CELL BIOLOGY

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ABSTRACTS

Bovine Thyroid Glutamate Dehydrogenase, John D. Larson and Arthur R. Schulz, Department of Biochemistry, Indiana University School of Medicine, Indianapolis, Indiana 46202.——Glutamate dehydrogenase is located in the mitochondrial matrix of liver, brain and kidney. In contrast, we have identified glutamate dehydrogenase activity in both the cytosol and mitochondrial fractions of the thyroid gland. The cytosol fraction of bovine thyroid tissue contains 20 to 30 times as much glutamate dehydrogenase as the mitochondria fraction. The presence of glutamate dehydrogenase activity in the cytosol of thyroid tissue does not appear to be due to rupture of mitochondria during isolation because NAD-specific isocitrate dehydrogenase and pyruvate dehydrogenase activities are found almost entirely in the mitochondrial fraction. Thyroid cytosol glutamate dehydrogenase catalyzes the reduction of α-keto-glutarate in the presence of ammonia with either NADH or NADPH as the electron donor, and the oxidation of glutamate with either NAD^+ or $NADP^+$ as the electron acceptor. The reaction in either direction is activated by ADP and inhibited by GTP. This enzyme has been partially purified. The possible physiological significance of thyroid cytosol glutamate dehydrogenase was discussed.

Elongation and Desaturation of Fatty Acids in *Aspergillus niger*. Rex Shellenbarger and Alice Bennett, Department of Biology, Ball State University, Muncie, Indiana 47306.——The elongation and desaturation of fatty acids was investigated by studying the fate of 1-^{14}C labeled lauric, myristic, and stearic acids added to submerged cultures of *Aspergillus niger*. The mycelium produced oleic and linoleic acids from 1-^{14}C-lauric and 1-^{14}C-stearic acids and to only a slight extent from myristic acid. Stearic acid was the principal labeled saturated fatty acid produced when lauric acid was the substrate, but both palmitic and stearic acids were produced in reduced amounts from myristic acid. Myristic acid has been reported to be a poor precursor for long chain fatty acids in *Penicillium chrysogenum* as well. A time study revealed that oleic acid was the initial C_{18} unsaturated fatty acid formed from all three precursors. The absence of label in fatty acids shorter than the added substrates indicated the oxidation followed by *de novo* synthesis did not occur. Periodate-permanganate oxidation data verified that *de novo* synthesis did not occur. When 1-^{14}C-lauric acid was the substrate, Schmidt decarboxylation data indicated that one-half of the label was in the 1-position of stearic acid, suggesting that direct elongation was not the predominant pathway. The results of these experiments suggest that the elongation of fatty acids is preceded by

removal of acetate groups which are used preferentially for chain elongation and not *de novo* synthesis.

Distribution and Characterization of Ganglioisdes in Mammary Gland and Milk. T. W. KEENAN and D. JAMES MORRÉ, Departments of Animal Sciences and Botany and Plant Pathology, Purdue University, Lafayette, Indiana 47907.——Gangliosides, which are sialic acid containing glycosphingolipids, were identified as constituents of bovine mammary gland and of the milk fat globule membrane (MFGM), a membrane which is derived directly from the apical plasma membrane of mammary secretory cells. Although gangliosides were enriched in MFGM, contrary to expectations, they were not specifically localized in the surface membrane. Gangliosides were found in all subcellular membrane fractions examined and in particulate free supernatant fractions. Milk contained approximately 5.6 nanomoles of ganglioside sialic acid per milliliter. About 90 per cent of the milk gangliosides were found in the MFGM; most of the remainder were in milk serum. Gangliosides from both mammary gland and the MFGM were predominantely of the monosialo-type. Both N-acetyl- and N-glycoyl-neuraminic acids were present in ganglioside fractions. Glucose, galactose and galactosamine were also present in ganglioside fractions. Major fatty acyl residues in gangliosides were 16:0, 18:0, 18:1, 22:0, 23:0 and 24:0. At least six chromatographically distinguishable gangliosides were present in both mammary gland and MFGM. *In vivo* experiments with ^{14}C-glucosamine revealed that mammary gland is the site of synthesis of gangliosides secreted with milk. Rat mammary carcinomas contained nearly twice as much ganglioside sialic acid as control tissue as well as elevated protein-bound sialic acid. The results support a role for gangliosides in tumorigenesis.

