CELL BIOLOGY

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Ultrastructure of the Anal Organ of the Larva of *Drosophila melanogaster*. Mohinder S. Jarial, Department of Physiology and Health Science, Ball State University, Muncie, Indiana 47306.——The anal organ of full-grown larva of *Drosophila melanogaster* (Oregon R, wild type) consists of two symmetrical plates located ventrolaterally on either side of the anus. Each plate consists of a thin cuticle which is closely applied to the underlying single layer of large, cuboidal, epithelial cells. The apical (external) plasma membrane of the epithelial cells is highly infolded and the infoldings are covered by a particulate coat on the cytoplasmic surface. The mitochondria lie adjacent to the apical region and small ones occassionally penetrate between the infoldings. On the basal surface (facing the haemocoele) the plasma membrane is also infolded with large mitochondria in close association to the membrandes. A thin basement membrane separates the epithelial cells from the haemocoele. The lateral borders of the cells are bridged by septate desmosomes. The cells are rich in mitochondria and contain rough endoplasmic reticulum and numerous multivesicular bodies. Tracheoles ramify the cells and penetrate deeply into the basal region.

When larvae are treated briefly with 1% silver nitrate solution, the anal organ becomes darkly stained and the silver grains after passing through the cuticle reach the apical regions of the eipthelial cells.

The observed ultrastructural features of the anal organ are characteristic of transporting epithelia and are consistent with an osmoregulatory function.

Role of a Calcium Binding Ribonucleoprotein in the Ontogenesis of Electrical Activity in the Developing Chick Embryo Brain. Z. Iqbal and N. C. Sharma, Indiana University School of Medicine, Indianapolis, Indiana 46202.——Electrical activity in the developing chick embryo brain has been found to appear around the 4th day and matures by 12th day of incubation. Our laboratory has been engaged in the evaluation of some key cellular components (Iqbal and Talwar, J. Neurochem. **18**:1261, 1971; Talwar and Iqbal *In* Macromolecules and Behavior, ed. J. Gaito, 1972) involved in the manifestation of this functional characteristic in the developing chick embryo brain. In this report, we present the data concerning an organ specific calcium binding ribonucleoprotein (NP) in relation to the development of electrical activity in growing chick embryo brain. NP purified from goat brain according to the method of Sharma (J. Sci. Industr. Res. **33**:662, 1974) was used as a marker and the brain extracts from chick embryos of various ages were anlayzed by

electrophoresis on 7.5% acrylamide gels. The protein in the chick embryo brain identical to NP as regards to its molecular weight and immunological properties appeared around the 6th day of incubation and increased in quantity with age of the embryo reaching a plateau on day 12. A parallelism between the onset and maturation of the electrical activity and the development of NP suggests that this protein is involved in the manifestation of the electrical activity in the developing chick embryo brain, probably by regulating the concentration of Ca^{2+} in the tissue.

Toxic Effects of the Vinca Alkaloids on Cultured Rat Midbrain: Correlation with Clinical Neurotoxicity. KATHLEEN L. KING and GEORGE B. BODER, Department of Cell Biology, Lilly Research Laboratories, Indianapois, Indiana 46206.——The three Vinca alkaloids currently in use as cancer chemotherapeutic agents differ in their clinical neurotoxic potential. Neurotoxicity is the dose limiting factor for Vincristine, but it is a less serious problem with Vinblastine. Vindesine appears to be intermediate. These effects may reflect pharmacokinetics and microtubular binding in nervous tissue and tumor cells. A system suitable for studying the factors underlying clinical differences and predictive of neurotoxicity would augment the development of novel non-neurotoxic Vinca molecules. Rodent animal models are inadequate for the study of this neurotoxicity because Vincristine produces a severe myopathy rather than neuropathy. Differences in pharmacokinetics can be avoided by studying the direct effects of the Vinca alkaloids on primary cultures of neuronal and glial cells. Vincristine at a dose as low as 0.004 μg/ml affects the cells with processes in cultures of dissociated newborn rat midbrain. In 3 day old cultures after 24 h of drug treatment, there is a loss of processes and swelling of the cell body. We have used this observation as the basis for a quantitative assay of the toxicity of a series of Vinca compounds, and have found that for a dose range of 0.1 to 0.004 μg/ml the relative toxicity of Vincristine, Vinblastine and Vindesine in this system correlates with their relative clinical neurotoxicity. Validation of the predictive elements of this system awaits clinical experience with novel Vinca compounds.

