CELL BIOLOGY

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ABSTRACTS

Effects of Retinoids on Phospholipid Bilayers. Mark Albrecht and William Stillwell, Department of Biology and Stephen R. Wassall, Department of Physics, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.——Retinoids representing each of the three oxidation states (all trans-retinol, retinal and retinoic acid) were incorporated into phospholipid bilayers and the effects on membrane permeability, acyl chain motion and phase transition temperature studied. Retinoic acid was shown to greatly enhance permeability of PC (phosphatidylcholine) bilayers to the non-electrolyte erythritol and to the fluorescent anion carboxyfluorescein. Retinol and retinal enhance permeability to a much less extent. This distinction between retinoid enhancement of permeability correlates with differences seen in the effects on ESR (electron spin resonance) order permaeter S and phase transition temperature T_c.

Development of the Neostriatum in the Rat Brain. Shirley A. Bayer, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.——Neurogenesis in the rat neostriatum was examined with [^{3}H] thymidine autoradiography, a quantitative method which allows the exact determination of the proportion of neurons originating in a given population over a 24 hr. period. The medium-sized neurons throughout the neostriatum are generated in a prominent ventrolateral-to-dorsomedial gradient so that ventrolateral cells originate mainly between embryonic days (E)14 and E18, dorsomedial cells between E18 and E21-22. Fewer than 10% originate between birth and postnatal day 4. Medium-sized neurons also show two other gradients. First, there is a superficial-to-deep gradient in the anterior part of the caudoputamen, while more posterior levels have a gradient in the opposite direction. Second, anterior parts have a caudal-to-rostral gradient while posterior parts have an opposite gradient. These shifts in neurogenetic gradients between anterior and posterior parts is developmental evidence that an anterior "caudate" can be separated from a posterior "putamen" in the rat. Finally, neurogenetic gradients in the medium sized caudoputamen neurons can be linked to the patterns of their anatomical interconnections with the substantia nigra.

A Comparative Study of the Hamster Kidney Cell Line BHK-21 (C-13) and Normal Hamster Kidney Tissues. Angela M. Burke and Pang Fai Ma, Center for Medical Education, Ball State University, Muncie, Indiana 47306.——Adenosine deaminase catalyzes the deamination of adenosine to inosine in the purine degradative pathway. Multiple molecular species of the enzyme have been found to be diversely distributed among the tissues of lower vertebrates and mammals. These molecular forms correspond to molecular weights of 200,000, 100,000 and 35,000 and have been designated as forms A, B and C,

respectively, estimated by gel filtration. All three forms appear to be prevalent among the tissues of lower vertebrates, while only the A and C form enzymes have been found in studies done on higher mammals. Previous studies have indicated that an increase in the activity of adenosine deaminase may be associated with some pathological conditions. In cancerous lung tissues both enzyme forms are present with an elevated ratio of forms C to A indicating an increase in the level of the C form with little or no A form present. This is in comparison with normal lung tissue in which there exists a much smaller such ratio. In this report, we present the results of a study done on the hamster kidney cell line BHK-21 (C-13) and normal hamster kidney tissues. The purpose of this study is to determine the enzyme activity and the relative proportions of the A and C form enzymes in the two systems mentioned.

Adhesion and Expansion of Epithelial Cell Sheets. E.A.G. CHERNOFF, Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.——Intact chick extraembryonic epiblasts were cultured on the inner face of the vitelline membrane in a chemically-defined serium-free medium. Epiblasts require the addition of hydrocortisone to attach, but will expand without it. Fusion occurs when edges contact each other. Microfilament organization (visualized by rhodamine-phalloidin fluorescence) changes abruptly between the specialized adhesive edge region and the unattached interior of the cell sheet. Flat (Type I) and ridged (Type II) transition regions are found, and may correlate with specific phases of the expansion process. Small microfilament-containing sites of substratum attachment are revealed by detachment of the epiblast. No exogenous fibronectin or laminin is required for adhesion and the vitelline membrane contains neither adhesive molecule (immunofluorescence, PAGE). The vitelline membrane inner face was separated from other layers using a freeze-thaw process. Disulfide-bond reducing agents are required to dissolve the membrane. Chemical analysis using PAGE and a color-based silver stain shows three principal components.

