CELL BIOLOGY

Chair: Robert J. Stark
Department of Zoology
DePauw University, Greencastle, Indiana 46135 (317) 653-4776

Chair-Elect: John W. Munford
Department of Biology
Wabash College, Crawfordsville, Indiana 47933 (317) 364-4202

ABSTRACTS

Role of Dietary Fatty Acids in Murine Mammary Tumors. A.S. Bennett, M.L. Richeson, and A. Foust, Ball State University, Muncie, Indiana 47306, and Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 47306.——Results of studies in our laboratory revealed that the latency period for the development of mammary adenocarcinomas in female Strain A/St mice was significantly increased when the dietary fat contained a high percentage of stearic acid (SA). The incidence of hyperplastic alveolar nodules (similar to preneoplastic structures) was increased in mice fed a diet rich in polyunsaturated fatty acids.

The fatty acid compositions of prelactating mammary gland fat pads, intraperitoneal adipose tissue, and non-lactating mammary glands of mice fed high fat diets containing stearic acid or linoleic acid (SAF) were compared to those of mice fed a low fat diet. All tissues of SAF fed mice contained a higher percentage of 18:2. Linoleic acid was significantly reduced in tissues removed from mice fed the SA diet. As much as 60% of fatty acids stored in adipose tissues from SAF fed mice was 18:2 compared to <5% in SA fed mice. These results support our suggestion that the availability of 18:2 plays a significant role in the tumorigenic process.

Lag Time of OH• Radical Production by Zymosan Stimulated Neutrophils. Bryon Bhagwandin, S.T. Barefoot and F.W. Kleinhans, Department of Physics, IUPUI, Indianapolis, Indiana, 46223 and Department of Medical Research, Methodist Hospital of Indiana, Inc., Indianapolis, Indiana 46202.——The rapid metabolic burst and production of OH• by stimulated neutrophils makes early time observations necessary. The OH• can be detected by using spin trapping techniques in which the OH• is trapped and stabilized with dimethyle pyrroline oxide (DMPO) and detected via electron paramagnetic resonance (EPR). Past procedures involved mixing the neutrophils with stimulant (Zymosan A) and spin trap (DMPO) on a lab table and then moving them to the EPR cavity for observation. This process takes 75 seconds, making early time observations impossible. An injection system was developed to allow the neutrophils, stimulant and spin trap to be mixed as they are injected into the EPR cavity. Collection of data is then possible from as early as 12 seconds after stimulation. Using this system we find that OH• production begins within 30 seconds after stimulation by zymosan.

Determination of OH• Production by Stimulated Neutrophils Using ESR Spectroscopy. Maureen Hill, F.W. Kleinhans and S.T. Barefoot, Department of Medical Research, Methodist Hospital of Indiana, Inc., Indianapolis, Indiana 46202 and Department of Physics, Indiana University-Purdue University at Indianapolis, Indiana 46223.——

The rate of OH• production by the neutrophil during the metabolic burst can be measured by ESR spectroscopy with the method of spin trapping. We report a method for quantitatively analyzing the ESR raw data and illustrate it with zymosan stimulated neutrophils. Peak ESR amplitudes for four concentrations of opsinized zymosan (ratios 1:1/2:1/4:1/8) occur from 3 to 25 minutes. If the effects of signal decay are removed from the raw spectrum, however, the actual profile of OH• production can be seen. The four above concentrations are demonstrated to differ only in magnitude of peak OH• production, all peaking at a time of three minutes. The signal decay time is determined assuming simple exponential decay of the ESR signal. The data, corrected for decay, can then be integrated to produce a cumulative OH• production curve. This permits comparison of OH• production data with that generated by spectrophotometric assays for superoxide. PMA (Phorbol 12-Myristate 13-Acetate) has also been used as a neutrophil stimulant and the effect of various sampling techniques and oxygen tension on OH• production have been examined.

