

CELL BIOLOGY

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ABSTRACTS

S-Adenosyl-L-Methionine Decarboxylase Activity in Mouse Mammary Adenocarcinomas. BETH AUGER and ALICE BENNETT, Department of Biology, Ball State University, Muncie, Indiana 47306.—In this study the activity of SAMD in non-tumorous mammary tissue and mammary adenocarcinomas in Strong Strain A female mice was determined.

S-adenosyl-L-methionine decarboxylase (SAMD) is one of the biosynthetic enzymes for the polyamines spermine and spermidine. SAMD activity is known to increase with the increase in intracellular concentration of spermidine in lactating mammary tissue in mice, is known to increase in rapidly proliferating tissues like regenerating rat liver and developing chick embryo, and has recently been found to increase in virus transformed cells.

The soluble particle-free supernate was isolated from prelactating and malignant mammary tissue by centrifugation. SAMD was assayed by liquid scintillation spectrometry using radioactively labeled ($^{14}\text{COOH}$)-S-adenosyl-L-methionine as the substrate to produce $^{14}\text{CO}_2$ as the measurable end-product of the enzyme catalyzed reaction.

The activity of SAMD in adenocarcinomas was found to be significantly higher than in normal tissues. The relationship between this activity and the types of phospholipids found in mammary tumors will be discussed.

Proteins Which Support Growth of *Paramecium multimicronucleatum* in Chemically Defined, Axenic Culture. THOMAS A. COLE, NICHOLAS E. LEAL and WILLIS H. JOHNSON, Department of Biology, Wabash College, Crawfordsville, Indiana 47933.—In 1980 this laboratory reported the culture of *Paramecium multimicronucleatum* on a chemically defined, axenic medium. Since that time, the supplier of chicken ovalbumin (5X crystallized) has discontinued this product which was used in culture for several years. In this report we detail attempts to substitute ovalbumins from other suppliers and a variety of other proteins (hemoglobin, myoglobin, ribonuclease, lysozyme, bovine serum albumin, concanavalin A, pepsin, trypsin, chymotrypsin) with and without added amino acids for the original ovalbumin preparation. Those proteins which support growth in an otherwise complete medium are concanavalin A, bovine serum albumin, myoglobin, hemoglobin and pepsin. Without added amino acids, three proteins (bovine serum albumin, myoglobin and hemoglobin) support growth of this ciliate through seven serial transfers.

Separation of Naturally Occurring Forms of Vitamin A by Reverse Phase HPLC in Tumor Bearing Rats. WILLIAM C. DOUGLASS, RICHARD E. ZOLLINGER and DOROTHY M. MORRÉ, Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana 47907.—Vitamin A and its derivatives have been

demonstrated to have a chemo-preventative effect on development of cancers in a number of experimental animals and organ cultures. In this study, we used reverse phase high pressure liquid chromatography (HPLC) to separate the naturally occurring forms of vitamin A in livers and tumors when rats were fed varying levels of vitamin A as retinyl acetate. Male weanling rats were fed basal AIN-76A diets supplemented with zero, adequate (4,000 IU/kg diet) or excess (100 X adequate) amounts of vitamin A. After two weeks, the rats were injected with transplantable hepatocellular carcinomas induced in syngeneic donor rats by the carcinogen, N-2-fluorenylacetamide. The liver and tumor samples were extracted by chloroform-methanol and aliquots were chromatographed on a C₁₈ reverse phase HPLC column using acetonitrile—1% ammonium acetate in water as the mobile phase. The retention times were approximately 5, 9, 11, 14 and 35 minutes, respectively, for retinoic acid, retinol, retinal, retinyl acetate and retinyl palmitate. The amounts of vitamin A in the tissues closely paralleled that fed in the diet. The HPLC profiles showed a general pattern of separation for all levels of vitamin A to be a small amount of retinoic acid and the fed retinyl acetate, moderate amounts of retinol and increased amounts of the various retinyl esters. Additional work is being done to determine the ester forms present in blood, particularly in the excess group where the observed symptoms of hypervitaminosis A toxicity are thought to be due to the circulating unbound retinyl esters.

