MEMBRANE TRANSPORT AND NEURORECEPTORS

Arthur Blume, Ivan Diamond and Dale Oxender, Organizers

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Membrane Transport and Transduction Systems

109 MEMBRANE PROTEINS INVOLVED IN SIGNAL TRANSDUCTION FROM MEMBRANE RECEPTORS TO FLAGELLA IN BACTERIAL CHEMOTAXIS. Julius Adler, Department of Biochemistry, University of Wisconsin, Madison, WI 53706.

The mechanism of bacterial chemotaxis is beginning to be understood. At the receptor end, we have considerable knowledge about the molecular properties of chemoreceptors. At the effector end, we know that bacterial flagella rotate and that the direction of rotation is determined by attractants and repellents, although we do not yet know the molecular features of the motor and the gear shift. Between the receptors and the effectors is a system for integrating the sensory information and transmitting a signal to the flagella. This system, sensory transduction, involves methylatable membrane proteins that generate the signal ("excitation") and that, upon a change in methylation, terminate the signal ("adaptation"). Further aspects of the mechanism of sensory transduction will be discussed, insofar as they are known.

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2. Protein methylation in behavioral control mechanisms and in signal transduction. M.S. Springer, M.F. Goy, and J. Adler. Nature 280, 279-284 (1979).

3. Attractants and repellents control demethylation of methylated chemotaxis proteins in Escherichia coli. Proc. Natl. Acad. Sci. USA. November, 1979.

110 CHEMICAL SIGNALS TO AND THROUGH THE ACETYLCHOLINE RECEPTOR, Micheal A. Raftery; California Institute of Technology, Pasadena, California 91125. Intact membrane vesicles containing only the acetylcholine receptor (AcChR) can be prepared from Torpedo californica electroplax. They are orientated such that neurotoxin binding sites are exclusively on the outside. Such preparations can be used to demonstrate that the AcChR is a transmembrane protein and that all four receptor subunits are present on the exterior membrane surface. These closed vesicles can be used to study cholinergic ligand binding and monovalent cation translocation into or out of the sealed compartments in response to acetylcholine or other agonists associating with the receptor. The AcChR protein is composed of four types of subunits and has a molecular weight of 255,000. It can be shown that this protein alone is responsible for both agonist binding and cation translocation using the purified membrane preparation or by reconstitution of detergent solubilized AcChR protein with lipids to form sealed vesicles. A variety of spectroscopic approaches are being used to delineate a molecular mechanism for the nicotinic cholinergic synapse using the in vitro system described here.

111 EVOLUTION OF MEMBRANE BIOENERGETICS T. Hastings Wilson and Edmund C. C. Lin
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One of the first problems encountered by primitive cells was that of osmoregulation; the continuous entry of ions (e.g., NaCl) and water in response to the internal colloid osmotic pressure threatened to destroy the cell by lysis. We propose that to meet this environmental challenge cells evolved an ATP-driven proton extrusion system plus a membrane carrier that would exchange external protons with internal Na^+. With the appearance of the ability to generate proton gradients additional mechanisms to harness this source of energy emerged. These would include proton-nutrient cotransport, nucleic acid entry, and motility. A more efficient system for the uptake of certain carbohydrates by vectorial phosphorylation via the PEP-phosphotransferase system probably appeared rather early in the evolution of these anaerobic bacteria.

The reversal of the proton-ATPase reaction to give net ATP synthesis became possible with the development of other types of efficient proton transporting machinery. Either light driven bacterial rhodopsin or a redox system coupled to proton translocation would have served this function. The availability of exogenous electron acceptors, such as fumarate, sulfate, and nitrate, resulted in the progressive elaboration of an electron transport chain. The stepwise growth of this chain required the accretion of one or more flavoprotein, quinone, or cytochrome. With only slight modifications of these three basic components a chlorophyll-dependent photosynthetic reductive system could be readily evolved. The oxygen which was generated by this photosynthetic system from water would eventually accumulate in the atmosphere of the earth. With molecular oxygen present, the emergence of cytochrome oxidase would complete the respiratory chain.

The proton economy of membrane energetics has been retained by most present day microorganisms, mitochondria, chloroplasts, and cells of higher plants. A secondary use of the energy stored as an electrochemical difference of Na^+ for powering membrane events probably also first evolved in microorganisms. The exclusive use of the Na^+-economy is distinctive of the plasma membrane of animal cells; the Na^+-K^+-ATPase sets up an electrochemical Na^+-gradient which provides the energy for osmoregulation, Na^+-nutrient cotransport, and the action potential of excitable cells

113 PHYSICAL AND BIOCHEMICAL PROPERTIES OF EGF RECEPTORS: RELATIONSHIPS TO RECEPTOR TURNOVER, C. Fred Fox, Peter, S., Linsley and Ken Iwata, Molecular Biology Institute and Department of Microbiology, University of California, Los Angeles, CA 90024

Incubation of cultured cells with EGF "down regulates" EGF receptors through their internalization and degradation. Our initial demonstration of EGF receptor internalization was based on affinity labeling. More recently we reported that EGF combines with its receptors forming a "direct linkage" complex without affinity labeling (1). Direct linkage occurs with cells, isolated membranes and detergent extracts. The complex formed on intact cells has an M_r of 160,000 - 170,000, but the major products with membranes are M_r = 145,000 and 115,000. This sensitivity to proteolysis has been used to probe EGF receptor structure as described by Linsley and Fox elsewhere in this abstract volume.

Treatment of cell membranes with nonionic detergent renders approximately half the EGF binding activity operationally "soluble". The other half readily sediments at low centrifugal force. Both the solubilized and sedimentable forms of receptor form the "direct linkage" complex. The soluble and sedimentable forms of receptor also had similar K_D's for EGF binding, but differed dramatically in their response to EGF in the membrane protein phosphorylation assay described by Carpenter et al. (2). The easily sedimentable form of the receptor is totally refractory to EGF-induced phosphorylation when measured after treatment with nonionic detergent and separation of the sedimentable and soluble fractions. The soluble fraction, on the other hand, had all the EGF-induced protein kinase activity, and the only major proteins phosphorylated were also acceptors for EGF in the "direct linkage" reaction. This indicates that EGF receptor proteins are the major phosphate acceptors. When membranes were treated with EGF and gamma-Pi^{32}-ATP before detergent addition, a significant fraction of the labeled receptor protein appeared in the pellet fraction. This indicates that the kinase activity and receptor protein are separable, or that EGF-induced phosphorylation promotes receptor aggregation. Gel filtration and sedimentation analyses of the detergent soluble EGF binding components reveal a heterogeneous array of supramolecular aggregates which bind EGF, while little EGF binding activity appears as a monodisperse entity. These data show that EGF receptors exist largely as aggregates on the membrane prior to EGF binding and may explain the ability of other polypeptide hormones (FGF, PDGF) to "down regulate" EGF receptors (3).

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Hypercholesterolemia

112 FAMILIAL HYPERCHOLESTEROLEMIA: A GENETIC RECEPTOR DISEASE, Michael S. Brown, M.D., Department of Molecular Genetics, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

The autosomal dominant disease familial hypercholesterolemia is an important cause of atherosclerosis and myocardial infarction in man. The disease is produced by a mutation in the gene encoding the structure of a cell surface receptor for plasma low density lipoprotein (LDL).

Recent studies relating the cell surface receptor mutation to the pathogenesis of the disease will be discussed.

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Joseph L. Goldstein, Y.K. Ho, Sandip K. Basu and Michael S. Brown, Binding Site On Macrophages that Mediates Uptake and Degradation of Acetylated Low Density Lipoprotein, Producing Massive Cholesterol Deposition, Proc. Natl. Acad. Sci. USA, 76, 333-337, 1979.

Regulation of Membrane Bound Receptors

114 RECEPTOR-MEDIATED INHIBITION OF ADENYLATE CYCLASE.

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It is now well established that extracellular information may be transferred across the plasma membrane via the regulation by specific membrane receptors of adenylate cyclase activity and thereby the intracellular concentration of cAMP. This coupling of membrane receptors with adenylate cyclase can result in either inhibition or activation of the enzyme. The vast majority of work with isolated membranes and solubilized constituents of these coupled systems has dealt with the mechanisms of receptor-mediated activation as the inhibitions observed in situ were not often detectable in vitro or only of limited magnitude. Recently, a neuroblastoma x glioma hybrid tissue culture was shown by Nirenberg and coworkers (1,2) to have opiate, alpha adrenergic, as well as muscarinic receptors, all of which cause a substantial inhibition of adenylate cyclase activity both in situ and in vitro. Using these cells as a model, we have investigated the mechanisms by which various receptors inhibit cyclase activity. Our studies with isolated membranes showed that both nucleotides and monovalent cations are required for the opiate (3) as well as the muscarinic (4) receptor-mediated inhibitions. A definite specificity with regard to both effectors is seen; GTP and ITP but not GMP, ATP, UTP or CTP will suffice as active nucleotide effectors, while Na^+ and Li^+ but not $choline^+$ or K^+ are active as the monovalent cation effectors. We have also found a similar requirement for extracellular monovalent cations for both opiate and muscarinic regulation of intracellular cAMP concentrations (5). The nucleotides and monovalent cations which are required for efficient coupling of opiate receptors to adenylate cyclase have also been found to produce decreases in ligand affinity to these opiate receptors (6,7). The effects of monovalent cations are agonist specific, whereas nucleotides appear to alter both agonist and antagonist interaction with these receptors. Studies with radioactive ligands have also shown heterogeneity of the opiate receptor population in these cells as approximately half the sites are Na^+ sensitive and the other half nucleotide sensitive. These studies will be discussed in terms of various membrane constituents which may play a role in regulating receptor-mediated inhibitions of adenylate cyclase.

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115 AMINO ACID TRANSPORT REGULATION,

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The branched-chain amino acids are transported into E. coli by two osmotic shock-sensitive high affinity systems. One of these, designated LIV I, is a general transport system whereas the second, LS, is leucine-specific. In addition there is an osmotic shock-insensitive low affinity general system, LIV II, that transports the branched-chain amino acids. Genetic studies indicate that there are at least 4 gene products specified by livJ, K, H, and G for the two high affinity transport systems, LIV I and LS. These four genes form a cluster which is located at min 74 of the E. coli linkage map. Two of these genes, livJ, and livK, encode the periplasmic LIV-binding protein and leucine-specific binding protein, respectively. These proteins serve as amino acid receptors and have been isolated and extensively characterized. Genes livH and livG are both required for high affinity transport and have only been identified genetically. The binding protein synthesis and transport activity are highly regulated and repressed when cells are grown on leucine. Regulatory mutations livR and lstR lead to the depression of binding protein synthesis suggesting the presence of a repressor type of regulation of initiation of transcription. Other studies have shown that the regulation also responds to the level of charged leucyl-$tRNA^{leu}$ and that the products of the hisT and relA genes are required for normal regulation. These results suggest that, in addition, an attenuator model of transcription regulation similar to the regulation of the biosynthetic operons for various amino acids may be operative. The four genes required for high affinity transport and their regulatory regions have been cloned into a multicopy plasmid vector. Strains with this plasmid show 5-to 10-fold increased expression of high affinity leucine transport which is also repressed when the cells are grown on leucine. This plasmid permits the study of the regulation of leucine transport gene expression in vitro and in vivo, in a minicell producing strain. Research supported by USPHS Grant GM 11024 (DLO) and Am. Heart Association (JJA).

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116 RECEPTOR FUNCTIONS OF THE ENZYMES II OF THE BACTERIAL PHOSPHOENOLPYRUVATE:SUGAR PHOSPHOTRANSFERASE SYSTEM. M.H. Saier, Jr., Department of Biology, Univ. of Calif. at San Diego, La Jolla, CA 92093.

The phosphotransferase system (PTS) in E. coli is a multi-functional, multi-component enzyme system (1). Its primary functions deal with carbon source acquisition while its secondary functions are concerned with the regulation of bacterial physiology (2). The 1° functions of the system include a) extracellular detection, b) transmembrane transport, and c) phosphorylation of the sugar substrates of the system. The 2° functions include a) regulation of the activities of adenylate cyclase and various non-PTS permeases, b) regulation of cellular motility, and c) regulation of the induced synthesis of several PTS enzymes. Both the 1° and 2° functions appear to be elicited by the binding of a sugar substrate to an Enzyme II complex. One of these integral transmembrane enzymes, the mannitol Enzyme II (II^{mtl}), has been solubilized with detergent, purified to homogeneity (3), and reconstituted in an artificial membrane system (4). II^{mtl} is a protein of 60,000 molecular weight. It possesses an extracellular sugar binding site and distinct intracellular combining sites for sugar phosphate and phospho-HPr. An essential sulfhydryl group and an antibody combining site are localized to the cytoplasmic surface of the enzyme. Genetic dissection of II^{mtl} reveals that the chemoreception, transport, and phosphorylation functions are distinct (4). Thus, mutants are available in which the transport and phosphorylation functions of II^{mtl} are reduced, but chemoreception activity is enhanced. Other mutants are defective for transport and chemoreception but retain phosphorylation activity. These studies emphasize the functional complexity of the PTS and its integral membrane protein constituents.

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Myasthenia Gravis

117 MOLECULAR ASPECTS OF EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS, Sara Fuchs, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel

Experimental autoimmune myasthenia gravis (EAMG) is induced in several animal species by the injection of purified electric fish acetylcholine receptor (AChR) and is an appropriate model for studying the molecular origin, immunological mechanism and therapy of myasthenia gravis. Molecular dissection of AChR is attempted by various chemical modifications and derivatizations in order to identify the molecular entity responsible for the pathological myasthenic activity of AChR.

Pharmacologically inactive denatured AChR preparation, obtained by reduction and carboxymethylation of AChR in 6M guanidine, does not induce myasthenia in rabbits although it elicits antibodies which cross react with the intact receptor (1). Denatured AChR has both preventive and therapeutic effects on EAMG (2).

The myasthenic as well as the pharmacological activity of AChR appear to reside within a conformational structure in the molecule which is sensitive to denaturation and resistant to mild enzymic digestion. Thus, tryptic digestion of AChR does not abolish the pharmacological specificity and myasthenic activity of the molecule. Trypsinated AChR shows on SDS acrylamide gel electrophoresis one major band with a molecular weight of 27,000 (3), which appears to result from the original 40,000 subunit in the receptor.

Antibodies to AChR can be fractionated according to their antigenic specificity by using appropriate immunoadsorbents (4). Another approach for obtaining anti-AChR antibodies with restricted specificity is by the production of monoclonal antibodies. Hybridoma lines secreting monoclonal anti-AChR antibodies directed against defined determinants were obtained by hybridization of mouse antibody producing cells with a mouse myeloma line (5).

Specific immunotherapy of AChR-induced myasthenia is being attempted based on the structural analysis of AChR and on the immunological analysis of EAMG.

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118 ANTIBODIES TO ACETYLCHOLINE RECEPTORS AS PROBES OF RECEPTOR STRUCTURE AND PATHOLOGICAL AGENTS IN MYASTHENIA GRAVIS, Jon Lindstrom, Salk Institute for Biological Studies, P.O. Box 85800, San Diego, CA 92138

Acetylcholine receptor (AChR) from the organ of marine ray Torpedo californica has the subunit structure $\alpha_2\beta\gamma\delta$. The α subunits are known to be involved in ligand binding. The cation-specific channel whose opening is regulated by ligand binding is an integral component of the AChR molecule, but it is not known which subunits compose it. Monoclonal antibodies have been used to show that although AChR subunits can be distinguished by molecular weight, peptide maps, affinity labeling, and reaction with subunit sera, α and β subunits share a similar structural feature as do γ and δ subunits.

AChR from the electric organs of the fresh water teleost Electrophorus electricus and from mammalian muscle have structures similar to torpedo AChR. Antigenic determinants corresponding to α, β, γ and δ have been identified in AChR from human and bovine muscle. Using antisera and monoclonal antibodies, subunits corresponding to each of the torpedo subunits have been identified in AChR from electrophorus.

Immunization with purified AChR results in antibodies which cross react with muscle AChR in vivo producing Experimental Autoimmune Myasthenia Gravis (EAMG). Monoclonal antibodies have been used to demonstrate that a small portion of the α subunit dominates the immunogenicity of native torpedo AChR in rats. However, EAMG can be caused by immunization with any of the 4 denatured AChR subunits. This indicates that there is no single myasthenogenic antigen. This is reasonable given the pathological mechanisms identified in EAMG and human MG. The muscular weakness characteristic of these diseases is caused by defective neuromuscular transmission due primarily to loss of AChR. The primary cause of AChR loss appears to be an increase in the rate of AChR destruction triggered by antibody crosslinking of AChR (antigenic modulation). Complement-mediated focal lysis of the postsynaptic membrane is important both in causing AChR loss and in modifying the structure of the postsynaptic membrane.

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Energy Coupling to Signal Transduction and Transport

119 THE ROLE OF ION CURRENTS IN CELL ORGANIZATION AND GROWTH. Franklin M. Harold, Division of Molecular and Cellular Biology, National Jewish Hospital and Research Center, Denver, Colorado 80206.

During the past decade a generalized version of the chemiosmotic theory (1) has received widespread acceptance as a framework for the bioenergetics of prokaryotic and eukaryotic cells (2,3). Ion currents circulating across the plasma membrane have been seen to provide driving force and molecular mechanisms for a wide range of cellular functions including oxidative phosphorylation, flagellar motility, regulation of cell volume and pH, and stimulus - response coupling. Best understood is the role of ion currents in the transport of metabolites across the cell membrane. Growth and morphogenesis can also be considered to be transport processes in that they require precursors of many kinds to be brought to a particular place, there to be assembled in a vectorial and highly ordered manner in accordance with the genetic instructions. Recent evidence, particularly from the work of L. F. Jaffe and his associates (3,4) has implicated transcellular ion currents in the localization of growth in space. The operative ion, at least in the Fucus egg, is apparently calcium. We have begun to study the role of ion currents in growth and development of the water mold Blastocladiella emersonii. The results indicate that the growing cells drive a current through themselves such that, positive charges (protons?) enter the rhizoids and leave from the thallus. During sporulation the pattern reverses, such that charge current enters the thallus (calcium?) and leaves from the rhizoid region. Separate experiments revealed that growth of the rhizoids can be polarized by proton-conducting ionophores. We shall discuss the proposition that a transcellular proton current is part of the mechanism by which growth is localized in space.

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120 BIOCHEMICAL AND ALLOSTERIC PROPERTIES OF NEUROTOXIN RECEPTOR SITES ASSOCIATED WITH VOLTAGE-SENSITIVE SODIUM CHANNELS. William A. Catterall and Daniel A. Beneski, Department of Pharmacology, University of Washington, Seattle, Washington 98195

Na^+ channels responsible for action potential generation in electrically excitable neuroblastoma cells contain three distinct neurotoxin receptor sites. These receptor sites are all missing in variant neuroblastoma clones which are electrically inexcitable. The first receptor site binds tetrodotoxin and saxitoxin which inhibit Na^+ channels noncompetitively with respect to the other neurotoxins. The second receptor site binds the lipid soluble neurotoxins batrachotoxin, veratridine, aconitine, and grayanotoxin. Occupation of this site causes persistent activation of Na^+ channels in the absence of an electrical stimulus. The third receptor site binds the polypeptide neurotoxins, scorpion toxin and sea anemone toxin. Occupancy of this receptor site enhances the activation of sodium channels by the lipid soluble toxins by a heterotropic allosteric mechanism. Binding to this site is highly dependent on membrane potential. Comparison of the voltage dependence of toxin binding with voltage clamp data reveals a close quantitative correlation between the voltage dependence of binding and of activation of the sodium channel. These results suggest that the scorpion toxin receptor site is located on a voltage-sensitive component of the sodium channel whose conformation changes during activation of the ion channel. This component may be an important voltage sensor of the sodium channel.

A photoactivable derivative of scorpion toxin, azido nitrobenzoyl mono[^{125}]iodo scorpion toxin, has been prepared which retains biological activity and the ability to bind specifically to receptor sites associated with sodium channels. This derivative can be covalently attached to its receptor site in electrically excitable neuroblastoma cells or synaptosomes by photolysis. A polypeptide of M_r ∿250,000 is specifically labelled in neuroblastoma cells. Labelling is blocked by unlabelled scorpion toxin and by depolarization. This polypeptide is not labelled in a variant neuroblastoma clone lacking voltage-sensitive sodium channels. Polypeptides of M_r ∿250,000 and M_r ∿32,000 are specifically labelled in synaptosomes. This labelling is also blocked by unlabelled toxin and by depolarization. These results identify specific polypeptides of M_r ∿250,000 and 32,000 that are components of the sodium channel.

121 THE LIGHT-DRIVEN SODIUM PUMP OF HALOBACTERIUM HALOBIUM, Janos K. Lanyi, Max-Planck-Institute for Biochemistry, D-8033 Martinsried bei Munich, Federal Republic of Germany

Light-induced sodium extrusion from *H. halobium* cell envelope vesicles proceeds largely through an uncoupler sensitive pathway involving bacteriorhodopsin and a proton/sodium antiporter. Vesicles from bacteriorhodopsin-negative strains also extrude sodium ions during illumination, but this transport is not sensitive to uncouplers, and has been proposed to involve a light-energized primary sodium pump (1). Proton uptake in such vesicles is passive, and under steady state illumination the large electrical potential (negative inside) is just balanced by a pH difference (acid inside), so that the protonmotive force is near zero (2). Action spectra indicate that this effect of illumination is attributable to a pigment absorbing near 585 nm (cf. 568 nm for bacteriorhodopsin). Bleaching of the vesicles by prolonged illumination with hydroxylamine results in inactivation of the transport; retinal addition causes partial return of the activity (3). Retinal addition causes also the appearance of an absorption peak at 588 nm, while the absorption of free retinal decreases. The 588 nm pigment is present in very small quantities (0.13 nmol/mg protein), and behaves differently from bacteriorhodopsin in a number of respects. These observations indicate the existence of a second retinal-protein, in addition to bacteriorhodopsin, in *H. halobium*, associated with primary sodium translocation. The initial proton uptake normally observed during illumination of whole *H. halobium* cells may be a passive flux, in response to the primary sodium extrusion by the second pigment.

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Endorphins and Opiate Receptors

122 BIOCHEMICAL AND AUTORADIOGRAPHICAL EVIDENCE FOR TYPE 1 AND TYPE 2 OPIATE RECEPTORS.
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Receptors which are coupled to adenylate cyclase in the "classical" manner in which receptor occupancy produces cyclic AMP production, show specific effects of GTP on receptor binding. Opiate receptors appear to fall into two very distinct groups. The Type I opiate receptor (in analogy with the classification of dopamine receptors recently proposed by John Kababian) are probably coupled to adenylate cyclase in the classical manner since low concentrations of GTP completely reverse the inhibition of tritiated diprenorphine binding by met-enkephalin. Type I opiate receptors are located in the guinea pig ileum, areas in the brain which mediate analgesia, and most other brain areas. Type II receptors show no effect of GTP, presumably because they are not coupled to cyclase or coupled in an unusual way so that occupancy by opiates causes an inhibition of cyclic AMP production. Type II opiate receptors are found in the vas deferens, the neuroblastoma-glioma cells, and many limbic brain regions including the amygdala, hypothalamus, substantia nigra, nucleus accumbens, and frontal cortex. Type I and Type II opiate receptors show markedly different drug specificities. Type I receptors have a much higher affinity for morphine than leu-enkephalin, while Type II receptors have a much higher affinity for leu-enkephalin and peptide opiates in general than morphine. The benzomorphans appear to owe their bizarre pharmacological properties to the fact that they are able to interact with both types of receptors. They differ in their relative affinity for the agonist and antagonist conformations of Type I receptors which can be experimentally determined by their sensitivity to sodium chloride.

Regulation of Membrane Bound Receptors II

123 BIOCHEMICAL STEPS IN VISUAL TRANSDUCTION.
M. Deric Bownds, Laboratory of Molecular Biology and Department of Zoology, University of Wisconsin, Madison, WI 53706.

Recent studies on the biochemistry and electrophysiology of the outer segments of amphibian retinal rod photoreceptors show promise of specifying pathways which underline excitation and adaptation. The prevailing hypothesis is that photoactivation of rhodopsin molecules in the internal disc membranes regulates the level(s) of diffusable internal transmitter(s) which control the permeability of the physically separate plasma membrane. Complete suppression of permeability is caused by a flash of light which activates only 100-200 of the 3×10^9 rhodopsin molecules present. Continuous illumination desensitizes the transduction mechanism so that permeability suppression occurs over 3-4 log units of light intensity. Recent experiments from this laboratory have studied several chemical changes which are relevant to these processes: (a) Light activation of a cyclic GMP phosphodiesterase which is sensitive to calcium concentration; (b) A resulting rapid drop in cyclic GMP levels which has stoichiometry and time course appropriate for the internal transmitter presumed to mediate between photon absorption in the disc membrane and the permeability decrease in the plasma membrane[1]; (c) A dephosphorylation of two small proteins whose phosphorylation is controlled by cyclic GMP levels[2]; and (d) A slower hydrolysis of GTP which may drive efflux of calcium from the outer segment[3]. On the basis of this work, it is suggested that the rapid decrease in sodium conductance which follows illumination is caused by the dephosphorylation of the two small proteins, with their dephosphorylation being controlled by the cyclic GMP decrease. In a slower reaction light activates a GTP-dependent extrusion of calcium from the cytoplasmic space. This lowering of internal calcium causes desensitization of the light-sensitive phosphodiesterase enzyme responsible for the cyclic GMP decrease, so that its intensity-response function now resembles that of the light-adapted rod photoreceptor. Thus, changes in plasma membrane conductance are regulated by cyclic GMP, and the sensitivity of the system is controlled by slower calcium movements which set the light-sensitivity of the phosphodiesterase enzyme. Finally, the light-initiated phosphorylation of rhodopsin may play a role in this process, with phosphorylated rhodopsin influencing inactivation of the phosphodieserase enzyme.

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124 PHOSPHORYLATION OF THE ACETYLCHOLINE RECEPTOR, Adrienne Gordon, Gil Magilen and Ivan Diamond, Dept. of Neurology, University of California, San Francisco, CA. 94143

The mechanism by which a receptor located in the plasma membrane acts as a transducer for metabolic signals, growth factors, hormones or neurotransmitters is of major interest in cellular biology today. We have found that the acetylcholine receptor (AChR) from the electric organ of Torpedo californica is phosphorylated in situ by an endogenous membrane protein kinase (1). Moreover, AChR-enriched membranes have phosphoprotein phosphatase activity which dephosphorylates the membrane-bound AChR (2). Therefore, it is possible that a reversible phosphorylation-dephosphorylation mechanism may play an important role in the regulation of receptor function at the synapse. Receptor phosphorylation may also be a general mechanism by which a signal is transduced at the cell membrane. We have therefore studied the properties of AChR phosphorylation and dephosphorylation in T. californica in order to better understand how receptor function is regulated in a model system where sufficient amounts of receptor and associated proteins are available for biochemical studies.

We have also investigated endogenous membrane protein phosphorylation in a mammalian muscle cell tissue culture line, L8. L8 myoblasts differentiate in culture to form myotubes with concomitant appearance of AChR and contractile proteins. When plasma membranes prepared from L8 myotubes are incubated with Mg^{+2} and [γ-^{32}P]ATP, the major phosphorylated polypeptide has a molecular weight of 41,000. Moreover, phosphorylation of this polypeptide is stimulated by K^+ but not by Na^+. Phosphorylation of this polypeptide (pp41K^+) appears to be correlated with development since membranes from myoblasts show comparatively little K^+-stimulated phosphorylation. Since the mammalian AChR has a polypeptide component of M.W. 41,000, it was possible that pp41K^+ could be a component of the AChR. However, using anti-mammalian AChR antibody, we have shown that pp41K^+ is not a component of AChR. Experiments with intact myoblasts in culture indicate that pp41K^+ is on the external surface of the plasma membrane. pp41K^+ may be associated with the AChR on the surface of myotubes since both molecules appear upon differentiation of L8 myoblasts to myotubes. The simultaneous appearance of AChR and pp41K^+ suggests that K^+-stimulated phosphorylation of muscle cell membranes may be involved in muscle cell function.

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125 ROLE OF PHOSPHOLIPID METHYLATION IN SIGNAL TRANSDUCTION, Fusao Hirata, John F. Tallman, Richard C. Henneberry[†], Pierre Mallorga, Warren J. Strittmatter, and Julius Axelrod, National Institute of Mental Health and [†]National Institute of Neurologic and Communicative Disorders and Stroke, Bethesda, Md. 20205

The conversion of phosphatidylethanolamine to phosphatidylcholine is catalyzed by two methyltransferases with S-adenosylmethionine as the methyl donor. Phosphatidylcholine formed by transmethylation is further metabolized by phospholipase A_2. The synthesis and degradation of methylated phospholipids are involved in regulating the number of the β-adrenergic receptors and their coupling to adenylate cyclase in rat reticulocytes, HeLa cells and astrocytoma cells. Methylation of the phospholipids in these cells is stimulated by binding of agonists to the β-adrenergic receptors. Accumulation of phosphatidyl-N-monomethylethanolamine causes an increase in membrane fluidity and enhances the coupling of the receptors to adenylate cyclase. Agents that inhibit phospholipid methylation decrease the number of receptors in intact HeLa cells. In rat reticulocyte ghosts, phospholipid methylation unmasks cryptic receptors with the concomitant formation of phosphatidylcholine. Conversely, the degradation of methylated phospholipids appears to be closely associated with desensitization of the β-adrenergic receptors following prolonged stimulation with isoproterenol. Inhibition and stimulation of phospholipase A_2 cause inhibition and stimulation of this desensitization process, respectively.

Energy Coupling Mechanisms

126 RECEPTORS AND SIGNAL TRANSDUCTION IN THE BACTERIAL MEMBRANE. D.E. Koshland, Jr., Department of Biochemistry, University of California, Berkeley. 94720

The bacterium senses its environment utilizing approximately 30 receptors located in its periplasmic space and inner membrane (1,2). Evidence for the periplasmic receptors has been obtained from behavioral studies, mutants, and isolation of the purified proteins. In this manner the galactose, ribose and maltose receptors have been identified and purified (3,4,5). Until recently the evidence for the inner membrane receptors has been entirely from behavioral studies. A technique has been developed for the measurement of binding to the inner membrane receptors (6). With this method the aspartate and serine receptor binding properties in isolated membranes have been correlated with their behavioral properties in vivo. The receptors are known to transmit their signals to proteins which are methylated and demethylated in the sensory process (7,8). Mutations in these proteins and purification procedures have made it possible to identify a transferase and an esterase which are involved in the methylation and are active in vitro (9,10). Methylation occurs sequentially over time and involves multiple methylation of the sensory transduction proteins (11). The entire process can be explained in terms of a response regulator which is altered in level in a transient manner. The level of the signal is dependent on both induced conformational changes and covalent modification of the sensory processing proteins.

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Membrane Biogenesis and Assembly

127 MECHANISMS FOR THE INTEGRATION OF PROTEINS INTO MEMBRANES, Günter Blobel, Department of Cell Biology, Rockefeller University, New York, NY 10021

Integral membrane proteins (IMPs) can be classified into monotopic, bitopic and polytopic (1). The polypeptide chain of monotopic IMPs exhibits a unilateral topology, i.e., it possesses hydrophilic domain(s) exposed to the hydrophilic environment on only one side of the membrane and a hydrophobic domain(s) which anchors the polypeptide to the hydrophobic core of the lipid bilayer. The polypeptide chain of bitopic and polytopic IMPs is bilateral in nature, containing two or multiple hydrophilic domains, respectively, exposed on opposite sides of the membrane.

The information for the asymmetric integration of IMPs is proposed to reside in discrete segments of the polypeptide chain. Thus, a "signal" sequence is required to initiate unidirectional translocation of hydrophilic domains across membranes that face the protein-biosynthetic compartments (pro and eukaryotic cytoplasm, chloroplast stroma, mitochondrial matrix). This signal sequence can be decoded only by a specific translocator. The translocator may consist of oligomeric IMPs. There are several types of signal sequences. Their corresponding translocators are restricted in their localization to distinct cellular membranes. A stop-transfer sequence interrupts the process of chain translocation that was previously initiated by a signal sequence, and, by excluding a distinct portion of the polypeptide chain from translocation, yields asymmetric integration of proteins into a translocation-competent membrane. An insertion sequence causes self-directed unilateral integration of the polypeptide chain into but not across the lipid bilayer. Unilateral integration proceeds without the mediation by a specific insertion apparatus in the membrane.

Using these discrete "topogenic" sequences one can formulate a set of rules for problem integration into membranes. Monotopic IMPs exposed on that side of the membrane that faces the protein-biosynthetic compartment (or a topological equivalent such as the exterior face of the outer membrane of gram-negative bacteria) only need an insertion sequence. Monotopic IMPs of all other locations need a signal sequence to effect their complete translocation and an insertion sequence to effect their subsequent integration. Bitopic IMPs need a signal sequence and a stop-transfer sequence. Polytopic IMPs need an internal signal sequence (2) or multiple signal sequences in combination with one or more stop-transfer sequence(s). Those IMPs which need a signal sequence for integration (which is most IMPs except for translocation-independent monotopic IMPs, see above) can be integrated therefore only into translocation-competent membranes. Translocation-incompetent membranes such as the Golgi, lysosomal and eukaryotic plasma membrane or the prokaryotic outer membrane have to receive their translocation-dependent IMPs from translocation-competent donor membranes, such as the rough endoplasmic reticulum or the prokaryotic plasma membrane, respectively. The existence of specific "sorting" sequences and of specific proteins that decode and process these sorting sequences to effect sorting is postulated. (1)Blobel, G. PNAS in press. (2) Lingappa, V.L. et al. Nature, 281:117-121.

128 GENETIC STUDIES OF OUTER MEMBRANE BIOGENESIS IN ESHCERICHIA COLI, Thomas J. Silhavy and Scott D. Emr, Frederick Cancer Research Center, P.O. Box B, Frederick, MD 21701.