Identification of Members of Species-groups of Genus *Paramecium* with Fluorescent, DNA-binding Dyes. THOMAS A. COLE, WILLIS H. JOHNSON AND RUCHIR SEHRA, Wabash College, Crawfordsville, Indiana 47933.——Within genus *Paramecium*, members of three species-groups are cylindrical and long, with tapered ends. Species-group *P. aurelia* is distinguishable, on the basis of size, from *P. caudatum* and *P. multimicronucleatum*, which overlap in size and are greater than 180 uM long. *P. caudatum* contains one micronucleus and *P. multimicronucleatum* contains four (or more) micronuclei. Staining with visible dyes is somewhat problematic. Recent confusion on species-group type of the larger forms has resulted from indiscriminate sampling by a biological supply house. The use of fluorescent, DNA-binding dyes to distinguish the micronuclei in these species-groups will be reported. These dyes include acridine orange, ethidium bromide, DAPI (4′, 6-Diamidine-2-phenylindole) and two *bis*benzimide compounds (Hoechst No. 33258 and No. 33342).

Degradation of Liver HMGCoA Reductase in Vitro. DAVID M. GIBSON, STEVEN J. MILLER AND TED GOODSON, III, Indiana University School of Medicine, Indianapolis, Indiana 46223.——HMGCoA reductase has a molecular weight of approximately 97,000. Simple freezing and thawing of lysosomal-contaminated microsomes releases a soluble portion of the enzyme (MW 53,000-62,000). Incubation of washed microsomes with a purified thiol protease, calpain, also results in the generation of an apparently identical fragment. This degradation product appears as a doublet on an SDS-polyacrylamide gel. Centrifugation of calpain-treated microsomes followed by electrophoretic and immunoblot analysis of the supernatents and pellets indicated that the lower mobility band was present in the supernatent. This has implications for the mechanism of reductase degradation.

In another experiment, the ability of chymostatin to inhibit the proteolysis of reductase was examined. This inhibitor is known to block degradation of reductase in hepatocytes, presumably by inhibition of lysosomal proteases. In a freeze-thaw system chymostatin prevented the release of reductase into the supernatent fraction, suggesting that lysosomal degradation is indeed blocked.

Effects of Extracellular Sodium and Calcium on Acetylcholine Induced Hyperpolarizations in Mouse Parotid Cells. A. GUBITOSI AND R.J. STARK. DePauw University, Greencastle, Indiana 46135.——Studies with the ionophore A23187 indicate that acetylcholine (ACh) induced membrane hyperpolarizations may be dependent on changes in cytosolic Ca^{++} or Na^{+}. To examine any ionic effects, acinar cell membrane potentials (Em) were measured during Ach (10^{-8} to 10^{-5}M) stimulation in a mammalian Ringer's, Ca-free Ringer's, or Na-free TRIS Ringer's sol'n. The induced changes in Em with and without extracellular calcium were not significantly different (P 0.2). The presence or absence of extracellular sodium (oNa) also had no effect on the Em hyperpolarizations at ACh conc. 10^{-6}M. Normally, higher ACh concs. fail to produce any further increase in Em. However, when oNa was removed, Em continued to increase. These results suggest that a sodium dependent negative feedback mechanism is activated at higher concentrations of ACh which inhibit the cell's response to the stimulus. This inhibition is eliminated in the absence of oNa or when the cells are stimulated with A23187 which bypasses the ACh activated ion channels. (Supported by a DePauw Faculty Development Grant)