The Effect of Endurance Training on the Ultrastructure of Diabetic Skeletal Muscle. LARRY R. GANION and DAVID COSTILL, Ball State University, Muncie, Indiana 47306.——To study the effect of endurance training on the morphology of the skeletal muscle fiber of the diabetic, muscle samples were obtained by needle biopsy from the gastrocnemius of subjects at the commencement and completion of a ten week training program and prepared for electron microscopy. The participants ran five days per week at a stress level of 75% of their maximum work load. The muscle samples were immersed in 3% glutaraldehyde for four hours, rinsed in 0.1M Sorenson's phosphate buffer (pH 7.2), postfixed for one hour in 1% osmium tetroxide, and embedded in Epon 812. Ultra-thin sections were cut on a Porter-Blum MT-2B ultramicrotome, stained with uranyl acetate and lead citrate, and viewed with an RCA EMU-3C electron microscope. Endurance training does alter the fine structure of the diabetic skeletal myofiber. These alterations include: a decrease in gylcogen particles, a definite increase in lipid vacuoles, and mitochondrial swelling. Dense

bodies are frequently seen at the periphery of the untrained diabetic myofiber. These pleomorphic structures, composed of aggregates of electron dense particles and lipid droplets, appear to decrease in number as a result of training. These morphological observations support the conclusion that training effects the physiology of the diabetic myofiber, particularly lipid metabolism. The significance of the dense body reduction in trained muscle is not known and merits further study.

Effects of Cytochalasins on Growing Fungal Hypahe. J. A. SWEIGARD, B. L. HOLAWAY and S. N. GROVE, Goshen College, Goshen, Indiana 46526.——Cytochalasin B(CB) at 50-100 μg/ml inhibits germination and hyphal growth in *Gilbertella persicaria*. When sporangiospores are germinated in the presence of CB the initial spherical growth (swelling) stage occurs as expected but the orderly initiation of germ tubes (polar growth stage) and continued hyphal growth does not occur. The germlings continue to enlarge (swell) and become giant cells sometimes with bulbous outgrowths instead of germ tubes. If CB is introduced after germ tube initiation subsequent growth results in knob-like swellings at the tips of existing structures. Our light and electron microscopic observations along with evidence from the literature may lead to a clarification of parts of the current model for hyphal tip growth. The model proposes that secretory vesicles produced in the subapical regions migrate to the apex and deposit the necessary materials for polarized growth but until now we have been unable to explain the mechanisms of this migration. If cytochalasins are active against actin microfibrils in fungal hyphae then any actin-dependent vesicle migrations would cease in the treated germlings. The result would be a non-polarized, spherical type of growth of the type we have observed. Therefore, we postulate that the vesicle transporting mechanism is actin dependent in these tip growing fungal hyphae.

Cytidine 5'-Monophosphosialic Acid Synthetase from Rat Liver. KIM E. CREEK, DIANE WEISMAN and D. JAMES MORRÉ, Department of Biological Sciences and Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907.——The incorporation of sialic acid into sialic acid-containing polymers, such as glycoproteins and glycolipids, requires CMP-sialic acid as the sialic acid donor. Cytidine 5'-monophosphosialic acid synthetase catalyzes the synthesis of CMP-N-acetylneuraminic acid (CMP-NAN) and inorganic pyrophosphate from cytidine triphosphate (CTP) and N-acetylneuraminic acid (NAN or sialic acid). Two assay methods were used to monitor the activity of CMP—NAN synthetase in isolated rat liver organelle fractions. A modified method of the thiobarbituric acid assay of Warren (J. Biol. Chem. 234, 1971, 1959) allowed the determination of CMP-NAN in the presence of large quantities of unreacted substrate. A second assay procedure used ^{14}C-NAN and separation of products and substrate by descending paper chromatography. Kinetic properties of CMP-NAN synthetase determined by either or both procedures may provide clues to the mechanisms that regulate the levels of sugar nucleotide within the cell and further our understanding of the processes involved in the biosynthesis of sialic acid-containing heteropolymers. Supported in part from a grant from the NIH CA 18801.

Does Cytindine 5'-Monophosphosialic Acid Synthetase Exist in Golgi Apparatus Isolated from Rat Liver? K. E. CREEK, D. WEISMAN and D. J. MORRÉ, Department of Biological Sciences and Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907.——Cytidine 5'-monophosphosialic acid (CMP-NAN) synthetase is widely distributed in animal tissues. A report by Kean (J. Biol. Chem, 245, 2301, 1970) has shown that the predominant subcellular location of the enzyme is the nucleus. If the sole location of the enzyme is the nucleus, a problem of cellular logistics arises since the predominant cellular location of the sialyltransferases of CMP-NAN utilization is the Golgi apparatus. An investigation was conducted to determine if Golgi apparatus isolated from rat liver exhibited significant CMP-NAN synthetase activity. Golgi apparatus incubated in the presence of 5 mM NAN and 5 mM CTP synthesized CMP-NAN at a rate of 45 nmoles/hr/mg protein. This activity apparently is not due to contamination of the isolated Golgi apparatus by cellular organelles other than nuclei. Studies are in progress to determine by several independent procedures more precisely the levels of potential contamination of the Golgi apparatus fractions by nuclear material. Supported in part by a grant from the NIH CA 18801.

