CELL BIOLOGY

Chairman: WILLIAM J. GRIFFIN, Eli Lilly and Company, Indianapolis, Indiana.

CHARLES W. GOFF, Life Sciences Department, Indiana State University, Terre Haute, Indiana 47809, was elected Chairman for 1972

ABSTRACTS

Electron Microscopic Study of *Anaplasma marginal* by Negative Staining. F. PADGETT and W. A. SUMMERS, Department of Microbiology, Indiana University School of Medicine, Indianapolis 46207.——Studies of the morphologic features and reproduction of the initial bodies of Anaplasma marginale were done by the negative contrast (phosphotungstic acid) technique of unfixed, dehemoglobinized, infected bovine erythrocytes. The internal structure appeared to be organized as a coiled, tubular, or ribbon-like structure. The reproduction process, reconstructed from observation of static preparations, began with the formation of a "budlike" structure in the initial body.

Growth Cycle of *Penicillium chrysogenum* Virus. L. F. ELLIS and R. J. DOUTHART, Eli Lilly and Company, Indianapolis, Indiana 46206.—— *Pennicillium chrysogenum* was grown in stainless steel fermenters with continuous agitation and aeration. During the fermentation aliquots were withdrawn and were centrifuged to harvest the cells. Either the virus particles of the double stranded RNA (dsRNA) was isolated from these cells. The amount of dsRNA per gram of mycelia increases until approximately 60 hours incubation and then declines. Physical and biological properties suggest this dsRNA is of viral origin. Other aspects of this virus were discussed.

Toxic Response of Mice to a DDT Diet, Its Supression by Feeding and the Effect of Estrogen. DOROTHY A. WERDERITSH, D. SHELTON, C. SWAN, C.-M. HAUN, W. YUNGHANS and D. J. MORRE, Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana 47907. ——Mice, 4 to 5 weeks old, of the C3H/HeJ strain or from the Purdue University colony received *ad libitum* water and laboratory chow to which corn oil (1:10, weight/weight) was added. For those animals receiving DDT, technical grade DDT (1, 1, 1-trichloro-2 2-*bis* (*p*-chlorophenyl) ethane) was added to the corn oil to give a final concentration of 900 parts per million DDT in the total diet. Overt signs of DDT toxicity appeared as early as the 5th day after DDT feed was offered. Most of the animals died within 3 days after the onset of severe symptom development. If the animals were withdrawn from the DDT feed and presented with control feed in the initial stages of tremor development, they recovered within 8 to 12 hours and exhibited normal behavior. Yet, if animals previously receiving DDT feed were fasted to starvation levels several weeks later, symptoms of DDT toxicity reappeared. Control animals fasted to the same weight levels did not develop tremors or other overt signs of DDT toxicity. It was not possible to prevent, reverse or significantly delay

the toxic response to DDT by administration of estrogen (subcutaneous injection of 5 micrograms estradiol cyclopentylpropionate in 100 microliters corn oil per animal). Apparently, a significant factor in effecting recovery of the animals from DDT toxicity is the removal of the source of the DDT and offering of a normal diet to reestablish a pattern of weight gain and caloric balance.

NADH Dehydrogenase Activity of Golgi Apparatus from Rat Liver. C. M. HUANG, D. J. MORRE, Department of Botany and Plant Pathology, and T. W. KEENAN, Department of Animal Sciences, Purdue University, Lafayette, Indiana 47907.——Highly purified rat liver Golgi apparatus (GA) fractions exhibited NADH dehydrogenase of specific activity approximately one-fourth that observed with endoplasmic reticulum (ER). With exogenous cytochrome *c* as electron acceptor, apparent Km's for NADH were 6.17 and 6.09 μM for GA and ER, respectively. The activity in both membrane fractions was insensitive to rotenone and Pieridine A, potent inhibitors of mitochondrial NADH dehydrogenase. With ferricyanide as acceptor, the apparent Km for ferricyanide was 26.7 μM for both ER and GA and excess of substrate inhibited activity with both membrane fractions. Maximal velocities for the NADH-ferricyanide reductase were 3.44 and 1.44 μM NADH oxidized/min/mg protein for ER and GA, respectively.

The high degree of similarity of these activities comparing ER and GA suggest that they are catalyzed by the same enzyme present in both membrane fractions. The results support the hypothesis that membrane material derived directly from ER is utilized for formation of GA cisterna.