Ultrastructural Correlates of the Spitzenkörper Core in *Rhizoctonia solani*.

STANLEY N. GROVE, Goshen College, Goshen, Indiana 46526.—Light and electron microscopic evidence indicates that a phase contrast—dark group of closely associated apical vesicles disappears immediately in hyphae of *R. solani* when polar growth is interrupted by the application of cytochalasins. When slide cultures are perfused with cytochalasins (CA or CE at 1 µg/ml in 0.1% DMSO) polar growth stops in 20-60 seconds accompanied by the loss of the dark portion of the Spitzenkörper (apical body). In control cultures perfused with 1% DMSO hyphae continue growing at pretreatment rates and maintain the typical morphology. In treated hyphae the phase contrast—bright core or inner zone of the Spitzenkörper enlarges and remains visible in the apical cytoplasm for several minutes. It retracts from the extreme apex and moves about in the apical cytoplasm closely associated with numerous mitochondria. The ultrastructural correlate of the persistent core may include tubules of endoplasmic reticulum which enlarge and become more prominent after treatment and cessation of apical growth.

Role of Calcium-binding Protein in Neuronal Function. ZAFAR IQBAL, Department

of Physiology and Biochemistry and Medical Biophysics Program, Indiana University School of Medicine, Indianapolis, Indiana 46223.—Calcium plays an important role in the regulation of a large number of cellular processes including the axoplasmic transport of materials in nerve fibers. Recent observations have indicated that the action of calcium is mediated through a calcium-binding protein now known as calmodulin. We have purified and characterized this protein from mammalian nerve and shown that the protein is transported in nerve fibers. In the presence of micromolar (10⁻⁶M) concentration of Ca²⁺, the protein was found to activate cyclic nucleotide phosphodiesterase (PDE), an enzyme responsible for the hydrolysis of ATP to liberate energy needed for the transport process. Stellazine, which binds to calmodulin blocks the activation of PDE and Ca-ATPase and axoplasmic transport as well. These observations suggest that calmodulin plays an important role in the axoplasmic transport which is an essential neuronal function required for the maintenance of normal physiological conditions.

Gamma Glutamyl Transpeptidase Activity Not a Reliable *In Vitro* Marker of Transformation in Cell Lines Derived from a Hepatocellular Carcinoma. LINDA B. JACOBSEN and PAMELA J. SWIATEK, Purdue Cancer Center, Purdue University, West Lafayette, Indiana 47907.—Many markers have been proposed to identify transformed cells in culture in order to avoid the inoculation of susceptible animals and the wait for tumor development. Anchorage independent growth (growth in soft agar) correlates with tumorigenicity in most systems, while the reliability of other markers depends on the system. Gamma glutamyl transpeptidase (γ GT) activity has been used in many *in vivo* studies to monitor tumor progression in hepatocellular carcinogenesis in rats. When transformed cell lines derived from liver tissue were tested they also were found to contain γ GT activity. Thus this marker has been proposed as a new method to identify transformed hepatocytes in culture.

A primary hepatocellular carcinoma was removed from a rat fed 2 acetylaminofluorene. A portion of the tissue was minced and inoculated into syngeneic recipients. The RLT₁ poorly differentiated metastatic transplantable tumor line was developed from this and was described previously. The remainder of the primary tumor was used to initiate an *in vitro* cell line, P CCL-H₂. Unique cell lines were derived from the parental P CCL-H₂ line by passaging through either syngeneic recipients or soft agar. There was no correlation between γ GT and ability to grow in soft agar in these related cell lines. This suggests that γ GT activity is not a reliable marker for transformation, and must, at present, be used in combination with other *in vitro* markers to evaluate the transformed phenotype.

