

CELL BIOLOGY

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ABSTRACTS

The Effects of a Fish Oil Diet on Rat Lipoproteins. DANIEL BELCHER, WILLIAM STILLWELL, STEPHEN WASSALL AND CAROL LANGSFORD, Department of Biology and Physics, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.—Rats were fed identical diets supplemented with hydrogenated coconut oil (HCO) or with menhaden oil (MO), a fish oil rich in N-3 long chain, polyunsaturated fatty acids. High density lipoproteins (HDL) and low density lipoproteins (LDL) were isolated from plasma, and fluidity and order were determined as a function of fatty acyl chain position in the lipoprotein monolayer, by fluorescence polarization and electron spin resonance (ESR). The HDL study showed a large difference in monolayer fluidity in the center region of the fatty acyl chains with the MO diet HDL being substantially less fluid than the HCO diet HDL. A parallel study with LDL indicated a small difference in monolayer fluidity in the center of the chains with the MO diet LDL being less fluid than the HCO diet LDL. These results were further substantiated by molecular modeling.

The Production of Accessory Limbs in Axolotls. MARGARET W. EGAR, Department of Anatomy, Indiana University School of Medicine, Indianapolis, Indiana 46223.—Accessory limbs may be the result of several types of trauma in the salamander. The stimulus of implants of various kinds are all made more successful if a large nerve is deviated to the wound site. In the present experiments, a high yield of ectopic limbs was sought with nerve deviation alone. Success was achieved when a 2 mm patch of skin was removed at the proximal site of the deviated nerve. However, not all resulting growths continued development.

Microscopic examination (light, TEM and SEM) of the early blastemata revealed an accumulation of mesenchymal type cells that was continuous with the deviated nerve. The accessory blastema differed from that which forms at an amputation stump in having a central core of axons with capillaries and debris intermingled. This work was supported by a grant from the American Cancer Society, Ohio Division, Cuyahoga County Unit.

Fine Structure of the Labium of *Cenocorixa bifada* in Relation to Ionic Transport. MOHINDER S. JARIAL, Center for Medical Education, Ball State University, Muncie, Indiana 47306.—The labium of water boatman *Cenocorixa bifada* was studied by light, scanning and transmission electron microscopy. The triangular labium dorsally bears a deep groove flanked by a series of cuticular plates. Each plate bears two rows of crescent shaped depressions. The cuticular depressions in one row are placed in between those of the other row. The epithelium underlying the cuticle consists of narrow, elongate cells. A sinus containing particulate material exists between the cuticle and the epithelium. The

basal plasma membrane of the epithelial cells exhibits extensive infoldings that are closely associated with large mitochondria. These infoldings on the cytoplasmic side are covered by a particulate coat. The epithelial cells have centrally placed nuclei and their cytoplasm is rich in ER, mitochondria, vesicles and dense bodies. Their apical membranes are thrown into delicate invaginations. The large intercellular spaces contain vesicles and electron dense material. The labium takes up silver ions from dilute silver nitrate solution and becomes darkly stained. On the basis of these observations, the labium of *Cenocorixa bifada* is worthy of consideration as the site of ionic transport.

Kinetic Assay for Primary Cytotoxic T-Lymphocyte Clones. LAURA J. JENSKI, Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223 and Beth Miller, Children's Hospital Research Foundation, Cincinnati, Ohio 45229.—Cytotoxic T-lymphocytes (CTL) aid in our defense against disease by killing abnormal cells such as virus-infected and cancer cells (targets). CTL and targets interact with an affinity amenable to analysis with methods designed for enzymes. We have developed a kinetic assay for primary murine CTL clones generated *in vitro*. We have shown that several assumptions underlying the Michaelis-Menten equation can be validated experimentally, and that there appears to be adequate justification for the use of " K_m " to estimate affinity. In normal naive spleen cell populations, we have found a broad range of affinities expressed by CTL clones responsive to foreign histocompatibility antigens. Selection or expansion of CTL clones with high target affinity, illustrated by a predominance of clones with small " K_m ", may in some instances play an important role in generation of protection immunity.

Effect of Pentoxifylline on ESR Measured RBC Fluidity. F.W. KLEINHANS, D.D. NGUYEN AND S.T. BAREFOOT, Department of Medical Research, Methodist Hospital, Inc. of Indianapolis, Indianapolis, Indiana 46202 and Department of Physics, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.—Pentoxifylline (Trental) is a hemorheologic agent useful for the treatment of patients suffering from intermittent claudication and is believed to work in part by increasing the flexibility of erythrocytes. Previous *in vitro* studies in which red blood cells were forced through capillary tubes demonstrated an increased flow rate after treatment of the RBC with Trental. Our purpose in the electron spin resonance experiments was to duplicate the previous *in vitro* treatment protocols and determine whether there were any detectable membrane modifications using ESR spin label techniques. 5 doxyl steric acid was used which is sensitive to modifications of the lipid regions of the RBC membrane. Cells were held in Hepes buffer (+ 8 mM Ca + +) and treated with 10 to 100 $\mu\text{g}/\text{ml}$ of Trental for 30 to 150 min. No differences ($\Delta S > 0.007$) were seen in the 5DS order parameter, S. These results suggest that Trental may act on some other component of the RBCs such as the structural proteins associated with the lipid bilayer.

