CELL BIOLOGY

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ABSTRACTS

Effects of Antibiotic A-23187 on Spore Germination and Apical Growth in Fungi. Philip A. Beachy, Jerry D. Smucker, James A. Sweigard, and Stanley N. Grove, Goshen College, Goshen, IN 46526.——Ion gradients and/or ionic currents have been implicated in many biological processes including apical growth. We have examined the influence of a divalent cationophore, antibiotic A-23187, on apical growth in fungal spores and hyphae. A-23187 when present between 0.01 and 10 μg/ml reversibly inhibits sporangiospore germination in the zygomycete, *Gilbertella persicaria*, with the degree of inhibition corresponding to the level of antibiotic in the medium. Apical growth of hyphae is also affected, as evidenced by the tendency of the germlings to branch more frequently and to form irregular hyphae in the presence of A-23187 at 1 μg/ml. The inhibitory effects of A-23187 on spore germination and apical growth are enhanced by the addition of external Ca^{++}, but not by external Mg^{++}. This is consistent with a role in growth for a Ca^{++} gradient. Additional evidence for a Ca^{++} gradient is provided by the reversal of La^{+++} inhibition of spore germination by external Ca^{++}. Our observations support the concept of a role for a Ca^{++} gradient in apical growth, although we are unable to demonstrate a Ca^{++} requirement for growth by serial transfer of cultures through presumed Ca^{++}-free environments.

Effects of Cytochalasin A on Spore Germination and Apical Growth in Fungi. James A. Sweigard, Alan R. Kurtz, and Stanley N. Grove, Goshen College, Goshen, IN 46526.——Spore germination can be separated into two stages with respect to the mode of growth. Stage I is characterized by spherical or general growth, while during stage II growth becomes polar resulting in a germ tube. Cytochalasin A (CA) at 0.01 μg/ml or higher inhibits stage II germination in *Gilbertella persicaria*. Upon continued incubation for about 5 hours, the germlings overcome the effect of CA and resume polar growth. With increasing levels of CA longer time periods are required for overcoming the inhibitory effects. This phenomenon can be used as a convenient bioassay for CA. If CA at 10 μg/ml is used for inhibition and the incubation medium is renewed prior to the expected time of overcoming the effect, the germlings will continue spherical growth, producing giant cells 50-90 μm diam. If CA at 10 μg/ml ca is introduced to germlings which have already entered stage II germination or to growing hyphal tips, polar growth stops immediately and spherical growth resumes. Results similar to these are obtained with conidia of *Choanephora curbitans*

in the presence of 1 μg CA/ml. Changes in physiological activities which have been correlated with our morphological observations of *G. persicaria* include dry weight, O_2 uptake, glucose uptake and metabolic rates. Fine structural correlates of CA inhibition include irregular deposition of cell wall material. These accumulations are at the cell periphery but often extend deep into the cytoplasm. Clearly the effect of this drug is to remove or uncouple polarity from the growth mechanism so that the typical apical growth which produces fungal hyphae is not possible.

Pattern Formation in Sexine Development of *Silene Alba* (Caryophyllaceae) Pollen. JANE R. SHOUP, Department of Biology, Purdue University Calumet, Hammond, Indiana 46323.——Sexine maturation in *Silene alba* (Caryophyllaceae) has been studied from meiosis to anthesis by light and transmission electron microscopy in a search for clues of patterning mechanisms and cellular sources of pollen wall materials. Exine formation is initiated while microspores are still enclosed within the callose wall of the tetrad, by the appearance of fine fibrous primexine material closely associated with the spore plasma membrane. Lamellae of unit membrane dimensions (ca. 7.5 mμ) are located primarily at the sites of future apertural pores. Electron-dense sporopollenin becomes associated with these unit membrane lamellae. The protectum, a vermiculate sheet elaborated by the primexine, occupies the position of the future sexine and seems to serve as a scaffold upon which sporopollenin will be deposited after dissolution of the tetrad. When the microspores are released, electron-dense particles in the intralocular cavity appear to contribute to sporopollenin accumulation in the protectum. To accommodate a threefold increase in pollen grain volume in the course of final maturation, further sporopollenin is added to the sexine. This process occurs by accumulation along white-line lamellae or "tapes" and is probably contributed at least in part by the tapetum. These processes are consistently correlated with another length.

