

MICROBIOLOGY AND MOLECULAR BIOLOGY

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ABSTRACTS

Stream Pollution from Coal Mine Waste Piles: Effect of Sulfur and Iron Oxidizing Bacteria. ROBERT RAMALEY and RICHARD KINDIG, Indiana University.—Analysis of 100 samples of stream water taken during hydrological studies of the Patoka (Pike County, Ind.) and the Busseron (Sullivan County, Ind.) watersheds showed a correlation between the acidity of the samples and the number of sulfur or iron oxidizing bacteria (*Thiobacillus-Ferrobacillus*).

Data obtained during local washouts of surface mined areas and experiments with sterilized mine waste material are consistent with the production of the acid pollution and perhaps some of the oxidized iron by the action of sulfur and iron oxidizing bacteria on sulfur bearing mine wastes and a subsequent washing out of both the acid and some of the bacteria into the streams following rainfall.

Core samples taken of land reclaimed in accordance with the 1967 Indiana Mining Act showed that such reclamation was effective in reducing the number of sulfur and iron oxidizing bacteria in the covered mine waste material and thereby decreasing the acid production to negligible amounts.

Fine Structure Changes during Germination of *Dictyostelium discoideum* Spores. DAVID A. COTTER, Indiana University Medical Center.—The earliest developmental stage in the life cycle of the cellular slime mold, *Dictyostelium discoideum*, is spore germination. Spores of this cellular slime mold can be induced to germinate by exposure to a mild heat shock of 45° C for 30 minutes. The spore germination process occurs in four well-defined stages: 1) dormant spore activation, 2) post-activation lag, 3) spore swelling, and 4) myxamoeba emergence.

Electron microscopy revealed significant changes in the fine structure of germinating spores during stages three and four. The mitochondria progressively became less dense, lost their peripherally attached ribosomes, and revealed more pronounced tubuli as germination proceeded. During spore swelling, the three-layered spore wall broke down in two stages: 1) the outer and middle layers were ruptured as a unit; and 2) the inner wall was breached. Crystals and dark (lipid) bodies seemed to disappear shortly before or during emergence of the myxamoebae. Autophagic vacuoles were found in dormant spores and throughout the entire germination process.

The addition of cycloheximide to germinating spores inhibited the loss of the crystals and dark (lipid) bodies. In addition, the drug inhibited the breakdown of the inner wall layer. Cycloheximide did not

prevent the formation of the water expulsion vesicle or the apparent function of the autophagic vacuoles. A hypothesis is offered to explain the inhibition of myxamoebae emergence by this drug.

Thermal Induction of Bacteriophage ϕ 1. S. S. LEE, Indiana University Medical Center.—The induction of a triply auxotrophic (thy⁻, ura⁻, met⁻) *Escherichia coli* lysogenic for ϕ 1 was studied under different nutritional and temperature conditions. At 37° C, as reported by others, induction occurs only under thymineless conditions. At 45° C, induction occurs under thymineless conditions, but also under conditions of amino acid deprivation. Although thermal induction appears to be a different process than thymineless induction, the fact that the two interact synergistically suggests that both involve repressor inactivation. Thermal induction is generally less complete than thymineless induction at 37° C. The fact that pre-treatment at 37° C under condition of amino acid deprivation lowers subsequent thermal induction under conditions where thymine, uracil and methionine are all withheld suggests that cells that have just completed one round of DNA replication and have not initiated another round are relatively insensitive to thermal induction. Finally, non-induced cells undergo little, if any, thymineless death at 45° C, although induced cells slowly lose plaque-forming ability at this temperature.

Computer-based Derivation of Rate Equations for Enzyme-catalyzed Reactions. ARTHUR R. SCHULZ and DONALD D. FISHER, Indiana University Medical Center.—An algorithmic process has been developed which provides for derivation of rate equations for enzyme-catalyzed reactions by a digital computer. An explicit notation system is employed which is adaptable to computer processing techniques. The sequence of an enzymic reaction is represented by two matrices. A connection matrix is used to determine the valid paths which connect the enzyme species, and a matrix of substrate and product names is used to append reactant symbols to the proper path vectors.

The denominator of a rate equation is given by the sum of the valid paths which connect the enzyme species. The algorithm provides for derivation of the numerator of the rate equation and for alphabetical sorting of the numerator and denominator terms. Reformulation of the rate equation from the "coefficient" form to the more useful "kinetic" form is accomplished by expressing each denominator term as a vector of reactant concentration exponents. This provides for computer-based definition of the Michaelis constants and for all possible inhibition constants, and for the reformulation of the equation using the definitions selected by the investigator.

Axoplasmic Transport of Materials in Nerve Fibers. S. OCHS, M. I. SABRI, and N. RANISH, Indiana University Medical Center.—Axoplasmic flow is present in nerve fibers. Our recent studies have shown that after lumbar seventh ganglion injection with H³-leucine, a crest of labelled activity is present in the sciatic nerve with the position of the crest

moving outward at a rate of 400 mm per day. Evidence that the labelled activity is intra-axonic was gained by freeze block. Extraction of labeled material showed it to be present in particulate and soluble form, as high molecular weight protein (450,000 and 65,000) and polypeptide (4-13,000). The precursor P^{32} -orthophosphate is incorporated into a slower moving material. The possible molecular basis of the fast transport system and other studies regarding the mechanism underlying intra-axonic transport were discussed.