Histological and Growth Characteristics of Transplantable Hepatomas and Squamous Cell Carcinomas Induced in the Rat by the Carcinogen N-2-Fluorenylacetamide. T. M. KLOPPEL, L. B. JACOBSEN, D. M. MORRÉ, P. FINK and D. J. MORRÉ, Departments of Biological Sciences and Foods and Nutrition and Purdue Cancer Center, Purdue University, West Lafayette, Indiana 47907.——Hepatocellular carcinomas and squamous cell carcinomas were induced by oral administration of the carcinogen N-2-fluorenylacetamide. Tumors arose 6 to 10 months following carcinogen ingestion when fed at a level of 0.025% for 16 weeks. Tumors were removed aseptically and transplanted subcutaneously to a syngeneic recipient or processed for tissue culture. The cytological and histological observations were contrasted and compared with growth rates and the ability to metastasize. The squamous cell carcinomas appeared as highly differentiated, fast-growing, keratinizing and non-invasive tissues which were readily cultured and transplanted back into the animal. The hepatomas were more difficult to grow in tissue culture and to transplant back into the animal. Hepatomas varied from poorly to highly differentiated and some showed metastasis to the lungs. Growth rates were variable and corresponded somewhat to degree of anaplasia. Work supported in part by a grant from the NIH CA 18801 and an Institutional Grant from the American Cancer Society.

The Effect of Electrically Induced Hyperthermia on Transplantable Tumors in the Rat. J. PEARCE, T. M. KLOPPEL, D. GOETZ, K. E. CREEK, J. WALTER, L. A. GEDDES, M. J. FREEMAN and D. J. MORRÉ, Biomedical Engineering Center, Institute of Interdisciplinary Engineering Studies and Purdue Cancer Center, Purdue University, West Lafayette, Indiana 47907.——Subcutaneous-transplantable hepatomas and squamous cell carcinomas originally induced in the rat by feeding N-2-fluorenylacetamide were treated with radio frequency electrical current at 500 KHz to induce hyperthermia. A typical current of 100 mA was required to establish a tumor temperature of 42 to 44 C. Electrical

contact was by means of gelled deformable electrodes. Temperature was monitored by an electrode contact thermistor, a subcutaneous thermocouple, and a calibrated thermograph. For each treatment, a temperature between 42 and 44.5 C was reached within 4 min. and was maintained for 11 min. Animals received 1, 2 or 3 treatments at 2 day intervals. Growth rates of various treated and untreated tumors were compared. The transplantable squamous cell carcinoma appeared to be more sensitive to heat than were the hepatomas. In some animals the treatments were tumoristatic, while there was complete remission in a few animals. Histological examination revealed necrotic areas in treated tissue with neutrophilic infiltration. The size of tumor mass at the time of initiation of the treatments seemed to influence the overall success. Smaller tumors responded most consistently to treatment. Supported in part by grants from the Indiana Elks and an Institutional Grant from the American Cancer Society.

The Distribution of Anionic Sites on Brush Border Membranes of Neonatal Rat Intestinal Absorptive Cells. RALPH A. JERSILD, JR., Department of Anatomy, Indiana University Medical Center, Indianapolis, Indiana 46202.——The distribution and behavior of anionic sites on the microvillous surface of newborn rat jejunal absorptive cells were studied using polycationic ferritin (PCF) as a visual probe, and compared with anionic sites previously described for adult intestine. Intestinal segments from 1 to 22 day old rats were incubated in PCF prior to fixation and preparation for electron microscopy. Anionic sites were randomly distributed along the length of microvilli. These sites did not show evidence of translational mobility within the membrane. In contrast, brush borders examined from animals at weaning (about 22 to 26 days) resembled those from the adult in which receptor sites were capable of mobility, and were induced by PCF to cluster into discrete patches. The random pattern of neonates was not affected by colchicine or cytochalasin B. It was altered by injections of cortisone which parallelled the premature cessation of pinocytosis and alternation of enzyme patterns described for glucocorticoids by others. It is suggested that the difference in mobility of anionic sites with age reflects the difference in absorptive function in the intestine of suckling and adult animals.

