CELL BIOLOGY

Chairperson: Ralph Jersild
Department of Anatomy
Indiana University School of Medicine
Indianapolis, Indiana 46202 (317)264-8730

Chairperson-Elect: Robert Stark
Department of Zoology, DePauw University
Greencastle, Indiana 46135 (317)653-4776

ABSTRACTS

Effect of Acetylcholine Stimulation on Cytosolic Chloride in Parotid Acinar Cells. Kathy Burek and Robert J. Stark, DePauw University, Greencastle, Indiana 46135.——In parotid salivary glands, acetylcholine stimulates fluid, electrolyte, and protein secretion and hyperpolarizes the basolateral membrane. To examine the ionic mechanisms involved in this process, we used ion-selective and conventional microelectrodes to measure the cytosolic chloride activity (a_{Cl}) and basolateral membrane potential (Em) during acetylcholine stimulation of mouse parotid glands. In unstimulated cells, a_{Cl} was 45.2 ± 1.1 mM (n=25) and Em was −33.8 ± 1.6 mV (n=66). Acetylcholine at concentrations of 2X 10-9, 1x 10-8, 1X 10-7, 1x 10-6 and 1x 10-5 M produced a decrease in a_{Cl} of 3.5 ± 0.3, 4.4 ± 0.7, 8.0 ± 0.5, 9.3 ± 0.7, and 9.5 ± 1.8 mM and hyperpolarized Em by 0.6 ± 0.1, 1.4 ± 0.2, 4.9 ± 0.2, 8.4 ± 0.3 and 8.4 ± 0.5 mV respectively. The inverse relationship observed between Em and log a_{Cl} suggests that the membrane hyperpolarizations occurring in response to acetylcholine stimulation may be related to the corresponding changes in cytosolic chloride. (Supported by a Research Grant from the Indiana Academy of Science)

Physiological Studies of *Azospirillum amazonense*. Edwin M. Goebel and Deborah A. McMahan, Department of Biological Sciences, Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 47805.——Members of the genus *Azospirillum* have been shown to fix nitrogen under microaerophilic conditions in both tropical and temperate regions. The microbe will fix nitrogen either in association with the roots of non-legume plants or free-living in the soil. Two species within the genus have been extensively studied. Neither of these species were able to utilize disaccharides for catabolism. A newly described species, *A. amazonense*, has been shown to utilize certain disaccharides, especially sucrose. This species shares the ability to use various five and six carbon sugars and organic acids with the other two members of the genus. This species has been shown by others to belong to the genus by means of comparing G+C ratio and morphological characteristics. The study reported here sought to determine which compounds could be used by the microbe grown under either nitrogen-fixing or fixed nitrogen conditions. Growth occurred in all conditions tested; however, the best growth occurred with glucose, sucrose, citrate, succinate and malate. Growth also occurred with galactose, rhamnose, xylose, fructose, ribose, and both D- and L-arabinose. Growth studies in a defined medium containing ammonium sulfate showed the doubling time to be shortest (1.5-2.5 hours) when either glucose or sucrose was provided. Growth with fructose or galactose was considerably slower. Attempts have also been made to isolate *Azospirillum* species from the soil in the midwestern area of the U.S. A semi-solid nitrogen-free malate medium was utilized for primary isola-

tion. Secondary isolation was accomplished by selecting characteristic colonies growing on a complex agar medium containing congo red.

A Brief History of the Cell Biology Section, Indiana Academy of Science. RALPH A. JERSILD, JR., Indiana University School of Medicine, Indianapolis, Indiana 46223.——The first meeting of the Cell Biology Section was held during the fall meeting of the Academy, October 21, 1967, at Indiana University, Bloomington. For two years prior to this, a number of scientists and technicians from throughout the state of Indiana and with common interests in electron microscopy had been meeting as a separate group. By 1967 this group was well-established, and it became clear that a more formal organization was needed. Informal discussions were initiated with Dr. A.A. Lindsey, then President of the Academy, for organization as a Section within the Academy. At the time, the formation of a Cell Biology Section had been under consideration by the Academy. At its spring 1967 meeting, therefore, the Academy offered to temporarily establish a Cell Biology Section through which our group could present and determine the extent of interest statewide. The idea was accepted enthusiastically. The divisional meeting in the fall of 1967 was considered a success, with 12 papers and 3 exhibits presented. The Executive Committee of the Academy subsequently voted at their spring 1968 meeting to make the Cell Biology Section permanent. It was an honor for me to serve as the Section's first chairperson. Others from the original group that were instrumental in organizing the Section include Dr. D. James Morre and Dr. Edward J. Hinsman, Purdue University; and Dr. James E. Carter, Indiana University School of Medicine. From 1967 through 1984, 13 different persons have chaired this Section, representing 11 institutional locations around the state. An average of 12 papers have been presented yearly during this period by persons with interests in Cell Biology.