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It is concluded that PLCB does *not* inhibit muscle cell fusion and, in contrast with PLCC, does *not* hydrolyze choline containing phospholipids on cultured chick embryo muscle cells. Supported by the NIH grant PHS SO7 RR 5371.

Elevated Uridinediphosphate Kinase and Cytidinetriphosphate Synthetase Activities in Transplantable Rat Hepatomas. WILLIAM L. ELLIOTT, D. JAMES MORRÉ and P. F. HEINSTEIN, Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, IN 47907.——Numerous biochemical and morphological differences contrast normal and cancerous cells. The resulting biochemical imbalances may contribute to the proliferative advantage commonly expressed by cancer cells. Weber (New England J. Med. 296, 486, 1977) has summarized evidence that the enzyme uridinediphosphate kinase (EC 2.7.4.6) is transformation linked, i.e. activity is elevated regardless of tumor

growth rate while cytidinetriphosphate synthetase (EC 6.3.4.2) is progression linked with activity proportional to growth rate. Our analyses of several lines of transplanted rat hepatomas induced initially by the chemical carcinogen 2-acetylaminofluorene show elevations of uridinediphosphate kinase (UDP kinase) and cytidinetriphosphate synthetase (CTP synthetase). In agreement with Weber, UDP kinase is uniformly elevated while CTP synthetase activity correlates with growth rate. A transplantable tumor arising from the jaw area also shows elevated UDP kinase but extremely low levels of CTP synthetase. Increased cellular amounts of UTP and CTP would provide increased nucleotide pools for DNA synthesis and as co-substrates for the production of UDP-sugars and CMP-sialic acid, important in the formation of glycoconjugates of the cell surface. They may represent an important potential determinant of cell growth and behavior. Work supported in part by a grant from the National Cancer Institute CA 18801.

Cinnamic Acid Derivatives Inhibit the Golgi Apparatus in Two Model Test Systems. Sue L. Deutscher, Kim E. Creek and D. James Morré, Department of Biological Sciences and Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, IN 47907.——The Golgi apparatus plays a central role in the packaging of secretory products destined for the cell's exterior. Availability of inhibitors of Golgi apparatus function will aid studies involving the role of this organelle in the growth and differentiation of plant and animal cells. However, few such inhibitors are known. We have developed two test systems for screening potential Golgi apparatus inhibitors. One system utilizes polysaccharide droplet formation by the outer root cap cells of maize. The droplet, a direct product of the Golgi apparatus, forms on the root tip and can be quantitated using a rating index. The second system employs secretory granule formation in the parotid gland of the rat, an activity also mediated by the Golgi apparatus. Parotid gland slices are stimulated with epinephrine to achieve 90% degranulation. The ability of the Golgi apparatus to package new secretory granules is then tested by incubating degranulated gland slices in the presence of putative inhibitors followed by restimulation with epinephrine. The amount of alpha-amylase secreted into the medium is used as a measure of secretory granule formation. Cinnamic acid, coumarin, 4-hydroxycoumarin, scopoletin and colchicine have shown substantial inhibition of Golgi apparatus activities in at least one of the two test systems. Current studies are directed toward determining the molecular requirements for maximal inhibition with a view toward developing an effective Golgi apparatus inhibitor.