The Effects of Calcium Deficiency and Calcium Replacement by Strontium on Cytokinesis in Cultured Plant Cells. JESSE WOOD* and CHARLES W. GOFF, Indiana State University, Terre Haute, Indiana 47809.——The effects of calcium (Ca^{2+}) deficiency and calcium replacement by strontium (Sr^{2+}) on growth and cell plate formation in cell suspension cultures of *Dacus carota* were studied. Ca^{2+} deficiency has been shown to inhibit fusion and/or coalescence of the vesicles which form the cell plate. In the absence of Ca^{2+}, Sr^{2+} has been shown to "detoxify" a nutrient solution in a manner similar to Ca^{2+}, but Sr^{2+} is reported to have minimal nutritional value. *Dacus carota* were grown in a modified Murashiege-Skoog's media (Eriksson, 1965). The media water was distilled twice and run through a mixed bed deionizing column. The conductivity measured 0.9×10^{-6} MHO. Ca^{2+} deficiency was induced by omission of Ca^{2+} from the nutrient media. For media with Sr^{2+} replacing Ca^{2+}, Sr^{2+} was added at the concentration that Ca^{2+} normally is supplied (120 ppm). Short term effects for a four day period were monitored. Micrographs of Ca^{2+} deficient cells show

inhibition of cell plate formation as reported earlier. However, micrographs of cells which have Sr^{2+} replacing Ca^{2+} show no inhibition of cell division. Sr^{2+} treated cells continue to divide after repeated transfers into media with Sr^{2+} replacing Ca^{2+}. Whether Sr^{2+} substitutes for Ca^{2+} or simply controls the toxic effects (ion imbalance?) of Ca^{2+} deficiency, thus allowing normal function with extremely low levels of contaminating Ca^{2+} from the chemical stocks, is not known.

The Effects of Caffeine on Certain Growth and Metabolic Parameters in *Allium cepa* L. T. K. SPAULDING, S. P. BREHM and C. W. GOFF, Indiana State University, Terre Haute, Indiana 47809.——The effects of caffeine on the mitotic index (MI) and on macromolecular synthesis, and the rate of uptake, retention, and metabolism of caffeine by onion root tips were studied. Caffeine causes both long and short term reduction of the MI. This effect is concentration dependent and reversible. The optimal caffeine concentration (0.1%) caused a 40-50% reduction in the MI after 1 hr treatment and an 80% reduction by the end of 12 hr continuous treatment. No further reduction was noted at the end of 24 hr treatment. Roots treated for 3 hr with 0.1% caffeine, then placed in distilled water for recovery, showed a marked decrease in MI at the end of treatment but complete recovery within 3 hr. A marked inhibition in the rate of DNA and RNA synthesis was noted during a 3 hr treatment with 0.1% caffeine, but the rate of protein synthesis showed no significant difference when compared to control rates. The uptake, retention, and metabolism of caffeine were studied using H^3-caffeine. Total uptake reached saturation levels within 1.25 hr. A 1 hr treatment with 0.1% caffeine followed by 3 hr continuous rinsing with distilled water showed no caffeine present at the end of 3 hr, but a marked presence of radioactivity. This suggests the caffeine was rinsed from the root and the radioactivity present in the post rinse homogenate represents incorporation of radioactive metabolites of caffeine. An attempt to identify these metabolites eliminated commonly accepted breakdown products of caffeine.

Novel Cells in the Uterus During Pregnancy. S. A. RHINE, Division of Medical Genetics, Methodist Hospital, Indianapolis, Indiana, A. MILUNSKY and P. STUBBLEFIELD, Harvard Medical School, Boston, Massachusettes and G. G. PALMER, Department of Medical Genetics, Indiana University, School of Medicine, Indianapolis, Indiana 46202.——A sampling device and laboratory procedures have been established for obtaining trophoblastic (fetal) tissue from the internal cervical os of the uterus during the first trimester of pregnancy. This potentiates the possibility of performing prenatal analysis concerning the genetic well being of the fetus via the endocervical canal as an earlier alternative to amniocentesis.

Phase contrast analysis of the tissue obtained consistently yields four types of cells which have not, to our knowledge, been described previously.

1. Epithelioid polygonal cells which usually occur in mosiac sheets.

2. Large singular ameboid cells which actively phagocytize erythorocytes and cellular debris.

3. Small fibroblastic amebocytes which migrate from the tissue fragments and occur in clusters with no obvious cell-to-cell contact.

4. Variable size, anucleate, multivacuolated giant 'cells', some which measure over 2100 μm in diameter.

Procedures have now been developed for the routine isolation of the cell types. Further study into the elucidation of their origin may yield new insights into the anatomy and physiology of early human pregnancy.