A series of strains have been isolated in which the gene coding for the outer membrane protein λ receptor (lamB) is fused to the gene coding for β-galactosidase (lacZ). These strains produce a hybrid protein comprised of an NH_2-terminal fragment of λ receptor and a functional COOH-terminal portion of β-galactosidase. Four classes of fusions have been identified based on the amount of lamB DNA present in the hybrid gene. Our results indicate that, provided a sufficient amount of lamB DNA present in the hybrid gene, the hybrid protein is exported to the outer membrane (1).

A number of mutations have been isolated that prevent export of one of the larger hybrid proteins. When recombined into an otherwise wild-type lamB gene, these mutations cause the accumulation of a λ receptor precursor in the cytoplasm (2). DNA sequencing analysis of these mutations (in collaboration with J. Hedgpeth, J.M. Clement and M. Hofnung) has revealed that all cause changes in the signal sequence of the λ receptor. The location of these mutations within this sequence defines regions that are critical for the correct cellular localization of λ receptor.

MET ILE THR LEU ARG LYS LEU PRO LEU ALA VAL ALA VAL ALA ALA GLY VAL MET SER ALA GLN ALA MET ALA

(VAL ↓ ASP, ALA ↓ GLU, ALA ↓ GLU, MET ↓ ARG)

A number of suppressor mutations have been isolated that allow export of the λ receptor protein containing a mutant signal sequence. Analysis of these mutants has revealed other components that are involved in the export process. For example, one class of suppressor mutations maps in a gene coding for a ribosomal protein.

While the above results demonstrate that importance of the signal sequence for export, a class of smaller lamB-lacZ fusions demonstrates that this signal is not, by itself, sufficient. The amino acid sequence of a small hybrid protein (in collaboration with M. Hall, F. Moreno, A. Fowler, I. Zabin and M. Schwartz) reveals that the entire signal sequence is present. Nevertheless, this hybrid protein remains in the cytoplasm.

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129 M13 PROCOAT AND THE MEMBRANE TRIGGER HYPOTHESIS, William T. Wickner, Koreaki Ito and Takayasu Date, Molecular Biology Institute, University of California, Los Angeles, California 90024

M13 coat protein is synthesized as a 5s soluble, precursor protein, termed procoat. It then binds to the inner face of the cytoplasmic membrane. In the presence of an electrical potential, an acidic central region of the procoat crosses the bilayer and is cleaved by leader peptidase to yield coat protein and leader peptide. This pathway has been elucidated by in vivo pulse-chase experiments, employing uncouplers to control the membrane potential and proteolysis to map the location of the procoat and coat proteins. Similar data are obtained in an in vitro reconstruction of these reactions.

Membrane Transport of Hormones and Toxins

130 DEFICIENCY OF RECEPTOR-CYCLASE COUPLING PROTEIN IN ALBRIGHT'S HEREDITARY OSTEODYSTROPHY, Henry R. Bourne, Zvi Farfel, Arnold Brickman*, Harvey Kaslow and Virginia Brothers, Departments of Medicine and Pharmacology & Cardiovascular Research Institute, Univ. of California, San Francisco, CA 94143 and (*) Department of Medicine, Univ. of California Los Angeles School of Medicine and Veterans Administration Hospital, Sepulveda, California.

Patients with Albright's Hereditary Osteodystrophy (AHOD) [also called Pseudohypoparathyroidism, Type I] exhibit skeletal abnormalities and resistance to parathormone-induced stimulation of phosphaturia and urinary cyclic AMP (cAMP) excretion. Because these patients often show partial resistance to effects of other hormones that work via cAMP, we investigated the possibility that the biochemical lesion of AHOD affects the guanine nucleotide regulatory component of adenylate cyclase, which functions as a Receptor-Cyclase Coupling Protein (RCCP).[1,2] RCCP in human erythrocyte membranes was assayed by: 1. Ability of detergent (Lubrol)-soluble membrane extracts to reconstitute isoproterenol- and guanine nucleotide-stimulable adenylate cyclase when mixed with genetically RCCP-deficient membranes of cyc^- S49 mouse lymphoma cells.[2] The assay of RCCP activity was linear with added erythrocyte extract. 2. Measurement of cholera toxin-catalyzed transfer of ^{32}P-ADP-ribose from ^{32}P-NAD to a 42,000-dalton erythrocyte membrane peptide subunit of RCCP.[2,3] We assayed RCCP in erythrocyte membranes of eight normal subjects, five patients with surgical or idiopathic hypoparathyroidism, and twelve patients with chemical evidence of AHOD (hypocalcemia, hyperphosphatemia, elevated serum parathormone and deficient phosphaturic and urinary cAMP responses to parathormone). Most AHOD patients showed abnormal responses to other hormones as well. RCCP activity and ^{32}P-transfer in erythrocytes of eight AHOD patients (five from one family, two from another, one isolated case) were indistinguishable in amount from those of normal and hypoparathyroid subjects. In contrast, erythrocytes of four AHOD patients (a mother and daughter from one family, plus two isolated cases)showed a 40-55% decrease in RCCP ($p<0.001$), measured by both the cyclase reconstitution assay and by incorporation of ^{32}P from NAD into the 42,000-dalton peptide. Relative RCCP levels were reproducible in individual AHOD and normal subjects on repeated blood drawing, and did not correlate with degree of hypocalcemia or administration of vitamin D. AHOD patients with low RCCP had skeletal abnormalities (brachydactyly, short metacarpals), while those with normal RCCP did not. Families with AHOD exhibited RCCP activities that were uniformly either high or low. Studies to date have not established a qualitative difference between RCCP in AHOD and normal subjects. We conclude that partial deficiency of erythrocyte RCCP is a biochemical marker of AHOD in a subset of individuals and families with the disease, and that RCCP deficiency may explain their resistance to parathormone and other hormones.

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131 CHOLERA TOXIN ENTRY AND MECHANISM OF ACTION, D. Michael Gill, Department of Molecular Biology and Microbiology, Tufts University, Boston, MA 02111.

Cholera toxin, and the related heat-labile enterotoxin of E. coli, activate adenylate cyclase by a complex sequence of events. 1) The toxin binds via its five B subunits to ganglioside G_{M1} exposed on the cell surface. 2) The active A subunit slowly traverses the plasma membrane by a process that is almost totally mysterious but may involve the creation of a channel. The A subunit must reach at least as far as the inner surface of the plasma membrane for it next interacts with intracellular molecules. 3) The A subunit is thiol reduced and, if not already, proteolytically nicked, releasing fragment A_1 that is an enzyme. 4) A_1 catalyzes the ADP-ribosylation of a number of soluble and membrane-bound proteins. The most readily-modified protein is the 42,000 MW GTP-binding regulatory subunit whose modification causes adenylate cyclase activity to increase.[1] Bound GTP is required for the modification to occur[2] and for the elevated cyclase activity to be apparent.

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Nerve Growth Factor

132 NERVE GROWTH FACTOR RECEPTORS ON PC12 CELLS: LIGAND INDUCED CONVERSION FROM LOW TO HIGH AFFINITY STATES, Eric M. Shooter and Gary E. Landreth, Dept. of Neurobiology, Stanford University Medical School, Stanford, CA 94305

A clonal line of pheochromocytoma cells (PC12) respond to nerve growth factor (NGF) by undergoing morphological and biochemical differentiation analogous to that of sympathetic neurons. The PC12 cells possess specific cell surface receptors which bind NGF with two distinct affinities, measured both at steady-state and kinetically. Analysis of NGF binding at steady-state revealed a low capacity, high affinity component ($K_d = 10^{-11}$ M, 25,000 sites/cell), as well as a high capacity, low affinity component ($K_d = 10^{-9}$ M, 500,000 sites/cell). Kinetic measurements of both association and dissociation rates also demonstrated two distinct binding affinities. The association kinetics at 37° demonstrated that ^{125}I-NGF initially bound to low affinity receptors, while high affinity binding appeared only after a 30 sec delay. The removal of ^{125}I-NGF from the incubation medium halted binding to the low affinity receptors, yet high affinity binding increased, indicating conversion of the NGF-receptor complex to a high affinity state. The change in receptor affinity was accompanied by the transfer of the NGF-receptor complex from a trypsin-sensitive to a trypsin-resistant state. We conclude that the binding of NGF to specific cell surface receptors on PC12 cells causes the conversion of a proportion of the low affinity receptors to a high affinity state accompanied by a concomitant positional and/or conformational change in the NGF-receptor complex.

133 BINDING AND SEQUESTRATION OF (125)I-β NERVE GROWTH FACTOR TO EMBRYONIC SYMPATHETIC NERVE CELLS, Robert W. Stach and Edward J. Olender, SUNY-Upstate Medical Center, Syracuse, New York 13210

Cell dissociates of embryonic day 11 (E11) sympathetic chain ganglia were incubated with various concentrations of (125)I-β nerve growth factor ((125)I-βNGF). The (125)I-βNGF was shown to bind to sympathetic nerve cells in a specific, saturable manner with high affinity. This binding is characterized by two binding sites. The higher affinity, type I, site has an equilibrium dissociation constant (KdI) of 1.1. x 10^{-11}M. The lower affinity (type II) site has an equilibrium dissociation constant (KdII) of 5 x 10^{-10}M. These binding constants are essentially the same as those found on sensory nerve cells (Sutter, et al., 1979, J. Biol. Chem. 254, 5972 and Olender, et al., submitted to J. Neurochem.). When E11 sympathetic nerve cells are incubated with (125)I-βNGF (10 ng/ml) for various times, then incubated with either an excess of βNGF or a trypsin solution, a certain amount of the specifically bound (125)I-βNGF can not be removed. The (125)I-βNGF is sequestered - it is inaccessible to the external milieu. The amount of (125)I-βNGF sequestered is time dependent. Under the conditions of the experiment, approximately 50% of the specifically bound (125)I-βNGF can be sequestered in one hour. The type I sites appear to be the ones that are involved in this sequestration. Since at low concentrations of (125)I-βNGF, a higher percentage of ligand is sequestered than at higher concentrations. Sequestration is an energy dependent process. Incubation with metabolic inhibitors eliminate the sequestration. However, there is no effect on the binding of (125)I-βNGF to sympathetic nerve cells under these conditions.
This work was supported by NIH grant number NS12325.

134 INTERACTION OF ^{125}I-NGF WITH CULTURED RAT SYMPATHETIC NEURONS, Edward Hawrot, Robert B. Campenot[†], Philippa Claude[††], and Paul H. Patterson, Harvard Medical School, Boston MA 02115, [†]Cornell University, Ithaca NY 14850, [††]University of Wisconsin, Madison WI 53706

Nerve Growth Factor (NGF) is important for the survival and maintenance of sympathetic neurons in vivo and in culture. In order to characterize the interaction of NGF with sympathetic neurons, the binding and uptake of ^{125}I-NGF was studied in low-density cultures of dissociated neurons obtained from neonatal rat superior cervical ganglia. In such cultures where the neurons are the only cell type present, the major portion of the cell's plasma membrane is in axonal processes. Binding studies performed at 25° on living neurons after 3-4 weeks in culture indicated a high affinity binding site with an apparent K_D of $\sim 6 \times 10^{-9}$M with $\sim 10^{7}$ binding sites per neuron. Autoradiographic studies show that the membrane binding sites are distributed both over the axons and the cell body. Upon dilution, bound ^{125}I-NGF dissociates slowly at 25° ($t_{1/2} \cong 5$ hrs.). Using a 3-chamber culture system where the neuronal cell bodies and their axons are located in separate compartments, it is possible to show that at 36°, ^{125}I-NGF is internalized and retrogradely transported from the axons to the cell bodies. The retrograde transport process appears to be saturated at $\sim 4 \times 10^{-9}$M, a concentration that is also the saturating dose for neuronal survival. Autoradiographic analysis of the subcellular distribution of ^{125}I-NGF after retrograde transport fails to show any localization to the nucleus at a time maximal for cellular accumulation.

Supported by the Helen Hay Whitney Foundation, MDA, NSF, and the NINCDS.

135 MODULATION OF EPIDERMAL GROWTH FACTOR (EGF) AND NERVE GROWTH FACTOR (NGF) INDUCED ADHESION AND GROWTH FACTOR BINDING OF PC-12 PHEOCHROMOCYTOMA CELLS BY THE TUMOR PROMOTER TETRADECANOYL PHORBOL ACETATE (TPA), Charles E. Chandler and Harvey R. Herschman, University of California, Los Angeles, CA 90024

NGF has previously been shown to increase the adhesion of PC-12 cells to cell culture dishes. We now report that EGF is also able to elicit increased adhesion of PC-12 cells. The dose response curve for EGF is bell shaped in contrast to the more classical dose response curve obtained with NGF. EGF does not cause neurite outgrowth although the increase in adhesion is of the same magnitude as that induced by NGF. Thus, an increase in cell adhesion is not sufficient to cause neurite formation.

TPA is able to block the EGF induced adhesion of PC-12 cells. TPA does not, however, alter the NGF-induced adhesion of these cells. TPA shifts the EGF binding curve to the right but does not alter maximal EGF binding at saturating concentrations of growth factor. NGF binding is not affected by TPA.

TPA is able to alter the expression of phenotypic characteristics in a number of cell culture systems. Process outgrowth of serum-deprived neuroblastoma cells and NGF exposed chick embryonic ganglia cells in culture have been reported to be delayed by TPA. NGF induced neurite formation by PC-12 cells, however, is unaffected by TPA.

TPA is able to block the decrease in short microvilli caused by EGF but not the decrease caused by NGF as seen by scanning electron microscopy. Long microvilli which are formed in response to both EGF and NGF are blocked in both cases by TPA.

136 STRUCTURE AND FUNCTION OF NERVE GROWTH FACTOR FROM GUINEA PIG PROSTATE AND MOUSE SUBMAXILLARY GLAND, Jeffrey S. Rubin, Nicholas V. Costrini and Ralph A. Bradshaw. Washington University School of Medicine, St. Louis, MO 63110.

Most studies performed with nerve growth factor (NGF) have utilized material obtained from the male mouse submaxillary gland. Recently a high concentration of NGF has been found in another androgen-sensitive tissue, the guinea pig prostate (1,2). Preliminary analysis indicates that the molecules from these two sources are structurally related and, like the protein isolated from the mouse submaxillary gland, guinea pig NGF migrates as a high molecular weight entity (≥100,000) on gel filtration chromatography at neutral pH. The possible exocrine function of these NGF molecules is unknown but presumably differs from their action on responsive neurons which is initially mediated by complex formation with a specific cell surface receptor that has been determined to be a minimally hydrophobic, asymmetric, intrinsic membrane protein with a calculated molecular weight of 135,000. While the two forms of NGF may be useful in further studies involving interaction with these receptors and those that occur within the cell, they have been of particular value in elucidating features of the biosynthesis of NGF, as it relates to the physiological production and transport of the hormone to target neurons, an aspect of NGF that has remained especially obscure. Supported by NIH research grant NS10229.

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137 SHORT-LATENCY EFFECTS OF NGF DEPRIVATION AND ADMINISTRATION ON GANGLIONIC CELLS, Silvio Varon and Stephen D. Skaper, University of California, San Diego, La Jolla, CA 92093

Nerve Growth Factor (NGF) is likely to exert its trophic action on dorsal root ganglion (DRG) and on sympathetic ganglion neurons by controlling a crucial function of these cells. Such a function would in turn regulate other cellular machineries and ultimately lead to traditional NGF consequences such as survival and neuritic growth. Thus, the key to NGF action must lie in short-latency events occurring within minutes of NGF administration. Chick embryo DRG dissociates have proved to be an effective experimental system to investigate short-latency responses to NGF, in that: i) measurable functional deficits develop with 6 hr of NGF deprivation and ii) delayed presentation of NGF promptly and fully restores the defective function. From the first deficit observed in this system, a decline in RNA labeling capability, we were led to the recognition that NGF controls the transports of selected exogenous substrates, all of which are Na gradient-dependent, and that NGF does so by regulating the neuronal ability to control its intracellular Na^+. Under NGF deprivation, the cells accumulate Na^+ to levels that reflect, and presumably equate, the extracellular Na^+ concentrations. On delayed NGF administration, the accumulated Na^+ is actively extruded to an extent and at a speed that depend on the NGF concentration. The "Na^+ response" is elicited by both Beta and 7S NGF, but not by other proteins tested. All ganglionic systems tested thus far have displayed the Na^+ response when they also display a requirement for exogenous NGF in culture. We believe that the regulation by NGF of Na^+ extrusion mechanisms is likely to occupy an early and fundamental place in the sequence of events underlying the mode of action of this factor.

Measurement of Membrane Potential and Energy Coupling

138 CHARACTERIZATION OF THE SEROTONIN TRANSPORTER OF PLATELET PLASMA MEMBRANE,
Gary Rudnick and Jane Talvenheimo, Yale University, New Haven, CT 06510
The mechanism of serotonin transport by platelets has been studied in isolated plasma membrane vesicles. Serotonin transport is coupled to gradients of Na^+, K^+ and Cl^- across the membrane and probably involves the coupled movement of one Na^+, one K^+, one Cl^- and one molecule of serotonin for each catalytic cycle. Binding of the specific inhibitor imipramine has been studied as a measure of the binding step during transport. The ion gradients which are responsible for driving serotonin accumulation are not required for imipramine binding, indicating that the binding step is independent of the energy-dependent steps in transport. The presence of Na^+ in the external medium, however, is required for imipramine binding, although Cl^- is not essential. Serotonin, the normal substrate, competitively displaces bound imipramine in a manner which suggests that both are bound at the same site, but only serotonin is transported. Imipramine binding has also been studied in detergent-disrupted plasma membranes. Some detergents inactivate imipramine binding activity reversibly, others inactivate irreversibly, and some do not inactivate. Of the detergents studied which do not inactivate the transporter, only digitonin solubilizes binding activity. Serotonin transporter solubilized in digitonin retains imipramine binding characteristics of intact platelet plasma membranes. Binding requires Na^+ and is completely displaced by serotonin. The K_D for imipramine binding, however, increases from about 10 nM in intact plasma membranes to approximately 150 nM in digitonin solution.

139 INTESTINAL Na:SUGAR TRANSPORT COUPLING STOICHIOMETRY, George A. Kimmich and Joan Randles, University of Rochester, Rochester, NY 14642
When a cation:solute coupling stoichiometry is measured for gradient-coupled transport systems it is necessary to take the difference between unidirectional cation fluxes measured in the presence and absence (basal) of the solute of interest. Typically basal cation flux is a high percentage of the total flux so that small changes in either value can markedly change the calculated coupling ratio. Furthermore, it has not been recognized that an ion-coupled solute flux may itself disturb the membrane potential with consequent adjustments in the flow of ion by other routes (i.e., basal cation flux). An accurate ratio can be determined in these instances only when appropriate corrections for the change in basal cation entry are made. Alternatively, accurate values can be determined under conditions in which the membrane potential is experimentally clamped at a fixed value regardless of the presence or absence of transported solute. We have found that conventional procedures applied to isolated intestinal epithelial cells provide a Na:sugar coupling ratio of 1:1. If cells in which the membrane potential is held near zero are employed, the measured coupling ratio is 2:1. This is consistent with the fact that basal Na^+ entry in these cells is a potential-dependent event which can change up to 30% when valinomycin-induced K^+ diffusion potentials are imposed. A 2:1 coupling stoichiometry means that theoretical sugar gradients as high as 400 fold can be achieved by these cells using only energy derived from the trans-membrane electrochemical potential for Na^+. In contrast the commonly accepted 1:1 coupling ratio would support theoretical sugar gradients somewhat less than the 70 fold gradients actually observed for these cells. Supported by NIH Grant #AM-15365 and Dept. of Energy, assigned Rept. #UR-3490- 1775.

140 TRANSMEMBRANE ELECTRICAL GRADIENTS AND $^{86}Rb^+$ UPTAKE BY GUINEA PIG ALVEOLAR MACROPHAGES, Andrij Holian and Ronald P. Daniele, University of Pennsylvania, Philadelphia, PA 19104
The transmembrane electrical potential (E) of the alveolar macrophage (AMØ) was determined from the uptake of (^3H)-triphenylmethyl phosphonium ($TPMP^+$). At 25°C in Hanks' balanced salt solution (HBSS), the E, which represents an upper limit, is -27 mV and is in agreement with reports of other phagocytic cells. The distribution of $TPMP^+$ was also measured for AMØ suspended in HBSS in which the extracellular [K^+] was 75 mM (equimolar K^+ for Na^+). The rate of $TPMP^+$ uptake was slower and gave a lower value of E, -6.5 mV. The addition of valinomycin (22.5 μM) did not affect the rate of $TPMP^+$ uptake or the final value of E. Equilibrium of $^{86}Rb^+$ across AMØ membranes was reached in one hour. It was completely inhibited (> 90%) by ouabain. Valinomycin also did not affect $^{86}Rb^+$ uptake. These studies indicate that (a) it is feasible to measure E in AMØ with lipophilic ions ($TPMP^+$); (b) E and K^+ permeabilities are unaffected by valinomycin; (c) the K^+ distribution is mediated mostly by the sodium-potassium ATPase. (Supported by NIH grants 07083-14, 1K04-HL-00210 and HL-15061.)

141 ELECTRICAL PROPERTIES OF GIANT ESCHERICHIA COLI, C.L. Slayman and H. Felle, Yale Medical School, New Haven, CT 06510 and University of Tübingen, D7400 Tübingen, G.F.R.
The major obstacle to be overcome in obtaining direct electrical measurements on intact energy-conserving cells or organelles is the finite leak at the point of insertion (damage) of the microelectrode. Increase of cell size (membrane area) and of lipophilicity of the electrode both tend to mitigate this leak. By combining a technique for growing giant *Escherichia coli* (spheres, 2-6 μm diameter), in mecillinam, with a technique for lipophilically coating electrode shanks, with paraffin ± phospholipid, we have been able to measure membrane potentials up to (-)170 mV (cell interior negative) in *E. coli* under standard conditions. And from the systematic relationship between apparent surface resistance and cell diameter, we have been able to calculate both the membrane resistivity and the leakage resistance around the microelectrode.

Depending on the exact method of electrode coating, and upon the ionic strength of the bathing solution, leakage resistances varied from 3 to 60 Gohms. Calculated membrane resistivities, ranging from 2 to 3 Kohms·cm^2, were insensitive to the electrode properties. Corrected membrane potentials were also insensitive to the electrode properties, but varied with cell size, averaging -170 mV in cells under 3 μm diam. and -120 mV in cells 4-5 μm diam. KCN or NaN_3 (1 mM) depolarized the cells rapidly, taking the membrane potential to about -40 mV. Cyanide, however, caused a 50% increase of membrane resistivity, while azide caused a 50% decrease of resistivity. Presumably, the cyanide effect reflects shut-down of respiratory transport, while the azide effect also reflects increased membrane conductance associated with uncoupler action.

142 MEASURING MEMBRANE POTENTIAL OF WHITE ADIPOCYTES AND HUMAN ERYTHROCYTES BY TRIPHENYLMETHYLPHOSPHONIUM$^+$ ACCUMULATION. Kang Cheng, Howard C. Haspel, Mary Lou Vallano, Babatunde Osotimehin and Martin Sonenberg, Memorial Sloan-Kettering Cancer Center, New York, NY 10021
The method of measuring potential (ψ) by the transmembrane distribution of [^{3}H]-triphenylmethylphosphonium$^+$ (TPMP$^+$) was validated and calibrated for rat white adipocytes (RWA) and human erythrocytes (RBC). A plot of ψ, determined from TPMP$^+$-uptake, versus log [Cl^-] (substituting tartrate$^-$) for RBC had a "Nernst slope" (58.5 mV). Plots of ψ vs log [K^+] (substituting choline$^+$) for RBC in the presence of valinomycin (2 μM), A23187 (2 μM) plus Ca^{++} (2 mM), and A23187 (2 μM) plus Ca^{++} (2 mM) and diisothiocyanostilbene sulfate (DIDS; 3 mg/ml) had slopes of 37, 48, and 57 mV ("Nernst slope"), respectively. For RWA, plots of ψ vs log [K^+] (substituting Na^+) had slopes of 57 mV ("Nernst slope") and 11 mV above and below 100 mM K^+, respectively. The resting ψ's for RBC and RWA, determined by TPMP$^+$-uptake were -8.4 ± 1.3 mV and -58.3 - 5.0 mV respectively. The ψ-response of RBC and RWA to ionophores, ion-transport inhibitors, and respiratory inhibitors were determined from TPMP$^+$-uptake. For RBC, TPMP$^+$-uptake responded rapidly (<1 min) to perturbations of ψ. Gramicidin-D (1 μg/ml), as expected, hyperpolarized the RWA (20 mV). It was concluded that TPMP$^+$-uptake is a theoretically valid method for measuring changing and steady state ψ's of RBC. Furthermore, for RWA, it was concluded that TPMP$^+$-uptake monitors a composite ψ which reflects the total electrochemical potential of the cell.

143 THE PROTON GRADIENT IN GROWING *STREPTOCOCCUS LACTIS* CELLS, Eva R. Kashket, William C. Metzger and Alaina Blanchard, Boston University School of Medicine, Boston, MA 02118
The aerotolerant anaerobe *Streptococcus lactis* offers the opportunity of determining in dividing cells the electrochemical gradient of hydrogen ions, which gives rise to the protonmotive force, Δp. The two components of Δp (Δψ, the membrane potential and ΔpH, the chemical gradient of H^+) were determined by the accumulation of tetraphenylphosphonium (TPP^+) and benzoate ions. The Δψ was calibrated with the K^+ diffusion potential in starved, valinomycin-treated cells. With resting, glycolyzing cells the Δp was also checked by the accumulation of the non-metabolizable sugar thiomethyl-β-galactoside. ^{3}H-TPP^+ and ^{14}C-benzoate were added at mid-exponential phase to *S. lactis* growing at 28° in two different media with glucose or galactose as energy source. As with resting cells, the ΔpH decreased as the medium pH increased. The Δψ was relatively constant at 85 mV at pH 5 and 90 mV at pH 7. The Δp thus decreased from 145 mV at pH 5 (ΔpH accounting for 60 mV) to 115 mV at pH 6.8 (ΔpH equivalent to 20 mV). The growth rate had no effect on Δp. These Δp values are acceptable in the light of the current ideas on the stoichiometry of the H^+-translocating ATPase. They are also similar to those of resting, glycolyzing cells incubated at similar pH's and K^+ concentrations, suggesting that these are the two principal determinants of the Δp in growing streptococci.

144 ELECTRICAL MEASUREMENTS OF MEMBRANE TRANSPORT IN A NEW METHOD OF RECONSTITUTION: A STUDY OF BACTERIORHODOPSIN. Juan I. Korenbrot and San-Bao Hwang. Dept. of Physiology, Univ. of California San Francisco.
We have assembled planar membranes separating two large aqueous compartments consisting of a sinle layer of purple membrane fragments and lipid. Membranes were assembled by transferring a lipid monolayer containing non-overlapped, oriented single sheet fragments of purple membrane from the air-water interface onto a thin, hydrophilic, electrically conductive support casted from nitrocellulose. The purple membrane-lipid film on the support was then transferred through a second interface film consisting only of phospholipids. The resulting membrane exhibits an electrical resistance of 10^5- 10^6 Ωcm^2 and is stable for several hours. Illumination of the membrane produces photocurrents and photovoltages whose action spectra match the absorption spectrum of bacteriorhodop. Simultaneous measurements of PH reveal that the photocurrent is carried only by protons. The peak amplitude of the photocurrent is proportional to membrane area. It is also linearly proportional to the log of the stimulus light energy The rate of rise of the photocurrent is also proportional to light energy. The photovoltage follows the photocurrent in amplitude and kinetics as expected of a simple RC network. Preceeding the proton photocurrent, transient photovoltages are detected which exhibit no detectable latency and reach peak in 100 μs. These probably arise from intramolecular charge displacements.

Outer Membrane Components

145 CO-REGULATION OF A NOVEL TRANSPORT SYSTEM FOR SN-GLYCEROL-PHOSPHATE AND THE OUTER MEMBRANE PROTEIN Ic (e) OF ESCHERICHIA COLI, Winfried Boos and Manfred Argast, Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz
Mutants derepressed for the novel outer membrane proteins Ic or protein e contain a recently discovered binding protein for sn-glycerol-3-phosphate. In addition, strains that were previously isolated as mutants derepressed for the sn-glycerol-3-phosphate transport system and carrying novel periplasmic proteins GP1-4 synthesize also a new outer membrane protein with the same electrophoretic mobility on SDS-polyacrylamide gels as protein Ic. A screen of different derepressed $G3P^+$ strains reveal the existence of three types in respect to the four novel periplasmic proteins GP1, 2, 3 and 4: i) containing all four; ii) containing only GP1, 2 and 3; iii) containing only GP1, 2 and 4. In confirmation of the observations by Lugtenberg we found that purified GP1 is identical to alkaline phosphatase, while purified GP3 has binding activity for inorganic phosphate and is identical to the phosphate-binding protein. Moreover, growth conditions that lead in a wild-type strain to the derepression of alkaline phosphate synthesis, also derepress the synthesis of the sn-glycerol-3-phosphate binding protein as well as the corresponding transport system. Thus, the new sn-glycerol-3-phosphate transport system is part of the alkaline phosphatase regulatory system.

146 SPECIFIC AND NON-SPECIFIC DIFFUSION CHANNELS IN THE OUTER MEMBRANE OF ESCHERICHIA COLI, Hiroshi Nikaido, Mary Luckey, and Emiko Y. Rosenberg, Dept. of Microbiology and Immunology, University of California, Berkeley, Calif. 94720

Some outer membrane proteins form transmembrane channels for the diffusion of nutrients and waste products. Recently we devised a method whereby the rates of diffusion of solutes through these channels are measured by the rates of swelling of liposomes containing the purified channel-forming protein (Luckey and Nikaido, Proc.Nat.Acad.Sci.U.S.A., in press). Using this method, we measured diffusion rates of various solutes through channels formed by porin la (synonym: Ia), as well as by the lamB protein (synonym: lambda receptor protein). The following results were obtained. (a) The lamB channels showed significant configurational specificity so that some disaccharides diffused at less than 3% of the rate of maltose, and some trisaccharides at less than 1% of the rate of maltotriose. In contrast, the porin channels showed little configurational specificity, and allowed the diffusion of the sugars of the same size class at more or less uniform rates. (b) The diffusion through porin channels was strongly influenced by solute size, so that disaccharides on average diffused at a rate 30-40 fold slower than hexoses. In contrast, the rates of diffusion (on weight basis) of maltose and maltotriose through lamB channel was similar to the diffusion rate of glucose. These results clearly show that the lamB channel is configurationally specific for sugars of maltose series, whereas such specificity is not found in porin channels.

147 INCREASED BINDING OF 1-AZIDOPYRENE TO ESCHERICHIA COLI CELLS WITH LOW MEMBRANE POTENTIAL, Marcia K. Wolf and Jordan Konisky, University of Illinois, Urbana, IL61801

1-Azidopyrene has been used to probe the interaction of small hydrophobic molecules with Escherichia coli cells. Upon photolysis of cells in the presence of [^{3}H] -1-azidopyrene, some of the probe molecules become irreversibly associated with the cells. It is shown that cells under energy stress from starvation, CCCP, KCN, or colicin Ia bind up to five times more 1-azidopyrene than energized cells. If starved cells are exposed to 1-azidopyrene but re-energized prior to photolysis, the cells retain low levels of 1-azidopyrene characteristic of energized cells. Using an UncA mutant energized by either D-lactate or glucose, it is apparent that low probe binding results from the energy derived from the generation of a membrane potential and not from ATP per se. Since the outer membrane is largely impermeable to hydrophobic molecules these results suggest that changes in the inner membrane $\Delta\Psi$ influence outer membrane function. The observation of reversible binding shows that the phenomenon is not simply a change in outer membrane permeability because such an explanation cannot account for how the probe is expelled.

148 PURIFICATION AND PROPERTIES OF THE F FACTOR OUTER MEMBRANE SURFACE EXCLUSION PROTEIN, Edwin G. Minkley, Jr., Carnegie-Mellon University, Pittsburgh, PA 15213

Conjugative plasmids can encode proteins which render cells containing them poor recipients in conjugation. This phenomenon is called surface exclusion and in the classic E. coli sex factor F is separately mediated by the products of two genes, traS and traT.

The product of traT is a 25 kd protein normally present in 10,000 copies in the outer membrane of an F$^+$ donor cell. The protein is in tight (but non-covalent) association with the underlying peptidoglycan layer of the cell. In such a state it resists solubilization by the detergent sodium dodecylsulfate at 70° (but not at 100°) and is resistant to many proteolytic enzymes. Using a mutant strain of E. coli which lacks the major outer membrane matrix (porin) protein, we have obtained electron micrographs of negatively stained preparations of the protein, in association with the peptidoglycan.

Using these unusual properties of the traT surface exclusion protein and a λptraSTD transducing phage (isolated in the laboratory of Neil Willetts of the Univ. of Edinburgh), we have developed a rapid and efficient purification procedure for the protein, and have determined its amino acid composition. We have also purified the protein from F traT missense mutants. Several models for the mechanism of surface exclusion by traT are now being tested.

We have also constructed a chimeric plasmid, pHUB2traTD, which can be heat-induced to give tremendous over-production of the traT protein from the λ P_L promoter. Contrary to published reports, we have found that the traT protein is first synthesized in a higher molecular weight precursor form. Cells containing pHUB2traTD can be induced to provide large quantities of this precursor.

149 REGULATION OF BIOSYNTHESIS OF MAJOR OUTER MEMBRANE PROTEINS 1a and 1b IN ESCHERICHIA COLI K-12, Michael N. Hall and Thomas Silhavy, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Mass. 02115, Frederick Cancer Research Center, P.O. Box B, Frederick, MD 21701.

Several insertion and spontaneous mutations in the genes controlling expression of major outer membrane proteins 1a and 1b have been isolated. These mutations have been mapped to three loci known to control protein 1 expression: ompF (1a), ompC (1b), and ompB (1a and 1b). Bacteriophage Mu insertions at ompC and ompF were used to construct fusions of the lac operon to these genes such that the level of β-galactosidase is a measure of the transcriptional activity of the gene to which lac is fused. Expression of β-galactosidase in these fusion strains reflects known regulatory properties of proteins 1a and 1b. The amount of β-galactosidase activity in the ompC-lac and ompF-lac fusion strains fluctuates depending on the growth medium. The introduction of ompB mutations affects both the absolute and relative amounts of β-galactosidase. The results suggest that 1) ompB is a regulatory locus controlling the transcriptional activity at ompC and ompF, 2) ompF and ompC are the structural genes for proteins 1a and 1b, and 3) the ompB locus codes for more than one function. Complementation analysis and detailed mapping studies have further elucidated the role of ompB.