Ultrastructural Organization of the Subcommissural Organ of Mongolian Gerbil *Meriones unguicalatus*. MOHINDER S. JARIAL, Center for Medical Education, Ball State University, Muncie, Indiana 47306.——The subcommissural organ (SCO) of adult mongolian gerbil has been examined by light and electron microsocopy. It is a specialized area of tall columnar ependymal and adjoining hypendymal cells, located in the roof of the caudal end of the third ventricle immediately below the posterior commissure. A network of capillaries with associated nerve endings exists between ependymal and hypendymal cells. Two cell types, namely light and dark epithelial cells are present. The ependymal cells have no basement membranes and their apical ends which often protrude into the ventricular lumen bear numerous slender microvilli and a few cilia. The lateral plasma membranes are fused distally by desmosomes and tight junctions. The cytoplasm of both ependymal and hypendymal cells contain numerous mitochondria which occur fairly evenly throughout the cytoplasm. Comparatively large nuclei with eccentric nucleoli, prominent Golgi apparatus, smooth and rough surfaced endoplasmic reticulum, secretory granules and lipid droplets are regular features of both cell types. The cells also display whorls and dilated cisternae of rough endoplasmic reticulum studded with widely spaced ribosomes, multivesicular bodies, dense bodies, lysosomes, vesicles and microtubules. A striking feature of the SCO is the presence of intercellular and intracellular canaliculi usually lined with microvilli and even cilia. Occasionally, membrane bounded granules and electron-lucent droplets apparently coalesce with membranes of these canaliculae and discharge their contents in their lumina. No quantitative differences were observed in the ultrastructure of the light and dark cells except for the density of the cytoplasm in the latter. They may represent the same cell in different stages of physiological activity.

The ultrastructural features suggest that the SCO has a secretary function, and its product is transported into the cerebrospinal fluid where some of it may be incorporated into the Reissner's fibre.

Using Cultured Fetal Mouse Salivary Glands to Detect Teratogenic Potential of Chemicals. R. DOUGLAS LYNG, Department of Biological Sciences, Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805.——During development of the mouse salivary gland, a spherical bud is converted into a multilobed organ by the interaction of several processes. These interactions, and the possibility of quantifying development by counting lobe production, suggested that this system may be useful for testing the teratogenic potential of chemicals. Mouse salivary glands from day 13 embryos were cultured in the presence of three known teratogens and in an

untreated control. A growth factor was determined by dividing the number of lobes at 48 hours by the number present at explanation. Cytochalasin B caused a growth reduction of over 75% at 1 ug/ml, the lowest concentration tested. Two niacin antagonists, 3-acetylpyridine (3-AP) and 6-aminonicotinamide (6-AN), showed a dose response reduction in growth. The most potent was 6-AN with over a 90% reduction in growth at 10^{-5}M, but only a 25% reduction was found with 3-AP at 10^{-5}M. These results indicate that this system has potential for testing chemicals for teratogenic properties. Supported by NIH Grant RR00169 to the California Primate Research Center and Indiana Academy of Science Research Grant.

A New Pharmacological Tool to Study Neurotransmitter Release at the Frog Neuromuscular Junction. RICHARD S. MANALIS, Department of Biological Sciences, Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805.——Recent neurochemical studies have shown that the drug AH5183 (2 – (4 – phenylpiperidino)cyclohexanol) blocks the incorporation of newly synthesized ACh into synaptic vesicles. The present experiments were undertaken because AH5183 might eventually permit electrophysiological studies of synaptic transmission to discriminate between cytoplasmic and vesicular ACh release. Sciatic nerve-sartorius muscle preparations from the frog (*Rana pipiens*) were mounted in a water-jacketed chamber which rested on the stage of a compound microscope. Neuromuscular junctions were identified visually; intracellular microelectrodes were used to record EPPs. A microcomputer system was used to digitize, average, and store the EPPs; software was available which permitted the EPP amplitudes to be measured and plotted against time. Preparations were bathed in normal Ringer solution containing (in mM): NaCl (111); KCl (2.5); $CaCl_2$ (2.0). The pH was buffered to 7.1-7.2 with tris maleate (4.0); the bath temperature was 12-14° C. d-tubocurarine chloride (3×10^{-3}mg/ml) was present in order to record subthreshold EPPs. 2-4 μM AH5183 lowered the EPP amplitude; in one experiment, the average EPP fell to less than 100 μV within 40 min after the drug was first added to the bath. AH5183 was also shown to decrease facilitated transmitter release. EPP amplitudes were compared immediately before and after tetanic stimulation (30 sec at 20 Hz) of the muscle nerve first during the control period and then in the presence of the drug: the control EPP increased from 0.62 mV to 0.91 mV while the AH5183-treated EPP only increased from 0.62 mV to 0.70 mV. (AH 5183 kindly supplied by Dr. S.M. Parsons.)

