MICROBIOLOGY AND MOLECULAR BIOLOGY

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ABSTRACTS

Searching for Intestinal Flora Involved in Secondary Bile Acid Production in the Rat. DAVID C. MADSEN, BERNARD S. WOSTMANN, and MARGARET BEAVER, Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana 46556.——The secondary bile acids hyodeoxycholate (HDC) and ω-muricholate (ω-MC) are present in conventional (CV) but not germfree (GF) rats and mice. HDC has been shown to be a precursor of ω-MC, formed by hepatic action. As these two bile acids constitute a major fraction of excreted bile acids, we have been searching for the bacterial species involved in HDC formation. We have examined defined combinations of common intestinal flora consisting of from 1-8 species: none of these have resulted in appearance of HDC or ω-MC. We were thus potentially facing a search involving innumerable permutations of combinations of intestinal species. If the responsible species were not a known or a common one, the task would be even more formidable. Bile acids of the gerbil are dissimilar to those of the rat. We used flora from the large intestines of gerbils to conventionalize GF rats. The subsequent appearance of HDC and ω-MC in feces would indicate that the species or capability involved was ubiquitous, and that the murine gut was favorable to HDC production. In fact, we have detected only ω-MC in feces from "gerbilized" rats. This is surprising, in view of the role of HDC in formation of ω-MC. Several possible explanations are discussed.

Selection of Mutants of Bacteriophage T4D Defective in Tail Fiber Morphogenesis. HAROLD L. EDDLEMAN, Indiana Biolab, Palmyra, Indiana 47164.——*In vitro* complementation was used to select mutants of phage T4D having tail fiber defects. Mutagenized wild type phage were grown at low multiplicity of infection (moi = .01) on *Escherichia coli* B. Cells infected by the desired mutants produced fiberless particles. Wild type phages were removed by low-speed centrifugation following their adsorption on bacteria.

Particles remaining in suspension were purified and concentrated by differential centrifugation. Fibers were attached to them by incubating the particles in a mixture containing fibers and any enzymes needed for their attachment. The product was plated on *E. coli* CR63 which permits growth of amber nonsense mutants.

Amber mutants were found in 7% of the plaques tested. Of 243 amber mutants isolated, 214 could be assigned to previously known genes. The remaining 29 strains appear to be defective in fiber synthesis but differ from previously described mutants and may represent mutations in one or more new genes.

Microbial Interactions in Soil Cropped to Beans. D. M. HUBER and A. L. ANDERSEN, Purdue University, West Lafayette, Indiana 47907, Michigan State University, East Lansing, Michigan 48823.——The most common association of microorganisms in the soil was one of compatibility, although mycoparasitism, necrosis, and lysis were observed. An apparent symbiotic association of *Fusarium* sp. with a bacterium was also observed. The intimate and compatible association of bacteria with most fungi may be an indication of a mycosphere phenomenon. Antibiosis of *Fusarium* was not demonstrated since most of the reported antagonists were either compatible with the *Fusarium* species encountered or not actively growing in the soil. *Streptomyces* sp. were the least compatible of all isolated organisms. These associations may influence the active growth, pathogenicity, germination, or survival of a specific organism in the soil.

The Elongation of Palmitic Acid by Cell-Free Extracts of Penicillium Chrysogenum. JILL ASHLEY and ALICE BENNETT. Results of previous research on whole-cell cultures of *Penicillium chrysogenum* have suggested that acetyl CoA, without being converted to malonyl CoA, supplies the two carbon units for the elongation of palmitic acid. The purpose of this study was to determine the mode of elongation of 1-^{14}C palmityl CoA by a 20,000 x g mitochondrial pellet from *P.* chrysogenum.

Acetyl CoA or malonyl CoA was incubated with radioactively-labeled palmityl CoA for 20 minutes. Avidin was added to some experimental reaction mixtures. The resulting fatty acids were saponified, extracted with hexane, methylated with diazomethane, and purified by thin layer chromatography. The methyl esters were separated and identified by gas-liquid chromatography. The radioactivity of each methyl ester was determined by liquid scintillation spectrometry.

Elongation of palmityl CoA was observed in the presence of acetyl CoA, but not in the presence of malonyl CoA. The addition of avidin produced a greater proportion of short-chained fatty acids at the expense of palmitic acid, but did not decrease the percentage of long-chained fatty acids produced.

A high proportion of label was recovered in the $C_{18:3}$ fatty acid, linolenic acid. This suggested that two pathways of linolenic acid synthesis may be operating in this organism.