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Subcellular Localization of the Early Enzymes of Glycosphingolipid Biosynthesis of Rat Liver. Vivian P. Walter, L. Seretto, K. E. Creek, M. Forman and D. James Morré, Department of Biological Sciences and Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, IN 47907.——Alterations in the amounts and

composition of neutral glycolipids in transformed cells and tissues have led to investigation of the early enzymes functioning in the pathway of glycosphingolipid biosynthesis. The first enzymes, uridinediphosphateglucose:ceramide glucosyltransferase (UDP-Glu:Cer transferase), and cytidine-monophosphate-sialic acid:lactosylceramide sialyltransferase (CMP-NAN:LacCer transferase) promote the sequential addition of sugar residues to ceramide (N-acylsphingosine). The subcellular location of these enzymes has been studied to better understand the flow of new membrane material to the cell surface.

The activity of the first enzyme in the glycosphingolipid pathway, UDP-Glu: Cer transferase, was found to be enriched 2.4-fold in endoplasmic reticulum (ER) and 1.6-fold in Golgi apparatus (GA) over that of total homogenate (TH) in rat liver. When both rough and smooth ER were isolated, the activity was most enriched in the rough ER. In the synthesis of lactosylceramide, 80% of the activity of the TH was in the rough and smooth ER. However, GA showed a 2-fold enrichment over TH suggesting that the activity may be present in this organelle. Lastly, the activity of CMP-NAN:LacCer transferase was shown to be concentrated in GA. Thus, the subcellular location of these three early enzymes of glycosphingolipid synthesis suggest a mechanism of sequential synthesis, first in ER and then in GA, as they progress to the cell surface.

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Degradation of Adenylate Nucleotides by Golgi Apparatus Membranes. SANDRA SCHILLER SMITH and D. JAMES MORRÉ, Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, IN 47907.——In studies of the distribution of phosphatase activities among rat liver endomembranes, some interesting observations were made concerning adenylate nucleotide catabolism. When Golgi apparatus isolated from rat liver were suspended in a buffered solution containing ^{14}C-AMP and incubated, there was nearly complete conversion of the AMP to adenosine followed by other products including adenine, inosine, inosine monophosphate and possibly adenyl succinate. The analyses involved precipitation of proteins with acid and removal by centrifugation, PEI-cellulose chromatography of the supernatants to separate different nucleotides, and autoradiography. The results suggest the existence of an enzyme system in Golgi apparatus capable of rapid and complete degradation of adenylate nucleotides.

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Altered Glycogen Accumulation During 2-Acetylaminofluorene-Induced Liver Tumorigenesis in the Rat. EMILY YEO, DOROTHY M. MORRÉ and WILLIAM L. ELLIOTT, Department of Foods and Nutrition and Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, IN 47907.——In contrast to normal livers, hepatocellular carcinomas induced by the carcinogen 2-acetylaminofluorene contain little or no glycogen. Glycogen in aqueous extracts was converted to

glucose using glycogen phosphorylase and glycogen content determined spectrophotometrically using a coupled reaction involving hexokinase and glucose-6-phosphate dehydrogenase. To determine the time course of stored glycogen during tumorigenesis, livers were analyzed at weekly intervals during an 8 week continuum of carcinogen administration. Comparisons were with animals fed basal diet lacking carcinogen. Loss of glycogen paralleled tumorigenesis with the half maximal decrease in stored glycogen corresponding to development of hyperplastic nodules. The findings suggest that loss of stored glycogen is among the earliest and most dramatic manifestation of 2-acetylaminofluorene-induced tumorigenesis in the rat.