150 STUDIES ON THE EXPORT OF THE λ RECEPTOR, AN OUTER MEMBRANE PROTEIN OF E.coli K12,
Maurice Hofnung, Catherine Breton, Jean Marie Clément, Joe Hedgpeth, Christian Marchal, David Perrin, Unité de Génie Génétique, Institut Pasteur, 75015 Paris, France
The lamB gene encoding the λ receptor, an outer membrane protein in E.coli K12 is used to study protein export. 1) In vitro synthesis of the precursor to the λ receptor - the pre λ receptor - has been obtained using DNA of a recombinant phage where lamB has been cloned under lac promotor control. Processing of the pre λ receptor occurs in vitro. The effects of a signal sequence mutation affecting export in vivo as well as the activity of the pre λ receptor towards the phage were examined in vitro. 2) Sequencing of gene lamB has already allowed to determine the sequence of the wild type signal sequence as well as that of signal sequence mutants (in collaboration with S. Emr and T. Silhavy). 3) Mutation located outside the signal sequence and affecting λ receptor presence in the outer membrane have been studied.

Anion Transport

151 COUPLING OF ANION GRADIENTS TO THE ACTIVE TRANSPORT OF METHOTREXATE IN L1210 CELLS,
Gary B. Henderson and Edward M. Zevely, Scripps Clinic & Res. Fndn., La Jolla, CA 92037
Transport of methotrexate, the 4-amino, 10-methyl analog of folic acid, by L1210 cells is highly dependent upon the ionic composition of the external medium. Half-maximal rates of methotrexate transport (K_t values) vary from 0.9 μM in cells suspended in potassium Hepes buffer containing Mg^{++} (Hepes-Mg), to 10 μM in phosphate-buffered saline (PBS). At saturating levels of substrate, however, transport rates approach the same maximum velocity (V_{max}) regardless of the buffering medium. The increased K_t value for methotrexate in PBS is due to the presence of the competitive inhibitors, phosphate (K_i = 0.87 mM) and Cl^- (K_i = 46 mM). Concentration gradients for methotrexate at the steady-state are also much lower in PBS (2-fold) than in Hepes-Mg (60-fold); the components of PBS that reduce this uptake parameter are phosphate, Cl^-, Ca^{++} and Na^+. Ions that decrease the influx rate or the steady-state level produce a corresponding increase in methotrexate efflux. The unusually high sensitivity of methotrexate transport to competitive inhibition by phosphate, Cl^-, and various other anions is consistent with the hypothesis that this system catalyzes the exchange of external methotrexate for an intracellular anion, and that the efflux of the latter down a concentration gradient is the driving force for active transport of methotrexate. The identity of this putative coupling ion has not been determined, although studies on the transport of phosphate show that this anion is concentrated by L1210 cells to an extent that would be sufficient to provide the needed energy for methotrexate uptake.

152 ATTEMPTS TO PHOTOLABEL THE RED CELL GLUCOSE TRANSPORT SYSTEM, *Donald F. Diedrich and*
Franklin F. Fannin, University of Kentucky College of Medicine, Lexington, KY 40536

The type of inhibition exerted by a new phloretin derivative (phloretin-3'-benzyl azide; PBAz) and its potency in the sugar transport system in human erythrocytes has been established. Transport measurements were made to test the asymmetry of the membrane transporter using the Ørskov technique under three conditions: (I) efflux of glucose into media containing variable concentrations of glucose (the Sen-Widdas method), (II) efflux of galactose into media containing no sugar, and (III) influx of galactose into cells containing no sugar. Inhibition of the initial rate of sugar transport by phloretin and PBAz was assessed in subdued light. Results indicated that the azide was consistently more potent than phloretin in all systems: (I) partially reversible but with complex kinetics, (II) reversible but noncompetitive, K_i < 1 μM and (III) competitive, K_i = 0.2 μM. Whereas exposure of the cells to UV *for 4 min had no apparent effect on the transport system, when irradiation was performed* in the presence of variable amounts of PBAz, an irreversible inhibition of glucose efflux was found. Unreacted free azide in the incubation medium and that bound non-covalently to the cells could be easily and quantitatively removed by passing the irradiated incubation mixture through a Sephadex G-10 column. The irreversible inhibition caused by the azide was dependent on azide level and hematocrit. Both ^3H- and ^{14}C-labeled PBAz are currently being used in photolysis experiments, with and without phloretin, cytochalasin B and D-glucose as protecting agents, to determine the specific membrane components to which the ligand is covalently bound.

153 ATP DEPENDENT PHOSPHATE TRANSPORT IN SARCOPLASMIC RETICULUM, William W. Carley and Efraim Racker, Section of Biochemistry, Cornell University, Ithaca, NY 14853

The uptake and storage of Ca^{2+} by rabbit sarcoplasmic reticulum (S.R.) is enhanced by the addition of Ca^{2+} precipitating anions such as P_i or oxalate. About 1 μmole of [$^{32}P_i$] P_i is taken up for each μmole of Ca^{2+} transported into S.R. The uptake of P_i is dependent on Ca^{2+}, Mg^{2+}, and ATP with K_m values of about 60 μM for Ca^{2+}, 2 mM for MgATP and 11 mM for P_i. In contrast to Ca^{2+} uptake which is abolished by A-23187, the uptake of P_i is not impaired provided sufficient Ca^{2+} (~1mM) is present. Co^{2+} substitutes for both Mg^{2+} and Ca^{2+} in P_i transport. Co^{2+} uptake into S.R. showed a rate that was less than 5% of the rate of Ca^{2+} uptake. These observations show that P_i uptake into S.R. is dependent on ATP but not dependent on an active transport of a divalent cation. Reconstitution of this ATP dependent P_i uptake was achieved with S.R. membrane fragments as well as with a soluble preparation obtained by cholate extraction.

154 A RIGID WATER COMPONENT OF BIOLOGICAL MEMBRANES, Mary D. Oldewurtel, Sloan-Kettering Institute, New York, New York 10021

Water is thought to be an effector in membrane transport, fusion and enzyme activity. Since the cell membrane is a selective barrier between two aqueous phases, water interaction with components may be a determining force in membrane structure. Using the fluorescent probe 1-anilinonaphthalene-8-sulfonate (ANS), which is sensitive to polarity and viscosity, hydration effects on red cell membranes compared to lecithin (with or without cholesterol) films have been measured. The ANS emission maximum in ghost films shifts from 460nm at 95% relative humidity (RH) to 450nm at 0% RH, accompanied by a large intensity increase, when the probe concentration is 40μM/mg protein. When a lower probe concentration (4μM/mg protein) is used, the emission maximum appeared at 450nm at either hydration level. This maximum is never observed in the lipid films, and energy transfer and excitation spectra studies show that the ANS environment in red cell membranes is in or near proteins. In fact, others have shown that ANS binds to the erythrocyte anion transport protein. In membrane suspensions, there is a D_2O isotope effect on ANS emission at all concentrations used, indicating exposure of the probe binding sites to the aqueous surroundings. These results demonstrate that the high affinity ANS sites in the erythrocyte membrane have water present in them in the hydrated state, but this water may be arranged in a rigid lattice so that it cannot stabilize the ANS excited state. The secondary sites must contain more liquid-like water. This study provides evidence for a viscous water component in the hydrated membrane, associated with proteins or their annular lipids.

155 KINETICS AND MECHANISM OF ANION EXCHANGE ACROSS THE HUMAN ERYTHROCYTE MEMBRANE, J.M. Salhany, Elizabeth D. Gaines, VA Medical Center and Depts. of Internal Medicine & Biomedicinal Chemistry Univ. of Nebraska Medical Center Omaha, NE. 68105

Despite the absence of information on the stoichiometry of anion binding to the exchange system of the erythrocyte, certain general mechanistic deductions can be made. Previous steady state measurements of divalent anion exchange have demonstrated apparent negative cooperativity with substrate inhibition. However, this result would seem at variance with equilibrium exchange measurements where observations indicated initial Michaelian kinetics followed by substrate inhibition. We suggest that these two results may not be contradictory if a half-of-the-sites reactivity mechanism is proposed. New, presteady state measurements for divalent exchange are presented. The results support a two substrate half-of-the-sites exchange mechanism. In this scheme, the two sites are thought to exchange anions in a reciprocating fashion via a slow (relative to anion binding and release) conformational isomerization. This slow conformational change can serve to accelerate exchange to a steady state level (lags) or produce burst kinetics when a very slowly permeable anion is incorporated into the cell. Furthermore, a half-of-the-sites mechanism can also explain the Michaelian - like behavior observed in equilibrium exchange measurements.

156 HEXOSE TRANSPORT IN VESICLES DERIVED FROM SKELETAL MUSCLE PLASMA MEMBRANES.
Michael F. Shanahan, Department of Physiology, University of Wisconsin, Madison, WI., 53706.

Plasma membranes were isolated from rat hind limb skeletal muscle using a modified proceedure of Schapira et al (1). Muscle homogenates were subjected to high salt extraction to remove contractile proteins, followed by a series of differential and rate zonal centrifugations. This proceedure yielded a subcellular fraction containing vesicles enriched in plasma membrane. This fraction exhibited the highest specific activity of the plasma membrane marker enzyme, (Na^+,K^+)-stimulated, Mg^{++}-dependent ouabain-sensitive ATPase, compared to all other subcellular fractions. In addition, these vesicles exhibited stereospecific uptake of D-glucose over L-glucose. D-glucose uptake exhibited saturation kinetics and was inhibited by cytochalasin B. Thus these vesicles exhibited uptake kinetics similar to muscle membrane vesicles described by Cheng et al (2). Preliminary experiments in this laboratory indicate that this D-glucose transport activity can be reconstituted into artificial liposomes following extraction of membrane proteins with sodium cholate and subsequent removal of detergent from the protein fraction in the presence of exogenous lipids. These reconstituted liposomes exhibit stereospecific uptake of D-glucose which is inhibited by cytochalasin B. Experiments are in progress to further characterize and purify this transport system.

1) Schapira,G.,Dobocz,I.,Piau,J.P., and Delain,E. (1974) Biochim. Biophys. Acta 345, 348-358.
2) Cheng, L.C., Rogus, E.M., and Zierler,K., Biochim. Biophys. Acta (1978) 513,141-155.

157 INSULIN RECEPTOR STRUCTURE: MULTIPLE DISULFIDE-LINKED FORMS IN ADIPOCYTE PLASMA MEMBRANE.
J. Massague, P.F. Pilch and M.P. Czech. Brown University, Providence, RI 02912

Insulin receptors in rat adipocyte and rat liver plasma membranes can be specifically labeled by crosslinking receptor-bound 125I-insulin with disuccinimidyl suberate (DSS) (Pilch & Czech, J. Biol. Chem. 254, 3375). When such cross-linked membranes are subjected to electrophoresis in polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS-PAGE) a major band of 125,000 daltons and a minor band of 225,000 daltons appear upon autoradiography if electrophoresis is carried out in the presence of dithiothreitol (DTT). A single band of 300,000 daltons is observed after electrophoresis in 5% polyacrylamide gels in the absence of reductant, indicating the native receptor(s) resides in the membrane as a disulfide-linked complex. However, when the acrylamide to bis-acrylamide ratio is increased from 37.5:1 to 100:1 in 5% gels, enhanced resolution showed the existence of at least three different bands upon autoradiography, with apparent molecular weights ranging from 250,000 to 350,000 daltons. These bands were obtained after ^{125}I-insulin treatment and DSS crosslinking of cell-free adipocyte plasma membrane preparations, but multiple high m.w. bands (250,000-350,000) were also observed when this protocol was performed with intact adipocytes. The three bands deriving from electrophoresis of the ^{125}I-insulin receptor complexes in the absence of DTT were subjected to second dimension SDS-PAGE in the presence of DTT. All three bands migrated as 125,000 dalton subunits in the second dimension gels. These findings suggest the existence of heterogeneous disulfide-linked insulin receptors and/or insulin receptors with different stoichiometry in subunit composition.

158 MONOCLONAL ANTIBODIES AGAINST RAT ADIPOCYTE INTRINSIC MEMBRANE PROTEINS MIMIC INSULIN ACTION ON HEXOSE TRANSPORT.
Joanna Beachy and Michael P. Czech.Brown U., Prov., RI

Antibodies produced in rabbits against rat intrinsic membrane proteins and in humans against the insulin receptor mimic the action of insulin on glucose oxidation in rat adipocytes. SJL mice were injected intraperitoneally with intrinsic membrane proteins isolated from the rat adipocyte. Three days after the boost injection, the spleens were removed and fused with the 8-azaguanine-resistant, non-secreting mouse myeloma cell line NS-1. The fused cells were distributed into 275 wells with approximately 10^6 cells per 0.1ml. Following HAT selection for hybrids 95% (261/275) of the wells contained clones. The cell medium of the hybrids was tested for its ability to mimic insulin action, specifically to stimulate the oxidation of glucose to CO_2. Sixty-eight wells (25%) stimulated glucose oxidation and this effect was abolished with preincubation of the cell medium with Staph. aureus and rabbit anti-mouse immunoglobulin IgG to remove the mouse immunoglobulins. Greater than 50% of the wells produced immunoglobulins that bound isolated plasma membranes of rat adipocytes although only half of these were biologically active on glucose metabolism. The hybrids produce both IgG and IgM which bind specifically to the adipocyte membrane. Thus, monoclonal antibodies specific for intrinsic membrane proteins of the rat adipocyte have been generated, some of which rapidly activate the D-glucose transporter in intact fat cells. The nature of the antigenic membrane proteins are under investigation.

159 STIMULATION AND INHIBITION OF PYRUVATE DEHYDROGENASE BY INSULIN-DEPENDENT, PLASMA MEMBRANE-GENERATED FACTORS, J. R. Seals and M. P. Czech. Brown University, Prov., RI 02912

Recent studies have demonstrated that addition of insulin to a mixture of plasma membranes and mitochondria from rat adipocytes stimulates the activity of the mitochondrial enzyme, pyruvate dehydrogenase (Seals, J.R. and Jarett, L., Proc. Natl. Acad. Sci., in press). These results suggested that interaction of insulin with its receptor rapidly generates a soluble factor which mediates intracellular transmission of the hormonal stimulus. Further studies have been carried out by incubating purified adipocyte plasma membranes at 37° with insulin (0-500 μU/ml) for various lengths of time (0-60 min). At the conclusion of this incubation, a sample of the plasma membrane mixture was either transferred directly to purified mitochondria or was centrifuged 1 min in an Eppendorf microfuge and the resulting supernatant added to mitochondria and pyruvate dehydrogenase activity assayed. In the absence of insulin, incubation of plasma membranes resulted in the slow production of a stimulating factor, the rate of production of which was progressively increased by increasing concentrations of insulin. The maximum stimulation observed was the same at all concentrations of hormone (22.7 ± 4.3%); insulin affected only the rate at which the maximum stimulation was reached. Further incubation of plasma membranes beyond the point of maximum stimulation resulted in inhibition of pyruvate dehydrogenase activity. Inhibition occurred at all concentrations of insulin but appeared earlier at higher levels of hormone. Identical results were obtained when only the 12,000g plasma membrane supernatant was transferred to the mitochondria, indicating that the insulin stimulus was transmitted by a soluble membrane-generated factor.

160 PEPTIDE MAPPING OF THE ADIPOCYTE INSULIN RECEPTOR, Paul F. Pilch, and Michael P. Czech. Brown University, Providence, RI 02912

The bifunctional, amino-specific reagent disuccinimidyl suberate can be used to covalently link 125I-insulin to its receptor in the plasma membrane of fat and liver cells (Pilch and Czech, J. Biol. Chem. 254, 3375). Analysis of this cross-linked complex on dodecyl sulfate-polyacrylamide gels (SDS-PAGE) in the absence of dithiothreitol followed by autoradiography reveals a disulfide-linked insulin receptor subunit structure (250,000-300,000 daltons) which in the presence of reductant migrates at 125,000 daltons. Exposure of intact fat cells to 10 μg/ml trypsin prior to binding and cross-linking of ^{125}I-insulin virtually abolishes the appearance of both the 300,000 and 125,000 dalton receptor bands. A major receptor fragment of molecular weight 150,000 (minus reductant) and several minor fragments are generated by trypsin and these retain the ability to specifically bind and cross-link to ^{125}I-insulin after proteolysis. Despite the apparent absence of labelled receptor, the trypsin treated adipocytes are able to elevate glucose oxidation in response to insulin suggesting that "nicked" receptor or receptor fragments can mediate insulin action. When trypsin is added after ^{125}I-insulin is bound and cross-linked to its receptor, a similar pattern of fragments is observed but with greatly different relative intensities. This change in relative intensities may be due to an insulin-induced conformational change in the receptor or may be a consequence of receptor modification by the cross-linking reagent. Little if any change is observed in the SDS-PAGE protein profile of the plasma membrane when considerable proteolytic digestion of the receptor has occurred, indicating that the insulin receptor of the adipocyte is particularly sensitive to trypsin action.

161 ASYMMETRY OF RED CELL INORGANIC ANION EXCHANGE, Michael L. Jennings, The University of Iowa, Iowa City, IA 52242

The functional symmetry of the inorganic anion exchange protein of the human red blood cell membrane has been examined by measuring the rate of net exchange of Cl for SO_4. This net exchange takes place as an electrically silent exchange of one Cl ion for one SO_4 plus one hydrogen ion. The Cl-SO_4 exchange process is rate-limited almost entirely by the transport events which involve SO_4. The partial reactions (binding, translocation, release) which result in anion influx may then be studied by measuring the net SO_4 influx from a Cl-free medium into Cl-containing cells; the partial reactions which result in anion efflux are studied by measuring the initial SO_4 efflux from Cl-free cells into a Cl medium. Experiments of this type have revealed the following asymmetries in the anion transport. The apparent dissociation constant for SO_4 binding to outward-facing transport sites is at least three-fold smaller than for binding to inward-facing transport sites. High anion concentrations have a larger inhibitory effect on SO_4 efflux than on influx. Low pH inhibits net SO_4 efflux into a Cl medium and SO_4 equilibrium exchange in an all-SO_4 medium, but does not inhibit SO_4 influx into Cl-containing cells. Extracellular DNDS (4,4'-dinitro-2,2'-stilbenedisulfonate) strongly inhibits influx, but has much less effect on efflux. Conversely, intracellular APMB (2-(4'-aminophenyl)-6-methylbenzene-thiazole-3',7-disulfonic acid) affects efflux much more than influx. These results suggest that extracellular DNDS inhibits the transport by binding to only the conformation of the protein which has a transport site accessible to extracellular anions, whereas intracellular APMB inhibits by binding to a form of the protein which has a transport site to which intracellular anions can bind. Supported by NIH Grant 1 R01 GM26861.

162 CHLORIDE-BICARBONATE EXCHANGE IN HUMAN RED CELLS: PREDICTIONS AND CALCULATIONS OF A SIMPLE PING-PONG MODEL, Robert B. Gunn, Otto Frohlich and Mark Milanick, University of Chicago, Chicago, IL 60637 Measurements of chloride and bromide exchange in human erythocytes are consistent with a ping-pong mechanism. The obligatory exchange of anions has asymmetrical kinetics when ion concentrations are varied separately on the two sides of the membrane. The ping-pong model makes several explicit predictions; e.g. half of all the transport mechanisms are empty on the side of the membrane at which the anion concentration is equal to $K_{½}$, the concentration giving the half maximal flux for a fixed trans-membrane anion concentration. The application of this model to the physiological heteroexchange of chloride and bicarbonate as occurs in the erythrocytes of the lung and tissue capillaries permits calculation of the percent occupancy of the mechanism by each ion on each side and the rates of transport. This calculation shows that the initial (maximal) heteroexchange is less then 4% of the maximum tracer exchange of chloride or bicarbonate. Extrapolation of these calculations from 0°C and pH 7.8 to physiological conditions indicates that this rate should be sufficient to complete the transport rate are not great. This rate is well within the 0.6 - 1.0 sec transit time in the lung. If this extrapolation is correct then Cl-HCO3 exchange by the red cell would not be rate limiting for CO2 excretion.

163 ENERGY TRANSFER DETERMINATION OF THE TRYPTOPHAN DISTRIBUTION OF BAND 3: A NEW APPROACH TO THE STUDY OF MEMBRANE PROTEIN FUNCTION. Alan M. Kleinfeld, Edmund D. Matayoshi, and A.K. Solomon, Biophysical Laboratory, Harvard Medical School, Boston, MA 02115. The tryptophan distribution of band 3 enriched in a preparation of human red cell vesicles (modified from Wolosin et al., J. Biol. Chem., 1977, 252, 2419), has been determined by measuring non radiative energy transfer between the distribution of tryptophan donors and a uniform distribution of anthroyloxy fatty acid acceptors. Both Coomassie blue absorption profiles and tryptophan fluorescence from SDS gels indicate that the vesicles are about 80% band 3, 15% band 4.5 and 5% band 7. Measurements of quenching of tryptophan intensity and lifetime and the sensitized emission of anthroyloxy fluorescence were performed. The results were analyzed by calculating transfer efficiencies for each tryptophan using the two dimensional theory of Förster. Several experiments have been carried out to determine changes in tryptophan distribution corresponding to changes in anion transport function. In the presence of 50 mM NaCl (pH 7.4) the tryptophan distribution changes from one which is slightly skewed towards the cytoplasmic side at 0° to one in which the tryptophan center of mass is probably 15 A from the center towards the cytoplasmic side at 25° C. At 25° C in the presence of 20 mM Na citrate, the anion exchange inhibitor H_2DIDS was found to skew the distribution even further towards the cytoplasmic side. Comparing the citrate case in the absence of H_2DIDS with the distribution in the presence of 35 mM SO_4 indicates that only the outermost tryptophan are affected and may imply that the SO_4- binding site is at the outer surface. This work is supported by NIH grants GM26350 and HL14820 and JRFA-15 from the Am. Cancer Soc.

Adenyl Cyclase Regulation

164 <u>QUATERNARY PROTEIN STRUCTURE OF ADENYLATE CYCLASE</u>, Thor Nielsen, Pramod Lad, Dermot Cooper, Constantine Londos, and Martin Rodbell, NIAMDD, NIH, Bethesda, MD 20205 The size of membrane enzymes and their components may be evaluated by target analysis following radiation inactivation, without disruption of the membrane structure. Use of target analysis has demonstrated that various hormone receptors form oligomeric complexes (of over 10^6 daltons) with GTP-regulatory proteins. In the rat liver and fat cell plasma membranes these oligomeric complexes restrict the ability of the GTP-regulatory proteins from interacting functionally with adenylate cyclase. Hormones bind to the receptors and relieve the restraints imposed by the oligomeric structure. Resultant interaction between GTP and the activated hormone receptor GTP-regulatory protein oligomer results in depolymerization of the oligomeric structure and "coupling" of these (receptor-GTP regulatory component) units with adenylate cyclase. By contrast, turkey erythrocyte adenylate cyclase appears to contain a simple complex: receptor-nucleotide regulatory unit-catalytic unit in a non-polymerized state. This difference in quaternary structure may reflect the different functional response to GTP shown by turkey erythrocyte compared to rat liver or fat cell adenylate cyclase. In adipocytes, the protein components associated with the hormone-mediated inhibition of adenylate cyclase are also multimeric complexes of receptor with regulatory proteins. Multimeric receptor-regulatory component complexes may interact with a variety of membrane-bound systems, of which adenylate cyclase may only be an example. The implications of quaternary structrue transitions for the overall signal transduction process will be discussed.

165 SELECTIVE EFFECTS OF CHARGED LOCAL ANESTHETICS ON THE GLUCAGON- AND FLUORIDE-STIMULATED ADENYLATE CYCLASE ACTIVITY OF RAT LIVER PLASMA MEMBRANES, Larry Gordon, Irene Dipple, Richard Sauerheber, Judy Esgate and Miles Houslay, Scripps Clinic and Res. Foundation, La Jolla, CA 92037 and the Univ. of Manchester Inst. of Science and Technology, Manchester, UK
The effects of charged anesthetics on the lipid fluidity of liver plasma membranes and the activities of glucagon- and fluoride-stimulated adenylate cyclase were examined. The cationic local anesthetics prilocaine, carbocaine and nupercaine can all increase the fluoride-stimulated adenylate cyclase up to a maximum level above which further increases in drug concentration inhibited the enzyme. At concentrations where this activity was stimulated, a fatty acid spin label detected an increase in bilayer fluidity, which may be responsible for the activation of the enzyme. A solubilized enzyme was unaffected by the drugs which would be consistent with this. These cationic drugs began to inhibit the glucagon-stimulated activity at concentrations where they activated the fluoride-stimulated activity. It is suggested that this is due to their effect on the coupling interaction between the receptor and the catalytic unit.

The anionic drugs, phenobarbital, pentobarbital and salicylic acid, all inhibited the fluoride-stimulated enzyme. This may be due in part to a direct effect on the protein and in part mediated by interaction of the drugs with the bilayer. The drugs had small inhibitory effects on the lubrol-solubilized enzyme. The glucagon-stimulated enzyme was initially inhibited by the anionic drugs at low concentrations, then activated and finally inhibited with increasing concentration. The reasons for such changes are complex, but there was no evidence from ESR studies to suggest that increases in activity were due to increases in bilayer fluidity.

166 CYLIC AMP LEVELS IN TOAD EPITHELIAL CELLS FOLLOWING TREATMENT WITH VASOPRESSIN, Stanley D. Hillyard and Mario B. Marrero, University of Nevada, Las Vegas, NV. 89154
When skin obtained from the ventral pelvic region of the toad, Bufo woodhouseii, was treated with arginine vasopressin (AVP, 36mu/ml), the rates of osmotic water flux (J_{H2O}) and active sodium transport (I_{Na}) were both elevated significantly. In ventral pectoral skin, however, AVP stimulated only I_{Na}. Treatment of pelvic and pectoral skin with collagenase (66mu/ml) did not affect this regional difference in AVP response and allowed the removal of epithelial cells from these skin regions for cyclic AMP analysis by radioimmunoassay. After adding AVP to the pelvic skin it was found that cyclic AMP levels in the epithelial cells from this region increased significantly from 1.4 to 6.6 pM/mg protein within one minute, reached a maximum of 11.4 pM/mg protein within five minutes and declined to control levels within twenty minutes. In the pectoral skin, cyclic AMP levels increased from 1.0 to 3.4 pM/mg protein after 5 minutes of AVP treatment and declined to control levels within 10 minutes. Unlike cyclic AMP levels, I_{Na} and J_{H2O} remained significantly elevated for at least 180 minutes following AVP treatment. These data suggest the existence of separate cyclic AMP pools for regulating J_{H2O} and I_{Na} with the larger pool regulating J_{H2O}. They also demonstrate that the prolonged elevation of J_{H2O} and I_{Na} following AVP treatment does not require continued elevation of cyclic AMP levels in the tissue.

167 A PHOTOAFFINITY LABEL FOR THE BETA ADRENERGIC RECEPTOR: EFFECTS ON ISOPROTERENOL STIMULATED ADENYLYL CYCLASE, Simeon M. Wrenn, J. and Charles Homcy, Baylor College of Medicine, Houston, Texas 77030 and Harvard Medical School, Boston, Mass. 02114.
An azide derivative of the beta adrenergic antagonist acebutalol has been synthesized and examined on isoproterenol stimulated adenylyl cyclase activity of rat reticulocytes. It was shown to be a fairly potent competitive antagonist (K_D = 0.2 uM) of the isoproterenol stimulated cyclase under non-photolysis conditions. However, when the reticulocyte preparation was photolyzed with the acebutalol azide, a non-competitive inhibition of isoproterenol stimulated cyclase was obtained. It was shown that photolysis of the azide did not convert it to a product of higher affinity or a slowly reversible ligand. The labeling of the beta receptor appeared to be irreversible since multiple washings could not reverse the inhibition produced during photolysis with the label, whereas washing would completely reverse the antagonism produced by the same concentration of label under non-photolysis conditions.

The effect appears specific for the beta receptor since the inhibition can be blocked stereoselectively by propranolol, and no effect is seen on fluoride or GMP-P(NH)P stimulated adenylyl cyclase. Furthermore, no effect is is observed in the glucagon stimulation of liver membranes, while the catecholamine response in the same membranes is inhibited upon upon photolysis with label.

168 DISTINCT ROLES OF TWO GUANINE NUCLEOTIDE SITES IN SIGNAL TRANSDUCTION BETWEEN RECEPTORS AND ADENYLYL CYCLASE. R. Iyengar, J. Abramowitz, T.L. Swartz, M.K. Bhat and L. Birnbaumer, Dept. of Cell Biology, Baylor College of Medicine, Houston TX 77030.

Not only GTP and its analogs but also GDP promote receptor-cyclase coupling leading to stimulation of ATP to cAMP cyclizing activity. This was shown for stimulation of the liver enzyme by glucagon, of the S49 cell enzyme by isoproterenol and of the NS-20 neuroblastoma enzyme by prostaglandin. The last of these systems does not respond to prostaglandin in the absence of nucleotide, is inhibited by GDP alone (50%), stimulated by GTP (100%) and stimulated by prostaglandin only in the presence of either GTP (600%) or GDP (400%). These findings suggest that: (a) nucleotides are required for receptor activation, and (b) activated receptors stimulate cyclizing activity regardless of whether the enzyme is occupied by stimulatory GTP or inhibitory GDP. In all systems studied dose-response curves for hormones or neurotransmitters in the presence of a more active nucleotide were to the left of those obtained with a less active nucleotide. However, dose-response curves for nucleotides either did not vary or were right-shifted by addition of hormone. Because of symmetry considerations and other experimental results the above data are inconsistent with involvement of a single nucleotide site. The data are best explained (1) if two nucleotide sites are involved: one responsible for activation of receptor and the other responsible for independent regulation of cyclizing activity, and (2) if the role of the active, hormone and nucleotide occupied receptor is to promote a general "activation" of the basic nucleotide regulated adenylyl cyclase sytem. The role of subunit association and concomittant isomerization reactions leading to "activation" is discussed in light of subunit distributions of solubilized components upon centrifugation.

Muscle, Membranes, Receptors and Disease

170 DEVELOPMENT OF BETA-ADRENERGIC RECEPTORS AND CATECHOLAMINE-RESPONSIVE ADENYLATE CYCLASE DURING MYOGENESIS, Peter H. Fishman and J. Brian Parent, National Institute of Neurological and Communicative Disorders and Stroke, NIH, Bethesda, MD 20205

Secondary cultures of embryonic quail muscle cells initially proliferate and then rapidly fuse to form myotubes. The myotubes contain ∿800 specific binding sites per diploid nucleus for ^{125}I-hydroxybenzylpindolol, a β-adrenergic antagonist. Binding is of high affinity (K_d of 10^{-10}M) and is stereoselectively inhibited by β-agonists and β-antagonists. For the agonists, the order of potency is (-)isoproterenol> (-)epinephrine> (-)norepinephrine, which is consistent with a beta 2 adrenergic receptor. In contrast, proliferating myoblasts contain <10 β-receptors per cell. As the myoblasts fuse, β-receptors appear in concert with fusion. Intact myotubes, but not myoblasts, accumulate cyclic AMP in response to isoproterenol while both cell types respond to cholera toxin. Membranes from both cell types contain an adenylate cyclase activity which is stimulated by NaF, GTP and cholera toxin. Only myotube membrane adenylate cyclase responds to isoproterenol. Thus, as the embryonic muscle cells fuse, they develop specific β-adrenergic receptors that become functionally coupled to pre-existing adenylate cyclase. Our results are consistent with current concepts of separate membrane components for β-receptors and adenylate cyclase. The catalytic and regulatory components of the β-adrenergic responsive adenylate cyclase are present early during muscle development whereas the specific receptors and thus hormone sensitivity are present in the mature myotube. This differential gene expression during myogenesis may have important implications for the abnormal catecholamine response observed in human muscular dystrophy. (JBP was a recipient of a Muscular Dystrophy Association Fellowship)

171 LITHIUM TRANSPORT IN HUMAN RED CELLS AND ITS ABNORMALITIES IN AFFECTIVE ILLNESS, Ghanshyam N. Pandey, Elizabeth Dorus, John M. Davis, and Daniel C. Tosteson

A large interindividual variation has been observed in the distribution of lithium levels between red cells and plasma (Li ratio) in patients treated with lithium. Li ratio has been related to clinical response to lithium therapy, clinical diagnosis and side effects of lithium therapy. In order to study the factors which cause such interindividual variations in the Li ratio, we studied the mechanism of lithium transport, and at least four distinct pathways of lithium transport in human red cells were characterized (Pandey et al., J. Gen. Physiology 72, 233-247, 1978). Of these pathways, Li transport mediated by a Li-Na countertransport mechanism (Li-Na exchange pathway) appeared to be lower in patients having a high Li ratio and a significant correlation was observed between them (Pandey et al., Proc. Nat'l. Acad. Sci. 36, 902-908, 1978; Arch. Gen. Psychiat. 36, 902-908, 1978). We also reported that the Li ratio in vitro was significantly higher in bipolar patients when compared to normal controls. Furthermore, we observed that the mean Li ratio in relatives of bipolar patients with a history of affective disorders was significantly higher than relatives with no such history (Dorus et al., Science 205, 932-934, 1979). These results suggested that a genetically controlled abnormality in the Li transport may play a role in the etiology of some forms of affective disorders. The data on the mechanism of Li transport in human red cells and its genetic and clinical aspects will be presented and discussed.

172 GENE(S) AT THE K END OF THE H-2 COMPLEX INFLUENCE SUSCEPTIBILITY TO EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS (EAMG) IN MICE, P. Christadoss, V.A. Lennon, E.H. Lambert & C.S. David, Depts. of Immunology & Neurology, Mayo Clinic, Rochester, MN 55901

We have shown previously that susceptibility to EAMG in mice is influenced by H-2-linked genes. To further map and define the(se) gene(s), congenic and recombinant mice were challenged with torpedo acetylcholine receptor (AChR) and tested for T cell responses to AChR, serum autoantibodies, muscle AChR complexed with antibody and electrophysiological evidence of EAMG.