Golgi Apparatus-Medicated Cytomembrane Differentiation in Rat and Bovine Mammary Gland. T. W. KEENAN, Department of Animal Sciences, and C. M. HUANG and D. J. MORRE, Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana 47907.——Golgi apparatus (GA), endoplasmic reticulum (ER) and plasma membrane (PM) fractions were isolated in highly purified form from both rat and bovine mammary glands and subjected to compositional analysis. Included in the analysis was the milk fat globule membrane (GM), a membrane which is derived directly from the secretory cell plasma membrane. With regard to lipid composition, GA was intermediate between ER at one extreme and the PM and GM at the other with regard to levels of total lipid phosphorus, phosphatidyl choline, sphingomyelin, cerebiosides and cholestrol. Fatty acid composition of GA polar lipids was intermediate between ER and PM. Polar lipids of ER contained higher levels of unsaturated fatty acids whereas PM and GM contained higher levels of saturated fatty acids.

Levels of membrane protein-bound sialic acid were lowest in ER, intermediate in GA and highest in PM and GM. Salt-washed ER, GA, PM and GM membranes displayed several protein bands with identical electrophoretic mobilities. Golgi apparatus was also intermediate between ER and the PM and GM with respect to specific activities of the enzymes NADH dehydrogenase, 5'-nucleotidase, and adenosine triphosphatase.

These results are compatible with the hypothesis of GA-mediated cytomembrane differentiation. Further, they confirm previous results obtained with rat liver cytomembranes and extend them to another cell type with different secretory functions.

Ultrastructure of Green Plastids in Leaves of Genetic Albino Tobacco. ANNE A. SUSALLA and PAUL G. MAHLBERG, Department of Botany, Indiana University, Bloomington, Indiana 47401.——Genetic green and albino tobacco seedlings were grown on agar with defined nutrients, growth substances and light conditions. Albino plants developed patches of green on the first and succeeding leaves even though the cotyledons were always white. In later stages of growth, these plants became highly variegated with some approaching the phenotype of normal green plants. In ultrastructural studies, some mutant-green plastids of the albino plants have grana that are massive in size with many appressed thylakoids and irregular margins. Other plastids are large with tapered margins to give a spindle effect while a few have only scattered thylakoids. Osmiophilic globules and DNA-like fibrils are present in the stroma. Starch is evident but photosynthetic activity is yet to be determined.

Use of the Scanning Electron Microscope in the Investigation of Cardiac and Pulmonary Tissue. SHIRLEY SIEW, Indiana University School of Medicine, Indianapolis 46202.——Scanning electron microscopic studies were performed on both fresh biopsy and autopsy specimens of cardiac and pulmonary tissue. The fresh material was obtained at open heart surgery and was fixed in Muir's paraformaldehyde electron microscopy fixative. After fixation, it was processed by three different methods for scanning electron microscopy:

1) placed in glycerine overnight
2) post-fixed in osmic acid and then placed in propylene glycol
3) treated with acetone

The specimens were then coated with gold under vacuum and were examined in a Cambridge Stereoscan Microscope. Chronic rheumatic aortic and mitral valves, papillary muscle and a biopsy of the left lingula were examined. The autopsy material comprised cardiac valves and lung. The latter was fixed under inflation. The best results were obtained in studies of valvular and pulmonary tissue by means of the second method. It was concluded that the scanning electron microscope will prove of value in the investigation of valvular and pulmonary disease.

Ultrastructure of Mouse Heart Cell Culture. G. B. BODER and L. F. ELLIS, The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206.——Newborn mouse ventricles were dispersed by short treatment with collagenase. Cultures derived by this method have a high proportion of beating cells which attach to collagen, glass or plastic within a few hours of isolation. Beating cells have been maintained in sterile culture in the absence of antibiotics for as long as 6 months and remain responsive to hormones. A photoelectric recording system was developed which is capable of measuring the rate and amplitude of contractions of a single myocardial cell or a colony of myocardial cells. The effects of

drugs on these cells was reported in 1971 by Boder *et al.* (Nature 231: 531-532). We have been studying the ultrastructure of the cultured cells.

This report compared the cultured cells to those of the intact myocardial tissue. Myofibrial filaments were observed in the cell cultures.

Ultrastructures of Neuronal Lipofuscin and Ceroid. ITARU WATANABE, VIMAL PATEL, and WOLFGANG ZEMAN[1], Department of Pathology, Indiana University School of Medicine, Indianapolis 46202.——Lipofuscin, an autofluorescent yellow pigment in the residual body, is considered to be indigestible by lysosomal acid hydrolases. This pigment is stained positively by conventional fat staining technique and hence the term "lipid pigment". The pigment is also called "wear and tear" or "age" pigment because it is found most abundantly in the cells of aged individuals. Actually, in the senile brain, the material occupies a large area of the nerve cell body by displacing the nucleus and other cytoplasmic organelles. Electron microscopy revealed that the lipofuscin bodies are surrounded by trilaminar limiting membrane and contain fine granular osmiophilic material. Using histochemical techniques for electron microscopy, we observed acid phosphatase activity in the granular matrix but not in the lipid portion. Our findings agree with observations by others. Recently, we also demonstrated some evidence of peroxidation in the lipid portion of this organelle, supporting the view that lipopigment is derived by peroxidation of fatty acids and proteins.