Influence of Dietary Fatty Acids on Murine Mammary Cell Types. MICHAEL I. LEE, SCOTT A. SMITH AND ALICE S. BENNETT, Ball State University, Muncie, Indiana 47306.——Experiments were performed to determine whether dietary induced alterations in the fatty acid distributions observed in intact mammary glands and white adipose tissues were reflected in distributions in mammary epithelium and adipocytes. Intraperitoneal white fat and mammary glands were removed from 5 mo. old Strain A/ST mice fed high fat diets rich in either stearic acid or linoleic acid or low fat diets. Mammary glands were enzymatically digested; epithelial cells and adipocytes were separated by differential centrifugation. Fatty acid distributions were determined by gas liquid chromatography. Results indicate that the fatty acid composition of mammary adipocytes was similar to the intraperitoneal white fat and intact mammary glands in each dietary group, but differed significantly from that of mammary epithelium.

Fatty acids have been demonstrated to affect the tumorigenic process. These studies should help determine whether the tumorigenic effect of dietary fatty acids is the result of alterations in the lipid structure of the epithelial cells or the availability of fatty acids in the closely associated adipocytes.

Con A Binding Sites in the Ciliate *Stentor coeruleus* Are Located in the Membranellar and Somatic Cilia. MICHAEL S. MALONEY AND JEANNETTE L. DANIEL, Department of Biological Sciences, Butler University, Indianapolis, Indiana 46208.——Previous studies had shown that Concanavalin A (Con A) inhibits the regeneration of a new oral feeding apparatus (oral regeneration) in the ciliate *Stentor coeruleus*. To extend these studies, we examined the initial location of the Con A binding sites by fixing nonregenerating and regenerating cells prior to exposure to FITC-Con A. When examined by fluorescence microscopy, the cells showed a bright fluorescence in the membranellar cilia that are a part of the oral apparatus and in the somatic (body) cilia. Regenerating cells revealed a striking fluorescence in the developing oral primodium at all stages of regeneration. Binding of the FITC-Con A was prevented by the presence of 50 mM α-D-methyl mannoside. The results demonstrate that membrane glycoproteins capable of binding Con A are present in the membranellar and somatic cilia in *Stentor* and that the effects of

Con A on oral regeneration may be related to these membrane proteins. Supported by grants from NSF and the Research Corporation and a Butler University Fellowship.

Diabetes-induced Decrease in Sodium Pump Activity in Rat Soleus Muscle. JOHN W. MUNFORD AND JOSEPH E. TRUMPEY, Wabash College, Crawfordsville, Indiana 47933.——The hypothesis that insulin regulates sodium pump activity *in vivo* was tested in this study by assessing the effects of streptozotocin-induced diabetes on the number and activity of sodium pump sites in rat soleus muscle. In rats made diabetic by a single intraperitoneal injection of streptozotocin (SZ), (75mg SZ/kg body wt.) plasma insulin and thyroid hormone (T_4) levels wre decreased to less than 50% of control levels. Associated with these hormonal changes were a 48% decrease in the rate of total ^{22}Na efflux and a 36% decrease in the rate of ouabain-sensitive ^{22}Na efflux. These decreases in the rates of ^{22}Na efflux seem to result from a decreased number of sodium pump sites since soleus muscles from diabetic rats bind 47% less ^{3}H-ouabain than muscles from control rats. The data of this study suggest that a complex relationship exists between the circulating levels of insulin and thyroid hormone and the number and activity of sodium pumps in mammalian skeletal muscle. This study was supported by a grant from The American Diabetes Association, Indiana Affiliate, Inc.

Effect of Caffeine on Contractility of Frog Skeletal Muscle. BRUCE PICKELHEIMER AND RICHARD S. MANALIS. Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805.——Experiments were performed on the frog sartorius neuromuscular preparation to determine the effects of caffeine on muscle contractility. The experiments were also designed to determine whether the effects were a result of presynaptic increases in the amount of neurotransmitter released or a result of postsynaptic increases in the amount of calcium released from the sarcoplasmic reticulum of the muscle. Indirect stimulation of the preparation showed increased contractility of the muscle as the caffeine concentration of the test solution increased. Experiments are currently being conducted by direct stimulation of the preparation in test solutions containing either d-tubocurarine chloride or a combination of high magnesium and low calcium to determine whether the observed effect is presynaptic, postsynaptic, or both.