Thioesterase II in Cell Free Fractions of Adenocarcinomas and Normal Mouse Mammary Tissue. ALBERT KENDRA and ALICE BENNETT, Department of Biology, Ball State University, Muncie, Indiana 47306.—Normal and tumorous rodent mammary tissue synthesize predominantly long chain fatty acids (C₁₄₋₁₈). The ability of rodent lactating tissue to synthesize medium chain fatty acids (C₈₋₁₂) correlates with the presence of thioesterase II, a chain terminating enzyme.

This research studied the ability of thioesterase II to affect a change in the types of fatty acids synthesized by cell free fractions of tumor tissue.

Thioesterase II was obtained by ammonium sulfate precipitation from the particle free supernatant and assayed spectrophotometrically. The fatty acids synthesized by the microsomal and soluble fraction of tumor, with and without thioesterase II were separated by gas liquid chromatography and the amount of incorporation of ¹⁴C-acetyl CoA was determined by liquid scintillation spectroscopy.

It was concluded that the normal and tumor fatty acids synthesized *de novo* were similar in distribution. The addition of thioesterase II to tumor tissue incubations did not affect a statistically significant change ($P < 0.05$) in fatty acid distribution.

It appears that thioesterase II does not contribute significantly to the distribution of fatty acids in tumor tissue, and that the major functional enzymes for *de novo* synthesis are acetyl CoA carboxylase and fatty acid synthetase.

Localization of Constitutive Heterochromatin in *Phlox drummondii*. ROMESCH C. MEHRA and GEORGE MARTIN, Department of Biology, Indiana University at South Bend, South Bend, Indiana 46615 and THOMAS FOGLE, Department of Biology, St. Mary's College, South Bend, Indiana 46556.—Several roles have been suggested for constitutive heterochromatin and its general correspondent, satellite DNA, in chromosome mechanics. Consequently, it has become important to localize con-

stitutive heterochromatin on chromosome complements of different taxa. *Phlox drummondii* ($2n = 14$), a member of the second largest genus in the family Polemoniaceae and native to Eastern and North Central Texas, was investigated. The modified BSG technique was utilized to localize constitutive heterochromatin. C-bands were more pronounced in the centromeric regions than the interstitial and terminal portions of the chromosomes. A total of 34 positively stained C-bands were delineated. On the basis of these bands and the length and centromeric index, a karyotype of the taxa has been prepared. The centromeric regions were more resistant to chemical degradation of the chromosomes which suggests that two types of constitutive heterochromatin are present.

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Effects of Vitamin A on Various Hematological Parameters of Rats Bearing Tumors. DENISE E. MORGAN, DOROTHY M. MORRÉ and GARY R. MATYAS, Departments of Foods and Nutrition and Biological Sciences, Purdue University, West Lafayette, Indiana 47907.—Patients with malignancies may have coagulative properties which differ from those of normal individuals. Previously, we have shown that vitamin A has the ability to prevent or alter the course of tumor establishment or metastasis in experimental tumorigenesis (J. Nutr. 110, 1629, 1980). It is not known to what extent this effect may be due to changes in hematological properties. In the present study, male weanling rats were fed diets containing either adequate (4,000 IU/kg diet) or excess (100 X adequate) amounts of vitamin A for two weeks. Then one-half of the rats in each group were injected with transplantable hepatocellular carcinomas induced in syngeneic donor rats by the carcinogen, N-2-fluorenylacetamide while the other half were not injected and served as positive controls. Hematological properties monitored included bleeding times, numbers of red blood cells, numbers of white blood cells, hematocrit, hemoglobin concentration and prothrombin rate. Tumors in the excess group were markedly smaller than those in the adequate group while livers of tumor bearing animals, in general, were larger than those of non-tumor bearers. Rats with tumors both in the excess and adequate groups exhibited below normal bleeding times. The excess groups, both with and without tumors, had slightly higher prothrombin rates than those in the adequate groups. Other hematological parameters were much less affected. Supported in part by Phi Beta Psi.