Relationship of Long Chain Fatty Acids in Sphingolipids to Membrane Stability. T. W. KEENAN, Department of Animal Sciences, Purdue University, Lafayette, Indiana 47907.——Subnormal levels of fatty acids with 20 to 26 carbons in sphingolipids are associated with defective myelination. This has led to the postulation that long chain fatty acids contribute to membrane stability and cohesiveness. To test this hypothesis various organs (rat heart, spleen, lung and liver and bovine mammary gland) were homogenized in isotonic sucrose and the homogenates were fractionated into total membrane and particulate-free supernatant fractions by centrifugation at 150,000 gravity for 90 minutes. It was reasoned that unstable membrane material would fragment during processing and would be present in supernatant fractions. Supernatant fractions contained from one-eighth (lung) to one-half (liver) of the total phospholipid of the organs. Sphigomyelin accounted for 3.6 to 13.4 per cent of the total phospholipid in membrane fractions and 3 to 7.3 per cent of the total phospholipids of supernatant fractions. The percentage of long chain (20 to 24 carbons) fatty acids in total membrane and supernatant sphingomyelin fractions, respectively, were: 63 and 60 per cent (spleen); 86 and 80 per cent (liver); 67 and 59 per cent (heart); 77 and 26 per cent (lung); and 69 and 53 per cent (mammary gland). Incubation of tissue for extended periods before fractionation increased the proportion of sphingomyelin

in supernatant fractions. After overnight incubation sphingomyelin from supernatant fractions contained only 9 per cent long chain acids whereas membrane fractions contained 44 to 60 per cent long chain acids. This suggests that lipoproteins containing sphingolipids with shorter chain fatty acids are released more readily from membranes.

Characterization of Nucleoside Diphosphatase Relative to Cytochemical Studies. WAYNE D. KLOHS, CHARLES W. GOFF, and ERNA BEISER, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.——The influence of the conditions and constituents of the cytochemical assay medium for nucleoside diphosphatase (NDPase) on the activity of this enzyme in Golgi apparatus enriched fractions was determined. The enzyme was normally assayed as inosine diphosphatase. Two millimoles of manganese chloride activated nucleoside diphosphatase maximally, although both calcium chloride and magnesium chloride also effectively supported enzyme activity. The greatest nucleoside diphosphatase activity occurred at pH 4.8 and 7.0, with considerable activity remaining between these peaks. Uridine diphosphate, guanosine diphosphate, and inosine diphosphate were hydrolyzed more rapidly than other substrates tested. Storage at 4° Centigrade for 4 days resulted in a doubling of the neutral pH nucleoside diphosphatase activity while the activity at pH 4.8 showed no such increase. Treatment with deoxycholate or Triton X-100 activates the pH 7.0 activity, and also appears to at least partially solubilize the enzyme from the membrane. Nucleoside diphosphatase can be partially inhibited by treatment with potassium chloride, sodium fluoride or uranyl nitrate, and enzyme activity is almost totally eliminated after exposure of the enzyme preparation to 60° Centigrade for 30 minutes. Glutaraldehyde and lead nitrate, two reagents to which nucleoside diphosphatase is exposed when studied cytochemically, greatly reduced enzyme activity. However, nucleoside diphosphatase activity of the Golgi enriched fraction was inhibited to a greater degree by glutaraldehyde fixation than was the activity of intact tissue fixed in glutaraldehyde.

A Cytochemical Survey of Nucleoside Diphosphatase in Certain Plant and Animal Cells. GARY DEVILLEZ and CHARLES W. GOFF, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.——The ultrastructural localization of nucleoside diphosphatase activity was compared in radish root hairs (Burpee white radish) and in ductus epididymidis and duodenum of the adult white mouse. The cytochemical medium used was essentially that of Novikoff and Goldfischeri (1961) except that the final concentration of both manganese and lead ions was two millimolar. Inosine diphosphate was utilized as substrate in all cases. In root hair cells reaction product was localized in the rough endoplasmic reticulum and the Golgi apparatus while reaction product was found in the Golgi apparatus, but not in endoplasmic reticulum, of epididymal secretory cells and duodenal absorptive cells. Reaction product was also found on the nuclear envelope of certain duodenal absorptive cells, the intensity of which often varied from one nucleus to another. The rough endoplasmic reticulum reaction product occurred consistently and uniformly along the entire

length of the root hair cell. Rough endoplasmic reticulum activity was also observed in the epidermal cell from which the root hair originated.

These localizations are consistent with those reported by other workers to contain nucleoside dephosphatase in either plant or animal cells. At this time it is not possible to suggest the significance of nucleoside dephosphatase localization, in terms of extent and distribution of reaction product, in the different cell types studied.