Concanavalin A Inhibits Oral Regeneration in *Stentor coeruleus* by Binding to the Cell Surface. MICHAEL S. MALONEY, Department of Zoology, 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. Cell surface glycoproteins seem to be involved in oral regeneration as Concanavalin A (Con A), which binds to such proteins, delays oral regeneration. Binding of Con A to the cell surface of *Stentor* is indicated by the fact that α-methyl mannoside completely reverses the effect of Con A on oral regeneration. Crosslinking of membrane bound Con A receptor molecules may also be involved as succinyl Con A, which does not crosslink these receptors in other cells, has no effect on oral regeneration. To provide a direct demonstration of Con A binding to the cell surface, cells were exposed to fluorescein isothiocyanate Con A (FITC-Con A) for 30 min, fixed, and then examined by fluorescence microscopy. Upon exposure to FITC-Con A, the Con A is localized on the cell surface as accumulations of fluorescent granules on the posterior one half of the cell. These granules are always localized in the pigmented stripes between the rows of body cilia. Smaller fluorescent granules were also found in a linear array at the base of the membranellar cilia in the gullet area. Quite often the entire membranellar band was diffusely stained. Fixed cells without FITC-Con A exposure show none of these features. When cells are treated simultaneously with FITC-Con A and α-methyl mannoside, there is no binding of Con A.

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The Effect of Fasting on Sodium Pump Activity in Rat Skeletal Muscle. JOHN W. MUNFORD AND THOMAS KOENIG, Department of Biology, Wabash College, Crawfordsville, Indiana 47933.——It has recently been reported that decreased circulating in-

sulin levels, resulting from either diabetes or fasting, are associated with a significant increase in intracellular sodium levels in rat skeletal muscle. It has been suggested that this increase in intracellular sodium results from decreased sodium pump activity. To test this hypothesis, the effect of fasting-induced hypoinsulinemia on the rate of ^{22}Na efflux from rat soleus muscle was investigated. In soleus muscles isolated from rats fasted for 72 hrs, the rates of both total ^{22}Na efflux and ouabain-sensitive ^{22}Na efflux were decreased by approximately 20% compared to the rates of ^{22}Na efflux of muscles from fed rats. However, it appears that soleus muscles from fasted rats retain their sensitivity to insulin since the *in vitro* treatment of soleus muscles from rats fasted for 72 hrs with insulin increased the rate of ^{22}Na efflux to the same level as in muscles from fed rats. The decreased rate of ^{22}Na efflux in muscles from fasted rats may be the result of a decreased number of sodium pump sites since preliminary data suggests that soleus muscles from rats fasted for 72 hrs have a decreased number of ^{3}H-ouabain binding sites compared to muscles from fed rats.

Increased Binding of Growth Hormone Following Cleavage by Rabbit Liver Plasmalemma. JEANETTE M. SCHEPPER AND JAMES P. HUGHES, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.——Several studies have shown that proteolytic cleavage can enhance the biological activity of the growth hormone (GH) molecule. It seemed possible therefore, that proteolytic modification of GH structure might be a normal function of GH-target tissues. Plasmalemma-enriched fractions isolated from rabbit liver were found to contain a proteinase(s) which cleaved the large disulfide loop of human (h) and rat (r) GH. The proteolytic activity was specific to plasmalemma-enriched fractions in that much lower activities were observed in microsomal-enriched fractions prepared from the same livers. The plasmalemmal proteinase(s) may be a trypsin-like enzyme because proteolytic activity was decreased by the two serine proteinase inhibitors. Inhibition by unlabeled hGH of [^{125} I] GH binding to receptors did not prevent cleavage of the tracer; therefore, hormone-receptor interaction was not required for cleavage of the GH molecule. In binding studies, cleaved GH associated more readily than did intact hormone with rabbit liver receptors. These studies suggest that plasmalemma-enriched fractions prepared from rabbit liver contain a proteinase which cleaves the GH molecule in a highly specific manner. Moreover, it is unlikely that inactivation of GH is the function of this limited proteolysis because cleaved hormone is bound preferentially by at least a subset of receptors in rabbit liver.