Response of Rat Liver Golgi Apparatus to the Sodium Ionophore Monensin. D. JAMES MORRÉ, S. PHANEUF, A. BEAUDOIN and H. H. MOLLENHAUER, Purdue University, University of Sherbrooke and Veterinary Toxicology and Entomology Research Laboratory, College Station, Texas.—Monensin, an antibiotic and ionophore, exhibits a high and specific complexing affinity for sodium ions. Among a variety of cellular effects of the compound is a tendency to inhibit Golgi apparatus-mediated phenomena. Associated with these inhibitions is a swelling of one or more intercalary Golgi apparatus cisternae. Results will be reported concerning effects of monensin on ultrastructure of isolated Golgi apparatus with emphasis on cisternal swelling.

A Survey of Selected Blood Parameters in Rats Bearing Transplanted Tumors. MASAME NAKANI, DENISE MORGAN, DOROTHY M. MORRÉ and D. JAMES MORRÉ. Purdue University, West Lafayette, Indiana 47907.—Rats bearing both metastatic and nonmetastatic transplantable hepatomas and squamous cell carcinomas were analyzed for blood coagulation times and various other blood parameters. The general tendency was for tumor bearing animals to exhibit shortened coagulation

times relative to normal animals without tumors but with reduced platelet numbers and reduced fibrinogen levels. Approximately $\frac{1}{3}$ of the animals bearing non-metastatic tumors or bearing tumors where metastasis had not occurred exhibited greatly increased coagulation times. Of these, all but one had markedly reduced platelet numbers and fibrinogen levels. Blood calcium and partial prothrombin times were not markedly affected by tumor presence. The shortened coagulation times of tumor bearing rats showed no simple correlation with tumor size or growth rate and preliminary results suggests that they may result from overproduction of one or more clotting factors.

Protein Kinase Activities Elevated During Hepatocarcinogenesis Induced by 2-Acetylaminofluorene in the Rat. JEAN ROSSIER, SANDRA SCHILLER-SMITH and D. JAMES MORRÉ. Department of Medicinal Chemistry and Pharmacognosy and Purdue Cancer Center, Purdue University, West Lafayette, Indiana 47907.—Hepatocarcinogenesis in the rat induced by the carcinogen 2-acetylaminofluorene is accompanied by a cascade of metabolic changes leading eventually to a loss of complex cell surface glycoconjugates and altered social behavior. The changes are cyclic and, for the first two major cycles at least, elevations in cyclic nucleotide independent protein kinases of the cytoplasm mark the beginnings of each cycle. To investigate the kinase changes in more detail, the cytoplasmic protein kinase activities were analyzed by chromatography on phosphocellulose at various times during a 50 day continuum of oral carcinogen administration. The results indicate that the elevations involve one or more specific kinases of relatively high molecular weight rather than an overall elevation of the complete kinase spectrum.

Chronology of Protein Kinase and Cyclic Nucleotide Changes During 2-Acetylaminofluorene Induced Hepatocarcinogenesis in the Rat. SANDRA SCHILLER-SMITH, DAVID NOWACK, WILLIAM L. ELLIOTT, PETER F. HEINSTEIN and D. JAMES MORRÉ. Department of Medicinal Chemistry and Pharmacognosy and Purdue Cancer Center, Purdue University, West Lafayette, Indiana 47907.—Livers of rats fed the carcinogen 2-acetylaminofluorene were analyzed for activities of both nuclear and cytoplasmic protein kinases as part of a more detailed study to establish the sequence of biochemical events accompanying tumorigenesis. Endogenous activities were determined with ^{32}P -ATP as substrate and casein or histone were added as co-substrates for determination of exogenous activities. Nuclear protein kinases, with exogenous histone or casein as substrate, were stimulated during the first week of carcinogen administration only. Protein kinases of the cytoplasm yielded maxima at about 25 and 42 to 49 days after the beginning of carcinogen administration. Cyclic AMP levels rose steadily to an approximately 4-fold elevation by day 49 in the livers of the animals receiving carcinogen with the increase beginning prior to the development of visible nodules (at about day 28). The findings provide further evidence for a biochemical cascade in hepatocarcinogenesis and provide useful reference and comparison points for more detailed investigations of individual protein kinases.