Strain	H-2 Complex K A B J E C S G D	Immunogen	T Cell Stim. Index with AChR	Serum Autoantibody $\pm$S.E. (x10^{-10}M)	Muscle AChR-Ig Complexes (% $\pm$S.E.M.)	Mepp Amplitude
B10/SN	b b b b b b b b b	AChR	15.5	39.1 $\pm$ 8.41	23 $\pm$ 8	0.85 $\pm$ 0.103
B10.BR	k k k k k k k k k	AChR	4.4	0.2 $\pm$ 0.10	<1	1.43 $\pm$ 0.117
B10.BR	k k k k k k k k k	nil	<1	0.1 $\pm$ 0.06	<1	1.54 $\pm$ 0.134
B10.A(4R)	k k b b b b b b b	AChR	1.1	0.0 $\pm$ 0.03	<1	1.39 $\pm$ 0.050
B10.A(4R)	k k b b b b b b b	nil	<1	0.0 $\pm$ 0.01	<1	1.43 $\pm$ 0.070
B10.A(5R)	b b b k k d d d d	AChR	15.4	8.16 $\pm$ 3.12	15 $\pm$ 4	1.21 $\pm$ 0.117
B10.A(5R)	b b b k k d d d d	nil	<1	0.0 $\pm$ 0.02	<1	1.58 $\pm$ 0.043

Only mice of B10/SN and B10.A(5R) strains developed weakness after inoculation with AChR and adjuvants. Susceptibility of these strains to induction of autoimmunity to AChR, and resistance of B10.BR and B10.A(4R) maps susceptibility to the K end of the H-2 complex. Further studies on the mode of segregation of susceptibility in F_1 and backcrosses, role of complementing genes and influence of non-H-2-linked genes in the induction of EAMG will be discussed.

173 THE MURINE MACROPHAGE Fc RECEPTOR FOR AGGREGATED IgG IS A LIPOPROTEIN. Clark L. Anderson, Department of Medicine, University of Rochester, Rochester, New York 14642.

Two distinct Fc receptors (FcR) for IgG are found on the plasma membrane of murine macrophages. One is specific for monomeric or aggregated IgG2a (FcRI). The other binds aggregates or immune complexes of two or three of the four IgG subclasses (FcRII). These two FcR have been solubilized by detergent lysis of a macrophage-like cell line (P388D1) and have been physically separated from one another by sucrose density gradient centrifugation and by affinity chromotography (Anderson and Grey, J Immunol 121:648, 1978). In soluble form the two receptors are trypsin-sensitive and are found in a fraction of the lysate with density 1.15 g/ml, but only FcRII is sensitive to phospholipase C digestion. The phospholipase C effect is abolished with 0.01M phenanthroline which chelates the enzyme co-factor Zn++. After inactivation by phospholipase C, soluble FcRII activity can be restored by the addition of liposomes prepared with purified phospholipids of specific type. Thus reconstituted, the soluble FcRII activity remains sensitive to trypsin digestion. The lipoprotein nature of the receptor therefore implies that purification of the receptor protein from detergent lysates by means of affinity chromatography will require incomplete solublization which may result in co-purification of other molecules.

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174 REGULATION AND PHARMACOLOGIC PROPERTIES OF Na^+ CHANNELS IN CULTURED RAT MUSCLE CELLS,
John C. Lawrence and William A. Catterall, Dept. of Pharmacology, Univ. of Wash., Seattle, WA 98195

Cultured rat skeletal muscle cells, like denervated skeletal muscle, have voltage-sensitive Na^+ channels that are 500-fold less sensitive to inhibition by tetrodotoxin (TTX). We have studied these Na^+ channels using $^{22}Na^+$ flux methods. The alkaloid batrachotoxin (BTX) is a full agonist in activating these Na^+ channels while veratridine and aconitine are partial agonists at the same receptor site. The polypeptides scorpion toxin and sea anemone toxin II do not themselves activate Na^+ channels but markedly enhance activation by the lipid-soluble toxins. The effects of both the alkaloid toxins and the polypeptide toxins are voltage-dependent. Depolarization increases V_{max} for BTX-dependent $^{22}Na^+$ influx 3-fold with no change in $K_{0.5}$. In contrast, depolarization increases $K_{0.5}$ for polypeptide toxin action 5- to 50-fold. Our results show that TTX-insensitive Na^+ channels in rat muscle cells have 3 separate receptor sites for neurotoxins as previously described for neuroblastoma cells, but differ in toxin affinity and in the voltage-dependence of alkaloid toxin action.

Cultured muscle cells, like denervated muscle, have a high density of nicotinic acetylcholine receptors (nAchR) which is reduced by electrical activity. Inhibition of spontaneous electrical activity of the cultured rat muscle cells caused a 2.5-fold increase in nAchR but had no effect on TTX sensitivity. Electrical stimulation caused a 30% reduction in AchR levels but had no effect on TTX sensitivity. Therefore, muscle electrical activity that is sufficient to regulate nAchR levels is not sufficient to alter TTX sensitivity. Different mechanisms may be involved in regulation of these two innervation-dependent muscle properties.

175 INDUCTION OF EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS (EAMG) SOLELY BY ACETYLCHOLINE RECEPTORS (AcChR) AND INHIBITION OF α-BUNGAROTOXIN (α-BuTx) BINDING TO RECEPTORS SOLELY BY ANTISERA FROM ANIMALS AFFLICTED WITH THIS DISEASE,
Toni R. Claudio and Michael A. Raftery, California Institute of Technology, Pasadena, CA 91125

EAMG has been induced in a wide variety of animals using AcChR purified from many sources. SDS polyacrylamide gels heavily loaded with purified AcChR often reveals the presence of minor contaminants. To test whether these contaminants or any other components present in *Torpedo californica* AcChR preparations could induce EAMG, solubilized *T. californica* membrane fragments were depleted of AcChR by passage over an α-BuTx conjugated resin and then injected into Lewis rats in an attempt to induce EAMG. The results demonstrated that some of the minor contaminants present in purified AcChR preparations were antigenic but EAMG could not be induced with preparations enriched in these contaminants or containing other non-AcChR components and lacking AcChR. Anti-*T. californica* AcChR antisera, prepared in New Zealand white rabbits and Lewis rats, were tested for the ability to inhibit $[^{125}I]$α-BuTx binding to membrane bound and detergent solubilized *T. californica* AcChRs. Similar inhibition studies were performed using rabbit antisera and Fabs prepared against each of the four isolated AcChR subunits. Antisera and Fabs prepared against intact AcChR could inhibit a maximum of 50% of the α-BuTx binding to solubilized AcChR. Rabbits and rats immunized with AcChR denatured by SDS all produced antisera which could bind to nondenatured AcChR but none of these animals developed EAMG. A strong correlation was observed between the presence of EAMG in animals and the ability of the antisera from these animals to inhibit 50% of the α-BuTx binding to solubilized AcChR.

176 RECONSTITUTION OF FUNCTIONAL ACETYLCHOLINE RECEPTOR INTO A MEMBRANE ENVIRONMENT.
Jose M. Gonzalez-Ros, Alex Paraschos and M. Martinez-Carrion, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

Acetylcholine receptor (AcChR) and total lipids were extracted from *Torpedo California* electroplax and were utilized to form fully functional, reconstituted vesicles. The AcChR, purified by α-cobratoxin affinity chromatography, could bind α-neurotoxins and specific ligands, and exist in a low carbamylcholine affinity state ("desensitized"). Isolated AcChR has four different polypeptide chains only (40,50,60 and 65,000 daltons) in $NaDodSO_4$-polyacrylamide gels. AcChR was reintegrated into the lipid environment by a detergent dialysis method and the formation of these vesicle-like structures was detectable through: 1) electron microscopy, 2) osmotic responses, and 3) loading with $^{22}Na^+$. The reconstituted vesicles could be fractionated by sucrose density gradient centrifugation. In addition, the membrane-like vesicles exhibited the following functional properties: 1) a "right-side-out" distribution of AcChR α-Bungarotoxin binding sites, 2) a lower K_d for cholinergic ligand binding compared to purified AcChR, 3) an agonist influenced "sensitized"-like state, and 4) an increase in Na^+ permeability due to the presence of agonists, which could be blocked by preincubation of the vesicles with either antagonists or α-neurotoxins. Non-specific $^{22}Na^+$ unloading could be induced by detergent solubilization of the vesicles or preincubation with gramacidin A. The role(s) of purified AcChR protein, pertaining to both neurotransmitter recognition and Na^+ translocation, will be discussed. (Supported by NSF grant BNS 77-24715)

177 MULTIPLE BINDING OF HISTAMINE IN RAT BRAIN, James W. Wells, Glenn H. Steinberg, Stephen I. Kandel, Marianne Kandel and Allan G. Gornall, Faculty of Pharmacy and Department of Clinical Biochemistry, University of Toronto, Toronto, Canada M5S 1A1.
To probe the relationship among histaminic binding sites in mammalian brain, three radiolabelled drugs have been studied in a synaptosomal preparation from rat cerebral cortex: the H_1 antagonist [^{3}H]mepyramine (MEP), the H_2 antagonist [^{3}H]cimetidine (CIM), and [^{3}H]histamine itself (HIS). In Krebs-Henseleit buffer at 30°, the direct binding profile for each probe contains a specific component inhibitable by various other histaminic drugs and describable by a rectangular hyperbola. Affinities measured directly (pK_d) agree well with those measured by autoinhibition (pIC_{50}) in the case of MEP (pK_d=8.50±.08, pIC_{50}=8.20±.13) and HIS (pK_d=7.84±.04, pIC_{50}=7.94±.07). Although direct estimates of CIM affinity are precluded by the long extrapolation involved, the value of pIC_{50} (6.72±.09) is essentially independent of the concentration of [^{3}H]CIM. Capacities were estimated assuming a single component of binding; pK_d was set equal to pIC_{50} only in the case of [^{3}H]CIM. While absolute values varied among groups of 30-40 animals, maximal specific binding expressed as pmol/g protein was found to yield a constant ratio of approximately 1:2:10 for [^{3}H]HIS, [^{3}H]MEP, and [^{3}H]CIM, respectively. Moreover, values of pIC_{50} obtained for MEP, CIM, and HIS depend upon the choice of radiolabelled probe, with all nine combinations yielding different apparent affinities. It thus appears that the three probes label different sites. If the binding of each probe is physiologically relevant, as suggested by previous authors, antagonistic relationships between histamine and antihistamines may not reflect simple competition for a common pool of sites. (Supported by the Banting Research Foundation, the Canadian Heart Foundation, and the MRC)

178 ALLOSTERIC EFFECTS OF VOLATILE ANESTHETICS ON THE MEMBRANE-BOUND ACETYLCHOLINE RECEPTOR Anthony P. Young, University of California, Los Angeles, CA 90024.
The central observation described here is that volatile anesthetics facilitate a structural transition of the membrane-bound acetylcholine receptor prepared from the electric organs of the marine ray Torpedo californica via an allosteric mechanism. The confromational change involves a transition from a state of low affinity for cholinergic agonists to one with a 100-1000 fold greater affinity for agonists. Pretreatment of membrane fragments with the volatile anesthetics halothane, chloroform, or diethylether or the primary alcohols ethanol, butanol, or octanol converts the receptor to the high affinity state. This heterotropic effect by volatile anesthetics can be reversed by passage of air over anesthetic-treated membranes. Because of the strong correlation between the high affinity conformation of the acetylcholine receptor and a desensitized (inactivated) postsynaptic membrane, this heterotropic effect provides the first neurochemical model for volatile anesthetic action at synapses, the postulated target for these agents.

179 ELECTROPHYSIOLOGICAL ABNORMALITIES OF MYOTONIC DYSTROPHY IN CULTURE, Michael Merickel, Rick Gray, Priscilla Chauvin and Stan Appel, Baylor College of Medicine, Houston, Texas 77030
Myotonic Muscular Dystrophy (MyD) is an inherited disease (autosomal dominant) which involves progressive muscular weakness and muscle degeneration. An inborn error of metabolism is expected to underly MyD but the specific defect has not been identified. We have approached the study of MyD by utilizing electrophysiological techniques to investigate the membrane properties of muscle fibers from normal and MyD patient biopsies which are grown in a primary tissue culture system. Other investigators and ourselves have attempted unsuccessfully to find morphological abnormalities in cultured MyD muscle at the EM or light microscope level which makes the investigation of possible electrical abnormalities particularly important. Cultures were prepared from muscle biopsy specimens by trypsin dissociation. Our results demonstrate that MyD myotubes have abnormalities in some of their fundamental membrane electrical properties when compared to control myotubes which include: 1) significantly decreased average resting potential of approximately 10 mV; 2) increased tendency to repetitively fire action potentials; 3) significantly decreased outward-going rectification: and 4) two-fold increase in specific membrane resistance. All of the observed MyD abnormalities can be accounted for by a decreased conductance to K^+. This demonstration indicates that an abnormality of MyD muscle membranes is propagated in culture. The culture model will permit detailed electrophysiological studies to further characterize the abnormality and provide an excellent system for future biochemical studies of the specific inborn error of metabolism.

180 REGULATION OF ACETYLCHOLINE RECEPTOR IN EMBRYONIC SKELETAL MUSCLE. Joav Prives[1,2], K. Olden[2], A. Fulton[3], S. Penman[3], M. Daniels[2] and C. Christian[2], SUNY at Stony Brook, NIH, Bethesda, Md. and MIT, Cambridge, Mass.

Profound changes in the amount and distribution of acetylcholine receptor (AChR) on the cell surface mark the differentiation and innervation of embryonic skeletal muscle. We are investigating the roles of, a) muscle cytoskeleton and b) protein glycosylation, in regulation of the distribution and stability of the AChR glycoprotein in cultured muscle cells. The association of AChR with underlying cytoskeleton was monitored using a detergent extraction that quantitatively removes membrane lipids and soluble proteins from cultured cells, leaving the intact cytoskeleton attached to the substratum. Using this procedure, we have identified two subpopulations of AChR. Our findings suggest that most of the diffusely distributed AChR is rapidly extracted from surface membranes of muscle cells by detergent treatment while aggregated AChR is predominantly retained by the cytoskeleton. Treatment of cultured muscle with medium previously conditioned by neuronal cells induces rapid formation of AChR aggregates and a subsequent increase in AChR retention on cytoskeleton. These results suggest that the aggregation of AChR precedes attachment of these receptors to underlying cytoskeleton.

Using tunicamycin, an inhibitor of protein glycosylation, we have investigated the role of glycosylation in the biosynthesis and turnover of AChR. We have found that inhibition of protein glycosylation markedly increases the degradation rate of AChR without blocking its biosynthesis or incorporation into the cell membrane.

181 ISOLATION AND CHARACTERIZATION OF SARCOLEMMA FROM NEWBORN AND EMBRYONIC CHICK HEARTS.

M. Marlene Hosey, Department of Biochemistry, University of Health Sciences/The Chicago Medical School, Chicago, Illinois 60612 .

Certain membrane receptors and transport functions exhibit variations in properties during the development of the chick myocardium. Large alterations in the intracellular concentrations of cyclic nucleotides and the activity of cAMP-dependent protein kinase parallel some of these developmentally related changes in membrane function. Thus the developing chick myocardium may be a useful system to determine if alterations in cAMP-mediated phosphorylation of membrane proteins can be correlated with specific alterations in membrane function. For such studies it is critical to obtain a well-defined sarcolemma fraction. Membrane fractions from hearts of newborn chicks and 9-10 day embryos have been purified using discontinuous sucrose gradients containing low ionic strength buffers. Sarcolemma from newborn chick hearts sedimented as "light membranes" at a 12:28% sucrose interphase (M_1). This fraction was enriched in ouabain-sensitive Na,K-ATPase and the acetylcholine receptor as determined by [^{3}H]-quinuclidinyl benzilate binding. This fraction accumulated [^{45}Ca]$^{2+}$ and contained cAMP-dependent protein kinase and several substrates of exogenous cAMP-dependent protein kinase catalytic subunit. Membranes isolated from hearts of 9-10 day embryos differed from the newborns in that Na,K-ATPase and the QNB binding fraction sedimented with heavier membranes while the protein kinase and its substrates remained localized in the M_1 fraction. The data suggest that structural as well as functional differences in the sarcolemma may occur during development. (Supported by the PMA Foundation, Chicago Heart Association and NIH).

182 PURIFICATION OF SODIUM CHANNELS FROM MUSCLE SARCOLEMMA. Robert L. Barchi, University of Pennsylvania, Philadelphia, PA 19104.

The component of the excitable membrane sodium channel which binds saxitoxin (STX) and tetrodotoxin (TTX) has been purified from rat sarcolemma. The channel could be solubilized with medium chain nonionic detergents (e.g. NP-40) and the toxin binding component (TBC) identified by specific equilibrium binding of ^{3}H-STX. The solubilized channel required phospholipid for stability and specific ^{3}H-STX binding was quite temperature sensitive. The solubilized channel bound toxin with an apparent K_d comparable to that in intact membranes, and binding was competitively blocked by monovalent cations and noncompetitively blocked by trimethyl oxonium tetrafluoroborate.

The solubilized TBC was purified from isolated sarcolemma by sequential chromatography on a weak ion exchange resin with guanidinium functional groups and a lectin column containing immobilized Wheat Germ Agglutinen. Specific ^{3}H-STX binding in the purified material averaged 1500 pmoles toxin/mg protein representing a 6000 fold purification over crude homogenate. The purified TBC had an apparent $S_{w,20}$ of 9.5 suggesting a size of 300,000 MW, although it behaved on sepharose 6-B as if it were a larger protein. SDS-PAGE indicated 3 major bands in the purified material.

Transport Binding Proteins

183 CLONING OF THE HISTIDINE TRANSPORT GENES OF *SALMONELLA TYPHIMURIUM*, Giovanna Ferro-Luzzi Ames, Feroza Ardeshir and Christopher F. Higgins, University of California, Berkeley, CA 94720.

High-affinity histidine transport in *Salmonella* involves three proteins, a periplasmic binding protein J, a membrane-bound protein P, and a third unidentified protein Q. The genes for these proteins, together with a promoter/regulatory region, are arranged as a single operon. A 12.4 kb *Eco*RI fragment of the *Salmonella* chromosome containing this operon has been cloned in a phage vector, λgt4. The presence of the histidine transport genes on this fragment has been confirmed by complementation and by the electrophoretic identification of the J and P proteins produced upon infection of UV-irradiated cells with the λ clone. A restriction map of the cloned fragment has been generated. The histidine transport genes have been positioned on this map by Southern transfer and hybridization to the DNA of deletion mutants of the transport region. The histidine transport operon has also been cloned in the single-stranded DNA phage vector, M13 mp2, as a prelude to sequencing by the chain termination method. We are presently introducing into the clone specific mutations permitting the identification of the Q protein, and also mutations causing overproduction of all three histidine transport proteins. The latter will allow the purification of these proteins and the study of the regulation of the operon.

184 A PRELIMINARY THEORETICAL COMPARISON OF THE SULFATE AND ARABINOSE BINDING PROTEINS, Robert W. Hogg and Hikaru Isihara. Case Western Reserve University, Cleveland, Ohio 44106

The primary amino acid sequence analysis of the sulfate binding protein and the arabinose binding protein have now been completed. Analysis of the Isoleucine-Leucine-Valine binding protein is available in the literature and a preliminary sequence analysis of the histidine binding protein is available in this laboratory. The four known sequences were used to test the ability to predict sequence similarities from compositional relatedness. Ten alignments of the two sequences A.B.P. and S.B.P. (chosen because of the similarity of residue numbers--306 and 310) were analyzed. An average probability value which reflects the degree to which the sequences may be related was calculated for each alignment. The method of Chou and Fasman was applied to both sequences (arabinose and sulfate) and a qualitative assignment of secondary structure was made to regions of each sequence.

185 ^{1}H NMR INVESTIGATION OF SUBSTRATE-INDUCED CONFORMATIONAL CHANGES OF HISTIDINE-BINDING PROTEIN J OF SALMONELLA TYPHIMURIUM, Chien Ho, Yueh-hua Giza, Seizo Takahashi, and Kenneth E. Ugen, Carnegie-Mellon University, Pittsburgh, PA 15213

The histidine-binding protein J (J protein) of Salmonella typhimurium binds L-histidine as a first step in the high-affinity active transport of this amino acid across the cytoplasmic membrane. High-resolution proton nuclear magnetic resonance (NMR) spectroscopy at 600 MHz has been used to investigate the conformations of J protein in the presence and absence of substrate. This protein contains 1 histidine, 2 tryptophans, 8 tyrosines, and 12 phenylalanines per mole. Thus, there are a large number of aromatic proton resonances together with numerous resonances due to protons on the α-carbon atoms, unexchangeable NH and backbone H-bonded protons over the spectral region from 1 to 6 ppm downfield from the proton resonance of residual HDO. Previous results reported by this laboratory have shown that there are extensive spectral changes over this region upon the addition of L-histidine. When resonances from individual amino acid residues can be resolved in the ^{1}H NMR spectrum of a protein, a great deal of detailed information on substrate-induced structural changes can be obtained. In order to gain a deeper insight into the nature of this type of structural changes, deuterated phenylalanines or tyrosines have been incorporated biosynthetically into bacteria. ^{1}H NMR spectra of selectively deuterated J proteins were obtained and compared to normal J protein. The relationship between the observed spectral changes and the transport of L-histidine will be discussed. (Supported by research grants from NIH and NSF.)

186 EFFECTS OF LOCAL ANESTHETICS AND PROTEASE INHIBITORS ON INDUCTION OF LACTOSE TRANSPORT ACTIVITY, Merna R. Villarejo, Sandra Granett and Sui-Lam Wong, University of California, Davis, California 95616

A local anesthetic has previously been shown to inhibit E. coli exported protein processing without interfering with protein synthesis. Proteolytic cleavage of the precursors of alkaline phosphatase, a periplasmic enzyme, and an outer membrane protein M2, was strongly inhibited by 20 mM procaine. Procaine also interfered with translocation of alkaline phosphatase through the E. coli inner membrane. Although the mechanism of procaine action is unknown, both lipid bilayer effects and direct protease inhibition have been suggested. To investigate the mechanism of membrane insertion of an integral inner membrane protein we have examined the effects of local anesthetics and protease inhibitors on the induction of lactose transport activity. We have found that lactose permease expression is not inhibited by 20 mM procaine or the protease inhibitors antipain and leupeptin. However, the more potent anesthetics, lidocaine and tetracaine, can abolish lactose transport at 10 mM and 5 mM respectively. Amino acid double labeling experiments show that the normal lactose permease protein (MW ≈ 30,000) is absent from the membrane in lidocaine treated cells.

187 CITRATE BINDING PROTEIN (C PROTEIN) OF *SALMONELLA TYPHIMURIUM*, Gaye D. Sweet, Jackie M. Somers, and William W. Kay, *University of Victoria, Victoria, British* Columbia, V8W 2Y2, Canada and Walter C. Mahoney, Purdue University, West LaFayette, Indiana, 47907, U.S.A.

Citrate grown cells of *S. typhimurium* produce a periplasmic citrate binding protein which is readily purified by conventional means. This protein, the C protein, appears to be dimeric and has a high affinity (μM) for the tricarboxylates citrate and isocitrate and for the analogue fluorocitrate. The binding of substrate requires monovalent cations, particularly Na^+. Mutants unable to either metabolize or transport citrate or isocitrate, and which were resistant to fluorocitrate were isolated. Shock fluids from these strains contain no detectable citrate binding activity and no cross reacting material. The defective gene (*tct* C) was localized at 59 units in a "silent" area between, but not cotransducible with, *srl* and *phe*A, and was accurately positioned relative to several nearby Tn10 insertions. The N terminal amino acid sequence has been determined and is compared with arabinose, ribose, and galactose binding proteins.

188 PERIPLASMIC BINDING PROTEINS FOR ACTIVE TRANSPORT AND CHEMOTAXIS: STRUCTURE AND FUNCTION, F.A. Quiocho, D.M. Miller, M.E. Newcomer, G.L. Gilliland, J.W. Pflugrath, and D.A. Charletta, Dept. of Biochemistry, Rice University, Houston, Texas 77001

The three-dimensional (X-ray) structure oi iive binding proteins specific for L-arabinose, D-galactose, maltose, Leu/Ile/Val and sulfate is currently being determined in our laboratory. The structure of arabinose-binding protein and stereochemistry of sugar-binding have been determined at 2.4 Å resolution. D-galactose-binding protein structure has been solved at 4 Å resolution.

We have also measured the kinetic of ligand-binding of galactose-, arabinose-, maltose- and histidine-binding proteins by stopped-flow rapid mixing techniques. The changes in tryptophan fluorescence that occur with binding to these proteins are described by a rapid, second-order process (k_1 = 1-4x10^7 $M^{-1}s^{-1}$ and k_2 = 1-100 s^{-1}); no additional phases were observed.

Using low angle X-ray scattering from solution of arabinose-binding protein, we find that the radius of gyration (Rg) decreases by 0.94 (±0.33) upon binding of arabinose. This change could be accounted for in terms of a sugar-induced cleft closure, resulting from one lobe rotating 18° relative to the other lobe about a "hinge" deep in the base of the cleft separating the two lobes. The cleft contains the sugar-binding site.

The structure and function of binding proteins will be discussed in light of these and other studies.

(Supported by NIH Grants GM21371 & GM26485 and the Robert A. Welch Foundation Grant C-581)

189 THE ROLE OF ACETYL-PHOSPHATE IN ACTIVE TRANSPORT, Jen-shiang Hong and Arthur G. Hunt, Brandeis University, Waltham, MA 02254

Previous results from this laboratory have suggested that acetyl-phosphate is required for osmotic-shock-sensitive active transport in E. coli. In order to further investigate the involvement of acetyl-phosphate in shock-sensitive transport, a micro-method for the measurement of acetyl-phosphate has been developed. This method, which involves the enzymatic reaction of acetyl-phosphate with tritiated ADP and subsequent separation and quantitation of labelled ATP, has a sensitivity of approximately 20 pmoles of acetyl-phosphate per ml. of cell-free extract. Applying this method to the system described in Proc. Natl. Acad. Sci. U.S.A. 76, 1213 (1979), we have verified the conclusion that E. coli must be able to synthesize acetyl-phosphate in order to transport shock-sensitive substrates. Further experiments regarding the kinetics of arsenate inhibition of transport have revealed that, over a period of time where transport ability decreases by a factor of ten to twenty, acetyl-phosphate levels do not change at all. Furthermore, ATP levels show, at most, a three-fold decrease over the same period of time. These results suggest that some other factor, in addition to acetyl-phosphate, is involved in shock-sensitive transport in E. coli.

190 IDENTIFICATION OF A CYTOPLASMIC MEMBRANE COMPONENT OF THE E. COLI MALTOSE TRANSPORT SYSTEM, Howard A. Shuman, Thomas J. Silhavy, and Jonathan R. Beckwith, Harvard Medical School, Boston, MA 02115

We have developed a general method for the identification of bacterial proteins which have been difficult to detect by conventional means. Specifically, the product of the malF gene has been identified. Fusions of the malF gene to the lacZ gene (which codes for β-galactosidase) have been constructed in vivo. These fusions code for hybrid proteins with a COOH-terminal region that retains β-galactosidase activity and an NH_2-terminal region that is coded for by the malF gene. A hybrid protein that contains a significant portion of the NH_2-terminal region of the malF protein was purified and antibodies to it were raised in rabbits. Some of the antibodies recognize determinants coded for by the malF gene. These were used in immune precipitation experiments to demonstrate that the product of the malF gene is a 40,000 mol. wt. protein that is located in the cytoplasmic membrane and present in approx. 500 copies per cell during growth on maltose. This protein has an essential but as yet undefined role in maltose and maltodextrin active transport. Studies are in progress to determine the orientation of the malF protein in cytoplasmic membrane.

191 THE RIBOFLAVIN-BINDING COMPONENT OF MEMBRANE VESICLES OF BACILLUS SUBTILIS, Gary Cecchini and Edna B. Kearney, University of California, San Francisco 94143 and Veterans Administration Hospital, San Francisco, California 94121.

The presence of a riboflavin-binding component in the cell membrane of Bacillus subtilis has been shown in studies of strain H52, a riboflavin requiring mutant. Evidence for its function in transport of riboflavin by the organism was adduced from the similarity of the K_D for riboflavin-binding and the K_m for uptake, and from studies with the analog 5-deazariboflavin, which inhibits effectively both uptake and binding of riboflavin in the same concentration range. The riboflavin-binding component has been solubilized and partially purified by first treating isolated membranes with 1% (w/v) sodium cholate in 10 mM Tris-50 mM NaCl, pH 8.0, centrifuging at 100,000 x g for 1 hr, and then reextracting the membranes with 2% Triton X-100 (w/v) in 10 mM potassium phosphate, pH 6.8. After again centrifuging the suspension at 100,000 x g for 1 hr, the supernatant is adsorbed and eluted from hydroxylapatite. The eluted material is purified about 16-fold in comparison with the membranes. Membrane vesicles from B. subtilis H52 have been shown to carry out exchange and counterflow of riboflavin under appropriate conditions. These findings further support the conclusion that riboflavin uptake in B. subtilis is carrier-mediated, although, thus far, it has not been possible to observe net accumulation of riboflavin by the vesicles under conditions adequate to support proline uptake. Transient uptake of riboflavin by the vesicles is observed occasionally but not predictably but valinomycin plus K^+ can be used to obtain transient uptake more consistently. Both binding and uptake vary with the level of riboflavin present during growth.

192 BIOSYNTHESIS OF CALCIUM-BINDING PROTEIN OF CHICK CHORIOALLANTOIC MEMBRANE,
Rocky S. Tuan, *Developmental Biology Laboratory, Massachusetts General Hospital, Boston, MA 02114*

The chick chorioallantoic membrane (CAM), which transports egg shell calcium into the embryonic circulation, contains a calcium-binding protein (CaBP) (J. Biol. Chem. 253, 1011, 1978) associated with the ectodermal cell surface (J. Cell Biol. 77, 743, 1978). CaBP biosynthesis has been studied in CAM organ cultures and by cell-free translation. The CaBP is a relatively stable protein ($t_{1/2}$ = 50-60 h) and is expressed in the CAM concomitantly with calcium transport as a function of embryonic development. Biosynthesis of the CaBP in the CAM involves microsomes and at least two post-translational modifications, glycosylation and vitamin K-dependent γ-glutamyl carboxylation. Cell-free translation of CAM mRNA in a rabbit reticulocyte lysate system revealed that CaBP is synthesized as a pre-protein containing a signal sequence which is cleaved during subsequent segregation of the polypeptide into microsomal vesicles. It appeared that the segregated lower-molecular-weight CaBP precursor is then post-translationally modified to form the mature CaBP. (Supported by NIH Grant AM03564 and Jane Coffin Childs Memorial Fund).

193 RECONSTITUTION OF BINDING PROTEIN DEPENDENT TRANSPORT IN BACTERIA, Frank Robb, Clement E. Furlong and Barnett B. Rosenblum, Departments of Medicine (Division of Medical Genetics, Center for Inherited Diseases) and Genetics, University of Washington, Seattle, Washington 98195.

In an earlier report (Galloway and Furlong, 1979) the reconstitution of binding protein dependent ribose uptake in spheroplasts of wild type E.coli K12 was described. This report noted that reconstitution did not take place in spheroplasts derived from a number of mutant strains deficient in ribose binding protein, or from cells which were not induced for ribose utilization. Recently we have been able to demonstrate reconstitution in one of the strains (DG1-9) with defective ribose binding protein. Residual uptake in the absence of added ribose binding protein was negligible. Antiserum directed against ribose binding protein prevented reconstitution completely. Ribose binding protein used in these studies was purified by a rapid method including a novel step, high pressure liquid chromatography. Highly purified binding protein thus obtained has proved effective, in preliminary studies, in the reconstitution of ribose uptake in spheroplasts of Salmonella typhimurium strain LT2. This finding that E.coli ribose binding protein is capable of interspecies reconstitution is important in establishing homology between the two systems. The reconstitution of ribose uptake in spheroplasts derived from Salmonella cells was eliminated in control experiments in which antiserum directed against E.coli ribose protein was added to the mixtures. We acknowledge support by a CSIR grant to F. Robb.
Reference: Galloway, D.R. and Furlong, C.E. (1979) Arch. Biochem. Biophys. 197:158-162

194 CLONING AND EXPRESSION OF LEUCINE TRANSPORT GENES IN ESCHERICHIA COLI,
Charles J. Daniels, James J. Anderson, Robert C. Landick and Dale L. Oxender, Department of Biological Chemistry, The University of Michigan, Ann Arbor, MI. 48103

We have cloned a 13 kB DNA fragment carrying the livG, H, J, K genes specifing high-affinity leucine transport in E. coli K-12. Appropriate subcloning has yielded a hybrid plasmid with a 2 kB piece encoding the complete structural gene (livK) for the leucine-specific binding protein, and the gene has been partially sequenced. Another plasmid carries the gene (livJ) for the liv binding protein on a 4 kB fragment. We have ordered the Hinc II sites in the latter, and have identified an internal (0.4 kB) piece with base homology to the amino terminus of the livK gene. We are determining the base sequence around this region. The closely linked genes livG, H whose products are presently unknown are being ordered relative to livJ, K by classical genetic techniques. We have employed hybrid plasmid DNA to generate precursor forms of the binding proteins in an in vitro transcription/translation system. Protein microsequencing of the precursor has verified a predicted 23 amino acid N-terminal extension of the leucine-specific binding protein, which can be removed by a crude S30 extract following translation. The mature and precursor forms are soluble and susceptible to papain. However, only the precursor is sensitive to trypsin. Processing activity in the S30 extract is sensitive to phenylmethylsulfonyl fluoride inhibition; other protolysis inhibitors are being tested. Preliminary characterization of mutants defective in producing mature binding proteins has yielded strains which accumulate fragments of binding protein. The relevance to protein secretion is under study. Supported by GM 11024 and the American Heart Association.

