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ABSTRACTS

Sulfatase Activity and Secondary Metalobism of *Cephalosporium Sp.* DIANE CARVER and DAVID W. DENNEN, Antibiotic Manufacturing and Development Division, Eli Lilly and Company, Indianapolis.—Methionine, added to culture media, induces a sulfatase in a number of microorganisms while its replacement by inorganic sulfate circumvents cellular requirements for this enzyme and it is normally repressed. Other investigators have shown that in the fungus, *Cephalosporium*, methioine also has a unique role in yield stimulation of the secondary metabolites penicillin-N and cephalosporin-C as well as supplying sulfur into the structure of these antibiotics. *Cephalosporium* cultures were therefore examined for the presence of a sulfatase and the possible relation of this enzyme to secondary metabolite regulation.

In particular, a wild-type strain (ATCC 11,550) was found to contain a sulfatase constitutive with respect to sulfate or methionine, while in a mutant strain the enzyme responded readily in a classical manner to the addition of these nutrients to the culture medium. The sulfatase of *Cephalosporium* has an approximate molecular weight of 50,000, a catalytic temperature optimum for p-nitrophenol sulfate (PNPS) at 68 C, and an activation energy of 7.8 KCal. Kinetic parameters which compare favorably with those in other organisms include an apparent K_m of 8 $x10^{-4}$ M and a pH optimum between 7-8. The enzyme, with PNPS as a substrate, is inhibited *in vitro* by taurine and choline sulfate suggesting its hydrolytic action on these metabolites *in vivo*.

Induction of Arginase by the Shope Papilloma Virus. L. E. BEATY, and M. E. HODES, Cancer Research, Departments of Medicine, Biochemistry and Medical Genetics, Indiana University Medical Center, Indianapolis. —The Shope papilloma contains high levels of arginase. Although papillomas are induced by the Shope papilloma virus, they are frequently contaminated with a second virus, the rabbit kidney vacuolating virus. Therefore, the induced arginase could be either the product of 1) the Shope virus genome, 2) the RKV virus genome, or 3) a derepression of the genetic apparatus of the host cell. In order to determine which of these mechanisms is responsible for the induced arginase, the two viruses have been separated one from another in density gradients of cesium chloride. The buoyant density of the Shope virus is 1.34 gm/cm^3 while the RKV virus has an apparent density of 1.32 gm/cm^3. In vitro infection of rabbit kidney cells with either of the purified viruses results in an elevation of arginase activity. However, the RKV virus induces an elevated activity of all the enzymes of the urea cycle. In order to exclude the possibility that virus infection serves only to derepress

the host cell, cells lacking arginase were sought. *Escherichia coli* spheroplasts infected with the Shope papilloma virus show an induced arginase activity which reaches a maximum level three hours after infection. This activity is not seen in control cultures. Since *Escherichia coli* is known to be devoid of arginase, these experiments strongly suggest that the Shope virus contains the genetic information necessary to direct synthesis of an arginase.

The Effect of Parenteral Mitogens on Tissue Cultivation. LUIZ HORTA BARBOSA and JOEL WARREN, Department of Biologics Research, Chas. Pfizer and Co., Inc. Terre Haute.—The blastogenic effects of phytohemagglutinin on leukocyte cultures have been described in several publications. Barker, *et al.*, recently reported that extract of pokeweed, *Phytolacca americana*, also prolongs the viability of leukocyte cultures. We have investigated the ability of pokeweed extract to enhance the growth of various tissues from laboratory animals. The addition of pokeweed extract to monolayer cultures of kidney, liver or lung had no effect on cell growth. However, when the donor animal was inoculated parenterally with 100 mg/kg two to six days prior to removal of the organ, these tissues grew more rapidly than those of control animals. Glucose consumption was increased in such cultures and higher yields of certain viruses were obtained.

Application of Latex-agglutination to the Measurement of Antibody Response to *M. pneumoniae* Vaccines. MEIR KENDE, Biologics Research Dept., Chas. Pfizer and Co. Terre Haute.—The use of inert carrier particles for adsorption of antigens has been reported by several investigators. Morton described a procedure for adsorbing live mycoplasma cells to latex and agglutination of the complex by antiserum. Vice versa latex-adsorbed mycoplasma antibodies could be used for detection of specific antigen. Because of a need for a mycoplasma serologic test which is not based on inhibition of metabolism (neutralization and tetrazolium reduction tests, respectively), latex agglutination procedures were standardized and used to measure the response to killed vaccines. Sera from guinea pig and monkey appeared to be the best for this purpose, while rabbit serum required pre-treatment with trypsin-periodate to eliminate non-specific agglutination. The latex agglutination was compared with complement fixation and tetrazolium reduction inhibition to determine its usefulness as an index of mycoplasmal immunity.

Serological Changes in Ex-germfree Rats Mono-associated with *Salmonella typhimurium*. B. S. WOSTMANN, Lobund Laboratory, University of Notre Dame, Notre Dame.—Germfree rats were inocculate *per os* with *Salmonella typhinurium* (ND 750 A) in order to study the activation of the complex of antimicrobial defense mechanisms. This paper describes changes in serum proteins as then relate to the stimulation of the reticuloendothelial system.

S. typhimurium established itself in the gut tract in a concentration approximating that of the total bacterial population in the conventional rat. The animals showed a pronounced loss in body weight and, depend-

ing on the diet, a death rate up to 25%. Spleen and lymph nodes demonstrated transitory hypertrophy, with a miaximum at approximately 10 days after association. Serum albumin showed a temporary loss, and serum α and β globulins transient increases in concentration. Minimum albumin and maximum α and β globulin values occurred at day 4. "Gamma globulin" (defined in the electrophoretic pattern as protein with electrophoretic mobility slower than that of transferrin) started to increase after 5 days. Total serum protein remained at approximately 6%.