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Adriamycin Effects on Plasma Membrane NADH Dehydrogenase. Warren C. MacKellar,* F. L. Crane, D. J. Morré, T. Ramasarma and H. Löw, Department of Biology and Medicinal Chemistry and Pharmacognosy, Purdue Univ., W. Lafayette, IN., Dept. of Biochemistry, Indian Institute of Science, Bangalore, India and Endocrinology Clinic, Karolinska Hospital, Stockholm, Sweden.—It is now clear that plasma membranes (P.M.) contain an NADH dehydrogenase, through which the response to hormones may be regulated and which may provide energy for transport (H. Löw and F. L. Crane, BBA *515*, 141, 1978). In liver P.M. adriamycin stimulates the NADH oxidase 180% above basal level. Erythrocyte P.M., which show very little NADH oxidase, do not show any evidence of stimulation by adriamycin. NADH cytochrome c reductase activity in these P.M. is not affected by adriamycin. NADH ferricyanide reductase is inhibited 27% of basal level in erythrocyte membrane and even more in liver P.M. Vanadate stimulated NADH oxidase is inhibited 34% of basal level. All these effects on NADH oxidation have a ½ maximum at about 3×10^{-5} M adriamycin. Adriamycin has been shown to stimulate superoxide production and induce lipid peroxidation in mouse heart tissue (C. E. Myers *et al.*, Science *197*, 165, 1977). If the stimulation of NADH oxidase involved the formation of superoxide by autooxidation of the adriamycin, then an increased rate of NADH cytochrome c reductase activity should be observed, since superoxide reduces cytochrome c. Also, vanadate stimulation of the NADH oxidase appears to proceed through superoxide, since the stimulation is abolished under anaerobic conditions or in the presence of superoxide dismutase. Since there is no stimulation of cytochrome c reductase, and there is an inhibition of vanadate stimulated oxidase, it would appear that the increased NADH oxidation rate in P.M. is not causing increased superoxide formation. It is possible that the increased NADH oxidation is accompanied by hydrogen peroxide formation. A balance of cAMP and cGMP has been suggested as a controlling factor in cell development, so an alteration of this balance by changing redox control of a cyclase may be a part of the growth control by adriamycin. If redox control functions are modified by adriamycin, then the effects on NADH oxidase may be involved in the

antitumor effect of this drug. Support by grants from NIH AM 25235, GMK6-21, 839 and CA 18801.

Ferritin Uptake from the Tubular Fluid in the Initial Segment of the Malpighian Tubules in *Cenocorixa bifida*. MOHINDER S. JARIAL, Department of Physiology and Health Science and Muncie Center for Medical Education, Ball State University, Muncie, Indiana 47306.——Ferritin uptake was studied by electron microscopy in the initial segment after the whole Malpighian tubules were exposed to ferritin solution in insect Ringer for 5, 10, 15 and 20 minutes.

Ultrastructure of the initial segment of the Malpighian tubules of *Cenocorixa bifida* show short basal plasma membrane in foldings associated with mitochondria, numerous vesicles and microtubules in the cytoplasm and small well separated microvilli at the luminal border which along with the latter are covered with fibrillar coat. The remaining segments of the tubule present elaborate basal plasma membrane infoldings, which penetrate deep into the cell, large mitochondria, excretory granules and well developed, closely packed microvilli.

After 5 minutes ferritin particles reach the tubular fluid in the lumen and by 10 minutes they become closely associated with the fibrillar coat of the luminal border and microvilli of the initial segment. Ferritin particles then appear in the pinocytotic vesicles which are pinched off from the luminal cell surface of the initial segment. Deeper in the cytoplasm, ferritin is found in small and large vesicles, the latter formed by the fusion of smaller ones. After 15 minutes ferritin particles appear in a very large membrane bound vacuoles, within intercellular channels and dilated plasma membrane infoldings and under the basement membrane ready to be released in the hemolymph. By 20 minutes very little ferritin is attached to the luminal cell surface and most of the ferritin-containing vesicles are located deeper in the cytoplasm. Free ferritin particles also occur in the cytoplasm of cells of the initial segment.

These observations suggest that the initial segment of the Malpighian tubules in *Cenocorixa* may be involved in the uptake of macromolecules like proteins and electrolytes from the tubular fluid.