195 DISPOSITION OF THE CYTOPLASMIC MEMBRANE TRANSPORT COMPONENTS OF THE DICARBOXLYATE TRANSPORT SYSTEM OF ESCHERICHIA COLI K12, K.M. Tippett, T.C.Y. Lo, University of Western Ontario, London, Ontario, Canada N6A 5C1.
The dicarboxylate transport system in E.coli K12 is responsible for the uptake of succinate, fumarate, and malate across the cell envelope. Both biochemical and genetic studies indicate that there is only one dicarboxylate transport system present in E.coli, and that at least one binding protein, and two cytoplasmic membrane transport components are involved in this process. The two cytoplasmic membrane transport components, SBP 1 and SBP 2, are coded by the dct B and dct A genes respectively. Reconstitution studies indicate that both components are essential for the transport process. Binding studies with both right-side-out and inside-out vesicles indicate that the substrate recognition sites of SBP 1 and SBP 2 are exposed on the inner and outer membrane surfaces respectively. The present investigation reports our recent findings on the disposition of these two components in the cytoplasmic membrane. Non-penetrating inhibitors, such as trypsin, chymotrypsin, pronase, and diazo-sulfanilic acid, were found to inhibit succinate transport by the cytoplasmic membrane vesicles. The effect of these reagents on the substrate recognition sites of the transport components, and on the integrity of the membrane vesicles will be reported. Surface labeling studies were also carried out with various membrane preparations to determine the disposition of the dicarboxylate transport components. (Supported by MRC)

Cation Transport Systems

196 THE INTERACTION OF Tl^+ WITH GASTRIC ATPASE VESICLES, G.Sachs, E Rabon, UAB, Birmingham, Alabama, 35294
The gastric ATPase catalyses an electroneutral exchange of K_{int} for H_{ext} generating a pH gradient of 4 units due to turnover of an EP intermediate. During turnover there is translocation of K from a high affinity internal COOH to a low affinity external COOH. The $K_{.5}$ for Tl is 80µM, for K it is 700µM. Tl is transported by the ATPase in the range 0.6 to 6mM. At the latter Tl first effluxes from the vesicles with ATP addition and then reenters. Provided external Tl is less than 2mM, high Tl internal also supports H transport. At external Tl concentrations 2mM and above Tl progressively inhibits H transport even in the presence of 150mM KNO_3 internally. Moreover these concentrations of Tl dissipate preexisting H gradients in gastric vesicles and synthetic liposomes. Thus although Tl interaction with the gastric ATPase fully mimics the physiological K ion, the lipid permeation of Tl at concentrations necessary for demonstrable H transport results in the induction of a Tl:H antiport with resultant uncoupling of ATPase activity from cation and H transport. (NIH, NSF support)

197 CHARACTERISTICS OF CANINE CARDIAC SARCOPLASMIC RETICULUM, H.M.Rhee, Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699. A modified method of Harigaya and Schwartz(Circ. Res. 25:781,1969) produced a highly active sarcoplasmic reticulum (SR) from the dog hearts. The yield of the SR was 1.27 ± 0.1 (means ± S.E.) mg per g of fresh cardiac left ventricle in seven dogs. ATP (5mM)-dependent Ca^{++} binding was 38.8 ± 2.8 nmol/mg SR at 37°C and the Ca^{++} uptake assayed in the presence of 3 mM Tris-oxalate was 1462 ± 104 nmol/mg SR after 10 minutes incubation. Ca^{++}-ATPase activity assayed in 50 µM $CaCl_2$ was 59.6 ± 5.8 µmol Pi/mg/hr. This active canine SR preparation has a low Na^+,K^+-ATPase activity (5.25 ± 0.75 µm Pi/mg/hr), which may indicate this canine microsomal preparation has very little contamination of sarcolemma. However, a treatment of the SR with sodium dodecylsulfate (SDS, 0.1 to 0.5 mg/ml) increased significantly the Na^+, K^+-ATPase activity. The treatment of SR with SDS also caused simultaneous loss of Ca^{++}-ATPase activity as well as Ca^{++} uptake ability. This indicates ATP-dependent Ca^{++} transport in the SR vesicles may couple tightly with Ca^{++} dependent ATP hydrolysis activity of the SR. A treatment of the SR with sulfhydryl agent such as dithio-bis-nitrobenzoic acid (DTNB) decreased the Ca^{++} uptake capacity (1462 vs. 277 nmol/mg for 10 min with 1 mM DTNB). This indicates the importance of SH group in SR for the transport of Ca^{++}. Several antiarrhythmic agents such as quinidine and dimethyl propanolol (UM-272) also decreased the ATP-dependent Ca^{++} uptake capacity of the SR preparation.

198 CONTROL OF STEADY-STATE FREE CA^{2+} BY MITOCHONDRIA, ENDOPLASMIC RETICULUM, AND DIGITONIN-TREATED CELLS, Fiskum, G., Becker, G., and Lehninger, A.L., Johns Hopkins Sch. of Medicine, Balt., MD 21205 and Univ. of Alabama, Birmingham, Ala. 35294

The cytosolic free Ca^{2+} concentration of most non-muscle cells is thought to be controlled to a large extent by Ca^{2+} transport systems present at the mitochondrial and endoplasmic reticulum membranes. In order to gain a better understanding of the roles that these systems play in regulating cellular Ca^{2+}, electrode measurements have been made of the steady-state free Ca^{2+} concentrations present in suspensions of energized isolated organelles or cells that have been made permeable to ions by treatment with digitonin. The concentrations of K^+, Mg^{2+}, phosphate and ATP in the suspending medium approximated those thought to be present in cytoplasm. Under these conditions mitochondria isolated from rat liver can buffer medium free Ca^{2+} to approximately 0.5 μM whereas in the additional presence of microsomes this value is near 0.2 μM. Results obtained with digitonin-permeabilized cells are similar to those obtained in the reconstituted systems. It is significant that the measured values vary with the type of tissue that is used. The steady-state Ca^{2+} concentrations are also influenced by the concentrations of Mg^{2+}, ATP, and phoshpate present in the medium; being higher with increasing phosphate and lower with increasing Mg^{2+} and ATP. These variations may reflect physiological modes of regulating cellular Ca^{2+} which involve the regulation of Ca^{2+} influx and efflux at the mitochondrial and endoplasmic reticulum membranes. G.F., USPHS National Research Service Awardee (IF32 GM97172-01). Supported by USPHS grant to A.L.L (CA25360).

199 MONENSIN AND A23187-INDUCED RELEASE OF AZUROPHILIC AND SPECIFIC GRANULES FROM HUMAN GRANULOCYTES, James D. Simon, Charles F. von Gunten, William E. Houck, and Maurice M. Albala, Rhode Island Hospital and Brown University, Providence, R.I. 02902.

Although it is well known that the secretion of a number of granule-associated substances from human granulocytes (PMN's) is Ca^{++}-dependent, the role of monovalent cations is unclear. In this study we have compared the release of unsaturated vitamin B12 binding capacity (UBBC), a specific (Sp) granule marker, to that of β-glucuronidase, a marker for azurophilic (Az) granules utilizing monensin, a Na^+ ionophore, nigericin, a K^+ ionophore, and A23187, a Ca^{++} ionophore. PMN's were isolated from buffy coats of ACD blood by density gradient centrifugation. Ten million cells were incubated for 30 minutes at 37°C in 3.0 ml. of phosphate-buffered saline with varying concentrations of ionophores. The cell-free supernatants were assayed for UBBC and β-glucuronidase. Ca^{++}-dependent release of UBBC was maximal (92% of total) at 1.0 μM A23187; only 5% of β-glucuronidase was secreted. At 10.0 μM monensin no UBBC and 7% of β-glucuronidase was released. Neither Ca^{++} nor Mg^{++} was required. Nigericin (10.0 μM) did not stimulate the release of either granule marker. At 0.1μM A23187, a suboptimal dose for UBBC release (25% of total), addition of 10.0 μM monensin resulted in synergistic release of UBBC (52% of total). No synergism was evident for β-glucuronidase release. In all experiments cell viability was not altered, as indicated by the lack of release of cytoplasmic lactate dehydrogenase. These results suggest that Ca^{++} and Na^+ exert differential effects on PMN Az and Sp granules and that both ions may act synergistically in stimulating Sp granule release.

200 THE ESCHERICHIA COLI TRKA K^+ TRANSPORT SYSTEM IS A SECONDARY K^+-H^+ SYMPORTER.
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Several genetically distinct K^+ transport systems have been described in Escherichia coli K-12, which all catalyze the electrogenic transport of K^+ ions into the cell. System TrkA, which is constitutive, is responsible for K^+ accumulation by the cells under most conditions of growth. It has peculiar energetics in that it requires for activity both a high transmembrane proton motive force (pmf) and a high intracellular ATP level. The aim of this work was to determine which of these two parameters is the driving force for K^+ transport. To this end EDTA-treated cells were prepared from mutant TK 1001, which only contains the TrkA K^+ transport system. With glucose as a substrate, the following parameters were measured in the steady state: the pmf and its components $\Delta\psi$ and ΔpH, the phosphate potential (ΔG_{ATP}) and the K^+ gradient across the cytoplasmic membrane ($\Delta\mu_{K^+}$). Under all conditions, but particularly at low external pH, $\Delta\mu_{K^+}$ was much higher than $\Delta\psi$. This indicates that $\Delta\psi$ alone is not driving K^+ transport. At increasing concentrations of protonophores (FCCP, SF 6847 or dinitrophenol) both $\Delta\mu_{K^+}$ and the pmf decreased dramatically. By contrast, ΔG_{ATP} hardly changed upon addition of protonophores, and n, the stoichiometry between the number of K^+ ions transported and moles of ATP broken down ($n = \Delta G_{ATP}/\Delta\tilde{\mu}_{K^+}$) even increased. The data from quantitative studies are compatible with a K^+-H^+ symport mechanism. Probably, ATP has only a regulatory function.

201 TRANSPORT OF K^+ AND NA^+ IN STREPTOCOCCUS FAECALIS, D. L. Heefner and F. M. Harold, National Jewish Hospital and Research Center, Denver, Colorado 80206

A basic characteristic of all living cells is that they accumulate K^+ and expel Na^+. In animal cells, Na^+/K^+ exchange is mediated by the Na^+,K^+-ATPase. In bacteria, and probably also in the lower eukaryotes, movements of K^+ and Na^+ are mediated by separate porters that are linked to the proton circulation. We have been studying the mechanism of energy coupling to cation transport in the fermentative bacterium Streptococcus faecalis.

Potassium uptake requires the cell to generate both ATP and a protonmotive force; uptake is electrogenic. The effects of inhibitors, and other features, suggest that the protonmotive force is the energy donor for K^+ uptake, but the K^+ concentration gradient (up to 50,000) is far too steep to be in equilibrium with the membrane potential. One solution to the dilemma is to invoke symport of K^+ with another cation, probably H^+. Adenosine triphosphate would, according to this scheme, serve as activator of the K^+,H^+ symporter.

Sodium transport apparently involves two pathways. As is the case with K^+ influx, Na^+ extrusion requires the cells to generate both ATP and a proton circulation. Most of the data from intact cells and membrane vesicles can be rationalized by invoking an electrogenic Na^+/H^+ antiporter, energized by the protonmotive force and activated by ATP. However, as is the case with K^+, under certain conditions the capacity for Na^+ extrusion is too great to be accounted for by measured values of ΔpH and $\Delta\Psi$. In addition to the antiporter there appears to be a pathway for Na^+ influx. Influx occurs in response to the membrane potential. These three independent pathways of cation transport allow one to account, qualitatively at least, for the movements of K^+ and Na^+ over a wide range of conditions.

202 AN ELECTROGENIC PROTON TRANSLOCATING ATPase WITH A PHOSPHORYL-ENZYME INTERMEDIATE, Gene A. Scarborough and John B. Dame, Univ. of North Carolina, Chapel Hill, NC 27514

In the presence of Mg-ATP, functionally inverted plasma membrane vesicles isolated from the eukaryotic microorganism, Neurospora, generate an interior positive membrane potential ($\Delta\Psi$), and/or an interior acid pH gradient (ΔpH), depending upon the experimental conditions employed. $\Delta\Psi$ can be measured as Mg-ATP dependent [^{14}C]-SCN^- uptake or anilinonaphthalene sulfonate fluorescence enhancement, and ΔpH can be measured as Mg-ATP dependent [^{14}C]-imidazole uptake or Mg-ATP dependent quenching of intravesicular fluorescein-labeled dextran fluorescence. Quantitative comparisons of the kinetic properties and inhibitor sensitivities of Mg-ATP hydrolysis and Mg-ATP dependent $\Delta\Psi$ and ΔpH generation show that all three processes are catalyzed by the same enzyme, indicating that the Neurospora plasma membrane contains only one principal ATPase and that this enzyme is an electrogenic proton pump. The hydrolytic moiety of the ATPase can be identified as a 108,000 dalton protein in SDS-polyacrylamide gels on the basis of its differential sensitivity to tryptic cleavage in the presence or absence of MgATP, and incubation of the membranes with γ-[^{32}P]-ATP, followed by acidic SDS-polyacrylamide gel electrophoresis and autoradiography, shows that the ATPase is phosphorylated. Importantly, quantitative isotope exchange experiments indicate that virtually all of the ATP hydrolysis catalyzed by the membranes occurs via phosphorylation and dephosphorylation of the 108,000 dalton protein. These studies demonstrate that electrogenic proton translocation catalyzed by the Neurospora plasma membrane ATPase takes place via a mechanism that involves a phosphoryl-enzyme intermediate. Possible implications of these findings will be discussed.

203 ELECTROGENIC PROTON PUMP OF NEUROSPORA: BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION, Carolyn W. Slayman and Barry J. Bowman, Yale University School of Medicine, New Haven, CT 06510, and University of California, Santa Cruz, CA 95064.

The plasma membrane of the fungus Neurospora crassa contains an ATP-driven electrogenic proton pump which is capable of generating large membrane potentials (on the order of -200 mV) and thereby of supplying energy to a variety of H^+-dependent cotransport systems. Isolated plasma membranes possess an ATPase activity which is the enzymatic expression of the pump. The ATPase has a pH optimum of 6.7 and a maximal specific activity of 3 to 5 μmol/min.mg membrane protein, and it shows a sigmoid dependence on ATP concentration, with a $K_{1/2}$ of 1.8 mM. It is sensitive to vanadate (which produces half-maximal inhibition at 0.45 to 1 μM, depending upon the ionic composition of the reaction medium) and to DCCD, but insensitive to oligomycin or ouabain. The ATPase has been solubilized by treatment of the membranes with deoxycholate in the presence of glycerol, and purified by glycerol gradient centrifugation. The purified enzyme, which displays an absolute requirement for added phospholipids, has a specific activity of 48 μmol/min.mg protein. Upon SDS-acrylamide gel electrophoresis, it exhibits two polypeptide bands: a major band of approximately 100,000 daltons and a minor band of approximately 30,000 daltons. The major band is strikingly similar in its electrophoretic mobility to the 100,000-dalton subunits of the [Na^+,K^+]- and [Ca^{2+}]-ATPases from animal cell membranes.

204 MECHANICAL REGULATION OF GENE EXPRESSION: OSMOTIC CONTROL OF THE KDP SYSTEM OF E. COLI
L.A. Laimins, D.B. Rhoads and W. Epstein, University of Chicago, Chicago, IL 60637
The Kdp system of Escherichia coli K-12 is a high affinity K^+ transport system consisting of three inner membrane proteins encoded in the kdpABC operon. Its expression is controlled by cellular need for K^+ and is mediated by a positive regulator coded by the kdpD gene. To examine how cells translate a need for K^+ into a genetic signal, we have used the method of Casadaban (Proc. Natl. Acad. Sci., USA 76:4530, 1979) to put the lacZ gene which codes for β-galactosidase, under kdp control by integrating it into kdpA, the first gene of the kdpABC operon. We find that the kdp operon is derepressed by growth in low K^+ media, but the concentration of K^+ below which derepression occurs depends on the osmolarity of the medium and on the presence of other K^+ transport systems. An increase in osmolarity alone without change in K^+ concentration derepresses the kdp operon. To explain these findings we suggest that the cells' quantitative requirement for K^+ reflects the role of K^+ in regulating internal osmolarity, and that cells sense their need for K^+ by sensing the turgor pressure, the osmotic pressure difference across the cytoplasmic membrane. A reduction in turgor pressure which should occur when K^+ uptake is inadequate is translated into a genetic signal by a membrane protein whose conformation is altered by the mechanical effects of a change in turgor pressure. This model is supported by our tentative identification of the product of the kdpD gene as a membrane protein.

205 TWO Ca^{2+} TRANSPORT SYSTEMS IN AZOTOBACTER VINELANDII VESICLES,
Piotr Zimniak and Eugene M. Barnes, Jr., Baylor College of Medicine, Houston, Texas 77030
Inverted and right-side-out membrane vesicles were prepared from A.vinelandii OP as described (E.M.Barnes,Jr., Arch.Biochem.Biophys.152,795,1972; P.Bhattacharyya and E.M.Barnes,Jr., J.Biol.Chem.251,5614,1976). Facilitated diffusion of Ca^{2+} (in absence of respiratory substrates or ATP) was studied. As found before (E.M.Barnes, Jr. et al., Membr.Biochem.1,73, 1978), a ΔpH-dependent component was observed at intravesicular pH 7.0 and external pH 8.5. Its behavior is consistent with a $Ca^{2+}/2H^+$ exchange model. The system is heat-labile, not affected by low concentrations of La^{3+}, inhibited by ruthenium red, and has an apparent K_m of 240 μM. The uptake of Ca^{2+} is abolished by A-23187 or by 2% Triton X-100. In addition, a second Ca^{2+} transport system is operative at low extravesicular pH (6.0-6.5). It is inhibited by La^{3+} and ruthenium red at concentrations lower than needed for the first system, is heat-stable (3 min at 100°C), is eliminated by 2% Triton X-100 and at high external pH, is stimulated by external tetraphenylboron, and has an apparent K_m of 68 μM. Its mode of action can be very tentatively described as electrogenic. At pH 6.5, Ca^{2+} uptake is diminished by certain anions (acetate, ethanesulfonate, MES) which might, however, act on a separate anion transport site needed for charge compensation.
Although most experiments were carried out with right-side-out vesicles, inverted vesicles behave generally in a similar way.
Both Ca^{2+} transport systems can be reconstituted into egg lecithin vesicles by the octyl glucoside dilution technique of E.Racker et al. (Arch.Biochem.Biophys., in press).

206 EFFECTS OF Na^+ AND Li^+ ON THE K^+ TRANSPORT SYSTEMS OF ESCHERICHIA COLI,
Erik N. Sorensen and Barry P. Rosen, University of Maryland School of Medicine, Baltimore, Maryland 21201

K^+ is actively accumulated in E. coli mainly via two K^+ transport systems: a repressible, high-affinity Kdp system and a low-affinity, constitutive TrkA system. Tl^+ is a substrate for each of these systems. We have studied the effects of Na^+ and Li^+ on each of these systems by observing the accumulation of $^{204}Tl^+$ and K^+.
When compared to choline, Na^+ stimulated the uptake of both Tl^+ and K^+ via the TrkA system. Li^+ was slightly inhibitory when compared to choline.
Tl^+ uptake via the Kdp system, on the other hand, was stimulated more by Li^+ than by Na^+ when compared to choline. In addition, Li^+ increased the effectiveness of Rb^+ as an inhibitor of Tl^+ uptake via the Kdp system. Na^+, however, was more effective than Li^+ as a stimulator of K^+ uptake via the Kdp system.
We suggest that Na^+ may be involved in the mechanisms of K^+ transport via the TrkA and Kdp systems in E. coli. Two models, an ATP-driven Na^+, K^+ antiport and an ATP-activated Na^+, K^+ symport, are under consideration to explain the Na^+ stimulation of K^+ transport via the TrkA and Kdp systems in E. coli.
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207 Ca^{2+}/H^+ ANTIPORTER OF MITOCHONDRIA, W. Dubinsky, A. Kandrach and E. Racker, Univ. of Texas Health Science Center at Houston, Houston, TX 77025 and Cornell Univ., Ithaca, NY 14853

A Ca^{2+}/H^+ antiport has been isolated from bovine heart submitochondrial particles by detergent extraction and differential centrifugation. Extracts were assayed for H^+-gradient dependent Ca^{2+} flux by reconstitution into phospholipid vesicles. Vesicles were reconstituted by sonication or by incorporation in the presence of 0.1% lysolecithin. Routinely, Na^+ transport was assayed as an indication of the degree of resolution of the Ca^{2+} transporter. Submitochondrial particles and 1% Triton X-100 extracts possessed both Ca^{2+} and Na^+ transport activities. Triton was removed by dilution of the extracts with phospholipids and cholate and precipitation of the protein with ammonium sulfate. A Na^+ transporter was preferentially extracted from the ammonium sulfate precipitate with the nonionic detergent octa-ethylene glycol dodecyl ether ($C_{12}E_8$). The Ca^{2+} transporter was then extracted from the $C_{12}E_8$ insoluble fraction with 2.5% lysolecithin. The Ca^{2+}/H^+ antiporter in the lysolecithin extract was fully resolved from all Na^+ transport activity on a sucrose density gradient run in the presence of lysolecithin and deoxycholate. Both the Na^+ and Ca^{2+} transport components were heat labile and inhibited by trypsinization. Ca^{2+} transport was completely independent of either a Na^+ or K^+ gradient. On the other hand, Ca^{2+} transport was dependent upon a proton gradient generated in pH jump experiments or artificially with a K^+ plus nigericin or a NH_4^+ gradient. Results are consistent with a Ca^{2+}/H^+ antiport mechanism. W.D., USPHS National Research Service Award (1F32-CA 05487). Supported by USPHS grant to E.R. (CA 08964).

208 SARCOPLASMIC RETICULUM MEMBRANE STRUCTURE AND CA^{++}ATP-ASE ACTIVITY, Philip L. Yeagle and Arlene D. Albert, Biochemistry Department, SUNY/Buffalo School of Medicine, Buffalo, N.Y. 14214.

Ca^{++} transport by the Ca^{++} ATPase of the sarcoplasmic reticulum and ATPase activity of the same protein have been presumed to be affected by the lipid environment of the sarcoplasmic reticulum membrane. An annulus of lipid has been suggested to surround the protein whose content controls activity, based on experiments in recombined systems. Recently we have discovered an analogous annulus of immobilized lipid around glycophorin from the erythrocyte membrane, but no such annulus around rhodopsin of the rod outer segment membrane, using a new independent approach to such studies employing ^{31}P NMR. this same approach has been applied to intact sarcoplasmic reticulum membranes isolated from rabbit hind leg muscle. Preliminary experiments do not support the concept of an immobilized annulus of 30 lipids around the Ca^{++} ATPase. However, the phase structure of the surrounding lipid membrane may be important to activity. The mitochondrial F_1F_0 ATPase appears to respond to the tendancy of the membrane lipids to form discontinuities in the membrane apparently consisting of inverted micelles ot lipid produced by high phosphatidylethanolamine contents. The possibility exists that the Ca^{++}ATPase may be similarly sensitive.

209 BLOCKADE OF A CALCIUM-DEPENDENT POTASSIUM CONDUCTANCE IN NEUROBLASTOMA CELLS BY QUININE, M. C. Fishman, I. Spector, Laboratory of Developmental Neurobiology, AND Biochemical Genetics, NIH, Bethesda, Md.

Slow oscillations in the membrane potential underly repetitive activity in several cell types. The origin of such oscillations remain unclear. In mouse neuroblastoma cells they may be enhanced by increasing extracellular calcium concentration or blocking the voltage-sensitive potassium channels with tetraethylanmonium (TEA) or both. In red blood cells and pancreatic cells quinine blocks a calcium-dependent potassium conductance. We have found in the mouse neuroblastoma NIE-115 line that (5×10^{-5}M) quinine reduces slow repetitive activity induced by calcium or TEA. The action potential that remains no longer has the usual pronounced slow after-hyperpolarization. Unless high dosages are used quinine does not alter the resting membrane potential, delayed rectification, or the component of the action potential due to calcium influx. The quinine effect does not require the presence of sodium in the medium. During washout of the quinine the after-hyperpolarization returns, as does repetitive activity. The slow after-hyperpolarization in NIE-115 has been shown to be due to a calcium-dependent potassium conductance. Our results suggest that quinine specifically blocks this conductance mechanism. Further, they suggest that in these cells this conductance is a critical component of membrance oscillations that underly repetitive activity.

210 THE Na^+/H^+ ANTIPORTER OF BACILLUS ALCALOPHILUS, Kenneth G. Mandel and Terry A. Krulwich, Mount Sinai School of Medicine of CUNY, New York, N.Y. 10029

B. alcalophilus grows optimally at pH 10.5 on L-malate. The organism generates a "reversed" ΔpH, interior acid, and a large $\Delta\psi$, interior negative (Guffanti, et al., 1978, J. Biol. Chem. 253, 708-715). Studies utilizing right-side-out vesicles demonstrated that only Na^+-loaded vesicles are capable of generating a "reversed" ΔpH upon energization. Energized everted vesicles exhibit concentrative uptake of $^{22}Na^+$; such vesicles also show an NADH-induced quenching of quinacrine fluorescence that is more than reversed by Na^+. Thus a Na^+/H^+ antiporter was indicated. A mutant strain, KM23, has been isolated on the basis of its ability to grow at neutral pH. Indeed, the non-alkalophilic mutant grows at pHs from 5 to 9, but no higher. This strain does not exhibit Na^+/H^+ antiporter activity and does not generate a "reversed" ΔpH. It maintains a ΔpH of 1.4 units, interior alkaline, at pH 5.5, decreasing to 0.5 units at pH 7.0. Non-alkalophilic KM23 fails to show Na^+-dependent α-aminoisobutyric acid (AIB) transport at pH 9.0, an activity that is present in the wild type strain. It is possible that this Na^+-translocating symporter shares a common subunit with the Na^+/H^+ antiporter. Several revertant strains of KM23 have been isolated. Some appear to be true revertants, but others still fail to exhibit Na^+/AIB symport. Perhaps another antiporter, subsequent to mutation, acidifies the cytoplasm in these strains.

211 K^+ REGULATION OF INTRACELLULAR pH IN ESCHERICHIA COLI. Barry P. Rosen, Robert N. Brey, Raymond H. Plack, and Erik N. Sorensen. University of Maryland School of Medicine, Baltimore, MD 21201.

The electron transport chain and H^+-translocating ATPase of bacteria and mitochondria are primary active transport systems which catalyze the formation of electrochemical proton gradients. The vectorial separation of H^+ and OH^- results in a continual alkalinization of the cytosol of bacteria. In order to maintain a constant intracellular pH, protons must be returned to the cytosol. Although both solute uptake and ATP synthesis result in proton uptake, neither would be expected to regulate cytosolic pH. An electroneutral proton/cation exchange could serve this purpose, returning protons to the cytosol at the expense of the ΔpH portion of the protonmotive force without dissipation of the $\Delta\psi$ component.

We have recently reported a K^+/H^+ antiport (KHA) system in E. coli, the function of which we postulate to be regulation of intracellular pH. In support of this hypothesis a mutant selected for inability to grow at alkaline pH was found to lack the KHA system. The KHA system was found to be extremely sensitive to trypsin, losing activity with a $t_{1/2} < 20$ seconds. A number of other inner membrane functions were not sensitive to trypsin under the same conditions. Prior formation of a protonmotive force protected the KHA system from trypsin inactivation. K^+, on the other hand, did not protect. Efforts are currently in progress to identify with two-dimensional gel electrophoresis a trypsin-sensitive membrane component related to the KHA system. (Supported by grants GM-21648 from the National Institute of General Medical Sciences and PCM 77-17652 from the National Science Foundation).

212 PHYSIOLOGICAL FUNCTION OF THE Mg^{++} ATPase ACTIVITY OF THE YEAST PLASMA MEMBRANE, Gail R. Willsky, Harvard University, Cambridge, MA 02138

Vanadate does not inhibit the mitochondrial Mg^{++} ATPase, while it does inhibit both the hydrolytic activity of the plasma membrane Mg^{++} ATPase and the formation of two phosphoprotein intermediates (Mr = 210,000 and 115,000) associated with that hydrolytic activity. The addition of vanadate to growing cells stopped growth and inhibited the transport of the following amino acids and bases: methionine, arginine, valine, glycine, uracil and adenine. The Ki for vanadate inhibition of cell growth and the transport of both adenine and arginine were similar. The finding that vanadate, a specific inhibitor of the yeast plasma membrane Mg^{++} ATPase, inhibits the transport of amino acids and bases through the plasma membrane supports the hypothesis that this enzyme is involved in the transport of small metabolites across that membrane. It has been shown in yeast that the transport of small metabolites involves changes in media pH. The Mg^{++} ATPase activity of the yeast plasma membrane may maintain a proton gradient needed for transport to occur.

ATPases and Energetics

213 β-ECDYSONE SENSITIVE (Na+,K+) ATPASE and CELL VOLUME ALTERATIONS IN MRRL-CH CELLS, Leigh H. English and Edwin P. Marks, Metabolism & Radiation Research Laboratory, USDA/SEA, Fargo, ND 58105.

β-ecdysone stimulates the (Na^+,K^+) ATPase of insect cells (MRRL-CH) in culture and in surface membranes isolated from these cells. Within 30-45 seconds after treatment with 10^{-8} M β-ecdysone, up to 25% of the internal Na^+ concentration was pumped out of the cells. Then, within five minutes, the internal Na^+ concentration returned to equilibrium. In response to 10^{-6} M β-ecdysone, an immedidate sevenfold stimulation of the enzyme in the surface membranes was observed, using the pseudosubstrate β-2-Puryl acryloyl phosphate (FAP). Both Na^+ efflux and FAP hydrolysis were inhibited by ouabain when the active tissues were preincubated with 10^{-3} ouabain in the absence of K^+. Rapid cell volume changes accompanied these phenomena. Ten percent of the cell population decreased in volume within 30 seconds after β-ecdysone treatment. The energetics of the β-ecdysone-stimulated (Na^+, K^+) ATPase are discussed in relation to cellular morphology and the molting process of insects.

214 ATP-INDUCED VESICLE LYSIS: POSSIBLE ROLE OF ATP IN EXOCYTOTIC RELEASE, Stephen G. Oberg, University of Washington, Seattle, Washington 98195

Secretory vesicles isolated from a variety of mammalian tissues are known to lyse and thereby release their secretory products when exposed to ATP. We report here that ATP causes the lysis of a highly purified preparation of rat parotid secretory granules (Anal. Biochem. 52, 589). ATP-induced lysis was characterized with respect to pH, temperature, osmolarity and the ionic composition of the media. The rate of vesicle lysis was measured spectrophotometrically. ATP-induced lysis is expressed as the increase in the rate of lysis (r = % lysis/min) when ATP is added. Assays were performed at 37°C in media containing 110mM KCl, 5mM MgCl2 and 25mM maleic acid. ATP-induced lysis of parotid granules was found to have the following properties in common with the extensively characterized chromaffin granule process (Arch. Biochem. Biophys. 176, 375): (1) it is a saturable function of ATP e.g. $K^1/_2$ = 0.5±0.1mM (2) it is inhibited in hyperosmotic media e.g. r = 5.2 at .3 OsM vs 0.8 at .4 OsM and (3) has an apparent chloride requirement. These findings, combined with reports of ATP-induced lysis of cholinergic, insulin and posterior pituitary secretory vesicles imply that ATP-induced lysis may reflect an ATP-dependent property of all secretory vesicles and as such could play a similar role in all mammalian exocytotic release processes. Using a model system, Miller and Racker (J. Mem. Biol. 30, 283) made a surprising finding that the extent to which liposomes fuse with a planar black lipid membrane depends on the osmotic gradient across the vesicle membrane. In view of the osmotic dependence of ATP-induced lysis of parotid and other secretory vesicle preparations, we postulate that ATP may serve to prime secretory vesicles for fusion with the plasma membrane by inducing and/or maintaining an osmotic gradient across the vesicle membrane.

215 DICYCLOHEXYLCARBODIIMIDE-REACTIVE PROTEINS FROM MUTANT STRAINS OF E. COLI - MOLECULAR IMPLICATIONS OF AMINO ACID REPLACEMENTS AND MODIFICATIONS. Karlheinz Altendorf, Gabriele Deckers, Hans H. Kiltz and Roland Schmid, Institut für Biochemie der Pflanzen, Ruhr-Universität Bochum, D-4630 Bochum, Germany

The hydrolysis of ATP by the bacterial ATPase complex is coupled to the translocation of protons. It was therefore reasonable to assign the BF_o component, especially the dicyclohexylcarbodiimide (DCCD) reactive protein, a role in the translocation of protons. We have purified and characterized the DCCD-reactive protein in DCCD-labelled and unlabelled form. The amino acid sequence (Wachter, Sebald) of the protein shows distinct stretches of hydrophilic and hydrophobic residues within the polypeptide chain. The inhibitor DCCD reacts with a single aspartic acid residue, located within a sequence of about 25 residues which is largely hydrophobic. We have found that in the DCCD-reactive protein derived from a DCCD-resistant strain only one isoleucine residue is replaced by one valine and, in the case of the protein obtained from an uncB mutant, only one aspartic acid residue is replaced by glycine. The locations of amino acid replacements were determined by amino acid sequence analysis. Furthermore, treatment of right-side out and everted vesicles with water-soluble carbodiimides revealed that the F_o-part could be closed for H^+-translocation only if the carbodiimides were applied from the medium site in right-side out vesicles. These experiments lend support to the notion that the N-terminus of the DCCD-reactive protein is facing the medium site of the membrane and that more than one carboxylgroup might be involved in the H^+-translocating mechanism. Further support for this view stems from the modification of the tyrosine residues by tetranitromethane.