Another pigment called "ceroid" is found to accumulate in brain cells of patients with Neuronal ceroid lipofuscinosis. Ultrastructural aspects of these two types of pigments, lipofuscin and ceroid, in the human brain were presented.

[1]The authors acknowledge the technical assistance of Vera Kolar, Janice Herring and Connie Aryea.

Effect of Beta-alanine on Glucose Catabolism and Growth of Ehrlich Ascites Tumor Cells. M. E. JACOBS, Department of Biology, Goshen College, Goshen, Indiana 46526.——Intraperitoneal injection of 0.1 ml 5 M beta-alanine at the time of infection, and daily thereafter, inhibits tumor-weight gain of Carworth female CF1 mice by 19% by the end of 14 days, as compared with infected mice injected with 0.1 ml 2.5 M NaCl. Incubation of the tumor cells at 37.5°C in 25 mM beta-alanine in Krebs-Ringers bicarbonate solution at pH 7.4 inhibits $^{14}CO_2$ excretion from the cells using 1-^{14}C-D-glucose as substrate. The inhibition is 18-25%, as compared with control samples containing no beta-alanine, or 25 mM of beta-aminoisobutyric or gamma-aminobutyric acids.

NOTE

Inhibition of Succinate Oxidation in Beef Heart Mitochondria by Derivatives of Pyridine Adenine Dinucleotide. P. V. BLAIR and L. Y. CHAO, Indiana University Medical Center, Indianapolis, 46202.——Succinate oxidation is inhibited by nicotinamide adenine dinucleotide (NAD+) in damaged or sonicated mitochondria. Two mechanisms to account for this NAD+-inhibition have been suggested. In 1948, Pardee and Potter (1) postulated that inhibition occurred because NAD+

stimulated the production of oxaloacetate an inhibitor of succinate oxidase, from succinate *via* fumarate and L-malate. In 1963 an alternative hypothesis was proposed by Neubert *et al.* (2) in which binding of NAD+ or one of its analogues to a site on succinate dehydrogenase would cause direct inhibition of succinate oxidation. Experiments to determine the most likely explanation for NAD+-inhibition of succinate oxidation were carried out on preparations of beef heart mitochondria.

All dinucleotide tested, NAD+, 3-acetylpyridine adenine dinucleotide (3-AcPAD+), 3-acetylpyridine deaminoadenine dinucleotide (3-AcPDeAD+), nicotinamide adenine dinucleotide phosphate (NADP+), NADH and NADPH with the exception of NADPH inhibited succinate oxidation. The inhibition had a lag period and increased with time in the presence of all inhibitory dinucleotides except NADP+. The increase in inhibition with time by NADP+ comes to an early steady (per cent inhibition does not change), probably because it is not a good coenzyme for malate dehydrogenase and because the reduced form (NADPH) can not be oxidized by the electron transfer chain. L-malate, which stimulates the production of oxaloacetate in the presence of NAD+ and its analogues, enhanced inhibition and compounds (glutamate, rotenone, NADH *plus* rotenone, and meso-tartrate) which remove oxaloacetate or stop its formation prevent or reverse NAD+-inhibition of succinate oxidation. The oxidized dinucleotides do not inhibit succinate oxidation in purified electron transfer particles. A supernatant fraction containing fumarase and malate dehydrogenase restores the inhibitory ability of NAD+ to these purified electron transfer particles. The addition of oxaloacetate to these purified particles inhibits succinate oxidation with apparent competitive kinetics.

Differential spectra and oxidation-reduction of the dinucleotides at absorption maxima were recorded in the presence of succinate and L-malate. The appearance of the reduced form of the NAD+-analogues was positively correlated with the per cent inhibition of succinate oxidation. The reduced analogues accumulated from malate dehydrogenation because they are not rapidly oxidized by the respiratory chain, whereas NADH does not accumulate because it is rapidly reoxidized by electron transfer. Our results are consistent with the postulate that oxaloacetate is the inhibitor of succinate oxidation and NAD+ only stimulates the accumulation of oxaloacetate. None of our evilence would indicate that NAD+ directly inhibits succinate dehydrogenation or the succinate dehydrogenase enzyme.

Literature Cited

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