The immunoelectrophoretic pattern of the germfree rat usually showed 5 protein fractions with mobilities equal to or slower than that of transferrin. The concentration of each of these fraction was affected by the association with *S. typhimurium*. One are in the slower gamma globulin range indicated a pronounced but a transient increase in concentration with a maximum approximately 3-4 days after association, and is speculated to represent an acute phase protein rather than an immunoglobulin. Specific agglutinins could be detected at the third day but did not reach maximum values until 2 weeks after association.

Specialization of Antibody Formation Among Individual Spleen Cells Responding to a Complex Antigen. JOSEPH S. INGRAHAM and BRUCE H. PETERSEN, Dept. of Microbiology, Indiana University Medical Center, Indianapolis.—Suspensions of spleen cells from mice or rabbits injected with sheep red blood cells (rbc) were examined for antibody forming cells by a localized hemolytic plaque assay (Ingraham and Bussard, J.E.M. *119:* 667). The resulting plaques could be separated into two groups: one clear in which almost all of the rbc were lysed, and one cloudy having a large fraction of unlysed rbc. With a given suspension of spleen cells the proportion of cloudy plaques, was quite constant. These plaques were as large as the clear plaques, they developed at the same rate, and did not clear up with prolonged incubation. Therefore these plaques appear to result from a difference in quality rather than quantity of antibody. Although they could possibly be the product of cells making hemolytically less efficient, presumably 7s, antibody, the fact that 40% of the plaques in a mouse spleen suspension 4 days after a single injection of rbc may be cloudy appears to argue against this. At present we believe that these cloudy plaques may result from heterogeniety in the antigenic composition of the sheep rbc such that some spleen cells make antibodies against an antigen present on only a fraction of the red cells.

Uncoating and Development of Vaccinia Virus in Tissue-Cultured Cell-Fragments Induced with Concentrated Extracts from Marine Algae. THEODORE J. STARR and OLE HOLTERMANN, University of Notre Dame, Department of Microbiology, Notre Dame.—This preliminary report evolved from continuing studies concerned with virus development in abnormal cell types. Recently, we described (Starr *et al, Tex. Rep. Biol. Med.*, 24:208) the phenomena of amitosis, micronucleation and multiple cytokinesis which were induced in tissue-cultured cells (McCoy) with concentrated extracts of marine algae. As observed by time-lapse cinematography and in fixed-preparations stained with acridine orange, multi-

ple cytokinesis provided a unique population of "miniature cells." Of those cells which were affected in this manner, three or more daughter cells were produced per mother cell. In effect, the progency of each cell contained only part of the total chromosonal complement. Such "cells" were subsequently grown on coverslips in Leighton tubes and were challenged with vaccinia virus. Development of inclusions or "factory areas" were noted in these "miniature cells." Our observations will be discussed in view of 1) the postulated role of the host-cell genome in the "uncoating" process (Joklik, *J. Mol. Biol.*, 8:277) and 2) the recent biochemical evidence on messenger RNA synthesis by a "coated" viral genome (Kates and McAuslan, *Proc. N.A.S.*, 58:134).

Identification of the Major ^{32}P Phosphohistidine Protein from *E. coli* as Succinyl CoA Synthetase. JAMES SEDMAK and ROBERT RAMALEY, Department of Microbiology, Indiana University.—Previous studies in Dr. P. D. Boyer's laboratory (Isolation and Properties of the Phosphorylated Form of Succinyl CoA Synthetase, R. F. Ramaley, W. Bridger, R. W. Moyer, and P. D. Boyer, J. Biol. Chem., in press) have shown that the only protein capable of being phosphorylated in partially purified preparations of Succinyl CoA Synthetase was Succinyl CoA Synthetase.

These studies have been further expanded by the use of crude cell extracts of *E. coli*. The cells were grown in a minimal salts medium containing 2.2% sodium succinate. The cells were disrupted by sonic oscillation, the 105,000 x g soluble extract dialysed and incubated at O°C with 0.1 mM ATP-γ-^{32}P, 10 mM Mg, and 50 mM Tris (pH 7.2). The reaction was terminated after 5 to 10 minutes by the additon of EDTA (pH 7.2) and the ATP and inorganic phosphate were removed by chromatography on Sephadex G-50 or Sephadex G-200.

The excluded or ^{32}P containing protein peak was then placed on DEAE-Sephadex and the proteins eluted with a linear KCl gradient in 0.05 M Phosphate (pH 7.2). The fractions were analysed for protein and for protein-bound phosphohistidine.

The results of these experiments with crude cell extracts suggest that, under the time and conditions employed, Succinyl CoA Synthetase is the predominant if not the only protein phosphorylated by ATP.

Other papers read

Hydrocarbon Metabolism by *Micrococcus cerificants*. R. MAKULA and W. R. FINNERTY, Indiana University Medical Center.

Intermediary Metabolism in a Hydrocarbon Oxidizing Microorganism. R. LERUD and W. R. FINNERTY, Indiana University Medical Center.

Quantitative Carbohydrate Changes during Schizophyllum commune Basidiospore Germination. BRENT AITKEN and DONALD J. NIEDERPRUEM, Indiana University Medical Center.

Ultrastructure and Nuclear Behavior in a Fir Mutant of *Schizophyllum commune*. DONALD J. NIEDERPRUEM and RALPH JERSILD, Indiana University Medical Center.

Temperature Limits of *Cyanidium*. WILLIAM B. DOEMEL and THOMAS D. BROCK, Indiana University.