216 Na^+-K^+ CO-TRANSPORT IN DOG KIDNEY EPITHELIAL CELLS (MDCK), Michael J. Rindler, James A. McRoberts and Milton H. Saier, Jr., Univ. of Calif., San Diego, La Jolla, CA 00008.
Confluent monolayer cultures of the differentiated kidney epithelial cell line, MDCK, have beer used to study ion transport mechanisms involved in trans-epithelial transport. We have investigated the previously reported K^+-stimulation of $^{22}Na^+$ uptake into confluent monolayers of Na^+ depleted cells (M.J. Rindler, et al., J. Biol. Chem. 254:11431-11439, 1979). This component of Na^+ uptake is insensitive to ouabain and amiloride, but is strongly inhibited by furosemide. Ouabain-insensitive $^{86}Rb^+$ uptake is also inhibitable by furosemide and stimulated by extracellular Na^+. The synergistic effect of Na^+ on $^{86}Rb^+$ uptake and K^+ on $^{22}Na^+$ uptake is reflected by an increase in the apparent V_{max} and a decrease in the apparent K_m as the concentration of the other cation is increased. The extrapolated K_m for either $^{86}Rb^+$ or $^{22}Na^+$ uptake in the absence of the other cation is 30 mM while the K_m in the presence of saturating concentrations of the other cation is 10 mM. The absolute V_{max} values for $^{22}Na^+$ and $^{86}Rb^+$ uptake suggest a co-transport system with a stoichiometry of $2Na^+:3K^+$, however, because of the experimental design, the actual ratio may be closer to 1:1. The co-transport system exhibited an absolute requirement for extracellular Cl^-. The effect of Cl^- was nonsaturating and could be replaced by Br^- but not F^-, I^-, acetate, thiocyanate, nitrate, sulfate or gluconate. Preincubation with agents which reduced intracellular ATP levels to 2% of normal abolished both K^+-stimulated $^{22}Na^+$ uptake and Na^+-stimulated $^{86}Rb^+$ uptake. The physiological significance of the transport system remains to be determined. [This work was supported by NIH grant #1 R01 AM21994-01A1.]

217 5'-FLUOROSULFONYLBENZOYLADENOSINE AS AN ATP SITE AFFINITY PROBE FOR Na^+,K^+-ATP-ASE. Charles G. Winter, University of Arkansas College of Medicine, Little Rock, AR 72201.
5'-Fluorosulfonylbenzoyladenosine (FSBA) reacts slowly with purified canine kidney Na^+,K^+-ATPase to inactivate it. At pH 8, in 0.1 M Na^+, about 50% of the enzyme activity is lost in 2 hours. ATP protects against this inactivation, with a half-maximal effect occurring at 5 µM ATP. Reaction with FSBA is irreversible, since ATP addition after partial inactivation by FSBA protects only against further inhibition. 100 µM ADP also protects the enzyme completely, but 100 µM AMP is ineffective, consistent with the known nucleotide specificity of the ATPase. Treatment with FSBA in 0.1 M K^+ causes a somewhat more rapid inactivation. This loss of activity is also prevented by ATP, but half-maximal protection requires 200 µM ATP. All of these results suggest that FSBA reacts at the high affinity ATP site of the enzyme, and are consistent with the previously described effects of K^+ and Na^+ on ATP affinity for this site. Attempts to measure an apparent K_i for FSBA interaction with the catalytic site indicate a value above 1 mM, the practical solubility limit of the compound. Measurements of the rate of enzyme inhibition at several FSBA concentrations confirm this conclusion. Control experiments rule out fluoride ion released as the cause of enzyme inhibition. Covalently-stabilized occupation of the catalytic site by sulfonylbenzoyladenosine is suggested by experiments showing a noncompetitive reduction of (^{3}H)ADP binding in proportion to loss of enzyme activity. Experiments are presently under way to determine the stoichiometry of reaction of FSBA with the Na^+,K^+-ATPase.

218 PROTON-CONDUCTING F_0 SECTOR OF THE H^+-ATPase OF E. COLI, Robert H. Fillingame, David L. Foster, Robert S. Negrin and Mary E. Mosher, U. Wisconsin, Madison WI 53706
The F_1F_0 ATPase of E. coli was purified and shown to contain eight polypeptide components (Foster and Fillingame, J. Biol. Chem. 254: 8230 [1979]). Five of these polypeptides are subunits of the F_1-ATPase, and the other three were thought to be subunits of the proton-translocating F_0-sector. The F_1F_0 complex has now been purified from a strain of E. coli carrying a lambda lysogen with the *unc* (ATPase) genes. Induction of the lysogen resulted in a six fold increase in ATPase activity. All eight of the previously identified components of the ATPase complex were overproduced in constant stoichiometric proportion. This result provides strong genetic evidence that all eight components are true subunits of the complex. The F_0 sector of the complex was prepared by treating a purified, particulate F_1F_0 preparation with EDTA to solubilize the F_1-ATPase. The resulting particulate F_0 fraction, which contained the expected three subunits, was incorporated into liposomes by sonication. Proton-efflux from these liposomes was measured with a pH electrode after imposition of a membrane potential with K^+ and valinomycin. The rate of proton-efflux from these liposomes was increased maximally ca. 100-fold on incorporation of the F_0 sector. The rate of H^+-efflux varied directly with the amount of F_0 added during reconstitution. Dicyclohexylcarbodiimide (DCCD) blocked F_0-mediated H^+-efflux with the F_0 from wild-type, but not with the F_0 from a DCCD-resistant mutant. The F_0 sector of several *unc* mutants was shown to have negligible proton-translocating activity using the liposome reconstitution assay.

219 CONFORMATION OF COUPLING FACTORS FROM E. coli, BEEF HEART AND CHLOROPLASTS, Hasko H. Paradies, Biochemie der Pflanzen, Freie Universität Berlin, Königin-Luise-Str. 12-16a, D-1000 Berlin 33, Germany

Crystallization of F_1 (space group $C222_1$) and CF_1 (space groups $C222_1$ and $P4_132$) yielded single crystals in the presence of 5 mM ATP, indicating perfect two-fold molecular symmetry. However, single crystals of CF_1 ($C222_1$) in the absence of ATP revealed a maximum largeness in solution of 150 Å, whereas in the presence of ATP hollow spheres of 100 ± 10 Å diameter were observed for all three coupling factors, obtained from small angle X-ray scattering measurements in solution. The state of the coupling factors is not modulated by ATP, only, but also by salt. Conformational changes were observed at high salt concentrations (0.1 M NaCl or 0.1 M KCl), as estimated by NMR measurements and polarization fluorescence measurements. The molecular correlation values increased by a factor of 1.8 at low salt concentration in the absence of ATP, whereas in the presence of ATP a tightening of the complex was detected. In ECF_1 the change in the physical parameters upon binding of ATP to the α-subunit changed the radius of gyration from 28.1 Å to 26.7 Å and the volume from 12.9×10^4 Å^3 to 9.56×10^4 Å^3. The conformation of the membrane portion of the coupling factor CF_o was investigated by small angle X-ray methods and revealed an uneven cylinder of molecular weight 75,000 with a length of 70 Å, an upper radius of 83.8 Å and a lower radius of 58.5 Å.

220 FUNCTIONS OF NUCLEOTIDE BINDING SITES ON MITOCHONDRIAL F1 ATP-ASE
Diether Recktenwald and Benno Hess, MPI Ern.Physiol., 4600 Dortmund, FRG

Three classes of nucleotide binding sites different with respect to binding-affinity, ligand-exchange-rate, and nucleotide specificity have been found on mitochondrial F1 ATPase from yeast: (I) sites for ATP which do not exchange with the medium, (II) tight exchangeable sites for ATP and ADP, and (III) loose sites for ATP, ITP, GTP, CTP, and other nucleotide-tri- and -diphosphates. Class II and III sites manifest themselves in an apparent negative cooperativity of the steady-state kinetics of ATP hydrolysis; the hydrolysis of ITP, GTP, and CTP by F1 ATPase shows Michaelis Menten type behavior, reflecting binding to type III sites only.
The existence of multiple binding sites is also apparent from the presteady-state kinetics of nucleotidetriphosphate binding and hydrolysis: The fluorescence change upon binding of the ATP-analog lin-benzo-ATP is multiexponential under pseudo-first-order conditions. The hydrolysis of ATP and -ATP is preceded by a lag-phase of 100 msec suggesting a nucleotidetriphosphate triggered inactive/active state transition.
These results are compatible with a dual site mechanism for ATP synthesis.
A reaction scheme for ATP hydrolysis by mitochondrial F1 ATPase will be presented.

221 REGULATION BY CALMODULIN OF THE PLASTICITY OF THE Ca^{2+} PUMP IN HUMAN ERYTHROCYTE VESICLES, Fred Larsen, Sidney Katz and Basil D. Roufogalis, University of British Columbia, Vancouver, B.C., V6T 1W5 (Canada)

Calmodulin is a low molecular weight acidic Ca^{2+}-binding protein which mediates many cellular responses to Ca^{2+}. We report here on the effects of calmodulin and Ca^{2+} on the kinetics of active Ca^{2+} transport across the plasma membrane of the red blood cell. Inside-out vesicles were prepared according to Larsen and Vincenzi [Science 204: 306, 1979]. Ca^{2+} transport showed one affinity for Ca^{2+} (apparent K_d = 2.0 μM) in the presence of saturating concentrations of calmodulin and two affinities for Ca^{2+} (apparent Kd's = 2 μM and 7 μM) in the absence of added calmodulin. To test whether endogenous calmodulin was contributing to these kinetics, the vesicles were incubated at 30°C for 30 min in a solution of 1 mM EDTA plus 20 mM Tris-glycylglycine (pH 6.8) to remove any endogenous calmodulin. Ca^{2+} uptake in EDTA-treated vesicles incubated in the absence of added calmodulin showed two distinct affinities (apparent Kd's = 2 μM and 50 μM Ca^{2+}). Calmodulin binding protein (5 μg/ml) almost completely inhibited Ca^{2+} transport in untreated vesicles, depending on the Ca^{2+} concentration. These data suggest that EDTA treatment unmasks a low affinity nature of the Ca^{2+} pump by removing at least some of the endogenous calmodulin. That the integrity of the pump was not affected by EDTA is suggested by the ability of added calmodulin to restore full transport activity and high affinity for Ca^{2+}. Therefore, calmodulin activates Ca^{2+} transport across plasma membranes by increasing both the V_{max} and the affinity of the pump for Ca^{2+}.

Supported by the Canadian Heart and Cytic Fibrosis Foundation and the MRC of Canada.

222 IRREVERSIBLE INHIBITION OF MITOCHONDRIAL RESPIRATION BY SALICYLANILIDE UNCOUPLERS,
Gary A. Rogers and Marta S. Hoffman, University of Texas at Dallas, Richardson, Tx 75080

A newly synthesized compound, 3-t-Butyl-3',5-dinitro-4'-fluorosalicylanilide (S-15), has demonstrated novel respiratory inhibition in rat liver mitochondria. At concentrations merely 50% greater than those required for maximal stimulation of respiration, S-15 completely inhibits the stimulated respiration. It is well-known that most uncouplers of oxidative phosphorylation also inhibit respiration, but none exhibit both stimulation and inhibition over such a narrow concentration range. Our studies indicate that respiratory inhibition takes place at the level of the flavin-linked dehydrogenases and occurs in two rather slow phases. The first phase is reversible by the addition of bovine serum albumin, while the second is not. From studies using 3-methyllumiflavin as a model, the irreversibility might be the result of formation of a covalent adduct between a flavin moiety in succinate or NADH dehydrogenase and a reduction product of S-15. Inhibition of respiration due to the oxidation of either malate/glutamate or succinate has been demonstrated. An artificial electron donor such as ascorbate/TMPD will release the inhibition. Furthermore, whichever substrate is not present when inhibition is induced will serve to restimulate respiration while addition of more of the same substrate will not.

223 ATPase ACTIVITY ON SECRETORY AMELOBLAST MEMBRANES AS SHOWN BY E. M. HISTOCHEMISTRY,
Alfred Weinstock and Toshihiko Inage, UCLA School of Dentistry, Dental Research Institute, and Department of Anatomy, Los Angeles, California 90024

The ameloblast secretes the enamel matrix and is involved in calcium deposition during mineralization. Since adenosine triphosphatase (ATPase) is known to be involved in cation transport, the localization of this enzyme in the ameloblast was investigated in rat teeth fixed by perfusion using 2.5% glutaraldehyde. After demineralization, incisors were chopped into 50 μ sections, preincubated in $MG(CL)_2$ or $CA(CL)_2$ and processed for the demonstration of ATPase (modified Wachstein and Meisel, 1957) using ATP as a substrate at PH 7.4 or 8.7. Secretory ameloblasts showed lead deposits on the outer leaflet of the plasma membrane around the entire cell. The basal membrane reaction was more intense than the lateral when $MG(CL)_2$ preincubation was used; the apical (Tomes') process reaction was most intense, especially during outer enamel secretion. In the apical process invaginating membranes on the distal, lateral, and proximal aspects showed lead deposits, as did coated vesicles located peripherally as well as near the secretory granules. The granules were unreactive, but multivesicular bodies showed reaction product. Most noticeable was the intense reaction over new enamel matrix called growth regions (Weinstock and Leblond, 1971) located near the proximal and distal portions of the apical processes. These results were not obtained when AMP or ADP were used as substrates. Heating tissue to 100°C, or adding 10 mM F^- to the substrate abolished the enzyme reaction. The observations suggest that a matrix ATPase may be involved in matrix mineralization. Membrane ATPase may regulate calcium transport for both mineralization and secretion. (Supported by USPHS Grant DE-3386)

Intracellular Protein Transport and Sorting

224 CHOLINERGIC SYNAPTIC VESICLES.
Regis B. Kelly, Steven S. Carlson, James Deutsch, Randall J. von Wedel & John Wagner. Department of Biochemistry & Biophysics, University of California, San Francisco, Ca 94143

The synaptic vesicle is a membrane enclosed sphere of 40 to 80 nm diameter which contains neurotransmitter at very high concentration. The membrane of the vesicle is made in the cell body of the neuron, transported often many centimeters down the axon of the neuron and inserted into the presynaptic plasma membrane during the exocytotic release of transmitter. To study this process we have isolated synaptic vesicles from the nerve terminals of the Narcine brasiliensis electric organ. After a greater than 250 fold purification, the vesicles are free of measurable contaminants. The pure vesicles have a very simple protein and lipid composition. To follow the membranes during biosynthesis, transport and fusion a marker is needed. We have succeeded in preparing antibodies to synaptic vesicle specific antigenic determinants. These antibodies bind not only to the electric organ but also to nerve terminals in the neuromuscular junctions of the rat and frog. By immunofluorescent techniques we have shown that stimulating transmitter release causes transfer of vesicle antigens to the presynaptic plasma membrane.

225 A LARGE PRECURSOR OF CYTOPLASMICALLY SYNTHESIZED PROTEINS OF COMPLEX III OF YEAST MITOCHONDRIA, Diana S. Beattie, Cynthia Battie and Robert A. Weiss, Mt. Sinai School of Medicine, New York, N.Y. 10029
Complex III purified from yeast mitochondria catalyzed antimycin-sensitive $coQH_2$-cytochrome _c_ reductase and separated after SDS-gel electrophoresis into 10 bands with molecular weights ranging from 6,000 to 50,000. Complex III was isolated by immunoprecipitation from mitochondria obtained from yeast cells labeled with (^{35}S)methionine using antiserum raised against the purified complex. Analysis of the immunoprecipitates on SDS gels revealed that cytochrome _b_ (30,000 daltons) and a 16,000 dalton protein were labeled in the presence of cycloheximide indicating that they are products of mitochondrial protein synthesis. Immunoprecipitates from mitochondria of cells labeled in the presence of chloramphenicol contained a new labeled band of 100,000 daltons coupled with significant decreases in the labeling of proteins of 50,000, 40,000, 30,000 and 16,000 daltons. When Complex III was isolated by immunoprecipitation from intact spheroplasts after a 5 min pulse, the 100,000 dalton protein was labeled whether chloramphenicol was present or not; however, after a 2 hr chase, decreased labeling of the 100,000 dalton protein was observed concomitant with increases in the labeling of the 50,000 and 40,000 dalton proteins. These results suggest that a protein with a molecular weight of 100,000 may be a precursor of other proteins of Complex III, probably the core proteins with molecular weights of 50,000 and 40,000. NIH-HD-04007

226 IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF VESICULAR STOMATITIS VIRUS GLYCOPROTEIN IN INFECTED CELLS, John E. Bergmann and S.J.Singer, Univ. of California at San Diego, La Jolla, Ca. 92093
Cell surface proteins are important in cell-cell recognition and communication and thus in such processes as embryonic development and growth control. Unfortunately, many details of the ultrastructural and biochemical pathway these proteins follow during maturation and transport to the cell surface have yet to be worked out. For example, biochemical experiments indicate that plasma membrane proteins pass through a smooth membrane-bound structure presumed to be the Golgi apparatus on their way to the plasma membrane. However, a definitive Golgi localization has never been directly demonstrated by electron microscopy and there has been no information whether proteins destined for the plasma membrane are found throughout the Golgi apparatus or are somehow sequestered in a specialized region of that organelle. We are presently utilizing immunocytochemical staining techniques in conjunction with ultra-cryotomy and electron microscopy to localize the VSV glycoprotein (G) within infected cells. So far, using a mutant of VSV blocked in a late step in G protein maturation, we have localized G within the Golgi apparatus where it appears to be uniformly distributed. After further characterizing the regions of accumulation of G protein in cells infected with wild type VSV and other mutants of VSV unable to complete transport of the G or M proteins at 39.5°C, we plan to use temperature shift experiments to define their pathways of maturation.

227 LYMPHOCYTE DEVELOPMENT AND PROCESSING OF MU HEAVY CHAINS, Charles Sidman, Basel Institute for Immunology, Basel, Switzerland.

Immunoglobulin-M (IgM) serves distinct and different roles in two stages of B lymphocyte development - a cell surface receptor in resting cells, and a secreted product in activated cells. The mu polypeptide chains from these two IgM populations have different apparent molecular weights in SDS-polyacrylamide gel electrophoresis, which raises the question of whether they are products of the same or separate genes. Biosynthetic studies on purified populations of lymphocytes at each of these developmental stages have shown one form of intracellular mu in common, as well as a second form unique to IgM-secreting cells. This second form unique to secreting cells appears to derive from the other (common) internal form, and to be the immediate precursor for secreted mu chains. Originally, a single, common non-glycosylated form is the ultimate mu precursor in both cell types. These results suggest that surface and secretory mu chains are not products of separate genes, but are forms of the same gene product which are processed differently for various final functions.

228 SECRETION AND PROCESSING OF -LACTAMASE: A GENETIC APPROACH, Douglas Koshland and David Botstein, Massachusetts Institute of Technology, Cambridge, MA 02139

The TEM-β-lactamase made by many kinds of penicillin-resistant gram-negative bacteria is normally found between the inner and outer cell membranes. The β-lactamase gene is usually found on plasmids, often within a transposon such as Tn1. Using a P22::Tn1insertion phage derivative as well as the fully sequenced plasmid pBR322, we have begun fine-structure genetic analysis of the β-lactamase.

Synthesis and transport of the intact wild-type enzyme and several shorter N-terminal polypeptide fragments specified by nonsense and frameshift mutants was studied taking advantage of specific labeling of P22::Tn1-specified proteins after infection of UV-irradiated hosts. From such studies we find 1) The removal of the N-terminal 23/amino acid residues (the "signal sequence") from the precursor to form mature enzyme occurs after synthesis of the precursor is completed; 2) all nonsense-mutant fragments examined also show maturation of a precursor by removal of about 23 amino acids; 3) the mutant fragments do not appear to be fully tranported to the periplasm (as judged by the Heppel osmotic shock technique) although wild-type enzyme is. These results suggest that the carboxyl terminus of the β-lactamase is somehow involved in transport to the periplasm and that removal of the 'signal peptide' may occur prior to transport of the protein across a membrane.

229 PURIFICATION OF SECRETORY GRANULES FROM A PITUITARY CELL LINE, Barry Gumbiner & Regis Kelly, University of California, San Francisco, Ca 94143

Secretory granules have been purified >70-fold from a mouse pituitary tumor derived clonal cell line, At-T20s. These cells synthesize adrenocorticotropic hormone (ACTH) and β-endophin as a common polypeptide precursor, and secrete the final hormone products after extensive glycosylation and proteolytic processing. Radioimmunoassayable ACTH sediments as a particulate fraction of ∿1000S and bands on Ficoll density gradients at 1.10 gm/ml. These values are appropriate for the 200 nm diameter secretory granules that have been observed by electron microscopy. Purification is achieved by differential centrifugation and subsequent equilibrium centrifugation on a D_2O density gradient. The predominant proteins of the secretory granules comigrate with ACTH, β-lipotropin, and β-endorphin on SDS-PAGE. A major band also migrates at the molecular weight range of the precursor protein. We conclude that the fraction is enriched in secretory granules because of the high specific activity of ACTH/protein and the content of processed secretory proteins. It is not yet known whether processing of the precursor occurs within secretory granules or prior to packaging into the secretory granule.

230 ANTIGENS SHARED BY SECRETORY CELLS. Steven S. Carlson, Joan E. Hooper & Regis B. Kelly. Department of Biochemistry & Biophysics, University of California, San Francisco, Ca 94143

We have raised antibodies in rabbits to cholinergic synaptic vesicles isolated from the electric organ of _Narcine brasiliensis_. After suitable adsorption these antibodies recognize antigenic determinants unique to synaptic vesicles in the electric organ. These determinants are found on both the internal and external faces of the vesicle membrane. The antisera can be used to detect and quantify vesicle antigens even when synaptic vesicles make up <0.5% of the total material present. Although the antibodies were raised against organelles from elasmobranchs they cross-react with antigens which are found in certain secretory tissues of mammals. Cross-reacting antigens are found by immunofluorescence in secretory cells such as the the chromaffin cell and the cells of the intermediate lobe of the pituitary. They are not found in all secretory cells, however, being absent from cells in the anterior lobe of the pituitary and from classes of nerve terminals found in the hippocampus, cerebellum and other areas. The nature of these elements shared by the cross-reacting tissues is not yet known.

231 BIOSYNTHESIS OF MAMMALIAN CYTOCHROME C OXIDASE: SITE OF SUBUNIT SYNTHESIS,
James F. Hare, University of Oregon Health Sciences Center, Portland, Ore., 97201, and Edwin Ching and Giuseppe Attardi, California Institute of Technology, Pasadena, Cal. 91125.

Cytochrome c oxidase, the terminal oxidase of the respiratory chain in eukaryotic cells, has been purified from human placental mitochondria. Seven polypeptides have been reproducibly resolved by electrophoresis of the enzyme through SDS-urea polyacrylamide gels. When HeLa cells, grown in suspension culture, were pulse labelled with ^{35}S-methionine in the presence of cycloheximide to inhibit cytoplasmic protein synthesis, and chased with an excess of unlabelled methionine in the absence of the drug, the mitochondrially synthesized polypeptides were resolved into at least 15 components by SDS-urea polyacrylamide gel electrophoresis. After mixing labelled HeLa mitochondria with human placental mitochondria and isolating the cytochrome c oxidase, three of the labelled components were found to co-purify with the three largest subunits of the complex. We conclude that human cytochrome c oxidase contains seven subunits, the three largest of which are synthesized on mitochondrial ribosomes, while the other four are synthesized in the cytoplasm.

232 INTRACELLULAR TRANSPORT OF ACETYLCHOLINESTERASE AND ACETYLCHOLINE RECEPTOR, Henry M. Smilowitz, University of Connecticut Health Center, Farmington, CT 06032
Most of the acetylcholinesterase (ACHE) that is synthesized by cultured chick embryo pectoral muscle cells is secreted into the culture medium by a process that is rapidly and reversibly inhibited by the monovalent ionophores (Smilowitz, H., Molecular Pharmacol., July 1979). The secretion of other continuously released glycoproteins such as collagen and fibronectin are similarly inhibited by the monovalent ionophores (Uchida, N., H. Smilowitz & M. Tanzer, P.N.A.S. 76: 1868, 1979). In contrast, we have shown that the rate of appearance of new acetylcholine receptors (ACHR) at α-bungarotoxin (α-BGT) accessible sites, the turnover of ACHR, the total number of ACHR (total α-BGT accessible sites) and the size of the intracellular pool are nearly the same in both control and monovalent ionophore treated cells. Since the monovalent ionophores reportedly block the flow of membranes out of the Golgi (Tartakoff, A. & P. Vassalli, J. Cell Biol. 79: 694, 1978) our data suggests that there are at least two types of membrane vesicles or carriers derived from the Golgi - one whose formation is inhibited by the monovalent ionophores (normally transporting secretory proteins such as ACHE) and one whose formation is unaffected by the ionophores (normally transporting integral membrane proteins such as the ACHR) (Smilowitz, H., Cell, in press). Recent data suggests that ACHR are transported to the plasma membrane by coated vesicles while the ACHE is transported from the Golgi by a non-coated vesicle mechanism. We propose that the ACHE is transported to cytoplasmic channels or the t-system of muscle. Once in the t-system, the enzyme is in communication with the external bathing medium. (Supported by NS13860.)

233 COATED VESICLES, CALMODULIN AND RECEPTOR MEDIATED TRANSPORT, Carol D. Linden[1], James G. Chafouleas, John R. Dedman, Anthony R. Means and Thomas F. Roth[1], [1]Dept. of Biological Sciences, University of Maryland Baltimore County, (UMBC) Catonsville, MD. 21228 and [2]Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030.
Coated vesicles (CV's) mediate the intracellular transport of a variety of receptor bound macromolecules (e.g. IgG, LDL, EGF, $_2$-macroglobulin). The binding of these molecules to their receptors is Ca^{++}-dependent and many of these receptors have been identified in coated pits on the plasma membrane. Calmodulin, a ubiquitous calcium binding protein, has been shown to regulate several Ca^{++}-dependent enymatic activities and cytoskeletal structures. We therefore examined the interactions of calmodulin with CV's purified from brain tissue. Coated vesicles purified in the presence of Ca^{++} contain almost 10 times more calmodulin than those purified using Ca^{++}-free buffers. Moreover, Ca^{++}-purified CV's contain additional polypeptides as detected by SDS-polyacrylamide gel electrophoresis. Radio iodinated calmodulin binding to CV's purified in the absence of Ca^{++} is specific and saturable and has an apparent Kd of 1×10^{-8}M. The binding is CA^{++} dependent; manganese will substitute for Ca^{++} but magnesium will not. Unlabelled calmodulin and troponin-C compete for ^{125}I-calmodulin binding with apparent Ki values of 3×10^{-8}M and 1×10^{-6}M respectively. The results of chromatography of solubilized CV's on a calmodulin-sepharose resin show that calmodulin binds to clathrin. A model for the role of calmodulin in coated vesicle uptake will be presented.

234 THE ORGANIZATION OF PROTEINS WITHIN THE SYNAPTIC VESICLE MEMBRANE, John A. Wagner and Regis B. Kelly, University of California, San Francisco, San Francisco, CA 94143. Synaptic vesicles have been purified to homogeneity from the electric ray Narcine brasiliensis (Carlson, S.S., Wagner, J.A. and Kelly, R.B., Biochemistry 17, 1188-1199, 1978). Six of the eight major prolypeptides found in these preparations copurify with synaptic vesicles during chromatography on CPG-10-3000 and are therefore thought to be vesicle specific. One of the proteins that does not copurify with the vesicles comigrates with actin on sodium dodecyl sulfate gels. Three complementary techniques were used to determine which of the eight proteins were exposed on the external (cytoplasmic) surface of the vesicle membrane: susceptibility to proteases, labelling by the membrane impermeable reagent diazotized [^{125}I]iodosulfanilic acid, and lactoperoxidase catalyzed iodination. The three techniques gave an internally consistant model of vesicle structure. Six of eight proteins are exposed on the surface of the intact vesicle while the other two are available only after the vesicles are lysed by detergents or by freezing and thawing. The actin-like protein is internal. The external proteins are candidates for interaction with the plasma membrane during the exocytotic process.

235 A MODEL FOR STIMULATION OF MEMBRANE MORPHOGENESIS, Gregory J. Brewer, University of Southern California School of Medicine, Los Angeles, CA 90033. Based on biochemical, genetic and morphological information on the formation of the membrane of bacteriophage PM2, a model for stimulation and control of membrane morphogenesis has been formulated. Discrete steps of the process include the following. (A)A toroidal amphipathic protein approaches the bilayer membrane from the cytoplasmic side of a membrane. (B) Initiation of membrane morphogenesis begins with the interaction of the hydrophobic face of the protein with the acyl chains of the lipids. (C) The distortion in the bilayer and lateral pressure in the membrane contribute to propagation of morphogenesis. (D) Further propagation is stimulated by the insertion of proteins into the newly forming membrane. (E) Limitation of size is achieved by the defined radius imposed by each inserted protein. (F) The new membrane pinches off from the host membrane. The physical-chemical justification for these events will be discussed.

236 DIPHTHERIA TOXIN FRAGMENT A DISULFIDE-LINKED TO ANTI-CON A IS TOXIC FOR 3T3 CELLS COATED WITH CON A, G. Gilliland and R.J. Collier, UCLA, 90024

A disulfide-linked conjugate between Fragment A from diphtheria and anti-Con A was toxic for 3T3 cells coated with Con A. The conjugate was made by derivatizing anti-Con A with cystamine in the presence of a water-soluble carbodiimide reagent and then reacting the modified anti-Con A with reduced Fragment A under conditions promoting disulfide interchange. The Fragment A-SS-[anti-Con A] conjugate was purified on Sephacryl S-200 and contained about 1.8 mol Fragment A per mole antibody. In control experiments, it was shown that the conjugate was not toxic for cells which had not been treated with Con A or for Con A treated cells which had been washed with buffer containing α-methyl-D-mannoside. Conjugates containing Fragment A disulfide-linked to anti-WGA or to nonimmune rabbit IgG were also non-toxic for cells pretreated with Con A. Fragment A-SS-[anti-Con A] was inactive on a toxin-resistant strain of CHO (Chinese hamster ovary) cells containing altered Elongation Factor-2, the target protein of Fragment A.

Cellular Receptor Regulation

237 BIOCHEMICAL CHARACTERIZATION OF THE β-ADRENERGIC RECEPTOR FROM TURKEY ERYTHROCYTE PLASMA MEMBRANES - A.D. Strosberg+, O. Durieu-Trautmann+, C. Delavier-Klutchko+, G. Vauquelin°, S. Bottari° and C. André°. + Groupe d'Immunologie Moléculaire - Institut de Recherche en Biologie Moléculaire - Tour 43 - 2, Place Jussieu - 75221 Paris Cédex 05 (France) ; ° Biochemical Pathology, Institute of Molecular Biology, Free University - V.U.B., 65 Paardenstraat - B-1640 Saint-Genesius-Rode (Belgium).

The β-adrenergic receptor from turkey erythrocyte plasma membranes was extensively characterized in our laboratory by the use of general chemical reagents such as dithiothreitol (DTT), N-ethylmaleimide. The binding properties of the membrane bound, the solubilized and the affinity purified receptor were compared in the presence and absence of these reagents and of guanyl nucleotides. Binding of either agonist or antagonists protects the receptor against inactivation by DTT. Alkylation by NEM is only observed after binding of agonists and is hampered by addition of GTP. The receptor was purified 12 000 fold by the use of an affinity adsorbent containing the antagonist alprenolol. SDS polyacrylamide electrophoresis of the iodinated rereceptor revealed a single major components with an apparent molecular weight of 32 000 daltons. Further characterization of the receptor is in progress.

238 COVALENT LABELING OF THE Na^+CHANNEL WITH A PHOTOACTIVABLE DERIVATIVE OF SCORPION TOXIN Daniel A. Beneski and William A. Catterall, Univ. of Washington, Seattle, WA 98195.

Scorpion toxin (Sctx) is a polypeptide neurotoxin which binds to voltage-sensitive Na^+ channels. The voltage dependence of binding is closely correlated with activation of the Na^+ channel. This report describes the use of a photoactivable derivative of ^{125}I-labeled Sctx to covalently label components of its receptor. When neuroblastoma cells with bound azido-nitrobenzoyl[^{125}I]Sctx (ANB-[^{125}I]Sctx) were irradiated, 50% of the specifically bound toxin was irreversibly attached to the cells. In a double label experiment, ANB-[^{131}I]Sctx was covalently attached to neuroblastoma cells. Subsequent [^{125}I]Sctx binding was inhibited to 82% of control values, indicating that at least 18% of the covalently attached toxin blocks its receptor site. Electrophoresis of covalently labeled neuroblastoma cell membranes or rat brain synaptosomes followed by autoradiography revealed a specifically labeled polypeptide of $M_r \sim 250{,}000$. A 32,000 dalton polypeptide was also specifically labeled in synaptosomes. In both systems all specific labeling was blocked by excess unlabeled Sctx and by depolarization. A variant neuroblastoma clone lacking the voltage-sensitive Na^+ channels had no specifically labeled bands. These results identify specific polypeptides that are components of the Na^+ channel.

239 EVIDENCE FOR CATECHOLAMINE-INDUCED INTERNALIZATION OF THE β-ADRENERGIC RECEPTOR. John P. Perkins, T. Kendall Harden, Calvin U. Cotton, Gary L. Waldo, John K. Lutton, University of North Carolina, Chapel Hill, North Carolina 27514

Isoproterenol (ISO) induces desitization of the β_2 adrenergic receptor (βAR)-linked adenylate cyclase (AC) of human astrocytoma cells by first uncoupling the receptor from the enzyme; only subsequently are receptors lost from the cell. Concommitantly with the uncoupling reaction ISO also selectively alters the sedimentation properties of the βAR. Cells were incubated with or without 1 μM ISO for a period of time (5-30 min) that resulted in a 50-60% loss of ISO-stimulated AC activity but no decrease in the number of βAR as measured by the radioligand, ^{125}IHYP. Following incubation, the cells were rapidly washed and exposed to 0.25 mg/ml concanavalin A. Cell lysates were placed on sucrose (30-60%) density gradients and centrifuged at 113,000 g for 60 min. ISO-and NaF-stimulated AC activities migrated as a single peak at 45-50% sucrose in both control and desensitized samples. βAR from control cells were predominately recovered in fractions corresponding to 45-50% sucrose. In contrast, βAR from desensitized cells migrated as two distinct peaks (30-35% sucrose and 45-50% sucrose). Incubation of desensitized cells for 30-45 min in the absence of ISO resulted in gradient profiles for the βAR that were indistinguishable from that of control, and also resulted in recovery of ISO-stimulated AC activity to control levels. The results suggest that alterations in the particulate form of the βAR occur during desensitization and that a catecholamine-induced internalization of receptors may occur prior to detection of a measurable loss of βAR from the cell.