Neurotransmitter Release Simulation. DANNY LEE ROBERSON AND RICHARD S. MANALIS. Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805.——A special purpose user-friendly interface program was developed in order to allow for the compartmental modeling of neurotransmitter release at the frog neuromuscular junction. A first step was to write a test program in PASCAL on a VAX computer and then to download the generated data to a personal computer. This simulation duplicated and confirmed the lumped parameter mathematical model reported by K.J. Gingrich and J.H. Byrne in the *Journal of Neurophysiology* of March 3, 1985. The data files generated were successfully downloaded to a personal computer and used as input into a statistical package. Future work is aimed at completing the user-friendly program and to constructing a complex neurotransmitter release model. Factors that determine the ionized $[Ca^{++}]$ present within the nerve terminal, the mobilization of transmitter, and the facilitation and depression of a transmitter release will be incorporated into the model.

Calmodulin Antagonists Inhibit Oral Regeneration in the Ciliate *Stentor coeruleus*. PATRICIA R. WALSH AND MICHAEL S. MALONEY, Department of Biological Sciences, Butler University, Indianapolis, Indiana 46208.——Loss of the oral feeding apparatus of the ciliate *Stentor coeruleus* results in the regeneration of a new one in 8-10 hrs., a process known as oral regeneration. The calcium regulatory protein, calmodulin, has been isolated from several protozoan species and it seemed likely that it might be present in *Stentor*

and involved in controlling oral regeneration. We investigated this possibility by examining the effects of several calmodulin inhibitors on oral regeneration. Both trifluoperazine and W-7, classic calmodulin antagonists, inhibited oral regeneration at concentrations as low as 4 uM. An inactive analogue of W-7, W-5, was used as a control for the specificity of action of these inhibitors. W-5 had no effect on oral regeneration even at concentrations up to 60 uM. Excess extracellular calcium could not modulate the effects of W-7 as it had no effect on the delays caused by W-7. These results provide evidence that calmodulin is involved in controlling oral regeneration in *Stentor*. Supported by a grant from the Research Corporation and a Butler University Fellowship.

Serum Angiotensin Converting Enzyme Changes in Rabbits Following Neutrophil Activation *in vivo*. Stephen P. Zimmer and Joan E. Lafuze, Indiana University School of Medicine, Indianapolis, Indiana 46223.——Intravenous infusion of N-formyl-methionyl-leucyl-phenylalanine (FMLP), synthetic analog of the endotoxin of E. coli, causes neutropenia *in vivo*. The sequestered neutrophils may block the microvasculature of the lung, adhere to the pulmonary endothelium and release toxic by-products of oxidative metabolism which may cause endothelial damage and respiratory distress. Because pulmonary endothelium produces angiotensin converting enzyme (ACE) which converts angiotensin I to angiotensin II and degrades bradykinin, we wished to determine whether the I.V. infusion of FMLP (0.2 ug/kg) would elevate serum levels of ACE. Results were calculated as percent change from pre-infusion ACE values and reported as Mean ± S.D. Changes in serum ACE levels were $+6.16 \pm 5.03\%$, $-5.89 \pm 8.16\%$, $+0.630 \pm 5.42\%$, $-11.6 \pm 7.38\%$ at 5, 10, 15, and 30 minutes post-infusion respectively, compared with controls (-6.70 ± 3.55, -0.910 ± 4.29, -6.06 ± 3.02, and -16.7 ± 6.32); $n = 3$ in each group. Thus FMLP-induced neutrophil activation in rabbits *in vivo* appears to increase serum ACE levels, a possible result of activated neutrophil mediated damage of pulmonary endothelium.