Effect of Amiloride on Insulin-stimulated Sodium Efflux from Rat Skeletal Muscle. JOHN W. MUNFORD. Department of Biology, Wabash College, Crawfordsville, Indiana 47933.——Insulin has been shown to stimulate active sodium efflux and active potassium influx in a number of tissues. It has been hypothesized that the increase in these ion fluxes is mediated by stimulation of the sodium pump by insulin. However, the mechanism by which insulin stimulates the sodium pump is unresolved. In order to test the hypothesis that insulin stimulation of sodium pump activity is secondary to an insulin-stimulated increase in Na^+/H^+ exchange, we investigated the effect of amiloride, a specific inhibitor of Na^+/H^+ exchange, on the rate of insulin-stimulated ^{22}Na efflux from rat soleus muscle. Insulin treatment of isolated soleus muscles increased the rate constant of ^{22}Na efflux by 18% compared to the rate constant of paired control muscles. The addition of 0.5mM amiloride to insulin-treated muscles reduced the degree of insulin stimulation of ^{22}Na efflux by approximately 30%. Therefore, it appears that only about one-third of the insulin-stimulated increase in sodium pump activity in rat soleus muscle is secondary to insulin stimulation of Na^+/H^+ exchange. Supported by a grant from the American Diabetes Association, Indiana Affiliate Inc.

Analysis of DNA Methylation in the Growth and Development of the Early Alaska Pea *(Pisum sativum)*. L.A. NEEB AND B.D. ALLAMONG, Department of Biology, Ball State University, Muncie, Indiana 47306.——The methylation of specific gene sites is thought to play a controlling role in gene expression in microorganisms, higher plants, and animals. The relationship between methylation of DNA and gene expression has been well documented in microorganisms and animals; however, the regulatory role of methylation in higher plants has remained relatively unresearched. The focus of this study was to investigate fluctuations in DNA methylation during the early development of the pea *(Pisum sativum)*.

Pea seeds were grown for 12 days in vermiculite in a growth chamber. Duplicate samples of 30 seedlings were harvested daily. The samples were pulse-labeled with S-Adenosyl-L-methionine, (methyl-$C3H_3$) for 10 hours. The labeled methyl group was allowed to be incorporated into the DNA as the samples continued to grow and differentiate. The methyltransferase action was stopped by freezing. DNA was then extracted, purified, and quantitated. Included in the analysis was the quantitation of RNA. Each methylated product was quantitated in the scintillation counter.

Analysis of the fluctuations in the methylated nucleic acids over the growth period of seedling differentiation was made. Methylated DNA was quantiated based on a comparison of radioactivity in DNA extracted versus the radioactivity contributed to the RNA extracted. Fluctuations of methylated DNA correlates to growth patterns observed. The results lend supporting evidence to the above stated hypothesis. This study indicated that differentiation in pea plants may be a product of methylated DNA masking the expression of selective genes. The results suggested that methyl group alterations on RNA follow the DNA cyclic pattern and also may play a significant role in gene expression.

Effect of Dietary Fats on the Incidence of Preneoplastic Nodules in Mammary Glands of Strain A/St Mice. M.L. RICHESON AND A.S. BENNETT Indiana University-Purdue University at Fort Wayne and Ball State University, Muncie, Indiana 47306.——The effect of high-fat (14%) stearic acid (SA) (saturated), high-fat (15%) safflower oil (SAF) (polyunsaturated) and low-fat (5%) corn oil (CO) diets on the occurrence of preneoplastic nodules in the mammary glands of Strain A/St mice was determined. Mammary neoplasms develop in a three stage process: normal cells----> preneoplastic nodules----> malignant tumors. Hyperplastic alveolar nodules (HAN) have been shown to be pre-malignant. Dietary polyunsaturated fats, specifically linoleic acid, promote tumor formation whereas stearic acid decreases tumorigenesis. The mechanisms involved in the promoter or inhibitor action are not known.

Mammary glands of 10 month old virgin females from the three dietary groups were stained, prepared in histologic whole mounts, and photographed with low power light microscopy. Morphological states of the mammary tissues were observed and numbers of HAN were recorded. Numbers of HAN in the SA fed mice was significantly lower than in either the CO or SAF fed mice. These results suggest that the tumor promoting effect of the dietary fat is at the normal cell to the preneoplastic transition.