240 PURIFICATION AND CHARACTERIZATION OF THE INSULIN RECEPTOR, Gönül Veliçelebi,
The Biological Laboratories, Harvard University, Cambridge, MA. 02138.
We have approached the problem of purification of the insulin receptor by preparing an insulin affinity column in which insulin is linked to agarose through a chemically cleavable bond, thus enabling the elution of the insulin receptor complex by means of a specific chemical reaction rather than the harsh methods used by other workers (Jacobs et. al.,(1977) Biochem. Biop. Res. Commun. 77, 981-88.). To this end, we have modified insulin by reacting it with methyl-4-mercapto butyrimidate (MMB). MMB is a bifunctional reagent that forms an amidine bond with an amino group of insulin while providing a free sulfhydryl for disulfide or mercaptide bond formation. The conditions of MMB-insulin reaction have been worked out and the resulting modified insulin has been demonstrated to successfully bind to liver membranes with the same specificity as unmodified insulin. Then MMB-insulin is reacted with organomercurial agarose to form agarose-Hg-S-MMB-insulin. Triton X-100 solubilized extract of rat liver membranes is passed through this affinity column to facilitate the binding of the solubilized receptor to immobilized MMB-insulin. The MMB-insulin-receptor complex is subsequently eluted by treatment with a reducing agent and is further characterized.

241 OXYTOCIN ACTION: LACK OF CORRELATION BETWEEN RECEPTOR NUMBER AND TISSUE RESPONSIVENESS, H.J. Goren, R.M. Geonzon, M.D. Hollenberg, K. Lederis, D. Morgan, University of Calgary, Calgary, Canada, T2N 1N4
Brattleboro rats exhibit diabetes insipidus (DI) because of a genetic autosomal recessive defect in the synthesis of vasopressin; oxytocin is synthesized normally, and preliminary work suggests that elevated circulating oxytocin levels may compensate for the absence of vasopressin (Endocrinology 77, 701 (1965)). In order to evaluate the consequence of presumed elevations of oxytocin levels, oxytocin binding and tissue responsiveness have been measured in the uterus and epididymal fat cells of homozygous-DI (HoDI), heterozygous-DI (HeDI) animals and Sprague-Dawley (SD) controls. Surprisingly, whereas membranes from HoDI uteri exhibit an 85% reduction in oxytocin binding, the biological response (contraction) to oxytocin is indistinguishable from HeDI or SD animals. The uterine response to vasopressin, angiotensin and carbachol is also normal in HoDI rats. In contrast, in adipocytes from HoDI animals the biological response to oxytocin (glucose oxidation) is abolished, whereas the binding of oxytocin is normal; insulin stimulated glucose oxidation is, however, normal. These results indicate that receptor binding, while critical to hormone action, is not the sole determining factor. With oxytocin action, post-receptor mechanisms are most important in determining oxytocin responsiveness.

242 CHANGES IN STRIATAL GABA TRANSPORT IN A NEUROLOGICAL MOUSE MUTANT, Anne Messer,
Division of Labs and Research, New York State Dept. of Health, Albany, NY 12201.
A previously undescribed neurological mouse mutant, nm 282, is characterized by a general unsteadiness of gait combined with hyperactivity, and a marked tendency to rear on to the hind legs and move the upper trunk in a circular motion.

To examine whether any of these effects might be due to changes in the striatum, purified synaptosomes were prepared (fraction 3), and assayed for transport of ^{3}H-GABA and ^{3}H-GABA inhibited by β-alanine. The ratio of total GABA uptake/mg protein in control vs. mutant was 0.63 ± 0.11 (n = 6), suggesting a higher density of synaptosomes which transport GABA in nm 282 than in control mice. However, when GABA uptake was done in the presence of β-alanine, there was no significant difference between mutant and control (ratio = 1.17 ± 0.15). Synaptosomes prepared from cerebellum showed no such differences for either case.

This suggests that mutant striata contain either an increase in the proportion of glial cells (actually gliosomes contaminating synaptosomes) or an increase in a population of "β-alanine neurons" (proposed as a distinct entity by Hitzemann & Loh, J. Neurochem. 30, 471-477, 1978). Further GABA analogs are being used to characterize the mutant changes.

243 N-(7-NITROBENZ-2-OXA-1,3-DIAZOLE)-23,24-DINOR-5-CHOLEN-22-AMINE-3β-YL LINOLEATE, A NEW FLUORESCENT CHOLESTERYL ESTER ANALOG, Louis C. Smith, I.F. Craig, D.P. Via, W.W. Mantulin, A.M. Gotto, Jr., and H.J. Pownall, Baylor College of Medicine, Houston, TX 77030
Little information is available about the intracellular fate of the lipid components of low density lipoproteins (LDL). To exploit microscopy systems designed for fluorescein and to monitor simultaneously the intracellular fate of lipoproteins with different fluorescent lipids, (7-nitrobenz-2-oxa-1,2-diazole)-23,24-dinor-5-cholen-22-amine-3β-ol (NBD-C) was synthesized. NBD-C was 50% as effective as cholesterol as a substrate for LCAT, but 60% more effective in inhibiting HMG-CoA reductase. r-(NBD-CE) LDL prepared by the reconstitution procedure of Kreiger et al. was only 72% as efficient as native LDL. Intracellular fluorescence from r-(NBD-CE)LDL had perinuclear distribution similar to that of lysosomes and was similar to that of normal LDL by anti-apo LDL immunofluorescence. LDL receptor-negative cells did not accumulate NBD-CE fluorescence. In addition, r-(NBD-CE)LDL uptake in normal fibroblasts was prevented by a 25-fold excess of LDL. After 18 hr, 97% of the extracted fluorescent material was NBD-C; the remainder NBD-CE. Experiments with ^{125}I-r-(NBD-CE)LDL gave fluorescence and isotopic data that were consistent. These analogs can be used to study lipoprotein-cell interaction, to select cell mutants of lipoprotein metabolism, to monitor phospholipid vesicle-cell interactions and to assess the immediate environment of the sterol in membranes. Video intensification microscopy (VIM) provides time resolution of about 250 msec. Simultaneous information about location, movement and the kinetics of changes in sterol concentration and lipoprotein structure inside the cell is now accessible. (HL-15648, HL-17269, HL-19459, Welch Q-343, AHA, TX Affiliate).

244 CHOLERA TOXIN AND COUPLING OF RECEPTORS TO ADENYLATE CYCLASE IN S49 LYMPHOMA CELLS, Frederick J. Darfler, Michael Kennedy, and Paul A. Insel, UCSD, La Jolla, CA 92093
Wild-type and variant S49 lymphoma cells have been useful for defining mechanisms by which membrane-active hormones, such as beta-catecholamines (β-CA) and prostaglandins (PGE_1) activate adenylate cyclase. Cholera toxin (CT) is another activator of adenylate cyclase in these and other mammalian cells. In order to explore the interaction between these two types of activators of cyclase, we have tested the effect of cholera toxin on cAMP generation of intact S49 cells subsequently treated with PGE_1 and β-CA. Pretreatment of wild-type S49 cells with CT enhances subsequent PGE_1 with β-CA-stimulated cAMP accumulation. Although the magnitude of the response varied, we find up to 6-fold increased maximal cAMP generation and a 2-4 fold increased potency of β-CA after CT treatment. This enhanced response occurs by an action distal to the β-CA receptor because the affinity (agonist and antagonist) and number of receptors (^{3}H-dihydroalprenolol binding) are unaltered by CT. In addition to enhancing response of wild type S49 cells, CT facilitates PGE_1 and β-CA stimulated cAMP accumulation in an S49 variant (UNC) that possesses receptors but that lacks hormone-stimulated cAMP in the absence of CT. These results are compatible with the idea that CT acts by inhibiting a hormone-sensitive GTPase that is distal to receptors. Moreover, the findings indicate that the uncoupled hormone receptors in UNC are at least partially recoupled to adenylate cyclase by CT treatment. This latter action--conferring hormonal responsiveness on cells with otherwise nonactive hormone receptors--may be useful in other hormonal systems and conceivably may be related to the pathogenicity of CT.

245 ISOLATION OF PLEIOTROPIC DIPHTHERIA TOXIN-RESISTANT MUTANTS: ELONGATION FACTOR 2 MUTANTS WITH REDUCED RECEPTOR LEVELS, Rebecca B. Dorland, John L. Middlebrook, and Stephen H. Leppla, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD 21701
Vero monkey kidney cells were mutagenized with ethyl methanesulfonate and 230 diphtheria toxin-resistant mutants were isolated following selection in toxin concentrations ranging from 0.02 to 100 ng/ml (2- to 10,000-fold the parental tissue culture mean lethal $dose_{50}$). Mutants were characterized by a variety of techniques, including (1) cytotoxicity and inhibition of protein synthesis assays, (2) binding, internalization, and degradation assays using radiolabeled diphtheria toxin, (3) growth rate and plating efficiency analyses, and (4) *in vitro* ADP-ribosylation assay. The predominant class of mutants is highly resistant (> 10,000 fold) and has an altered elongation factor 2 that can not be ADP-ribosylated by toxin; the mutation appears to reside in a post-translational modification step. These mutants show a concomitant 75% reduction in toxin-specific cell surface receptors. A second rare mutant type was identified that displayed an altered toxin uptake pattern.

246 APPARENT INDUCTION OF HEPATIC BILE ACID CARRIERS, Francis R. Simon, Univ Colorado School of Medicine, Denver, CO 80262.
Administration of bile acids to man and animals is associated with increased biliary secretory capacity. To examine the mechanism for this increase, cholic acid (CA) (1 mg/g BW) was given to rats for 1 to 4 days. After 4 days the bile acid pool size was increased 8 fold and the maximum capacity to excrete taurocholate (T_m) was 2.3 fold increased compared to controls ($p<0.01$). This increase in Tm is apparently due to induction of putative bile acid carriers for: (1) the composition of phospholipid, cholesterol and their molar ratio was unchanged in liver plasma membrane fractions (LPM), (2) increased bile acid transport is time dependent -- 4 hours after CA administration Tm was unchanged, but it was increased to 135% at 16 hours and to 250% at 2 days, (3) the number of specific ^{14}C-cholic acid binding sites is increased 2 fold ($p<0.005$) at 4 days, and this increase is also time dependent -- 4 hours after CA administration the number of binding sites in LPM fractions is unchanged, while it is increased to 165% at 16 hours, 209% at 24 and 181% at 48 hours. Since 5'-nucleotidase activity is unaltered these studies suggest that bile acids may selectively regulate their own hepatic transport through induction of plasma membrane carriers.

247 INTERACTION OF NUCLEOTIDES WITH DIPHTHERIA TOXIN, S. Lory, R. J. Collier, Dept. of Microbiology, University of California, Los Angeles, CA 90024
We have employed the techniques of flow dialysis and affinity chromatography to show high affinity binding of nucleotides and several other phosphate-containing compounds to diphtheria toxin. These results indicate the presence of a new site on diphtheria toxin, that may be involved in receptor binding. The affinity of the phosphate-containing compounds depends on the degree of phosphorylation and to the lesser extent on the presence of a purine or pyrimidine ring. When chromatographed on a column of ATP-Sepharose, a fraction of the toxin in any given preparation is retained. The fraction of the toxin that is not retained does not bind nucleotides, while the retained fraction binds one mole of nucleotides per mole of toxin. In addition, diphtheria toxin binds NAD and exhibits significant levels of NAD-glycohydrolase activity. However, the kinetic parameters of both NAD binding and hydrolysis are markedly different from those observed for fragment A moiety of the toxin.

248 DIFFERENTIAL INDUCTION OF BETA-ADRENERGIC AND CHOLERA TOXIN RECEPTORS: A MODEL FOR REGULATION OF CELL SURFACE RECEPTOR EXPRESSION AND FUNCTION, R.C. Henneberry, J.F. Tallman, and P.H. Fishman, NINCDS and NIMH, NIH, Bethesda, MD 20205
We have previously shown that short-chain fatty acids, especially butyrate, are effective inducers of both beta-adrenergic and cholera toxin receptors in HeLa cells. Both classes of receptor are fully functional with respect to the ability of the appropriate ligand to activate adenylate cyclase (AC), but the cellular content of AC is not increased. This system now proves to be of value for comparative studies on the regulation of cell surface receptor expression. Whereas both beta-adrenergic receptors and cholera toxin receptors are inducible in HeLa, the dose response curves for the two receptors differ; 0.8 mM butyrate suffices for full induction of beta-adrenergic receptors, but 5 mM is needed to fully induce the toxin receptors. Similarly, the time courses for induction of the two receptors are very different; beta-adrenergic receptors are fully induced within 10 hrs of butyrate addition while full induction of toxin receptors requires 48 hr. Comparison of receptor turnover rates also indicates major differences between the two receptor classes. We have previously described induction of beta-adrenergic receptors in 2 forms, either coupled to or uncoupled from AC, as well as the stimulation of phospholipid methylation in response to occupancy of the receptor by agonists. We now take advantage of this feature of the system to examine the effect of several phospholipase inhibitors on receptor maintenance and function and relate the results to the phenomenon of desensitization.

249 STUDIES ON THE BIOSYNTHESIS OF ACETYLCHOLINE RECEPTORS: EFFECTS OF INHIBITION OF GLYCOSYLATION AND THE MEMBRANE TOPOLOGY OF NEWLY SYNTHESIZED RECEPTORS, John M. Gardner, Carnegie Institution of Washington, Baltimore, Md. 21210
Tunicamycin was used to investigate the possible involvement of lipid-saccharide intermediates in the biosynthesis of acetylcholine receptors by chick embryonic muscle cells in culture. Muscle cultures were labeled with ^{2}H, ^{13}C, ^{15}N-amino acids both in the presence and absence of tunicamycin and the numbers of newly synthesized receptors were determined by application of ^{125}I-α-bungarotoxin to detergent extracts and subsequent velocity sedimentation of the solubilized toxin-receptor complexes. Also, the effects of tunicamycin on the incorporation of ACh receptors into the plasma membrane were tested at levels which did not inhibit protein synthesis significantly, but almost totally abolished the incorporation of ^{14}C glucosamine into macromolecules. The data indicate that tunicamycin completely blocks the incorporation of ACh receptors into the plasma membrane and this effect is substantiated by an 80% reduction in the number of detectable, newly-synthesized ACh receptors in the precursor receptor pool. These results implicate the participation of lipid intermediates in the biosynthesis of ACh receptors. We have also used α-bungarotoxin as an impermeable affinity probe to explore the polarity of organization of newly synthesized receptors present in the intracellular membrane system. The results, which indicate the newly synthesized receptors are oriented predominantly toward the lumen of intracellular membrane vesicles, support current models of membrane biogenesis and increase our understanding of the mechanisms by which the acetylcholine receptor acquires its asymmetry in the plasma membrane of excitable cells.

250 NEURO-ENDOCRINE CELLS IN ORGAN CULTURES OF FETAL RABBIT LUNGS. K. Sonstegard and E. Cutz, NIEHS, Res. Triangle Park, NC, and Hospital for Sick Children, Toronto, Ontario
Neuro-endocrine cells dispersed as single cells or as innervated organoid structures referred to as neuro-epithelial bodies (NEB) have been identified by histochemical and ultrastructural studies of the bronchial mucosa in lungs of human and other mammalian species. These specialized lung cells may be involved in the production of amine and polypeptide hormones and the innervated NEB may act as hypoxia-sensitive intrapulmonary chemoreceptors. Little is known regarding the development of lung neuro-endocrine cells, their secretory products, physiologic function, and the relationship of the two types. Lung neuro-endocrine cells may be a specific target for environmental carcinogens. We have developed an _in vitro_ organ culture system for the isolation of neuro-endocrine cells free from complexing neurohumoral mechanisms but maintained in organotypic form. This presents a new approach to morphologic, metabolic and physiologic studies of lung neuro-endocrine cells. Lung explants prepared from fetal rabbits, 20 days gestation and near term, were maintained up to 22 days. NEB retained their ultrastructural integrity; amine and polypeptide components were demonstrable. Nerve terminal axons within NEB degenerated _in vitro_. Neuro-secretory granules were larger, pleomorphic, electron-dense and distributed throughout the cytoplasm. These changes together with the hypertrophy of golgi zones suggest increased synthesis and storage of secretory products during culture. Reserpine and Ca^{++}-ionophore depleted amine content of cultured NEB. Nerve growth factor contributed to the maintenance of nerve terminal axons and may be necessary for membrane potential.

Neuroreceptor Regulation

251 ENKEPHALIN AND ENKEPHALIN RECEPTORS IN PRIMARY CULTURES FROM FETAL RAT BRAIN, James A. Weyhenmeyer, Robert E. Fellows, and Mohan K. Raizada, University of Illinois College of Medicine, Urbana, Illinois 61801, University of Iowa College of Medicine, Iowa City, Iowa 52242
Studies from several laboratories have demonstrated the presence of opiate-like peptides in mammalian brain. To investigate the role of specific receptors in the CNS action of enkephalin, immunohistochemical and biochemical studies were performed to characterize intrinsic leu-enkephalin and leu-enkephalin receptors in substratum attached brain cells from fetal rat. Using a specific antiserum for immunohistochemical localization of leu-enkephalin, approximately 2% of the neurons demonstrated positive staining in nonnuclear portions of the soma and neuronal processes. Staining was completely eliminated by preabsorption of the primary antiserum with 2 mM leu-enkephalin. Receptor binding studies, using ^{3}H leu-enkephalin, were performed in either isotonic sucrose buffer or isotonic choline buffer in the absence of Na^{+}. Binding of ^{3}H leu-enkephalin is 75% specific, rapid, reversible, and inhibited in the presence of Na^{+}. It is time dependent and follows first-order kinetics. Nonlinear Scatchard analysis suggests the presence of 10,000 binding sites per cell with a high affinity constant of 1.2×10^{10} M^{-1} and a low affinity constant of 0.8×10^{9} M^{-1}. These data demonstrate the presence of leu-enkephalin and its receptor in neurons cultured from fetal rat brain and provide a unique system for studying the role of leu-enkephalin and its receptor in the CNS. (Supported by NIH grant HD 11184-01. J.A.W. was a PMA Foundation Fellow.)

252 PHOTOAFFINITY INACTIVATION OF THE ENKEPHALIN RECEPTOR, Theodore T. Lee, Robert E. Williams and C. Fred Fox, Department of Microbiology, Molecular Biology Institute, University of California, Los Angeles, California 90024

The discovery of endorphins and enkephalins, endogenous polypeptides with opiate properties, has not only greatly stimulated the study of the physiological roles of opiates in the brain and possibly other parts of the body, but also has facilitated the study of the opiate receptor. Attempts have been made in the past to identify the receptor. These include gel filtration of detergent-solubilized receptor-etorphine complex (1,2) and photoaffinity labeling with an arylazide derivative of levorphanol (3). It is now possible to design photolabile derivatives of enkephalin and endorphins, that are probably less hydrophobic (i.e. membrane-soluble) and more specific than the fat-soluble opiates, and which are more likely to label the receptor specifically. With this objective in mind, we have synthesized an arylazide enkephalin derivative, [D-Ala^2,Met^5]enkephalin-Tyr-N-(2-nitro-4-azidophenyl)ethylenediamine. In the dark, it inhibited the binding of [^{3}H]-enkephalin to enkephalin-receptor-rich NG-108 cell membranes with an I_{50}=2.2 x 10^{-8}M, or K_I= 7x10^{-9}M assuming competitive inhibition. Photolysis of membranes in the presence of ETN caused irreversible inactivation of the enkephalin receptor, but inactivation was prevented by the addition of enkephalin, the half effective concentration being 3x10^{-9}M. Thus our enkephalin analogue [D-Ala^2,Met^5]enkephalin-Tyr-N-(2-nitro-4-azidophenyl)ethylenediamine appears to be an excellent photoaffinity label for the enkephalin receptor. References: (1) Simon, E.J., Hiller, J.M. and Edelman, I. (1975) Science, 190,389. (2) Zukin, R.S. and Kream, R.M. (1979) PNAS 76, 1593-1597. (3) Winter, B.A. and Goldstein, A. (1972) Mol. Pharmacol. 8, 601

253 RECONSTITUTION OF PURIFIED ACETYLCHOLINE RECEPTOR, R.L. Huganir, E.S. Levitan and E. Racker, Section of Biochemistry, Cornell University, Ithaca, NY 14853

The acetylcholine receptor from *Torpedo californica* was purified by affinity chromatography with a gel containing carboxymethyl choline (Reynolds and Karlin, *Biochemistry*, *17*, 2035, 1978). The preparation showed 4 major polypeptide bands in SDS electrophoresis (39,000, 41,000, 52,000 and 65,000 daltons) and was reconstitutively fully active. The carbamylcholine-stimulated ion flux was sensitive to α-bungarotoxin and exhibited the phenomenon of desensitization. Specific antibodies against the receptor inhibited reconstitution of active vesicles. Reconstitution was performed with a crude mixture of soybean phospholipids which contain a light-sensitive component that is required for the reconstitution of active vesicles. Experiments with proteolytic enzymes indicate that the preparation can be exposed to considerable cleavage without loss of reconstitutive activity.

254 RELEASE OF ADENOSINE BY NEUROBLASTOMA CELLS AND ITS EFFECT ON CYCLIC AMP METABOLISM, *Richard D. Green, Department of Pharmacology, School of Basic Medical Sciences, University of Illinois, Chicago, Illinois 60612.*

Adenosine, in the presence of nonxanthine phosphodiesterase inhibitors such as Ro 20 1724, produces large elevations in the cyclic AMP content of neuroblastoma cells. Previous work in our laboratory on the N2a clone of neuroblastoma led us to postulate that these cells release adenosine in the growth medium where it acts at the level of the extracellular adenosine receptors to modulate the sensitivity of the cells to the cyclic AMP-elevating effect of adenosine (J. Pharmacol. Exp. Ther. 203:610-620,1977). We now report that erythro-9-(2-hydroxyl-3-nonyl)adenine (EHNA), a potent adenosine deaminase inhibitor, elevates the cyclic AMP content of N2a cells if Ro 20 1724 is present. This effect of EHNA is inhibited by dipyridamole, an inhibitor of nucleoside transport. A HPLC procedure for quantitating adenosine has been developed and used to measure the adenosine content of growth medium. Unused growth medium is devoid of detectable adenosine while media from moderately dense cultures contain >10nM adenosine. The levels of adenosine in growth medium 30 min after the addition of Ro 20 1724 + EHNA are elevated approximately 10 fold. This elevation is prevented by the simultaneous presence of dipyridamole. These results show that adenosine is released by N2a cells into the growth medium via the nucleoside transport system and give support to the hypothesis that adenosine in the growth medium modulates the sensitivity of the cells to the cyclic AMP-elevating effect of adenosine.

255 DIFFERENTIAL EFFECTS OF GUANINE NUCLEOTIDES ON CNS NEUROPEPTIDE RECEPTORS, Terry W. Moody, Duncan B. Taylor and Candace B. Pert, NIMH, Bethesda, MD 20205
Bombesin and Vasoactive intestinal polypeptide (VIP) represent two classes of neuropeptides active in the central nervous system (CNS) and gastrointestinal (GI) tract. The action of each peptide after interaction with its receptor may be mediated by a second messenger. In acinar cells derived from the guinea pig pancreas bombesin increases the intracellular levels of c-GMP (Jensen et al., 1978, PNAS 75, 6139) whereas VIP increases c-AMP levels (Christophe et al., 1976, JBC 251, 4629). Similarly, numerous investigators have determined that VIP increases c-AMP levels in brain slices and adenylate cyclase activity in brain homogenates. Therefore VIP receptors in the CNS may be coupled to adenylate cyclase. Other investigators have determined that guanine nucleotides affect receptor-ligand interactions of CNS neurotransmitter receptors coupled to adenylate cyclase. Therefore the effects of guanine nucleotides of CNS neuropeptide receptor binding was investigated. Micromolar concentrations on GTP inhibited binding of radiolabelled VIP but not tyrosine-4-bombesin to crude rat brain homogenates. VIP binding was inhibited in a dose dependent manner but only 60% of the total specific binding was inhibited. GMP-PNP was a more potent inhibitor than was GTP whereas GMP, ATP, ITP and UTP were less effective. These data indicate that guanine nucleotides may interact with a membrane bound component and affect the conformation of the VIP receptor such that the receptor binds VIP with lower affinity. Currently, we are investigating the regional distribution of VIP receptors to determine if there are different receptor subtypes with different linkages to cyclase.

256 USE OF [^{3}H]SPIROPERIDOL IN BRAIN RECEPTOR ASSAYS, Philip G. Strange, Raymond M. Withy and R. John Mayer, Nottingham University Medical School, Nottingham NG7 2UH, England
[^{3}H]Spiroperidol has been widely used to study dopamine receptors in brain using ligand-binding assays. It has recently been realised that this ligand will also bind to serotonin receptors. We have investigated the binding of [^{3}H]spiroperidol to bovine caudate nucleus (a major component of the striatum which is usually assumed to contain mainly the dopaminergic class of [^{3}H]spiroperidol binding site) in order to quantitate the various binding interactions using displacement studies with ligands selective for dopamine or serotonin receptors. There seem to be roughly equal numbers of dopaminergic and serotoninergic binding sites for [^{3}H]spiroperidol in the caudate nucleus and this may complicate the interpretation of binding experiments using this ligand. However, binding of [^{3}H]spiroperidol to dopaminergic sites may be defined as the binding displaceable by 0.1 mM dopamine and to serotoninergic sites as the binding displaceable by 0.3 μM mianserin. We are currently investigating the properties of these sites after solubilization.

257 GUANINE NUCLEOTIDE AND SODIUM ION REGULATION OF AGONIST MUSCARINIC CHOLINERGIC RECEPTOR BINDING, William R. Roeske, Frederick J. Ehlert, Yvon Dumont, Lois B. Rosenberger, David J. Triggle, and Henry I. Yamamura, University of Arizona, Tucson, Az 85724.
We have shown that the guanine nucleotides shift the agonist oxotremorine sesquifumarate to lower affinity in competition curves with the muscarinic antagonist [^{3}H]quinuclidinyl benzilate. In the heart, ileum, and certain brain regions this shift of affinity of about one log unit is observed for superhigh and high affinity sites and is synergistic with a similar shift produced by Na^+. We now report the use of the muscarinic agonist ligand [^{3}H] cismethyldioxolane (CD) in brain and ileum. [^{3}H]-CD bound to homogenates in a complex fashion was analyzed for two binding sites in the nanomolar range. At a concentration of 10 nM [^{3}H]-CD, the binding was stereospecifically displaced by the muscarinic antagonist dexetimide (IC_{50}=0.1nM) and its stereoisomer levetimide (IC_{50}=1 μM) and by the muscarinic agonist 1-acetyl-β-methylcholine (IC_{50}=0.11 μM) and its stereoisomer (IC_{50}=57 μM). In the absence of Gpp(NH)p, the binding was 106±6 fmol/mg protein in the forebrain and 44.7±3.2 fmol/mg protein in the ileum, while in the presence of 100 μM Gpp(NH)p, the binding was reduced to 10% of the control value ($p<0.001$). 140 mM Na^+ also reduced agonist binding to <10% of control value ($p<0.001$) in both brain and ileum. These data directly indicate that the muscarinic agonist state is regulated by guanine nucleotides and sodium ion.

General Topics Related to Membrane Transport and Neuroreceptors

258 EVIDENCE FOR A CA-CHANNEL MUTATION IN THE K^+-RESISTANT MEMBRANE-EXCITATION MUTANTS OF PARAMECIUM, Helen G. Hansma, Department of Biological Sciences, University of California, Santa Barbara, CA 93106

The K^+-resistant mutants of Paramecium tetraurelia were isolated for their ability to survive high concentrations of K^+ that kill wild type (Shusterman et al. (1978) Proc. Natl. Acad. Sci. U.S.A. 75: 5645). These mutants have a normal turnover of the K-analog ^{86}Rb and do not appear to be defective in their K-regulation. Instead, the following evidence suggests that these mutants have a defective Ca channel that carries a lower-than-normal current: (1) Two K-resistant mutants survive longer than wild type in Ba^{++}, which enters Paramecium through the Ca channel during an action potential. (2) The most resistant mutant swims backward longer in Ba than wild type and has a much slower uptake of ^{133}Ba per second backward swimming. (3) Only the influx of Ba^{++} is altered in this mutant; the efflux of Ba^{++} is normal. All of the phenotypic differences of these mutants, including their lack of "adaptation" to high K^+, can be explained by the postulated Ca-channel defect.

259 DIFFERENCES IN OUABAIN-BINDING BETWEEN THE TWO LARGE SUBUNITS OF BRINE SHRIMP NA^+,K^+-ADENOSINE TRIPHOSPHATASE, Lynn Churchill, Clifford C. Hall, Gary L. Peterson, Lowell E. Hokin, and Arnold E. Ruoho, Dept. Pharmacology, Univ. of WI Med. Sch., Madison, WI 53706

In brine shrimp Na,K-ATPase, there are two large subunits, which function as catalytic sites, since they are both specifically phosphorylated from ATP in the presence of Na and Mg and dephosphorylated in the presence of K (Peterson et al (1978) J. Biol. Chem. 253: 4762). Both subunits are cross-linked by copper phenanthroline, but other proteins are not affected (Peterson, unpublished obs.). Ouabain-binding in the two large subunits of brine shrimp Na,K-ATPase has been studied using photoactivatable derivatives of digitoxin and digitoxigenin. Some of these derivatives have been demonstrated to specifically label the ouabain-binding site in the large subunit of eel Na,K-ATPase (Hall and Ruoho (1978) Fed. Proc. 37: 1511a). Photolysis of brine shrimp Na,K-ATPase with ^3H-4'-ethyl-diazomalonyl digitoxigenin monodigitoxiside in the Mg, Pi complex resulted in specific labeling of the larger of the two large subunits. Less than 7% of the specific radioactivity observed for the large subunit was present in the lower large subunit. The differences in ouabain-binding between large subunits of brine shrimp Na,K-ATPase have also been observed in mammalian brain Na,K-ATPase (Sweadner (1979) J. Biol. Chem. 254: 6060). In brine shrimp Na,K-ATPase the smaller of the two large subunits is more prominent during initial development of Na,K-ATPase and then nearly disappears in the older nauplii. This subunit may be a precursor or an isozyme of Na,K-ATPase. The differences in ouabain-binding between these subunits suggests that ouabain-binding is not fully developed in the precursor or the isozyme lacks a ouabain-binding site.

260 GENDER DIFFERENCES IN BENDING OF GONADAL STEROIDS BY THE In Vitro HIPPOCAMPAL SLICE PREPARATION, Richard M. Vardaris and Timothy J. Teyler, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272

Studies conducted with the in vitro hippocampal slice system have shown that there are gender differences in the electrophysiology of CA1 pyramdal cells when gonadal steroids are added to the superfusate. In male hippocampus 17-beta-estradiol (E_2) increased throughput of the Schaffer collateral/CA1 pyramid synapse. This effect was seen in slices from non-proestrous females only when treated with testosterone (T). Proestrous slices exhibited a pattern of response which was similar to that of male slices. Specificity of binding by steroid receptors was assessed by administering 100 pM concentrations of the steroids in the sequences E_2-T-E_2 or T-E_2-T to slices obtained from males or proestrous and non-proestrous females. The results indicated that the response to the first steroid in a sequence could be reversed by administration of the next (different) steroid. A second administration of the original gonadal hormone did not restore the initial pattern of response. The time course of these effects was such that significant changes were evident at 10 minutes after administration of the hormone and the effects were asymptotic at 20 minutes. Washout experiments revealed that the effects persisted in of steroid-free bathing medium. These results suggest that the two steroids interacted with different receptors. The evidence favors the existence of high-affinity saturable receptor binding of gonadal steroids in the hippocampus. The rapid onset of the effects, however, is not consistent with classical receptor binding models. Such results could be explained by gender-specific receptors on the cell surface, whose affinities for gonadal steroids change in the presence of endogenous hormones.

261 ION EFFLUX DURING T5 BACTERIOPHAGE INFECTION, Donna Duckworth, Jerry Glenn and D.J. McCorquodale*, University of Florida, Gainesville, FL, 32610 and *Medical College of Ohio, Toledo, OH, 43614.

During T5 bacteriophage infection of E. coli, intracellular K^+ and Mg^{++} are rapidly released from the cells. In a productive infection the ion release lasts only six-eight minutes, while if the E. coli contains a colicin Ib plasmid so that the infection is abortive, the ion release continues for at least twenty minutes. Uptake of K^+ and Mg^{++} are not inhibited at any time during the productive infection but do become inhibited at about 8 min post infection during an abortive infection. The rate of loss of K^+ during the initial stages of infection is dependent on multiplicity and does not represent a true decrease in intracellular ion concentration. The rate of loss during the abortive infection is not multiplicity dependent and does represent a decrease in the concentration of intracellular ions. These results are not consistent with a damage-repair hypothesis, but do support the idea that the abortive infection is caused by ion pore formation and may be caused by the colicin Ib protein itself.