Fast Transported Calcium-Binding Protein is Similar to Calmodulin *Zafar Iqbal*, Departments of Physiology and Biochemistry and Medical Biophysics Program, Indiana University School of Medicine, Indianapolis, Indiana 46223 U.S.A.——Calcium, an essential element for the axoplasmic transport of materials in nerve fibers has been found to be transported at a fast rate in cat sciatic nerve on injection of $^{45}Ca^{2+}$ into the L7 dorsal root ganglia. On analysis of the nerve segments corresponding to regions of transported activity, the fast transported $^{45}Ca^{2+}$ was found bound to a protein of 15,000 dalton. Using ^{3}(H)-leucine as a precursor, a labeled calcium binding protein (CaBP) was found located at the same position in elution volumes from the columns (Sephadex G-100 and Bio Gel A5m) as was the protein-bound $^{45}Ca^{2+}$, indicating that Ca^{2+} is fast transported in association with the CaBP. A comparison of CaBP with calmodulin showed them to be similar in

regard to elution profiles from gel filtration columns, activation of cyclic nucleotide phosphodiesterase, isoelectric points determined on analytical isoelectrofocusing gels and electrophoretic mobility on SDS-polyacrylamide gels. The presence of a fast transported CaBP in nerve and its similarities with calmodulin raises some interesting possibilities for its involvement in the axoplasmic transport of materials in nerve fibers. CaBP can be conceived as contributing to the regulation of Ca^{2+} levels in nerve fibers. Another more direct interaction is that CaBP could modulate the Ca^{2+}-Mg^{2+}-ATPase of nerve which, in our transport filament model, is required for the hydrolysis of ATP to supply energy for transport. Thus, the protein itself may be acting as an integral part of the transport filament mechanism.

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Influence of Omega Amino Acids in Production of Tanning Pigments in Integuments. M. E. Jacobs, Goshen College, Goshen, Indiana 46526.——During cuticular sclerotization in *Drosophila melanogaster*, beta-alanine is transported into the cuticle where it produces primary setting by uniting with the chitinous microfibrils. Meanwhile, N-acetyl-dopamine, derived from dopamine, passes into the cuticle to meet the phenol oxidase, which oxidizes it to the tanning quinone. This oxidation proceeds rapidly, if the highly reactive quinone finds a complexing site. Beta-alanine provides such a site, and, reacting quickly, produces tanning pigments. In the absence of beta-alanine, the precursor dopamine accumulates by feed-back inhibition to produce melanization. The inverse relationship between beta-alanine tanning and melanization in insects is thereby explained. Similar reactions occur between dopamine and beta-aminoisobutyric acid with ultraviolet light substituted for phenol oxidase. This may account for racial differences in pigmentation of human skin.

Syngeneic Spleen Cell Therapy of a Transplantable Rat Myeloma. *Lisa Anselmino and Kara Eberly, Department of Biology, Saint Mary's College, Notre Dame, Indiana 46556.——Louvain rats develop readily transplantable spontaneous illeocecal immunocytomas (myelomas), which secrete immunoglobulin. In the course of radiation therapy of the transplantable IgE-secreting myeloma IR162, it was observed that syngeneic spleen cells alone prolonged the life of tumor bearing animals. Therefore rats were injected subcutaneously with 3×10^6 IR162 myeloma cells, which is 30 times the dose required to initiate subcutaneous tumors. Eight days later the rats were injected intravenously with Eagle's minimum essential medium (controls) or 5×10^7 Louvain spleen cells in Eagle's MEM. In two experiments the spleen cell treatment delayed the appearance of tumors and prolonged survival. Five of 14 spleen cell treated rats remained tumor free for more than three months. If transplanted myelomas do not appear within three months, the rats remain tumor-free. Tumor burden and injection protocol were critical to the success of this treatment. No rats remained tumor free when the tumor cell inoculum was doubled. Weekly injections

of spleen cells delayed tumor appearance longer than a single injection. A single spleen cell injection was as effective in producing cures as 900 R whole body irradiation followed by bone marrow transplantation. Therefore, spleen cell injections appear to provide a valuable approach to the therapy of rat myelomas, and efforts are currently underway to determine the cell type(s) responsible for this effect.