CELL BIOLOGY

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ABSTRACTS

Computer-based Method for Calculation of the Utilizable Energy of Proteins. Arthur R. Schulz, Indiana University, Indianapolis, Indiana, 46202.——An aspect of protein nutrition which has not been resolved in a satisfactory manner is the metabolizable caloric equivalence of proteins. The existing methods involve determinations of the difference in the heats of combustion of a protein and the nitrogenous excretory products, and these methods over-estimate the utilizable energy of proteins. A more accurate method of calculating the utilizable energy of proteins is to calculate the moles of adenosine triphosphate formed during the complete oxidation of a given amount of protein to carbon dioxide, water and urea. The moles of adenosine triphosphate formed can be calculated from a knowledge of the amino acid composition and knowledge of the metabolic pathway for each amino acid. A computer program is described which provides the bookkeeping required for these calculations. The metabolizable energy of a protein is calculated in this computer-based method by adjusting the caloric value of the protein so that it is equivalent to that of carbohydrates and fats in providing the energy for adenosine triphosphate formation. The computer-based method has been employed to calculate the utilizable energy of a group of proteins of known amino acid composition. The utilizable energy varied from 3.02 kcal/g for collagen to 3.71 kcal/g for the protein of raw cow's milk.

Low Level Microwave Effects on the Thyronine-Binding Capacity in Rats. William D. Travers and Richard J. Vetter, Department of Bionucleonics, Purdue University, West Lafayette, Indiana 47907.——A study conducted in the U.S.S.R. and present work at Purdue by the authors indicates that low-level chronic exposure to microwave radiation affects serum protein levels of laboratory animals. Thyro-binding capacity (TBC) was tested in rats exposed to 5 and 25 mW/cm^2 for periods of 10 and 20 minutes daily for 15 days. The animals were irradiated in an anechoic chamber designed to insure uniform power distribution. Following the last exposure blood serum was analyzed for TBC using an I-125 radioimmunoassay procedure and the results were normalized with a human serum standard. Analysis of the data indicated a significant increase in the TBC index at the 25 mW/cm^2 level. The results suggest that thyroid hormone levels may be affected by chronic microwave exposure.

Ultrastructural Observations on Endocytosis (Secondary Vacuolation) in Plant Cells, Paul G. Mahlberg and F. R. Turner, Department of

Plant Sciences, Indiana University, Bloomington, Indiana 47401.—— The plasma membrane of plant cells possesses invaginations, or secondary vacuoles, of variable size that project into the adjacent cytoplasm. Secondary vacuoles which were present in thin sections of cells at different phases in vacuolation were numerous in some cells while fewer in others. In vacuolated cells enlarged secondary vacuoles protrude into the primary vacuole but are delimited from the tonoplast by an intermembrane zone of variable width. The plasma membrane at the orifice of an invagination may fuse and detach the secondary vacuole from the membrane to form a structure in the cytoplasm bounded by a single membrane. Complex accumulations of membranes consisting of spherical, tubular, and laminar structures, possibly containing cytoplasm, may develop within secondary vacuoles. Contents of many of these vacuoles arise from folds along its limiting membrane which pinch off into the interior of the secondary vacuole. Measurements of the secondary vacuoles attached to and detached from the plasma membrane are significantly similar to those of the plasma membrane and differ significantly from other cytomembranes (dictyosome vesicles, endoplasmic reticulum, mitochondrion outer membrane, tonoplast). The width of the membranes of the contents within secondary vacuoles indicates that most of these contents are derived from the plasma membrane. A selective stain, reportedly specific for the plasma membrane, stains attached and detached secondary vacuoles and their membranous contents as well as the plasma membrane, and suggests that secondary vacuoles are derived from the plasma membrane. Endocytosis appears to occur in various cells of the plant body and may represent a phenomenon of general occurrence in many plant cells.

Cadmium Inhibition of Renal Amino Acid Transport. THOMAS H. GIESKE, Department of Biology, Indiana University, South Bend, Indiana 46615. ——Properties of amino acid transport systems at the peritubular (PTM) and luminal membranes (LM) of the proximal convoluted tubule of control (mercaptoethanol) and cadmium-mercaptoethanol (2.5 μmoles Cd and 200 μmoles mercaptoethanol) treated rabbits were studied. Mutual inhibition studies led to the conclusion that at least four separate systems are present for transport of L-amino acids at the PTM. These systems are similar in their substrate specificities to those at LM, which are responsible for reabsorption from the filtrate in the proximal tubule. However, the mechanisms at the PTM show a different pattern of sensitivity to inhibition by cadmium. At the PTM cadmium inhibits transport of the dicarboxylic amino acids without significantly depressing transport of neutral and basic amino acids. In contrast, cadmium strongly depresses transport of all amino acids at the LM. The results support the hypothesis that peritubular transport plays no role in renal reabsorption of amino acids. The physiological significance of the peritubular systems remains unclear.