262 ATP-DEPENDENT CALCIUM TRANSPORT BY A GOLGI MEMBRANE-ENRICHED FRACTION FROM MOUSE MAMMARY GLAND. M.C. Neville, F. Selker, K.S. Semple, University of Colorado Health Sciences Center, Denver, Colorado 80262 and C.D. Watters, Middlebury College, Vermont 05753

Membrane fractions obtained by homogenization and differential centrifugation of mammary glands from lactating mice accumulated calcium in the presence of ATP. Rapid accumulation was observed at micromolar calcium concentrations in the presence of mitochondrial inhibitors. Steady state concentrations were achieved within 15 minutes when membrane fractions were incubated at 37°C in the presence of ATP. The accumulated calcium was released by the ionophore A23187 indicating transport against an electrochemical gradient. The highest specific activity for calcium transport was found in a partially purified Golgi membrane fraction as identified by the activity of the marker enzyme, galactosyl transferase. This fraction contained minimal numbers of mitochondria as estimated by the succinate dehydrogenase activity. When the Golgi fraction was incubated for 20 seconds at 0°C with γ-[^{32}P]-ATP and then fractionated on SDS-polyacrylamide gels, calcium-dependent, hydroxylamine-sensitive phosphorylation of a 100,000 Dalton protein was observed. These data provide evidence that active, ATP-dependent transport of calcium is the mechanism by which calcium is transferred from the cytoplasm of the mammary alveolar cell to the Golgi-derived secretory vesicles. They further suggest that a calcium ATPase similar to that present in sarcoplasmic reticulum is found in Golgi membranes from mammary glands.

(Supported by NIH grant #15807 to Margaret C. Neville).

263 EPITHELIAN Na^+-CHANNEL: IRREVERSIBLE BLOCKADE BY TYROSINE-SPECIFIC REAGENTS. Chun Sik Park* and Darrell D. Fanestil. Division of Nephrology, Univ of Calif., San Diego, La Jolla, California 92093.

This study sought to elucidate the molecular mechanism involved in the entry of Na^+ across the apical membrane of the toad's urinary bladder. Tyrosine-specific reagents were were used to react covalently with Na^+-channel protein on the apical membrane. The reactivity of reagents was evaluated by measuring Na^+transport using the short-circuit current (SCC) method. SCC was inhibited 50% by N-acetylimidazole, tetranitromethane and 7-chloro-4-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl) at 4.6×10^{-2}, 1.8×10^{-4}, and 3.4×10^{-5} M, respectively. The irreversible inhibition of SCC was accompanied by an increase in the transepithelial electrical resistance. The functional specificity of the reagents to block Na^+ entry via the Na^+-channel was confirmed by the following findings: 1) amphotericin B produced complete recovery of the inhibited SCC to the control level; 2) vasopressin only increased SCC in proportion to the SCC of the control of reagent-inhibited tissues; 3) comparable fractional inhibition of SCC was produced by NBD-Cl under both aerobic and anaerobic conditions; 4) the apparent $K_{\frac{1}{2}}$ for Na^+ was not altered by the reagents; 5) the half time for the inhibition of SCC by NBD-Cl varied as a function of amiloride concentration, being 19 min. in the absence and 46 min. in the presence of 2μM amiloride ($p = .005$). Based on the reactivity and specificity of these tyrosine-specific reagents and on the protection against modification by amiloride, these reagents are believed to be useful chemical probes for the identification and characterization of Na^+-channel protein.

264 AMINO ACID TRANSPORT RATE NORMALIZATION BY FATTY ACID SUPPLEMENTATION IN PANTOTHENATE-DEFICIENT LACTOBACILLUS PLANTARUM, Joseph T. Holden, Joyce Easton, James Bolen and John DeGroot, City of Hope National Medical Center, Duarte, CA 91010.

A pantothenic acid deficiency substantially reduces the lipid content of Lactobacillus plantarum. There are concomitant changes in amino acid transport. The steady state accumulation capacity for all amino acid is reduced. This effect can be reversed osmotically or by allowing such cells to synthesize additional lipid. Lipid depletion appears to reduce accumulation capacity by increasing membrane permeability. In contrast to the effect of a pantothenate deficiency on accumulation capacity, initial transport rates were not uniformly affected. Several systems (alanine, lysine, asparagine) were not altered, whereas others (glutamic acid, aspartic acid, leucine) functioned at a greatly increased rate. This effect cannot be accounted for by increased membrane permeability since the amino acids were transported against substantial concentration gradients. To determine whether this change was attributable solely to the reduced lipid content, transport rates were studied using cells which had been grown in pantothenate-deficient media supplemented with various unsaturated fatty acids. Several fatty acids (cis-vaccenic acid, oleic acid) restored glutamate transport rates to normal in cells which remained markedly pantothenate deficient. The effects of exogenous fatty acids on transport rate and capacity and on lipid content normalization were generally concordant. Therefore, the alteration of transport rates by a pantothenate deficiency reflects the changes in lipid content and is not significantly affected by other metabolic disturbances associated with this deficiency. The mechanism by which reduced lipid levels produce selective amino acid transport rate increases has not yet been established.

265 REVERSAL BY TRYPSIN OF THE INHIBITION OF ACTIVE TRANSPORT BY COLICIN E1. W. A. Cramer, J. Dankert, and S. M. Hammond, Dept. of Biological Sciences, Purdue University, W. Lafayette, Indiana 47907.

The time required for inhibition of proline transport by colicin E1 and the decay of cell viability after incubation in trypsin was measured as a function of temperature using a thermostated flow dialysis system. Complete inhibition of proline transport at 33° and 13° occurs in 0.5 min and 3-5 min., respectively, after addition of colicin E1 at an effective multiplicity of about 4. At these times the fractional cell viability, assayed by dilution directly from the flow dialysis vessel into trypsin, ranges from 35% to 80%, with viability always greater than 50% at the lower incubation temperatures.

The direct addition of trypsin to the flow dialysis vessel, after an addition of colicin E1 that causes complete inhibition of proline or glutamine transport, results in restoration of the transport rate. The restored level is typically about 40% of the control rate, which is very similar to the fractional cell viability measured after incubation in trypsin in the same vessel.

It is concluded that inhibition of active transport by colicin E1, although it occurs very rapidly, is not the physiological event that, by itself, determines the irreversible lethal effect of colicin E1. A structural interpretation of these data would be that after colicin E binds to the receptor, it passes through an intermediate state in which it can span the cell envelope. The effects of colicin would become irreversible when it is no longer accessible to trypsin.

266 K^+ INFLUX COMPONENTS IN ASCITES CELLS: EFFECTS OF AGENTS INTERACTING WITH THE $(Na^+ + K^+)$-PUMP, Tilly Bakker-Grunwald, J. Scott Andrew & Margaret C. Neville, University of Colorado Medical Center, Denver, Co 80262.

The steady-state flux of $^{86}Rb^+$ (as a tracer for K^+) across the ascites cell plasma membrane was differentiated into a ouabain-inhibitable 'pump' component, Cl^--dependent and furosemide-sensitive exchange diffusion, and a residual 'leak' flux. Agents known to interact with the $(Na^+ + K^+)$-pump (ouabain; quercetin; oligomycin; phosphate) all affected both the 'pump'- and the Cl^--linked $^{86}Rb^+$ flux in our strain of ascites cells. The results suggest some, probably indirect, linkage between the $(Na^+ + K^+)$-pump and a KCl symporter; they also illustrate the complications that may arise in the evaluation of K^+ pump rates from unidirectional tracer flux measurements.

267 A MUTANT OF CHINESE HAMSTER OVARY CELLS ALTERED IN AMINO ACID TRANSPORT. Anne H. Dantzig, Carolyn W. Slayman, and Edward A. Adelberg, Departments of Human Genetics and Physiology, Yale University School of Medicine, New Haven, CT 06510
A mutant of Chinese hamster ovary cells was isolated using tritium suicide by allowing cells to incorporate ^{3}H-proline into protein. Survivors were screened by replica plating and autoradiography. The mutant (isolated from a proline auxotroph CHO PEOT/1) showed a reduced ability to grow on growth limiting concentrations of proline when compared to the parent, but showed the parental ability to grow on growth limiting concentrations of potassium. Uptake measurements were determined using Earle's balanced salt solution. The mutant showed a 50 percent reduction in the steady-state accumulation of α-methylaminoisobutyric acid, a model compound for the A system. The initial uptake rate of α-aminoisobutyric acid, but not of other typically A system substrates (alanine, serine, glycine, and proline), was reduced by 50 percent. The initial uptake rate of leucine in sodium-free medium appeared to be enhanced. A model will be presented.

268 ROLE OF Ca^{2+} TRANSPORT IN ERYTHROID DIFFERENTIATION, Linda Chapman, University of Missouri, Columbia, MO 65211
Friend erythroleukemia cells (FV cells) are permanent cell lines which, upon treatment with appropriate chemicals, can be induced to mature along the erythroid pathway. Induction of hemoglobin synthesis by butyrate is inhibited by changing the ratio between internal and external Ca^{2+} concentrations. The concentration ratio can be successfully manipulated by addition to the growth medium of 1) Ca^{2+} channel blocker D600 ($9x10^{-8}$M - $4x10^{-7}$M), 2) Ca^{2+} ionophore A23187 ($1x10^{-7}$M - $2x10^{-7}$M), 3) EGTA at molar concentrations comparable to the Ca^{2+} concentration of the medium formulation ($3x10^{-4}$M). Addition of D600 at concentrations inhibitory to erythropoesis decreased cellular Ca^{2+} levels two fold. Intracellular Ca^{2+} increased only 20% upon addition of A23187 in the absence of inducer. Simultaneous addition of inducer and ionophore produced a five fold increase (compared with the intracellular concentration in the presence of inducer alone). Since induction can be inhibited either by a compound which raises the intracellular Ca^{2+} concentration or by a compound which decreases it, the indication is that a specific Ca^{2+} gradient is required for erythroid differentiation to proceed. In many cell types, maintenance of a low intracellular level of Ca^{2+} relative to the medium enables the transient entry of Ca^{2+} into the cell to be used as a "second messenger." Membrane preparations capable of active transport of Ca^{2+} have been developed to study the mechanism for generating the gradient in FV cells.

269 EFFECT OF FUROSEMIDE ON Na^+ and K^+ FLUXES IN MOUSE L CELLS, David W. Jayme, John J. Gargus, Carolyn W. Slayman and Edward A. Adelberg, Departments of Human Genetics and Physiology, Yale University School of Medicine, New Haven, Connecticut 06510.
Potassium transport mutants have been selected for their ability to grow at 0.2 mM K^+, a concentration too low to sustain growth of the parent strain. These mutants maintain high intracellular potassium when grown in potassium-deficient medium and are unaltered in ouabain-insensitive Na/K pump activity. One class (LTK-5) exhibited an enhanced ouabain-insensitive furosemide-sensitive K^+ influx mode. We examined the growth of the parent strain and LTK-5 in medium containing potassium concentrations at or below the threshhold required for growth and in the presence or absence of furosemide. Rather than inhibiting growth of LTK-5 at 0.2 mM K^+, furosemide enhanced the growth rate of the mutant and allowed the parent strain to proliferate in potassium-deficient medium. Furosemide also greatly increased the efficiency of plating of the parent strain at reduced potassium concentrations. Steady state levels of Na^+ and K^+ of the two strains grown at reduced potassium concentrations in the presence or absence of furosemide, and their associated ouabain-insensitive, diuretic- sensitive potassium fluxes will be discussed.

270 ADENINE TRANSPORT IN NORMAL AND APRT-DEFICIENT L1210 CELLS, M. R. Suresh and N. G. Srinivasan, Scripps Clinic and Research Foundation, La Jolla, California 92037.
The kinetics of adenine transport have been examined in normal and adenine phosphoribosyltransferase (APRT)-deficient L1210 cells. In the former, uptake of [8-^{3}H]adenine is linear for 90 s, and reaches a steady-state level within 120 s. K_t and V_{max} values for adenine transport are 17 μM and 200-400 pmoles/min/mg protein, respectively; these cells also appear to have a higher affinity transport system for adenine (70 nM and 30 pmol/min/mg protein). APRT, the enzyme that converts adenine to AMP, was purified to homogeniety by a procedure that includes chromatography on AMP-Sepharose; the purified enzyme had a K_m value of 167 μM for adenine. Pre-incubation of the L1210 cells with 10 mM inosine or glucose substantially increased the levels of 1-pyrophosphorylribose-5-phosphate and stimulated adenine transport. An L1210 subline, capable of growing in the presence of 20 μM 2,6-diaminopurine, contained less than 10% of the APRT found in normal cells (∿ 300 pmoles/min/mg protein) and transported adenine poorly (K_t ≈ 2.8 mM). These results suggest that adenine transport in L1210 cells is greatly facilitated, but not obligatorily linked to, APRT.
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271 "Mechanism of Cyclic AMP Action on Nutrient Transport in CHO CELLS: a Genetic Approach," Alphonse Le Cam, Michael Gottesman, and Ira Pastan, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

The wild type strain of Chinese Hamster Ovary (CHO) cells responds to a sustained increase in intracellular cAMP levels by a change in cell shape and a decrease in growth rate. We have isolated several mutant cell lines resistant to these effects of cAMP; most of these mutants have altered cAMP-dependent protein kinase activity. In this study we analyzed the effect of cAMP on nutrient transport in both wild type and mutant cell lines. In the wild type cells, exposure to 8 Br-cAMP for 10 to 24 hours resulted in a decrease of 3-O-Me-glucose and AIB transport. Kinetic analysis demonstrated that cAMP reduced the maximal velocity of transport by 1.5 to 2 fold without significantly changing the Km. The same proportion of sugar transport could be inhibited by cytochalasin B in both control and 8 Br-cAMP-treated cells. Using conditions that allowed the discrimination of the three major transport systems (A, ASC and L), we observed that 8 Br-cAMP decreased their activity to the same extent. In the cAMP resistant mutants, no effect of 8 Br-cAMP on sugar or amino acid transport was observed. This study proves that in CHO cells the cAMP effects on transport processes, as well as on cell shape and growth rate, are mediated by cAMP-dependent protein kinase.

272 PAMAMYCIN, A SPECIFIC INHIBITOR OF NUCLEOSIDE, PURINE AND PYRIMIDINE UPTAKE IN STAPHYLOCOCCUS AUREUS, Wen-Gang Chou and Burton M. Pogell, DEPARTMENT OF MICROBIOLOGY, ST. LOUIS UNIVERSITY, ST. LOUIS, MO 63104
Pamamycin is a new alicyclic antibiotic isolated from *Streptomyces alboniger* (J. Antibiotics. 1979. 32: 673-678). It is active against Gram-positive bacteria, Mycobacteria and *Neurospora crassa*. It also stimulates aerial mycelium formation in the producing organism. *In vivo* studies in *S. aureus* show that pamamycin inhibits nucleoside and nucleic acid base uptake but, under the same conditions, has no effect on amino acid or 2-deoxyglucose uptake, protein synthesis, cell wall synthesis or glucose utilization. The fact that accumulation of uridine into the TCA-soluble pool is inhibited more than RNA synthesis suggests that a specific transport process is affected. Isolated membrane vesicles from *S. aureus* grown in brain heart infusion broth accumulate nucleosides when L-lactate, α-glycerol-P or PMS + ascorbate are used as the electron donor. Accumulated uridine remains unchanged chemically. Pamamycin inhibits these transport systems at similar concentrations which inhibit *in vivo* uptake. Adenine and uracil are not accumulated in the above three systems. Amino acids are accumulated in the presence of L-lactate or PMS + ascorbate; however, pamamycin inhibits *in vitro* amino acid transport, though to a lesser extent than that seen with nucleosides. Since pamamycin binds to membranes and does not inhibit membrane-bound α-glycerol-P dehydrogenase or NADH oxidase, its site of action is probably on a specific component of the energy coupling system.

273 IRON TRANSPORT IN CULTURED HEART MUSCLE CELLS, B. R. Byers, C. V. Sciortino and P. G. Cox, University of Mississippi Medical Center, Jackson, Mississippi 39216.
Primary cultures of beating heart muscle cells (obtained from 2-5 day old rats) were used to study iron transport, cellular iron distribution, iron overload, and effects of chelators on iron transport. Rates of iron uptake from various concentrations of 100% radioiron-saturated transferrin indicated a saturable transport/assimilation system. Conversely, uptake from $^{55}FeCl_3$ or [^{55}Fe]ferric ammonium citrate appeared non-saturable and yielded high cellular iron levels. From any of the 3 iron sources ferritin synthesis was rapid, and ferritin was visualized after 48 hours in lysosomes by electron microscopy. Fractionation of cells labelled for 24 hours with [^{55}Fe]transferrin showed ^{55}Fe distributed in the 34,000 x g pellet (32%), in the 100,000 x g pellet (9%), in ferritin (46%) and in a non-ferritin cytosol component(s) (17%). Pulse/chase experiments revealed rapid movement of iron from the non-ferritin cytosol component to both ferritin and to the 34,000 x g pellet; this cytosol component may be a "pool" of iron. The iron-chelating drug, Desferal, did not significantly lower uptake of [^{55}Fe]transferrin; Desferal did shift some ^{55}Fe from ferritin to the non-ferritin cytosol fraction. Desferal blocked uptake of $^{55}FeCl_3$. One chelator (a phenolate of microbial origin) inhibited uptake of both [^{55}Fe]transferrin and $^{55}FeCl_3$; drugs based on this structure may be useful in treating iron-storage diseases in which heart damage can be serious and sometimes fatal. Uptake of high levels of iron from ferric ammonium citrate may have caused some cell damage as evidenced by loss of activity for both the marker enzymes lactate dehydrogenase and creatine phosphokinase.

274 MODULATION OF D-GLUCOSE TRANSPORT ACTIVITY IN ADIPOCYTE PLASMA MEMBRANES BY AGENTS AFFECTING BILAYER FLUIDITY. Peter A. Thompson, Paul F. Pilch, and Michael P. Czech. Brown University, Providence, RI 02912
Plasma membranes were prepared from untreated and insulin-treated rat adipocytes ("untreated" and "insulin-treated" membranes), incubated with a saturated or a monoenoic fatty acid, and D-^{3}H glucose uptake was measured in the resultant vesicles. Incorporation of fatty acids was accomplished by incubating plasma membranes (protein ca 3 mg/ml) in 10mM Tris, 1mM EDTA, pH 7.4, with a solution of 100mM fatty acid in ethanol, or ethanol alone, to final concentration of 2.4mM fatty acid and 2.4% ethanol for 30 min at either 0° (monoenoic) or 25° (saturated). This resulted in incorporation of ca 70-90% of the fatty acid added. Incubation of untreated membranes with either of the cis-monoenoic fatty acids, oleate or cis-vaccenate, resulted in markedly increased rates of D-^{3}H glucose into membrane vesicles over control (ethanol incubation) vesicles. Hexose uptake in cis-vaccenic acid-treated vesicles was stimulated to levels observed in insulin-treated membranes; moreover, incubation of insulin-treated membranes with cis-vaccenate did not result in further stimulation of glucose uptake. In contrast, incubation of membranes with a saturated fatty acid (palmitic or stearic) greatly diminished the stimulated levels of D-glucose uptake in insulin-treated membranes without inhibiting transport in untreated membranes. Increasing the fluidity of isolated liver plasma membranes, in which hexose transport is not insulin-sensitive, did not affect D-glucose uptake. Since the incorporation of unsaturated fatty acids is known to increase and saturated fatty acids to decrease membrane fluidity, these results suggest a possible role for bilayer fluidity in the modulation of hexose transport by insulin.

275 CHROMATOGRAPHIC RESOLUTION OF THE ADIPOCYTE HIGH AFFINITY INSULIN RECEPTOR FROM THE INSULIN SENSITIVE D-GLUCOSE TRANSPORTER, Christin Carter-Su, Paul F. Pilch, and Michael P. Czech, Brown University, Providence, RI 02912
^{125}I-Insulin was covalently linked to high affinity receptors in dimethylmaleic anhydride-extracted rat adipocyte plasma membranes using the cross-linking reagent disuccinimidyl suberate as previously described. Hydroxylapatite chromatography resolved the cholate-solubilized proteins into three main peaks. The largest peak contained about 50% of the protein, 75-85% of the bound 125I-insulin and no transport activity as assessed by the ability of the proteins to exhibit cytochalasin B-sensitive D-glucose transport activity subsequent to their reconstitution into phospholipid vesicles. The second major peak contained 35-40% of the protein, only 15-25% of the bound ^{125}I-insulin and all of the D-glucose transport activity. Autoradiograms of these column fractions electrophoresed on sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE) confirm the presence of only a small fraction of the insulin receptor in the second peak as well as a significant amount of 125I-insulin linked to low affinity binding proteins. Using DEAE anion exchange chromatography, less than 5% of the bound ^{125}I-insulin was recovered in the tightly bound protein fraction (elution between 1M and 4M NaCl) which contained all the recoverable transport activity. Autoradiography of SDS-PAGE gels of the protein revealed no high affinity receptors in this fraction. These data indicate that the insulin receptor and D-glucose transporter represent distinct polypeptides in the fat cell membrane, and that some membrane transduction mechanism must mediate hexose transport activation due to insulin-receptor interaction.

276 FREEZE-FRACTURE PATTERNS OF RETINAL PHOTORECPTOR MEMBRANES FROM ULTRA-RAPIDLY FROZEN ISOLATED ROD OUTER SEGMENTS. M.J. Costello, Dept. of Anatomy, Duke University Medical Center, Durham, NC 27710.

Freeze-fracture electron microscopy can provide a partial description of the organization of membrane components in retinal rod outer segment (ROS) disks, once the locations of the fracture planes have been established. Suspensions of isolated ROS were sandwiched between thin copper strips and quenched (passing from 0°C to -100°C at rates in excess of 10,000°C/s) by rapidly plunging streamlined sample holders into liquid propane (-190°C). Excellent preservation of the regular arrangement of disk membranes without ice crystal damage was obtained in the absence of fixatives or cryoprotectants. It was concluded that the fracturing of ROS disks normally occurs within membranes (as opposed to occuring along membrane/aqueous interfaces), based on the symmetry of the fractures through stacked disks and on the presence of small, distinct steps from aqueous compartments onto membrane fracture faces. The rough fracture face, therefore labeled PF, displays an irregular pattern of interconnecting ridges, and the relatively smooth face, labeled EF, contains a similar distribution of crevices. In rare cases, extensive longitudinal fractures of ROS reveal that some disks are entirely pulled out during fracturing, leaving deeply scalloped impressions of the cytoplasmic membrane PS-face in the adjacent aqueous compartment. Such observations suggest that the bulk of the principal structural unit, probably a rhodopsin/lipid complex, is located at the cytoplasmic interface and that a significant portion of the complex which spans the disk membrane is exposed during fracturing.

277 MECHANISM OF ACTION OF PLASMA FIBRONECTIN AS A RECOGNITION FACTOR FOR PHAGOCYTOSIS OF NEUTRAL COLLOIDS, J. Molnar*, M.Z. Lai*, L. Gabelman*, P. Gudewicz", G.E. Siefring,Jr. ¶, Departments of Biochemistry* and Physiology", University of Illinois at the Medical Center, Chicago, IL. 60612 and Department of Biochemistry and Molecular Biology¶, Northwestern University, Evanston, IL. 60201.

Fibronectin was isolated from human plasma by affinity chromatography on a gelatin-Sepharose-4B column by using arginin for specific elution. The product showed high purity by electrophoresis on polyacrylamide in presence of SDS and had high activity in the phagocytosis assay systems. The phagocytosis promoting activity was tested by a liver slice assay as well as via rat peritoneal macrophages by using ^{125}I-gelatin coated latex particles (g-Ltx*). Phagocytosis by macrophages was inhibited by agents known to disrupt the cytoskeleton system (Colchicin, vincristin and cytochalasin B). Drugs, causing increased intracellular cyclic AMP levels (dibutyril-cAMP, aminophyllin, isoproterenol and prostaglandin E_1) also reduced significantly the amount of gLtx* uptake. Fibronectin was shown to bind specifically to g-Ltx*, heparin enhanced while free gelatin inhibited this binding. The opsonized particles after centrifugation and washing could be used in phagocytic experiments without further requirement for fibronectin, however, addition of heparin was needed for optimal phagocytosis. Gelatin or gelatin-fibronectin complex did not cause inhibition of phagocytosis of preopsonized g-Ltx*. The data showed that fibronectin mediated phagocytosis requires an intact cytoskeleton system and modulated by intracellular cyclic AMP. The surface receptors of macrophages recognize fibronectin-gelatin heparin complexes only when these are attached to larger particles.

278 Transepithelial Transport of Organic Cations, Peter D. Holohan and Charles R. Ross, SUNY/Upstate Medical Center, Syracuse, N.Y. 13210

The mammalian kidney actively secretes drugs by two separate and distinct systems. One for organic cations and the other for organic anions. The mechanism of organic cation transport was studied (using N^1-methylnicotinamide (NMN) as the prototype drug) in separated luminal (or brush border) and antiluminal (or basal-lateral) membrane vesicles prepared from dog kidney cortex. Specific transport processes were found in both membranes. The two systems differed however, on the basis of: kinetic parameters (the K_m and V_{max} values in the two membranes were different); and countertransport studies (counterflow of NMN was observed only in the luminal membrane). These features were incorporated into a model to explain renal secretion (JPET, 209, 443, 1979). Additionally, it was hypothesized that transport across the luminal membrane was the "active" step in the movement of cationic drugs from blood to urine. Further studies on the luminal membrane suggest: 1)transport is "secondarily-active", with the energy supplied by a proton gradient which in turn is created by a Na^+:H^+ antiport system (i.e., two functionally-linked antiport systems drive secretion); 2) translocation is "carrier" mediated; 3)a series of drugs known to be secreted by the kidney are all transported by the same carrier but are translocated at different rates; 4)the carrier is kinetically asymmetrical in that the V_{max} for influx and efflux differ; and 5)the unloaded carrier is negatively charged and therefore in the cell is probably orientated to the exterior. Transport across the antiluminal membrane does not appear to be via a "carrier" but rather displays properties more consistent with a "gated channel" as described by Deves and Krupka (BBA, 513, 156, 1978).

279 STUDIES ON THE MECHANISM OF THE GLUCAGON ELICITED CHANGES IN MITOCHONDRIAL Ca^{2+} UPTAKE. Naomi Friedmann, University of Texas School of Medicine, Houston, Texas 77025.

It has been demonstrated previously that glucagon administration to the perfused liver results in an immediate efflux of Ca^{2+} and activation of protein kinase(s) (Friedmann, N., Biochem. Biophys. Acta 423:495, 1976). This is followed by a hyperpolarization of the cell membrane increase in mitochondrial calcium uptake, which is probably the result of the increased respiration also observed (Yamazaki, R.K., J. Biol. Chem. 250:7924, 1974). In order to gain some insight into the mechanism by which the mitochondrial changes are elicited, livers were perfused in a variety of conditions known to have differential effects on ion fluxes and activation of protein kinase(s). Administration of insulin prior to glucagon blocked the effect of glucagon on Ca^{2+} efflux, hyperpolarization and mitochondrial Ca^{2+} uptake. Perfusion of the liver with Na^+-free perfusate (sodium being replaced with either Li^+ or choline) reduced the efflux of Ca^{2+}, blocked hyperpolarization and the changes in mitochondrial Ca^{2+} uptake. 106mM K^+, had similar effects. Because neither tetracaine nor Na^+-free perfusate blocks the activation of adenylate cyclase, or the activation of protein kinase(s), it is concluded that the changes in mitochondrial behavior are probably the consequence of the prior changes in ion fluxes and membrane potential.

280 Transport of amino acid mediated by γ-glutamyl transpeptidase in reconstituted liposomes. Vijay K. Kalra and Suresh C. Sikka, Dept. of Biochemistry, Univ. of Southern California, School of Medicine, Los Angeles, CA 90033.

γ-glutamyl transpeptidase, an enzyme, that catalyzes the transfer of γ-glutamyl moiety of glutathione and other γ-glutamyl compounds to a variety of amino acids and peptide acceptors has been postulated by Meister and his coworkers to play a role in the amino acid transport via γ-glutamyl cycle. γ-glutamyl transpeptidase purified from hog kidney was incorporated into phospholipid liposomes of different charge and fluidity. Studies show that detergent purified enzyme can be incorporated into neutral and acidic liposomes but not in basic liposomes. Moreover, γ-glutamyl transpeptidase reconstituted liposomes exhibited the uptake of glutamic acid only when the liposomes contained intravesicular glutathione. The uptake was inhibited (80-90%) by serine-borate (20 mM) which inhibit γ-GTase activity. Data will be presented showing that the uptake of glutamic acid represents transport into the liposomes rather than the binding. Our studies support the hypothesis that the translocation of amino acid can be mediated by γ-glutamyl transpeptidase. Supported by NIH grant GM 26013.

281 PEPTIDE TRANSPORT AND UTILIZATION IN EUKARYOTIC CELLS, Lloyd Wolfinbarger, Jr. Old Dominion University, Norfolk, VA 23508

Studies in the transport and hydrolysis of radiolabeled peptides suggest that eukaryotic microorganisms possess the ability to transport peptides larger than dipeptides but smaller than hexapeptides. The system(s) responsible for peptide transport has been designated as an oligopeptide transport system and this system requires the presence of a free alph-amino group on the small peptide, but does not appear to discriminate between the residue amino acids in the peptide. Peptide transport is sensitive to metabolic poisons and the peptide is hydrolyzed to its residue amino acids during or immediately after the membrane translocation step. Intracellular peptidohydrolytic activity possesses a broad range of specificities and essentially all peptides transported are hydrolyzed. It is suggested that the differential mechanisms by which eukaryotic microorganisms respond tolarge and small peptides may serve as a model system to explain how eukaryotic mammalian cells respond to large and small peptides as hormones.

282 PHORBOL ESTER AND PROSTAGLANDIN $F_{2\alpha}$ SIGNAL TRANSDUCTION MEDIATED BY THE $(Na^{+}+K^{+})$-PUMP IN MOUSE FIBROBLASTS, C. E. Wenner, J. Cheney and L.D. Tomei, Department of Experimental Biology, Roswell Park Memorial Institute, Buffalo, NY 14263

In postconfluent 3T3 mouse fibroblasts, Tetradecanoyl phorbol acetate (TPA) or $PGF_{2\alpha}$ as well as serum stimulate ^{3}H-TdR incorporation into DNA and an early ouabain-sensitive $^{86}Rb^{+}$ influx. Ouabain also prevents the initiation of DNA synthesis after serum addition to postconfluent 3T3 cells. This early increase in $(Na^{+}+K^{+})$ pump-mediated activity by these agents which are also mitogenic raises the question as to the relationship of K^{+} movements to other metabolic events and the subsequent initiation of DNA synthesis.

The hypothesis that an increase in $(Na^{+}+K^{+})$-ATPase is responsible for the altered metabolic events which leads to rapid proliferation was examined with the use of the C3H 10T½ fibroblasts, where cell cycle is also activated by serum in quiescent cultures. The introduction of either 10^{-7}M TPA or 10^{-7}M $PGF_{2\alpha}$ stimulated ouabain-sensitive $^{86}Rb^{+}$ influx at 30 mins. in postconfluent C3H 10T½ cultures by 33-50% as did serum. However, serum or TPA but not PGF_2 at these concentrations enhanced by over 50-fold the incorporation of ^{3}H-TdR into DNA (specific activity as determined by fluorometric measurement of DNA with a modified method of Kissane and Robbins). Thus it is concluded that while modulation of membrane events such as the $(Na^{+}+K^{+})$ pump activity in fibroblasts may be necessary for initiation of DNA synthesis, this membrane change is not sufficient in itself to set in motion the proliferative response. (Supported by USPHS - CA-13784)

283 THE HEXITOL TRANSPORTSYSTEMS AS CHEMORECEPTORS IN E. COLI K12, Joseph W. Lengeler, University of Regensburg, Regensburg, Germany

In E. coli K12, there exist three enzymeII-complexes (EII) of the PEP-dependent phosphotransferase system (PTS), specific for D-mannitol, D-glucitol, and galactitol respectively. They apparently consist of a single protein each, coded for by the genes mtlA, gutA and gatA, whose synthesis and activity is regulated by a series of control mechanisms. The EII's are the molecules responsible for substrate specificity and affinity at the same time in uptake/phosphorylation and in the chemotaxis towards these substrates. As for other carbohydrates, it is not essential that the attractants are metabolized, but it is sufficient that they are transported/phosphorylated. The properties of the EII's tested thus far (substrate specificity or affinity and binding capacity; regulation of synthesis and activity) all are identical, wether tested in transport or in chemotaxis assays. During uptake and phosphorylation of substrates, the EII's alternate between a phosphorylated-high-affinity, and a dephosphorylated- low-affinity state. This conformational switch could be the signal which triggers a transductional signal and eventually the chemotactical response, since mutants able to bind the substrate, but unable to phosphorylate the EII's no longer respond chemotactically to hexitols.

284 STRUCTURAL STUDIES OF THE METHYL ACCEPTING CHEMOTAXIS PROTEINS OF ESCHERICHIA COLI: EVIDENCE FOR MULTIPLE METHYLATION SITES, Frederick W. Dahlquist and Daniel Chelsky, Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403

Two methyl-accepting chemotaxis proteins, MCP I and MCP II in Escherichia coli, are necessary for the behavioral response to two complementary sets of sensory stimuli. Two-dimensional analysis of tryptic peptides from [^{35}S]-methionine labeled MCP I and MCP II demonstrates a high degree of homology between the two proteins. After labeling the methylation sites with S-adenosyl-L-[(Methyl-^{3}H)]-methionine, three peptides in each protein were found to carry a methyl group. These multiple methylations appear to be responsible in part for the observed multiple banding patterns on SDS-polyacrylamide slab gels.

285 ADAPTIVE ALTERATION IN PHOSPHOLIPID COMPOSITION OF PLASMA MEMBRANES FROM A SOMATIC CELL MUTANT DEFECTIVE IN THE REGULATION OF CHOLESTEROL BIOSYNTHESIS, Michael S. Sinensky, Eleanor Roosevelt Institute for Cancer Research, Denver, CO 80262

A somatic cell mutant (CR1) of a Chinese hamster ovary cell (CHO-K1) which has previously been shown to be defective in the regulation of cholesterol biosynthesis accumulates more cholesterol than the parental cell line in plasma membranes. Although such an increase in membrane cholesterol should lead to an increase in the order parameter of these membranes, as measured with an ESR spin probe, the order parameter of mutant and wild-type plasma membranes are identical - apparently due to an adaptive alteration in membrane phospholipid composition. The phospholipid compositions of mutant and wild-type cell plasma membranes are compared and the mutant is shown to have a 3-fold higher level of oleic acid and a 2-fold lower level of phosphatidyl ethanolamine than the wild-type. These results are consistent with model studies which show that these compositional changes lead to lower order parameters for phospholipid dispersions.