Protein Degradation after Eccentric Exercise. A.C. SNYDER, A.R. COGGAN AND J.J. UHL, Human Performance Laboratory, Ball State University, Muncie, Indiana 47306.——Net degradation of proteins in skeletal muscle and liver occurs after exhaustive exercise. Similarly, increases in muscle protein degradation and structural alternations occur following nonexhaustive eccentric muscular contractions (force produced in lengthening muscles). The purposes of this study were to determine: 1) if increasing muscle protein, but not liver protein degradation, occurred following a single bout of nonexhaustive eccentric exercise, and 2) the association between this muscle protein breakdown and the activity of the calcium activated factor (CAF), a muscle protease. METHODS: Male rats were randomly assigned to one of two groups: 1) sedentary or 2) exercised for 90 minutes down a 16° decline on a treadmill at 16 m/min. Animals were sacrificed 24 hours following the exercise bout and the appropriate tissues were removed. RESULTS: Following the exercise, muscle protein degradation was significantly increased; however, no change in liver protein content was observed. The activity of the CAF enzyme was not increased in any of the muscles examined following the exercise bout. CONCLUSIONS: 1) Muscle protein but not liver protein degrada-

tion increases following a single nonexhaustive eccentric exercise. 2) As the CAF enzyme is thought to be the initiating enzyme of protein degradation, the exact mechanism causing the increased degradation following nonexhaustive eccentric exercise remains unknown.

Calmodulin Stimulation of ATP-Dependent Ca^{2+} Uptake in Maize Root Microsomes. MARTIN A. VAUGHAN, TIMOTHY J. MULKEY AND CHARLES W. GOFF, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.——The ATP-dependent uptake of Ca^{2+} by microsomal membrane fractions prepared from 1 cm segments of maize root tips was assayed in the presence of added bovine calmodulin and calmodulin antagonists. Increased concentrations of bovine calmodulin resulted in increased ATP-dependent Ca^{2+} uptake by the microsomal vesicles. The magnitude of calmodulin stimulation over calmodulin depleted controls ranged from 200-400%. The very specific calmodulin antagonist R24571 inhibited the ATP-dependent Ca^{2+} uptake by 90% at a concentration of 10^{-4}M. A concentration of 0.5mM chlorpromazine, a phenothiozine drug, was required to affect a similar level of inhibition. Contrary to previous reports, these data strongly suggest that the ATP-dependent Ca^{2+} uptake of maize root microsomes is a calmodulin mediated process.

The Effect of Illumination on the Rat Pineal as Measured by MSH Activity. HENRY C. WOMACK, Ball State University, Muncie, Indiana 47306.——Albino rats were kept in constant light or constant darkness for a period of 24 hours. The animals were then decapitated and their pituitary glands removed, weighed, and homogenized. The melanocyte-stimulating hormone (MSH) activity of these glands was assayed by injecting the test material into the dorsal lymph sacs of hypophysectomized frogs. Pinealectomized and sham-pinealectomized animals were subjected to these same experimental procedures. MSH levels were higher in the pituitaries of those rats kept in constant light regardless of the age or sex of the animal. The pituitary MSH content of rats kept in constant darkness elevated significantly about eight hours after the animals were exposed to light; about twice this amount of time was required for significant decreases in MSH levels when light-adapted animals were placed in the dark. When pinealectomized rats were placed in darkness there was no subsequent fall in MSH levels as in the controls. It is felt that the pineal hormone melatonin may influence pituitary MSH release by blocking the action of a MSH-release inhibiting factor (MIF) known to be produced by the rat hypothalamus; the release of melatonin itself is suppressed by illumination.