Changes in Cytosolic Calcium During Stimulus-secretion Coupling in Mouse Pancreatic Acinar Cells. ROBERT J. STARK, Department of Zoology, DePauw University, Greencastle, Indiana 46135 and J. O'DONERTY, Department of Physiology, Indiana University School of Medicine, Indianapolis, Indiana 46223.—In the exocrine pancreas, natural secretagogues act at the serosal surface of the acinar cells while stimulating fluid and enzyme release from the luminal surface. As changes in cytosolic calcium ($[\text{Ca}]_i$) have been proposed to mediate this process, Ca -selective and conventional microelectrodes were used to examine the relationship between

the secretory response and $[Ca]_i$. By continuously measuring the acetylcholine (ACh) induced changes in intracellular potentials, we determined the effect of varying the stimulus conc. on $[Ca]_i$ and secretion. Stimulation with conc. of ACh ranging from $10^{-8}M$ to $10^{-5}M$ increased $[Ca]_i$ from $0.4 \mu M$ to between 0.5 and $1.0 \mu M$. ACh-stimulation of amylase release also followed a definite dose response curve with maximal secretion at $10^{-7}M$ ACh. Increasing the stimulus concentration above the optimal conc. reduced enzyme release to near basal levels. Use of the Ca-ionophore A23187 mimicked the action of ACh on both enzyme release and $[Ca]_i$ during stimulation of pancreatic acinar secretion and provide further evidence in support of the concept that $[Ca]_i$ mediates stimulus-secretion coupling in these cells. Supported by USPHS NIH AM 26246.

Insulin Control of Transplasma Membrane NADH Dehydrogenase. I.L. SUN and F.L. CRANE, Department of Biological Sciences, Purdue University, West Lafayette, Indiana.—Plasma membranes from many animal cells have been shown to contain NADH dehydrogenase. The maximum plasma membrane NADH dehydrogenase activity is seen with ferricyanide as electron acceptor. Using oriented vesicles of erythrocyte membranes it can be shown that there are three types of NADH ferricyanide reductase activities. One is on the outside of the membrane, another is exclusively inside and the third is transmembranous. The transmembrane activity can be measured using intact cells by measuring external ferricyanide reduction since ferricyanide is impermeable. Insulin at physiological concentration partially inhibits (20%) of the activity of open membranes, but it gives up to 45% inhibition of the exclusively transmembrane activity measured with whole cells. Insulin controls this transmembrane activity which can cause proton movement or membrane potential changes. Maximum inhibition with pig erythrocytes is at $20 \mu U$ insulin per ml.

Effect of Retinol on Sister Chromatid Exchanges in Chinese Hamster V79 Cells Treated with Cyclophosphamide. JAMES C. TAN, Department of Biology, Valparaiso University, Valparaiso, Indiana 46383.—Sister chromatid exchanges (SCE), observed through the fluorescence plus Giemsa technique, was used to study the inhibitory action of retinol (RA, 32 mcg/ml) on SCE frequency in cultured V79 cells, treated for one hour with the indirect mutagen cyclophosphamide (CPP, 3 mcg/ml) and activated S9 mix, after two rounds of DNA duplications. The induction of SCE by CPP + S9 was either preceded or followed by one hour of retinol treatment. Preliminary results indicate that the treatment combinations CPP + S9, RA + (CPP + S9) and (CPP + S9) + RA showed significantly higher frequencies of SCE than the controls (none or S9 only). Furthermore, both the treatment combinations with retinol showed significantly fewer SCE than the CPP + S9 combination, which suggest an inhibitory effect. However, the SCE frequencies of the treatment combinations with retinol introduced before and after the CPP + S9 induction are not statistically different. This result suggests that the inhibitory action of retinol is not so much affected by whether it is present before or after the mutagenic induction by CPP.