An Improved Method for Measuring Lysozyme. STEVEN C. SALARIS AND STEVEN T. BAREFOOT, Department of Medical Research, Methodist Hospital of Indiana, Inc., Indianapolis, Indiana 46202.——The neutrophil is a white blood cell that is important in host defense. During the killing of invading microorganisms, various enzymes are released, including lysozyme. Lysozyme is measured by observing a spectrophotometric decrease in the 515 nm absorbance of a *Micrococcus Lysodeikticus* suspension. Typically, this change in absorbance is assumed to a linear relationship. We have found that

the rate of change may be better described by exponential decay. Changes in absorbance of a *Micrococcus Lysodeikticus* suspension were measured in the presence of known lysozyme concentrations (0-20 ug/ml) for 10 minutes. The resultant graph of change in absorbance vs. time did more closely resemble exponential decay. A plot of the natural log of this data was nearly linear and therefore supported this concept. Using a standard curve generated by this new method is a significantly more accurate method of determining lysozyme concentration in a given solution. This new assay was applied to the determination of lysozyme released by stimulated neutrophils and was found to be a reliable accurate method of measuring lysozyme.

Muscle Glucose-6-Phosphate Dehydrogenase Activity Following Various Durations of Eccentric Exercise. A.C. SNYDER, S.B. KAISERAUER AND S. GRIFFTH. Human Performance Laboratory, Ball State University, Muncie, Indiana 47306.——Muscle inflammation has been shown to occur following activities which involve eccentric exercise. The purpose of this study was to determine if this inflammatory response was dependent on the eccentric exercise duration. METHODS: Five groups of male rats (mean body weight = 554.3 g) were run on a treadmill for 60 minutes. The groups differed in the percentage of time (0, 25, 50, 75 or 100%) of the run which was downhill (16 degrees, an eccentric activity). For each group the remainder of the run was performed on the level. The inflammatory response was determined by analysis of the enzyme glucose-6-phosphate dehydrogenase (G6PDH) activity in the soleus, plantaris, triceps brachii and vastus intermedius muscles 60 hrs. post-exercise. RESULTS: The plantaris G6PDH activity was significantly elevated following all eccentric exercise bouts, while that of the vastus intermedius was only elevated following the longer bouts of eccentric work. The soleus and triceps brachii muscles G6PDH activity was not significantly elevated above control values in any exercise group. CONCLUSION: Inflammation appears to occur following any eccentric exercise with the degree of inflammation dependent on the muscle fiber type and muscles involved.

Effects of Ionophore A23187 on Acinar Cells of Mouse Parotid Salivary Glands. ROBERT J. STARK, Department of Biological Sciences, DePauw, University, Greencastle, Indiana 46135.——The ionophore A23187 is frequently used to increase cytosolic calicum ([Ca]i) to examine calcium's role in the regulation of cell function. In this study, ion-selective and conventional microelectrodes were used to measure the effects of A23187 (10^{-7}, $5x10^{-7}$, 10^{-6}, and 10^{-5} M) on [Ca]i, cytosolic sodium ([Na]i), and the basolateral membrane potential (Em) in parotid acinar cells from ICR mice. These concentrations of A23187 induced membrane hyperpolarizations of 0.9 ± 0.1, 2.7 ± 0.3, 3.9 ± 0.6, & 6.8 ± 0.9 mV; increased [Na]i from 9.2 ± 0.4 to 10.4 ± 0.4, 11.3 ± 0.4, 13.4 ± 0.4, & 14.9 ± 0.6 mM; and increased [Ca]i from 0.44 ± 0.04 to 0.63 ± 0.01, 0.98 ± 0.04, 0.92 ± 0.04, & 0.94 ± 0.06 uM respectively. When these changes were compared to those induced by the natural secretagogue acetylcholine (ACh), both produced similar Em hyper-polarizations but different dose-dependent patterns with regard to the increases in [Ca]i and [Na]i. This suggests the presence of a mechanism for limiting the increase in these ions during stimulation with ACh. This regulation was absent when the ionophore was employed indicating that the regulation may be associated with the ACh-receptor. (supported by grants from the Indiana Academy of Science and DePauw University).