Morphogenesis of Glandular Hairs of *Cannabis sativa* L. from Scanning Electron Microscopy. CHARLES T. HAMMOND and PAUL G. MAHLBERG, Department of Plant Sciences, Indiana University, Bloomington, Indiana 47401.——Three distinct types of glandular hairs of increasing morphological complexity which occur on flowering tops of *Cannabis sativa* (marihuana) are described from scanning electron microscopy. These gland types termed bulbous, capitate-sessile, and capitate-stalked, occur on pistillate plants in greatest abundance on the outer surface of bracts ensheathing the ovary. Bulbous and capitate-sessile glands which arise at an early stage in bract development are scattered over the bract surface. Mature bulbous glands have a small swollen head on a short stalk whereas capitate-sessile glands have a large globular head attached directly to the bract surface. Because of their numbers and large size, capitate-sessile glands are the most conspicuous gland type during the early phase of bract development. Capitate-stalked glands which have a large globular head on a tall, angled stalk arise during subsequent bract development. These stalked glands first differentiate along the bracteal veins and then over the entire bract surface. A voluminous, fluid secretory product accumulates in the glandular head of all three gland types. These glands are believed to be a primary site of localization of the marihuana hallucinogen, tetrahydrocannabinol.

Histochemistry and Scanning Electron Microscopy of Starch Grains from Latex of *Euphorbia terracina* L. and *Euphorbia tirucalli* L. PAUL MAHLBERG, Department of Plant Sciences, Indiana University, Bloomington, Indiana 47401.——The morphology and distribution of starch grains in the various tissues of the embryo and the morphology of the starch grains isolated from the latex of two species of *Euphorbia* was compared by histochemistry and scanning electron microscopy. In *Euphorbia terracina* they are elongated and greater in diameter at the midregion than at the tips while in *Euphorbia tirucalli* they are osteoid. Small accessory grains may be fused to the elongated grain in *Euphorbia terracina*. Starch grains varied in size in both species although in *Euphorbia tirucalli* the largest grains which measured 49u were approximately twice the length of those in *Euphorbia terracina* (27u). The enlarged ends on the osteoid grain may vary in shape or size on individual grains. The morphology of starch grains in adjacent parenchyma cells in both species is round and dissimilar from that in laticifers. Plastid specialization suggests that the morphology of the starch grain is species specific in laticifers and as a character may be useful for taxonomic analyses or for interpreting plastid phylogeny in laticifers in different species and possibly genera within a family.

A Radiation-Induced Paracentric Inversion in *Aedes aegypti* (L.) I. Cytogenetics and Interchromosomal Effects. JAMES J. MCGIVERN and KARAMJIT S. RAI, Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556.——A paracentric inversion in one of the autosomes (Linkage Group 2) in the yellow fever mosquito, *Aedes aegypti*, was induced by gamma irradiation. This inversion was originally detected through suppression of recombination in a certain segment of Linkage Group 2 and confirmed by cytological analysis. Female inversion heterozygotes showed normal fertility (approx. 85 per cent) while the fertility of the male heterozygous for the inversion was approximately 45 per cent. Attempts to isolate inversion homozygotes were unsuccessful. Backcrosses involving females heterozygous for the inversion and certain markers on Linkage Groups 1 and 3 and karyotypically normal multiple marker stocks, showed a significant increase in crossing over indicating interchromosomal effects of this inversion on recombination.

An Assay for GABA Receptors of the Rat Cerebellum. J. M. SCHAEFFER, J. H. CLARK, and E. J. PECK, JR., Department of Biological Sciences, Purdue University, Lafayette, Indiana 47907.—— Autoradiographic studies have revealed that γ-aminobutyric acid (GABA) is accumulated and stored in the nerve-endings of two cell types in the rat cerebellar cortex, the basket and stellate cells. In the present investigation techniques analogous to those used in studies of bacterial transport have been employed to examine the capacity of isolated nerve-ending particles or synaptosomes of the cerebellar cortex to bind ^{3}H-GABA. Synaptosomes were isolated from rat cerebellar cortical tissue by standard techniques. After incubation with varying concentrations of ^{3}H-GABA at 0-4° Centigrade in the presence or absence of the phthalide isoquinoline, bicuculline (BIC), the synaptosomes were filtered and washed using Millipore filters (0.8 micron pore size). The filters were solubilized and bound ^{3}H-GABA was measured using a scintillation counter. A rectangular hyperbolic relationship is observed for GABA binding as a function of the concentration of GABA in the media. Double reciprocal analyses indicate that the binding of BIC is strictly competitive with GABA binding. Estimates of the K_d for the receptor•GABA and receptor•BIC complexes are 0.9-2.5x10^{-5}molar and ~2x10^{-5}molar respectively.