The Developmental Toxicity of Nicotine on the Planula of the Marine Hydrozoan, *Halocordyl disticha*. KATHLEEN J.S. KOLBERG AND VICKI J. MARTIN. Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556.—Nicotine concentrates around the embryo and fetus in mammals and its developmental toxicity is of interest. This study examines the effects of nicotine on developmental processes in a simple invertebrate system, hydrozoan embryos. Nicotine administered at gastrulation and after development of the first nerve cells caused several developmental changes: 1) alteration in the ratios of derivatives of interstitial stem cells, 2) reversal of phototactic behavior, 3) premature accelerated metamorphosis, 4) the polyps formed by the early, rapid metamorphosis showed several morphological abnormalities: irregular tentacle spacing, stunted tentacles and wrinkled head surface. The last two effects were mimicked

by exposing mature control planulae to a low dose of exogenous norepinephrine. Planulae tested with sucrose-phosphate-glyoxylic acid exhibited a fluorescence indicative of catecholamines. Treatment of planulae with several neuroactive drugs substantiated the presence of endogenous catecholamines. The effect of nicotine on metamorphosis could be mediated by the release of endogenous catecholamines. This study was funded by research grants from the Indiana Academy of Science.

The Effects of Vitamin C on Histone Acetylation and Phosphorylation in Mouse Fibroblasts. KYOUNGSOOK PARK AND DAVID A. PRENTICE, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—Antioxidants such as vitamin C (L-ascorbic acid, AA) show protective effects on cellular aging and enhance cell growth *in vitro*, but the mechanism of action is unknown. One mechanism may be alteration of gene expression. Because changes in chromatin structure via histone modifications can affect gene expression, mouse fibroblasts (L cells) grown in serum-free medium were treated with AA to investigate its effect on histone modifications. AA (50 $\mu\text{g}/\text{mL}$) did not affect L cell growth. Cells were labeled with [^3H] lysine or [^{14}C] lysine to label histone proteins and then pulse labeled with [^{32}P] phosphate or [^3H] acetate to detect phosphorylation or acetylation, respectively. Extracted histones were resolved by SDS-PAGE, gel lanes sliced and labels detected by double-label liquid scintillation counting. Phosphorylation of histones H1 and H2A decreased in AA-treated cells, while acetylation of histones H3 and H4, as well as that of H2A and H2B, increased. Based on these results, one of the effects of AA may be alteration of histone modifications such as phosphorylation and acetylation thereby altering chromatin structure and gene expression.

Effect of Dietary Stearate and Linoleate on Prostaglandin Concentrations of Plasma in Normal and Tumor-bearing Strain A/ST Female Mice. TIMOTHY A. TRUE AND ALICE S. BENNETT. Department of Biology, Ball State University, Muncie, Indiana 47306.—Prostaglandins (PGs), more specifically PGE₂, have been implicated in the evolution of breast cancer and may indicate the metastatic potential of that cancer. Dietary linoleic acid (18:2), a precursor of PGs, promotes the formation of mammary tumors whereas stearic acid (18:0) decreases tumorigenesis. The mechanisms involved in the promoter or inhibitor action are not known. In this study the effect of dietary 18:0 and 18:2 on PG concentrations of plasma removed from normal and mammary tumor-bearing female Strain A/ST mice was examined. Four high fat (15%) and two low fat (5%) diets containing varying percentages of 18:0 or 18:2 were used in this study. PGE₂ concentrations were determined by HPLC analysis of their panacyl PG ester derivatives, using a fluorescent detector. Variations of PG levels in plasma from mice fed experimental diets will be presented. Supported in part by a Sigma Xi Grant.

Phytohemagglutinin Binds to the Same Sites in the Ciliate *Stentor* as Does Con A. PATRICIA R. WALSH AND MICHAEL S. MALONEY, Department of Biological Sciences, Butler University, Indianapolis, Indiana 46208.—The plant lectins Con A and PHA are known to delay oral regeneration in the ciliate *Stentor coeruleus* and Con A appears to do so by binding to ciliary membranes only. Studies were initiated to determine whether PHA, which has effects identical to Con A, binds to the same sites on the cell surface. Cells were fixed and then exposed to 100 $\mu\text{g}/\text{ml}$ of FITC-PHA for 30 min. Observations of these cells by fluorescence microscopy showed that PHA bound to the membranellar and body cilia exactly as Con A did. Furthermore, when regenerating cells were treated in the same manner, FITC-PHA also bound to the developing oral primordium as did Con A. Despite the similar binding patterns of the two lectins, α -methyl mannoside could not reverse the binding of FITC-PHA. Supported by NSF and a Penta Corporation Grant of the Research Corporation.

