicron* antigen, prepared as above, and showed one band of apparent identity with *B. fragilis*. The band of apparent identity for both organisms was lost if the antigens were heated at 80°C for 30 minutes before reacting with antibody. Antiserum prepared as above against *B. thetaiotaomicron* did not react with *B. fragilis* antigen. A precipitin curve was performed using anti- *B. fragilis* antiserum and *B. thetaiotaomicron* antigen and optimal precipitate was obtained using an antibody to antigen ratio of 3:1. This ratio was used to precipitate a quantity of *B. thetaiotaomicron* heat labile antigen. This precipitate was dissolved in 1.5M acetic acid and was separated by gel filtration on an EM gel 1000 A column. Two peaks, measured at 280 nm, were seen on elution of the column with 1.5M acetic acid. One peak found at the void volume gave a reaction, after dialysis and concentration, in Ouchterlony plates like the heat labile antigen above. The other peak did not react with *B. fragilis* antiserum.

Investigation of Bacterial Mouth Flora of Ball State University Students. M. LANGONA, D. HENDRICKSON, J. HENDRIX, D. GOBBLE, Ball State University, Muncie, Indiana 47306.——The occurrence of pathogenic and non-pathogenic bacteria isolated from the throats of thirty Ball State University students was investigated in a surveillance study utilizing weekly throat swabbings. The study was conducted during the Winter Quarter of the 1977-78 academic year. The reference population was divided into three groups; the control; the semi-control; and the experimental group. The experimental group was exposed to numerous bacterial pathogens and non-pathogens bi-weekly during one academic quarter. The objective of the study was to determine if the throat flora of the individuals within the experimental group underwent changes in relation to the bacterial organisms to which the individual was exposed. The results of

the study indicated that bacterial changes did not occur within the throat region following bacterial exposure.

Implications of Undesired Effects of Antibiotic Use in Rodents. D. MADSEN, B. WOSTMANN, and M. BEAVER, Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana 46556.——In murine rodents, such as the laboratory rat, the major fecal, secondary bile acids (BAs) are hyodeoxy-cholic (HDC) and ω -muricholic (ω -MC), both being derived from the BA, β -muricholic acid. The ratio of HDC/ ω -MC in feces is a sensitive indicator of perturbations of the intestinal microecology. For example, treatment of rats with aureomycin, penicillin, neomycin or polymyxin for only 5 days results in a lowering of the HDC/ ω -MC ratio from the control value of around 1.9 to values below 0.90. This effect is termed the "HDC/ ω -MC shift", and results in ω -MC replacing HDC as the major fecal BA. Two undesired "side effects" of antibiotic treatment have been observed. The first is a persistence of the lowered HDC/ ω -MC ratio for at least 4 months after cessation of treatment. The second side effect, seen to date only with polymyxin, is a gradual spreading of the HDC/ ω -MC shift to untreated control rats housed nearby; this is seen only after 1-2 months beyond treatment of the experimental animals. Implications of the results are that antibiotically altered microflora can persist long after their induction, and undesired floras may be produced by such treatment. Spreading of the phenomenon suggests that the altered flora can spread to control animals and express their new potential, thus changing the microecology of untreated animals.

Isolation of a Calcium Binding Protein from Cat Brain and Spinal Cord and its Comparison with the Fast Transported Calcium Binding Protein of Cat Sciatic Nerve. ZAFAR IQBAL, Department of Physiology and Medical Biophysics Program, Indiana University School of Medicine, Indianapolis, Indiana 46202.——Fast axoplasmic transport of calcium in cat sciatic nerve has been shown to be associated with a calcium binding protein (CaBP) of 15,000 daltons (Iqbal & Ochs, *J. Neurochem.* **31**:409-418, 1978). Using a column gel chromatographic techniques, brain and spinal cord of cat have been found to contain a protein of molecular weight similar to that of nerve CaBP. The tissue (brain or spinal cord) was homogenized in a 10 mM phosphate buffer of pH 7.5, the homogenate centrifuged at 105,000 g for 1 hour and the supernatant so obtained was concentrated by freeze drying. The proteins in the supernatant fractions were fractionized on a column (2.4 x 40 cm) packed with Sephadex G-100 using an automatic fraction collector. The column was equilibrated and eluted with a 10 mM phosphate buffer of pH 7.5 and the first peak, Peak I, was further analyzed on BioGel A5m (1.6 x 60 cm) using the same system as with Sephadex G-100 column. The fractions corresponding to the third peak, I_c, were pooled, reduced in volume by lyophilization and dialyzed against 0.1% glycine. The properties of the proteins isolated from brain and spinal cord were compared with that of nerve CaBP by electrophoresis on 10% acrylamide gels with or without 0.1% SDS. The proteins of brain and spinal cord were found to comigrate with the nerve CaBP suggesting that all three proteins are similar in nature as regards to their molecular weight and electrophoretic mobilities. Supported by the NIH grant PHS RO1 NS 8706-09.

An Unusual Organism Suggestive of *Beijerinckia* spp. in Stream Leaf-Litter.
GARY W. PETTIBONE, Department of Biology, Bowling Green State University, Bowling Green, Ohio 43403 and DONALD A. HENDRICKSON, Department of Biology, Ball State University, Muncie, Indiana 47306.——An organism suggestive of the genus *Beijerinckia* was found in association with stream leaf-litter in Bell Creek (Delaware County, Indiana). Leaf material was recovered from the stream and homogenized aseptically in the laboratory. Serial dilutions were performed and triplicate spread plates were placed to extinction on Tryptone Glucose Yeast Extract Agar. Incubation was at ambient temperatures (20-24° C.) for up to twenty-one days. Typically convoluted colonies possessing a hard tenacious slime were observed on all plates exhibiting bacterial growth. These colonies comprised between twenty and thirty per cent of the heterotrophic bacterial population recovered by the spread plate technique. The cells were purified and tentatively identified as members of the genus *Beijerinckia*. This organism is a non-symbiotic nitrogen fixer and is usually considered to be restricted to acidic tropical soils although it has been isolated from temperate regions in a few cases. The presence of this organism in stream leaf-litter could be important in supplying fixed nitrogen for cellulose decomposing microorganisms.