Light-induced Ultrastructural Change in the Protein Body of Mung Bean Plastids. WILLIAM J. HURKMAN, Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47906.——The etiolated primary leaf of mung bean contains plastids which are modified exclusively for protein storage (proteoplasts). The proteoplast has a

single protein body 3 um in diameter containing a granular matrix and bounded by a single membrane. Proteoplasts of this type are located only within a one-celled layer separating the meristematic leaf mesophyll cells.

Differentiation of the proteoplast containing layer occurs within a few days after germination. Proplastid precursors are distinguished by the development of an electron-transparent tubular complex. Eventually the tubular complex swells to form a single protein body. Development of the proteoplast-containing zone is maximal in 6 days of darkness.

Upon illumination the proteoplast can divide and acquire starch. The protein body also divides repeatedly and blackens. Within two days the shrunken proteoplast remains are deposited on the cell wall. During this time adjacent cells enlarge and develop chloroplasts of normal structure. Over a ten-day period, in the absence of light, there is little change in proteoplast fine structure.

Glycosyl transferases of ganglioside biosynthesis in rat liver hyperplastic nodules and hepatomas induced by N-2 flourenylacetamide. WILLIAM D. MERRITT, T. W. KEENAN, and D. JAMES MORRÉ, Departments of Biological Sciences, Animal Sciences, and Botany and Plant Pathology, Purdue University, Lafayette, Indiana 47907.——Hyperplastic liver nodules and hepatomas were induced in rats using a low-protein diet containing 0.05% N-2 flourenylacetamide. Nodules and hepatomas from animals on the diet for varying times were excised, measured and weighed. Portions of each tissue were fixed in formalin and the degree of malignancy was determined from hematoxalin and eosin-stained sections. Remaining portions of the nodules or hepatomas were homogenized in 0.32 M sucrose containing 14 mM mercaptoethanol, and the total particulate fraction was analyzed for levels of 5′-nucleotidase and glycosyl transferases of ganglioside biosynthesis. In non-malignant and hyperplastic nodules, an increase in the activities of G_{M^2}:UDPGal galactosyltransferase and G_{M^1}:CMP-NANA sialyltransferase was directly correlated with the size of the nodule. In hepatomas, the levels of these enzymes were directly proportional to the degree of malignancy of the tissues. The level of AMPase was inversely related to the size of hyperplastic nodules but directly proportional to the degree of malignancy in hepatomas. Total, bound and ganglioside sialic acid were increased in hepatomas relative to control tissue; levels in hyperplastic nodules were intermediate between hepatoma and control levels. The results are consistent with the theory of the progressive nature of alterations of cellular functions during the course of tumorigenesis.

Work supported in part by a grant from the National Institutes of Health CA13145.

Molecular Weight Differences in Polypeptides From Type A and B Trichomonad Costae. FLORENCE JUILLERAT, Indiana University-Purdue University, Indianapolis, Indiana 46202.——Cells of *Tritrichomonas augusta* were lysed in Triton X-100, sonicated, and centrifuged at 270 x g to yield pellets of isolated costae. Washed pellets of the organelle contained approximately 1.6% of the total cell protein. Sodium

dodecyl sulfate electrophoresis in 5% polyacrylamide gels revealed one major polypeptide band with a molecular weight of 143,000 daltons. Five minor bands at 119,000, 109,000, 102,000, 96,000 and 34,000 were seen. The periodate-Schiff test revealed no glycoprotein in the gels. No ribonucleic acid was detected with methylene blue staining.

Costae isolated from other trichomonads for comparative studies required a 3020 x centrifugation to pellet organelles. Electron micrographs of *Trichomonas suis* costa pellets revealed organelles with Honigberg's type A periodicity. Samples of known type A costae were also obtained from *Tritrichomonas foetus* and *Trichomitus batrachorum.* Sodium dodecyl sulfate gel polypeptide patterns of costae from all three organisms were similar to the pattern of *T. augusta.*

Electron micrographs of isolated *Trichomonas gallinarum* costae revealed a striation pattern resembling that previously seen in Honigberg's type B costa of *Trichomonas gallinae.* Gel electrophoresis of costae from these two organisms showed a major polypeptide component with a molecular weight of 122,000 daltons. The pattern of minor bands was also different from that seen is gels of Type A costae.

These studies have begun the dissection of the complex electrophoretic pattern of polypeptides visible in sodium dodecyl sulfate gels of isolated trichomonad mastigont systems. These comparative investigations also illustrate the usefulness of this technique for biochemical studies of the evolution of trichomonads, complementing the many electron microscopic papers that have been published.